Modification Form for Permit BIO-UWO-0194

Permit Holder: Lynne-Marie Postovit

Approved Personnel
(Please stroke out any personnel to be removed)

Giovanni Poggenpoel

Kevin Kania

Alia Cloutier-Bosworth

Amelia Nuhn

Dylan Dieters-Castator

Courtney Brooks

Chris Hughes

Michael Jewer

Scott Findlay

Padmalaya Das

Guihua Zhang

Jeff Law

Meghan Taylor

Daniela Quail

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms	E.coli top 10	
Approved Primary and Established Cells	Human [established]: T47D, MCF7, Hs578T, Hs578Bst, MDA-MB-231, MDA-MB-468, MDA-MB-435, JEG-3, JAR, BeWO, HuVEC, NIH 3T3, C8161, C81-61, H1 hESC, H9 hESC, CA1, CA2, MCF-10A, MCF-10A-	
Approved Use of Human Source Material	human organs or tissues (preserved)	
Approved Genetic Modifications (Plasmids/Vectors)	pGIPZ, pGL3-Promoter Vector, pGL3-Control Vector, pGL4.70(hRUC) Vector, pGFP-V-RS, pReceiver-M13, pTRIPZ, pCMV6-XL4, pCR 4-TOPO, pCDNA3, pCMV6-XL5, ptdTomato	FRE5, FRE5CF, RG6 (plasmids)

Approved Use of Animals	HSD;ATHYMIC NUDE-FOXN1NU/FOXN1	
Approved Biological Toxin(s)		

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: Lynne-Marie Postovit

Current Classification: 2 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Nov 15, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

Description of use (from MTA): The research material and unmodified derivatives thereof ("Research Material") will be used only for non-commercial research uses in the laboratory of Recipient's Principal Investigator and in connection with the following research project ("Research Project") (or use an attachment page):
Investigating the alternative splicing of a novel exon for human Nodal transcript. This will include studying how patterns of alternative splicing of Nodal differ between cell types, how the Nodal splicing decision is regulated, and what genetic elements are involved.

The attached paper describes the plasmids.

Published online 16 November 2006

Nucleic Acids Research, 2006, Vol. 34, No. 22 ***e148***
doi:10.1093/nar/gkl967

A bichromatic fluorescent reporter for cell-based screens of alternative splicing

James P. Orengo[1,2], Donnie Bundman[1] and Thomas A. Cooper[1,2,*]

[1]Department of Pathology and [2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA

Received September 26, 2006; Revised October 16, 2006; Accepted October 17, 2006

ABSTRACT

Alternative splicing is the primary source of proteome complexity in metazoans and its regulation shapes the proteome in response to shifting physiological requirements. We developed a bichromatic splicing reporter that uses a peculiar feature of some fluorescent protein coding regions to express two different fluorescent proteins from a single alternative splicing event. The mutually exclusive expression of different fluorescent proteins from a single reporter provides a uniquely sensitive approach for high-throughput screening and analysis of cell-specific splicing events in mixed cell cultures and tissues of transgenic animals. This reporter is applicable to the majority of alternative splicing patterns and can be used to quantify alternative splicing within single cells and to select cells that express specific splicing patterns. The ability to perform quantitative single-cell analysis of alternative splicing and high-throughput screens will enhance progress toward understanding splicing regulatory networks and identifying compounds that reverse pathogenic splicing defects.

INTRODUCTION

Alternative splicing exponentially increases the number of proteins expressed from a surprisingly limited number of genes. Beyond simply generating proteome diversity, the regulation of alternative splicing is responsible for a recently recognized level of complexity of post-transcriptional regulation of gene expression. Recent biocomputational and microarray analyses have demonstrated the extent to which regulation of alternative splicing modulates proteome diversity in response to dynamic cellular requirements and indicate the presence of regulatory networks interconnected with the intricate control mechanisms of gene expression [reviewed in (1)]. There is also growing awareness of the significant role that the disruption of splicing plays in human disease (2,3).

Identification of splicing regulators has been accomplished primarily through biochemical and genetic approaches (4). The combination of this gene-by-gene approach with global approaches would greatly enhance progress toward deciphering signaling events and regulatory networks that control splicing. Recently, several reports have demonstrated the utility of a fluorescent protein read out for alternative splicing in cell culture and transgenic mice (5–12). These splicing reporters have been used in screens for RNA binding proteins or upstream signaling pathways that modulate specific splicing events or for identifying cells within a mixed population that express a splicing pattern that differs from the majority of the cells in the population. This approach typically utilizes alternative splicing to control on–off expression of GFP such that inclusion or skipping of a variable region puts GFP in frame. The downside to using a monochromatic readout for alternative splicing is that the splicing pattern producing GFP cannot be quantified relative to the other splicing pattern(s) expressed from the reporter. Detection of GFP does not determine whether the GFP mRNA represents a majority or a small minority of the mRNAs from the reporter. In addition, monochromatic reporters require all-or-none splicing decisions for on or off expression of GFP because it is difficult to compare fluorescence intensity of different cells without an internal control. As the majority of alternative splicing events are not regulated as all or none, splicing events must be manipulated to fit into an all-or-none output. One successful approach that has been utilized in cell cultures and transgenic animals is to co-express GFP and mRFP1 from two separate reporters (10,12).

We developed a novel approach to quantify the ratio of two alternative splicing pathways expressed from a single reporter in which enhanced green fluorescent protein (EGFP) is expressed from one splicing pathway and dsRED is expressed from the other. The approach uses an unusual feature of some fluorescent proteins that contain an alternate reading frame that lacks stop codons. The reporter described here contains dsRED upstream of EGFP such that dsRED and EGFP are expressed from the two different reading frames; one for dsRED, and the other for the dsRED alternate reading frame fused with EGFP. Inclusion or skipping of a variable region of the appropriate size located upstream of dsRED allows toggling between the dsRED and EGFP reading

*To whom correspondence should be addressed. Tel: +1 713 798 3141; Fax: +1 713 798 5838; Email: tcooper@bcm.tmc.edu

frames. The reporter can be used with all alternative splicing patterns that generate internal variability within an mRNA (cassette and mutually exclusive exons, alternative 3′ and 5′ splice sites and retained introns). The resulting mutually exclusive expression of dsRED and EGFP proteins provides high sensitivity and a quantitative measure of the ratio of splicing patterns. We demonstrate that this reporter can be used to quantify the ratio of alternative splicing events within individual cells, for flow cytometry and is applicable to Fluorescence Activated Cell Sorting (FACS) analysis, and can be used to identify cells expressing different splicing patterns within a mixed cell culture. The reporter is engineered such that genomic segments containing variably spliced regions of interest can be easily 'cut and paste' into convenient restriction sites. This alternative splicing reporter will be useful for high-throughput screening to identify direct regulators and the signaling pathways that modulate a specific splicing event or agents that reverse aberrant splicing events that are the cause of disease.

METHODS AND MATERIALS

Plasmid construction

The FRE5 expression plasmid was constructed by PCR amplifying EGFP from pEGFP-N1 (Clontech) using primers that removed the EGFP translation initiation codon. The EGFP open reading frame (ORF) was place into pcDNA3.1-HisC (Invitrogen) in which the Xpress tag had been replaced by the FLAG epitope tag and a downstream nuclear localization signal (NLS). The primers used to PCR amplify EGFP were designed such that EGFP was flanked by restriction enzymes that are unique to the final RG6 plasmid. The dsRED ORF was PCR-amplified from pCX-dsRed (obtained from Dr A. Nagy) without its initiation codon. The dsRED primers also flanked the ORF with restriction enzymes that are unique to the final RG6 plasmid (Figure 3A). The dsRED sequence was placed upstream of EGFP such that the alternate ORF of dsRED was in frame with the downstream EGFP. The reading frame was also designed such that the initiating ATG for FLAG–NLS was in frame with EGFP in FRE5 and that addition of 2 nt (filled in ClaI site generating FRE5Cf, Figure 1B) shifted the reading frame to dsRED.

The RG6 minigene was derived from FRE5 by inserting PCR-generated segments containing introns 4 and 5 of chicken cardiac troponin T (13). The chicken cTNT genomic fragment was amplified in two segments to introduce the natural introns 4 and 5, an artificial alternative exon in place of exon 5 and the 3′ splice site of intron 5 + 35 nt of exon 6 which contains purine rich motifs resembling an exonic splicing enhancer (14). The artificial exon is flanked by restriction sites that are unique to the plasmid. Several exons of different size and sequence composition were tested to generate a construct that produced 1:1 exon skipping:inclusion in COSM6 cells. The complete sequence of the RG6 plasmid is posted at http://www.bcm.edu/pathology/labs/cooper/reagreq.htm.

RG6ME (minus exon) and RG6PE (plus exon) constructs contain cDNAs of the RG6 mRNAs that lack and include, respectively, the alterative exon. The XbaI/AgeI fragments

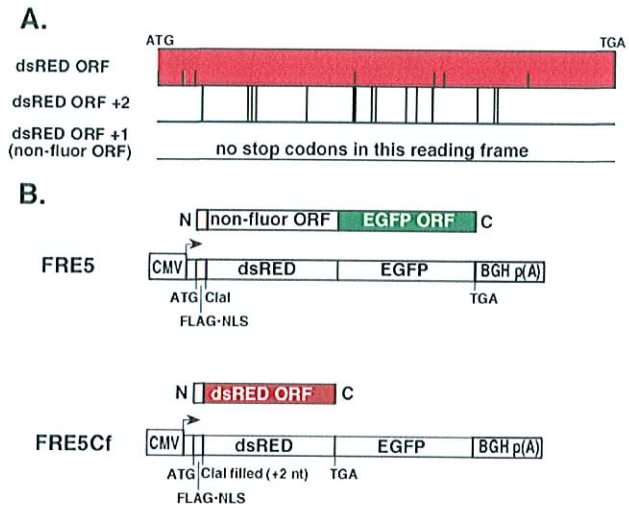


Figure 1. Strategy for bichromatic expression of dsRED and EGFP from alternative splicing pathways. (**A**) The three reading frames of dsRED showing the dsRED ORF (red) and illustrating that the +1 reading frame lacks translation stop codons. Lines that extend across the whole reading frame indicate translation stop codons and lines that extend to the bottom third of the reading frame indicate ATG codons. (**B**) Expression constructs to determine whether the alternate ORF of dsRED is efficiently expressed in mammalian cells. FRE5 and FRE5Cf differ by insertion or deletion of 2 nt (ClaI filled in) between the NLS and dsRED coding regions. The predicted proteins are shown above the gene diagram. Transcription initiation is indicated by the arrows.

from the amplified PCR products from the spliced RG6 mRNAs that include or exclude the alternative exon were cloned into the XbaI/AgeI sites of the RG6 plasmid (see Figure 1A). Therefore, RG6ME and RG6PE express mRNAs identical to those from the spliced mRNAs from RG6 that lack and include the alternative exon, respectively.

Transfection, RNA extraction and RT–PCR

Cell cultures for COSM6, C2C12 and primary chicken breast muscle cultures were prepared, maintained and transiently transfected as described previously (15,16) except that 1 μg of plasmid DNA was used. RT–PCR was performed as described previously (15) using RGf (CAAAGTGGAGGAC-CCAGTACC) and RGr (GCGCATGAACTCCTTGATGAC).

Fluorescence microscopy

COSM6 cells were plated at a density of 1.5×10^5 cells per well on No. 1 coverslips (Fisher brand) for transfection. Forty-eight hours post-transfection, coverslips were washed once with chilled 1× PBS (phosphate-buffered saline). Cells were fixed in 4% paraformaldehyde for 30 min on ice, followed by three washes with 1× PBS at RT. To minimize autofluorescence, cells were quenched with 0.1 M ammonium chloride in 1× PBS for 10 min at room temperature, followed by two washes with 1× PBS. Cells were then permeabilized with 0.5% Triton X-100 for 5 min at room temperature, followed by three washes with 1× PBS, and a final rinse with ddH_2O. Fixed coverslips were mounted on VWR micro slides (Superfrost Plus) 25 × 75 × 1 mm with Vectasheild hard set

mounting media containing DAPI (Vector). Slides were allowed to dry in the absence of light for 15 min at RT.

Images were collected using FITC channel 488 nm and Texas Red channel 594 nm. Healthy cells with intact nuclei as visualized by DAPI staining and cells which expressed both red and green were selected. Images were collected with the exposure time kept constant between the red and green channels for each individual cell. The collected images were exported as TIFF files and opened with Image J software. Nuclei were identified by DAPI staining and a region of interest (ROI) was drawn by hand to incorporate the entire nucleus. Image J software was used to compute the average red and green intensity for the ROI. The percent green expression was calculated by dividing the green intensity by the sum of the red and green intensity multiplied by 100.

Flow cytometry analysis

A total of 1.5×10^5 cells were plated per six well plate, transiently transfected with RG6 alone or with Xpress-ETR-3 or FLAG-MBNL3 (transfection efficiency ~45%). Forty-eight hours following transient transfection, cells were dissociated in 0.25% trypsin with vigorous pipetting to completely dissociate cells, centrifuged a 800× g for 5 min with slow stop and resuspended into 0.5% FBS/1× PBS. Cells were spun down again and resuspended in 1% paraformaldehyde in PBS for flow cytometry. Flow cytometry was performed on FACScan (Becton Dickinson), and was analyzed using FlowJo software (Tree Star).

RESULTS

Our goal was to develop a splicing reporter in which each of two alternative splicing pathways would express different fluorescent proteins. We noticed a peculiar feature of dsRED in that the +1 reading frame contains no stop codons (Figure 1A). This feature allowed us to design an expression construct with EGFP placed downstream from dsRED in mutually exclusive reading frames: one for dsRED and the other for a fusion protein between the dsRED alternate reading frame and EGFP (Figure 1B). Insertion or removal of a variably spliced region located upstream of dsRED and EGFP can be used to toggle between the dsRED and EGFP reading frames based on the size of the variable region.

To test whether this was a viable strategy, and in particular to determine whether the individual reading frames will express the expected proteins, we generated two expression plasmids for either the dsRED or EGFP reading frames (FRE5Cf and FRE5, respectively, Figure 1B). Of particular concern, it was unclear whether the dsRED alternate reading frame would be expressed in mammalian cells. In the mRNAs from both plasmids (which represent the two reading frames from the planned bichromatic reporter), translation initiates from the same start codon at position 90 of the mRNA and both proteins contain an N-terminal FLAG epitope tag for detection by western blotting and a NLS to concentrate the fluorescent proteins in the nucleus for increased sensitivity. The predicted protein expressed from FRE5 contains the dsRED alternate reading frame (231 amino acids) fused to EGFP for a total of 498 amino acids (MW = 55.7 kDa). FRE5Cf was derived from FRE5 by filling in a unique ClaI restriction site between the FLAG–NLS and the dsRED ORF to add 2 nt and switch the reading frame to dsRED (MW = 29.4 kDa). FRE5 and FRE5Cf plasmids were transiently transfected in COSM6 cells. Forty-eight hours after transfection, cell cultures were used to prepare whole cell protein extracts for western blotting or mounted for fluorescence microscopy. Western blot analysis using anti-FLAG antibodies demonstrated that FLAG-tagged proteins of the expected size were expressed from FRE5 and FRE5Cf (Figure 2A). Trace amounts of additional proteins were detected upon overexposure of the blot, suggesting expression of minor amounts of longer (FRE5Cf) and truncated (FRE5) proteins containing the FLAG epitope. The minor FRE5 protein was slightly larger than GFP. Antibodies to EGFP detected only the expected protein from the FRE5 plasmid (Figure 2A) indicating that the smaller protein was not due to internal initiation upstream of EGFP and will not produce interfering GFP fluorescence.

Fluorescence microscopy demonstrated that cells transfected with FRE5 and FRE5Cf expressed green and red fluorescence, respectively, which was localized to nuclei (Figure 2B). Equal exposures of cells transfected with either plasmid demonstrated that red fluorescence was not detected in cells transfected with FRE5 and green fluorescence was only minimally detected in cells transfected with FRE5Cf. We conclude that both the dsRED and EGFP fluorescent proteins are efficiently expressed and expression of the two reading frames is mutually exclusive. Specifically, the alternate reading frame of dsRED efficiently expresses an EGFP fusion protein of the expected size. We also demonstrated that FLAG-NLS-tagged protein from the dsRED alternate reading frame lacking EGFP that was readily detectable by western blotting was not detectable by fluorescence microscopy using filters to detect DAPI, dsRED or EGFP (data not shown). Therefore, the protein from the alternate reading frame of dsRED does not interfere with detection of dsRED or EGFP by fluorescence microscopy.

Quantification of alternative splicing pathways using a bichromatic reporter

Having demonstrated that the different fluorescent proteins from mutually exclusive reading frames can be expressed, we next generated a construct in which alternative splicing would toggle between the two reading frames. We chose to use the well characterized chicken cardiac troponin T (cTNT) regulated alternative splicing event. Two genomic fragments containing the introns and exons flanking the cTNT alternative exon (exon 5) were placed downstream of the FLAG–NLS coding segment and upstream of the dsRED/EGFP cassette to generate the RG6 reporter plasmid (Figure 3A). Splicing of cTNT exon 5 responds strongly to co-expressed CUG-BP and ETR-3 Like factors (CELF) and muscle-blind like 3 (MBNL) proteins which promote exon inclusion and skipping, respectively (16,17). The natural cTNT alternative exon is 30 nt and would maintain the reading frame. Therefore, the natural exon was replaced by an artificial exon of 28 nt that shifted the reading frame from dsRED (exon skipping) to EGFP (exon inclusion) (Figure 3A). Previous results have demonstrated that the specific exon sequence is not required for cTNT exon 5 regulation (14). The alternative

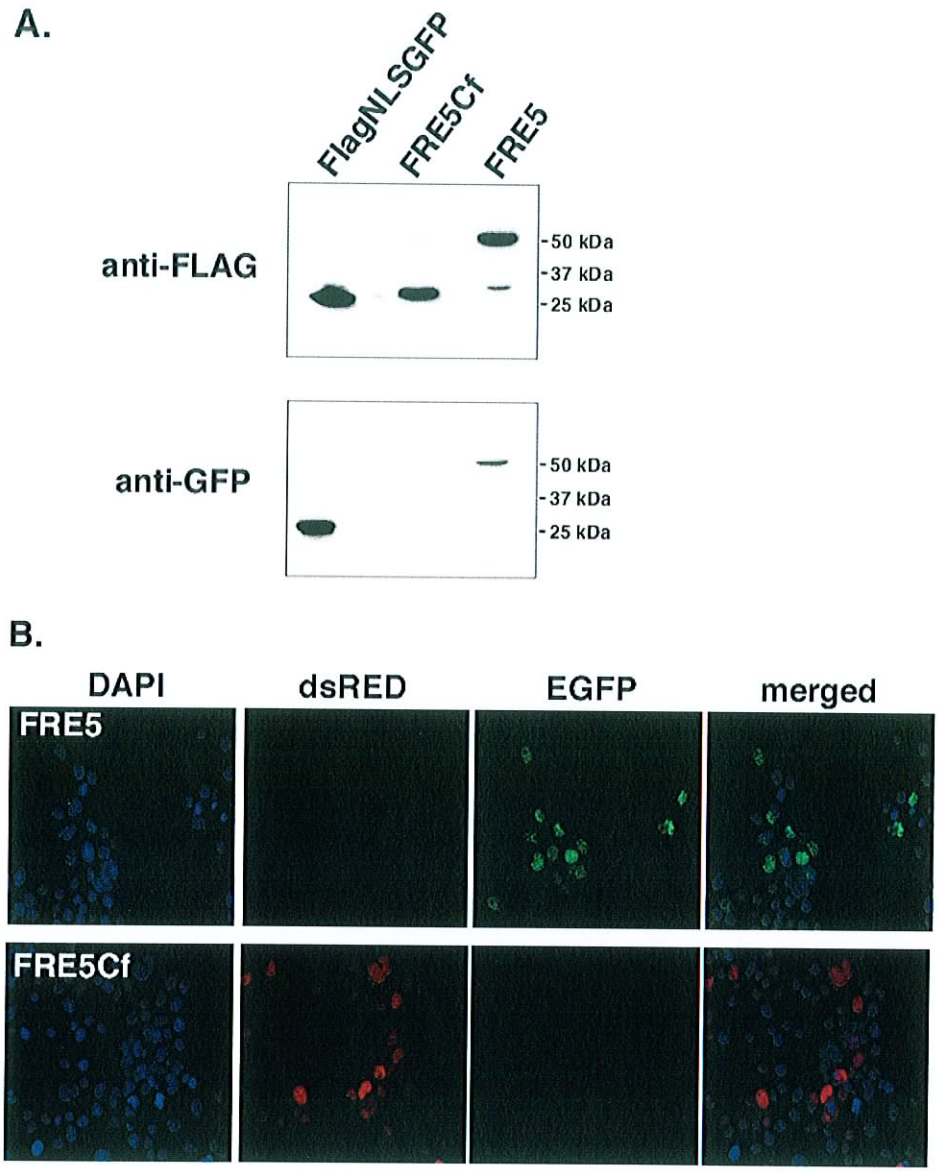


Figure 2. Expression of dsRED and EGFP from FRE5 and FRE5Cf mRNAs with alternate open reading frames. (**A**) Western blot analysis demonstrated efficient expression of FRE5 (EGFP) and FRE5Cf (dsRED) FLAG-tagged proteins. FlagNLSGFP is a FLAG-tagged version of GFP used for a positive control. (**B**) Nuclear expression of dsRED from FRE5Cf and EGFP from FRE5. Fluorescence microscopy demonstrates that the FLAG–NLS-alternate dsRED ORF–EGFP fusion protein is expressed in nuclei with enhanced nucleolar localization while FLAG–NLS–dsRED shows more diffuse nuclear distribution. Nucleolar localization of the EGFP fusion protein is variable between cell lines (data not shown).

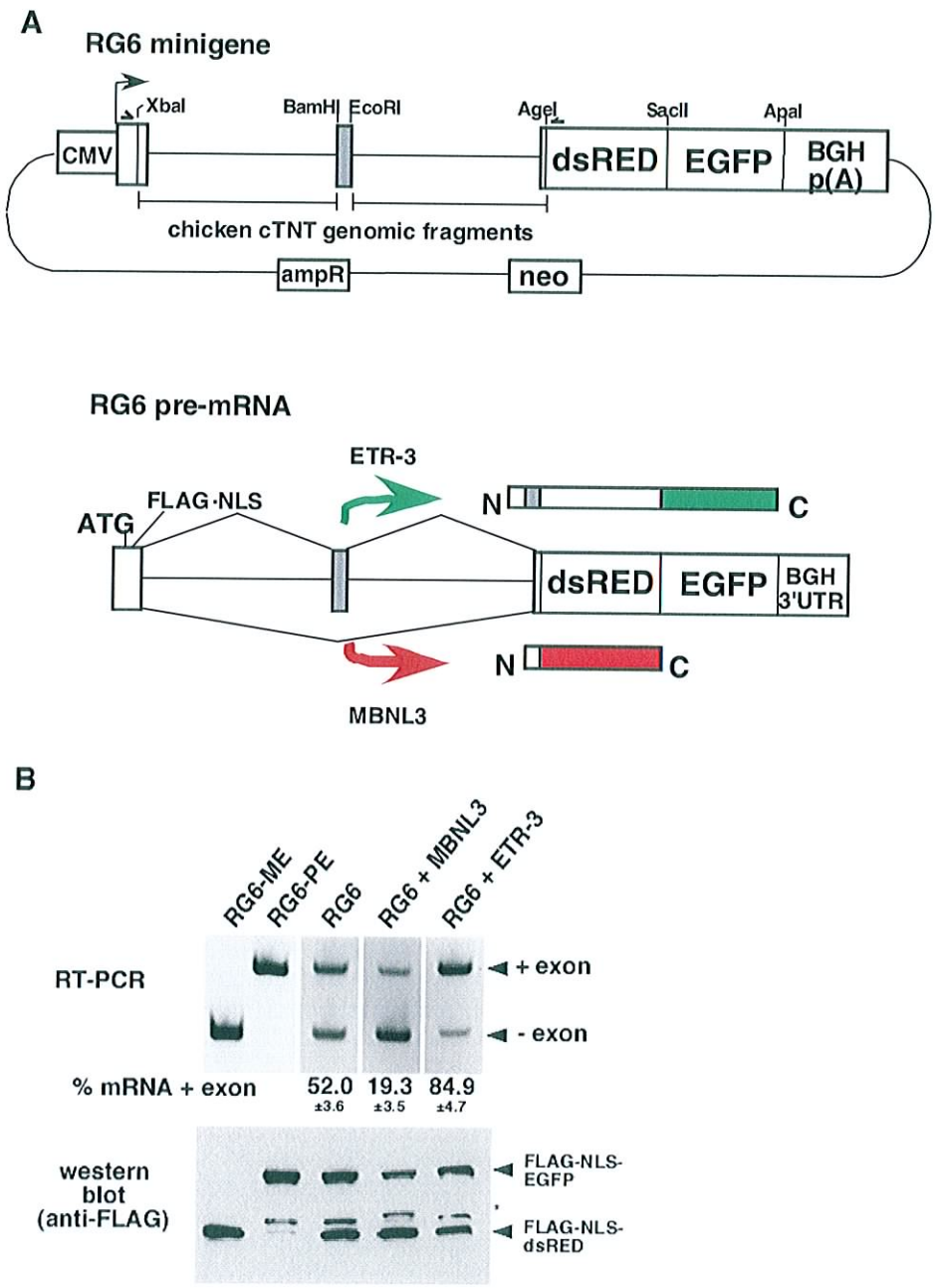
exon is flanked by unique restriction sites to allow easy manipulation of the region to be spliced. The length and composition of the alternative exon was designed to balance splicing such that equivalent levels of exon inclusion and skipping was expressed in the COSM6 cells used for this analysis.

RG6 was transfected alone or with ETR-3 or MBNL3 expression plasmids. The effects on exon inclusion were examined using three assays: RT–PCR using primers located in the flanking exons (positions indicated in Figure 3A), western blot analysis for FLAG-tagged dsRED and EGFP proteins, and fluorescence microscopy. RT–PCR analysis demonstrated that the RG6 minigene expressed ~1:1 exon inclusion to skipping and, as expected, ETR-3 promoted exon inclusion and MBNL3 promoted exon skipping (from 52 to 85% and 19% inclusion, respectively, Figure 3B). Shifts in the ratios of the two reading frames were also detectable by western blotting of FLAG-tagged dsRED and EGFP (Figure 3B).

An important potential feature of this bichromatic reporter is the ability to quantify the ratio of alternative splicing in individual cells using quantitative fluorescence microscopy. The majority of cells transfected with the RG6 minigene alone contained both nuclear dsRED and EGFP fluorescence (Figure 3C). Coexpression of either ETR-3 or MBNL3 significantly altered dsRED and EGFP expression consistent with promotion of exon inclusion by ETR-3 and exon skipping

by MBNL3 (Figure 3C). Nuclear fluorescence intensity for individual nuclei was quantified using ImageJ software and the results are presented as percent green fluorescence intensity (resulting from alternative exon inclusion) in Table 1. This analysis indicated that the different splicing patterns induced by ETR-3 and MBNL3 are detected by quantitative fluorescence of individual nuclei. We conclude that the ratio of red and green fluorescence expressed from the RG6 minigene can be used to quantify the ratios of splicing patterns within individual cells. The loss of one fluorescent protein and gain of the other due to mutually exclusive use of alternate reading frames provides a highly sensitive assay.

To determine whether this reporter was applicable to cell sorting, transiently transfected COSM6 cells were trypsinized and used for flow cytometry. Consistent with results from fluorescence microscopy above, cells transfected with the RG6 minigene contained a mixture of cells expressing red and green fluorescence, however there was a large population exhibiting predominantly green fluorescence which did not respond to ETR-3 or MBNL3 expression (RG6 alone, Figure 4). This population could represent cells that are expressing both dsRED and EGFP but dsRED is below the level of detection of FACscan. The population expressing relatively high levels of both red and green fluorescence



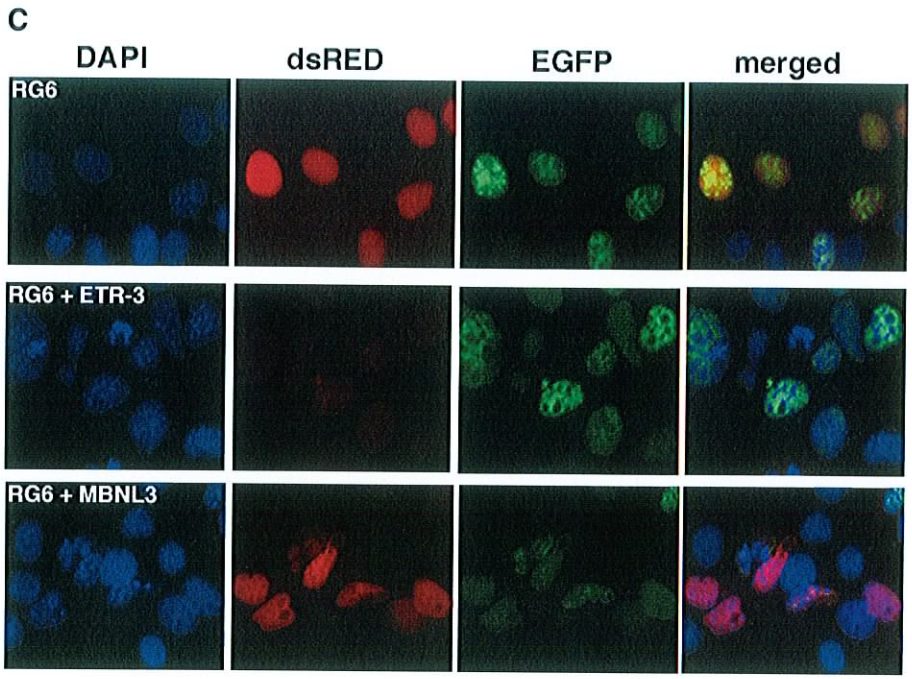


Figure 3. A bichromatic alternative splicing reporter for cTNT. (**A**) Diagram of RG6 minigene. Alternative splicing of a 28 nt cassette exon shifts the reading frame between dsRED and EGFP. Restriction sites shown are unique to the plasmid. (**B**) RG6 was transiently transfected into COSM6 either alone or co-expressed with Xpress-ETR-3 (promotes exon inclusion) or FLAG–MBNL3 (promotes exon skipping). RT–PCR analysis demonstrates exon repression by MBNL3 and activation by ETR-3. Percent exon inclusion was calculated by intensity of the top band divided by the sum of the intensities of the top and bottom bands × 100. The results are from four independent transfections. Western blot analysis using anti-FLAG antibodies demonstrates expression of FLAG-tagged EGFP and dsRED proteins. RG6PE (plus exon) and RG6ME (minus exon) are expression constructs for the spliced mRNAs containing and lacking the alternative exon, respectively. The additional band in the RG6+MBNL3 lane (indicated by asterisk) is FLAG–MBNL3. (**C**) Fluorescence microscopy of the RG6 reporter plasmid. Cells within cultures expressing RG6 alone contain nuclear staining for both dsRED and EGFP consistent with ~1:1 exon inclusion:skipping detected by RT–PCR in panel B. RG6+ETR-3 clearly exhibited a shift towards nearly exclusive expression of EGFP and RG6+MBNL3 exhibited a strong shift toward dsRED. Quantification of EGFP versus dsRED fluorescence is presented in Table 1.

was divided in half into boxes A and B to approximate the 1:1 ratio of dsRED and EGFP mRNAs detected by RT–PCR. Cultures expressing MBNL3 exhibited a significant shift in cell population expressing higher red fluorescence and lower green fluorescence while cultures expressing ETR-3 shifted toward higher green and lower red fluorescence (Figure 4). By setting the RG6 plasmid results at 1:1 and then calculating the percentage of cells in box A (green = exon inclusion) the flow cytometry analysis indicated that ETR-3 induced 82.6% of the cells into box A and MBNL3 reduced the fraction of cells in box A to 8.3%. These results are consistent with the results obtained from RT–PCR (88.9 and 17.3%, respectively, shown in Figure 3B). We conclude that the bichromatic reporter provides a sensitive assay for sorting cell populations based on predominant alternative splicing patterns.

Analysis of cell-specific splicing patterns in mixed cell populations

To determine whether the RG6 minigene could be used to detect splicing changes driven by endogenous regulatory programs, we tested its expression in differentiated and undifferentiated C2C12 mouse skeletal muscle cultures. C2C12 myoblasts proliferate as mononucleated cells in high serum medium and differentiate into multinucleated myotubes after 3–4 days in low serum medium. Analysis of chicken

Table 1. Percent green fluorescence in individual nuclei within cultures transfected with RG6 alone or with MBNL3 or ETR-3

RG6	41.7 ± 3.8 (n = 16)
RG6 + MBNL3	15.1 ± 2.7 (n = 16)
RG6 + ETR-3	65.4 ± 3.7 (n = 22)

cTNT minigenes previously indicated that both exon 5 inclusion and skipping was detected in undifferentiated C2C12 myoblast cultures while exon inclusion was strongly favored in differentiated myotubes (16). In undifferentiated C2C12 cultures transfected with the RG6 plasmid, the majority of transfected cells expressed both red and green nuclear fluorescence indicating that both splicing patterns are expressed in each cell (RG6, undiff; Figure 5A). In contrast, chains of nuclei located within differentiated myotubes contained only nuclear EGFP, indicating that the splicing switch to the exon inclusion pathway is readily detectable based on the bichromatic read-out (Figure 5B). Differentiated C2C12 cultures also contain a subpopulation of cells that have lost the ability to undergo myogenic differentiation. In addition to the exclusively green nuclei consistently observed in myotubes, we detected mononucleated cells that expressed both red and green fluorescence consistent with a failure to differentiate and expression of both splicing patterns (lower panel, open arrows, Figure 5C). This result indicates that

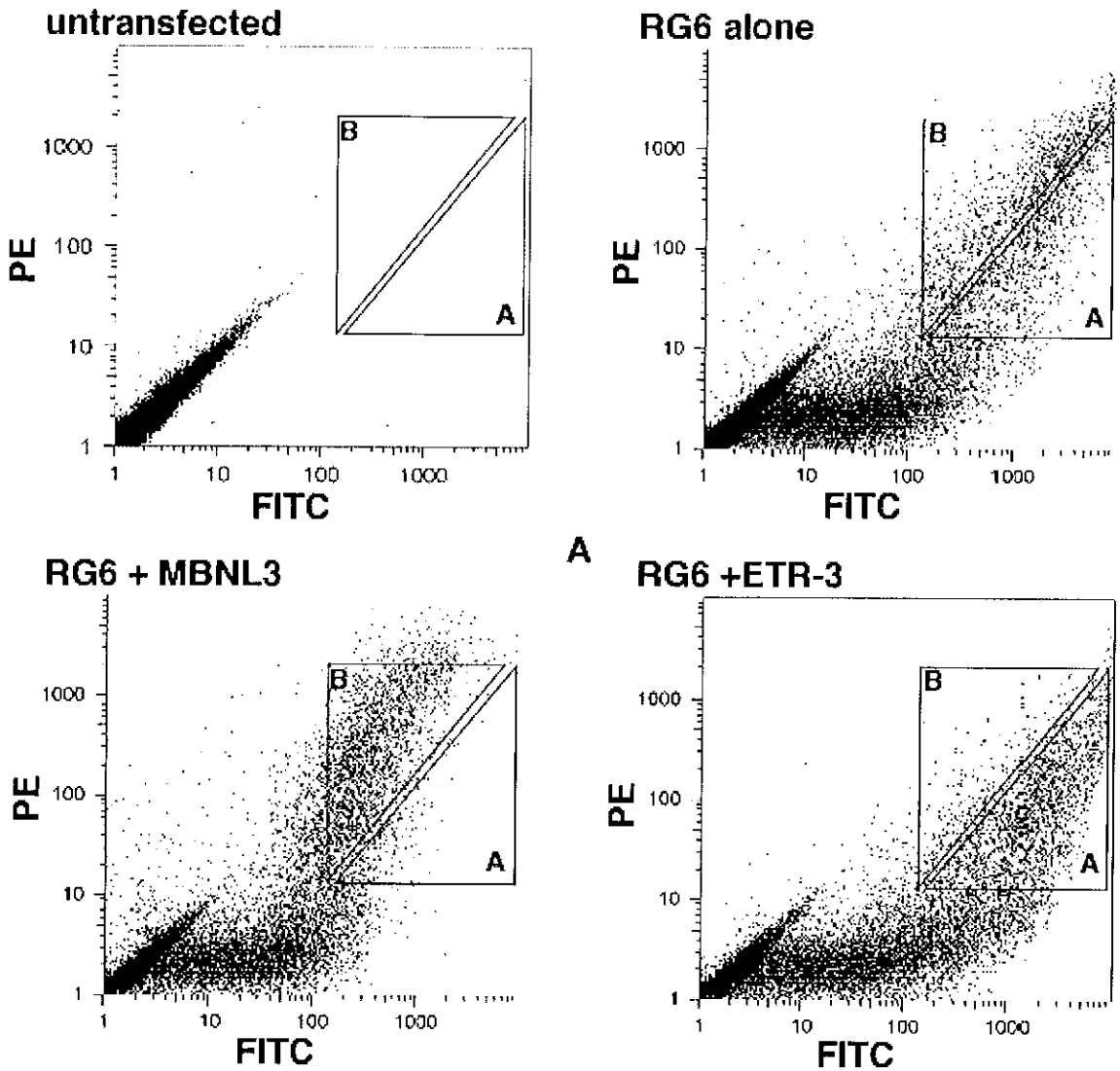


Figure 4. Analysis of RG6 expressing cells by flow cytometry. Cells determined to be expressing both red and green were gated in the RG6 alone sample so that 50% of cells are located in box A (green) and the other 50% in box B (red). The percent of cells favoring box A was calculated for cells co-transfected with MBNL3 or ETR-3. This percentage represents the relative shift of cells expressing more green and less red indicative of an alteration in splicing regulation. The y axis (PE) is intensity of red fluorescence and the x axis (FITC) is the intensity of green fluorescence.

the red:green fluorescence output can be used to readily identify cells within a mixed cell population that express different splicing patterns.

To further address the resolution with which the RG6 minigene can be used to detect individual differences between cells in mixed cell populations, we transfected RG6 into chicken primary breast muscle cultures and allowed these cultures to differentiate. Differentiated primary cultures contain a mixture of differentiated myotubes and fibroblasts. Avian fibroblasts have previously been shown to express predominantly exon skipping and a low level of exon inclusion (14). Fluorescence microscopy demonstrated that all chains of nuclei found in differentiated myotubes expressed only EGFP and no detectable dsRED (Figure 6A). In contrast, non-muscle cells were detectable by nuclear fluorescence from both dsRED and EGFP (open arrows, Figure 6B). These results demonstrate the utility of this reporter to identify individual cells expressing divergent splicing patterns within a mixed cell population.

DISCUSSION

Using a peculiar property of dsRED (also found in EGFP), we have developed a bichromatic readout for alternative splicing in which a single reporter expresses dsRED from one alternative splicing pathway and EGFP from the other. Inclusion or skipping of variable regions introduced into the mRNA by alternative splicing upstream of the dsRED/EGFP cassette allows toggling between the dsRED or EGFP reading frame. The system is amendable to the majority of alternative splicing patterns and requires only that the variable

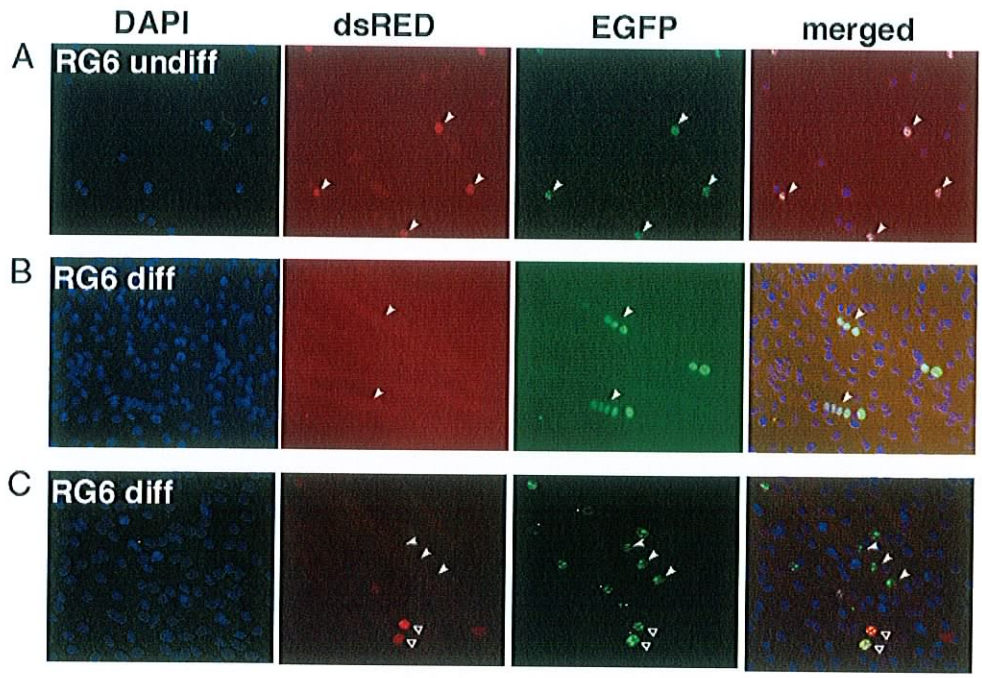


Figure 5. Expression of the RG6 bichromatic reporter in undifferentiated (**A**) or differentiated (**B** and **C**) C2C12 mouse skeletal muscle cultures. The cTNT alternative exon shifts from exon skipping and inclusion to exon inclusion during differentiation of C2C12 myoblasts (16). The vast majority of cells in undifferentiated cultures expressing the RG6 minigene contain red and green nuclear fluorescence (panel A). Differentiated cultures contain chains of nuclei in differentiated myotubes which express exclusively EGFP (exon inclusion) (B and C, solid arrows). Undifferentiated mononucleated cells in the same culture express nuclear red and green fluorescence indicative of both splicing patterns (panel C, open arrows).

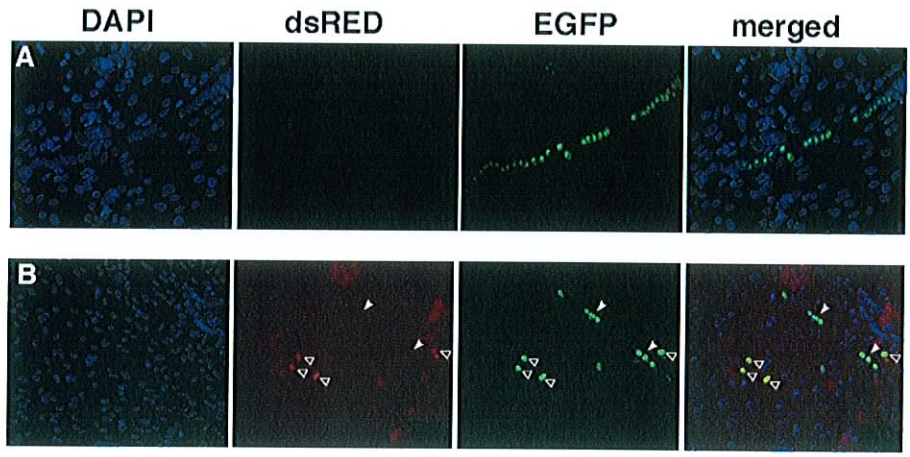


Figure 6. Expression of the RG6 bichromatic reporter in primary skeletal muscle cultures (both **A** and **B**). Chicken breast muscle cultures were transiently transfected with RG6 then examined for fluorescence. (A) nuclear chains found within differentiated myotubes express EGFP with no detectable dsRED indicative of the expected exon inclusion splicing pattern. (B) Exclusive expression of EGFP in myotubes (solid arrows) and both red and green fluorescence in fibroblasts (open arrows).

region not be a multiple of three and that it lacks a translation stop codon.

Expression of different fluorescent proteins from each of the two alternative splicing patterns expressed from a single reporter has several advantages compared to a monochromatic read-out or the use of two separate constructs to perform a bifluorescent read-out. First, expression of dsRED and EGFP is mutually exclusive so that a splicing transition results in both a gain of one fluorescent signal and the loss of the other enhancing the change in signal. Second, having a read-out for both of two splicing pathways allows quantification of the complete output of a gene. This is particularly useful for quantitative assessment of alternative splicing within single cells. Third, using a single bichromatic reporter removes the variability associated with coexpression of two separate reporters that express different fluorescent proteins.

This reporter is directly applicable for high-throughput analyses of libraries of cDNAs, shRNAs and small molecules

that alter splicing pattern of a specific alternative splicing event. In addition, a large fraction of human diseases are caused by mutations that disrupt normal splicing and high-throughput screens can be used to identify compounds that restore correct splicing patterns. For example, reversing an exon skipping event of the SMN2 gene could reverse, in a large fraction of cases, the protein deficit in spinal muscular atrophy (SMA) (18). While compounds that affect this splicing event have been identified (19), a high-throughput screen would enhance the likelihood of identifying compounds with enhanced specificity for the aberrant splicing event and reduce potential side effects from a general disruption of splicing.

This reporter will also be useful to identify the range of alternative splicing patterns expressed by individual cells within cultured cells and tissues of transgenic animals. Single cell RT–PCR has directly demonstrated that different cells within a tissue express different splicing patterns (20,21). A GFP reporter was used in transgenic mice to demonstrate appropriate cell-restricted expression of the smooth muscle specific exon of alpha tropomyosin (6). Recently, an RFP splicing reporter for FGFR2 used in AT3 prostate tumors transplanted into syngenic animals revealed an unexpected minor population of cells expressing a splicing pattern indicative of a mesenchyme to epithelial transition (12). The ability to use a bichromatic reporter to quantify the ratios of alternative splicing patterns in individual cells will enhance our understanding of the diversity of splicing patterns in individual cells with in tissues and mixed cell culture.

Both dsRED and EGFP proteins contain the identical N-terminal FLAG–NLS. Nuclear localization concentrates the fluorescent signal for enhanced sensitivity and also rules out spurious expression of fluorescent proteins from internal translation initiation. Earlier versions of these constructs were found to express low levels of cytoplasmic dsRED and EGFP protein that were not detectable on western blots by FLAG antibodies but were detectable by antibodies to dsRED or GFP (data not shown). Cytoplasmic localization and the absence of the FLAG epitope indicated an inappropriate internal translation initiation at the dsRED and EGFP translation initiation codon. The current reporter lacks the initiating methionines of both dsRED and EGFP and does not express detectable levels of internally initiated proteins.

The variable region upstream of the dsRED–EGFP cassette must be of the appropriate size to change reading frames between dsRED and EGFP. For variable regions that are a multiple of three, this can be accomplished by altering the size of the variable region by one nucleotide. Variable regions that contain stop codons in either of the reading frames required for expression of dsRED or EGFP would need to be converted to an ORF. Such changes simply require single nucleotide substitutions or insertion/deletion.

It is of interest that two fluorescent proteins from very different sources, EGFP from the *Aequorea* genus of jelly fish and dsRED from the *Discosoma* genus of coral, both have an alternate reading frame that is devoid of both translation stop codons and start codons. In addition to providing the opportunity to generate a bichromatic readout for alternative splicing, it is likely that this unusual property has biological implications with regard to how fluorescent proteins or adjacent or overlapping genes are expressed and regulated.

ACKNOWLEDGEMENTS

We thank Andrew Sharabi for help establishing and analyzing flow cytometry, Adam Szafran with microscopy advice, and Dr Andras Nagy for the pCX-DsRed plasmid. This work was supported by funding from the NIH (R01HL45565 and R01AR45653) to T.A.C. Funding to pay the Open Access publication charges for this article was provided by R01HL45565 and R01AR45653.

Conflict of interest statement. None declared.

REFERENCES

1. Blencowe,B.J. (2006) Alternative splicing: new insights from global analyses. *Cell*, **126**, 37–47.
2. Faustino,N.A. and Cooper,T.A. (2003) Pre-mRNA splicing and human disease. *Genes Dev.*, **17**, 419–437.
3. Cartegni,L., Chew,S.L. and Krainer,A.R. (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nature Rev. Genet.*, **3**, 285–298.
4. Black,D.L. (2003) Mechanisms of alternative pre-messenger RNA splicing. *Annu. Rev. Biochem.*, **27**, 27–48.
5. Sazani,P., Gemignani,F., Kang,S.H., Maier,M.A., Manoharan,M., Persmark,M., Bortner,D. and Kole,R. (2002) Systemically delivered antisense oligomers upregulate gene expression in mouse tissues. *Nat. Biotechnol.*, **20**, 1228–1233.
6. Ellis,P.D., Smith,C.W. and Kemp,P. (2004) Regulated tissue-specific alternative splicing of enhanced green fluorescent protein transgenes conferred by alpha-tropomyosin regulatory elements in transgenic mice. *J. Biol. Chem.*, **279**, 36660–36669.
7. Wang,Z., Rolish,M.E., Yeo,G., Tung,V., Mawson,M. and Burge,C.B. (2004) Systematic identification and analysis of exonic splicing silencers. *Cell*, **119**, 831–845.
8. Wu,J.Y., Kar,A., Kuo,D., Yu,B. and Havlioglu,N. (2006) SRp54 (SFRS11), a regulator for tau exon 10 alternative splicing identified by an expression cloning strategy. *Mol. Cell. Biol.*, **26**, 6739–6747.
9. Levinson,N., Hinman,R., Patil,A., Stephenson,C.R., Werner,S., Woo,G.H., Xiao,J., Wipf,P. and Lynch,K.W. (2006) Use of transcriptional synergy to augment sensitivity of a splicing reporter assay. *RNA*, **12**, 925–930.
10. Newman,E.A., Muh,S.J., Hovhannisyan,R.H., Warzecha,C.C., Jones,R.B., McKeehan,W.L. and Carstens,R.P. (2006) Identification of RNA-binding proteins that regulate FGFR2 splicing through the use of sensitive and specific dual color fluorescence minigene assays. *RNA*, **12**, 1129–1141.
11. Wagner,E.J., Baines,A., Albrecht,T., Brazas,R.M. and Garcia-Blanco,M.A. (2004) Imaging alternative splicing in living cells. *Methods Mol. Biol.*, **257**, 29–46.
12. Oltean,S., Sorg,B.S., Albrecht,T., Bonano,V.I., Brazas,R.M., Dewhirst,M.W. and Garcia-Blanco,M.A. (2006) Alternative inclusion of fibroblast growth factor receptor 2 exon IIIc in Dunning prostate tumors reveals unexpected epithelial mesenchymal plasticity. *Proc. Natl Acad. Sci. USA*, **103**, 14116–14121.
13. Cooper,T.A. and Ordahl,C.P. (1985) A single cardiac troponin T gene generates embryonic and adult isoforms via developmentally regulated alternate splicing. *J. Biol. Chem.*, **260**, 11140–11148.
14. Xu,R., Teng,J. and Cooper,T.A. (1993) The cardiac troponin T alternative exon contains a novel purine-rich positive splicing element. *Mol. Cell. Biol.*, **13**, 3660–3674.
15. Singh,G. and Cooper,T.A. (2006) Minigene reporter for identification and analysis of cis elements and trans factors affecting pre-mRNA splicing. *Biotechniques*, **41**, 177–181.
16. Ladd,A.N., Charlet-B.,N. and Cooper,T.A. (2001) The CELF family of RNA binding proteins is implicated in cell-specific and developmentally regulated alternative splicing. *Mol. Cell. Biol.*, **21**, 1285–1296.
17. Ho,T.H., Charlet-B., N., Poulos,M.G., Singh,G., Swanson,M.S. and Cooper,T.A. (2004) Muscleblind proteins regulate alternative splicing. *EMBO J.*, **23**, 3103–3112.

18. Sumner,C.J. (2006) Therapeutics development for spinal muscular atrophy. *NeuroRx*, **3**, 235–245.
19. Jarecki,J., Chen,X., Bernardino,A., Coovert,D.D., Whitney,M., Burghes,A., Stack,J. and Pollok,B.A. (2005) Diverse small-molecule modulators of SMN expression found by high-throughput compound screening: early leads towards a therapeutic for spinal muscular atrophy. *Hum. Mol. Genet.*, **14**, 2003–2018.
20. Wang,Z.H. and Grabowski,P.J. (1996) Cell- and stage-specific splicing events resolved in specialized neurons of the rat cerebellum. *RNA*, **2**, 1241–1253.
21. Graf,E.M., Bock,M., Heubach,J.F., Zahanich,I., Boxberger,S., Richter,W., Schultz,J.H. and Ravens,U. (2005) Tissue distribution of a human Ca v 1.2 alpha1 subunit splice variant with a 75 bp insertion. *Cell Calcium*, **38**, 11–21.

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Lynne-Marie Postovit
DEPARTMENT	Anatomy & Cell Biology
ADDRESS	MSB 438
PHONE NUMBER	80524
EMERGENCY PHONE NUMBER(S)	519-601-3661
EMAIL	Lynne.postovit@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) __MSB_____ Room(s)_437__

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES:_CIHR; CRS_____
GRANT TITLE(S): _Role of Oxygen as a Regulator of Tumour Cell Plasticity and Metastatic Potential; Role of Nodal in Breast Cancer Metastasis
List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Guihua Zhang	gzhang4@uwo.ca	June 13, 2006
Padmalaya Das	Padmalaya.Das@schulich.uwo.ca	Oct. 5, 2010
Daniela Quail	dquail@uwo.ca	June, 2006
Scott Findlay	sfindla@uwo.ca	Jan. 16, 2008
Michael Jewer	mjewer@uwo.ca	Oct. 2009
Chris Hughes	chughe7@uwo.ca	May 14, 2007
Courtney Brooks	cbrook8@uwo.ca	Sept.7, 2010
Dylan Dieters-Castator	ddieters@uwo.ca	June, 2009
Amelia Nuhn	anuhn2@uwo.ca	Oct. 2, 2008
Alia Cloutier-Bosworth	aclouti7@uwo.ca	Oct. 13, 2010
Jeff Law	jlaw42@uwo.ca	June 6, 2008
Kevin Kania	kkania@uwo.ca	Sept. 30, 2008
Giovanni Nicolas Poggenpoel	gpoggenp@uwo.ca	Aug. 11, 2010

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

We are a cell biology lab. As such, our biohazards consist of previously derived cell lines as well as molecular biology tools (i.e. bacteria and plasmids). Cells are used to conduct cell biological and biochemical experiments including Western blotting, PCR, and functional assays such as invasion, proliferation and tube formation. We use animal models, but not in the main laboratory space. We also use tissue that has been archived (i.e. paraffin embedded). Such archived specimens are not hazardous. Cells are cultured in a level 2 tissue culture facility, as per standard operating procedures. This includes the use of certified class II A2 biosafety cabinets and HEPA filtered incubators. All glass and waste is sterilized with bleach and 75% alcohol is used to disinfect all surfaces before and after use. When not in culture, cells are stored in a liquid nitrogen repository located in the level 2 lab space. Glycerol stocks of plasmid containing bacteria are similarly stored in the repository, and are transformed, selected, grown, and stored in the main level 2 lab space. Plasmid DNA is purified from bacteria and then used to transfect mammalian cells. These plasmids include shRNA constructs and overexpression constructs. As is the case with any biohazards, all equipment is bleached following use, as are disposables. Following bleaching, items are autoclaved and then disposed of, or reused as appropriate.

Please include a one page research summary or teaching protocol

Bidirectional communication between cells and their microenvironment is a hallmark of both cancer progression and embryological development. Indeed, in all physiological instances, cells do not survive autonomously, but rather rely on extracellular cues to direct functions as diverse as proliferation, apoptosis, invasion and differentiation. The past decade has seen an explosion of research on cells with the capacity to differentiate in response to specific microenvironmental cues. During embryogenesis, these "stem cells" are the source of all cell lineages and in adulthood they function in tissue repair and rejuvenation. Recent studies have found that cancers may similarly develop from stem cell populations, and that these rarely occurring cells are likely responsible for tumour formation, drug resistance and metastasis. ***The unifying goal of my research program is to determine what types of microenvironments regulate normal and cancer stem cell plasticity and function, and to elucidate the mechanisms by which such microenvironments elicit their effects.*** It is my hope that these studies will lead to the development of methods to maintain normal stem cell pluripotency and to inhibit cancer cell plasticity and metastasis. Over the next 5 years, this research program will be comprised of the following integrative projects:

1. Role of Oxygen as a Regulator of Tumour Cell Plasticity and Metastatic Potential

Low oxygen levels enhance tumour cell metastasis and highly metastatic cancer cells tend to be dedifferentiated, expressing many stem cell markers, such as Nodal. Furthermore, recent studies have revealed that low oxygen levels promote the pluripotency of hESCs. We propose that decreased oxygen levels enhance the metastatic potential of cancer cells by selecting for less differentiated, more plastic cell types; cells that are able to survive in secondary tissue sites. As such, we plan to examine the effects of hypoxia on the expression of stem cell markers (with an emphasis on Nodal) as well as the contribution of these factors to tumour progression and metastasis. ***This is the project described in the current research proposal, and will be given the most emphasis.***

2. Role of Nodal in Metastatic Progression

This project will complement project 1, by specifically interrogating the mechanisms by which Nodal (a potent microenvironmental mediator) promotes cancer progression. Studies will interrogate the role of Nodal in each step of the metastatic cascade, from invasion in the primary site to proliferation in the secondary site. We will also determine the effects of Nodal on tumour-associated stromal cells. Finally, we will examine the utility of Nodal as prognostic indicator for breast cancer patients.

3. Role of Embryonic Microenvironments in the Regulation of Cell Fate

Embryonic stem cells do not subsist autonomously, but rather exist in a dynamic microenvironment, composed of oxygen gradients, extracellular matrices and growth factors. While many of these entities are derived from exogenous sources, it is likely that stem cells actively sustain a niche that is permissive to a balance of self renewal and differentiation. This project will implement a novel 3-D model that was developed during my post-doctoral studies, to determine the factors that human embryonic stem cells (hESCs) deposit into their microenvironment. Specifically, hESCs will be allowed to condition the microenvironment on select extracellular matrices. We will then remove the cells and use proteomics to discover factors deposited by the undifferentiated hESCs into the microenvironment. Specific components of this microenvironment will then be purified and their effects on stem cell pluripotency and phenotype will be determined. We will eventually tie this project to the other projects by examining the roles that oxygen and Nodal play in the ability of hESCs to self-regulate their phonotype.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO

If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____

_____

_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
TOP10 Chemically Competent *E. coli* Cells	O Yes X No	O Yes X No	O Yes X No	1 L	Invitrogen	X 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes x No		Not applicable
Rodent	O Yes x No		
Non-human primate	O Yes x No		
Other (specify)	O Yes x No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	X Yes O No	T47D, MCF7, Hs578T, Hs578Bst, MDA-MB-231, MDA-MB-468, MDA-MB-435, JEG-3, JAR, BeWO, HuVEC, NIH 3T3	ATCC
		C8161, C81-61	Dr. Mary Hendrix, Northwestern
		H1 hESC, H9 hESC	WiCell
		CA1, CA2	Cheryle Seguin, UWO

		MCF-10A, MCF-10A-T1KCl2, MCF-10CA1h, MCF-10CA1aCl1 HTR-8/SVneo	Karmanos Cancer Center Dr. Peeyush Lala, UWO
Rodent	X Yes O No	CF-1 irradiated mEF	Global Stem
Non-human primate	O Yes X No		
Other (specify)	O Yes X No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 x 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? X YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	N/A JS.	O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)	OICR	Not Applicable Unknown JS	Paraffin embedded tissue sections	Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? X YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
TOP10 Chemically Competent *E. coli* Cells	*PLEASE SEE ATTACHMENT*			.

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below X NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen O YES X NO
- E1A oncogene O YES X NO
- Known oncogenes O YES, please specify _____ X NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

4.5 Will virus be replication defective? O YES X NO

4.6 Will virus be infectious to humans or animals? O YES X NO

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____N/A_____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES X NO

5.3 How will the biological agent be administered? _____N/A_____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:___N/A_____

5.5 Has human ethics approval been obtained? O YES, number: _____ X NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? X YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used__ HSD:ATHYMIC NUDE-FOXN1NU/FOXN1_____

6.3 AUS protocol # ___2008-101_____

6.4 Will any of the agents listed in section 4.0 be used in live animals x YES, specify: potentially all_ O NO

6.5 Will the agent(s) be shed by the animal: O YES x NO, please justify:
__agents are non-replicating and inserted into the genome of the tumour cells only_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE Lynn-Marie Pastoret

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest

HC or CFIA Containment Level required. O 1 X 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
YES, permit # if on-campus_BIO-UWO-0194_
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Anne-Marie Pastoriza Date: 10-27-2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

_____
_____

See E-mail

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 10 Nov '10

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: NOV 12/10

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: BIO-UWO-0194 Expiry Date (3 years from Approval): ~~Nov~~ 9 NOV 2013

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Biological Agents Registry Form (Postovit)
Date: Tue, 02 Nov 2010 17:37:34 -0400
From: Lynne Postovit <Lynne.Postovit@schulich.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer:

Please see my answers below.

Best regards,

Lynne

Lynne-Marie Postovit, PhD
Assistant Professor
438 Medical Science Building
Dept. of Anatomy & Cell Biology
The Schulich School of Medicine
University of Western Ontario
London, Ontario
Canada N6A 5C1
Phone: (519) 661-2111 x 80524
Fax: (519) 661-3936

E-mail

Dr. Postovit -

Thank you for your recent submission - I received it in the mail today.

Please note that two questions were not answered (please respond by e-mail):

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

In addition to standard Level 2 measures, we limit access to our facility using a deadbolt on the door to our main tissue culture facility (inside our main lab). We also maintain spill kits, inclusive of bleach. Finally several hand sanitizing stations are maintained.

14.3 Please outline what will be done if there is an exposure to the biological agents listed,
such as a needlestick injury, accidental splash or accidental injection:

_In the case of exposure, the exposed areas will be disinfected an flushed with water as per standard operating procedures. Occupational health will also be notified and medical examination will proceed as needed.

Regards,
Jennifer

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 500257
Product name TOP 10 - ONE SHOT

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Suspension

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability Stable.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available

Cell Line Designation: T-47D
ATCC® Catalog No. HTB-133™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast ductal carcinoma; derived from metastatic site: pleural effusion
Age: 54 years
Gender: female
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 11,13
D13S317: 12
D16S539: 10
D5S818: 12
D7S820: 11
TH01: 6
TPOX: 11
vWA: 14
Morphology: epithelial
Growth properties: adherent
DoublingTime: 32 hrs
Receptors: calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin; estrogen receptor, positive.
Isoenzymes: AK-1, 1; ES-D, 2; G6PD, B; GLO-I, 1-2; PGM1, 1; PGM3, 1
Tumorigenic: forms colonies in soft agar
Depositors: I. Keydar
Comments: The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast.
This differentiated epithelial substrain (T-47D) was found to contain cytoplasmic junctions and receptors to 17 beta estradiol, other steroids and calcitonin.It will form colonies in soft agar.
The cells express the WNT7B oncogene [PubMed: 8168088].
Cytogenetic analysis: This is a hypotriploid human cell line. The modal chromosome number is 65 occurring at 50% and polyploidy at 0.8%. 18 marker chromosomes are common to most cells, of which 7 are paired and 11 are single-copied. The t(8q14q), t(9q17q), t(10q17p) are among 7 paired markers common to most cells. N7, N9, and N10 are absent and N11 is generally present in 4 copies. DM's occurred, but infrequently. Q-band examination did not show the presence of a Y chromosome.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA: from this line is available as ATCC HTB-133D (10µg).
Total RNA from this line is available as ATCC HTB-133R (100µg)

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., 1995) the ATCC manual on quality control (Hay et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, ***Biosafety in Microbiological and Biomedical Laboratories*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of the cell suspension to new culture vessels.
Subcultivation Ratio: 1: 3 to 1: 5

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001.
To make the complete growth medium, add the following components to the base medium:

- 0.2 Units/ml bovine insulin;
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Keydar I et al. **Establishment and characterization of a cell line of human breast carcinoma origin**. Eur. J. Cancer 15: 659-670, 1979 PubMed: 80068971

Judge SM and Chatterton RT Jr. **Progesterone-specific stimulation of triglyceride biosynthesis in a breast cancer cell**

line (T-47D). Cancer Res. 43: 4407-4412, 1983 PubMed: 83259030

Lamp SJ et al. **Calcitonin induction of a persistent activated state of adenylate cyclase in human breast cancer cells (T-47D).** J. Biol. Chem. 256: 12269-12274, 1981 PubMed: 82053097

Sher E et al. **Whole-cell uptake and nuclear localization of 1,25-dihydroxy-cholecalciferol by breast cancer cells (T-47D) in culture.** Biochem. J. 200: 315-320, 1981 PubMed: 82182106

Freake HC et al. **1,25-Dihydroxyvitamin D3 specifically binds to a human breast cancer cell line (T-47D) and stimulates growth.** Biochem. Biophys. Res. Commun. 101: 1131-1138, 1981 PubMed: 82068379

Faust JB and Meeker TC. **Amplification and expression of the bcl-1 gene in human solid tumor cell lines.** Cancer Res. 52: 2460-2463, 1992 PubMed: 92233404

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue.** Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

Bellet D et al. **Malignant transformation of nontrophoblastic cells is associated with the expression of chorionic gonadotropin beta genes normally transcribed in trophoblastic cells.** Cancer Res. 57: 516-523, 1997 PubMed: 97164677

Hoppe HC et al. **Identification of phosphatidylinositol mannoside as a mycobacterial adhesin mediating both directand opsonic binding to nonphagocytic mammalian cells.** Infect. Immun. 65: 3896-3905, 1997 PubMed: 97427981

Burfeind P et al. **Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo.** Proc. Natl. Acad. Sci. USA 93: 7263-7268, 1996 PubMed: 96293512

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines.** 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

Centers for Disease Control (1993), **Biosafety in Microbiological and Biomedical Laboratories** Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 3rd Edition U.S. Government Printing Office Washington D.C.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org/

07/07

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

Product Information Sheet for ATCC® HTB-22™

Cell Line Designation: MCF-7
ATCC® Catalog No. HTB-22™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 69 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Doubling time: about 29 hours
Growth Properties: adherent
Oncogene: wnt7h +
Antigens Expressed: Blood Type O; Rh+
Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
TH01: 6
TPOX: 9,12
vWA: 14,15

Depositor: C.M. McGrath
Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.
Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Karyology: modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%.
There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases.
No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA from this line is available as ATCC® HTB-22D™ (10µg)
Total RNA from this line is available as ATCC® HTB-22R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cell pellet to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Note: Present batches of MCF7 cells are exhibiting the following growth pattern:
The cells usually attach as three-dimensional clusters and eventually grow to a 80-90% confluent monolayer. However, we are finding that most of the clusters remain in suspension until after the 2nd subculture.
After first subculture all the cells will not attach. There will be clusters in suspension. Break up the clusters the best you can by gently pipetting with a small bore pipette (5 ml or smaller). After a few days incubation, the cells should reattach as three-dimensional islands (there will be some clusters that do not reattach). Growth will eventually spread out from the islands and the culture should, after the second subculture, flatten and become 70-80% confluent.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 10 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Transfer the cell suspension to the centrifuge tube with the medium and cells from Step #1 and spin at approximately 125 xg for 5 to10 minutes. Discard the supernatnant.

6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Maintain cultures at a cell concentration between 2x10(4) and 2 x 10(5) cells/cm2.
   Subcultivation Ratio: 1:3 to 1:6.

7. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- 0.01mg/ml bovine insulin
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) or ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog at www.atcc.org)

Sugarman BJ et al. **Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro**. Science 230: 943-945, 1985 PubMed: 86044518

Takahashi K and Suzuki K. **Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7** cells. Int. J. Cancer 55: 453-458, 1993 PubMed: 93388025

Brandes LJ and Hermonat MW. **Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol**. Cancer Res. 43: 2831-2835, 1983 PubMed: 83206536

Lan MS et al. **Polypeptide core of a human pancreatic tumor mucin antigen**. Cancer Res. 50: 2997-3001, 1990 PubMed: 90242270

Pratt SE and Pollak MN. **Estrogen and antiestrogen modulation of MCF7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media**. Cancer Res. 53: 5193-5198, 1993 PubMed: 94036798

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue**. Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

Soule HD et al. **A human cell line from a pleural effusion derived from a breast carcinoma**. J. Natl. Cancer Inst. 51: 1409-1416, 1973 PubMed: 74054239

Bellet D et al. **Malignant transformation of nontrophoblastic cells is associated with the expression of chorionic gonadotropin beta genes normally transcribed in trophoblastic cells**. Cancer Res. 57: 516-523, 1997 PubMed: 97164677

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma**. Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Komarova EA et al. **Intracellular localization of p53 tumor suppressor protein in gamma-irradiated cells is cell cycle regulated and determined by the nucleus**. Cancer Res. 57: 5217-5220, 1997 PubMed: 98053886

van Dijk MA et al. **A functional assay in yeas for the human estrogen receptor displays wild-type and variant estrogen receptor messenger RNAs present in breast carcinoma**. Cancer Res. 57: 3478-3485, 1997 PubMed: 97413630

Landers JE et al. **Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein**. Cancer Res. 57: 3562-3568, 1997 PubMed: 97413643

Umekita Y et al. **Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride**. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996 PubMed: 97030277

Zamora-Leon SP et al. **Expression of the fructose transporter GLUT5 in human breast cancer**. Proc. Natl. Acad. Sci. USA 93: 1847-1852, 1996 PubMed: 96312501

Geiger T et al. **Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice.** Anti-Cancer Drug Des. 13: 35-45, 1998 PubMed: 98134516

Jang SI et al. **Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene**. J. Biol. Chem. 271: 24105-24114, 1996 PubMed: 96394543

Lee JH et al. **The proximal promoter of the human transglutaminase 3 gene**. J. Biol. Chem. 271: 4561-4568, 1996 PubMed: 96224044

Chang K and Pastan I. **Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers**. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996 PubMed: 96133892

Zhu X et al. **Cell cycle-dependent modulation of telomerase activity in tumor cells**. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996 PubMed: 96234095

Bacus SS et al. **Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen.** Mol. Carcinog. 3: 350-362, 1990 PubMed: 91119659

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

05/09

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	HTB-126™	Order this Item	Price:
Designations:	Hs 578T		
Depositors:	AJ Hackett		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	Organ: mammary gland; breast Disease: carcinoma		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Receptors:	estrogen receptor, not expressed [1119]		
Tumorigenic:	No		
DNA Profile (STR):	Amelogenin: X CSF1PO: 13 D13S317: 11 D16S539: 9,12 D5S818: 11 D7S820: 10 THO1: 9,9.3 TPOX: 8 vWA: 17		
Cytogenetic Analysis:	Number of cells examined = 50; Modal Chromosome Number = 59 with a range of 50 to 77; Polyploidy Rate = 33.8% Composite karyotype: 50-77 <3n> X, -1, del(1)(q12), -2, del(2)(?q36), der(3)t(3;15)(q10;p10),-4, -5,der(5)t(5;8)(p10;q10),-6, i(6)(p10), +8, -9, -10, -11, del(11)(p12), -12, -13, -14, -15, -15, -16, -17, -17, -17, i(17)(q10), -18, -19,der(19)(19pter<-q13::5q13<-qter), +22, +3 mar[cp12]		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 0 PGM1, 1 PGM3, 1		
Age:	74 years adult		
Gender:	female		
Ethnicity:	Caucasian		

$273.00

Related Links ▸

NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products
Product Information Sheet

Comments:

The Hs 578T cell strain was derived from a carcinoma of the breast.
It was originated by A.J. Hackett, et al. along with the Hs 578Bst (see ATCCHTB-125), which is a normal fibroblast-like line from the same patient.
The Hs 578T line had a mixed polygonal morphology initially, but a stellate cell type was selected for during passage and by cloning.
Aggregates of casein protein granules, desmosomes, tight junctions, lipid droplets and vesicularized smooth endoplasmic reticulum were observed by electron microscopy.
As with Hs 578Bst, no estrogen receptors or endogenous viruses were detected.
Cytogenetics: Derivatives and Markers
There are 8 consistent derivative chromosomes: del(1)(q12), del(2)(?q36), der(3)t(3;15)(q10;p10), der(5)t(5;8)(p10;q10), i(6)(p10), del(11)(p12), i(17)(q10), der(19)(19pter<-q13::5q13<-qter) plus two markers of unknown origin and one minute chromosome.
Four other markers, including two derivative chromosome 1s were noted are lower frequency.
Cytogenetics: Comments
This is a hypotriploid human cell line with a modal chromosome number of 59.
QM staining verified the absence of a Y chromosome.
The rate of polyploidy in excess of the modal number is 33.8%.
There were 8 consistent derivative chromosomes: del(1)(q12), del(2)(?q36), der(3)t(3;15)(q10;p10),der(5)t(5;8)(p10;q10), i(6)(p10), del(11)(p12),i(17)(q10),
der(19)(19pter<-q13::5q13<-qter) plus two markers of unknown origin and one minute chromosome.
Normal chromosome 17's were absent and only a single normal 15 was seen in most cells.
No brightly fluorescent Y chromosomes were detected with QM staining.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
normal (or near-normal) cell line established from the same patient:ATCC HTB-125
purified DNA:ATCC HTB-126D
purified RNA:ATCC HTB-126R

References:

1119: Hackett AJ, et al. Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs 578T) and the diploid myoepithelial (Hs 578Bst) cell lines. J. Natl. Cancer Inst. 58: 1795-1806, 1977. PubMed: 864756
22543: Smith HS. In vitro properties of epithelial cell lines established from human carcinomas and nonmalignant tissue. J. Natl. Cancer Inst. 62: 225-230, 1979. PubMed: 283258
32275: Littlewood-Evans AJ, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. Cancer Res. 57: 5386-5390, 1997. PubMed: 9393764

Return to Top

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	HTB-125™	Order this Item	Price: $329.00
Designations:	Hs 578Bst		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	fibroblast		
	PHOTO		
Source:	Organ: mammary gland; breast		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Receptors:	epidermal growth factor (EGF)		
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 11,13 D16S539: 9,12 D5S818: 11,13 D7S820: 10 THO1: 9,9.3 TPOX: 8 vWA: 17		
Cytogenetic Analysis:	modal number = 46; range = 42 to 48.. This is a diploid human cell line with 46,XX karyotype. Polyploidy occurred at 6.9%. Both X chromosomes were normal. The chromosome N9 pair was heteromorphic for the centromeric heterochromatin having one with the normal size and the other about twice the size of the normal.		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 0 PGM1, 1 PGM3, 1		
Age:	74 years		
Gender:	female		
Ethnicity:	Caucasian		
Comments:	Hs 578Bst was derived by A.J. Hackett, et al. from normal breast tissue peripheral to an infiltrating ductal carcinoma which was the source for Hs 578T (see ATCCHTB-126). Hs 578Bst may have been myoepithelial in origin since the cells possessed microfilaments and clusters of pinocytotic vesicles similar to those seen in myoepithelia in vivo. No desmosomes were observed, estrogen receptor protein was not present, and no endogenous viruses were detected. Note: A frozen ampule at unknown population doubling (PDL) was received at the ATCCin 1983. Cells had the potential to reach approximately 22 more population doublings before the onset of senescence. See Batch Specific information for PDL of current Distribution freeze.		
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC Hybri-Care Medium, Catalog No. 46-X. Hybri-Care Medium is supplied as a powder and should be reconstituted in 1 L cell-culture-grade water and supplemented with 1.5 g/L sodium bicarbonate. To make the complete growth medium, add the following components to the base medium: 30 ng/ml mouse EGF; fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C Atmosphere: air, 95%; carbon dioxide (CO2), 5%		

Related Links
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products
Product Information Sheet

Subculturing:	Protocol: Remove medium, and rinse with 0.25% trypsin, 0.53 EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended Medium Renewal: 2 to 3 times per week
Preservation:	Freeze medium: Complete growth medium, 95%; DMSO, 5% Storage temperature: liquid nitrogen vapor phase
Related Products:	recommended serum:ATCC 30-2020 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 46-X tumor cell line established from the same patient:ATCC HTB-126
References:	1119: Hackett AJ, et al. Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs 578T) and the diploid myoepithelial (Hs 578Bst) cell lines. J. Natl. Cancer Inst. 58: 1795-1806, 1977. PubMed: 864756 21947: Hancock ME, et al. Method for predicting chemosensitivity of anti-cancer drugs. US Patent 4,937,182 dated Jun 26 1990 24344: Stampfer MR, et al. Enhanced growth medium and method for culturing human mammary epithelial cells. US Patent 4,423,145 dated Dec 27 1983

Return to Top

Cell Line Designation: MDA-MB-231
ATCC® Catalog No. HTB-26™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 51 years
Gender: female
Ethnicity: Caucasian
Tumorigenic: yes, in nude mice also in ALS treated BALB/c mice; forms poorly differentiated adenocarcinoma (grade III)
AntigenExp: Blood Type O; Rh-
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
TH01: 7,9.3
TPOX: 8,9
vWA: 15,18

Morphology: epithelial
Growth properties: adherent
Receptors expressed: epidermal growth factor (EGF); transforming growth factor alpha (TGF alpha)
Depositors: R. Cailleau
Comments: The cells express the WNT7B oncogene [PubMed: 8168088].
Karyology: The cell line is aneuploid female, with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al., Cancer Genet. Cytogenet. 3: 61, 1981.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA: from this line is available as ATCC Catalog HTB-26D™ (10µg)
Total RNA from this line is available as ATCC HTB-26R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this*

point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a new culture flask.

5. Incubate the culture at 37°C in a suitable incubator. **(without CO2)**

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a free gas exchange with atmospheric air until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37° in a free gas exchange with atmospheric air until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of cell suspension to new culture vessels.
Subcultivation Ratio: 1:2 to 1:4.

6. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

2 to 3 times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO_2 and air mixture is detrimental to cells when using this medium for cultivation
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references are available in the catalog at www.atcc.org)

Brinkley BR et al. **Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro.** Cancer Res. 40: 3118-3129, 1980 PubMed: 81042058

Cruciger Q et al. **Morphological, biochemical and chromosomal characterization of breast tumor lines from pleural effusions**. In Vitro 12: 331, 1976

Siciliano MJ et al. **Mutually exclusive genetic signatures of human breast tumor cell lines with a**

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

common chromosomal marker. Cancer Res. 39: 919-922, 1979 PubMed: 79146816

Cailleau R et al. **Breast tumor cell lines from pleural effusions.** J. Natl. Cancer Inst. 53: 661-674, 1974 PubMed: 75007579

Cailleau R et al. **Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization.** In Vitro 14: 911-915, 1978 PubMed: 79087497

Bates SE et al. **Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells**. Endocrinology 126: 596-607, 1990 PubMed: 90092004

Dickstein B et al. **Increased epidermal growth factor receptor in an estrogen-responsive, adriamycin-resistant MCF-7 cell line**. J. Cell. Physiol. 157: 110-118, 1993 PubMed: 94012995

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue**. Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

Satya-Prakash KL et al. **Cytogenetic analysis on eight human breast tumor cell lines: high frequencies of 1q, 11q and HeLa-like marker chromosomes**. Cancer Genet. Cytogenet. 3: 61-73, 1981 PubMed: 82001960

Katayose Y et al. **Promoting apoptosis: a novel activity associated with the Cyclin-dependent kinase inhibitor p27**. Cancer Res. 57: 5441-5445, 1997 PubMed: 98069835

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma**. Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Sheng S et al. **Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells**. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996 PubMed: 97030253

De Vincenzo R et al. **Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines.** Anti-Cancer Drug Des. 13: 19-33, 1998 PubMed: 98134515

Soker S et al. **Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain**. J. Biol. Chem. 271: 5761-5767, 1996 PubMed: 96215040

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods **11**:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Cell Line Designation: MDA-MB-468
ATCC® Catalog No. HTB-132™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: adenocarcinoma; mammary gland; breast
Age: 51 years
Gender: female
Ethnicity: Black
Morphology: epithelial
Growth Properties: adherent
Tumorigenic: Yes, tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells.
Antigens Expressed: Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)
Receptors expressed: epidermal growth factor (EGF); transforming growth factor alpha (TGF alpha)
DNA profile: (STR analysis)

Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 9
D5S818: 12
D7S820: 8
TH01: 7
TPOX: 8,9
vWA: 18

Depositor: R. Cailleau
Comments: The MDA-MB-468 cell line was isolated in 1977 by R. Cailleau, et al., from a pleural effusion of a 51-year-old Black female patient with metastatic adenocarcinoma of the breast. Although the tissue donor was heterozygous for the G6PD alleles, the cell line consistently showed only the G6PD A phenotype.
EGF receptor is present at 1 X 10 exp6 per cell.
There is a G -> A mutation in codon 273 of the p53 gene resulting in an Arg -> His substitution.
Karyotype: The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.
Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.
A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes. Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.

4. Resuspend the cell pellet with the recommended complete medium and dispense into a culture flask.

5. Incubate the culture at 37°C in a suitable incubator in a free gas exchange with atmospheric air

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a free gas exchange with atmospheric air until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a free gas exchange with atmospheric air until cells are ready to be subcultured

Subculturing Procedure

Volumes used in this protocol are for a 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of the cell suspension to new culture vessels.
   Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended.

6. Incubate cultures at 37°C; in a free gas exchange with atmospheric air.

   Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in ***Culture of Animal Cells: A Manual of Basic Technique*** by R. Ian Freshney, 4th edition, published by Wiley - Liss, N.Y., 2000.

Medium Renewal

Every 2-3 days.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO_2 and air mixture is detrimental to cells when using this medium for cultivation.
ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) and ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references are available in the catalog at www.atcc.org)

Brinkley, B.R., et al. **Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro.** Cancer Res. 40: 3118-3129, 1980 PubMed: 81042058

Siciliano, M.J., et al. **Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker.** Cancer Res. 39: 919-922, 1979 PubMed: 79146816

Pathak, S., et al. **A human breast adenocarcinoma with chromosome and isoenzyme markers similar to**

those of the HeLa line. J. Natl. Cancer Inst. 62: 263-271, 1979 PubMed: 79112569

Cailleau, R., et al. **Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization**. In Vitro 14: 911-915, 1978 PubMed: 79087497

Nigro, J.M., et al. **Mutations in the p53 gene occur in diverse human tumour types**. Nature 342: 705-707, 1989 PubMed: 90081846

Bates, S.E., et al. **Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells**. Endocrinology 126: 596-607, 1990 PubMed: 90092004

Avila, M.A., et al. **Quercetin mediates the down-regulation of mutant p53 in the human breast cancer cell line MDA-MB468**. Cancer Res. 54: 2424-2428, 1994 PubMed: 94215181

Littlewood-Evans, A.J., et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma**. Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Zamora-Leon, S.P., et al. **Expression of the fructose transporter GLUT5 in human breast cancer**. Proc. Natl. Acad. Sci. USA 93: 1847-1852, 1996 PubMed: 96312501

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods **11**:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

07/07

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	HTB-129™ Order this Item	Price:	$264.00
Designations:	MDA-MB-435S		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	spindle shaped		
	PHOTO		
Source:	Organ: previously described as: mammary gland; breast Disease: previously described as ductal carcinoma Derived from metastatic site: pleural effusion		
Cellular Products:	tubulin; actin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Isolation:	Isolation date: 1976		
Tumorigenic:	No		
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18		
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 2 PGM3, 1		
Age:	31 years adult		
Gender:	female		
Ethnicity:	Caucasian		

Related Links
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Comments: This cell line was originally described as a spindle shaped variant of the parental MDA-MB-435 strain isolated in 1976 by R. Cailleau, et al. from the pleural effusion of a 31 year old female with metastatic, ductal adenocarcinoma of the breast. However, recent studies have generated questions about the origin of the parent cell line, MDA-MB-435, and by extension HTB-129. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast [PubMed ID: 10700174, PubMed ID: 15150101, PubMed ID: 15679052]. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCChas responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line [PubMed ID: 12354931 and PubMed ID: 17004106].
Derivatives of HTB-129 with identities in question:
M4A4, ATCC® CRL-2914
M4A4 GFP, ATCC® CRL-2915
M4A4 LM3-2 GFP, ATCC® CRL-2916
M4A4 LM3-4 CL 16 GFP, ATCC® CRL-2917
NM2C5, ATCC® CRL-2918
NM2C5 GFP, ATCC® CRL-2919

Propagation: ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium:

- 0.01mg/ml bovine insulin
- 0.01mg/ml glutathione
- fetal bovine serum to a final concentration of 10%

Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing: Protocol: Remove medium, add fresh 0.25%trypsin - 0.53 mM EDTA, rinse and remove. Place flask at room temperature (or incubated at 37C) for approximately 10 minutes or until the cells detach. Add fresh medium, aspirate and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: Freeze medium: Culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-129D
purified RNA:ATCC HTB-129R

References:
1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
22429: Siciliano MJ, et al. Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker. Cancer Res. 39: 919-922, 1979. PubMed: 427779
22656: Cailleau R, et al. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro 14: 911-915, 1978. PubMed: 730202
32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194
32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: 8650224
49803: Ross DT, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nature Genetics 24: 227-235, 2000. PubMed: 10700174
89918: Ellison G, et al. Further evidence to support the melanocytic origin of MDA-MB-435. Mol. Pathol. 55: 294-299, 2002. PubMed: 12354931
90826: Sellappan s, et al. Lineage infidelity of MDA-MB-435 cells: expression of melanocyte proteins in a breast cancer cell line. Cancer Res. 64: 3479-3485, 2004. PubMed: 15150101
90828: Rae JM, et al. Common origins of MDA-MB-435 cells from various sources with those shown to have melanoma properties. Clin. Exp. Metastasis 21: 543-552, 2004. PubMed: 15679052
16173093: Rae JM, et al., MDA-MB-435 cells are derived from M14 Melanoma cells - a loss for breast cancer, but a boon for melanoma research. Breast Cancer Res. Treat. 104:13-19, 2007. PubMed: 17004106.
16173545: Chambers AF. MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 69(13): 5292-5293, 2009. PubMed: 19549886.

Return to Top

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	HTB-36™	Order this Item	Price: $273.00
Designations:	JEG-3		
Depositors:	G Kohler		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	Organ: placenta Disease: choriocarcinoma		
Cellular Products:	human chorionic gonadotropin (hCG), human chorionic somatomammotropin (placental lactogen); progesterone		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Applications:	transfection host (Roche FuGENE® Transfection Reagents)		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9,11 D16S539: 13,14 D5S818: 10,11 D7S820: 10,12 THO1: 9,9.3 TPOX: 8 vWA: 16		
Cytogenetic Analysis:	This is a hypertriploid human cell line. The modal chromosome number is 71, occurring at 34%, and polyploidy at 2.6%. The t(4;11)(p15;q13), i(13q), t (10p15q), del(18)(q21), and 6 other markers are common to most cells, and two other markers are found in some. Giant satellites are seen in one N14, and two N22. N2, N5, and N9 have 4 copies, and N7, N13, N18, N21 and X a single copy. A single Y chromosome is detected by Q-band examination.		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1-2 PGM1, 1 PGM3, 1-2		
Comments:	This is one of six clonally derived lines isolated from the Woods strain of the Erwin-Turner tumor by Kohler and associates. The cells are able to transform steroid precursors to estrone and estradiol		
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C		
Subculturing:	Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended Medium Renewal: 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.		
Preservation:	Freeze medium: Culture medium, 95%; DMSO, 5% Storage temperature: liquid nitrogen vapor phase		
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020		

Related Links ▸
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

References:

22536: Fogh J, et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. 58: 209-214, 1977. PubMed: 833871
22539: Fogh J, et al. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59: 221-226, 1977. PubMed: 327080
23377: . . Acta Endocrinol. Suppl. 153: 137-153, 1971.
32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029
32564: Roesler WJ, et al. The alpha-isoform of the CCAAT/enhancer-binding protein is required for mediating cAMP responsiveness of the phosphoenolpyruvate carboxykinase promoter in hepatoma cells. J. Biol. Chem. 271: 8068-8074, 1996. PubMed: 8626491
58051: Kohler PO, Bridson WE. Isolation of hormone-producing clonal lines of human choriocarcinoma. J. Clin. Endocrinol. 32: 683-687, 1971.

Return to Top

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	HTB-144™	Order this Item	Price: $287.00

Designations:	JAR
Depositors:	RA Pattillo
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	Organ: placenta Disease: choriocarcinoma
Cellular Products:	estrogen; progesterone; human chorionic gonadotropin (hCG); human chorionic somatomammotropin (placental lactogen); hCG production averages 22.5 ng/ml after reculturing
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 7,10 D13S317: 11 D16S539: 9,10 D5S818: 10,11 D7S820: 10,11 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	This is probably a pseudotriploid human cell line with the modal chromosome number of 68, occurring in 24% of cells, but cells with both 69 (22%) and 70 (18%) chromosome counts also occurred frequently. Cells with higher ploidies occurred at 7.0%. Karyotypes were extremely complex. Consistently there were 20 to 25 marker chromosomes (>30% of total chromosome content) per cell. Most marker chromosomes had complex structural rearrangements, and the origin of chromosome segments of these markers often defied identification. Among the frequently found markers were 8p+, 11p, a large metacentric [?t (3qter--3q12::?--C--?::3q12--3qter)] der(?13)T(1;?13) (p13;?q14), and many others. There was only one normal X chromosome. Normal N3 and N13 were not found.
Isoenzymes:	AK-1, 1 ES-D, 2 G6PD, B GLO-I, 1 PGM1, 1-2 PGM3, 1-2
Age:	fetus
Gender:	male
Ethnicity:	Caucasian
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37.0°C

Related Links ▸
NCBI Entrez Search
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Subculturing: Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.03% (w/v) EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.
   Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
   Medium Renewal: Twice per week

Preservation: Freeze medium: Culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020

References: 2156: Pattillo RA, et al. The Jar cell line -- continuous human multihormone production and controls. In Vitro 6: 398-399, 1971.

Return to Top

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number:	CCL-98™	Order this Item	Price: $264.00
Designations:	BeWo		
Depositors:	RA Pattillo		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	Organ: placenta Disease: choriocarcinoma		
Cellular Products:	hormones; progesterone; human chorionic gonadotropin (hCG); human chorionic somatomammotropin (placental lactogen); estrogen; estrone; estriol; estradiol; keratin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Virus Susceptibility:	Human poliovirus 3 Vesicular stomatitis virus		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9,11 D16S539: 13,14 D5S818: 10,11 D7S820: 10,12 THO1: 9,9.3 TPOX: 8 vWA: 16		
Cytogenetic Analysis:	modal number = 86; range = 71 to 178. Stemline number is hypotetraploid. Karyotype relatively stable within stemline number.		
Isoenzymes:	G6PD, B		
Gender:	male		
Comments:	The cells are positive for keratin by immunoperoxidase staining.		
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C		
Subculturing:	Protocol: 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended Medium Renewal: 3 to 4 times per week		
Preservation:	Freeze medium: Complete growth medium 95%; DMSO, 5% Storage temperature: liquid nitrogen vapor phase		

Related Links
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

1219: Pattillo RA, et al. Human hormone production in vitro. Science 159: 1467-1469, 1968. PubMed: 5753554
22139: Pattillo RA, et al. Control mechanisms for gonadotrophic hormone production in vitro. In Vitro 6: 205-214, 1970. PubMed: 5535575
22343: Pattillo RA, et al. Estrogen production by trophoblastic tumors in tissue culture. J. Clin. Endocrinol. Metab. 34: 59-61, 1972. PubMed: 4332667
26271: Pattillo RA, Gey GO. The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. Cancer Res. 28: 1231-1236, 1968. PubMed: 4299001
26272: Hertz R. Choriocarcinoma of women maintained in serial passage in hamster and rat. Proc. Soc. Exp. Biol. Med. 102: 77-81, 1959. PubMed: 14401422
26273: Pattillo RA, et al. The hormone-synthesizing trophoblastic cell in vitro: a model for cancer research and placental hormone synthesis. Ann. N.Y. Acad. Sci. 172: 288-298, 1971. PubMed: 5289994
32279: Schar BK, et al. Simultaneous detection of all four alkaline phosphatase isoenzymes in human germ cell tumors using reverse transcription-PCR. Cancer Res. 57: 3841-3846, 1997. PubMed: 9288797
32858: Heckert LL, et al. The cAMP response elements of the alpha subunit gene bind similar proteins in trophoblasts and gonadotropes but have distinct functional sequence requirements. J. Biol. Chem. 49: 31650-31656, 1996. PubMed: 8940185

Return to Top

Cell Line Designation: HUVEC-CS
ATCC® Catalog No. CRL-2873™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculture Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: umbilical vein; vascular endothelium
Doubling Time: approximately 36 hours
Morphology: endothelial
Growth Properties: adherent
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 11,12
D13S317: 9,11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,12
TH01: 6,9.3
TPOX: 8,11
vWA: 16

Depositor: Bird I.
Comments: HUVEC-CS was derived from HUV-EC-C (ATCC CRL-1730™) by initially passaging in tissue culture plastic flasks without additional growth factor like ECGS. Subsequently the HUVEC-CS cell line was plated on gelatin-coated flasks.
It exhibits positive acetylated low-density lipoprotein (AcLDL) uptake and expresses eNOS, CD31 and ve-cadherin (classical markers of endothelial cells. It spontaneously forms capillary-like structures when grown on Matrigel. Receptors were detected for angiotensin II (AII), bradykinin and ATP.[PubMed: 15350190].
Distribution freeze lots have a doubling potential of 5 to 8 PDLs
Parent item: HUV-EC-C (ATCC CRL-1730™)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*
Note: porcine gelatin coated flasks have to be used. (Depositor recommends Becton Dickinson Labware 75 cm^2 flask Cat No. 356654 or 100 mm dish Cat No. 354653 or 6 well plates Cat No 354652).

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 10 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a **porcine gelatin coated** culture flask (*It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Culture

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to **porcine gelatin coated** flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin- 0.53 mM EDTA solution (GIBCO Cat# 25300-054) to remove all traces of serum that contains trypsin inhibitor.

3. Add 1.0 to 2.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. To remove trypsin-EDTA solution, transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to10 minutes.

6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new **porcine gelatin coated** culture vessels.
   Subcultivation Ratio: 1:3 to 1:4
   An inoculum of 1X 10(4) to 2X 10(4) viable cells/cm^2 is recommended.
7. Place culture vessels in incubators at 37°C. Subculture when reaching a cell concentration between 3 X 10(4) and 5 X 10(4) cells/cm^2.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal:

Every 2-3 days

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 20%

This medium is formulated for use with a 5% CO_2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 in air atmosphere is then recommended).
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 10% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Gifford, S.M et al. **Functional Characterization of HUVEC-CS: Ca^{2+} signaling, ERK ½ activation, mitogenesis and vadodilator production.** Journal of Endocrinology 182: 485-499 (2004). PubMed: 15350190

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

09/08

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Cell Line Designation: HUV-EC-C
ATCC Catalog No. CRL-1730™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal Procedure
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: umbilical vein; vascular endothelium
Morphology: endothelial
Growth Properties: adherent
Tumorigenic: the cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 11,12
D13S317: 9,11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,12
TH01: 6,9.3
TPOX: 8,11
vWA: 16

Products: factor VIII
Depositors: H. Hoshi
Comments: Endothelial Cell Growth Supplement (ECGS) and unidentified factors from bovine pituitary, hypothalamus or whole brain extracts are mitogenic for this line.
The cells have a life expectancy of 50 to 60 population doublings.
Karyotype: This is a hypodiploid human cell line. The modal chromosome number was 45 occurring in 72% of cells counted. The rate of polyploid cells was 15.8%. All cells had monosomic N13 and the subclone with additional monosomic N15 predominates. Other coexisting subclones include those with 46,XX,-11,-13,i(11p),i(11q) and 46,XX,+11,-13 karyotypes. Both X chromosomes appear normal.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into

a new culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all of the growth medium except for approximately 5 to 10 ml to cover the floor of the flask. The old medium can be saved for reuse. Incubate the cells at 37°C in a 5% carbon dioxide gas phase until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes to spin down the suspended cells into a soft pellet. Remove all but 5 ml of supernatant medium, then resuspend the cells in the remaining medium and add back to a 25 cm^2 flask. The old medium can be saved for reuse. Incubate at 37°C in a 5% carbon dioxide gas phase until they are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of the cell suspension to new culture vessels.
Subcultivation ratio : 1:2 to 1:3.

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated of F-12K Medium, Catalog No. 30-2004.
To make the complete growth medium, add the following components to the base medium:

- 0.1mg/ml heparin
- 0.03-0.05 mg/ml endothelial cell growth supplement (ECGS)
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% carbon dioxide gas phase.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Technical Information site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog at www.atcc.org)

Hoshi H. And McKeehan W.L. (1984), **Brain- and liver cell-derived factors are required for growth of human endothelial cells in serum-free culture.** Proc. Natl. Acad. Sci. USA 81:6413-6417. PubMed: 6333682.

Zahedi K. (1997), **Characterization of the binding of serum amyloid P to laminin**. J. Biol. Chem. 272:2143-2148. PubMed: 97152982.

Soker S. et al. (1996), **Characterization of novel vascular endothelial growth factor (VEGF) receptors on**

tumor cells that bind VEGF165 via its exon 7-endoded domain. J. Biol. Chem. 271:5761-5767. PubMed: 96215040.

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods **11:**223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Cell Line Designation: NIH/ 3T3
ATCC Catalog No. CRL-1658™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal Procedure
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Mus musculus* (mouse)
Strain: NIH/Swiss
Tissue: embryo
Morphology: fibroblast
Growth properties: adherent
VirusSuscept: murine sarcoma viruses; murine leukemia viruses
Depositors: S.A. Aaronson
Comments: The NIH/3T3, a continuous cell line of highly contact-inhibited cells was established from NIH Swiss mouse embryo cultures in the same manner as the original random bred 3T3 (ATCC CCL-92™) and the inbred BALB/c 3T3 (ATCC CCL-163™). The established NIH/3T3 line was subjected to more than 5 serial cycles of subcloning in order to develop a subclone with morphologic characteristics best suited for transformation assays. These cells are useful for DNA transfection and transformation studies.
Tested and found negative for ectromelia virus (mousepox).

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure For Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes

handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Never allow the culture to become completely confluent. Subculture at 80% confluency or less.
Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of the cell supension to new culture vessels. Use 3-5 x 10(3) cells/cm^2 and subculture about every 3 days.

Note: In order to maintain this property of **high contact inhibition** it is necessary to transfer routinely at only high dilutions, otherwise variants tend to be selected having reduced contact inhibition. Such low density make culture vessels appear sparse and cell growth sensitive to sub-optimal temperature and media conditions.

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two times per week.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:

- bovine calf serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 in air atmosphere is then recommended).
The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references are available in the catalog at www.atcc.org)

Copeland NG and Cooper GM. **Transfection by exogenous and endogenous murine retrovirus DNAs.** Cell 16: 347-356, 1979 PubMed: 79211204

Loffler S et al. **CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus.** J. Virol. 71: 42-49, 1997 PubMed: 97138295

Berson JF et al. **A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains.** J. Virol. 70: 6288-6295, 1996 PubMed: 96323150

Jones PL et al. **Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB.** Mol. Cell. Biol. 17: 6970-6981, 1997 PubMed: 98038766

Gonzalez Armas JC et al. **DNA immunization confers protection against murine cytomegalovirus infection.** J. Virol. 70: 7921-7928, 1996 PubMed: 97048074

Siess DC et al. **Exceptional fusogenicity of chinese hamster ovary cells with murine retrovirus suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process.** J. Virol. 70: 3432-439, 1996 PubMed: 96211474

Jang SI et al. **Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene.** J. Biol. Chem. 271: 24105-24114, 1996 PubMed: 96394543

Medin JA et al. **Correction in trans for Fabry disease: expression, secretion, and uptake of alpha-**

galactosidase A in patient-derived cells driven by a high-titer recombinant retroviral vector. Proc. Natl. Acad. Sci. USA 93: 7917-7922, 1996 PubMed: 96353919

Lee JH et al. **The proximal promoter of the human transglutaminase 3 gene**. J. Biol. Chem. 271: 4561-4568, 1996 PubMed: 96224044

Chang K and Pastan I. **Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers**. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996 PubMed: 96133892

Cranmer LD et al. **Identification, analysis, and evolutionary relationships of the putative murine cytomegalovirus homologs of the human cytomegalovirus UL82 (pp71) and UL83 (pp65) matrix phosphoproteins**. J. Virol. 70: 7929-7939, 1996 PubMed: 97048075

Shisler J et al. **Induction of susceptibility to tumor necrosis factor by E1A is dependent on binding to either p300 or p105-Rb and induction of DNA synthesis**. J. Virol. 70: 68-77, 1996 PubMed: 96099415

Cavanaugh VJ et al. **Murine cytomegalovirus with a deletion of genes spanning HindIII-J and -I displays altered cell and tissue tropism**. J. Virol. 70: 1365-1374, 1996 PubMed: 96190530

Westerman KA and Leboulch P. **Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination**. Proc. Natl. Acad. Sci. USA 93: 8971-8976, 1996 PubMed: 96392350

Jainchill J.L. et al. (1969), **Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells**. J. Virol. 4:549-553. PubMed:70064316.

Andersson P. et al. (1979), **A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection.** Cell 16:63-75. PubMed:79126035.

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

Plasmid Record

Plasmid	Source	Gene Transfected	Changes that Result from the Transfection
pGIPZ	open Biosystems	shRNA-Control	Control; no effect
pGIPZ	open Biosystems	shRNA-Nodal	Knock down of Nodal
pGL3-Promoter Vector	open Biosystems	Nodal MNDE-WT	To test Nodal enhancer activity via luciferase reporter
pGL3-Promoter Vector	Promega	Nodal MNDE-M1	To test Nodal enhancer activity with site 1 mutation via luciferase reporter
pGL3-Promoter Vector	Promega	Nodal MNDE-M2	To test Nodal enhancer activity with site 2 mutation via luciferase reporter
pGL3-Control Vector	Promega	Control Firefly Luciferase	Control Firefly Luciferase acitivity
pGL4.70 (hRUC) Vector	Promega	Control Renilla Luciferase	Control Firefly Luciferase acitivity for normalization
pGFP-V-RS	OriGene	empty vector	Control; no effect
pGFP-V-RS	Origene	scrambled shRNA	Control; no effect
pGFP-V-RS	Origene	HuSH 29mer shRNA Constructs against NODAL (4 sequences)	Knockdown Nodal expression
pReceiver-M13	GeneCopoeia	TGFBRII	Overexpress Type II receptor
pReceiver-M13	GeneCopoeia	ALK4	Overexpress ALK4
pReceiver-M13	GeneCopoeia	ALK7	Overexpress ALK7
pReceiver-M13	GeneCopoeia	EX-EGFP-M13	Express eGFP
pTRIPZ	open Biosystems	Nodal shRNA	Conditionally Knock out Nodal using Tet on and Tet Off
pTRIPZ	open Biosystems	Nodal shRNA	Conditionally Knock out Nodal using Tet on and Tet Off
pTRIPZ	open Biosystems	Nodal shRNA	Conditionally Knock out Nodal using Tet on and Tet Off
pTRIPZ	open Biosystems	RHS4743	Conditionally express turbo RFP using Tet on and Tet off
pTRIPZ	open Biosystems	sFRP2 shRNA	Conditionally Knockout sFRP2
pTRIPZ	open Biosystems	sFRP2 shRNA	Conditionally Knockout sFRP2
pTRIPZ	open Biosystems	sFRP2 shRNA	Conditionally Knockout sFRP2
pTRIPZ	open Biosystems	sFRP2 shRNA	Conditionally Knockout sFRP2
pTRIPZ	open Biosystems	sFRP2 shRNA	Conditionally Knockout sFRP2
pCMV6-XL4	OriGene	Nodal-DDK-MYC	Nodal Overexpression and the outcome from Nodal
pCR 4-TOPO	Invitrogen	pCR 4-TOPO 3956bp	PCR product as portion of Nodal Transcript
pCDNA 3	Invitrogen	GFP	Express GFP
pCMV6-XL5	OriGene	empty vector	Control; no effect
pCMV6-XL5	OriGene	XL5-HIF 1a	Express HIF-1a
ptdTomato	Clontech	TdTomato	Express TdTomato

Use of the miR30 design also allowed the use of **'rules-based' designs** for target sequence selection. One such rule is the destabilizing of the 5' end of the antisense strand which results in strand specific incorporation of miRNAs into RISC.

The proprietary design algorithm targets sequences in coding regions and the 3'UTR with the additional requirement that they contain greater than 3 mismatches to any other sequence in the human or mouse genomes.

Each shRNA construct has been sequence verified to ensure a match to the target gene. To assure you the highest possibility of modulating the gene expression level, each gene is represented by multiple shRNA constructs, each covering a unique region of the target gene.

Vector Information

Versatile Vector Design

Features of the pGIPZ lentiviral vector (Figure 2-3, Table 1) that make it a versatile tool for RNAi studies include:

- Ability to perform transfections or transductions using the replication incompetent lentivirus (Shimada, *et al.* 1995)
- TurboGFP and shRNAmir are part of a bicistronic transcript allowing the visual marking of shRNAmir expressing cells
- Amenable to *in vitro* and *in vivo* applications
- Puromycin drug resistance marker for selecting stable cell lines
- Molecular barcodes enable multiplexed screening in pools

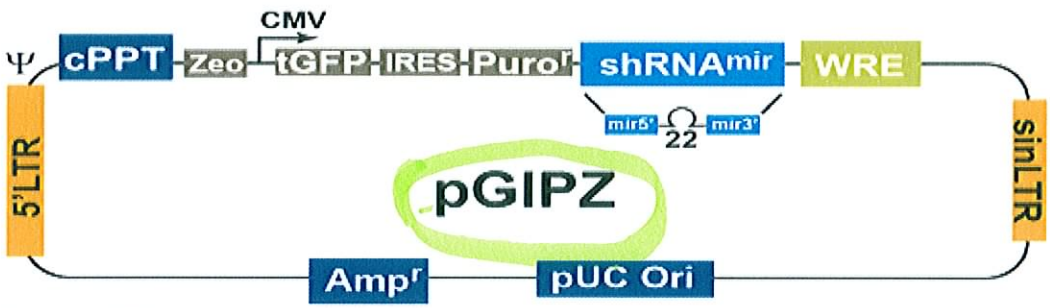


Figure 2. pGIPZ lentiviral vector

Table 1. Features of the pGIPZ vector

Vector Element	Utility
CMV Promoter	RNA Polymerase II promoter
cPPT	Central Polypurine tract helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
TurboGFP	Marker to track shRNAmir expression
IRES-puro resistance	Mammalian selectable marker
Amp resistance	Ampicillin (carbenicillin) bacterial selectable marker
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in *E. coli*
SIN-LTR	3' self inactivating long terminal repeat (Shimada, et al. 1995)
RRE	Rev response element
Zeo resistance	Bacterial selectable marker

Vector Map

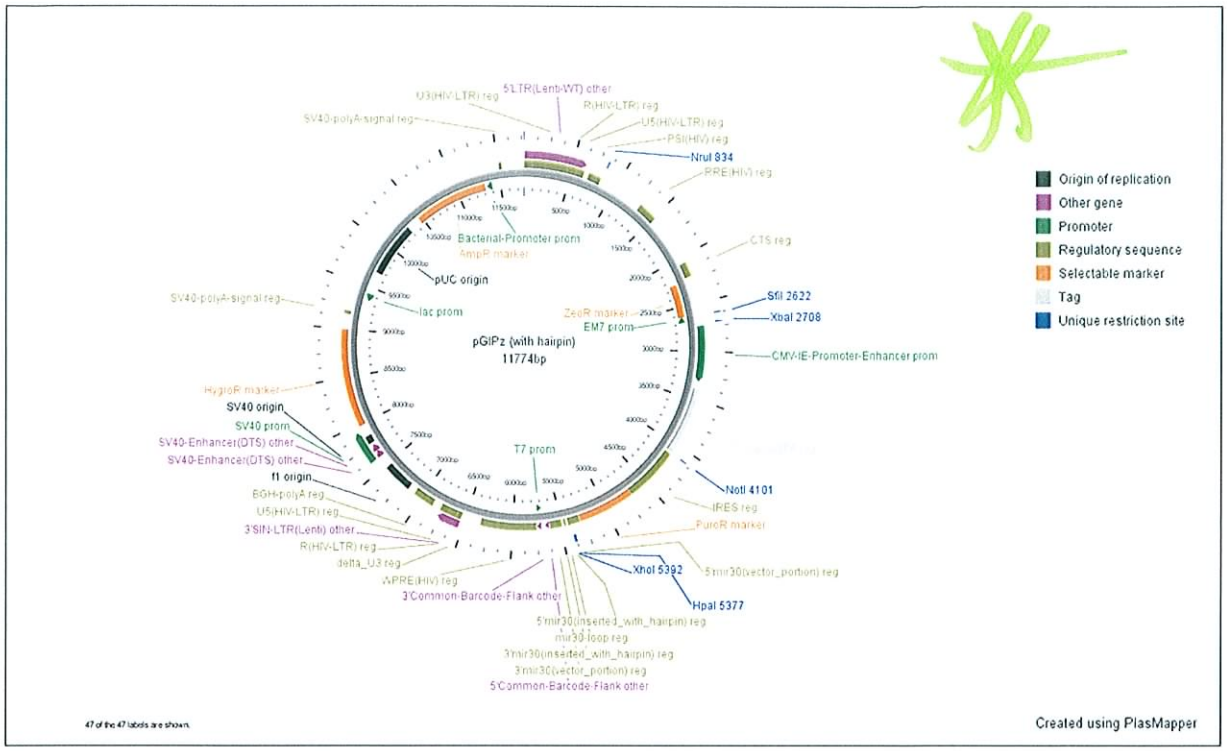


Figure 3. Detailed vector map of pGIPZ lentiviral vector.

Antibiotic Resistance

pGIPZ contains 3 antibiotic resistance markers (Table 2).

Quality Control

Table 2. Antibiotic resistances conveyed by pSM2

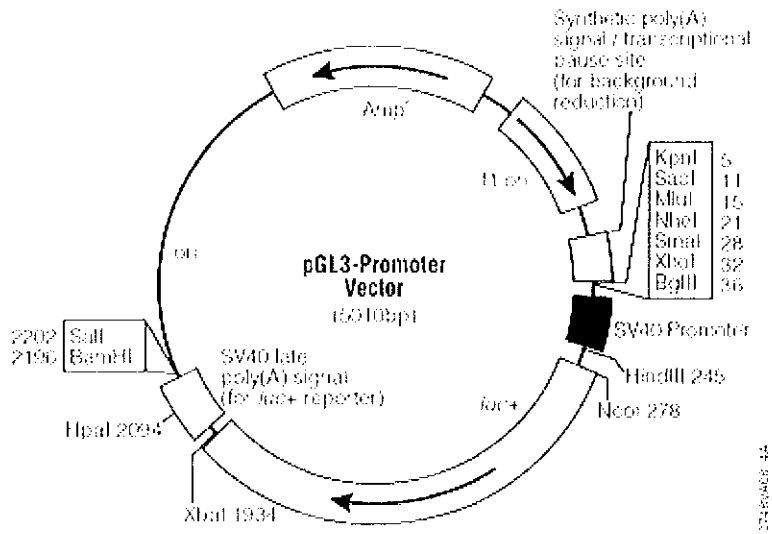
Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100 µg/ml	Bacterial selection marker (outside LTRs)
Zeocin	25µg/ml	Bacterial selection marker (inside LTRs)
Puromycin	Variable	Mammalian selectable marker

The GIPZ Lentiviral shRNAmir Library has passed through internal QC processes to ensure high quality and low recombination (Figures 4 and 5).

Figure 4. Representative shRNAmir containing pGIPZ lentiviral clones grown for 16 hours at 30°C and the plasmid isolated and normalized to a standard concentration. Clones were then digested with *Sac*II and run out on a gel. The expected band sizes are **1259 bp, 2502 bp, 7927 bp.** No recombinant products are visible. 10 kb molecular weight ladder (10 kb, 7 kb, 5 kb, 4 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb)

pgl3-promoter vector

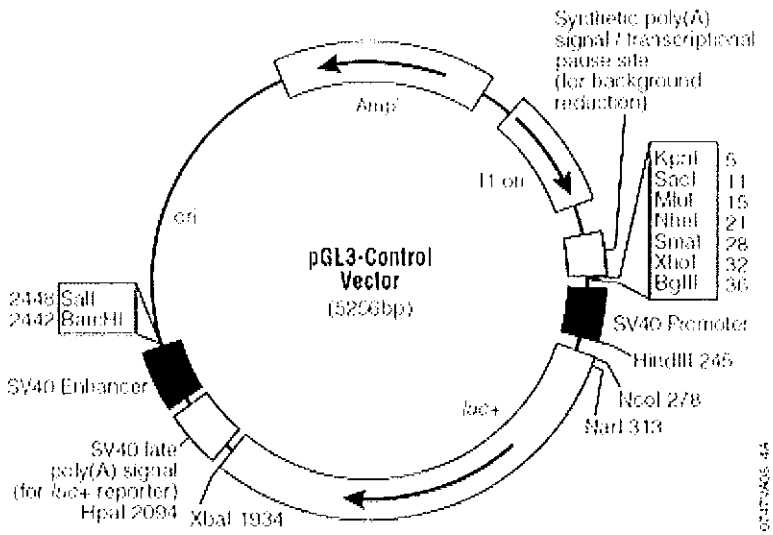
e1761



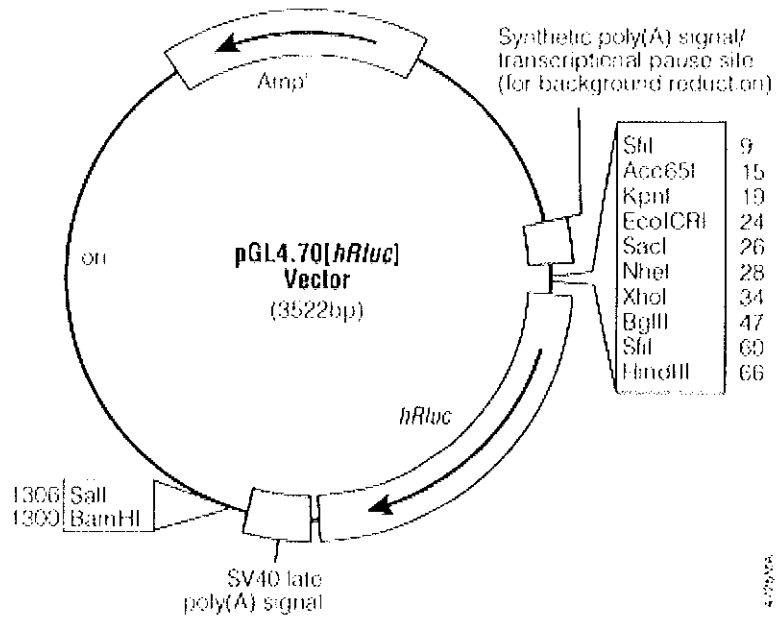
Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

pgl3-control vector

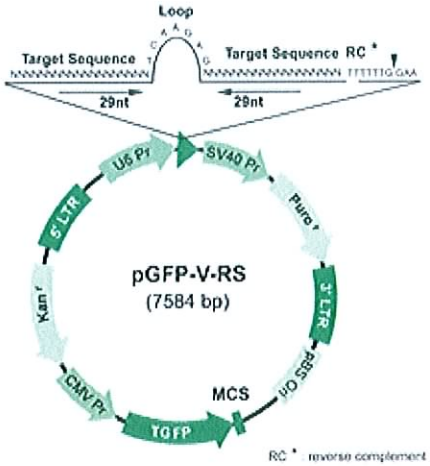
e1741



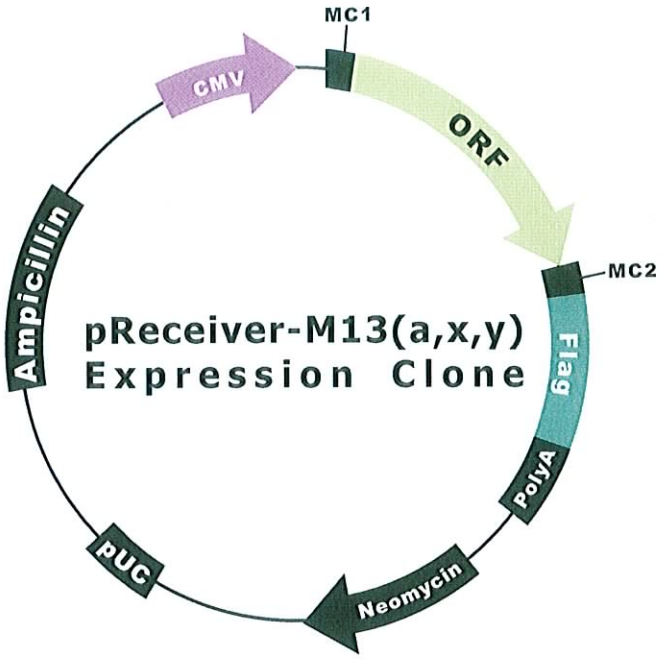
Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330



Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330


Loop
Target Sequence
Target Sequence RC *
29nt
29nt
U6 P
SV40 P
Puro r
3' LTR
pGFP-V-RS
(7584 bp)
Kan r
5' LTR
CMV P
MCS
TGFP
pBS ORI
RC * reverse complement

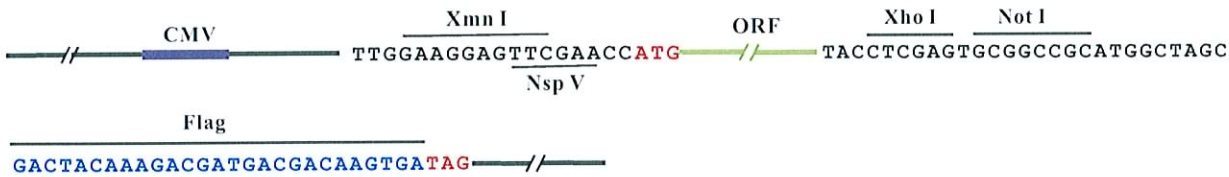
OmicsLink™ Expression Clone (CMV Promoter)



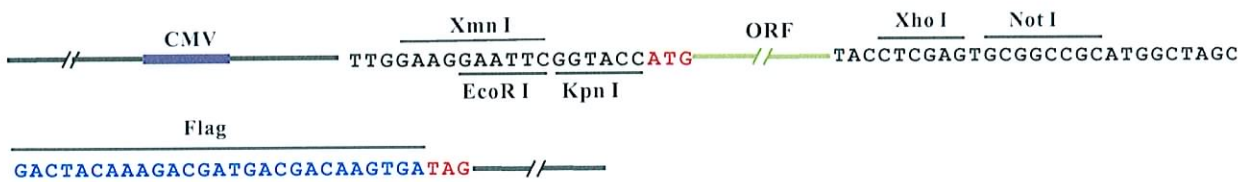
Vector Features

Promoter	CMV
Host Cell	Mammalian
Bacterial selection antibiotic	Ampicillin
Mammalian selection marker	Yes
Tag	Flag

pReceiver-M13a



pReceiver-M13x



pReceiver-M13y

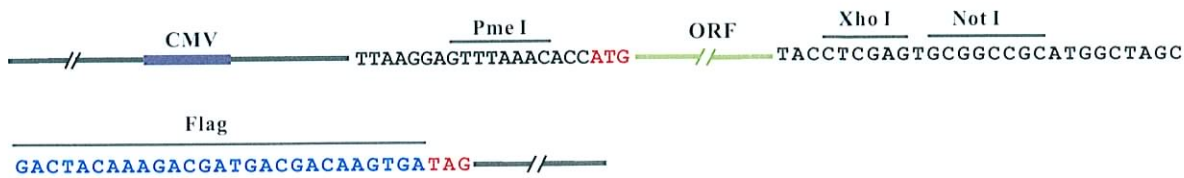


Table 1. Features of the pTRIPZ vector

Vector Element	Utility
TRE-minCMV promoter	Tetracycline responsive RNA Polymerase II promoter
UBC promoter	Drives expression of rtTA3 and IRES-puro
rtTA3	Reverse tetracycline transactivator
cPPT	Central Polypurine tract helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
TurboRFP	Marker to track shRNAmir expression
IRES-Puro resistance	Mammalian selectable marker
Amp resistance	Ampicillin (carbenicillin) bacterial selectable marker.
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in *E.coli*
SIN-LTR	Self inactivating long terminal repeat (Shimada, et al. 1995)
RRE	Rev response element
Zeo resistance	Bacterial selectable marker

VECTOR MAP

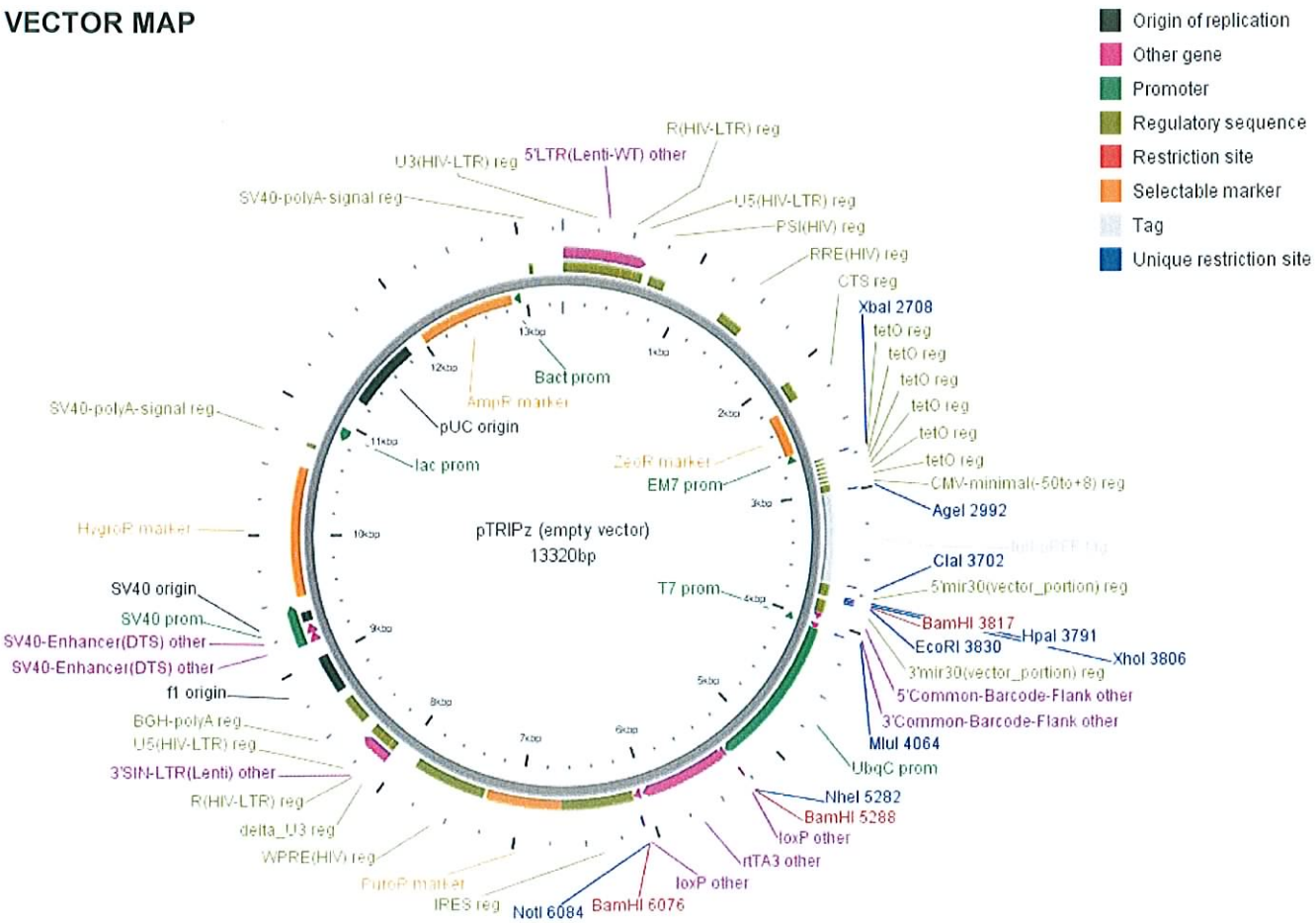


Figure 3. Detailed Vector Map of the pTRIPZ™ lentiviral vector (without hairpin). The empty vector is 13320bp in size

from its natural state to consist of a string of operators fused to the CMV minimal promoter, exhibits reduced basal expression and tighter binding to the second component, the transactivator. The pTRIPZ transactivator, known as the reverse tetracycline transactivator 3 (rtTA3) binds to and activates expression from TRE promoters in the presence of doxycycline. The rtTA3 transactivator is a modified version of the wildtype in two ways. First, unlike the original tetracycline transactivator the rtTA3 is modified to bind to the TRE in the presence of doxycycline rather than in its absence. Secondly, there are three mutations within the transactivator that increase its sensitivity to doxycycline by 25-fold over the initial rtTA without increasing background activity (Das, Zhou et al. 2004).

Use of TurboRFP in the pTRIPZ™ vector

As an added feature of the pTRIPZ vector, the TRE drives the expression of a TurboRFP reporter in addition to the shRNAmir. This induced expression of TurboRFP enables the user to easily observe expression from the TRE promoter, allowing quick assessment of factors such as: basal expression, viral titer, transduction efficiency/efficacy and overall technical success.

Tet-On® or Tet-Off® configuration is possible

The pTRIPZ vector is versatile in that it can be easily converted to a Tet-Off® capable vector using Cre/loxP technology or classical restriction digest. The rtTA3 is flanked by loxP sites allowing *in vitro* or *in vivo* excision of the rtTA3 by exposure to Cre recombinase. The rtTA3 is also flanked by a pair of *Bam*HI restriction sites allowing for straightforward cleavage and ligation of the vector to remove the rtTA3. Without the rtTA3 present on the vector a tetracycline transactivator (tTA) can be added extraneously to the system allowing it to function as Tet-Off®; where expression of shRNAmir and TurboRFP are alternatively induced in the absence of doxycycline. The functionality and versatility of the pTRIPZ vector is thus unsurpassed in the field of RNAi.

VECTOR INFORMATION

Versatile vector design

Features of the pTRIPZ inducible lentiviral vector (Figure 2-3, Table 1) that make it a versatile tool for RNAi studies include:

- Ability to use the vector in either a Tet-On® or Tet-Off® configuration
- TurboRFP and shRNAmir are part of a single transcript allowing the visual marking of shRNAmir expressing cells
- Amenable to *in vitro* and *in vivo* applications
- Inducible RNAi expanded to include both dividing and non-dividing cell lines
- Puromycin drug resistance marker for selecting stable cell lines
- Molecular barcodes enable multiplexed screening in pools

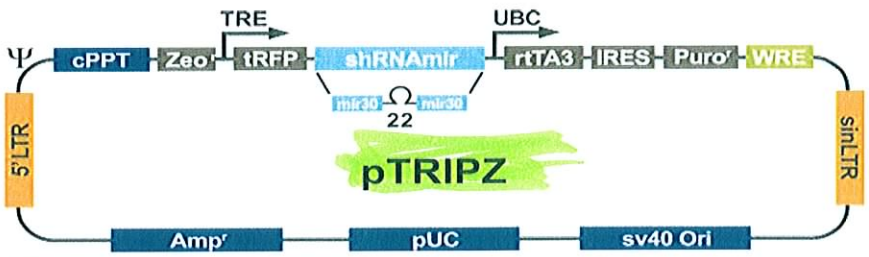
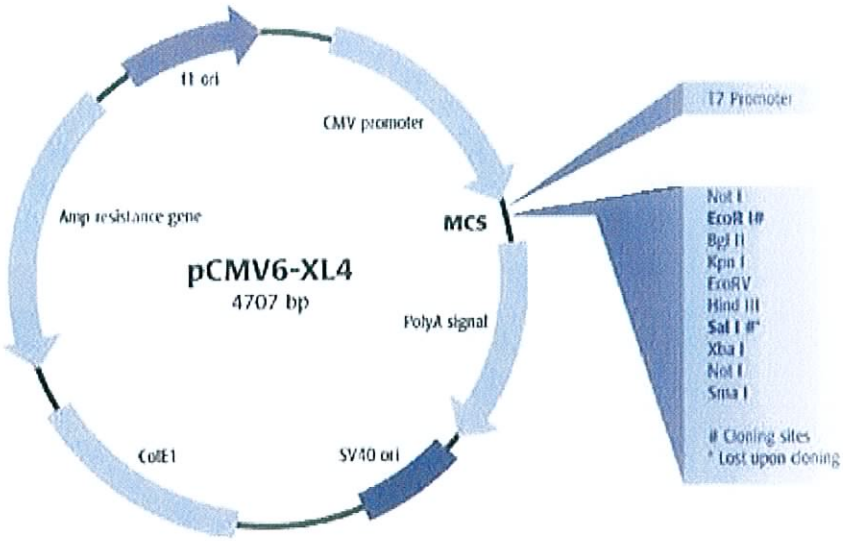
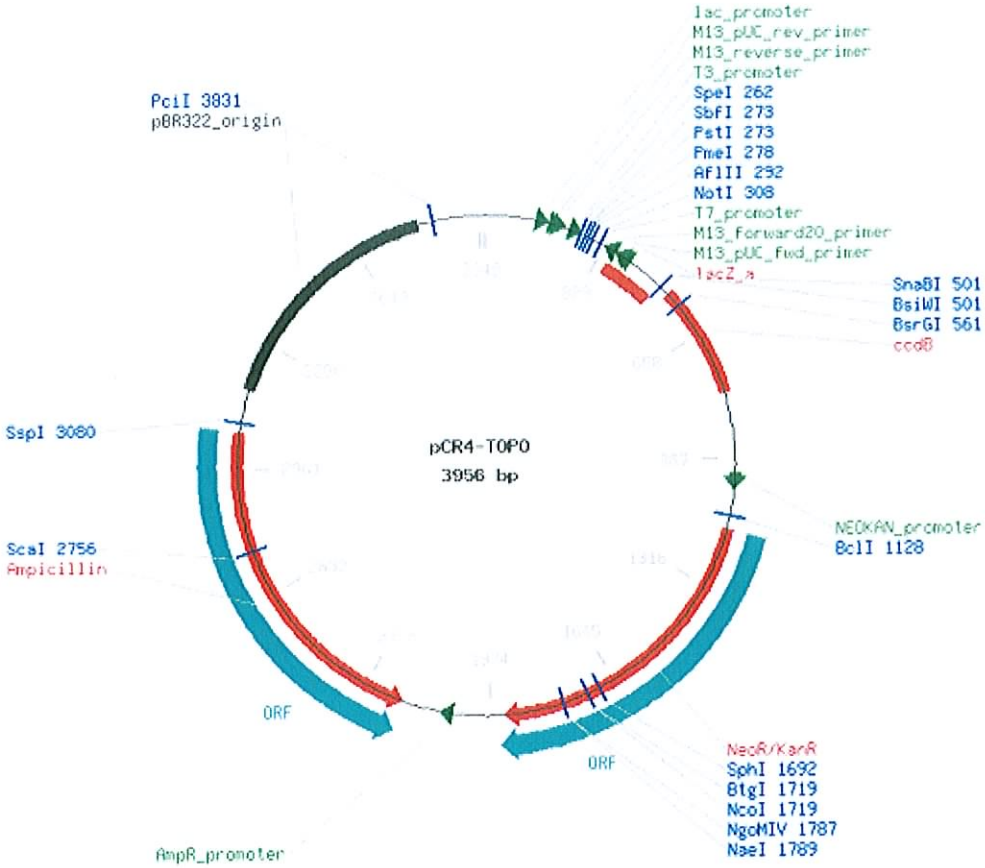


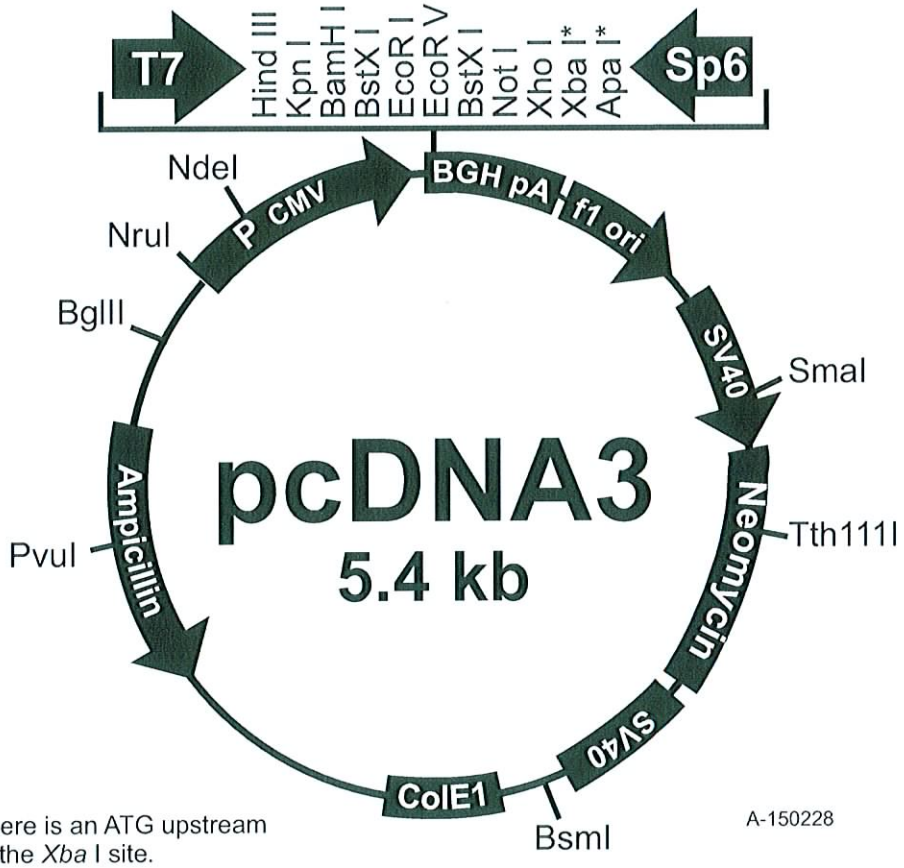
Figure 2. pTRIPZ lentiviral vector


f1 ori
CMV promoter
T7 Promoter
Amp resistance gene
MCS
pCMV6-XL4
4707 bp
Not I
EcoR I#
Bgl II
Kpn I
EcoRV
Hind III
Sal I #*
Xba I
Not I
Sma I
Cloning sites
* Lost upon cloning
PolyA signal
ColE1
SV40 ori


lac_promoter
M13_pUC_rev_primer
M13_reverse_primer
T3_promoter
SpeI 262
SbfI 273
PstI 273
PmeI 278
AflII 292
NotI 308
T7_promoter
M13_Forward20_primer
M13_pUC_Fwd_primer
lacZ_a
SnaBI 501
BsiWI 501
BsrGI 561
ccdB
PciI 3831
pBR322_origin
SspI 3060
pCR4-TOPO
3956 bp
NEOKAN_promoter
BclI 1128
ScaI 2756
Ampicillin
ORF
ORF
NeoR/KanR
SphI 1692
BtgI 1719
NcoI 1719
NgoMIV 1787
NaeI 1789
AmpR_promoter

Comments for pcDNA3:
5446 nucleotides

CMV promoter: bases 209-863
T7 promoter: bases 864-882
Polylinker: bases 889-994
Sp6 promoter: bases 999-1016
BGH poly A: bases 1018-1249
SV40 promoter: bases 1790-2115
SV40 origin of replication: bases 1984-2069
Neomycin ORF: bases 2151-2945
SV40 poly A: bases 3000-3372
ColE1 origin: bases 3632-4305
Ampicillin ORF: bases 4450-5310



* There is an ATG upstream of the *Xba* I site.

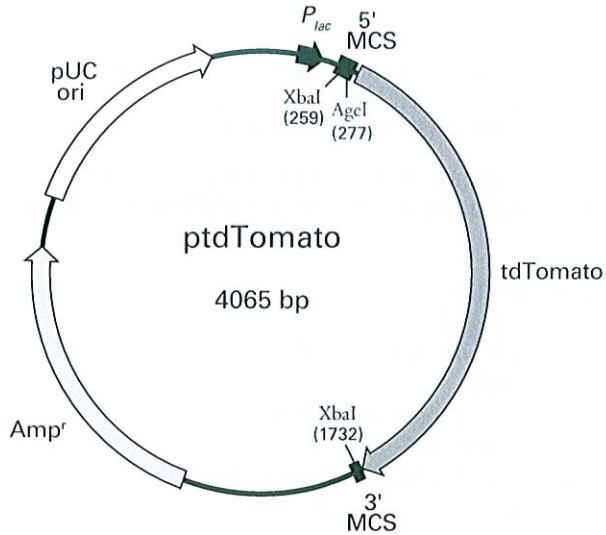
A-150228

The sequence of pcDNA3 has been compiled from information in sequence databases, published sequences, and other sources. This vector has not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department.

Vector Information

ptdTomato Vector Information PT4066-5

Catalog No. 632531



5' MCS

Asp718
KpnI
SmaI
XmaI
SalI
AccI
HindIII
BamHI
AgeI

234 GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGG TCGCCACCAT
CGGTTCGAAC GTACGGACGT CCAGCTGAGA TCTCCTAGGG GCCCATGGCC AGCGGTGGTA

3' MCS

NotI
EcoRI

1720 TACAAGTAGG CGGCCGCGAC TCTAGAATTC
ATGTTCATCC GCCGGCGCTG AGATCTTAAG

ptdTomato Vector Map and Multiple Cloning Sites (MCS).

Description

ptdTomato encodes tdTomato, a member of the family of fruit fluorescent proteins (1) derived from the *Discosoma sp.* red fluorescent protein, DsRed (2). Because the Tomato protein has a tendency to dimerize, the vector was designed with two copies of the Tomato coding region linked together to allow intramolecular dimerization. As a result, each tdTomato RNA transcript encodes a tandem dimer of the Tomato protein (excitation and emission maxima equal 554nm and 581nm, respectively).

In ptdTomato, the tdTomato coding sequence (2) is flanked by restriction sites (5' MCS and 3' MCS) that make it easy to excise the gene for use in other cloning applications. In *E. coli*, tdTomato is expressed from the *lac* promoter (P_{lac}) as a fusion with several amino acids, including the first five amino acids of the LacZ protein. Note, however, that if the tdTomato coding sequence is excised using a restriction site in the 5' MCS, the protein will no longer be expressed as a fusion (as it is when expressed from the *lac* promoter).

(PR882588; published 10 October 2008)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com