



The University of Michigan Transgenic Animal Model Core

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Mouse Embryonic Stem (ES) Cell Training

The purpose of the class is to provide training in all aspects of ES cell culture manipulation and to provide the scientific background needed to make a gene targeted (gene knockout) mouse. You will both methods and the principles behind the methods.

The Mouse Embryonic Stem Cell Training Course is designed to instruct researchers in the art of pluripotent mouse ES cell culture and the methodologies of gene targeting. The training includes daily laboratory sessions on culture techniques. Daily seminars cover key papers in the field of gene targeting, conditional gene knockouts, and genetic background (see bibliography). Trainees are expected to read papers, participate in discussions, and present at least one seminar. Special topics specific to trainee research are welcome. The course will take place in room 2578 Medical Science Research Building II, and will last two weeks. In order to provide as much hands-on experience as possible, ES cell training class size is limited to four or fewer trainees.

A. Purpose: To instruct personnel in the art of ES cell culture and gene targeting protocols.

B. Training Overview: Discussion and lab experience in ES cell culture methods and techniques. This includes: setting up a laboratory for stem cell work, evaluation of fetal bovine serum lots for ES cell culture, mycoplasma testing, media preparation, train eye for stem cell differentiation, normal ES culture, preparation of mouse embryonic fibroblast (MEF) for co-culture, freezing and thawing of ES cells, ES cell electroporation with DNA, ES cell clone picking, cryopreservation and recovery of ES cell clones in 96-well plates, DNA preparation from cells cultured on 96 well plates, preparation of chromosome spreads from ES cells and chromosome counting.

C. Preparation:

C1. Highly Recommended:

Gene Targeting: A Practical Approach. 2000. Joyner AL, ed. Oxford University Press; ISBN: 019963792X. Read the entire book.

Or

“Advanced Protocols for Animal Transgenesis: An ISTT Manual.” 2011. Pease S and Saunders TL, eds. Springer. ISBN 364220791X. Read Chapters 3, 4, 14, and 15.

C2. Optional: Read Chapters 8, 9, and 10 in "Manipulating the Mouse Embryo: A Laboratory Manual." 3rd ed. 2003. Nagy, Gertsenstein, Vintersten, and Behringer. Cold Spring Harbor Laboratory Press. pp. 359-451.

C3. Optional: read Chapters 49 through 53 in "Guide to Techniques in Mouse Development." 1993. Wassarman and DePamphilis. Methods in Enzymology, Volume 225:803-900.

C4. Novices in tissue culture are expected to read "Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications." 6th ed. 2010. Freshney. Wiley-Blackwell. We expect that trainees will be familiar with basic tissue culture procedures.

D. Example Schedule (subject to revision)

Week One

- Mon. Introduction, watch MEF preparation, set up laboratory, thaw ES cells. Media preparation.
- Tue. Watch Passaging of ES cells, feed ES cells.
- Wed. Passage ES cells, set up 96-well plates and dishes for colonies.
- Thu. Investigator passages, thaws MEFs.
- Fri. Investigator passages, watch training video, passage and freeze 96-well plate
- Sat. Renew
- Sun. Passage ES cells, set up for chromosome preparation.

Week Two

- Mon. Renew ES cells. MEF prep. Chromosome prep.
- Tue. Passage ES cells. Freeze ES cells. Analyze chromosomes. Thaw 96-well plate.
- Wed. Test thaw ES cells. Start DNA prep.
- Thu. Finish DNA prep. Clone picking.
- Fri. Evaluate test thaw, 96-well plate thaw, clones. Lab clean-up.

E. ES Culture Training Follow-up: This consists of the ability of the trainee to produce gene-targeted germline competent embryonic stem cells. This is beyond the scope of a two-week training session. Please let us know your results as you proceed in your project.

Sample Bibliography: Papers Discussed Subject to Revision
Special Topic Suggestions Welcomed

Class Reading List

As part of the ES class, you will receive a course packet when you arrive for class containing references and protocols. We will discuss a number of relevant papers, please read them in advance. You will be assigned to present the papers for one of the days below. If you have interest in a special topic, please let us know. If you have any questions, comments or concerns please feel free to contact us. We will have some books available for you on the shelf during the class. Please sign them out if you remove them from the lab. We look forward to seeing you Monday morning at 8:30am, in the Stem Cell Lab (Room 2578, MSRB II).

Discussion Papers:

Day 1: Overview of Gene Targeting

Capecchi MR. 2005. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet.* 6:507-512.

Capecchi MR. 1994. Targeted gene replacement. *Sci Am.*270:52-59.

Day 2: ES Cell Biology

Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC.1993. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc Natl Acad Sci U S A.* 1993 Sep 15;90(18):8424-8.

Fedorov LM, Haegel-Kronenberger H, Hirchenhain J. A comparison of the germline potential of differently aged ES cell lines and their transfected descendants. *Transgenic Res.* 1997 May;6(3):223-31.

Longo L, Bygrave A, Grosveld FG, Pandolfi PP. 1997. The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism. *Transgenic Research* 6:321-328.

Rebuzzini P, Neri T, Mazzini G, Zuccotti M, Redi CA, Garagna S. 2008. Karyotype analysis of the euploid cell population of a mouse embryonic stem cell line revealed a high incidence of chromosome abnormalities that varied during culture. *Cytogenet Genome Res.* 121:18-24.

Sugawara A, Goto K, Sotomaru Y, Sofuni T, Ito T. 2006. Current status of chromosomal abnormalities in mouse embryonic stem cell lines used in Japan. *Comp Med.* Feb;56(1):31-4.

Day 3: Isogenic Gene Targeting

Zhou L, Rowley DL, Mi QS, Sefcovic N, Matthes HW, Kieffer BL, Donovan DM. Murine inter-strain polymorphisms alter gene targeting frequencies at the mu opioid receptor locus in embryonic stem cells. *Mamm Genome.* 2001 Oct;12(10):772-8.

te Riele, H, Maandag, ER, Berns, A. 1992. Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. *Proc. Natl. Acad. Sci. U.S.A.* 89:5128-5132.

Van Deursen J, Wieringa B. 1992. Targeting of the creatine kinase M gene in embryonic stem cells using isogenic and nonisogenic vectors. *Nuc. Acids Res.* 20(15):3815-3820.

Day 4: Genetic Polymorphisms of 129 Mice and ES Cells

Simpson EM, Linder CC, Sargent EE, Davisson MT, Mobraaten LE, Sharp JJ. 1997. Genetic variation among 129 substrains and its importance for targeted mutagenesis in *Nat Genet.* 16:19-27.

Threadgill DW, Yee D, Matin A, Nadeau JH, Magnuson T. 1997. Genealogy of the 129 inbred strains: 129/SvJ is a contaminated inbred strain. *Mamm Genome.* 8:390-3.

Jax Bulletin, no.1. New 129-Nomenclature – revised. June 1999; revised June 2001.

Day 5: C57BL/6 ES Cell Lines

Hughes ED, Qu YY, Genik SJ, Lyons RH, Pacheco CD, Lieberman AP, Samuelson LC, Nasonkin IO, Camper SA, Van Keuren ML, Saunders TL. 2007. Genetic variation in C57BL/6 ES cell lines and genetic instability in the Bruce4 C57BL/6 ES cell line. *Mamm Genome.* 18:549-558.

Pettitt SJ, Liang Q, Rairdan XY, Moran JL, Prosser HM, Beier DR, Lloyd KC, Bradley A, Skarnes WC. 2009. Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat Methods.* 6:493-5.

Poueymirou WT, Auerbach W, Friendewey D, Hickey JF, Escaravage JM, Esau L, Doré AT, Stevens S, Adams NC, Dominguez MG, Gale NW, Yancopoulos GD, DeChiara TM, Valenzuela DM. 2007. F0 generation mice fully derived from gene-targeted embryonic stem cells allowing immediate phenotypic analyses. *Nat Biotechnol.* 25:91-99.

Poueymirou et al. (ibid) Supplemental Figure 1 and Tables.

Schuster-Gossler K, Lee AW, Lerner CP, Parker HJ, Dyer VW, Scott VE, Gossler A, Conover JC. 2001. Use of coisogenic host blastocysts for efficient establishment of germline chimeras with C57BL/6J ES cell lines. *Biotechniques.* 31:1022-4, 1026.

Day 8: Breeding Strategies for ES Cell-Mouse Chimeras

Mutant mice and neuroscience: recommendations concerning genetic background. Banbury Conference on genetic background in mice. 1997. 19:755-9.

Markel P, Shu P, Ebeling C, Carlson GA, Nagle DL, Smutko JS, Moore KJ. Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nat Genet.* 1997 Nov;17(3):280-4.

Hoffert JD, Pisitkun T, Miller RL. 2011. Conditional Allele Mouse Planner (CAMP): software to facilitate the planning and design of breeding strategies involving mice with conditional alleles. *Transgenic Res.* 2011 Aug

Day 9: Introduction to Cre/LoxP and FLP/FRT

Nagy A. Cre recombinase: the universal reagent for genome tailoring. *Genesis.* 2000 Feb;26(2):99-109.

Soriano P. 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet.* 21:70-1.

Kranz A, Fu J, Duerschke K, Weidlich S, Naumann R, Stewart AF, Anastassiadis K. 2010. An improved Flp deleter mouse in C57Bl/6 based on Flpo recombinase. *Genesis.* 48:512-520.

Day 10: International Knockout Mouse Consortium: Gene Targeting Strategies

Schnutgen F, De-Zolt S, Van Sloun P, Hollatz M, Floss T, Hansen J, Altschmied J, Seisenberger C, Ghyselinck NB, Ruiz P, Chambon P, Wurst W, von Melchner H. 2005. Genomewide production of multipurpose alleles for the functional analysis of the mouse genome. *Proc Natl Acad Sci U S A.* 102:7221-6.

Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A. 2011. A conditional knockout resource for the genome-wide study of mouse gene function. *Nature.* 474:337-342.

Singla V, Hunkapiller J, Santos N, Seol AD, Norman AR, Wakenight P, Skarnes WC, Reiter JF. 2010. Floxin, a resource for genetically engineering mouse ESCs. *Nat Methods.* 7:50-52.

Osterwalder M, Galli A, Rosen B, Skarnes WC, Zeller R, Lopez-Rios J. 2010. Dual RMCE for efficient re-engineering of mouse mutant alleles. *Nat Methods.* 7:893-895.

Day 11. Gene Targeting Pitfalls in Genetics, ES Cells, and Mice

Gajovic S, Mitrecic D, Augustincic L, Iaconig A, Muro AF. 2006. Unexpected rescue of alpha-synuclein and multimerin1 deletion in C57BL/6J OlaHsd mice by beta-adducin knockout. *Transgenic Res.* 15:255-9.

Kumar RA, Chan KL, Wong AH, Little KQ, Rajcan-Separovic E, Abrahams BS, Simpson EM. 2004. Unexpected embryonic stem (ES) cell mutations represent a concern in gene targeting: lessons from "fierce" mice. *Genesis.* 38:51-7.

Nagy A, Moens C, Ivanyi E, Pawling J, Gertsenstein M, Hadjantonakis AK, Purity M, Rossant J. 1998. Dissecting the role of N-myc in development using a single targeting vector to generate a series of alleles. *Curr Biol.* 1998 May 21;8(11):661-4.

Scacheri PC, Crabtree JS, Novotny EA, Garrett-Beal L, Chen A, Edgemon KA, Marx SJ, Spiegel AM, Chandrasekharappa SC, Collins FS. 2001. Bidirectional transcriptional activity of PGK-neomycin and unexpected embryonic lethality in heterozygote chimeric knockout mice. *Genesis.* 30:259-63.

Schulz TJ, Glaubitz M, Kuhl D, Thierbach R, Birringer M, Steinberg P, Pfeiffer AF, Ristow M. 2007. Variable expression of Cre recombinase transgenes precludes reliable prediction of tissue-specific gene disruption by tail-biopsy genotyping. *PLoS One.* 2:e1013.

Day 12. Nucleases and Genome Editing

Cui X, Ji D, Fisher DA, Wu Y, Briner DM, Weinstein EJ. 2011. Targeted integration in rat and mouse embryos with zinc-finger nucleases. *Nat Biotechnol.* 29:64-67.

Meyer M, de Angelis MH, Wurst W, Kühn R. 2010. Gene targeting by homologous recombination in mouse zygotes mediated by zinc-finger nucleases. *Proc Natl Acad Sci U S A.* 107:15022-15026.

de Souza, N. 2012. Primer: genome editing with engineered nucleases. *Nature Methods* 9, 27/

Boch J. 2011. TALEs of genome targeting. *Nature Biotechnology.* 29: 135–136

Supplemental Reading:

Adams DJ, van der Weyden L. 2008. Contemporary approaches for modifying the mouse genome. *Physiol*

Genomics. 34:225-238.

Branda CS, Dymecki SM. 2004. Talking about a revolution: The impact of site-specific recombinases on genetic analyses in mice. *Dev Cell*. 6:7-28.

Camper SA, Saunders TL, Kendall SK, Keri RA, Seasholtz AF, Gordon DF, Birkmeier TS, Keegan CE, Karolyi IJ, Roller ML. 1995. Implementing transgenic and embryonic stem cell technology to study gene expression, cell-cell interactions and gene function. *Biol Reprod* 52:246-57.

Carlson CM, Largaespada DA. 2005. Insertional mutagenesis in mice: new perspectives and tools. *Nat Rev Genet*. 6:568-80.

Copeland NG, Jenkins NA, Court DL. 2001. Recombineering: a powerful new tool for mouse functional genomics. *Nat Rev Genet*. 2:769-79.

Culture of Animal Cells: A Manual of Basic Technique. Freshney, RI. 1994. Wiley-Liss. New York.
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Embryonal Stem Cells: Introducing Planned Changes into the Animal Germline. Hooper, ML. 1992. Harwood Academic Publishers. Philadelphia.
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Farley FW, Soriano P, Steffen LS, Dymecki SM. 2000. Widespread recombinase expression using FLP_{eR} (Flipper) mice. *Genesis* 28:106-110.

Fiering S, Epner E, Robinson K, Zhuang Y, Telling A, Hu M, Martin DIK, Enver T, Ley TJ, Groudine M. 1995. Targeted deletion of 5'HS2 of the murine beta-globin LCR reveals that it is not essential for proper regulation of the beta-globin locus. *Genes and Development* 9:2203-2213.

Guide to Techniques in Mouse Development. Wassarman, PM, DePamphilis, MR, eds. *Methods in Enzymology*, vol. 225. Academic Press, New York.
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Manipulating the Mouse Embryo: A Laboratory Manual. Nagy, A, Gertsenstein, M, Vintersten, K, Behringer, R. 2003. Cold Spring Harbor Press. New York.

Mouse Genetics: Concepts and Applications. Silver, LM. 1995. Oxford University Press. New York.
SCIENCE Book Stacks - QH 432 .S561 1995
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Teratocarcinomas and Embryonic Stem Cells; A Practical Approach. Robertson EJ, ed., IRL Press at Oxford University Press, 1987.

Lobe CG, Koop KE, Kreppner W, Lomeli H, Gertsenstein M, Nagy A. Z/AP, a double reporter for cre-mediated recombination. *Dev Biol*. 1999 Apr 15;208(2):281-92.

Novak A, Guo C, Yang W, Nagy A, Lobe CG. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis*. 2000 Nov-Dec;28(3-4):147-55.

Olson EN, Arnold HH, Rigby PW, Wold BJ. Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene MRF4. *Cell*. 1996 Apr 5;85(1):1-4.

O'Shea KS. 2004. Self-renewal vs. differentiation of mouse embryonic stem cells. *Biol Reprod.*71:1755-65.

Vasquez KM, Marburger K, Intody Z, Wilson JH. Manipulating the mammalian genome by homologous recombination. *Proc Natl Acad Sci U S A.* 2001 Jul 17;98(15):8403-10.

Wolfer DP, Lipp HP. 2000. Dissecting the behavior of transgenic mice: is it the mutation, the genetic background, or the environment? *Exp Physiol.* 85:627-34.