



Standard Operating Procedure

Protocol:	ESC03	Version:	1.0
Protocol Section:	Embryonic Stem Cells	Effective Date:	01-Oct-2007
Protocol Title:	Quarterly pathogen testing of embryonic stem cell clones	Date Reviewed:	01-Oct-2007
		Date Revised:	26-Mar-2008

This standard operating procedure (SOP) was developed by the Canadian Mouse Mutant Repository (CMMR) of The Hospital for Sick Children and the Transgenic Core and Specialty Resources (TgCore) of the Toronto Centre for Phenogenomics (TCP). This SOP describes the protocol for propagation and submission of mouse embryonic stem cells (ESCs) for IMPACT pathogen testing to MU Research Animal Diagnostic Laboratory (RADIL), Columbia, Missouri.

Due to the high throughput nature of ES cell clone expansion and distribution, testing each individual clone for murine pathogens is cost prohibitive and impractical. However, it is essential to monitor ESCs propagated, distributed and/or used for the generation of chimeric mice for pathogens. Thus, quarterly testing of a random sample of ESC clones grown in the laboratory is used to monitor the health of the ESCs at the CMMR. These testing protocols ensure that clones grown from the NorCOMM (North American Conditional Mouse Mutagenesis project) resource at the CMMR are considered “approved for chimeric mouse generation” at the TgCore of the TCP without additional pathogen testing.

Related Protocols:

Protocol ESC01 describes the materials and SOPs for the cryorecovery and expansion of ESC clones.

Appendices:

- I. MU RADIL Pathogen Testing Profiles
- II. MU RADIL Sample Submission Form

Materials:

As for Protocol ESC01
 Cryovials
 Insulated shipping box
 Dry ice

Protocol:

Cell sampling:

1. Samples of ES cells should be collected every two (2) weeks or from each large-scale transfection, if these occur less frequently than bi-weekly. Samples from up to five independent clones/plates can be selected and pooled.
2. A sample identification number should be assigned to the pool, with records kept of which clones/plates served as the source of cells for the pool. Pool identification numbers should have the standard form: YYYYQ#.A, YYYYQ#.B, etc.

3. When the CMMR is growing cells from different source labs (*e.g.* CMHD or MFGC), cell pools should not be of mixed sources. In these cases, it may be necessary to sample two pools during a sample period.

Cell Passage:

4. Passage ES cells at least 3 times in media without antibiotics, expanding to have $\sim 2 \times 10^7$ cells, about two 10-cm plates. Cells can be grown on gelatin, morphology is not important at this point and medium does not need to be changed as often as for regular culture.

Cell Freezing:

5. Trypsinize the cells to release from plate. Stop trypsinization by addition of antibiotic-free ES cell media.
6. Aseptically transfer 1×10^7 cells each into two sterile centrifuge tubes.
7. Collect cells by centrifugation at $\sim 300 \times g$ for 5 minutes at room temperature. Aspirate media.
8. Aseptically re-suspend each pellet in 0.5 ml of antibiotic-free ES cell media or 1X PBS (DMSO is not necessary). Transfer cell suspension to a labeled cryovial and freeze.
9. Store in liquid nitrogen until sample submission or clean test results have been received for the associated quarter's tests.

Sample Submission:

10. Complete sample submission form with appropriate information and ensure that results are reported to Marina Gertsenstein at the TgCore of TCP.
11. Select one sample pool from mid-quarter and one sample pool from the end of the quarter.
12. Place the cryovials into conical tubes or plastic bags clearly marked with the sample ID exactly as recorded on the sample submission form.
13. On the sample submission form, specify **TCP Profile IV** for routine quarterly testing. Specify **IMPACT profile I** for testing of new lots of MEFs or expanded parental cell lines. Complete separate sample submission forms for each type of test.
14. Package cells into a box with a minimum of 2 kg of dry ice and ship by overnight express courier to:

Dr. Lela Riley,

University of Missouri Research Animal Diagnostic Laboratory

MU RADIL, 1600 E. Rollins, Columbia, MO 65211

573 884-7521 (FAX)

1-800-669-0825 (Toll Free)

573 882-5983 (Customer Service)

Additional contact in RADIL is Linda Brokamp: 573-884-9472 brokamp@missouri.edu

Appendix I: MU RADIL Pathogen Testing Profiles

Source:

<http://www.radil.missouri.edu/info/DiagTesting/services/molecularbiology.asp>

RADIL developed and validated a PCR-based alternative to MAP testing. Contamination of biological specimens, such as cell lines, hybridomas and tumor cells, with rodent pathogens can result in devastating outbreaks of disease in laboratory animals implanted with these materials and confounding and deleterious effects on tissue culture-based experiments. The traditional method for testing biological specimens for murine pathogens has been the Mouse Antibody Production (MAP) test. The major disadvantage of MAP testing is the 6 to 8 weeks required to get results. To address this issue, our laboratory has developed the **Infectious Microbe PCR Amplification Test** or **IMPACT**, which is a panel of PCR assays that detects murine pathogens. Comparison of IMPACT results with MAP testing results for representative DNA and RNA viruses indicated that the sensitivity of the IMPACT was equal to or greater than that of MAP testing. Turnaround time for IMPACT results in 5 business days. The cost of testing by the IMPACT is markedly lower than commercial MAP testing.

IMPACT Profile I (22 Agent Tests)	IMPACT Profile II (19 Agent Tests)	IMPACT Profile III (16 Agent Tests)	IMPACT Profile IV (9 Agent Tests)
<i>Mycoplasma</i> spp.	<i>Mycoplasma</i> spp.	<i>Mycoplasma</i> spp.	<i>Mycoplasma</i> spp.
Sendai virus	Sendai virus	Sendai virus	Sendai virus
Mouse hepatitis virus	Mouse hepatitis virus	Mouse hepatitis virus	Mouse hepatitis virus
Pneumonia virus of mice	Pneumonia virus of mice	Pneumonia virus of mice	Pneumonia virus of mice
Minute virus of mice	Minute virus of mice	Minute virus of mice	Minute virus of mice
Mouse parvovirus (MPV1, MPV2, MPV3)	Mouse parvovirus (MPV1, MPV2, MPV3)	Mouse parvovirus (MPV1, MPV2, MPV3)	Mouse parvovirus (MPV1, MPV2, MPV3)
Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus
Murine norovirus	Murine norovirus	Murine norovirus	TCP Profile IV (11 agent tests)
Reovirus 3	Reovirus 3	Reovirus 3	
Mouse rotavirus	Mouse rotavirus	Mouse rotavirus	
Ectromelia virus	Ectromelia virus	Ectromelia virus	
Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus	
Polyoma virus	Polyoma virus	Polyoma virus	
Lactate dehydrogenase-elevating virus	Lactate dehydrogenase-elevating virus	Lactate dehydrogenase-elevating virus	
Mouse adenovirus (MAD1, MAD2)	Mouse adenovirus (MAD1, MAD2)		
Mouse cytomegalovirus	Mouse cytomegalovirus		
K virus			
Mouse thymic virus			
Hantaan virus			

Appendix II: MU RADIL Mouse Sample Submission Form

MOLECULAR BIOLOGY SERVICES ACCESSION FORM
 University of Missouri Research Animal Diagnostic Laboratory
 http://www.radil.missouri.edu

SHIP SAMPLES TO: Dr. Lela Riley, MU RADIL, 1600 E. Rollins, Columbia, MO 65211

573 884-7521 (FAX)

800-669-0825 (Toll Free)

573 882-5983 (Customer Service)

<p>MAIL REPORT TO: NAME: Marina Gertsenstein INST/FIRM: Toronto Centre for Phenogenomics ATTN: Marina Gertsenstein, Transgenic Core ADDRESS: 25 Orde street CITY: Toronto ST: Ontario ZIP: M5T 3H7 COUNTRY: Canada PHONE #: 647-837-5811 ext. 4302 FAX #: 647-837-5834 E-MAIL: gertsenstein@mshri.on.ca marina.gertsenstein@phenogenomics.ca</p>	<p>BILL TO: NAME: INST/FIRM: ATTN: ADDRESS: CITY: ZIP: COUNTRY: PO Number: Credit Card: VISA <input type="checkbox"/> Master Card <input type="checkbox"/> Discover <input type="checkbox"/> Card #: EXP: / Check (enclosed) <input type="checkbox"/></p>
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USE A SEPARATE ACCESSION FORM FOR EACH SPECIES AND TYPE OF PROFILE/TEST(S)

SHIPPING DATE:

TOTAL # OF SAMPLES:

SPECIES: **mouse**

PROFILES AVAILABLE:

mice - IMPACT I, IMPACT II, IMPACT III, IMPACT IV; TCP IMPACT Profile IV

NUMBER SPECIMENS	SPECIMEN ID	INVESTIGATOR	TYPE OF SPECIMEN OR TISSUE	ASSAY REQUESTED
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
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_____	_____	_____	_____	_____
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HISTORY/CLINICAL SIGNS: (This information will appear on page 1 of report)
