University of Toledo

Institutional Biosafety Committee

J. David Dignam Ph.D - Chair (David.Dignam@utoledo.edu)
Monika DeGregorio B.S. - Administrator (Monika.DeGregorio@utoledo.edu)
http://www.utoledo.edu/research/RC/biosafety.html
Form: RSP601 - IBC - General Information - Rev. 7/2011

IBC Protocol Form

(Research involving Recombinant DNA, Infectious Agents, and/or Hazardous Biological Materials)

Please submit via email to Monika DeGregorio in Research and Sponsored Programs
*(must be sent from PI's UT email address, **DO NOT SUBMIT A SIGNED HARD COPY** at the time of application)*
MUST BE FILLED OUT WITH ADOBE ACROBAT READER 9.0 OR PROFESSIONAL VERSION 8.0 OR LATER
Using earlier versions will cause errors in the form and result in the administrative withdrawal of the protocol

IBC # Office Use Only	[redacted]				
Title of Protocol	Role of Formins in cancer cell amoeboid-type morphology and migration --MDeG Submitted				
PI Name	[redacted]			Rocket# R	[redacted]
Department	Biochemistry and Cancer Biology	Office Phone#	[redacted]	Home Phone#	[redacted]
Office Building	Block	Office Room#	390		
Lab Building	Block	Lab Room#	371		
Campus	☐ Main Campus ☒ Health Science Campus	UT-Email Address (will be used for all revision correspondence)	kathryn.eisenmann@utoledo.edu		

A. Personnel - Please list all other personnel working on this research project

Add Line	Name	Rocket#	Highest Biosafety Containment Level
Delete Line	[redacted]	R [redacted]	BSL-2
Delete Line	[redacted]	R [redacted]	BSL-2
Delete Line	[redacted]	R [redacted]	BSL-2
Delete Line	[redacted]	R [redacted]	BSL-2

B. Protocol Objectives - State Briefly the general objectives of the project for which recombinant DNA will be employed. Be sure to include a discussion of the type of experiments to be done (ie Northern Blot, Protein production, Expression Studies etc) (Right click for Bold, Italic, Super/ Subscript. Control-click on Mac. Greek letters can be pasted from Word, Field will expand for content)

This project will address the role of the formin family of actin nucleators in the transition of certain types of cancer cells towards the amoeboid-type of cell migration.

Types of vectors to be used:
-replication incompetent lentivirus for the stable expression of genes of interest. The lentiviral platform is the widely utilized pSLIKneo system.
-standard plasmids for transfection by lipid, and CaPO4-based methods.

Standard techniques to be employed during this work:
-generation of lentiviral particles in HEK293T cells
-infection of cancer cells for generation of stable, inducible cells lines expressing gene of interest
-fluorescent microscopy of fixed or live cells
-western blotting

-production and purification of recombinant protein in DH5alpha bacteria for microinjection
-adhesion, migration and invasion assays
-flow cytometry to assess protein expression levels, F-actin content
-orthotopic injection of lentiviral modified tumor cells into mice
-necropsies of mice with infected tumor cells

C. General Details

○ Yes
◉ No — 1.) Plant subjects are involved

◉ Yes
○ No — 2.) Animal subjects are involved

☐ 2a.) Animals will be a source of biohazardous/infectious material

☒ 2b.) Biohazardous/infectious agents will be administered to animals

2c.) List all IACUC Protocol Numbers approved for this work (**required** if animals are being utilized)

+	Approved UT-MC or HSC protocol#
-	[redacted]
-	[redacted]

○ Yes
◉ No — 3.) Human subjects are involved

○ Yes
◉ No — 4.) Agent(s) will be used in this work that is on the HHS, HHS/UDSA or USDA list of select agents/toxins **The Department of Safety and Health must approve an SOP for the use of all Select Agents before approval will be granted**

D. Recombinant DNA

◉ Yes
○ No — Does this work involve Recombinant DNA?

1. Provide the following Information about the DNA Source(s), (DO NOT LIST VECTORS, only genetic material to be inserted into vectors)

Add Line	Source (dropdown list)	Classification	Genus/Species/Subtype	Fraction of Genome (dropdown list)
Delete Line	Lab Derived-Describe Below mDia2, mDia1, DIP were PCR cloned out from HeLa cells and subcloned into commercially available fluorescent fusion vectors (Clontech). Point mutations were generated in some instances to generate constitutively (in) active versions of the proteins	◉ Eukaryote ○ Prokaryote ○ Viral ○ Other-specify in next field	human	Single Gene mDia1, mDia2, DIP

Delete Line	Lab Derived-Describe Below mDia2, mDia1 and Dip protein domains generated as above subcloned into commercially available pGexKT bacterial expression vectors.	○ Eukaryote ○ Prokaryote ○ Viral ○ Other-specify in next field	human	Single Gene mDia2, mDia,1 DIP (full-length proteins and individual protein domains)

2. Information - Please consult the NIH guidelines for work involving recombinant DNA Molecules 2002 in answering the following. A link can be found here http://www.utoledo.edu/research/RC/link1.html

○ Yes
◉ No a.) Consult Appendix B - Are any of the source organisms pathogenic to humans or other vertebrates?

○ Yes
◉ No b.) Are you introducing antibiotic resistance into an infectious pathogen?

○ Yes
◉ No c.) Are you creating genetically engineered plants or using plants with microorganisms or insects containing recombinant DNA.?

3.) Experiments - Check all the categories below that apply to the work you intend to do with recombinant DNA

☒ a.) Expression Studies

☒ b.) Virus Production

☒ c.) Protein Production

c1.) List ALL proteins to be produced and their known activity:

Add Line	Protein	Protein Activity/Enzymatic Activity
Delete Line	DIP	enhances branched actin nucleation; inhibits mDia2-dependent non-branched actin nucleation and bundling; induces amoeboid-type motility in cancer cells
Delete Line	mDia2	nucleates and bundles non-branched actin filaments; involved in cytoskeletal rearrangements during cell motility, cell division, vessicular trafficking
Delete Line	mDia1	nucleates non-branched actin filaments; involved in cytoskeletal rearrangements during cell motility, cell division, vessicular trafficking

○ Yes
◉ No c.) Are any of the above proteins known vertebrate toxins?

4.) Vectors and Cell Lines - Provide the following Information about the Vectors(s) being employed for this work

◉ Yes
○ No a.) Are commercially available vectors being used on this protocol?

Provide the following information about each of the commercially available vectors(s) or those derived from commercially available vectors used for this project

Add Vector

Remove this Vector	Vector Type (Drop Down List)	Vector Name and Vendor Name	Transmissible? (Drop-down List)
	Plasmid	pGEX-KT	No

Add a cell line to be used with this vector

Remove this Cell Line		Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	(•) Amplification Cell Line	E Coli	DH5alpha
	( ) Host Cell Line		
	( ) Packaging Cell Line		
Remove this Cell Line	( ) Amplification Cell Line	Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	(•) Host Cell Line	E coli	Rosetta
	( ) Packaging Cell Line		

Remove this Vector	Vector Type (Drop Down List)	Vector Name and Vendor Name	Transmissible? (Drop-down List)
	Plasmid	pCMV C1-EGFP/EYFP/ECFP/Cherry (Clontech)	No

Add a cell line to be used with this vector

Remove this Cell Line		Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	HeLa
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	MDA-MB-231
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	M2 melanoma
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	SKOV3
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	ES-2
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	U87MG
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	U87251
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	MDA-MB-231 LN Luc
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	HEK293T
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	Ovca429
	○ Amplification Cell Line ◉ Host Cell Line ○ Packaging Cell Line	Human	MDA-MB-435S

Remove this Vector	Vector Type (Drop Down List)	Vector Name and Vendor Name	Transmissible? (Drop-down List)
	Plasmid	pCMV-HA	No

Add a cell line to be used with this vector

Remove this Cell Line		Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	MDA-MB-231
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	HeLa
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	HEK 293
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	M2
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	OVCA429
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	ES2
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	SKOV3
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	U87MG
Remove this Cell Line	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	U251

Remove this Vector	Vector Type (Drop Down List)	Vector Name and Vendor Name	Transmissible? (Drop-down List)
	Plasmid	pCMV-FLAG 3X	No

Add a cell line to be used with this vector

Remove this Cell Line		Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	MDA-MB-231
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	HEK 293T
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	Hela
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	OvCa429
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	ES2
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	SKOV3
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	U87MG
	○ Amplification Cell Line ● Host Cell Line ○ Packaging Cell Line	Human	U251

Remove this Vector	Vector Type (Drop Down List)	Vector Name and Vendor Name	Transmissible? (Drop-down List)
	Plasmid	pMD2.G (ATCC)	No

Add a cell line to be used with this vector

Remove this Cell Line		Species (ie Human, E. coli)	Type/Subtype (ie HeLa, BL21)
	○ Amplification Cell Line ○ Host Cell Line ● Packaging Cell Line	Human	HEK 293T

Remove this Vector | Vector Type (Drop Down List): Plasmid | Vector Name and Vendor Name: pSLIK Neo Venus (ATCC) | Transmissible? (Drop-down List): No

Add a cell line to be used with this vector

Remove this Cell Line | ◯ Amplification Cell Line ◯ Host Cell Line ◉ Packaging Cell Line | Species (ie Human, E. coli): Human | Type/Subtype (ie HeLa, BL21): HEK293T

Remove this Vector | Vector Type (Drop Down List): Plasmid | Vector Name and Vendor Name: psPAX2 (ATCC) | Transmissible? (Drop-down List): No

Add a cell line to be used with this vector

Remove this Cell Line | ◯ Amplification Cell Line ◯ Host Cell Line ◉ Packaging Cell Line | Species (ie Human, E. coli): Human | Type/Subtype (ie HeLa, BL21): HEK293T

◯ Yes
◉ No b.) Are non-commercially available vectors being used on this protocol? (e.g. generated *de novo* by your lab)

◯ Yes
◉ No c.) Are cell lines **OTHER THAN THOSE LISTED IN 4a and 4b above used** for any purpose on this protocol?

5.) Volume - What is the maximum volume of culture to be grown at any time? Be advised that volumes greater than 10L will require more stringent oversight.

◯ More than 10 Liters

◉ Less than 10 Liters

6.) Risk Assessment - Consult the NIH Guidelines http://www.utoledo.edu/research/RC/link1.html. Consult Section II. Which Risk Group applies to the work described in thisProtocol? Provide the specific citation to justify your choice. FAILURE TO PROVIDE CITATION FROM FEDERAL REGULATIONS WILL RESULT IN THIS PROTOCOL BE ING ADMINISTRATIVELY WITHDRAWN FROM COMMITTEE CONSIDERATION.

☐ Risk Group 1 (RG1): Agents not associated with disease in healthy adult humans

☒ Risk Group 2 (RG2): Agents associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available

☐ Risk Group 3 (RG3): Agents associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available

☐ Risk Group 4 (RG4): Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions *are not usually* available

Citation: lentivirus: Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work. A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to

human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

List all health risks associated with this research

potential for lentiviral infection in exposed/broken skin, eyes or mucous membrane. Exposure is limited by using 3 plasmids to produce virus in HEK 293T cells and using replication incompetent virus. Recombination of the virus producing a replication competent virus is possible, but not a common risk.

7.) NIH Classification - Consult the NIH Guidelines linked above. Consult Section III. Which experimental classification citation applies to the work proposed in this protocol? Provide the specific citation to justify your choice. FAILURE TO PROVIDE CITATION FROM FEDERAL REGULATIONS WILL RESULT IN THIS PROTOCOL BE ING ADMINISTRATIVELY WITHDRAWN FROM COMMITTEE CONSIDERATION.

Citation: Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

8.) Biosafety Containment Level - Consult Appendix G of the NIH Guidelines linked above. Give the specific citation that applies to the Biosafety Containment level appropriate for your work and choose the appropriate level from the drop-down box.Provide the specific citation to justify your choice. FAILURE TO PROVIDE CITATION FROM FEDERAL REGULATIONS WILL RESULT IN THIS PROTOCOL BE ING ADMINISTRATIVELY WITHDRAWN FROM COMMITTEE CONSIDERATION.

Citation: Appendix G-II-B. Biosafety Level 2 (BL2)

Biosafety Level for procedures, equipment, and facilities:	BSL2/PBSL2
BSL1 Lab Campus/Building/Room#	Health Sci Campus , Block 371
BSL2 Lab Campus/Building/Room#	Health Sci Campus, Block 361
Final Disposal Location Campus/ Building/Room#	Health Sci Campus, Block 361

9.) Waste/Disposal/Elimination - Select the appropriate disposal methods for materials used in this work

- ☒ 10% Household Bleach solution <24 hours old
- ☐ Standard Bin
- ☒ Tied or Taped Red Bag, Biohazard Bin
- ☒ Autoclave
- ☐ Other described below:

tissue culture performed in biosafety cabinet

10.) Decontamination - Select the appropriate methods decontaminating instruments and work surfaces for this protocol

- ☒ 10% Household Bleach solution <24 hours old
- ☒ Ethanol
- ☒ Other described below:

Tissue culture hood exposed nightly with UV light 15'

E. Infectious/Biohazardous Material

(•) Yes ( ) No Does this work involve infectious (e.g. pathogenic bacteria or viruses) or biohazardous(e.g. body fluids, select agents) material?

1.) Infectious Materials

(•) Yes ( ) No a.) Are infectious materials used on this project?

Provide the following information for each source of infectious material

Add Line	Source	Classification	Genus/Species/Subtype
Delete Line	Lab Derived-Describe Below Replication-incompetent Lentiviral vectors expressing miRNAs or gene fusions	( ) Eukaryote ( ) Prokaryote (•) Viral ( ) Other-specify in next field	lentivirus

2.) Biohazardous Materials

( ) Yes (•) No a.) Are biohazardous materials used on this project?

3.) Risk Assessment - Please consult Section II of the CDC-Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition Link: http://www.utoledo.edu/research/RC/link2.html. Which Risk Group applies to the work described in this protocol? Provide the specific citation to justify your choice. FAILURE TO PROVIDE CITATION FROM FEDERAL REGULATIONS WILL RESULT IN THIS PROTOCOL BEING ADMINISTRATIVELY WITHDRAWN FROM COMMITTEE CONSIDERATION.

☐ Risk Group 1 (RG1): Agents not associated with disease in healthy adult humans

☒ Risk Group 2 (RG2): Agents associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available

☐ Risk Group 3 (RG3): Agents associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available

☐ Risk Group 4 (RG4): Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions *are not usually* available

Citation: Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

List all health risks associated with this research

potential for lentiviral infection in exposed/broken skin, eyes or mucous membrane. Exposure is limited by using 3 plasmids to produce virus in HEK 293T cells and using replication incompetent virus. Recombination of the virus producing a replication competent virus is possible, but not a common risk.

4.) Biosafety Containment Level - Consult Section IV of the CDC-BMBL linked above. Give the specific citation that applies to the Biosafety Containment level appropriate for your work and choose the appropriate level from the drop-down box.FAILURE TO PROVIDE CITATION FROM FEDERAL REGULATIONS WILL RESULT IN THIS PROTOCOL BE ING ADMINISTRATIVELY WITHDRAWN FROM COMMITTEE CONSIDERATION.

Citation: Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment.

Biosafety Level for procedures, equipment, and facilities:	BSL2/PBSL2
BSL1 Lab Campus/Building/Room#	Health Sci Campus, Block Health Sciences 371
BSL2 Lab Campus/Building/Room#	Health Sci Campus, Block Health Sciences 361
Final Disposal Location Campus/ Building/Room#	Health Sci Campus, Block Health Sciences 361

5.) Waste/Disposal/Elimination - Select the appropriate disposal methods for materials used in this work

- ☒ 10% Household Bleach solution <24 hours old
- ☐ Standard Bin
- ☒ Tied or Taped Red Bag, Biohazard Bin
- ☒ Autoclave
- ☐ Other described below:

6.) Decontamination - Select the appropriate methods decontaminating instruments and work surfaces for this protocol

- ☒ 10% Household Bleach solution <24 hours old
- ☒ Ethanol
- ☐ Other described below:

7.) Contingency Plans - Describe all contingency plans in place in the event of spill/escape/personnel exposure. State if you have received your UT Safety and Health-issued spill kit.

No, we have not received a Spill Kit yet.

INJURY/EXPOSURE PROCEDURE

Eye exposure

Rinse eye/s for a minimum of 15 minutes in the eyewash. Report all eye exposures to the Department of Occupational Health and Safety/Emergency Department.

Needlestick and/or Sharps Exposure

Wash the affected area for a minimum of 15 minutes with soap and water. **Immediately** report the Incident to your PI or Lab Manager **and** to the Department of Occupational Health and Safety/Emergency Department.

Skin Contamination

Wash the affected area for a minimum of 15 minutes with soap and water. Report the Incident to your PI and the Laboratory Safety Manager. If the skin is broken, treat the Incident as a Needlestick (above).

Spill Containment: adsorbent is added to larger liquid spills and then disposed of in red biohazard bags. The remaining area is disinfected with 10% bleach solution followed by 70% ethanol. All wipes and gloves are disposed of in red biohazard bags which are then sealed and autoclaved.

F. Animals

◯ Yes

(•) No Will this protocol involve recombinant DNA-derived, biohazardous, or infectious materials; or Select Agents/Toxins to be used in or on living vertebrate animals?

I agree to comply with the NIH and Institutional requirements pertaining to shipment and transfer of recombinant DNA materials, and will obtain the proper training related to the safe packaging and shipping of the biological materialsI am familiar with and agree to abide by the provisions of the current NIH *Guidelines* and other specific NIH instructions pertaining to the proposed project as well as provisions of the University of Toledo Biosafety Manual.I have read the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (April 2002) and the current CDC Biosafety Manual, and am willing to abide by them. I take full responsibility for ensuring others in my laboratory have been appropriately trained and will comply with these guidelines. Any unusual illness of any personnel engaged in this research will be reported to the IBC and if appropriate NIH.

All of the above answers are true and complete to the best of my knowledge.

I acknowledge that upon approval, this protocol will be active for either 5 years, subjecting my work to all required oversight for the full duration or until I choose to formally terminate the protocol.

Signature: _____ Date: [redacted]

[redacted]