

Index

A

Adam22, 193

B

BAC. *See* Bacterial artificial chromosome

Backcrossing, congenic mouse strain
generation

backcross, 85

materials, 83–84

number of backcrosses, 85

outcross, 84

overview, 83–84

troubleshooting, 85–86

Bacterial artificial chromosome (BAC),
253–254

Balancer chromosome, maintaining
recessive lethal or sterile
mutations, 79–80

Biological safety, 289

Blastocyst. *See* Chimera; Preimplantation
lethality phenotypic analysis

Bone. *See* Skeleton

Books. *See* Resources

Brachyury, 217

Brca1, 245

Breeding. *See also* Mutant colony
maintenance

backcrossing. *See* Backcrossing,
congenic mouse strain
generation

CRISPR-Cas recovering targeted
mutations breeding schemes

indel mutations, 59–60

knock-in mutations, 60

missense mutations, 60

overview, 59

homologous recombination recovering
targeted mutations breeding
schemes

coat color, 57–58

male versus female chimeras, 56

strain selection, 56–57

test breed chimeras, 57, 59

inbreeding depression, 76

mutation analysis

complementation testing, 88–89

genetic interaction testing

biochemical pathway compo-
nents, 90–92

interpretation, 90

overlapping expression
patterns, 92

phenotypes caused by
downstream gene

overexpression, 92–93

rationale, 89

redundancy and compensation,
90–91

similar phenotypes caused by
unrelated genes, 92

homozygous mutant frequency
enhancement, 93–94

hypomorphic allele testing, 89

overview, 87–88

C

Cannibalism, prevention, 47, 53–54

Cardiovascular defects

embryonic phenotypes lethal after E9.5,
150–152

perinatal lethality phenotypic analysis,
178, 180

Cer1, 193

Chi square test, Mendelian segregation,
97–98, 101, 242

Chimera

blastocyst injection for
lineage-restricted chimeras, 240

cell distribution due to mutant gene
effect, 233, 235

checklist for experiments, 234

composition control techniques, 237

developmental potential

limitations, 237

phenotypic analysis, 170

dominant mutation effects in

embryonic stem cell

chimeras, 221–224

embryonic stem chimera production
corpora lutea counting to determine

ovulated oocyte number,
50–52

flow chart, 41

overview, 41–42

planning, 45–46

troubleshooting

no mice born after chimeric

embryo transfer, 46–47

pups born with no or low

chimerism, 47–48

fluorescent protein transgenes for

distinguishing cells, 233, 236,
240–242

generation strategies, 232–233

genotyping, 242–243

homologous recombination recovering
targeted mutations breeding
schemes

coat color, 57–58

male versus female chimeras, 56

test breed chimeras, 57, 59

intersex chimeras, 61

lethal phenotype circumventing,
231–234

morula aggregation

lineage-restricted chimeras

embryonic stem cell aggregation,
238

trophoblastic stem cell

aggregation, 238–239

mix of cells in all tissues, 235,
237–238

tetraploid morula aggregation

diploid embryonic stem cell,
239–240

diploid morula, 239

transgene rescue, 222

Cited2, 162

CL. *See* Corpora lutea

Cleavage phenotypic analysis. *See*
Preimplantation lethality
phenotypic analysis

Cleft palate, perinatal lethality phenotypic
analysis, 180, 182

Coat color

chimera test-breeding programs, 57–58

common markers, 63

Complementation, testing, 88–89

Conditional gain-of-function allele

overview, 245–246

recombinase mouse strains, 247–248

Conditional null allele

characterization of mouse lines

inducible mouse lines, 249–250

tissue-specific expression, 248–249

CRISPR-Cas generation, 36–38

homologous recombination generation,
34–36

indications for use, 5, 246

principles for generation, 33–35

recombinase mouse strains, 247–248

Index

- Conditional null allele (*Continued*)
 testing, 246–247
 use and analysis, 251–253
- Confocal microscopy, three-dimensional
 imaging of embryos,
 160–162
- Congenic strain. *See* Backcrossing, con-
 genic mouse strain generation
- Corpora lutea (CL), counting to determine
 ovulated oocyte number,
 50–52
- Cranial nerve defects, perinatal lethality
 phenotypic analysis, 179,
 181–182
- Crb1*, 206
- Cre/loxP* system, 23–25, 33–34, 78–79,
 89, 93–94, 246, 248–253,
 255
- CRISPR-Cas
 conditional null allele generation,
 36–38
 embryonic stem cells, 3, 7, 14, 22, 44
 fluorescent protein tagging, 118
 indications for mutant generation, 13
 mosaics, 219
 overview, 1–2
 preimplantation embryos, 3, 7, 14, 26
 principles
 knock-in allele generation, 15,
 31–32
 null allele generation, 14, 27–28, 30
 point allele generation, 15, 32–33
 recovering targeted mutations
 breeding schemes
 indel mutations, 59–60
 knock-in mutations, 60
 missense mutations, 60
 overview, 59
 mutant allele transmission
 troubleshooting
 gene targeting construct design,
 62–63
 impatience, 62
 lost potential of embryonic stem
 cells, 63–64
 phenotype issues, 64
 no offspring troubleshooting
 dominant sex reversal, 62
 infertility, 60–61
 intersex chimeras, 61
 overview, 55–56
 sexing of mice
 genotyping by polymerase chain
 reaction, 69–70
 morphology, 65–66
 single sex offspring troubleshooting
 dominant effect on
 gametogenesis, 67
 dominant mutation, 67
 dominant sex reversal, 68
 sex-limited dominant effect, 67
 wild-type allele instead of mutant
 recovery troubleshooting
 haploinsufficiency, 65, 67
 technical glitches, 64–65
 segregation of alleles from founders, 26
- Cross-fostering, newborn mice, 53–54
- Crybb*, 206
- D**
- Developmental delay, perinatal lethality
 phenotypic analysis,
 178–179, 181
- Developmental potential phenotypic
 analysis
 cell line establishment from organ
 culture, 169–170
 embryo culture, 166–167
 organ culture
 cell marking, 168–169
 examples, 167
 technique, 167–168
 overview, 165–166
 teratoma differentiation, 171–172
 testing in chimeras, 170
 transplantation of cells and organs, 171
- Diaphragm defects, perinatal lethality
 phenotypic analysis, 181
- Dominant mutant phenotypic analysis
 chimera dominant effects, 221–224
 embryonic stem cell dominant effects,
 220–221
 heterozygous mice and dominant
 effects
 development, 224
 morphology, 224
 reproduction, 224–225
 imprinted gene considerations,
 226–227
 mechanisms of dominant effects,
 218–220
 overview, 217–218
 transgene rescue, 223
 XO subclone isolation from XY
 embryonic stem cells,
 228–229
- Downs and Davies staging, 131
- Dre/rox* system, 23, 25, 246
- Drgl1*, 207
- E**
- Edinburgh Mouse Atlas Project (eMAP),
 161
- EFIC. *See* Episcopic fluorescence image
 capture
- Egfr*, 210
- Electroretinogram (ERG), 206
- eMAP. *See* Edinburgh Mouse Atlas Project
- Embryo culture. *See* Developmental
 potential phenotypic analysis;
 Preimplantation lethality
 phenotypic analysis
- Embryonic stem (ES) cell
 cell line establishment from organ
 culture, 169–170
 chimera production
 corpora lutea counting to determine
 ovulated oocyte number,
 50–52
 flow chart, 41
 overview, 41–42
 planning, 45–46
 troubleshooting
 no mice born after chimeric
 embryo transfer, 46–47
 pups born with no or low
 chimerism, 47–48
- chimeras. *See* Chimera
- clonal line isolation
 expansion and freezing
 number of cells required, 44
 number of clones required, 44
 troubleshooting
 death of cells, 43
 phenotype resulting in death,
 43–44
 selectable marker problems, 43
 targeting construct problems, 43
- lost potential in mutant allele
 transmission, 63–64
- mistakes in gene-targeting experiments,
 42
- morula aggregation. *See* Chimera
- recovering targeted mutations
 breeding schemes
 male versus female chimeras, 56
 strain selection, 56–57
 test breed chimeras, 57, 59
 coat color, 57–58
- mutant allele transmission
 troubleshooting
 gene targeting construct design,
 62–63
 impatience, 62
 lost potential of embryonic stem
 cells, 63–64
 phenotype issues, 64
- no offspring troubleshooting
 dominant sex reversal, 62
 infertility, 60–61
 intersex chimeras, 61
 overview, 55–56
 sexing of mice
 genotyping by polymerase chain
 reaction, 69–70

- morphology, 65–66
single sex offspring troubleshooting
 dominant effect on
 gametogenesis, 67
 dominant mutation, 67
 dominant sex reversal, 68
 sex-limited dominant effect, 67
wild-type allele instead of mutant
 recovery troubleshooting
 haploinsufficiency, 65, 67
 technical glitches, 64–65
techniques for mutagenesis. *See specific techniques*
transgene rescue of mutants, 222
XO subclone isolation from XY
 embryonic stem cells,
 228–229
- En2*, 190–191, 209
ENU. *See* Ethylnitrosurea
Epifluorescence microscopy,
 three-dimensional imaging of
 embryos, 160–162
Episcopic fluorescence image capture
 (EFIC), three-dimensional
 imaging of embryos,
 160, 162
ERG. *See* Electroretinogram
ES. *See* Embryonic stem
EST. *See* Expressed sequence tag
Estrous female, selection, 61
Ethylnitrosurea (ENU), mutagenesis
 embryonic stem cells, 9
 expression pattern evaluation, 19
 frequency, 8
 gene evaluation before mutagenesis,
 17–19, 22
 male spermatogenesis, 8
 overview, 1, 8
 test crosses for recessive mutations,
 9–10
EUCOMM. *See* European Conditional
 Mouse Mutagenesis Program
European Conditional Mouse
 Mutagenesis Program
 (EUCOMM), 12
Expressed sequence tag (EST),
 databases, 19
Extraembryonic endoderm (XEN)
 stem cell, culture,
 169–170
- F**
F10, 183
Fgf8, 251
Fgf10, 159
flox, 252
Flp/*FRT* system, 23–25, 78–79, 89, 246,
 248, 251
- Folliculogenesis, stages, 197
Foxn1, 190–191
- G**
Gait analysis, 209
Gene Expression Omnibus (GEO), 19
Genetic interaction testing. *See* Breeding
Gene trap, insertional mutagenesis, 11
Genotyping
 chimeras, 242–243
 errors, 202
 prenatal lethality timing, 99–100
 sexing by polymerase chain reaction,
 69–70
GEO. *See* Gene Expression Omnibus
Glossary of terms, 273–283
Gnrhr, 195–196
Grip strength test, 208
- H**
Hearing, testing, 207
Hematopoietic defects, embryonic
 phenotypes lethal after E9.5,
 148–150
Homologous recombination
 conditional null allele generation, 34–36
 elements for idealized targeting
 construct, 23
 indications for mutant generation, 13
 knock-in allele generation, 30–31
 null allele generation, 27
 point mutation generation, 32
 principles for gene targeting in
 embryonic stem cells, 13–14,
 22–23
 random integration negative selection, 25
 screening, 25–26
 vector incorporation positive selection,
 24–25
Hot plate test, 207
Hoxa1, 179
Hprt, 201
HSV *tk*, toxicity of expression in males, 25,
 62–63
Husbandry. *See* Mutant colony
 maintenance
Hypomorphic allele, testing, 89
- I**
ICM. *See* Inner cell mass
ICSI. *See* ICSI
IKK2, 250
IKMC. *See* International Knockout Mouse
 Consortium
Il1r1, 204
IMPC. *See* International Mouse
 Phenotyping Consortium
- Imprinting
 dominant mutant phenotypic analysis
 considerations, 226–227
 effect on embryo, 16
 heredity of autosomal mutant gene, 18, 219
 X-linked mutant gene, 17
Inbreeding depression, 76
Incisor, growth, 194–195
Induced pluripotent stem (iPS) cell,
 culture, 169–170
Infertility
 assisted reproduction
 females, 81–82
 males, 81
 overview, 79, 81
 female, 198–199
 male, 194–198
 no offspring troubleshooting, 60–61
Inner cell mass (ICM), 108, 114–115, 125,
 129, 239, 242
Internal ribosome entry site (IRES), 29
International Mouse Phenotyping Consor-
 tium (IMPC), 2–3, 12, 33
International Knockout Mouse
 Consortium (IKMC), 3, 12,
 45, 57, 72
Intracytoplasmic sperm injection (ICSI),
 81–82
In vitro fertilization (IVF), 81–82, 204
iPS cell. *See* Induced pluripotent stem cell
IRES. *See* Internal ribosome entry site
IVF. *See* In vitro fertilization
- J**
Jag1, 130
- K**
Kit, 225
Knock-in allele
 characterization of mouse lines, 248–251
 CRISPR-Cas generation, 15, 31–32, 60
 general strategies, 28–30
 homologous recombination generation,
 30–31
Knockout Mouse Project (KOMP), 12, 19
KOMP. *See* Knockout Mouse Project
Krt5, 178
- L**
Lethality
 analysis. *See* Phenotypic analysis;
 Preimplantation lethality
 phenotypic analysis;
 Preimplantation to mid-
 gestation phenotypic analysis;
 Prenatal lethality timing
 mutations, 3

Index

Lhx1, 177, 253*Lif*, 193

M

Mbn1, 161*Mbn2*, 161MBP. *See* Myelin basic protein*Mdm1*, 117*Mdm2*, 117MGI. *See* Mouse Genome Informatics

Micro computed tomography, perinatal

lethality defects, 178,

180–181

MMRRC. *See* Mutant Mouse Resource and

Research Centers

Molecular Embryology of the Mouse

course, 2

Morula aggregation. *See* Chimera

Mouse Genome Informatics (MGI),

12–13, 19

Msx1, 180

Mutant colony maintenance

backcrossing for congenic mouse strain

generation

backcross, 85

materials, 83–84

number of backcrosses, 85

outcross, 84

overview, 83–84

troubleshooting, 85–86

balancer chromosomes for maintaining

recessive lethal or sterile

mutations, 79–80

breeding, 76–77

facilities, 72–74

goals, 75–77

inbreeding depression, 76

infertility and assisted reproduction

females, 81–82

males, 81

overview, 79, 81

overview, 71–72

resources for husbandry, 257–258

selection cassette deletion in vivo,

77–79

strain effects on phenotype, 74–75

Mutant generation

ethylnitrosurea mutagenesis, 1, 8–10

existing mutant resources, 12–13

flow chart, 4

gene targeting. *See* CRISPR-Cas;

Homologous recombination

insertional mutagenesis

gene traps, 11

principles, 10–11

transgenes, 11

transposons, 11–12

viruses, 11

overview, 7–8

predictions and expectations, 16

spontaneous mutation, 8

transgenic mice, 12

X-ray mutagenesis, 10

Mutant Mouse Resource and Research

Center (MMRRC), 73–74

Myelin basic protein (MBP), 193

Myelin proteolipid protein (PLP), 193

Myf5, 210*Myo15*, 253*MyoD*, 210

N

Naming

mouse nomenclature resources, 262

novel mutant alleles, 72

Nociception, testing, 207

Notch2, 204

No visible phenotype analysis

age-related pathologies

skeleton, 204–205

tumor formation, 203–204

balance and coordination testing

gait analysis, 209

grip strength test, 208

rotarod test, 209

swim test, 208–209

tail suspension test, 208

causes

functional mutant, 202

genotyping error, 202

no overt abnormality, 203

variable penetrance phenotype,

202–203

environmental challenges, 211

genetic challenge experiments,

209–211

overview, 201–202

sense testing

hearing, 207

nociception, 207

smell, 207

taste, 207

touch, 207

vision, 205–206

transgenic strain crosses, 211

Null allele

conditional allele. *See* Conditional null

allele

CRISPR-Cas generation, 14, 27–28, 30

general strategies, 26–27

homologous recombination, 27

O

OCT. *See* Optical coherence tomographyOPT. *See* Optical projection tomography

Optical coherence tomography (OCT)

retina, 206

three-dimensional imaging of embryos,
160–162

Optical projection tomography (OPT),

three-dimensional imaging of
embryos, 160–162Organ culture. *See* Developmental

potential phenotypic analysis

Ovca1, 178, 182

P

Pancreas, detection in newborn mice, 189

Pde6b, 206

Perinatal lethality phenotypic analysis

causes of death at birth

cardiovascular defects, 178, 180

catastrophic abnormalities, 177–178

cleft palate, 180, 182

cranial nerve defects, 179, 181–182

developmental delay, 178–179, 181

diaphragm defects, 181

miscellaneous causes, 181, 183

skeletal defects, 179, 181

overview, 175–176

time of death determination, 176–177

Phenotypic analysis

chimeras for early lethal phenotypes,

231–234

developmental potential. *See*

Developmental potential

phenotypic analysis

dominant mutants. *See* Dominant

mutant phenotypic analysis

examples by book chapter, 265–269

flow chart, 4

gonadal mutants, 196

gross anatomical assessment, 186–187

growth curves, 190

infertility

female, 198–199

male, 194–198

lethality. *See* Chimera; Perinatal

lethality phenotypic analysis;

Preimplantation lethality

phenotypic analysis;

Preimplantation to

mid-gestation phenotypic

analysis; Prenatal lethality

timing

mammary glands, 188

mid- to late-gestation phenotypes

cell proliferation and death analysis,

159

embryonic phenotypes lethal after

E9.5

cardiovascular defects, 150–152

hematopoietic defects, 148–150

placental insufficiency, 152–155

histological analysis, 155, 157

- imaging of embryos, 159–162
 - molecular characterization, 158–159
 - morphological analysis, 155, 157
 - overview, 147–148
 - sexing of embryos, 156–157
 - skeletogenesis analysis, 157–158
 - morphological abnormalities, 190–191
 - motor dysfunction, 192–194
 - neurological problems, 191–192
 - no phenotype challenge. *See* No visible phenotype analysis
 - overview, 15–16
 - pups
 - growth and vigor abnormalities, 194
 - lethality before weaning, 189
 - marking, 186
 - overview, 185–186
 - visible phenotypes before weaning, 186, 188
 - reproductive organs, 188
 - resources, 261–262
 - Pitx3*, 205
 - Placental insufficiency, embryonic
 - phenotypes lethal after E9.5, 152–155
 - PLP. *See* Myelin proteolipid protein
 - Plxnd1*, 161
 - Point mutation
 - CRISPR-Cas generation, 15, 32–33
 - homologous recombination generation, 32
 - Preimplantation lethality phenotypic analysis
 - blastocyst and implantation analysis
 - failure to attach, 113
 - failure to hatch, 112–113
 - failure to outgrow, 113–114
 - implantation delay analysis, 115–116
 - inner cell mass analysis, 114–115
 - zona pellucida removal, 111–113
 - cell counting in embryos using fluorescent dyes
 - differential inner cell mass/trophoectoderm cell counts, 125–126
 - materials, 124, 126
 - overview, 123
 - total cell count, 124–126
 - troubleshooting, 125–126
- cleavage stages, 108
- early stage analysis
 - blastocyst morphology
 - abnormalities, 111
 - cleavage arrest or delay, 110–111
 - compaction abnormalities, 111
 - embryo culture
 - culture, 121–122
 - materials, 120–122
 - overview, 109–110, 120
- troubleshooting, 122
- no mutants, 110
- gene expression in whole embryos, 117–118
- immunocytochemistry, 117
- in situ hybridization, 117
- overview, 107–109
- superovulation, 116
- Preimplantation to mid-gestation phenotypic analysis
 - gastrulation to allantoic fusion lethality (E6.5–E9.5)
 - cell death measurement, 139–140
 - cell proliferation measurement, 138–139
 - embryo staging, 133–134
 - gross morphology, 130–133
 - histological assessment, 135–136
 - molecular characterization
 - cell type-specific gene expression, 137–138
 - strategy, 136
 - overview, 130
 - photodocumentation of embryos, 134–135
 - histological sectioning of embryos
 - dissected embryos, 144–146
 - fixative recipes, 146
 - implanted embryos, 143–144
 - materials, 143
 - overview, 142
 - overview, 127–128
 - preimplantation death (E4.5–E5.5), 128–130
- Prenatal lethality timing
 - control frequency of loss establishment, 104–106
 - development stages, 98
 - developmental landmarks between E4.5 and term, 101–103
 - dissection, 99–101
 - genotyping, 99–100
 - homozygous mutant detection at birth, 96
 - overview, 95–96
- Pup phenotyping. *See* Phenotypic analysis
- ## R
- Radioactive safety, 288
 - rd1*, 206
 - Resources
 - development, 258–259
 - essentials, 257
 - existing mouse mutants, 12–13
 - husbandry, 257–258
 - methods, 259–261
 - phenotypic analysis, 261–262
 - safety information, 285
 - web-based resources, 262–263
 - ROSA26*, 248
 - Rotarod test, 209
 - RU-486, 250
- ## S
- Safety
 - biological safety, 289
 - general cautions, 286–287
 - hazardous chemical properties, 289–290
 - radioactive safety, 288
 - resources, 285
 - waste disposal, 287–288
 - Selection cassette
 - deletion in vivo, 77–79
 - removal from embryonic stem cells in vitro, 24
 - Sex determination
 - genotyping by polymerase chain reaction, 69–70, 156
 - morphology, 65–66, 157
 - SHIRPA protocol, 192
 - Skeleton
 - age-related pathologies, 204–205
 - perinatal lethality phenotypic analysis, 179, 181
 - phenotypic analysis, 157–158
 - Smell, testing, 207
 - Soft mouse chow, 194
 - Sp7*, 179
 - Specific pathogen-free (SPF) facility, 72–73
 - Sperm
 - haploid problem circumvention, 65
 - spermatogenesis
 - ethylNitrosourea mutagenesis, 8
 - stages, 197
 - SPF facility. *See* Specific pathogen-free facility
 - Strain
 - compatibility and chimera production from embryonic stem cells, 48
 - effects on mutant phenotype, 74–75
 - Swim test, 208–209
 - Sys*, 197
- ## T
- Tail flick test, 207
 - Tail suspension test, 208
 - Tamoxifen, 250
 - Taste, testing, 207
 - Tbx1*, 182, 225
 - Tbx4*, 242, 245
 - Tbx6*, 88
 - Teratoma. *See* Developmental potential phenotypic analysis
 - Texas A&M Institute for Genomic Medicine (TIGM), 12
 - TIGM. *See* Texas A&M Institute for Genomic Medicine

Index

- Tissue-specific knockout,
regulatory mechanisms,
254–255
- Tk* mutant, 191
- Touch, testing, 207
- Transgene rescue
chimeras, 222
dominant mutant phenotypic analysis,
223
embryonic stem cell mutants, 222
lethal phenotype circumvention,
253–254
- Transposon, insertional mutagenesis,
11–12
- Trophoblast stem (TS) cell
culture, 169–170
morula aggregation, 238–239
- Trophoectoderm. *See* Preimplantation
lethality phenotypic analysis
- Trp53*, 203
- TS cell. *See* Trophoblast stem cell
- Tumors. *See* No visible phenotype analysis
- TUNEL assay, 139
- V**
- Vasculogenesis defects, embryonic
phenotypes lethal after E9.5,
151–152
- Vika/vox*, 246
- Vision, testing, 205–206
- von Frey filament test, 207
- Vsx1*, 206
- W**
- Waste disposal, 287–288
- X**
- XEN stem cell. *See* Extraembryonic endo-
derm stem cell
- X inactivation, effect on embryo, 16,
219–220
- XO subclone, isolation from XY
embryonic stem cells,
228–229
- X-ray microcomputed tomography,
three-dimensional imaging of
embryos, 160–162
- X-ray mutagenesis, 10
- Y**
- Y-linked gene, dominant mutant in males,
219
- Z**
- Zic3*, 221
- Zona pellucida (ZP), 109, 111–113, 129
- ZP. *See* Zona pellucida