

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: April 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 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/

PRINCIPAL INVESTIGATOR Dr G.M. Kelly
DEPARTMENT Biology
ADDRESS 359 WSE
PHONE NUMBER 83121
EMERGENCY PHONE NUMBER(S) 661-7535
EMAIL g.kelly@uwo.ca

Location of experimental work to be carried out: Building(s) WSE Room(s) 355

*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: NSERC
GRANT TITLE(S): Cell signaling cross-talk in Development

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Jason Huang - Ph.D student
Shaun Symons - M.Sc student
Leanne Sandison - M.Sc student

* 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? YES 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? YES 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
<i>E. coli</i>	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	1. l. the Inhibitor	Inhibitor	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

MSDS for One Shot ...
 E. coli provided

*Please attach a Material Safety Data Sheet or equivalent from

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES 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	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

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	<input checked="" type="radio"/> Yes <input type="radio"/> No	U87m6	WWD
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	F9 cells	ATCC
Non-human primate	<input checked="" type="radio"/> Yes <input type="radio"/> No	COS-7	ATCC
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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 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 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		<input type="radio"/> Yes <input checked="" type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input checked="" type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input checked="" type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		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? 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
E. coli	pCMV SP0AT6 pCDNA3.1+	Origene	Gratac, Foxa2, Fzd7	plasmid propagation

* 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

* 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 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 Danio rerio

6.3 AUS protocol # 2007-109

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: injected into embryos. NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

See attached email.

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)? YES No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? 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 LD₅₀ (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? YES, Please provide details: _____ 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? 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? Initiate and maintain colony, give location: _____
 "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? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? 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? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES 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? YES 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? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

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

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

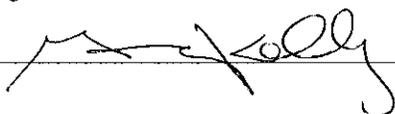
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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.

SIGNATURE  _____

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 O 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-C081
 NO, please certify
 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 [Signature] Date: July 12/10

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 biohazards listed, such as a needlestick injury:

15.0 Approvals

1) UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

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

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

Special Conditions of Approval:

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

Product code C505003
Product name ONE SHOT TOP10 P3 CHEMICALLY COMPETENT E COLI

Contact manufacturer
 INVITROGEN CORPORATON
 1600 FARADAY AVENUE
 PO BOX 6482
 CARLSBAD, CA 92008
 760-603-7200

Material Safety
Data Sheet (S)

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
Suspension

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

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
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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

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Contact manufacturer
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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
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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
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

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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

Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product contains the following HAPs:

U.S. State Regulations

California Proposition 65

This product contains the following Proposition 65 chemicals:

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 required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified 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 caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

Cell Biology

ATCC® Number: **HTB-14™** [Order this Item](#) Price: **\$256.00**

Designations: U-87 MG

Depositors: J Ponten

[Biosafety Level:](#) 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)
epithelial

Morphology:



Organ: brain

Source: **Tumor Stage:** classified as grade IV as of 2007

Disease: glioblastoma; astrocytoma

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC

Permits/Forms:

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 ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Tumorigenic: Yes

Antigen Expression: Blood Type A, Rh+

Amelogenin: X
CSF1PO: 10,11
D13S317: 8,11
D7S820: 8,9

DNA Profile (STR): D5S818: 11,12

D16S539: 12
vWA: 15,17
THO1: 9.3
TPOX: 8

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Cytogenetic Analysis: This is a hypodiploid human cell line with the modal chromosome number of 44 occurring in 48% of cells. The rate of higher ploidy was 5.9%. Twelve markers were common to all cells, including der(1)t(1;3) (p22;q21), der(16)t(1;16) (p22;p12), del(9) (p13) and nine others. The marker der(1) had two copies in most cells. There was only one copy of normal X. N1, N6 and N9 were not found.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 1
Me-2, 1
PGM1, 2
PGM3, 1

Age: 44 years

Gender: female

Ethnicity: Caucasian

Comments: This is one of a number of cell lines derived from malignant gliomas (see also ATCC [HTB-15](#) and ATCC [HTB-16](#)) by J. Ponten and associates from 1966 to 1969.

Mycoplasma contamination was eliminated in September 1975.

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: 5% CO₂ in air recommended

Temperature: 37.0°C

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:5 is recommended

Medium Renewal: 2 to 3 times per week

Subculturing: 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: Culture medium, 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC [30-2003](#)
recommended serum: ATCC [30-2020](#)

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[BioStandards](#)

[Biological
Reference
Material and
Consensus
Standards for
the life science
community](#)

- 22159: Beckman G, et al. G-6-PD and PGM phenotypes of 16 continuous human tumor cell lines. Evidence against cross-contamination and contamination by HeLa cells. Hum. Hered. 21: 238-241, 1971. PubMed: [4332744](#)
- 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](#)
- 23094: Olopade OI, et al. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res. 52: 2523-2529, 1992. PubMed: [1568221](#)
- 23128: Ponten J, Macintyre EH. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. 74: 465-486, 1968. PubMed: [4313504](#)
- 32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: [8855306](#)
- 16173472: Clark MJ, et al. U87MG decoded: The genomic sequence of a cytogenetically aberrant human cancer cell line. PLoS Genetics 6 (1) : e1000832, 2010.

References:

[Return to Top](#)

Cell Biology

ATCC® Number: **CRL-1720™** [Order this Item](#) Price: **\$365.00**

Designations: F9

Depositors: S Strickland

[Biosafety Level:](#) 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: epithelial

Source: **Organ:** testis

Strain: 129

Disease: embryonal carcinoma; testicular teratoma

Cellular Products: plasminogen activator; laminin; type IV collagen

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC

Permits/Forms:

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](#))

Age:

embryo

F9 cells can be stimulated to differentiate into parietal endoderm in the presence of retinoic acid and dibutyryl cyclic AMP (cAMP). Differentiating cells synthesize plasminogen activator, laminin and type IV collagen.

Comments:

cAMP is active only on cells that have been treated with retinoic acid.

The cells maintain three copies of the beta 1 integrin gene.

Tested and found negative for ectromelia virus (mousepox).

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: fetal bovine serum to a final concentration of 10%.

Propagation:

Temperature: 37.0°C

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Protocol: NOTE: culture vessels must be coated with 0.1% gelatin prior to use. To do so, cover the surface of the vessel with 0.1% gelatin (Difco) in sterile distilled water for 2 hours at 4C, then wash three times with sterile distilled water. Treated flasks and dishes can be stored at room temperature.

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[Standards for](#)
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Subculturing:

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 coated culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:10 is recommended

Medium Renewal: Every 2 to 3 days

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2002](#)
recommended serum: [ATCC 30-2020](#)

References:

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Cell Biology

ATCC® Number: **CRL-1651™** [Order this Item](#) Price: **\$269.00**

Designations: COS-7

Depositors: Y Gluzman

[Biosafety Level:](#) 2 [Cells Contain SV-40 viral DNA sequences]

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Cercopithecus aethiops*
fibroblast

Morphology:



Source: **Organ:** kidney
Cell Type: SV40 transformed

Cellular Products: T antigen

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 ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Comments: This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region. The line was derived from the CV-1 cell line (ATCC® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.

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: fetal bovine serum to a final concentration of 10%.

Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

Temperature: 37.0°C

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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.

Subculturing:

Subcultivation Ratio: A subcultivation ratio of 1:4 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

Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2002](#)

recommended serum: [ATCC 30-2020](#)

Related Products:

parental cell line: [ATCC CCL-70](#)

0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca⁺⁺, Mg⁺⁺): [ATCC 30-2101](#)

Cell culture tested DMSO: [ATCC 4-X](#)

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CELL SIGNALING CROSSTALK IN DEVELOPMENT

Overview: Signal transduction affects every facet of cell physiology and with the myriad of biochemical processes responding to a signal, the task of teasing apart a particular pathway is daunting. My research goal is to elucidate how signaling pathways are modified and modulated by the cell. In particular, I am interested in Wnt proteins, which are involved in almost every aspect of development and at the centre of numerous diseases⁽¹⁾. When a Wnt, of which there are 19 in mammals, is secreted from a cell a signaling cascade is initiated when it binds to at least one of 10 different Frizzled (Fz) receptors or to a non-Fz receptor. Ligand-receptor selectivity acting through Dishevelled (Dvl) diverts the signal toward inducing changes in gene expression or toward the restructuring of the cytoskeleton.

We reported recently on Wnt6 in a cell model that recapitulates extraembryonic endoderm formation in the mouse embryo⁽²⁾. In chick and *Xenopus* embryos Wnt6 signals to both Dvl-directed pathways⁽³⁻⁵⁾ and its up-regulation in murine stem cells correlates to self-renewal⁽⁶⁾. *Wnt6* and *Wnt6a* are present in the zebrafish genome⁽⁷⁾, but there has been no definitive report on their expression or function. Many unanswered questions remain regarding the role of Wnt6 in mammalian and Wnt6 and 6a in zebrafish development and a series of experiments to address this are proposed within this application.

Background Literature: Wnts are secreted lipoglycoproteins that signal through G-protein coupled Fz receptors and LDL-receptor related protein (LRP) co-receptors^(8,9). Three individual signaling pathways have been described as canonical Wnt/ β -catenin, non-canonical Wnt/cGMP/Ca²⁺ and Wnt/c-Jun N-terminal kinase (JNK)/planar cell polarity (PCP), but their individual nature is in question. Common players exist in each pathway, some forming scaffolds comprised of the proteins Dvl, Axin and β -arrestin^(10,11). In the canonical pathway, Wnt bound to Fz and LRP5/6 uses Dvl-1, -2 or -3 to dismantle a complex that includes the APC tumour suppressor, glycogen synthase kinase-3 (GSK-3 β), Axin and β -catenin⁽¹¹⁾. Without the complex β -catenin translocates to the nucleus where it acts with T-cell factor (Tcf) to regulate the expression of target genes⁽⁸⁾. Activation of the PCP pathway causes Dvl to signal to Rho and Rho-kinase or through Rac to JNK, which reorganizes the cytoskeleton effecting changes in cell polarity and motility⁽¹²⁾. Activating these multi-functional proteins has more extensive ramifications. For instance, Rac1 controls actin polymerization, but it also affects gene expression by JNK and p38 MAPK signaling, generates reactive oxygen species (ROS) by NOX activation and facilitates nuclear translocation of β -catenin via JNK⁽¹³⁻¹⁵⁾. Thus, an extensive network forms as a result of Wnt signaling through Dvl(s) to numerous downstream effectors.

Dissecting the Wnt network has been difficult, partly as a result of having a number of ligands in excess of available receptors. In fact, multiple Wnts bind to a given Fz. For example, in zebrafish embryos Wnt11 binds to Fz7 for PCP signaling, whereas in chick embryos Wnt6 binds to Fz7 to initiate canonical signaling^(5,16). In contrast, Wnt5a activation of PCP is mediated by Fz2 in zebrafish embryos, whereas Wnt5a signals via Fz3 in melanoma cells^(17,18). Thus, caution must be exercised when assigning definitive pathways to a ligand or receptor. With over 20 suspected Dvl-interacting proteins⁽¹¹⁾ the potential for extensive crosstalk from other signaling pathways only adds to the confusion. Crosstalk converges on Wnt/Dvl signaling networks at many places and is not limited to protein:protein interactions. ROS for instance, including superoxides, hydroxyl radicals and H₂O₂, are active participants in signaling and like Wnts are linked to proliferation and differentiation in normal development and tumour progression^(19,20). ROS are by-products of aerobic respiration, but are also produced in response to G-protein coupled receptor signaling from G $\alpha_{12/13}$ subunits leading to Rac1 and NOX activation⁽²¹⁾. ROS activation of the β -catenin and PCP pathways is mediated in part by the direct oxidation and subsequent release of nucleoredoxin from Dvl^(22,23). H₂O₂ also inactivates the PTEN tumour suppressor leading to increased Akt activity⁽²⁴⁾, which negatively regulates GSK-3 causing gastrulation defects in zebrafish⁽²⁵⁾. Finally, PTEN and GSK-3 negatively regulate Wnt target genes required for mitosis⁽²⁶⁾. However, Wnt activation of Akt⁽²⁷⁾, which acts directly on GSK-3 or indirectly by activating the Rac1/NOX axis to produce ROS, can also inhibit PTEN.

$G\alpha_{13}$ signaling and ROS and their involvement with Wnt pathways have been overlooked. In contrast, $G\alpha_{13}$ is well known for its ability to signal through RhoA, Rac1 and Cdc42 to JNK, which affects cytoskeletal changes during cell proliferation and migration. The importance of $G\alpha_{13}$ in development is evident by the response of F9 cells to retinoic acid (RA), which recapitulates a step in primitive endoderm (PrE) formation in mouse^(28,29). RA, a potent morphogen in vertebrate embryos, has pleiotropic effects ranging from inducing the GATA and forkhead box (Fox) transcription factors required for PrE formation to stimulating ERK1/2 and PI3K activities through non-genomic mechanisms^(30,31). In F9 cells, RA signaling from $G\alpha_{13}$ through p115RhoGEF to JNK is necessary for PCP and is accompanied by Akt activation^(32,33). In zebrafish, $G\alpha_{13}$ signaling acts in parallel to PCP signaling and is required to coordinate cell movements during gastrulation⁽³⁴⁾. Details coupling $G\alpha_{13}$ activity with agonist-bound receptors are unclear, but evidence exists that RA increases $G\alpha_{13}$ activity⁽²⁸⁾. RA also up-regulates GATA-6 and Foxa2 in F9 cells⁽³⁵⁾ and in silico analysis reveals putative GATA-6 and Foxa2 binding sites in the mouse Wnt6 promoter. Wnt6 activates PCP and Wnt/ β -catenin pathways during chick and *Xenopus* organogenesis^(4,5,36) and although it signals to β -catenin in RA-treated F9 cells⁽²⁾, we are proposing it also participates in PCP in these cells. In F9 cells there is precedence for a one Wnt/one receptor/two pathway response, where Wnt3a signaling to ectopically expressed rat Fz1 and the $G\alpha_{q/0}$ subunits, stimulates β -catenin and PCP pathways⁽³⁷⁾. One caveat, however, is that the application of Wnt3a-conditioned media (CM) to untransfected RA-treated F9 cells blocks activation of RA-responsive genes⁽³⁸⁾. Nevertheless, the ability of F9 cells to respond to RA by activating Wnt/ β -catenin, Wnt/PCP and PI3K/Akt signaling pathways, while expressing endo-A intermediate filaments, which are criteria for PrE differentiation, provides a unique platform by which one can investigate the crosstalk that occurs between signaling pathways. Extending these studies to the zebrafish will reveal how common this crosstalk is in development. *My long term goal is to elucidate how crosstalk from other pathways converges on the Wnt6 network to influence how cells are induced in embryos.*

Progress & Research Activities: We use two models to investigate signaling events during embryonic development^(2,25,29,39-42). Cell movements in zebrafish embryos are affected following the knockdown of the myosin regulatory light chain interacting protein (Mir)⁽³⁹⁾. Two-hybrid analysis revealed Mir and Annexin V act downstream of Wnt11/Fz7 in PCP signaling^(39,40). We have also used transient transgenesis and pharmacological inhibitors to identify an Akt-PTEN feedback mechanism that affects gastrulation⁽²⁵⁾. Reduced levels of p-Akt allow GSK-3 to target PTEN for inactivation, whereas high levels inhibit GSK-3, allowing PTEN activity to increase, thereby resetting pro-proliferation and pro-apoptotic signaling required in gastrulating embryos.

In F9 cells we showed *Wnt6* is up-regulated by RA during PrE formation⁽²⁾. In the absence of RA, F9 cells expressing *pCMV-Wnt6* or treated with Wnt6-CM accumulate nuclear β -catenin. We cloned the mouse *Wnt6* minimal promoter into a firefly luciferase vector for a Dual Reporter Assay and are in the process of stably expressing it and a non-GATA responsive *Renilla* reporter in COS-7 cells. In our assay we will test GATA-6 and Foxa2 constructs, which induce PrE markers when expressed in F9 cells. A *Wnt6* cDNA is being cloned behind the *Wnt6* promoter and will be transfected in the presence or absence of the GATA-6/Foxa2 constructs in F9 cells stably expressing the Super8XTOPFLASH reporter to detect β -catenin/Tcf-dependent transcription. Luciferase activity will confirm that nuclear β -catenin accumulation seen in Wnt6-CM-treated F9 cells partakes in Tcf-dependent transcription. In the search to define the RA-Wnt6- $G\alpha_{13}$ signaling axis, we reported on the influence p115RGS, a dominant negative mutant of p115RhoGEF, has on blocking RA- $G\alpha_{13}$ signaling, and the ability of LiCl (a GSK-3 inhibitor) to promote PrE⁽⁴²⁾. We also found RA up-regulates *Fz2* and *7*, but have not confirmed that they encode bona fide Wnt6 receptors. Finally, we found that H_2O_2 causes F9 cells to undergo morphological changes and express PrE markers, and in zebrafish embryos H_2O_2 causes gastrulation defects. Together, we will use the zebrafish embryo and F9 cell model to identify common and unique features of the Wnt6 network that are influenced by crosstalk between ROS and the PI3K/Akt/PTEN signaling axis.

Hypothesis & Objectives: I hypothesize that a *Wnt6* signaling network is augmented and fine tuned by the activities of ROS and the coordinated regulation of PTEN by PI3K/Akt. To address this hypothesis we will find answers to the following questions:

- 1) What are the consequences of altering *Wnt6* expression on PrE formation?
- 2) What effect does altering *Wnt6* expression have on zebrafish embryogenesis?
- 3) To what extent do ROS have on augmenting Wnt signaling?
- 4) How much influence does Akt/PTEN crosstalk have on the Wnt/ β -catenin pathway?

Proposed Research Plan:

Aim 1: What are the consequences of altering *Wnt6* expression on PrE formation?

Since Wnts signal through $G\alpha$ subunits and *Wnt6* is up-regulated in response to RA, I hypothesize that the RA-*Wnt6* signaling axis extends to $G\alpha_{13}$, activating β -catenin and PCP pathways for PrE formation. There is no precedent for Wnt signaling to $G\alpha_{13}$, which makes my programme very exciting yet controversial. To investigate how crosstalk influences RA-*Wnt6* signaling, it is important to identify the effect on PrE when levels of *Wnt6* and its bona fide receptor(s) are depleted. *J. Hwang* and *B. Cadesky* (M.Sc. yr. 2) will use a knockdown approach to address these questions. To begin, three siRNAs will be tested for their efficacy in suppressing *Wnt6* expression. Knockdown efficiency will be determined by RT-PCR. These studies will benefit from *Wnt6* antibodies as the ones currently available are unreliable. Towards that end, antibodies will be made using a commercial facility and then tested on immunoblots and by immunocytochemistry. Cells transfected with *Wnt6* siRNAs or sham transfected will be treated with RA and assayed by immunocytochemistry for endo-A intermediate filaments and β -catenin (canonical signaling) and by immunoprecipitation and immunoblot analysis to measure JNK activity (EZ-Detect, Pierce) indicative of PCP signaling. Rescuing the *Wnt6* knockdown will be achieved using *Wnt6*-CM. The absence of differentiation markers following knockdown will suggest *Wnt6* is necessary for activating β -catenin and PCP pathways, whereas the absence of nuclear β -catenin alone will indicate RA induces an alternate pathway to stimulate PCP. *Wnt5a* is a candidate for its involvement in PCP and Wnt/cGMP/ Ca^{2+} pathways, but there are conflicting reports about it being up-regulated by RA in F9 cells^(43,44). If *Wnt5a* is involved in PrE formation, the p115RGS results showing constraint at the $G\alpha_{13}$ branch point would suggest *Wnt5a* and 6 utilize the same receptor or different receptors, but each signaling to $G\alpha_{13}$. Eliminating crosstalk from other $G\alpha$ subunits including $G\alpha_{q/11}$ will be done using pertussis toxin or YM-254890 (Astellas) to selectively inhibit $G\alpha_{q/11}$. The knockdown results and eliminating the involvement of other $G\alpha$ subunits will influence our decision as to whether to first silence the putative *Wnt6* receptors (Fz7/2) or *Wnt5a*. Fz7 silencing is expected to phenocopy the *Wnt6* knockdown and to prevent nuclear β -catenin accumulation, but we have no preconceived notions for Fz2. Together, a systematic knockdown of Wnt and Fz will define what in the *Wnt6* network participates in and is accessible to signaling crosstalk. I remain confident *Wnt6* signals to two pathways via $G\alpha_{13}$, but in the event my hypothesis is wrong, progress on the grant will not be affected as the focus remains on crosstalk converging on the *Wnt6* network, specifically the β -catenin component.

Aim 2: What effect does altering *Wnt6* expression have on zebrafish embryogenesis?

If *Wnt6* expression in zebrafish embryos is as widespread as it is in chick and *Xenopus*⁽³⁻⁵⁾, disrupting its activity should cause a broad spectrum of defects. *H. Struthers* (M.Sc. yr. 1) will use RT-PCR and in situ hybridization to elucidate the temporal and spatial expression of the two *Wnt6* mRNAs in embryos. cDNAs encoding full-length FLAG-tagged *Wnt6* and HA-tagged *Wnt6a* protein will be cloned into *pT7TS* for in vitro transcription of mRNA or into *pEGFP-N* for expression from injected DNA. *Wnt6/6a* mRNA or DNA will be injected into 1-2 cell embryos to evaluate the effects of ectopic protein levels before and after the onset of zygotic transcription. These experiments are straightforward providing insight into the pathway(s) and organ system(s) requiring *Wnt6/6a*, but potentially misleading

if one or both aberrantly activate pathways before the endogenous genes are expressed. Clarification will come with results from the *Wnt6/6a* knockdowns. Once expression profiles are known, embryos will be injected with translational or splice-site targeting *Wnt6* or *6a* morpholinos (MOs), or with a “scrambled” MO as control. Off-targeting effects will be determined by TUNEL assay and for rescue, embryos will be co-injected with MO-insensitive *Wnt6* mRNA and a *Wnt6* MO. Morphants are expected to have defects in many organ systems and preference to investigate one over another will depend on factors including, but not limited to, the availability of molecular markers, antibodies and the accessibility of tissue(s) to target *Wnt6/6a* and *Fz7/2* MOs or mRNAs to specific sites by in vivo electroporation⁽⁴⁵⁾. Wnt antibodies would complement this and subsequent studies, prompting us to generate them in years 1 (*Wnt6*) and 2 (*Wnt6a*). I am confident these studies will yield novel results, especially for *Wnt6a*, and will expose players in the *Wnt6* network that may have been overlooked in the F9 model. We have experience with microinjection/electroporation and have used MOs and shRNAs⁽²⁹⁾.

Aim 3: To what extent do ROS have on augmenting Wnt signaling?

N. Alkahlout (M.Sc. yr. 1) found H₂O₂-treated F9 cells express PrE markers. ROS activate MAPK pathways converging on Wnt networks, with p38 MAPK promoting β -catenin accumulation by negatively regulating GSK-3⁽⁴⁶⁾, and JNK facilitating β -catenin translocation to the nucleus⁽¹⁵⁾. In a ROS-independent manner *Wnt3a* activation of p38 MAPK, in F9 cells expressing rat *Fz1*, inhibits GSK-3 and promotes β -catenin signaling⁽⁴⁷⁾. However, these authors note that this activation is not an integral step in β -catenin signaling as Tcf-dependent transcription occurs even when p38 MAPK activation is abolished⁽⁴⁷⁾. With this evidence, I hypothesize that the changes seen in H₂O₂-treated F9 cells are due to ROS-crosstalk acting on JNK and p38 MAPKs to increase β -catenin levels. To test this, H₂O₂-treated or untreated cells will be assayed for endo-A intermediate filaments, nuclear vs. cytoplasmic levels of β -catenin (NE-PER, Pierce), and measuring JNK and p38 MAPK activities (EZ-Detect, Pierce).

If my hypothesis is correct and ROS play a role in PrE formation, then RA must trigger the increase endogenously. With the evidence for a RA/ $G\alpha_{13}$ /Rac1 axis, we will test whether or not Rac1 activation of NOX is responsible for producing ROS. ROS levels, in response to RA, DMSO, and *Wnt6*-CM or in cells transfected with constitutively active V12Rac1 or the empty vector, will be measured in live cells using (CM)H₂DCFDA and fluorometry. Rac1, JNK and p38 MAPK activation following the aforementioned stimuli, will be measured by immunoprecipitation-immunoblotting (Cytoskeleton; Pierce), as will p-GSK-3 (inactive) and nuclear vs. cytoplasmic β -catenin levels, to determine if a correlation exists between ROS levels and PrE formation. Identifying elevated levels of ROS and p-GSK-3 in response to RA or *Wnt6*-CM, and especially in V12-Rac1 expressing cells, would be novel. Likewise, if nuclear β -catenin levels in V12-Rac1-expressing cells exceed that in controls, this would support the notion of Rac1 crosstalk augmenting the β -catenin pathway. Based on a previous study, RA-treated P19 embryonal cells expressing dominant negative Rac1 do not express endo-A intermediate filaments or form PrE⁽³²⁾, or more specifically do not undergo PCP based on our criteria. Thus, it will be interesting to see if low JNK activity in RA- or *Wnt6*-CM-treated cells expressing dominant negative N17Rac1 correlates to low levels of ROS, p-GSK-3 and nuclear β -catenin relative to controls. The Super8XTOPflash assay will be used to measure Tcf-dependent transcription in cells expressing V12-Rac1 or N17Rac1. This assay will be repeated for RA-stimulated cells treated with SB203580 to inhibit JNK activity or SP600125 to block p38 MAPK activity, clarifying the role each MAPK has in response to RA. If MAPK crosstalk converges on the RA-*Wnt6* network, Tcf-dependent transcription should be reduced when JNK activity is inhibited and severely compromised when Rac1 or p38 MAPK activity is blocked.

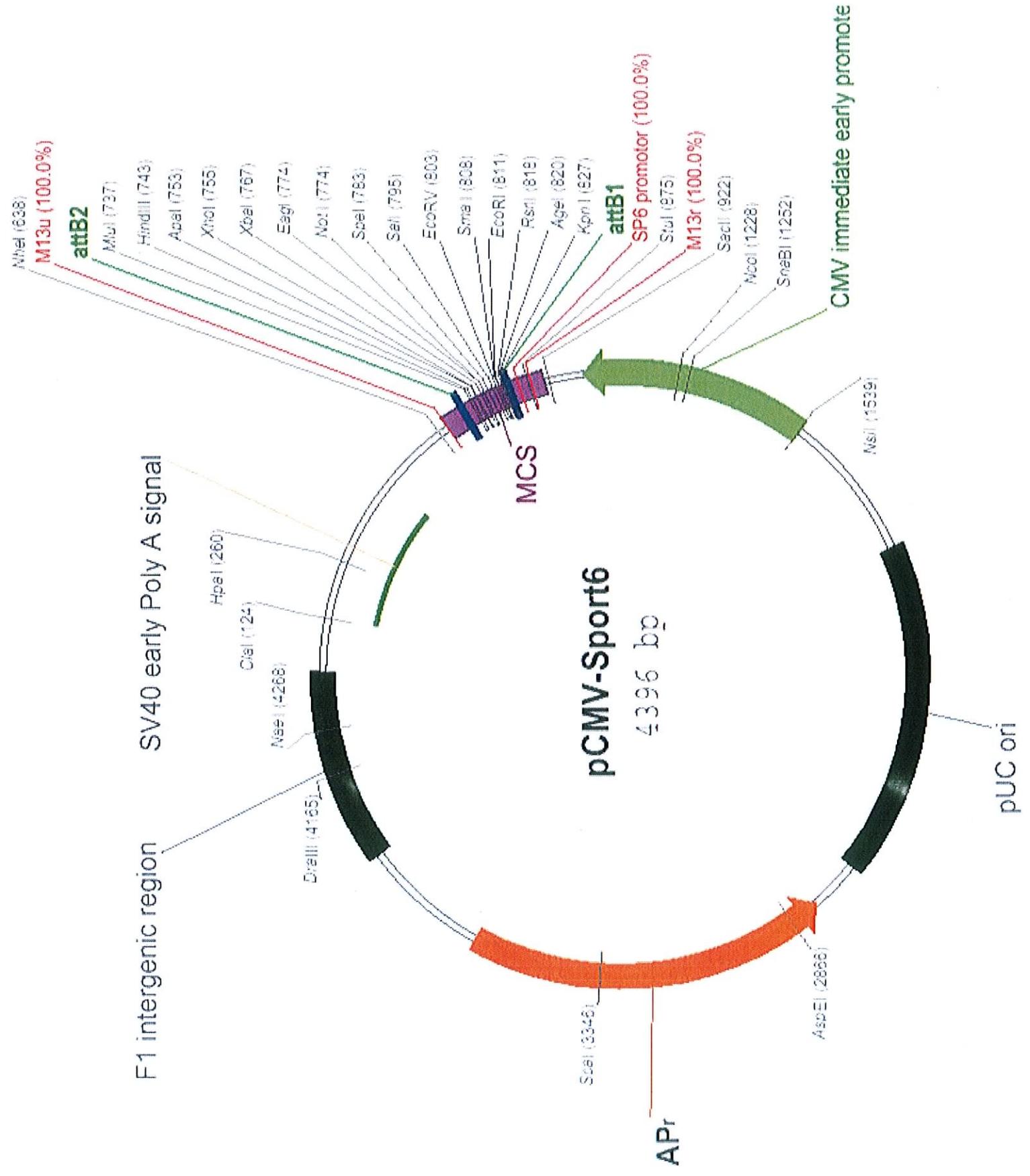
If my hypothesis that crosstalk from ROS on Wnt signaling influences differentiation, then treating cells with antioxidants should block PrE formation. To test this *J. Wen* (M.Sc. yr. 1) will treat F9 cells with Trolox or N-acetyl cysteine, followed by challenge with RA or *Wnt6*-CM. Levels of JNK and p38 MAPK activity will be measured on immunoblots, and immunocytochemistry used to assay for endo-A

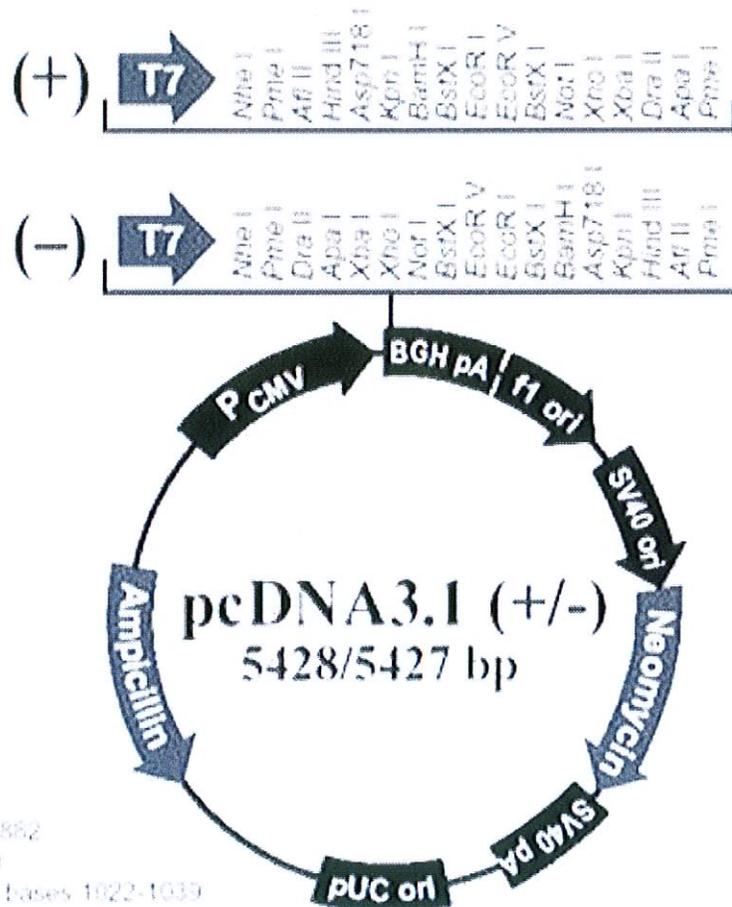
intermediate filaments and β -catenin localization. ROS levels will be detected by (CM)H₂DCFDA and fluorometry. Results from the Super8XTOPflash assay will uncover the extent of β -catenin signaling. I expect RA- or Wnt6-CM-treated cells exposed to antioxidants will not have the elevated p38 MAPK or JNK activity seen in controls, but will show Tcf-dependent transcriptional activity for two reasons: 1) the Wnt6 negative regulation of GSK-3 is the obvious source; and 2) a ROS-independent mechanism involving PI3K/Akt (Aim 4). Results from these experiments will be very insightful and directional, providing the basis to explore ROS signaling in zebrafish. This is uncharted territory and although the prospect for discovery is great, at present it would be imprudent to propose experiments without clear hypotheses. To begin, however, we will use (CM)H₂DCFDA and fluorometry to measure ROS in embryos cultured under normal and hyperoxic conditions (assisted by R. Cumming, Biology, UWO) or in the presence of H₂O₂, which affects Akt/PTEN signaling and gastrulation. The relative transparency of the developing embryo/larva will allow us to use (CM)H₂DCFDA with confocal microscopy and digital imaging to identify relative levels of ROS in developing organs, pointing us toward a system in the Wnt6 project (Aim 2). Together, this information will provide background for a proposal to investigate the effects of environmental pollutants on the Wnt6 network and to promote zebrafish as a model for high throughput screening to identify environmental sources of oxidative stress.

Aim 4: How much influence does Akt/PTEN crosstalk have on the Wnt/ β -catenin pathway?

A. Finkielstein (Ph.D. yr. 5) has shown GSK-3 activity decreases as Akt activity increases, allowing PTEN to counterbalance PI3K/Akt signaling⁽²⁵⁾. This trend would explain why the sharp increase in Akt activity in RA-treated F9 cells is followed by a marked decrease in activity. The ability of Akt to negatively regulate GSK-3 and to stimulate the Rac1/NOX axis to produce ROS, places it as a “crosstalker” in Wnt signaling. I hypothesize that increasing Akt activity either directly or in response to Wnt6 leads to elevated Tcf-dependent transcription. To test this *Shaun Symons* (summer/M.Sc. student) will treat F9 cells with RA, Wnt6-CM, or with 740Y-P to specifically activate PI3K/Akt, then assay for changes in the levels of p-Akt (active), p-PTEN (inactive), p-GSK-3 (inactive) and Rac1 activity. Repeating these assays with lysates from Wnt6-silenced, RA-treated cells (Aim 1), will allow us to compare PI3K/Akt activity due specifically to Wnt6 signaling. The Super8XTOPflash reporter assay is required to compare PI3K/Akt and Tcf-transcriptional activities. If my hypothesis is correct, high p-GSK levels and elevated Rac1 activity under all treatments, especially for 740Y-P, should correlate to high levels of Tcf-dependent transcription. Likewise, if PI3K/Akt crosstalk augments β -catenin signaling, then we will expect to see a synergistic effect in Tcf-dependent transcriptional activity in Wnt6-CM/740Y-P-treated cells. Blocking PI3K activity with wortmannin or LY294002 will produce the opposite effect, reducing p-GSK3 and inactivating Rac1 thereby compromising Tcf-dependent transcription. Our transient transgenic and inhibitor studies have taught us a great deal about Akt/PTEN signaling in zebrafish, but we have not linked this information to a specific Wnt pathway. Thus, the results from the F9 model will be significant and extending these studies to zebrafish will provide the opportunity to test whether or not this crosstalk is a positive regulator on Wnt6 signaling in embryos. In 5 years I expect that we will know how ROS, MAPK and PI3K/Akt crosstalk converges on the RA/Wnt6/G α ₁₃ axis to effect PrE formation. Teasing apart the mechanism(s) and uncovering other sources of crosstalk in multicellular zebrafish and eventually mouse embryos will take longer.

Significance & Training: This proposal addresses questions focused on the characterization of a complex Wnt signaling network. Wnt signaling is fundamental to almost every aspect of development and altering any facet of the circuitry has severe consequences in embryos and adults. We have made significant progress towards understanding how cells communicate and I am confident this new research will provide insight into how the Wnt6 signaling network influences cell differentiation under normal and disease conditions. Towards that end my research programme provides an environment adequately suited for training at all levels and allows trainees to blend together a range of cell, molecular, biochemical and genetic approaches to answer basic biological questions.





Comments for pcDNA3.1 (+)
 5428 nucleotides

- CMV promoter: bases 232-819
- T7 promoter/priming site: bases 863-882
- Multiple cloning site: bases 895-1010
- pcDNA3.1 BGH reverse priming site: bases 1022-1039
- BGH polyadenylation sequence: bases 1026-1252
- f1 origin: bases 1298-1726
- SV40 early promoter and origin: bases 1731-2074
- Neomycin resistance gene (ORF): bases 2136-2930
- SV40 early polyadenylation signal: bases 3104-3234
- pUC origin: bases 3617-4287 (complementary strand)
- Ampicillin resistance gene (bla): bases 4432-5438 (complementary strand)
- ORF: bases 4432-5292 (complementary strand)
- Ribosome binding site: bases 5300-5304 (complementary strand)
- oriA promoter (PB): bases 5327-5333 (complementary strand)

n 8/12/2010 8:09 AM, Greg Kelly wrote:

> Hi Jennifer:

>

>

> I didn't make a copy so can you please tell me the name of the plasmid

> in 4.2. We did include the MSDS on the plasmids we routinely used, but not

> certain about the one(s) you mentioned. As for #2, we inject those plasmids

> into embryos, but they are for transient expression analysis, i.e., they are

> not incorporated into the fish's genome.

> Let me know if there is anything else.

>

> Cheers,

>

> Greg

Section 6.0