THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 biohazards 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 Biohazard 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

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Location of experimental work to be carried out: Building(s) RRI Room(s) 4274

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: HSFO
GRANT TITLE(S): Rapid Vascular Effect of Steroids

* **PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.**

Names of all personnel working under Principal Investigators supervision in this location:
QingMing Ding
Jozef Chorazyczewski

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES ⊗ NO
(including but not limited to 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 O 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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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? ⊗ 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	⊗ Yes O No	both	Not applicable
Rodent	⊗ Yes O No	primary	
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ 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	☒ Yes ○ No	HEK 293	
Rodent	☒ Yes ○ No	Rat Vascular Smooth Muscle Rat Endothelial Cells	
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ 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 ○ 1 ☒ 2 ○ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒ YES ○ 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 Known to Be 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	Human blood	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		○ Yes ☒ No		○ 1 ○ 2 ○ 3

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? ☒ YES ○ NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α JM109	PDC315 316	Microbix Biotech	ACS. AC6 MR GPR30	

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
adenovirus	adeno MR, GPR30, GFP, ShMR	Microbix Biosystem	AC5, AC6 MR, GFP, GPR30	

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ☒ YES ○ NO

4.6 Will virus be infectious to humans or animals? ☒ YES ○ NO

4.7 Will this be expected to increase the containment level required? ○ YES ☒ NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? ○ YES ○ 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: _____
Please attach a full description of the biological agent.

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

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals ○ YES, specify: _____ ○ NO

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7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ⊗ 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*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗ 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 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ☒ 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 NO, please forward the permit to he Biosafety Officer when available.

10.9 Please 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_____
If no, please proceed to Section 12.0 ☒ NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

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SIGNATURE 

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ☒ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
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  Date: 1 Sept 09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: 
Date: September 02, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

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GRANT SUMMARY

Aldosterone and other vasoactive steroids are important physiological and pathophysiological regulators of cardiovascular function. The traditional view of the cardiovascular actions of the vasoactive steroids, like aldosterone, estrogen and testosterone has focused on their roles as regulators of transcription via activation of their "classical" receptors (Androgen Receptors -AR, Mineralocorticoid Receptors -MR and Estrogen Receptors -ER). However, based on a series of observations going back more than half a century, scientists have speculated that a range of steroids, including estrogens and aldosterone, might have effects on smooth muscle to regulate both vascular tone and cell growth and differentiation mediated by signalling mechanisms (like MAP kinase activation) that are too rapid to be accounted for by transcriptional regulation. Studies performed in our laboratory over the past several years have begun to elucidate the mechanisms by which steroids regulate peripheral resistance by rapid pathways.

At the single cell level, in vascular smooth muscle cells, aldosterone and estrogen both demonstrate rapid regulation of cell contraction/myosin light chain phosphorylation and regulation of MAP kinases. Further, we have most recently demonstrated that GPR30- a newly appreciated "orphan receptor" that has been implicated as the receptor mediating the rapid effects of estrogen, "promiscuously" mediates the rapid effects of both estradiol and aldosterone- as assessed at the single cell level. Further, we have shown that GPR30 expression reverses the effects of estradiol on ERK activation and apoptosis, to parallel the profile of aldosterone's rapid actions.

Based on these findings we now propose a series of studies to assess the role of GPR30 (vs. the role of ER and MR) in mediating the rapid effects of aldosterone and estrogen in vascular smooth muscle cells, vascular endothelial cells, and cardiac fibroblasts. All of this studies will be performed under control condition using adenovirus-expressing GFP,ER,MR or GPR30. These studies will be critical in understanding the overall cardiovascular effects of these steroids at a cellular level.

As noted above some of the common effects of aldosterone and estrogen are mediated by activation of GPR30. However, our initial studies were performed in isolated vascular smooth muscle cell systems. Whether this mechanism is important in the physiological regulation of vascular responses in whole organ systems or in vivo is unknown and is an important focus of this application.

Additionally we propose to elucidate the mechanism underlying the rapid vascular effects of testosterone. Previous studies have reported inconsistent effects of testosterone on rapidly-mediated vascular function that are vascular bed-/species-dependent. We hypothesize that this inconsistency may be related to testosterone mediating its rapid effects through multiple receptors- including GPR30. Thus we propose a series of studies to elucidate thereceptor-mediated mechanism underlying the rapid vascular effects of testosterone.

Lastly, we will determine whether GPR30-mediated responses (as well as ER-, AR and MR-mediated responses) are altered in hypertension.

These proposed studies, utilizing a range of integrative, cellular techniques and molecular techniques, will be important in understanding the mechanism of the rapid regulation of vascular function by aldosterone, estrogen and testosterone and may be critical in the development of new therapeutic approaches to modulate these pathways.

Generation of adenoviral constructs. Adenoviral constructs were generated with AdMax^{TM} adenovirus vector creation kit as per manufacturer's instructions (Microbix BioSystems Inc, Toronto). Briefly, GFP (used as control infections), MR or GPR30 cDNA were generated by PCR using plasmid templates of GFP cDNA (Clontech), hGPR30 (ATCC) or hMR (kindly provided by Dr Marc Lombes, INSERM, Paris, France). Resultant cDNAs were subcloned into shuttle vector pDC316 and purified. The recombinant plasmid was then co-transfected into Human Embryonic Kidney (HEK) 293 cells with adenoviral DNA pBHGlox (delta) E1, 3Cre. Recombinant adenovirus was harvested by lysis of transfected HEK293 cells using 3 freeze/thaw cycles.

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Cell Biology

ATCC® Number: CRL-1573™ Order this Item **Price:** $256.00

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
	PHOTO
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
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.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9

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D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der (1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments: Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

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%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

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.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

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

Related Products: derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006

References:

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92579: Standard Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash and Handrub Agents Using the Fingerpads of Adult Volunteers. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 1838-02.
92587: Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.

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ElectroMAX™ DH5α-E™ Cells
Cat. No. **11319-019**

ElectroMAX™ DH5α-E™ cells are derived from the DH5α™ strain and are suitable for transformation by electroporation. They may be used in procedures requiring high transformation efficiencies, such as generation of cDNA.

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Material Safety Data Sh

Revisio

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

Product code 54357
Product name pUC 19 Control DNA

Company/Undertaking Identification

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

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

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

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

3. HAZARDS IDENTIFICATION

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

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

3. HAZARDS IDENTIFICATION

Ingestion No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

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.

Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

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
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information requ... the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human ... diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qu... to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with ca... Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company sha... liable for any damages or losses resulting from the handling or from contact with the product as described h... INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR ... IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

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ElectroMAX™ DH5α-E™ Cells
Cat. No. **11319-019**

ElectroMAX™ DH5α-E™ cells are derived from the DH5α™ strain and are suitable for transformation by electroporation. They may be used in procedures requiring high transformation efficiencies, such as generation of cDNA.

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312.00

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AdMax

"We continue to have great success with the AdMax™ system for producing a wide range of vectors for our research. We can go from plasmid clone to high titre vector ready for in vitro or in vivo evaluation in just a few weeks."
Michael Parr Ph.D., Scientist, Gene Therapy/Delivery Group, Validation Biology, Biogen Inc.

"Our lab uses the AdMax™ system for construction of E1/E3-deleted Adenovirus vectors routinely, with excellent results. We have found this technology to be easy and reliable."
C.S.H. Young, Professor of Microbiology, Columbia University, New York, NY USA.

"This method works really great, it proved to be efficient and reliable. We think that so far this is the best available method for constructing the recombinant viruses."
Dr. Elena Burova, Associate Manager, Adenovirus Facility, Regeneron Pharmaceuticals, Inc.

Download a pdf file on AdMax™ Vector Creation Kits (file size 133 kB)

Get Acrobat Reader

You will require the ACROBAT plug-in to view PDF files. If you do not have the plug-in, please download the latest version here.

Clone, cotransfect and GO!
Small shuttle plasmids, single cloning step, cotransfections without restriction, 95% reliability. The simplest, most efficient, most flexible system for construction of adenovirus expression vectors.

How fast?
How fast can you clone your gene into a small pUC based shuttle plasmid and prepare 100ug plasmid DNA? Add 7 to 10 days to that!

How efficient?
Approximately 100 fold more plaques rescued than with previous two plasmid methods.

How reliable?
If your expression cassette is less than 7-8 kb and your transgene product is nontoxic, 95% of recombinant viruses should contain and express the transgene. Use your favourite promoter or use the high efficiency MCMV IE promoter provided with our kits.

How simple?
Only two steps. No homologous recombination in difficult to handle bacterial systems; use your favourite bacterial strain. No transfer of candidate plasmids from one bacterial strain to another. No need for expensive, exotic restriction enzymes or for linearization of plasmid DNA prior to cotransfection of 293 cells. The system does not require lambda packaging or yeast technologies that are not standard procedures in the majority of labs.

How flexible?
Cassettes can be inserted in E1 or E3 or transgenes can be cloned into both regions. For example a transactivator can be inserted

in E3 and a regulated expression cassette in E1. Vectors can be designed with an E3 deletion, a wild type E3 region or, if the transgene in E1 is small, a stuffer sequence can be inserted in E3 to prevent formation of RCA. You have a choice of two site specific recombinases: Cre or FLP, with similar high rescue efficiencies.

How expensive?

The initial cost of our kits is competitive with other systems, but unlike other kits ours allow for an infinite number of vector rescues. If you can grow plasmid DNA there is no need to purchase our kits more than once.

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System Overview

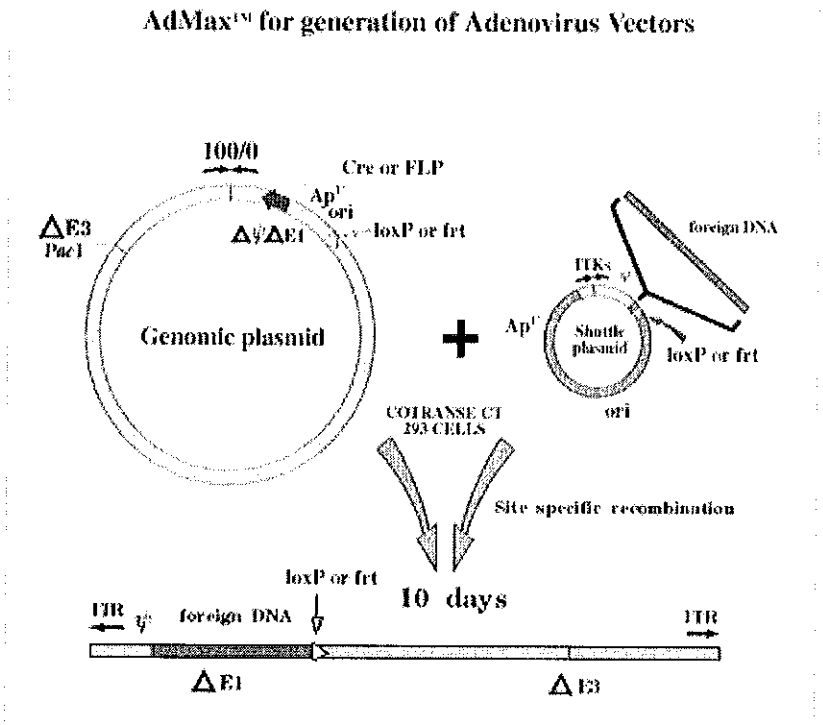

Figure 1 outlines the principles of the AdMax™ system with Cre-lox as an example. Recombination in cotransfected cells introduces the gene of interest into infectious Ad DNA while simultaneously excising the recombinase gene (Ng et al., 1999, 2000).

Neither the small shuttle plasmid nor the genomic plasmid need be digested with restriction enzymes prior to cotransfection. Any E1 complementing cell line such as 293 cells (Graham et al., 1977), 911 cells (Fallaux et al., 1996) or PERC6 cells (Fallaux et al., 1998) can be used for cotransfections.

Although rescue of viral vectors is highly efficient (over 100 fold greater than with the original two plasmid method of Bett et al., (1994)), and 95% of viruses generated by cotransfection should carry the transgene, it is good laboratory practice to build up working stocks of virus from plaque isolates before extensive experimentation.

Microbix provides low passage 293 cells that are especially cultured to maintain the strong adherence and plaque forming properties of the original 293 cells. For rapid production of vectors to be used in preliminary experiments, it may be possible to produce recombinant viruses by incubating cell cultures under liquid medium following cotransfections.

Transgenes are cloned into one of our small high copy number shuttle plasmids (Figures 2 and 4) which are then cotransfected with an Ad genomic plasmid (Figures 3 and 5) into 293 cells. High efficiency site specific recombination catalyzed by Cre or FLP recombinase results in "rescue" of the expression cassette into the left end of an E1 deleted (first generation) Ad vector.

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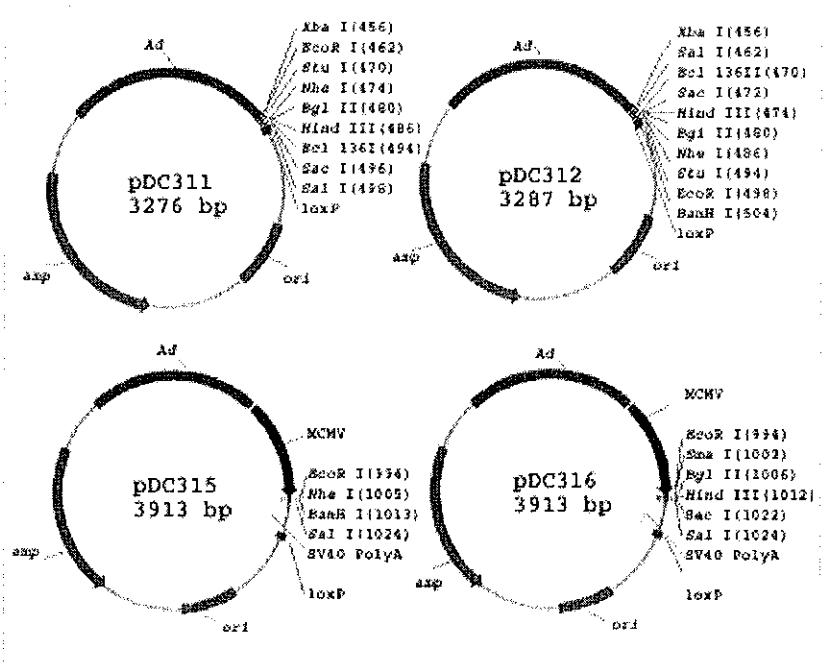

Figure 2. Shuttle plasmids for Cre-lox Ad vector construction

Shuttle plasmids **(Figure 2)** designed for insertion of the transgene are small, simple and pUC based for high yields. Promoterless plasmids with polycloning sites comprising recognition sites for 8 enzymes are only 3.2 kb in size. Plasmids containing an expression cassette utilizing the Murine Cytomegalovirus Immediate Early Gene promoter (MCMV Pr) are only 3.9 kb and have up to 6 restriction enzyme cloning sites. The genomic plasmids containing most of the Ad genome plus cassettes expressing recombinase and carrying the recombinase recognition site are approximately 34 kb in size. Two recombination systems are available, based on Cre-lox or FLP-frt.

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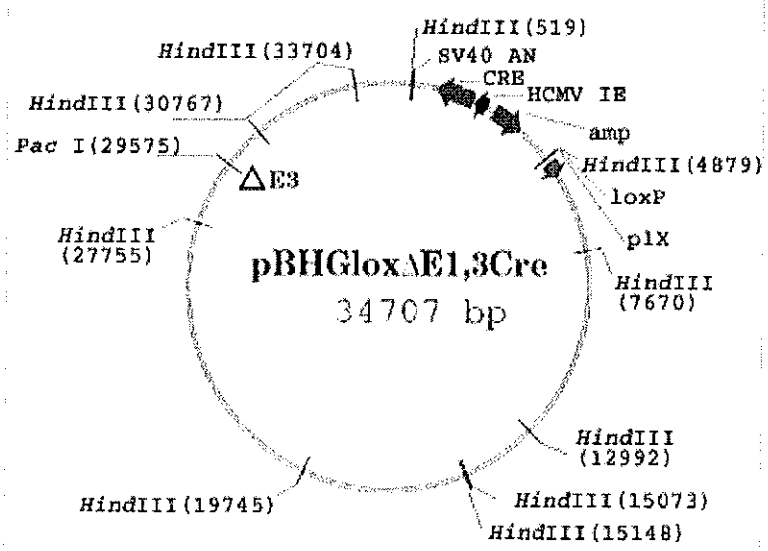

Figure 3. Ad genomic plasmid for construction of Ad vector by Cre-lox recombination

Figure 3 shows an example of one of the available Ad genomic plasmids containing a Cre expression cassette (which is excised during recombination with the shuttle plasmid). This plasmid can be purified and aliquoted and stored frozen for multiple vector rescue cotransfections. As little as 2 ug DNA/dish suffices to generate numerous plaques following cotransfection of 293 cells with a shuttle plasmid.

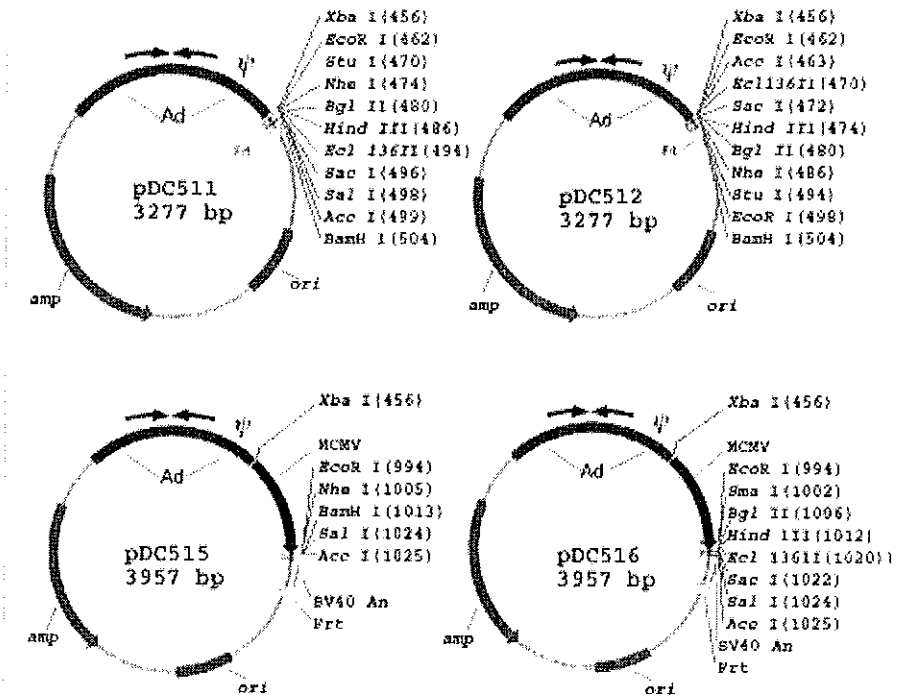

Figure 4 illustrates a set of shuttle plasmids analogous to those shown in Figure 2 but containing frt sites for recombination by the site specific recombinase, FLP, encoded by the yeast 2u plasmid (O'Gorman et al. Science 251, 1351, 1991).

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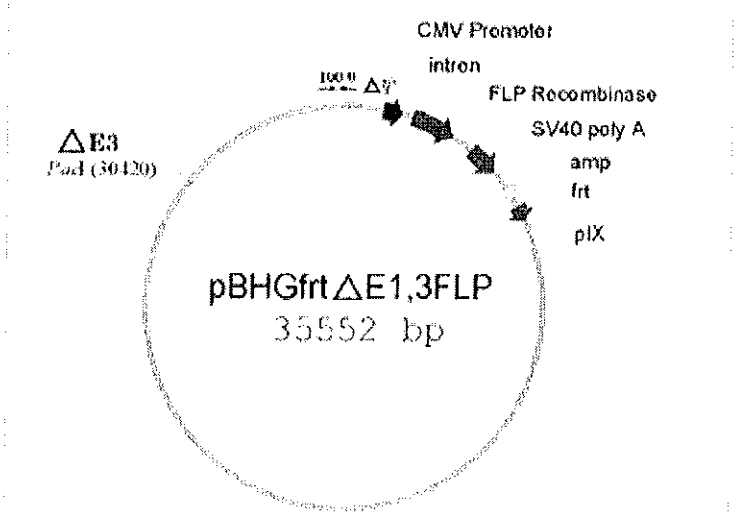

Figure 5. Ad genomic plasmid for construction of Ad vectors by FLP-frt recombination

The genomic plasmid encoding FLP and carrying an frt site for FLP mediated recombination with the shuttle plasmids of Figure 4 is illustrated in Figure 5. FLP functions as efficiently as Cre for production of adenovirus recombinants by site specific recombination between two cotransfected plasmids (Ng., et al., submitted). Plasmids can be propagated in any of the common bacterial strains such as DH5 alpha.

For recombinant DNA cloning any commonly used protocols will suffice but it is recommended that plasmid DNA to be used in cotransfections be prepared using the protocol provided with the kits.

Also we recommend that the simple cotransfection protocol provided with the kits be followed as closely as possible at least initially. Once the users have successfully rescued a number of transgenes and feel comfortable with the system, they are invited to try other plasmid DNA purification protocols and transfection methods.

For beginners we recommend that initial transfections be done using pFG140 (Graham, 1984), an infectious Ad genomic plasmid that serves as a positive control and which is provided free with all kits.

Because the only restriction enzymes required with the AdMax™ system are common enzymes used for cloning into the small shuttle plasmids the AdMax™ system is simpler and more economical than methods requiring rare cutters (Chartier et al., 1996; He et al., 1998; Mizuguchi & Kay, 1998).

Moreover those rescue protocols typically use enzymes such as Pac I or SwaI to linearize plasmid DNA prior to transfection. If the transgene contains these sites then these methods are not practical. PacI sites, for example, are found surprisingly often in eukaryotic DNA. (There is one PacI site in the Murine Cytomegalovirus Immediate Early Gene promoter (one of the strongest viral promoters available (Addison et al., 1997)) and one also in the gene encoding luciferase, a popular reporter gene.)

The E3 deleted genomic plasmids contain a unique PacI cloning site in E3. It is possible to insert a reporter gene (Parks et al., 1996) or a gene for a transactivator in the E3 region to create a modified genomic plasmid that can then be combined with cassettes inserted in the E1 shuttle plasmid. Thus, for example, a series of vectors expressing genes under regulation by tet or by RU486 can be readily constructed using the AdMax™ system.

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Ordering Information

AdMax™ Kits Available	
Catalogue#	**Microbix Product**
PD-01-64	Kit D (contains pDC311, pDC312, pDC315, pDC316, pBHGloxΔE1,3Cre, and pFG140)
PD-01-65	Kit E (contains pDC511, pDC512, pDC515, pDC516, pBHGfrtΔE1,3FLP, and pFG140)
PD-01-67	Kit F (contains pDC411, pDC412, pDC415, pDC416, pBHG10, pBHGE3 and pFG140)

AdMax™ Plasmids must be ordered in complete kits. Each plasmid is priced at 10 ug per vial.

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Individual AdMax™ Plasmids	
Catalogue#	**Microbix Product**
PD-01-29	pDC411
PD-01-30	pDC412

PD-01-31	pDC415
PD-01-32	pDC416

AdMax™ is covered by US patents 7,132,290; 6,855,534; 6,756,226; and 6,379,943

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