Modification Form for Permit BIO-UWO-0237

Permit Holder: Rodney DeKoter

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

Additional Personnel
(Please list additional personnel here)

Marek Gruca (Student)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Ecotropic retrovirus	
Approved Cells	human (primary), BM, spleen, thymus, rodent (primary, BM, spleen, thymus, human (established), rodent (established), continued in Notes	WEHI-279 * (ATCC CRL-1704)
Approved Use of Human Source Material	blood (whole)	
Approved GMO	E. coli DH5 alpha, XL1-blue, XL10-gold, TOP10, Ecotropic retrovirus MIGR1, pBABE-puro, pBABE-EGFP, pBABE-EGFP-Pu.1 wt, MSCV, MSCV-neo, MSCV-hygro, MSCV-pac, MSC-pac-PU.1 wt, MSCV-EGFP,	
Approved use of Animals	Mouse	
Approved Toxin(s)		

* Mouse lymphoma cell line. Will be used for transient + stable transfections and Facs analysis.

Rodney DeKoter

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

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

Signature of Permit Holder: Rodney DeKoter

Classification: 2

Date of Last Biohazardous Agents Registry Form: Apr 7, 2009

Date of Last Modification (if applicable):

BioSafety Officer(s):

Chair, Biohazards Subcommittee:

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Product Description

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Cell Biology

ATCC® Number: CRL-1704™ Order this Item **Price:** $417.00

Designations: WEHI-279

Depositors: NL Warner, LL Lanier

Isotype: IgM (surface); kappa light chain

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension

Organism: *Mus musculus* (mouse)

Morphology: lymphoblast

Source: **Disease:** lymphoma
Strain: NZC
Cell Type: B lymphocyte;

Cellular Products: immunoglobulin

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Comments: Does not secrete immunoglobulin.
Tested and found negative for ectromelia virus (mousepox).

Propagation: **ATCC complete growth medium:** Dulbecco's modified Eagle's medium with 4.5 g/L glucose and 0.05 mM 2-mercaptoethanol, 90%; fetal bovine serum, 10%

Subculturing: **Medium Renewal:** 2 to 3 times per week

References:

22846: Warner NL, et al. Flow cytometry analysis of murine B cell lymphoma differentiation. Immunol. Rev. 48: 197-243, 1979. PubMed: 161906
26146: Norris JS, et al. Autocrine regulation of growth: II. Glucocorticoids inhibit transcription of c-sis oncogene-specific RNA transcripts. Biochem. Biophys. Res. Commun. 122: 124-128, 1984. PubMed: 6743325
58054: Gutman GA, et al. Immunoglobulin production by murine B-lymphoma cells. Clin. Immunol. Immunopathol. 18: 230-244, 1981. PubMed: 6781803

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- Make a Deposit
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- Material Transfer Agreement
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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Rodney DeKoter
SIGNATURE Rodney DeKoter
DEPARTMENT Microbiology and Immunology
ADDRESS 3003 Dental Sciences Building
PHONE NUMBER
EMAIL rdekoter@att.net

Location of experimental work to be carried out: Building(s) Dental Sciences Room(s) 3003

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Regulation of myeloid versus lymphoid cell fate by PU.1 (pending)
Functions of related Ets transcription factors in B cells (pending)

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

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

TBA

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

Summary of Biohazards – Rodney DeKoter, Ph.D.

Our laboratory studies gene regulation in the immune system. Our goal is to learn the mechanism(s) of how genes are turned on or off by proteins called transcription factors. To do these experiments, we rely on two experimental approaches. First, we use transgenic or gene targeting technology to modify genes in mice. Our laboratory maintains a number of lines of mice in which genes encoding transcription factors have been genetically modified. Second, we grow primary or transformed cells in culture to study gene expression. We use standard recombinant DNA technology to modify genes in plasmid vectors. We also use replication-incompetent retroviral vectors to transfer genes into cultured cells. Our laboratory does not work with retroviral vectors capable of replicating in culture or in live organisms. Our laboratory does not work with infectious microorganisms.

Summary – Biohazards in our laboratory include

- Recombinant DNA technology (using plasmids grown in E. coli)
- Genetically modified mice
- Replication-incompetent retroviral vectors

Responses to Reviewers -- Rodney DeKoter, Ph.D.

2.3 - please identify the specific cell lines

-a list of all cell lines used in the DeKoter laboratory is provided as an Excel spreadsheet (DeKoter_Cell_Lines.xls)

3.2 - what is the source of the human blood? (ie healthy hospital patients???)

-the source will be healthy hospital patients. This is now written on the form.

4.2 - please give details asked in the table such as the strain(s) of E. coli used for cloning, plasmids, etc.

-a list of all E. Coli strains used in the DeKoter laboratory is provided as an Excel spreadsheet (DeKoter_Bacteria.xls)

-a list of all plasmids used in the DeKoter laboratory is provided as an Excel spreadsheet (DeKoter_Plasmids.xls)

4.3 - please give more details asked in the table such as the specific ecotropic retrovirus (is it Moloney murine leukemia virus?).

-the retroviral vectors used are all based on Moloney murine leukemia virus. I have included a new reference for you (DeKoter_reference.pdf)

Sincerely,

Rod DeKoter

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ◉ YES O NO

If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O 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	Health Canada or CFIA Containment Level
Ecotropic Retrovirus	O Yes ◉ No	O Yes ◉ No	O Yes ◉ No	N/A	N/A	O 1 ◉ 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ◉ YES O NO

If no, please proceed to Section 3.0

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

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⊘ Yes O No	Various	ATCC
Rodent	⊘ Yes O No	Various	ATCC, Mice
Non-human primate	O Yes ⊘ No		
Other (specify)	O Yes ⊘ No		

See Attached Excel Spreadsheet

*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 HC or CFIA containment level required ⊘ 1 O 2 O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Healthy Hospital Patients	O Yes ⊘ No		O 1 ⊘ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli.	Various	Various	Various	Various

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

See Attached Excel Spreadsheet

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
Ecotropic Retrovirus	MIGR1	Clontech	Various	Various

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ⓞ YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⓞ NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mouse

6.3 AUS protocol # In progress

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

Retroviral-infected cells will be transplanted into mice. Virus will be replication-incompetent, non-infectious, and will not be directly given to animals.

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊘NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

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

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

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ ⊘ NO
If no, please proceed to Section 10.0

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE Rodney DeKoter

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

13.0 Containment Levels

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

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

O YES, permit # if on-campus_____
O NO
O NOT REQUIRED

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 have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Rodney DeKoter Date: 1-13-2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G M Kidder
Date: 7 April 2009

Safety Officer for Institution where experiments will take place: SIGNATURE:_____
Date:_____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: J Stanley
Date: April 7/09

Approval Number: BIO-UWO-0237 Expiry Date (3 years from Approval): Apr. 6, 2012

Special Conditions of Approval:

Level 2+ required for this work (in vivo, in vitro) unless shown that virus is not spreading (by PCR). Level 2 only per note from Dr. Kidder April 14/09.

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

Dekoter Lab Cell Lines

Row	Cell Line Name	Cap Color	Date Frozen	Number	Annotation	Link to ATCC
	Box #1 Packaging Cell Lines					
	Retroviral Lines					
1	Plat-E	yellow	8/22/08 8/7/03	9 1	Platinum-E Ecotropic Packaging Cell Line reselected	
1	PA317	none	2/17/97	4	retroviral packaging cell line	ATCC
2	ϕ-NX Eco	red			Phoenix Ecotropic Retroviral Packaging Cell Line	
	ϕ-NX Ampho				Phoenix Retroviral Packaging Cell Line	
2	GP+E-86	yellow	3/13/01	3	Ecotropic 3T3-based packaging cells (gpt reselected)	
	Stable GP+E-86 Producers					
	MIG-based PU.1 vectors					
3	GP+E-86 MIGR1	yellow	7/18/00	2		
3	GP+E-86 MIG-PU.1 wt	green	3/9/01	5		
4	GP+E-86 MIG-PU.1 ΔN2-30	orange	9/12/00	1		
4	GP+E-86 MIG-PU.1 ΔN33-74	yellow	9/12/00	0		
4	GP+E-86 MIG-PU.1 ΔN75-100	blue	9/28/00	1		
5	GP+E-86 MIG-PU.1 ΔN118-167	pink	12/20/00	1		
5	GP+E-86 MIG-PU.1 Ala 30-34	white	12/21/00	1		
5	GP+E-86 MIG-PU.1 Ala 35-39	gray	12/21/00	1		
6	GP+E-86 MIG-PU.1 Ala 40-44	brown	12/21/00	1		
6	GP+E-86 MIG-PU.1 Ala 45-49	yellow	5/7/01	3		
7	GP+E-86 MIG-PU.1 Ala 50-54	orange	5/7/01	3		
7	GP+E-86 MIG-PU.1 Ala 55-59	blue	12/22/00	1		

8	GP+E-86 MIG-PU.1 Ala 60-64	red	4/30/01	1
8	GP+E-86 MIG-PU.1 Ala 65-69	purple	4/30/01	1
8	GP+E-86 MIG-PU.1 Ala 70-74	gray	4/30/01	1
9	GP+E-86 MIG-PU.1 S41A	brown	9/30/00	2
9	GP+E-86 MIG-PU.1 S37, 41, 45A	gray	9/30/00	2
9	GP+E-86 MIG-PU.1 R232, 235A	green	12/22/00	2
	GP+E-86 MIG-Spi-C	none		1 unsorted 5-24-02
	GP+E-86 MIG-PU.1ΔN100	none		1 unsorted 5-24-02

Box #2

Other MIG-Based Vectors

1	GP+E-86MIG-Spi-B	orange	11/15/00	3
1	GP+E-86MIG-Spi-B T670C ("v.1")	white	8/31/00	3 Ets domain mutant of Spi-B
2	GP+E-86 MIG-EBF	purple	5/7/01	3
2	GP+E-86 MIG-Pax5	pink	3/7/00	4
3	GP+E-86 MIG-IL-7Ra			

MSCV-based lines

4	GP+E-86 MSCV-EGFP clone 3	white	2/17/00	1 Clone 3 was subcloned for higher titer
4	MEPT	orange	1/25/00	2 MSCV-EGFP-PU::ER fusion
4	GP+E-86 MSCV-EGFP-PU.1 wt	red	6/13/00	2
5	GP+E-86 MSCV-EGFP-PU.1 ΔN100	none	3/30/98	2
5	GP+E-86 MSCV-EGFP-PU.1 ΔN74	none	3/30/99	5
6	GP+E-86 MSCV-EGFP-PU.1 ΔN75-100	none	3/30/98	4
6	GP+E-86 MSCV-EGFP-PU.1 S148A	none	3/30/98	5

7	GP+E-86 MSCV-EGFP-PU.1 SΔN	none		3/30/99	4 Both ΔN100 and S148A	
7	GP+E-86 MSCV-IL-7Ra	white		3/18/00	0 used up?	
8	GP+E-86 MSCV-EGFP-IL-7Ra	none	?		5	
8	GP+E-86 MSCV-pac-IL-7Ra	none	?		4	
9	GP+E-86 MSCV-EGFP-hFes-FLAG	none		9/29/98	5 FLAG-tagged human Fes cDNA retrovirus	
	Other					
9	φ2-FMS	none		2/4/98	4 Rohrschneder's lab's FMS cDNA-producing virus	

Box #3

General Cell Lines

Fibroblasts / Stromal Cells

1	NIH-3T3	yellow		12/2/05	9	ATCC
1	HeLa	brown		12/14/04	1	ATCC
2	S17	orange/none		9/3/08	5 also in row 4 position 5-9, row 6 position 5-9	
	OP9			8/26/03	9	ATCC
3	ST2-Puro	none		6/13/08	6 puromycin-resistant stromal line (Rolink)	

Myeloid Cells

4	WEHI-3B	none		10/14/03	3	ATCC
4	J774.16	none		1/24/98	macrophage cell line	

B cells

5	WEHI-231	brown		10/14/03	7	ATCC
5	70Z/3	none	?		1	ATCC
6	AA4.1	none	?		1 Antibody-producing hybridoma	

	VP245			6/10/02	2	
6	38B9	blue		9/21/04	3 Abelson-transformed pro-B cells	
7	A20	none	?		2	ATCC
7	CESS-SKW+	none	?		2	ATCC
8	1-8	pink		8/22/01	6 Abelson-transformed pre-B cells	
5	J558-IL-7	blue		8/26/06	4 IL-7-producing plasmacytoma	ATCC
8				8/26/06	4	
	Mast Cells					
9	MC/9	none		5/5/97	4	ATCC
	Other					
9	MEL	none		6/14/06	3 from Cliff Takemoto	

Box #4

Factor-Dependent Cell Lines

IL-3-dependent lines

1	Null2	none	5/2/97	1
1	Null2 + PU-ER	none	3/16/06	4
2	Null2 clone 1	green	12/13/99	3
2	Null2 clone 2	gray	12/16/99	3
2	Null2 clone 3	red	12/22/99	3
2	Null3	none	6/24/97	2
3	FDCP-1	none	1/19/98	3
3	BN/BN	none	1/25/08	4 IL-3-dependent BN/BN cell lines
4	PU.1-/- #2	none	10/24/07	3 new IL-3-dependent cell lines

4	PU.1-/- #9	none	10/24/07	4 new IL-3-dependent cell lines
4	PU.1-/- #10	none	10/24/07	3 new IL-3-dependent cell lines
	GM-CSF-dependent lines			
4	FDCP-1 GM-CSF-dependent	none	8/6/98	3 adapted to GM-CSF
5	PUN1	none	10/29/97	2 PU.1-/- transduced with MSCV-EGFP-GMCSFRa
5	PUN2	none	11/7/97	2
6	GM-CSFR-transduced clone 7	none	10/3/98	2
6	GM-CSFR-transduced clone 9	none	10/3/98	2
7	Blac/Blac	none	1/25/08	2 Blac/Blac IL-3-dependent cell lines
7	KO/KO	none	1/25/08	2 KO/KO IL-3-dependent cell lines
7	GM clone 1	none	8/26/02	1 PU.1-immortalized cell line #1 (Amberly Davidson)
8	Blac/Blac	none	4/5/07	4 Blac/Blac IL-3 dependent cells
8	PIP	none	9/13/06	3 PIP cells (Isaac)

Box #5

IL-7-dependent lines

1	B wt 2	none	1/25/06	3 wt fetal liver-derived pro-B cell lines
1	B wt 3	none	9/20/06	3
2	PUB pro-B	none	10/10/06	3 PU.1-/-Spib-/- pro-B
3	BN/BN pro-B	none	5/6/06	3
3	BN/BN pro-B	none	4/14/06	2
3	Blac/Blac pro-B	none	3/16/06	1
3	PUB pro-B + MIGR1	none	3/3/06	91% GFP after 1x sorting
3	PUB pro-B + MIG-PU.1-ER	none	3/3/06	97% GFP after 1x sorting

3	PUB pro-B + MiG-S148A-ER	none	3/3/06	98% GFP after 1x sorting
3	BN/Blac pro-B one each	none	8/3/06	
4	P8	yellow	1/26/00	2 PU.1-/- Rescued with PU.1 retrovirus
5	P9	purple	1/26/00	4
5	P10	green	1/31/00	3
6	MEPT1	brown	2/22/00	3 PU.1-/- Rescued with MEPT retrovirus
6	MEP12	blue	3/4/00	4
7	PUB-PU.1 #1	blue	3/14/01	3 PU.1-/-Spi-B-/- rescued with PU.1
7	PUB-PU.1 #3	yellow	5/17/01	1
7	BN/BN pro-B #2	none	4/4/07	3
7	PU.1 +/+ MIGR1	none	11/26/08	7 MIGR1-infected WT pro-B cells
7	PU.1-/- Mef2c	none	11/26/08	8 Mef2c rescued pro-B cells
8	M7R1	red	3/13/00	2 PU.1-/- Rescued with MSCV-IL-7R retrovirus
8	M7R2	none	5/13/00	2
9	M7R3	none	5/13/00	2
9	M7R4	none	5/13/00	2
9	Null-MIGR1	none	5/21/02	1 IL-3-dependent cells adapted to IL-7
9	Null-PU.1 #1	none	5/21/02	1 IL-3-dependent cells adapted to IL-7
9	Null-PU.1 #2	none	5/21/02	1 IL-3-dependent cells adapted to IL-7

Box #6

1	M7R5	yellow	6/29/00	2
1	M7R6	orange	1/11/03	3 tested as 95% CD19+

3	PUB-EBF	none	4/13/07	4 EBF-rescued PU.1-/-Spib-/- pro-B cells
4	EBF-1	blue	7/12/00	2 PU.1-/- Rescued with MIG-EBF retrovirus
6	PUB-EBF #1	gray	3/14/01	3 PU.1-/-Spi-B-/- rescued with EBF retrovirus
6	PUB-EBF #2	pink	3/13/01	2
7	Spi-B1	none	?	2
8	Spi-B3	red	?	2
9	M7R10	none	11/22/03	5 PU.1-/-Spi-B+/+ rescued with IL-7Ra virus #10

Box #7 Various

1	Nuli2	none	various	6
1	S17	orange	4/4/01	3
2	PUN1	none	?	4
2	PUN2	none	?	2
3	E12 clone 1	none	?	2
3	E12 clone 2	none	?	2
3	P12 clone 1	none	?	2
3	P12 clone 2	none	?	2
4	M7R2 clone 1	gray	6/16/00	2
4	M7R4 clone 1	green	6/16/00	2
5	"c8" myeloid	blue	6/16/00	1 CD19- clones
6	M7R5 clone 1	purple	7/24/00	2
6	M7R5 clone 2	pink	7/24/00	2
7	MIG-IL-7Ra	none		1
8	OP9	none	12/17/07	2 ATCC

9	OP9-GFP	none	12/17/07	6	Obtained from Toronto	ATCC
9	OP9-DLL	none	12/17/07	6	Obtained from Toronto	ATCC

Box #8 Various

1	OP9 Jagged1 WT	none	6/3/03	3		ATCC
	38B9 + pSIREN	none	2/23/06	1	38B9 cells transduced with siRNA knockdown vectors	
	38B9 + pSIREN-GABP1	none	2/23/06	1		
	38B9 + pSIREN-GABP3	none	2/23/06	1		
2	38B9-pac	none	? ?		38B9 cells transduced with MSCV-pac	
	38B9-Gfi1	none	? ?		38B9 cells transduced with MSCV-Gfi1	
3	OP9-MIGR1	none	6/26/03	1	OP9 transduced with MIG vectors encoding	ATCC
	OP9-MIG-DL!	none	6/26/03	1	Jagged-1 mutants	ATCC
	OP9-MIG-Jagged1 wt	none	6/26/03	1	Collaboration with Capobianco lab	ATCC
	OP9-MIG-Jagged1ΔPL	none	6/26/03	1		ATCC
	OP9-MIG-Jagged1ΔDSL	none	6/26/03	1		ATCC
4	RAW264.7 macrophage cells	none	7/25/03	7	DEMEM-dependent macrophage cell lines	
5	PU.1-/- IL-3-dependent new	none	2/17/04	4		
6	38B9-hCAR	none	4/14/04	7	38B9 cells transduced with truncated human adenovirus receptor	
7	D1 thymocyte line	none	7/23/04	5	IL-7-dependent thymocyte line, Durum lab	ATCC
8	EL4	none	7/29/04	7	T cell lines	ATCC
9	38B9	none	9/21/04	5	Abelson-transformed pro-B cell lines	
	HEK tet-off	none	7/6/06	1		ATCC

Box #9 Inducible Cell lines (located in unmarked lab position)

1	38B9 + TMP-GABP miR30	none	9/15/06	5	GFP+, worted
2	TMP + pRevTet-ON	none	10/26/06	4	G418-selected

	38B9 + TMP-GABP	none	10/26/06	4 20% GFP-positive
3	38B9 + pRevTet-ON, G418-selected	none	10/12/06	6
4	TMP + pRevTet-ON, G418-selected	none	10/27/06	4 100% GFP, resorted
5	TMP + pRevTet-ON, G418-selected	none	10/31/06	4 old
6	TMP + pRevTet-ON clone25	none	11/17/06	3
	TMP + pRevTet-ON clone28	none	11/17/06	3
7				
8	TMP + pRevTet-ON clone 6	none	12/2/06	3
9				

Rod's Lab Stocks

1	Null2	brown	8/18/01	2
2	38B9	blue	8/18/01	4

TOTAL 457

DeKoter Laboratory Competent E. Coli Strains

Strain Name	Company	MSDS Link
DH5 alpha	Protein Express	link
XL1-blue	Stratagene	link
XL10-gold	Stratagene	link
TOP10	Invitrogen	link

DeKoter Lab Plasmids

Plasmid	Annotation	Box / Row
Box 1 - Retrovirus / PU.1		
pBABE-puro	pBABE is a MMLV-based retroviral vector	1-1
pBABE-EGFP		1-1
pBABE-EGFP-PU.1 wt		1-1
MSCV	MSCV-neo with PGK::neo removed by BglII/BamHI digestion	1-1
MSCV-neo	missing	1-1
MSCV-hygro	Vectors obtained from Robert Hawley	1-1
MSCV-pac	missing	1-1
MSCV-pac-PU.1 wt		1-1
MSCV-EGFP	EGFP substituted for pac in MSCV-pac vector	1-2
MIGR1	Vector obtained from Warren Pear's lab.	1-2
MSCV-EGFP-PU.1 wt		1-2
MSCV-EGFP-PU.1 DN100		1-2
MSCV-EGFP-PU.1 DN75		1-2
MSCV-EGFP-PU.1 DN75-100		1-2
MSCV-EGFP-PU.1 S148A		1-2
MSCV-EGFP-PU.1 SDN	DN100 and S148A	1-2
MSCV-EGFP-hFes-FLAG	FLAG-tagged human c-Fes cDNA	1-3
MSCV-EGFP-mGMCSFRa	murine GMCSFRa cloned by Takara	1-3
MSCV-pac-mGMCSFRa		1-3
MIG-PU.1 wt		1-3
MIG-PU.1 DN100		1-3
MIG-PU.1 DN118-167		1-3
MIG-PU.1 R232, 235A	DNA binding mutant (from Abe Brass)	1-3
MFG-IL-7Ra	Vector is from Ashok Venkitaramen	1-4
MSCV-pac-IL-7Ra		1-4
MSCV-IL-7Ra		1-4
MSCV-EGFP-IL-7Ra		1-4
MIG-Pax5	Pax-5 cDNA from Meinrad Busslinger	1-4

MIG-EBF(T7)	EBF from Grosschedl, T7-tag done by Dan N	1-4
MIG-Spi-B	Murine Spi-B cloned by Takara	1-4
MIG-Spi-B T670C	T670C mutation in Spi-B Ets domain	1-4
MIG-Spi-C		1-4
MIG-IL7Ra	Kay Medina	1-4
MILR1	IRES-b-lactamase vector	1-5
MIL-PU.1	IRES-b-lactamase-PU.1 vector	1-5
MSCV-lib	cDNA library from pro-B, 0.7 mg/ml	1-5
MSCV-Cre	Cre virus without GFP	1-5
MIGE3-Cre	Cre virus with EGFP (David Williams Lab)	1-5
pBlue-HA-PU.1		1-6
pGEM-PU.1		1-6
pCDNA3-PU.1 wt		1-6
pCDNA3-PU.1 DN75		1-6
pCDNA3-PU.1 DN75-100		1-6
pCDNA3-PU.1 DN118-167		1-6
pCDNA3-PU.1 R232, 235A		1-6
pCDNA3-PipPU	Abe Brass	1-6
pTRE-PU.1 wt	Jon Walsh	1-7
pTRE-DN100	Jon Walsh	1-7
pTRE-S148A	Jon Walsh	1-7
pTRE-SDN	Jon Walsh	1-7
PU.1 transgene	Ed Scott	1-9
PU.1 Cosmid	Ed Scott	1-9
PU.1 Cosmid 5 kb EcoRI frag		1-9
PU.1 transgene Cassette	Celeste Simon	1-9
PU.1 100 kb P1 phage clone	Celeste Simon (never transformed)	1-9
pCR2.1 PU.1 Site II	site 2 from IL-7Ra promoter 1.1 mg/ml	1-9
MIG-Cre-ER(t)	Isaac Houston	1-8
MIG-PU.12lox	Isaac Houston	1-8

MiPU.1-2lox	Isaac Houston	1-8	has IRES-GFP removed

Box 2 - Retrovirus / PU.1

MIG-PU.1 Δ2-30	Kelly Huang	1-1	
MIG-PU.1 Δ33–74	Kelly Huang	1-2	
MIG-PU.1 Δ75–100	Kelly Huang	1-3	
MIG-PU.1 aa30-34	Kelly Huang	1-4	
MIG-PU.1 aa35-39	Kelly Huang	1-5	
MIG-PU.1 aa40-44	Kelly Huang	1-6	
MIG-PU.1 aa45-49	Kelly Huang	1-7	
MIG-PU.1 aa50-54	Kelly Huang	1-8	
MIG-PU.1 aa55-59	Kelly Huang	1-9	
MIG-PU.1 aa60-64	Kelly Huang	2-1	
MIG-PU.1 aa65-69	Kelly Huang	2-2	
MIG-PU.1 aa70-74	Kelly Huang	2-3	
LXSN-hCAR	human adenovirus receptor retrovirus	2-5	
LXSN-ΔhCAR	truncated receptor	2-6	
B7	pBANSHEE-Thy1.1 SIN retrovirus	3-1	
Bantu	same as B7 but with U6 promoter	3-2	
Bantim	same as B7 but with U6-Bim siRNA	3-3	
pDK-Thy1.1	pBABE-Thy1.1	3-4	
pDK-TU	pBABE-Thy1.1 with U6 promoter	3-5	
pDK-Tim	pBABE-Thy1.1 with U6p-Bim siRNA	3-6	
MSCV-pac-Gfi1	Lee Grimes Gfi1 retrovirus	4-1	
MIG-Gfi1	My Gfi retrovirus	4-2	
MIG-GABPalpha	Kelly Huang	4-3	
MIG-GABPbeta1	Kelly Huang	4-4	
pBABE-puro	Alan Friedman	4-6	
pBABE-C/EBPalpha	Alan Friedman	4-7	
pBABE-C/EBPalpha-ER	Alan Friedman	4-8	
MIG-C/EBPalpha-ER	Alan Friedman	4-9	

pSCA-PU.1 wt	Cloned wt PU.1 transcript (no tag)	5-1
MIG-PU.1 wt	wt PU.1 transcript - no tag	5-2
pSCA-BN	Cloned Pu.1 BN transcript	5-3
MIG-BN	BN PU.1 retrovirus	5-4
pCR-TOPO-BLADD	Isaac's targeting vector!	5-6
pCR-TOPO-PU.1DN31	clone DN31 PU.1	6-1
MIG-PU.1DN31	HA-tagged PU.1 lacking aa 1-31	6-2
pVSV-g	pseudotyping vector	6-4

Box 2: Cloning / Expression / cDNAs

pFlox	Jamey Marth	2-1
pMC-Cre	Jamey Marth	2-1
pCDNA3	Invitrogen	2-1
pCDNA3-HA	Abe Brass	2-1
PUC19		2-1
pBlueScript KS(+)	Stratagene	2-1
pGEM-7Z		2-1
pCITE-2(a)+		2-1
pDNR-1	Clontech	2-1
IL-7Ra probe 1	DNASE HS Assay probes	2-2
IL-7Ra probe 2	DNASE HS Assay probes	2-2
pCR4-TOPO-IL-7Ra probe 2	DNASE HS Assay probes	2-2
pEμ-SR	EiH transgene vector	2-2
IL-7Rα-e2	enhancer 2 from the IL-7Ra gene	2-2
pUC19-EiH	IgH intronic enhancer	2-2
CMV-EGFP	CMV promoter inserted into pEGFP	2-4
pEGFP-1	Clontech. Note: both vectors Kan-R	2-4
CMV-EGFP		2-4
c-fms	From ATCC	2-4
pCDNA3-fms		2-4
pBluescript-PU.1 gene .65	from PU.1 cosmid	2-4
pBluescript-PU.1 gene 1.5 kb		2-4

pBlue-hFes-FLAG		2-4
hFes-FLAG	from Renee Hackenmiller	2-4
pBlue-mIL-7Ra	From Ashok Venkitaramen	2-5
pBlue-mGMCSFRa		2-5
pKW10-Pax-5	Vector from Meinrad Busslinger	2-5
pBlue-Pax5		2-5
pCR-II TOPO IL-7Rp old	original	2-5
pCR2.1-TOPO IL-7Rp new	extended sequence cloned Nov 2003	2-5
Olf-1 (Rat EBF)		2-5
pBlue-Olf-1 (Rat)		2-5
pBlue-EBF (mouse)		2-5
CMV-EBF (mouse)	Vector from Rudi Grosschedl	2-5
pCR-II TOPO EBF (T7)		2-5
pCR-II TOPO IL-7Ra endo	pcr product	2-6
pCR-II TOPO JH4	pcr product	2-6
pCR-II TOPO-Mu(o)	pcr product	2-6
pCR-II TOPO iMu	pcr product	2-6
pCR-II TOPO hprt probe	pcr product	2-6
PV.B4	probe for J558 rearrangement	2-6
pCR-II TOPO Spi-B c3	cloned murine Spi-B cDNA	2-7
pCDNA3-mSpi-B		2-7
pCDNA3-mSpi-B T670C		2-7
pCDNA3-hSpi-B	human Spi-B cDNA from Celeste Simon	2-7
pCR-II-topo-SpiC frag (probe)		2-8
pCR-II-SpiC		2-8
pBlue-Spi-C		2-8
pCDNA3-HA-Spi-C		2-8
pCR-4-topo-LoxP		2-8
pBlue-LoxP		2-8
pDNR-1-LoxP		2-8
pGFP cDNA vector	Clontech	2-9

pEGFP-1 promoter reporter vector	Clontech	2-9
BLAM(+)-pUC	Aurora Biosciences	2-9
BLAM(-)-pUC	Aurora Biosciences	2-9
IL-7Rα BAC	< 20 ng / ml, chloramphenicol resistant	2-9
IL-7Ra probe 1	DNASE HS Assay probes	
IL-7Ra probe 2	DNASE HS Assay probes	
pCR4-TOPO-IL-7Ra probe 2	DNASE HS Assay probes	
3' alpha 1.3	tgH probe for Southern-Birshtein	2-9
pCRE-ER(t)	Chambon	
pSCA-BLNKp	Cloned 1159 bp BLNK promoter!	2-9

Box 3: Luciferase Reporter Vectors

pGL3-basic		3-1
pGL3-control		3-1
pGL3-promoter	Promega	3-1
pGL3-enhancer		3-1
pRL-TK	missing	3-1
RSV-luc		3-1
pGL2-IL7Rp BsaI sense	IL-7Rp fragment HindIII - BsaI	3-3
pGL2-IL7Rp BsaI antisense		3-3
pGL3-basic-IL7Rp 1378-2495		3-3
pGL3-pro-IL7R PU.1(3x) wt	PU.1 wt binding site multimer	3-3
pGL3-basic IL-7Rp	IL-7R promoter (PU.1 site II removed)	3-3
pGL3-enh IL-7Rp		3-3
pGL3-pro SmaI Acc1-BsaI wt		3-4
pGL3-pro Bamc8 Acc1-BsaI wt		3-4
pGL3-pro Bamc9 Acc1-BsaI wt		3-4
pGL3-pro SmaI Acc1-BsaI mut		3-4
pGL3-pro Bamc8 Acc1-BsaI mut		3-4
pGL3-pro Bamc9 Acc1-BsaI mut		3-4
pGL3-I5 enhancer	from O'Riordian / Grosschedl	3-6

pGL3-pro-IL-7Rp WT c7	made fall 2001	3-7
pGL3-pro-IL-7Rp MUT c6	made fall 2001	3-7
pGL3-pro-IL-7Renh WT	made fall 2001	3-7
pGL3-pro-IL-7Renh MUT	made fall 2001	3-7
pGL3-IL-7Rp enh WT	made fall 2001	3-7
pGL3-IL-7Rp enh MUT	made fall 2001	3-7

Box 4: Vector and cDNA Box 2

	These vectors all from Francis Stewart:	4-1
irtTA-AR	Androgen receptor irtTA vector	4-1
irtTA-GR	Growth Hormone Receptor irtTA vector	4-1
Ins-Ad1-Ad2	Insulator vector - no selection	4-1
Ins-puro	puromycin selectable Insulator vector	4-1
Ins-Hygro	hygromycin selectable insulator vector	4-1
KS-tet tk-pA	tet-regulatable casette	4-1