

Chapter 2

Global Resources: Including Gene Trapped ES Cell Clones – Is Your Gene Already Knocked Out?

Lluís Montoliu

Abstract

The design of any new mouse genetic modification today should start with careful scrutiny of the resources that are already available, through the internet, for information relating to your gene of interest. International mouse consortia are constantly providing new genetically modified alleles of virtually any gene in the mouse genome. Therefore, unless a very specific knock-in allele is required, it is more than likely that the envisaged mutation has already been obtained somewhere and made available in the form of embryonic stem (ES) cell clones, live animals, or cryopreserved sperm or embryos. In this chapter, I will review the current (November 2010) global resources that are available through the internet, where the most updated information about any given mouse gene should be examined, before any new experiment is planned or conducted. The knowledge and adequate use of all these global resources should speed up the acquisition of knowledge in the fields of biology, biomedicine, and biotechnology, while avoiding the redundant use of animals for experimentation and optimizing the use of limited funding resources. In this chapter, I will try to respond to two basic questions: where is my mouse? and what is known about my gene?

Abbreviations

CMMR	Canadian mouse mutant repository
CREATE	Coordination of resources for conditional expression of mutated mouse alleles
EBI	European Bioinformatics Institute
EMAP	Edinburgh Mouse Atlas Project
EMBL	European Molecular Biology Laboratory
EMMA	European Mouse Mutant Archive
EMPreSS	European mouse phenotyping resource of standardized screens
ENSEMBL	A joint project between EMBL – EBI and the Wellcome Trust Sanger Institute to develop a software system which produces and maintains automatic annotation on selected eukaryotic genomes
ES	Embryonic stem

ESPCR	European Society of Pigment Cell Research
EUCOMM	European Conditional Mouse Mutagenesis
EuMMCR	European mouse mutant cell repository
EUMODIC	European Mouse Disease Clinic
EUMORPHIA	European Union Mouse Research for Public Health and Industrial Applications
EUROPHENOME	Open source project to develop a software system for capturing, storing, and analyzing raw phenotyping data from SOPs contained in EMPReSS
FP6	Framework Programme 6
ICS	Institut Clinique de la Souris
IGTC	International Gene Trap Consortium
IKMC	International KnockOut Mouse Consortium
IMSR	International Mouse Strain Resource
ISTT	International Society for Transgenic Technologies
JAX	The Jackson Laboratory
KOMP	Knock-Out Mouse Project
KORC	Knock-Out Rat Consortium
MGI	Mouse Genome Informatics
MMRRC	Mouse Mutant Regional Resource Centres
NBRP	National BioResource Project for the Rat
NCBI	National Center for Biotechnology Information
NIH	National Institutes of Health
NorCOMM	North-American Conditional Mouse Mutagenesis
OMIM	Online Mendelian Inheritance in Man
RGD	Rat genome database
RRRC	Rat Resource & Research Centre
SNP	Single nucleotide polymorphism
SOP	Standard operating procedures
TIGM	Texas A&M Institute for Genomic Medicine
UCSC	University of California, Santa Cruz
ZFIN	Zebrafish model organism database
ZGC	Zebrafish gene collection
ZIRC	Zebrafish International Resource Center

2.1 Has My Favorite Gene Already Been Knocked-Out? Where Should I Start?

After sequencing of the human [1] and mouse [2] genomes, strategies were needed to reveal gene function. Since human and mouse genes share 95% homology, it was established that mouse genes could serve as tools for understanding human gene function. This can be achieved due to the ease by which the mouse

genome can be genetically manipulated with the available genetic toolbox, by knocking-out the corresponding murine homologous loci and interpreting the associated phenotypes generated. Globally, this process is known as mouse functional genomics.

Several approaches were initiated with intent to produce embryonic stem (ES) cell lines carrying gene mutations. At first, several gene trap consortia were arranged worldwide, with collaborative intent to saturate the mouse genome with gene trap vector insertions in mouse ES cells. This was based on the proposition that most genes could be mutated and the corresponding mouse mutants derived from these ES cell clones, carrying such random insertions. Eventually, all gene trap projects merged into the International Gene Trap Consortium (IGTC) [3] (Fig. 2.1).

Independently, three additional consortia were organized in Europe, USA, and Canada. In Europe, the European Conditional Mouse Mutagenesis (EUCOMM) project [4] was formed; in Canada the North-American Conditional Mouse Mutagenesis (NorCOMM) project came to be, and in the USA, the Knock-Out Mouse Project (KOMP) [5] was set up. Their orchestrated purpose was to systematically knockout all mouse genes using gene targeting approaches. These consortia used different approaches to vector design. Eventually, all three projects merged under the umbrella of the International KnockOut Mouse Consortium [6]. Later, the Texas A&M Institute for Genomic Medicine (TIGM) joined in as the fourth project of this type [7].

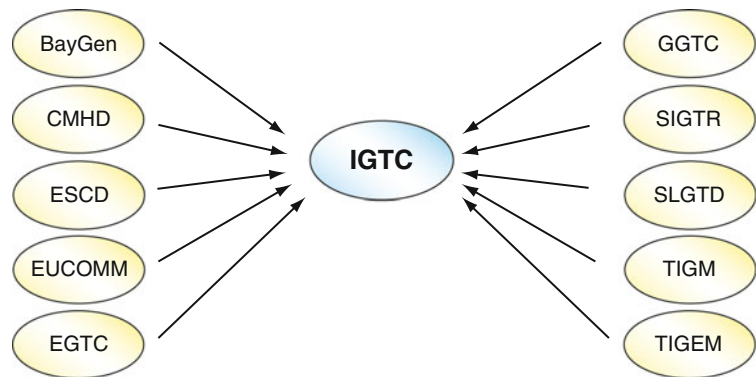


Fig. 2.1 The *International Gene-Trap Consortium* (IGTC) is constituted by the following ten members: [BayGen] BayGenomics (USA); [CMHD] Centre for Modeling Human Disease (Toronto, Canada); [ESCD] Embryonic Stem Cell Database (University of Manitoba, Canada); [EUCOMM] European Conditional Mouse Mutagenesis (European Union); [EGTC] Exchangeable Gene Trap Clones (Kumamoto University, Japan); [GGTC] German Gene Trap Consortium (Germany); [SIGTR] Sanger Institute Gene Trap Resource (Cambridge, UK); [SLGTD] Soriano Lab Gene Trap Database (Mount Sinai School of Medicine, New York, USA); [TIGM] Texas Institute for Genomic Medicine (USA); and [TIGEM] TIGEM-IRBM Gene Trap (Naples, Italy). The entire contents of the IGTC database can be browsed and searched via <http://www.genetrap.org>

2.1.1. Recommended Web Sites

All available ES cell clones from the various gene-trap consortia can be searched and browsed, at once, from IGTC at: <http://www.genetrap.org/>. Simply typing in the gene of interest will give an indication of whether there are any gene-trapped ES cell clones already generated for that gene and from where they can be obtained.

The EUCOMM project can be accessed at: <http://www.eucomm.org> and all the associated EUCOMM ES cell clones and vectors can be searched for and ordered from the European Mouse Mutant Cell Repository (EuMMCR) at: <http://www.eummc.org/>. Live mice and cryopreserved embryos derived from EUCOMM ES cell lines can be searched and ordered through the European Mouse Mutant Archive (EMMA) [8] at: <http://www.emmanet.org>.

The NorCOMM Project is available at: <http://www.norcomm.org/>, the KOMP Project from: <http://www.nih.gov/science/models/mouse/knockout/>, and the TIGM Project from: <http://www.tigm.org/>. Global resources made available by the merging of EUCOMM, NorCOMM, and KOMP and the formation of IKMC are available from: <http://www.knockoutmouse.org/>. Biological material from KOMP (ES cell clones, live mice, and cryopreserved embryos) can be obtained through the Mouse Mutant Regional Resource Centres (MMRRC) <http://www.mmrrc.org/>. Similarly, biological material from NorCOMM is available through the Canadian Mouse Mutant Repository (CMMR) at: <http://www.cmmr.ca/>. The description of the ES cells used by IKMC has been reported [9] and details are available from: http://www.eummc.org/products/wild_type_cells.php. All the international knockout mouse consortia data are based on the C57BL/6N inbred mouse strain, in contrast to the C57BL/6J inbred mouse strain, classically used in the previous generation of many transgenic and knockout animal models. Therefore, specific genetic polymorphisms should be taken into account where they differ between these and other related C57BL/6 mouse substrains ([10]; <http://www.cnb.csic.es/~montoliu/C57/>).

Today, if anyone needs to verify whether a given mouse gene has been already knocked out, one could start by searching the contents of two independent databases: the IGTC database (<http://www.genetrap.org/>) and the IKMC database (<http://www.knockoutmouse.org/>).

However, there may be other previously made mouse models or spontaneous mutants available relating to the gene of interest, not necessarily hit by the IGTC and/or not included by the IKMC. How could we look for them? The best global resource to find any mouse mutant strain, to browse whether a given mouse gene has been mutated or not, to eventually obtain biological material, in the form of ES cell clones, cryopreserved embryos,

cryopreserved sperm, or live animals, is the International Mouse Strain Resource (IMSR), available from Mouse Genome Informatics (MGI), within The Jackson Laboratory (JAX) web site (<http://www.jax.org>), at: <http://www.findmice.org/>. Searching IMSR does, in one single step, a systematic search of most available databases, the contents of which have been merged. This includes IKMC, EMMA, MMRRC, CMMR, JAX, and all major mouse archives worldwide. The only exception would be the contents of the IGTC database (gene-traps), which is not entirely directly searchable through the IMSR database (Fig. 2.2). However, gene-trapped ES cell clones from some IGTC members are already included in the IMSR, such as those distributed by TIGM.

Therefore, submitting search requests through the IGTC (<http://www.genetrap.org>) and the IMSR databases (<http://www.findmice.org>) should be the first two steps in any experimental planning for a new mouse mutation, in order to explore whether mouse strains, ES cell clones (targeted or gene-trapped), or cryopreserved material already exist for the envisaged mutation in our favorite mouse gene.

Where a gene symbol has been used as search term, a typical IGTC search would bring up a list of ES cell lines where the

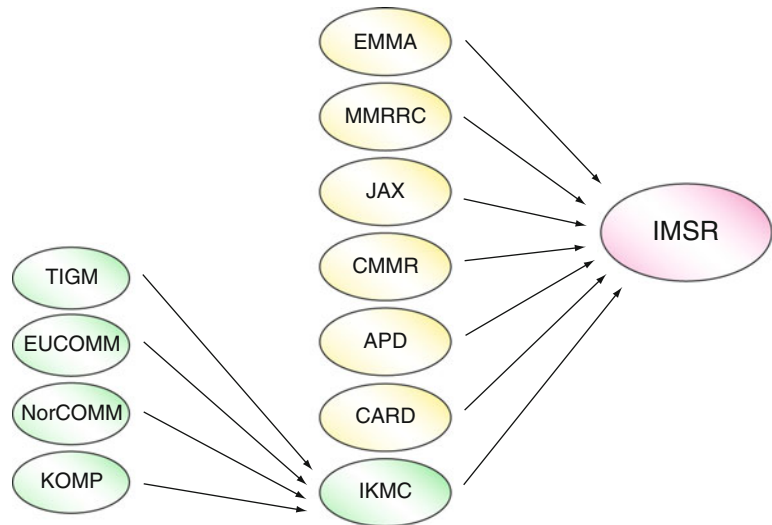


Fig. 2.2 The *International Mouse Strain Resource* (IMSR) provides information about mice, cryopreserved material, and ES cell lines contributed by a number of international repositories, including: [EMMA] European Mouse Mutant Archive, Monterotondo, Italy; [MMRRC] Mutant Mouse Regional Resource Centers, USA; [JAX] The Jackson Laboratory, Bar Harbor, Maine, USA; [CMMR] Canadian Mouse Mutant Repository, Toronto, Canada; [APD] Australian Phenome Bank, Acton, Australia; and [CARD] Centre for Animal Resources and Development, Kumamoto, Japan. In addition, the IMSR includes ES cell lines produced through the International KnockOut Mouse Consortium (IKMC). Additional repositories contributing to IMSR can be identified at the corresponding web site: <http://www.findmice.org>

gene-trapped locus is the gene of interest. The list indicates which gene-trap repository banks each ES cell line and from where the cells can be obtained. All potentially useful ES cell lines should be explored, and their gene-trap events understood in great detail, prior ordering any clone, for assessment of whether the insertion is likely to result in a knockout or knockdown effect. Each ES cell line is associated with plenty of genetic and mapping information that is absolutely required for analysis of the relevance of each gene-trap event. An example of IGTC output is shown in Fig. 2.3. A typical IMSR search would produce a list of mouse strains, in the form of (1) ES cell clones, indicating the particular project within the mouse consortium that has generated the biological resource; (2) live mice; or (3) cryopreserved embryos or sperm, linked to the repository where the mouse line is held. All suggested mouse mutant strains should be explored in detail. Usually, there will be several genetic backgrounds available, out of which the best suitable strain for our purposes should be ordered. In addition, not all mutant mouse strains will be available in the form of live mice. Most strains will be cryopreserved, as embryos or sperm, making them specially suited for shipping purposes. An example of IMSR output is shown in Fig. 2.4.

2.2 The Mouse Genome Informatics Web Site and Related Web Pages

If you are interested in exploring all that is currently known about any given mouse gene, its corresponding mutant alleles and associated mouse mutant strains, the best starting point is currently the “Mouse Genome Informatics” (MGI) web site (<http://www.informatics.jax.org>), available from The Jackson Laboratory (JAX) web site (<http://www.jax.org>). Whether you are interested in known gene alleles at this locus, gene expression patterns, genomic location, or associated mouse mutant strains, etc., all the information will be nicely arranged and organized on the corresponding web page at MGI (Fig. 2.5).

In particular, MGI interfaces its genomic information with popular genome browsers, such as ENSEMBL (<http://www.ensembl.org>), NCBI (<http://www.ncbi.nlm.nih.org>), or UCSC genome browser (<http://genome.ucsc.edu>), where a greater amount of genetic detail can be searched for, researched and downloaded.

There are many sections with information and useful links in every single gene card (Fig. 2.6). Information about the corresponding human disease associated with each gene is linked through the OMIM (Online Mendelian Inheritance in Man) database (<http://www.ncbi.nlm.nih.gov/omim>). Information about all known alleles and mouse strains available which carry

Browse Cell Lines

Search Field: genesymbol
Search Term: FGFR2

Export All Matching Results

Gene Description Index: # A B C D E F G H I J K L M N O P Q R S T U V W X Y Z - All

Showing 1 - 21 out of 21 records First Prev Next Last						
Cell Line Name	Source	Chromosome	Gene Description	Gene Symbol	Identification Status ²	Process Date
3SE044C04	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-05-23
3SE325E08	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-05-29
5SE044C04	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-05-23
5SE325E08	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-05-24
EUCG0003f03.q1k5SPK	EUCOMM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-04-23
G002B04	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-05-17
G002FJ5	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-05-12
G019B03	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-05-11
G019D03	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-05-14
G020C11	GGTC	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-05-16
IST11773B10BBF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I T	2010-06-04
IST12266H2BBF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-07-06
IST12266H2HMF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-06-24
IST12363E10BBF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-06-06
IST12395C2HMF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-03-29
IST12407C2BBF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-07-10
IST12407C2IMF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-06-02
IST12542E12BBF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-06-04
IST12542E12HMF1	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-05-22
IST12723D2HMF2	TIGM	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-07-09
PST14349-NL	ESDB	7	fibroblast growth factor receptor 2	Fgfr2	I	2010-04-12
Showing 1 - 21 out of 21 records First Prev Next Last						

Fig. 2.3 Typical results from a search at the IGTC. Using *Fgfr2* (gene encoding fibroblast growth factor receptor 2) as the search term, up to 21 different gene-trap ES cell lines are listed, from various programs and centers (GGTC, TIGM, ESDB, EUCOMM). Clicking on each of the ES cell line names will provide additional useful information of the gene-trap event.

Fig. 2.4 Typical results from a search at the IMSR. Using *Fgf2* (gene encoding fibroblast growth factor receptor 2) as the search term, up to 17 different mouse strains appear as available, in the form of live mice, frozen embryos, frozen sperm, or ES cell lines, from various repositories (JAX, MMRRC, TIGM, EM, CMMR) and on different genetic backgrounds. Clicking on each of the mouse strain names will provide additional useful information of the associated mutation. Please note that some (but not all) of the IGTC ES cell lines (i.e., from TIGM) are also included in the IMSR.

Fig. 2.4 Typical results from a search at the IMSR. Using *Fgf2* (gene encoding fibroblast growth factor receptor 2) as the search term, up to 17 different mouse strains appear as available, in the form of live mice, frozen embryos, frozen sperm, or ES cell lines, from various repositories (JAX, MMRRC, TIGM, EM, CMMR) and on different genetic backgrounds. Clicking on each of the mouse strain names will provide additional useful information of the associated mutation. Please note that some (but not all) of the IGTC ES cell lines (i.e., from TIGM) are also included in the IMSR.

Take our short survey

MGI About Help FAQ

Mouse Genome Informatics

Search Download More Resources Submit Data Find Mice (IMSR) Analysis Tools Contact Us

? Keywords, Symbols, or IDs Quick Search

Explore MGI All Search Tools

Genes
MGI Mouse Genome Informatics
Genes, Transcripts, Proteins
MGI Mouse Genome Informatics
Genes, Transcripts, Proteins

Phenotypes

Expression

Recombinases (cre)

Function

Pathways

Strains / SNPs

Variation Type	SNP	STR	Indel	Copy Number	Allele Summary (all strains)
SNP	G	A	A	A/G	
SNP	C	T	T	C/T	

Orthology

Tumors

FAQs

How do I...

- .. search for genes by genomic interval? [FAQ](#)
- .. find mutations for phenotypes or diseases? [FAQ](#)
- .. find expression data? [FAQ](#)
- .. view a structural genomic map? [FAQ](#)

[More FAQs](#) [MGI tutorial \(OpenHelix\)](#)

News July 6, 2010

- Help MGI serve you better. Please [take our short survey](#).
- For MGI 4.35, the Quick Search now returns the alleles most closely associated with a query. [Read more...](#)
- For the 4.34 release, MGI is retiring several query forms. See [FAQ](#) for alternative ways to find the same information on the MGI site.
- MGI now represents all alleles from KOMP and EUCOMM; the 4.33 release adds Vega & Ensembl transcript/protein sequences and identifies any gene model associations with gene/pseudogene discrepancies. [Read more...](#)
- MGI 4.32 introduces [MGI BioMart](#), a database warehouse for querying MGI markers and joining results with other BioMarts. [Read more...](#)

[More MGI news](#) [MGI Statistics](#)

Fig. 2.5 The *Mouse Genome Informatics* (MGI) web site (<http://www.informatics.jax.org>) at The Jackson Laboratory. Main menu of the MGI web pages leading to various sections with different, but linked, types of information. If you are interested in statistics and would like to see the progress of mouse genome coverage in the form of gene targeting events, number of mouse models created, etc., simply click on “MGI statistics” (*bottom right corner* of this main menu page).

an allele at the locus of interest is also linked through the IMSR database, as described before, or through the Phenotypic Alleles summary. If you are interested in genetic polymorphisms (i.e., single nucleotide polymorphisms, SNPs) that could be used to differentiate the same gene in different mouse genetic backgrounds these are also indicated. The best collection of known mouse genome SNPs is found at the Mouse Phenome Database (<http://phenome.jax.org>) where a whole section is devoted to SNPs (<http://phenome.jax.org/SNP>). Regarding expression data there are various links to resources detailing where this gene is expressed. In this regard, complementary information can be

MGJ [Take our short survey](#) [Keywords, Symbols, or IDs](#) [Quick Search](#)

[About](#) [Help](#) [FAQ](#) [Home](#) [Genes](#) [Phenotypes](#) [Expression](#) [Recombinases](#) [Function](#) [Pathways](#) [Strains / SNPs](#) [Orthology](#) [Tumors](#)

[Search](#) [Download](#) [More Resources](#) [Submit Data](#) [Find Mice \(IMSR\)](#) [Analysis Tools](#) [Contact Us](#)

Fgfr2 Gene Detail

Your Input Welcome

Symbol	Fgfr2																				
Name	fibroblast growth factor receptor 2																				
ID	MGJ:95523																				
Synonyms	Bek, Fgfr-2, Fgfr-7, Fgfr7, KGFRT, sv5																				
Genetic Map	Chromosome 7 62.0 cM Detailed Genetic Map ± 1 cM Mapping data(14)																				
Sequence Map	Chr7:137305965-140315033 bp, - strand (From VEGA annotation of NCBI Build 37) VEGA ContigView Ensembl ContigView UCSC Browser NCBI Map Viewer																				
Mammalian homology	human; chimpanzee; cattle; dog, domestic; rat (Mammalian Orthology) Comparative Map (Mouse/Human Fgfr2 ± 2 cM) Protein SuperFamily: fibroblast growth factor receptor TreeFam: TF316307																				
Sequences	<table border="0"> <thead> <tr> <th></th> <th>Representative Sequences</th> <th>Length</th> <th>Strain/Species</th> <th>Flank</th> </tr> </thead> <tbody> <tr> <td><input type="checkbox"/> genomic</td> <td>OTTMUSG00000031222 VEGA Gene Model MGJ Sequence Detail</td> <td>3009069</td> <td>C57BL/6J</td> <td>± 0 Kb</td> </tr> <tr> <td><input type="checkbox"/> transcript</td> <td>OTTMUST00000077352 VEGA MGJ Sequence Detail</td> <td>4429</td> <td>Not Applicable</td> <td></td> </tr> <tr> <td><input type="checkbox"/> polypeptide</td> <td>OTTMUSP00000040878 VEGA MGJ Sequence Detail</td> <td>334</td> <td>Not Applicable</td> <td></td> </tr> </tbody> </table> <p>For the selected sequences download in FASTA format Go</p> <p>All sequences(171) RefSeq(4) UniProt(23)</p>		Representative Sequences	Length	Strain/Species	Flank	<input type="checkbox"/> genomic	OTTMUSG00000031222 VEGA Gene Model MGJ Sequence Detail	3009069	C57BL/6J	± 0 Kb	<input type="checkbox"/> transcript	OTTMUST00000077352 VEGA MGJ Sequence Detail	4429	Not Applicable		<input type="checkbox"/> polypeptide	OTTMUSP00000040878 VEGA MGJ Sequence Detail	334	Not Applicable	
	Representative Sequences	Length	Strain/Species	Flank																	
<input type="checkbox"/> genomic	OTTMUSG00000031222 VEGA Gene Model MGJ Sequence Detail	3009069	C57BL/6J	± 0 Kb																	
<input type="checkbox"/> transcript	OTTMUST00000077352 VEGA MGJ Sequence Detail	4429	Not Applicable																		
<input type="checkbox"/> polypeptide	OTTMUSP00000040878 VEGA MGJ Sequence Detail	334	Not Applicable																		
Alleles and phenotypes	All alleles(95) : Targeted, knock-out(11) Targeted, other(13) Gene trapped(70) Spontaneous(1) Mice homozygous for null mutations die as embryos. Isoform IIIb deficient mutants die at birth with defects in multiple organs and tissues. Isoform IIIc deficient mutants have defects in osteoblast and chondrocyte lineages, producing dwarfism. Associated Human Diseases (4) Alleles Annotated to Human Diseases (7) Phenotype Images (15)																				
Polymorphisms	RFLP(2) SNPs within 2kb(13347 from dbSNP Build 128) SNPs within 2kb including multiple locations(13385)																				
Gene Ontology (GO) classifications	All GO classifications: (136 annotations) Process: angiogenesis , axonogenesis ... Component: cell surface , cytoplasm ... Function: ATP binding , fibroblast growth factor 1 binding ... External Resources: FuncBase																				
Expression	Literature Summary: (214 records) Data Summary: Assays (65) Results (595) Tissues (303) Images (177) Theiler Stages: 2,3,4,5,6,8,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,28 <table border="0"> <thead> <tr> <th>Assay Type</th> <th>Assays</th> <th>Results</th> </tr> </thead> <tbody> <tr> <td>RT-PCR</td> <td>30</td> <td>172</td> </tr> <tr> <td>RNA in situ</td> <td>30</td> <td>391</td> </tr> <tr> <td>Immunohistochemistry</td> <td>4</td> <td>28</td> </tr> <tr> <td>Northern blot</td> <td>1</td> <td>4</td> </tr> </tbody> </table>	Assay Type	Assays	Results	RT-PCR	30	172	RNA in situ	30	391	Immunohistochemistry	4	28	Northern blot	1	4					
Assay Type	Assays	Results																			
RT-PCR	30	172																			
RNA in situ	30	391																			
Immunohistochemistry	4	28																			
Northern blot	1	4																			

Fig. 2.6 Typical example of MGJ search results regarding available information on the *Fgfr2* gene (list of topics covered is longer than shown, but it has been truncated for illustrative purposes). Each section is linked to additional sources of information.

obtained from Genepaint (<http://www.genepaint.org>), a digital atlas of gene expression patterns in mice, determined by nonradioactive in situ hybridization on serial tissue sections and associated with each gene, all available through their web site. Particularly interesting, for neuroscientists, are the new links to the Allen Brain Atlas (<http://www.brain-map.org>) for adult mouse brain and developing mouse brain sections, where the expression of every gene is annotated.

MGJ collects and annotates expression and activity data for cre recombinase-containing transgenes and knock-in alleles. All these

very useful cre-mouse lines can be browsed and searched through a specific site (<http://www.creportal.org>).

At MGI, they also provide links to complete reference books in the mouse field that are out of print. These valuable online books include: “The Biology of the Laboratory Mouse” Earl L. Green (ed.) (<http://www.informatics.jax.org/greenbook>); “Mouse Genetics” by Lee Silver (<http://www.informatics.jax.org/silverbook>); “The Anatomy of the Laboratory Mouse” by Margaret J. Cook (<http://www.informatics.jax.org/cookbook>); “The coat colors of mice” by Willys K. Silvers (<http://www.informatics.jax.org/wksilvers>); and the “Origins of Inbred Mice” Herbert C. Morse III (ed.) (<http://www.informatics.jax.org/morsebook>). Additional information on the genetics of pigmentation, or genes whose function affect coat color pigmentation, can be obtained from the “Color Genes” web site, at the European Society of Pigment Cell Research (ESPCR), at: <http://www.espcr.org/micemut/>.

One of the most useful sections within MGI is the “Mouse Nomenclature Home Page” (<http://www.informatics.jax.org/mgihome/nomen>), where the guidelines for nomenclature of genes, genetic markers, alleles, and mutations in the mouse and rat are found. The Mouse Genome Informatics (MGI) Database is the authoritative source of official names for mouse genes, alleles, and strains. Nomenclature follows the rules and guidelines established by the International Committee on Standardized Genetic Nomenclature for Mice. Recently, from the International Society for Transgenic Technologies (ISTT) (<http://www.transtechsociety.org>) and the scientific journal Transgenic Research (Springer) (<http://www.springer.com/biomed/molecular/journal/11248>), a combined position paper has been recently published, encouraging the use of standard nomenclature to adequately name transgenes, knockout gene alleles, and any mutation associated to a genetically modified mouse strain [11].

The MGI is fully interconnected with ENSEMBL and NCBI. At NCBI, one all-in-one bioinformatic resource can complement the information obtained from a given mouse gene. This is the “all databases” feature of NCBI (global query: <http://www.ncbi.nlm.nih.gov/gquery/>) that provides all the known information about a gene, interfacing with all NCBI databases, including published articles from PubMed.

2.3 Additional Databases for Mouse Transgenesis

Besides MGI, the reference for all mouse databases, there are additional bioinformatic resources available which are worth being aware of, since they also provide useful information.

Most of these additional databases are already compiled at the “General Links” page of the ISTT web site (<http://www.trans-techsociety.org/link.php>).

Of outstanding interest are several independent databases that account for different Cre-transgenic mouse lines created for use in combination with mice carrying *floxed* (flanked-by-loxP-sites) alleles, for mouse conditional gene mutagenesis. Besides the creportal at MGI already mentioned, an additional database for cre-mouse lines is an initiative pioneered by Andras Nagy, the Cre-X-Mice database (<http://nagy.mshri.on.ca/cre/>). Other transgenic mouse cre lines can be obtained from the crezoo database (<http://bioit.fleming.gr/crezoo/>), originating at the Fleming Institute (Vari, Greece) and from the MouseCre database (<http://www.ics-mci.fr/mousecre/>), at the *Institut Clinique de la Souris* (ICS, Strasbourg-Illkirch, France). All worldwide databases collecting Cre transgenic mouse lines are coordinated through the CREATE consortium (<http://creline.org/>), a Cre recombinase portal organized by the European Bioinformatic Institute (EBI, Hinxton-Cambridge, UK).

Information on existing ES cell lines (name and mouse strain of origin) can be downloaded from MGI (ftp://ftp.informatics.jax.org/pub/reports/ES_CellLine.rpt). The diverse 129 mouse substrains follow revised nomenclature, indicated by Simpson et al. [12] and now are available through a useful web site at the MGI (http://www.informatics.jax.org/mgihome/nomen/strain_129.shtml).

Specific details on the use of the popular R1 mouse ES cell line [13] is available from a web site devoted to the topic (<http://www.mshri.on.ca/nagy/r1.htm>).

With regard to web sites oriented toward phenotyping of mice, those from the EUMORPHIA European Project (<http://www.eumorphia.org/>) should be mentioned, since that led to the EMPReSS initiative (<http://empress.har.mrc.ac.uk/>), a database of Standard Operating Procedures (SOPs) for procedures that can be used to characterize the phenotype of a mouse, and to EUROPHENOME (<http://www.europhephenome.org/>), a database for collection of phenomic data obtained from the EMPReSS SOPs. The interaction of these phenotyping projects with the international knockout consortia can be followed with EUMODIC (<http://www.eumodic.org/>), a new project funded by the European Commission under Framework Program 6 (FP6) to generate phenome data on 650 mutant mice generated by EUCOMM, using the EMPReSS SOPs.

The Edinburgh Mouse Atlas Project (EMAP, <http://genex.hgu.mrc.ac.uk>) is another great resource for a 3D-mouse embryo anatomy atlas and its corresponding expression database. Again, for those focused on neuroscience, you will find The Mouse Brain Library (MBL, <http://www.mbl.org>) a very useful resource,

consisting of high-resolution images and databases of brains from several inbred mouse strains.

On the subject of mouse welfare issues, several projects have been initiated associated with their corresponding web sites, including “Mouse Welfare Terms” (<http://www.mousewelfare-terms.org/>), a site dedicated to standardizing the way different characteristics which may impact on the welfare of laboratory mice, are described. Also the COST B24 Action on “Laboratory Animal Science and Welfare” (http://www.cost.esf.org/domains_actions/bmbs/Actions/B24-Laboratory-Animal-Science-and-Welfare-End-date-April-2009) that recently published The COST Manual of Laboratory Animal Care and Use. Refinement, Reduction and Research.

Finally, from the ISTT web site, it is possible to reach many transgenic cores, facilities, and/or units producing genetically modified mice and rats in many countries all over the world (<http://www.transtechsociety.org/linkstg.html>).

2.4 Resources on Additional Animal Models

Mice are the most frequently used animal models in vertebrate functional genomics and for experiments involving mammalian genetic modification, but they are not the only species that might be used. Other species to consider as candidates for genetic modification are rats, zebrafish, flies, worms, etc., and, correspondingly, web sites listing such resources provide lots of interesting and useful information about these alternative and additional animal models. In this section, I will review some of these web sites, the most important for each species, where additional global resources can be readily explored and information obtained.

2.4.1. Rats

Some might still consider rats to be “bigger” mice, but this is not so. Rats are truly a different rodent species, with a specific reproductive system physiology that has precluded their routine use in most transgenic facilities for many years. Fortunately, several recent efforts and methods of investigation have resulted in the establishment of robust protocols that allow the generation of transgenic rats with efficiency comparable to that currently obtained in mice [14, 15]. Rats are the animal model of choice for most toxicological and pharmacological studies. For many years, the rat genome was not available to investigators for gene targeting, as is often used in mice. Despite the initial excitement generated with the cloning of rats [16], the nuclear-transfer technique has proven to be difficult to reproduce in this species [17]. Recently, true rat ES cells were obtained [18, 19] providing the tools for the generation of future knockout rats through standard

gene targeting in ES cells. The first gene knockout engineered by homologous recombination in rat ES cells has been published [20]. However, a totally different method, using Zinc-finger nucleases, has been reported to produce the first gene-specific knockout rats [21, 22].

2.4.1.1. *Recommended Web Sites*

The reference entry point for almost anything related to rat genetic and genomic research is the Rat Genome Database (RGD), at: <http://rgd.mcw.edu/>. Complementary resources can be obtained from the NIH Rat Genomics and Genetics web site (<http://www.nih.gov/science/models/rat/>). In addition, rat genome information can also be obtained from the specific ENSEMBL (http://www.ensembl.org/Rattus_norvegicus/) and NCBI (<http://www.ncbi.nlm.nih.gov/genome/guide/rat/>) project web sites. Specific archives for obtaining rat strains are also available, such as The National BioResource Project for the Rat in Japan (NBRP: <http://www.anim.med.kyoto-u.ac.jp/nbr/>) [23], the Rat Resource & Research Centre (RRRC) at the University of Missouri (<http://www.nrrrc.missouri.edu/>), or the Michael Festing's collection of rat inbred strains (http://www.informatics.jax.org/external/festing/search_form.cgi). Finally, the standard nomenclature rules and guidelines to name genes, alleles, or strains are also available for rats at the Mouse Genome Informatics web site of The Jackson Laboratory (<http://www.informatics.jax.org/mgihome/nomen/>). A few transgenic core facilities are also producing transgenic rats by request, such as the University of Michigan Transgenic Core (<http://www.med.umich.edu/tamc/rats.html>) and the Transgenic Rats common facility of IFR26 and Biogenouest in Nantes, France (<http://www.ifr26.nantes.inserm.fr/ITERT/transgenese-rat/>).

Recently established, the Knock-Out Rat Consortium, (KORC; <http://www.knockoutrat.org>) is pledged to the creation of knockout mutations in rats by means of multiple technologies.

KORC is a consortium, with goals similar to that of KOMP. Additional rat global resources can be found linked to any of these web sites.

2.4.2. *Other Mammals*

Global information on genetic, genomic, and biological resources relating to various other mammalian species are available from NCBI. They include the following. For the pig, at (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/>), for sheep (<http://www.ncbi.nlm.nih.gov/genome/guide/sheep/>), for the cow (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>), the rabbit (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/rabbit/>), the goat (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/goat/>), and the horse (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/horse/>). These are a few

among other mammals where genetic modification methods can be applied.

2.4.3. Zebrafish

Zebrafish have become a reference animal model for early vertebrate genomic research. The ease by which genetic modification can be accomplished and the visual transparency and short duration of zebrafish embryo development make them unique for many exploratory experiments or genetic screening. The genetic toolbox available for zebrafish includes standard transgenesis, through the use of *Tol2* transposon-mediated methods [24], gene targeting in zebrafish ES cells [25], site-specific recombination using the Cre/lox [26], or Flp/frt technologies [27], among other techniques.

Furthermore, most of the mammalian genes have their homologous counterpart in the zebrafish genome. The essential functions of most loci, especially if they are relevant during embryo development, are evolutionarily conserved, hence genetic studies in zebrafish are of value and provide a more efficient approach to understanding corresponding gene function in mammals [28].

2.4.3.1. Recommended Web Sites

The reference gate to access to all zebrafish biological and genetic resources is ZFIN, the Zebrafish Model Organism Database [29], available at: <http://zfin.org>. The ZFIN database is interconnected with many other useful resources for zebrafish, such as the specific web site for the Zebrafish genome project within ENSEMBL, at: http://www.ensembl.org/Danio_reio/ or its equivalent web site at the NCBI server: <http://www.ncbi.nlm.nih.gov/genome/guide/zebrafish/>. Additional web sites with helpful information are the NIH Zebrafish Gene Collection (ZGC) database, at: <http://zgc.nci.nih.gov/> and the Zebrafish International Resource Center (ZIRC), at: <http://zebrafish.org/zirc>. Additional zebrafish resources can be found linked to any of these web sites already mentioned.

2.4.4. Flies

The fruit fly, *Drosophila melanogaster*, has been a classical animal model for genetic studies for more than a century. Even though flies and mice are very distantly evolutionary related, many fundamental gene functions have proven to be surprisingly similar [30, 31], therefore genetic modification studies conducted in *Drosophila* have been, and will continue to be, instrumental for the understanding of mammalian genomes.

2.4.4.1. Recommended Web Sites

The essential reference entry point for all genetic, genomic, and biological information and resources currently available for *Drosophila* is the FlyBase (<http://flybase.org/>) [32]. This impressive resource offers links to almost everything in existence relating to *Drosophila* genetics. The corresponding *Drosophila* genome

web sites in ENSEMBL (http://www.ensembl.org/Drosophila_melanogaster/) and NCBI (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/fly/>) can also be used to access supplementary information.

2.4.5. Worms

The nematode *Caenorhabditis elegans* (*C. elegans*) was introduced by Sydney Brenner in 1974 as a new model organism for biology and genetic studies. Due to its apparent simplicity and rapid and transparent embryo development, the entire fate map for the approximately thousand cells that constitute an adult individual was known quite soon. The sequencing of this genome triggered many comparative studies of genomes and the use of worm models in the study of complex biological processes such as ageing [33].

2.4.5.1. Recommended Web Sites

Essential global resources for genetic, genomic, and biological information about *C. elegans* are WormBase (<http://www.wormbase.org/>) and WormBook (<http://www.wormbook.org/>). Additional helpful information on behavioral and structural anatomy can be obtained from the WormAtlas (<http://www.wormatlas.org/>).

References

1. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG et al (2001) The sequence of the human genome. *Science* 291:1304–1351
2. Mouse Genome Sequencing Consortium et al (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420:520–562
3. Skarnes WC, Von Melchner H, Wurst W, Hicks G, Nord AS, Cox T, Young SG, Ruiz P, Soriano P, Tessier-Lavigne M, Conklin BR, Stanford WL, Rossant J, International Gene Trap Consortium (2004) A public gene trap resource for mouse functional genomics. *Nat Genet* 36:543–544
4. Auwerx J, Avner P, Baldock R, Ballabio A, Balling R, Barbacid M, Berns A, Bradley A, Brown S, Carmeliet P, Chambon P, Cox R, Davidson D, Davies K, Duboule D, Forejt J, Granucci F, Hastie N, de Angelis MH, Jackson I, Kioussis D, Kollias G, Lathrop M, Lendahl U, Malumbres M, von Melchner H, Müller W, Partanen J, Ricciardi-Castagnoli P, Rigby P, Rosen B, Rosenthal N, Skarnes B, Stewart AF, Thornton J, Tocchini-Valentini G, Wagner E, Wahli W, Wurst W (2004) The European dimension for the mouse genome mutagenesis program. *Nat Genet* 36:925–927
5. Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M, Collins FS, Dove WF, Duyk G, Dymecki S, Eppig JT, Grieder FB, Heintz N, Hicks G, Insel TR, Joyner A, Koller BH, Lloyd KC, Magnuson T, Moore MW, Nagy A, Pollock JD, Roses AD, Sands AT, Seed B, Skarnes WC, Snoddy J, Soriano P, Stewart DJ, Stewart F, Stillman B, Varmus H, Varticovski L, Verma IM, Vogt TF, von Melchner H, Witkowski J, Woychik RP, Wurst W, Yancopoulos GD, Young SG, Zambrowicz B (2004) The knockout mouse project. *Nat Genet* 36:921–924
6. International Mouse Knockout Consortium, Collins FS, Rossant J, Wurst W (2007) A mouse for all reasons. *Cell* 128:9–13
7. Collins FS, Finnell RH, Rossant J, Wurst W (2007) A new partner for the International Knockout Mouse Consortium. *Cell* 129:235
8. Wilkinson P, Sengerova J, Matteoni R, Chen CK, Soulat G, Ureta-Vidal A, Fessele S, Hagn M, Massimi M, Pickford K, Butler RH, Marschall S, Mallon AM, Pickard A, Raspa M, Scavizzi F, Fray M, Larrigaldie V, Leyritz J, Birney E, Tocchini-Valentini GP, Brown S, Herault Y, Montoliu L, de Angelis MH, Smedley D (2010) EMMA—mouse mutant resources for the international

- scientific community. *Nucleic Acids Res* 38 (Database issue):D570–D576
9. 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* 6:493–495
 10. Zurita E, Chagoyen M, Cantero M, Alonso R, González-Neira A, López-Jiménez A, López-Moreno JA, Landel CP, Benítez J, Pazos F, Montoliu L (2010) Genetic polymorphisms among C57BL/6 mouse inbred strains. *Transgenic Res* 2011, 20:481–489
 11. Montoliu L, Whitelaw CB (2011) Using standard nomenclature to adequately name transgenes, knockout gene alleles and any mutation associated to a genetically modified mouse strain. *Transgenic Res* 20(2):435–440
 12. 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 mice. *Nat Genet* 16:19–27
 13. 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 USA* 90:8424–8428
 14. Filipiak WE, Saunders TL (2006) Advances in transgenic rat production. *Transgenic Res* 15:673–686
 15. Charreau B, Tesson L, Soullillou JP, Pourcel C, Anegón I (1996) Transgenesis in rats: technical aspects and models. *Transgenic Res* 5:223–234
 16. Zhou Q, Renard JP, Le Friec G, Brochard V, Beaujean N, Cherifi Y, Fraichard A, Cozzi J (2003) Generation of fertile cloned rats by regulating oocyte activation. *Science* 302:1179
 17. Popova E, Bader M, Krivokharchenko A (2009) Efficient production of nuclear transferred rat embryos by modified methods of reconstruction. *Mol Reprod Dev* 76: 208–216
 18. Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A (2008) Capture of authentic embryonic stem cells from rat blastocysts. *Cell* 135:1287–1298
 19. Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL (2008) Germ-line competent embryonic stem cells derived from rat blastocysts. *Cell* 135:1299–1310
 20. Tong C, Li P, Wu NL, Yan Y, Ying QL (2010) Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. *Nature* 467:211–213
 21. Geurts AM, Cost GJ, Freyvert Y, Zeitler B, Miller JC, Choi VM, Jenkins SS, Wood A, Cui X, Meng X, Vincent A, Lam S, Michalkiewicz M, Schilling R, Foeckler J, Kalloway S, Weiler H, Ménoret S, Anegón I, Davis GD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jacob HJ, Buelow R (2009) Knockout rats via embryo microinjection of zinc-finger nucleases. *Science* 325:433
 22. Rémy S, Tesson L, Ménoret S, Usal C, Scharenberg AM, Anegón I (2010) Zinc-finger nucleases: a powerful tool for genetic engineering of animals. *Transgenic Res* 19:363–371
 23. Serikawa T, Mashimo T, Takizawa A, Okajima R, Maedomari N, Kumafuji K, Tagami F, Neoda Y, Otsuki M, Nakanishi S, Yamasaki K, Voigt B, Kuramoto T (2009) National BioResource Project-Rat and related activities. *Exp Anim* 58:333–341
 24. Burket CT, Montgomery JE, Thummel R, Kassen SC, LaFave MC, Langenau DM, Zon LI, Hyde DR (2008) Generation and characterization of transgenic zebrafish lines using different ubiquitous promoters. *Transgenic Res* 17:265–279
 25. Fan L, Moon J, Crodian J, Collodi P (2006) Homologous recombination in zebrafish ES cells. *Transgenic Res* 15:21–30
 26. Pan X, Wan H, Chia W, Tong Y, Gong Z (2005) Demonstration of site-directed recombination in transgenic zebrafish using the Cre/loxP system. *Transgenic Res* 14: 217–223
 27. Wong AC, Draper BW, Van Eenennaam AL (2011) FLPe functions in zebrafish embryos. *Transgenic Res* 20:409–415
 28. Haga Y, Dominique VJ 3rd, Du SJ (2009) Analyzing notochord segmentation and intervertebral disc formation using the twhh:gfp transgenic zebrafish model. *Transgenic Res* 18:669–683
 29. Sprague J, Bayraktaroglu L, Clements D, Conlin T, Fashena D, Frazer K, Haendel M, Howe D, Mani P, Ramachandran S, Schaper K, Segerdell E, Song P, Sprunger B, Taylor S, Van Slyke C, Westerfield M (2006) The Zebrafish Information Network: the zebrafish model organism database. *Nucleic Acids Res* 34:D581–D585
 30. Mercader N, Leonardo E, Azpiazu N, Serrano A, Morata G, Martínez C, Torres M (1999) Conserved regulation of proximodistal limb axis development by *Meis1/Hth*. *Nature* 402:425–429
 31. Giraldo P, Martínez A, Regales L, Lavado A, García-Díaz A, Alonso A, Busturia A, Montoliu L (2003) Functional dissection of the mouse tyrosinase locus control region

- identifies a new putative boundary activity. Nucleic Acids Res 31:6290–6305
32. Tweedie S, Ashburner M, Falls K, Leyland P, McQuilton P, Marygold S, Millburn G, Osumi-Sutherland D, Schroeder A, Seal R, Zhang H, The FlyBase Consortium (2009) FlyBase: enhancing *Drosophila* Gene Ontology annotations. Nucleic Acids Res 37: D555–D559
33. Kenyon CJ (2010) The genetics of ageing. Nature 464:504–512

Advanced Protocols for Animal Transgenesis

An ISTT Manual

Pease, S.; Saunders, Th.L. (Eds.)

2011, XV, 669 p. 210 illus., 65 illus. in color., Hardcover

ISBN: 978-3-642-20791-4