Application for Exempt Dealings: Version 1 dated Oct 2012

Austin Health
Institutional Biosafety Committee
ABN: 96 237 388 063
P.O Box 5555 Heidelberg 3084
Telephone: 03 9496 4090
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Office Use Only
IBC Project number:

IBC	**2**	**0**			/					

1.1 TITLE

Title of proposed dealing *(the title should be concise and convey the purpose of the dealing).*

Characterization of genes, expression and protein activity in the gastrin pathway (Gastrin Releasing Peptide, Gastrin and P21-Activated Kinase).

Will this Exempt Dealing replace another approval for work with GMs? If yes, provide the IBC or OGTR Reference Number

No

1.2 VERSION DATE & NUMBER

Please provide a version date and number for your application.

Version number: 1
Version date: 11/11/12

1.3 PRINCIPAL INVESTIGATOR

The Principal Investigator will have legal responsibility for any approved projects and will be the person to whom the Austin Health IBC will send correspondence.

NAME (Title, Given Name, Family Name)		[redacted]	
NAME OF EMPLOYING INSTITUTION			
NAME OF INSTITUTION ADMINISTERING FUNDS			
DEPARTMENT			
EMAIL ADDRESS			
PHONE NUMBER			

1.4 RESPONSIBLE RESEARCHER

The Responsible Researcher will be the person submitting the proposal and proposing to undertake the dealings.

NAME (Title, Given Name, Family Name)		[redacted]	
NAME OF EMPLOYING INSTITUTION			

NAME OF INSTITUTION ADMINISTERING FUNDS	[redacted]
DEPARTMENT	[redacted]
EMAIL ADDRESS	[redacted]
PHONE NUMBER	[redacted]

1.5 CO-INVESTIGATORS

Please list all co-investigators on the project.

	Name	Position	Department/Institution	Email
Co-investigators (Title, Given Name, Family Name)	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
Person to act in Principal investigator's absence	[redacted]	[redacted]	[redacted]	[redacted]

2.1 PROJECT DESCRIPTION

(i) Describe the dealing to be undertaken

- Use **only** a few sentences
- Use plain, simple language and explain all technical terms and acronyms
- **Do not** list all the GMOs here – these details are to be provided in Part 2.1 (iii)

(Consider the breadth and scope of the all activities in relation to the dealings including any importation, transport, storage or disposal of the GMO – refer to Section 10 of the *Gene Technology Act 2000* for a definition of "deal with" in relation to dealing with a GMO)

Our group studies growth factors including gastrins, gastrin-releasing peptides and associated proteins. We need to generate plasmids that express, target, or regulate these genes in cultured cells to better characterize and understand their growth factor activities. Plasmid/vector DNA will be inserted and amplified in bacteria to express proteins or purified as plasmid/vector DNA to be later inserted into cultured mammalian cell lines. All of the targeted human genes have been knocked out in mouse cells and either made into viable knockout mice (gastrin and p-21 activated kinase) or are in the process (gastrin-releasing peptide). However, we require these genes to be knocked-out (deleted) in human cells to continue our research.

(ii) Classification of the GMO(s)

Explain how these dealings meet the Exempt Dealing criteria as described in Attachment 1.

The bacterial work will use *Escherichia coli* (BL21 for protein expression and DH5α for plasmid/vector amplification) and non-conjugative plasmids/vectors. The same plasmids will be used for mammalian tissue culture cell lines. These plasmids/vectors will not contain viral components, lead to pathogenicity, confer the ability of the host or vector to cause harm, nor allow the spontaneous generation of a whole animal. They will have selection resistance to standard antibacterials like tetracycline or ampicillin to allow growth selection in bacteria and standard selection markers in mammalian cells, like puromycin and G418. All inserted DNA into plasmids will comply with exempt dealing regulations further outlined in the Australian government's GMO exempt dealings publication.

2.1 (iii)	**RECORD OF GMO(S)** This table is intended to be a concise, accurate record of **all** the GMOs to be generated or used. This details should not be so narrow as to preclude foreseeable and intended work (which would then need a new approval), nor so broad as to lead to confusion about what dealings with GMOS are covered.

COMMON NAME OF THE HOST ORGANISM (e.g. Mouse, Bacteria etc.)	**SCIENTIFIC NAME OF THE HOST ORGANISM** (organism that is / will be genetically modified)	**VECTOR(S) & METHOD OF TRANSFER**[1] (if applicable)	**EXEMPT HOST/ VECTOR SYSTEM**[2]	**DONOR NUCLEIC ACID**[3]**:** **1. IDENTITY** **2. FUNCTION** **3. ORGANISM OF ORIGIN** (Address 1-3)	**KIND OF DEALING**[4] (numerical category only)
Example: *Bacteria*	*Escherichia coli* *K12 derived strain*	*Standard non-conjugative plasmid by electroporation*	*YES*	*Expression of green fluorescent protein (GFP) from Aequorea victoria*	*4*
Bacteria	*Escherichia coli* (DH5α)	Non-conjugative plasmids by heat-shock or electroporation (such as: pTALs in pUC57-ΔBsal plasmid backbones and pJDS in modified T7 vectors)	YES	Non-pathogenic DNA from *Xanthomonas spp*. in pUC57-ΔBsal plasmid backbones sold as a kit from Addgene. The DNA inserts are comprised of transcription activator-like (TAL) effectors, which specifically bind certain nucleic acids. Combining a series of these plasmids through restriction enzyme digestion and ligation can generate DNA specific targeting regions. We will use these constructs to target gastrin, gastrin-releasing peptide and p21-activated kinase in mammalian cells, primarily in human cancer cell lines. These targeting constructs can then be cloned into modified T7 vectors (pJDS plasmids) that contain a Fok1 nuclease (which only cleaves specific sequences of DNA as a dimer when co-transfected with a like plasmid).	1
Bacteria	*Escherichia coli* (BL21)	Non-conjugative plasmids by heat-shock or electroporation (such as: proEXHtb and pGEX2T)	YES	Fragments of DNA that encode various forms (wild type, mutated or truncated) of gastrin, gastrin-releasing peptide and p21-activated kinase will be cloned into expression vectors that will allow the expression and purification of human proteins from bacteria, in addition to control empty vectors.	1

Bacteria	*Escherichia coli* (DH5α)	Non-conjugative plasmids by heat-shock or electroporation (such as: pCDNA3, pBSK (bluescript), and pGeneClip)	YES	Empty vectors and vectors containing fragments of human DNA that encodes various forms (wild type, mutated or truncated) of gastrin, gastrin-releasing peptide and p21-activated kinase genes will be amplified and purified in bacteria for use in cultured mammalian cells. These vectors may also contain siRNA against the same proteins.	1
Mammalian cultured cell lines	Human cancer cell lines such as gastric cancer (AGS, AGSCCK2R, MKN), human colon cancer (DLD-1, HCT116, HT29, SW480, SW1222, Colo320, Colo320CCK2R, Lovo), human prostate cancer (PC3, LNCap), human renal cancer (ACHN, Caci-1, Caci-2), human T-cell leukaemia (Jurkat), pancreatic cancer (PANC-01, MiaPaca-01) Mouse cancer cell lines such as pancreatic cancer (PAN02)	Non-conjugative, non viral and non-pathogenic plasmids will be transfected into cell lines using electroporation or lipofectamine reagents (such as: pCDNA3, pJDS, pGeneClip, pTurbo-Cre)	YES	Empty vectors and vectors containing fragments of human DNA that encode various human forms (wild type, mutated or truncated) of gastrin, gastrin-releasing peptide and p21-activated kinase genes will be transfected into human cancer cell lines to study the effects they have on the cells. These vectors may also contain siRNA against the same proteins. Targeting vectors comprised of non-pathogenic TAL effectors in T7 vectors (pJDS plasmids) with the Fok1 endonuclease (TALENs) will be co-transfected into human cells in order to generate specific double stranded DNA breaks. Non-pathogenic donor vectors containing fragments of human DNA that encode various human forms (wild type, mutated or truncated) of gastrin, gastrin-releasing peptide and p21-activated kinase genes will be used in combination with the TALENs above to provide a template for homologous recombination, allowing specific targeting of the above mentioned genes. These may contain loxP sites flanking the resistence markers and require transient transfection with a CRE plasmid to 'pop out'.	4

[1] You don't need to specify the name of the vector (e.g. "standard non-conjugative cloning vectors", "lamda bacteriophage" are adequate).
[2] The answer to this question must be "yes". Refer to Attachment 1 - the host and vector must be included in the list of host/vector systems for Exempt Dealings.
[3] Categories or classes of genes are acceptable but cannot be too broad (e.g. "human genes" is too broad). Remember to list marker and reporter genes (e.g. GFP, antibiotic resistance).
[4] Refer to Attachment 1 – the dealings must meet the Exempt Dealing criteria.

2.1 (iv)	**FACILITIES TO BE USED** List all the facilities to be used for this dealing. For OGTR-certified facilities, the requested information can be found on the OGTR sign displayed at the entry to the facility.

BUILDING NAME	**ROOM NUMBER**	**TYPE** e.g. PC2 lab (if applicable)	**OGTR certification number** (if applicable)
Lance Townsend Building	Room 8.24	PC2 lab	Cert-1694
Lance Townsend Building	Room 9.11	PC2 lab	Cert-1695
Lance Townsend Building	Room 8.22	PC1 lab	N/A

3.1 LICENCE HOLDER SIGNATURE

The signature of the nominated licence holder is required from the institution you are an employee of for the purposes of this research project.

Nominated Licence	Licence Name & Designation	Licence Nominee	Licence Nominee Signature	Date
☒	Austin Health	Sianna Panagiotopoulos Phone: (03) 9496 5088 Email: sianna@unimelb.edu.au	<<insert electronic signature>>	11/11/2012
☐	University of Melbourne at Austin Health	Helen Dedman Phone: (03) 9496 3602 or (03) 9035 7056 Email: hld@unimelb.edu.au		_ _ / _ _ / _ _ _ _
☐	Ludwig Institute for Cancer Research – Austin Branch	Mark Frewin Phone: (03) 9496 5299 Email: mark.frewin@ludwig.edu.au		_ _ / _ _ / _ _ _ _
☐	Florey Institute of Neuroscience & Mental Health	Phone: Email:		_ _ / _ _ / _ _ _ _

3.2 PRINCIPAL INVESTIGATOR DECLARATION

I declare that:

- To the best of my knowledge the information provided in this form is accurate and true;
- work on this project will not start without written permission from the organisation named in section 1.4 of this form;
- the dealings will be conducted in accordance with legislative and regulatory requirements as they apply to gene technology and GMOs;
- only the dealings described in this document will be undertaken;
- the dealings will only be conducted in the facilities listed in this application or as amended from time to time by the organisation named in section 1.4 of this form;
- I will ensure the dealings are properly supervised and a record of the details of the dealings retained;
- I will ensure personnel under my supervision have the appropriate qualifications, experience and training before they start work on the dealings;
- Signed and dated training records for all personnel under my supervision will be made available for auditing purposes.

Printed Name	Professor X
Signature:	<<insert electronic signature>>
Date:	11/11/2012

3.1 IBC DECLARATION (OFFICE USE ONLY)

I declare that:

- I am duly authorised to sign this form
- The Austin health IBC has assessed the dealings in this form to be an Exempt Dealing under the amended *Gene Technology Regulations 2001.*

Name of IBC	Austin Health IBC
Chair:	
Date of IBC Assessment:	_ _ / _ _ / _ _ _ _
Signature:	
Date:	_ _ / _ _ / _ _ _ _

Attachment 1: Part 2 of Schedule 2 – Host/vector systems for Exempt Dealings

Please use for section 2(iii) of this form. Excerpt from the *Gene Technology Regulations 2001*, effective from 1 September 2011.

Item	Class	Host	Vector
1	Bacteria	*Escherichia coli* K12, *E. coli* B, *E. coli* C or *E. coli* Nissle 1917 — any derivative that does not contain: (a) generalised transducing phages; or (b) genes able to complement the conjugation defect in a non-conjugative plasmid	1. Non-conjugative plasmids 2. Bacteriophage (a) lambda (b) lambdoid (c) Fd or F1 (eg M13) 3. None (non-vector systems)
		Bacillus — specified species — asporogenic strains with a reversion frequency of less than 10^{-7}: (a) *B. amyloliquefaciens* (b) *B. licheniformis* (c) *B. pumilus* (d) *B. subtilis* (e) *B. thuringiensis*	1. Non-conjugative plasmids 2. Plasmids and phages whose host range does not include *B. cereus*, *B. anthracis* or any other pathogenic strain of *Bacillus* 3. None (non-vector systems)
		Pseudomonas putida — strain KT 2440	1. Non-conjugative plasmids including certified plasmids: pKT 262, pKT 263, pKT 264 2. None (non-vector systems)

Item	Class	Host	Vector
		Streptomyces — specified species: (a) *S. aureofaciens* (b) *S. coelicolor* (c) *S. cyaneus* (d) *S. griseus* (e) *S. lividans* (f) S. parvulus (g) *S. rimosus* (h) *S. venezuelae*	1. Non-conjugative plasmids 2. Certified plasmids: SCP2, SLP1, SLP2, PIJ101 and derivatives 3. Actinophage phi C31 and derivatives 4. None (non-vector systems)
		Agrobacterium radiobacter *Agrobacterium rhizogenes* — disarmed strains *Agrobacterium tumefaciens* — disarmed strains	1. Non-tumorigenic disarmed Ti plasmid vectors, or Ri plasmid vectors 2. None (non-vector systems)
		Lactobacillus *Lactococcus lactis* *Oenococcus oeni* syn. *Leuconostoc oeni* *Pediococcus* *Photobacterium angustum* *Pseudoalteromonas tunicata* *Rhizobium* (including the genus *Allorhizobium*) *Sphingopyxis alaskensis* syn. *Sphingomonas alaskensis* *Streptococcus thermophilus*	1. Non-conjugative plasmids 2. None (non-vector systems)

Item	Class	Host	Vector
		Synechococcus — specified strains: (a) PCC 7002 (b) PCC 7942 (c) WH 8102 *Synechocystis* species — strain PCC 6803 *Vibrio cholerae* CVD103-HgR	
2	Fungi	*Kluyveromyces lactis* *Neurospora crassa* — laboratory strains *Pichia pastoris* *Saccharomyces cerevisiae* *Schizosaccharomyces pombe* *Trichoderma reesei* *Yarrowia lipolytica*	1. All vectors 2. None (non-vector systems)
3	Slime moulds	*Dictyostelium* species	1. *Dictyostelium* shuttle vectors, including those based on the endogenous plasmids Ddp1 and Ddp2 2. None (non-vector systems)

Item	Class	Host	Vector
4	Tissue culture	Any of the following if they cannot spontaneously generate a whole animal: (a) animal or human cell cultures (including packaging cell lines); (b) isolated cells, isolated tissues or isolated organs, whether animal or human; (c) early non-human mammalian embryos cultured *in vitro*	1. Non-conjugative plasmids 2. Non-viral vectors, or replication defective viral vectors unable to transduce human cells 3. Baculovirus (*Autographa californica* nuclear polyhedrosis virus), polyhedrin minus 4. None (non-vector systems)
		Either of the following if they are not intended, and are not likely without human intervention, to vegetatively propagate, flower or regenerate into a whole plant: (a) plant cell cultures; (b) isolated plant tissues or organs	1. Non-tumorigenic disarmed Ti plasmid vectors, or Ri plasmid vectors, in *Agrobacterium tumefaciens, Agrobacterium radiobacter* or *Agrobacterium rhizogenes* 2. Non-pathogenic viral vectors 3. None (non-vector systems)

Part 1 of Schedule 2 – Exempt Dealing criteria

Excerpt from the *Gene Technology Regulations 2001,* effective from 1 September 2011.

Item	Description of dealing
1	There is no Item 1
2	A dealing with a genetically modified *Caenorhabditis elegans*, unless: (a) an *advantage* is conferred on the animal by the genetic modification; or (b) as a result of the genetic modification, the animal is capable of secreting or producing an infectious agent.
3	A dealing with an animal into which genetically modified somatic cells have been introduced, if: (a) the somatic cells are not capable of giving rise to infectious agents as a result of the genetic modification; and (b) the animal is not infected with a virus that is capable of recombining with the genetically modified nucleic acid in the somatic cells.
3A	A dealing with an animal whose somatic cells have been genetically modified *in vivo* by a replication defective viral vector, if: (a) the *in vivo* modification occurred as part of a previous dealing; and (b) the replication defective viral vector is no longer in the animal; and (c) no germ line cells have been genetically modified; and (d) the somatic cells cannot give rise to infectious agents as a result of the genetic modification; and (e) the animal is not infected with a virus that can recombine with the genetically modified nucleic acid in the somatic cells of the animal.

Item	Description of dealing
4	(1) Subject to subitem (2), a dealing involving a host/vector system mentioned in Part 2 of this Schedule and producing no more than 25 litres of GMO culture in each vessel containing the resultant culture. (2) The donor nucleic acid: (a) must meet either of the following requirements (i) it must not be derived from organisms implicated in, or with a history of causing, disease in otherwise healthy: (A) human being; or (B) animals; or (C) plants; or (D) fungi; (ii) it must be characterised and the information derived from its characterisation show that it is unlikely to increase the capacity of the host or vector to cause harm; *Example: Donor nucleic acid would not comply with subparagraph (ii) if its characterisation shows that, in relation to the capacity of the host or vector to cause harm, it:* *(a) provides an advantage; or* *(b) adds a potential host species or mode of transmission; or* *(c) increases its virulence, pathogenicity or transmissibility;* (b) must not code for a toxin with an LD_{50} of less than 100 μg/kg; and (c) must not code for a toxin with an LD_{50} of 100 μg/kg or more, if the intention is to express the toxin at high levels; and (d) must not be uncharacterised nucleic acid from a toxin-producing organism; and (e) must not include a viral sequence, unless the donor nucleic acid (i) is missing at least 1 gene essential for viral multiplication that: (A) is not available in the cell into which the nucleic acid is introduced; and (B) will not become available during the dealing; and (ii) cannot restore replication competence to the vector.

Item	Description of dealing
5	A dealing involving shot-gun cloning, or the preparation of a cDNA library, in a host/vector system mentioned in item 1 of Part 2 of this Schedule, if the donor nucleic acid is not derived from either: (a) a pathogen; or (b) a toxin-producing organism.

TAX INVOICE:
New Protocol
(Upfront payment required)

Austin Health
Institutional Biosafety Committee
ABN: 96 237 388 063
P.O Box 5555 Heidelberg 3084
Telephone: 03 9496 4090
Fax: 03 94964103

Please complete the appropriate section (A or B). Please note that GST does not apply when requesting a transfer of funds from an internal department (section A). GST is applicable for all other forms of payment (Section B). Please contact the Research Ethics Office on 9496 4099 if you have any queries.

Upon payment this document becomes a Tax Receipt.
Please retain a copy, as no further receipts will be issued.

1. Principal Investigator
Professor X

2. Project Title
Characterization of genes, expression and protein activity in the gastrin pathway (Gastrin Releasing Peptide, Gastrin and P21-Activated Kinase).

Please tick the appropriate box:

☒ Exempt Dealings	$ No Charge
☐ Non Commercially Sponsored Notifiable Low Risk Dealings	$275 (including GST)
☐ Commercially Sponsored Notifiable Low Risk Dealings	$2,200 (including GST)
☐ Non Commercially Sponsored DNIR or DIR	$660 (including GST)
☐ Commercially Sponsored DNIR or DIR	$6050 (including GST)

If you have to pay both an IBC and AEC charge there may be some grounds for a discount. Please contact Research Ethics to discuss this.

Section A - For Internal Projects you must quote a Y3000 or above SPF number

Austin Health SPF No	Name of Dept/SPF	Expense Classification	Charge (see fee schedule)
			$ (not including GST)

Authorised by	Title & Printed Name	Signature

Section B - Payment by Cheque or Credit Card (including GST)

☐ Cheque (made out to "Austin Health)
☒ Credit Card (see details below)

Type (please tick)
☒ Visa ☐ MasterCard ☐ BankCard

Credit card number															
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Exp Date			
x	x	1	5

Name on card
Prof X

Amount	$0 (including GST)

Signature	<<insert electronic signature>>