Chapter 2

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

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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

S. Pease and T.L. Saunders (eds.), *Advanced Protocols for Animal Transgenesis*, Springer Protocols,
DOI 10.1007/978-3-642-20792-1_2,

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].

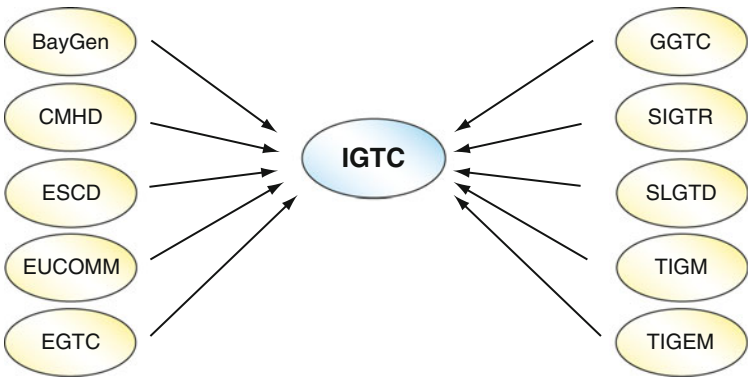


Fig. 2.1 The *International Gene-Trap Consortium* (IGTC) is constituted by the following ten members: [BayGen] BayGenomics (USA); [CMHD] Centre for Modeling Human Disease (Toronto, Canada); [ESCD] Embryonic Stem Cell Database (University of Manitoba, Canada); [EUCOMM] European Conditional Mouse Mutagenesis (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.eummcr.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.eummcr.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

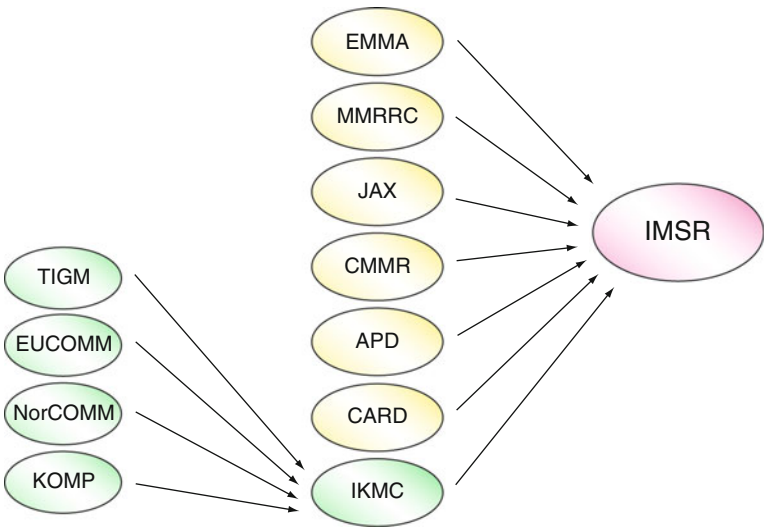


Fig. 2.2 The *International Mouse Strain Resource* (IMSR) provides information about mice, cryopreserved material, and ES cell lines contributed by a number of international repositories, including: [EMMA] European Mouse Mutant Archive, Monterotondo, Italy; [MMRRC] Mutant Mouse Regional Resource Centers, 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

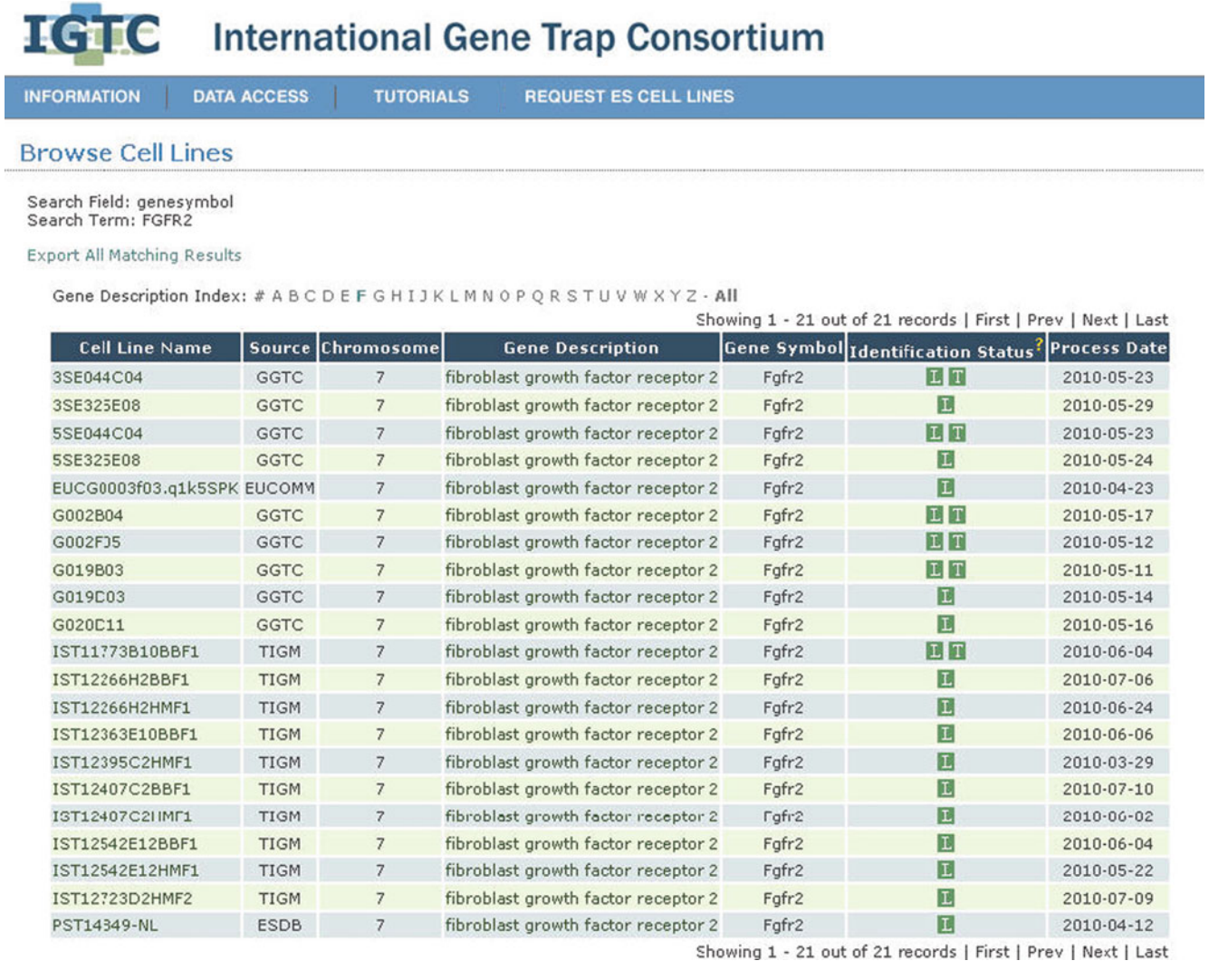
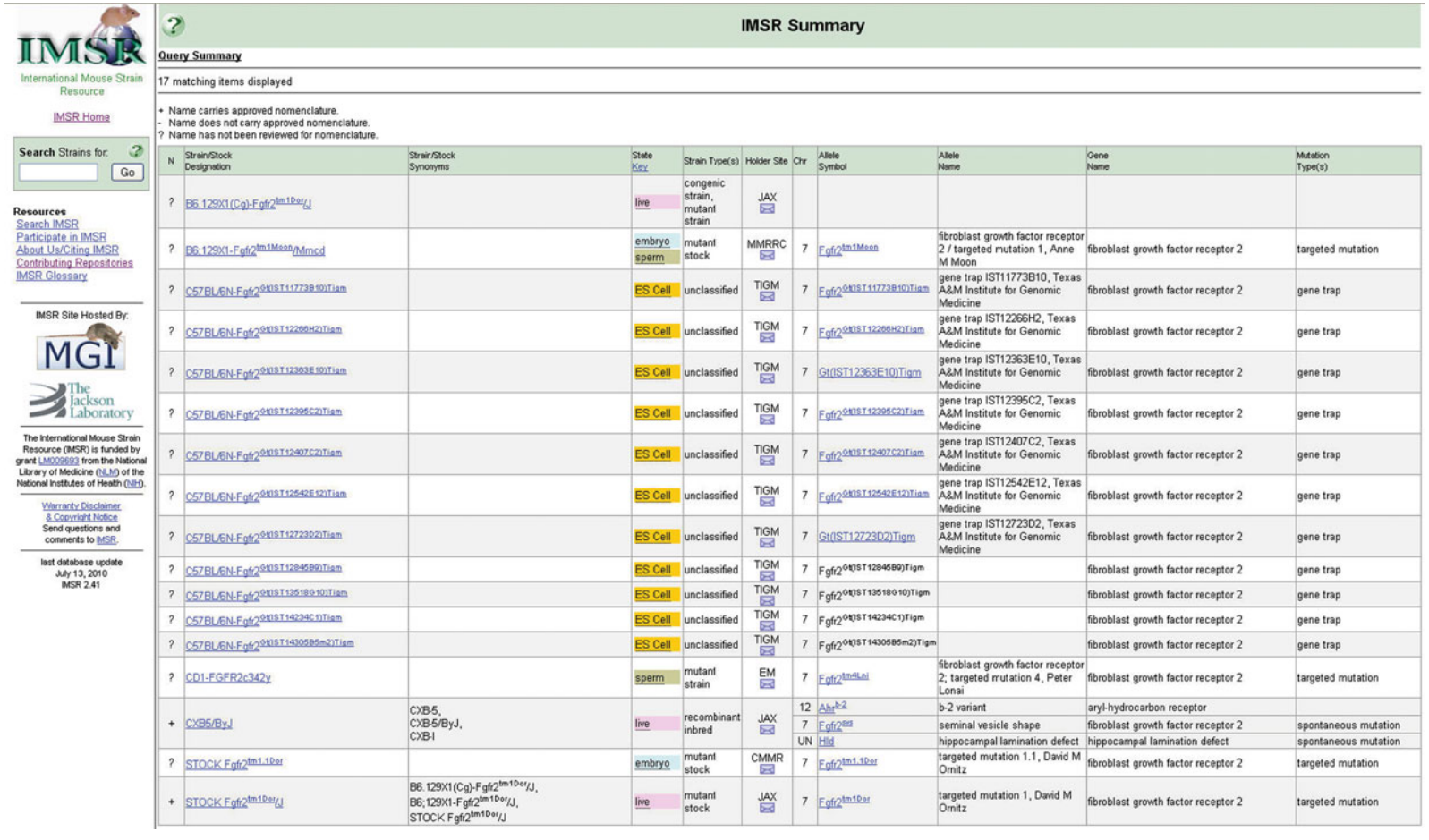


Fig. 2.3 Typical results from a search at the IGTC. Using *Fgfr2* (gene encoding fibroblast growth factor receptor 2) as the search term, up to 21 different gene-trap ES cell lines are listed, from various programs and centers (GGTC, TIGM, ESDB, EUCOMM). Clicking on each of the ES cell line names will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



IMSR

International Mouse Strain Resource

IMSR Home

Search Strains for: Go

Resources
Search IMSR
Participate in IMSR
About Us/Citing IMSR
Contributing Repositories
IMSR Glossary

IMSR Site Hosted By: MGI, The Jackson Laboratory

The International Mouse Strain Resource (IMSR) is funded by grant LM009693 from the National Library of Medicine (NLM) of the National Institutes of Health (NIH).

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Send questions and comments to IMSR.

last database update
July 13, 2010
IMSR 2.41

IMSR Summary

Query Summary

17 matching items displayed

\+ Name carries approved nomenclature.
\- Name does not carry approved nomenclature.
? Name has not been reviewed for nomenclature.

N	Strain/Stock Designation	Strain/Stock Synonyms	State Key	Strain Type(s)	Holder Site	Chr	Allele Symbol	Allele Name	Gene Name	Mutation Type(s)
?	B6.129X1(Cg)-Fgfr2^{tm1Dor}/J		live	congenic strain, mutant strain	JAX					
?	B6;129X1-Fgfr2^{tm1Moon}/Mmcd		embryo sperm	mutant stock	MMRRC	7	Fgfr2^{tm1Moon}	fibroblast growth factor receptor 2 / targeted mutation 1, Anne M Moon	fibroblast growth factor receptor 2	targeted mutation
?	C57BL/6N-Fgfr2$^{Gt(IST11773B10)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST11773B10)Tigm}$	gene trap IST11773B10, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12266H2)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST12266H2)Tigm}$	gene trap IST12266H2, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12363E10)Tigm}$		ES Cell	unclassified	TIGM	7	Gt(IST12363E10)Tigm	gene trap IST12363E10, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12395C2)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST12395C2)Tigm}$	gene trap IST12395C2, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12407C2)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST12407C2)Tigm}$	gene trap IST12407C2, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12542E12)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST12542E12)Tigm}$	gene trap IST12542E12, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12723D2)Tigm}$		ES Cell	unclassified	TIGM	7	Gt(IST12723D2)Tigm	gene trap IST12723D2, Texas A&M Institute for Genomic Medicine	fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST12845B9)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST12845B9)Tigm}$		fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST13518G10)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST13518G10)Tigm}$		fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST14234C1)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST14234C1)Tigm}$		fibroblast growth factor receptor 2	gene trap
?	C57BL/6N-Fgfr2$^{Gt(IST14305B5m2)Tigm}$		ES Cell	unclassified	TIGM	7	Fgfr2$^{Gt(IST14305B5m2)Tigm}$		fibroblast growth factor receptor 2	gene trap
?	CD1-FGFR2c342y		sperm	mutant strain	EM	7	Fgfr2^{tm4Lni}	fibroblast growth factor receptor 2; targeted mutation 4, Peter Lonai	fibroblast growth factor receptor 2	targeted mutation
+	CXB5/ByJ	CXB-5, CXB-5/ByJ, CXB-I	live	recombinant inbred	JAX	12	Ahr^{b-2}	b-2 variant	aryl-hydrocarbon receptor	
						7	Fgfr2^{ovs}	seminal vesicle shape	fibroblast growth factor receptor 2	spontaneous mutation
						UN	Hld	hippocampal lamination defect	hippocampal lamination defect	spontaneous mutation
?	STOCK Fgfr2$^{tm1.1Dor}$		embryo	mutant stock	CMMR	7	Fgfr2$^{tm1.1Dor}$	targeted mutation 1.1, David M Ornitz	fibroblast growth factor receptor 2	targeted mutation
+	STOCK Fgfr2^{tm1Dor}/J	B6.129X1(Cg)-Fgfr2^{tm1Dor}/J, B6;129X1-Fgfr2^{tm1Dor}/J, STOCK Fgfr2^{tm1Dor}/J	live	mutant stock	JAX	7	Fgfr2^{tm1Dor}	targeted mutation 1, David M Ornitz	fibroblast growth factor receptor 2	targeted mutation

Fig. 2.4 Typical results from a search at the IMSR. Using *Fgfr2* (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.

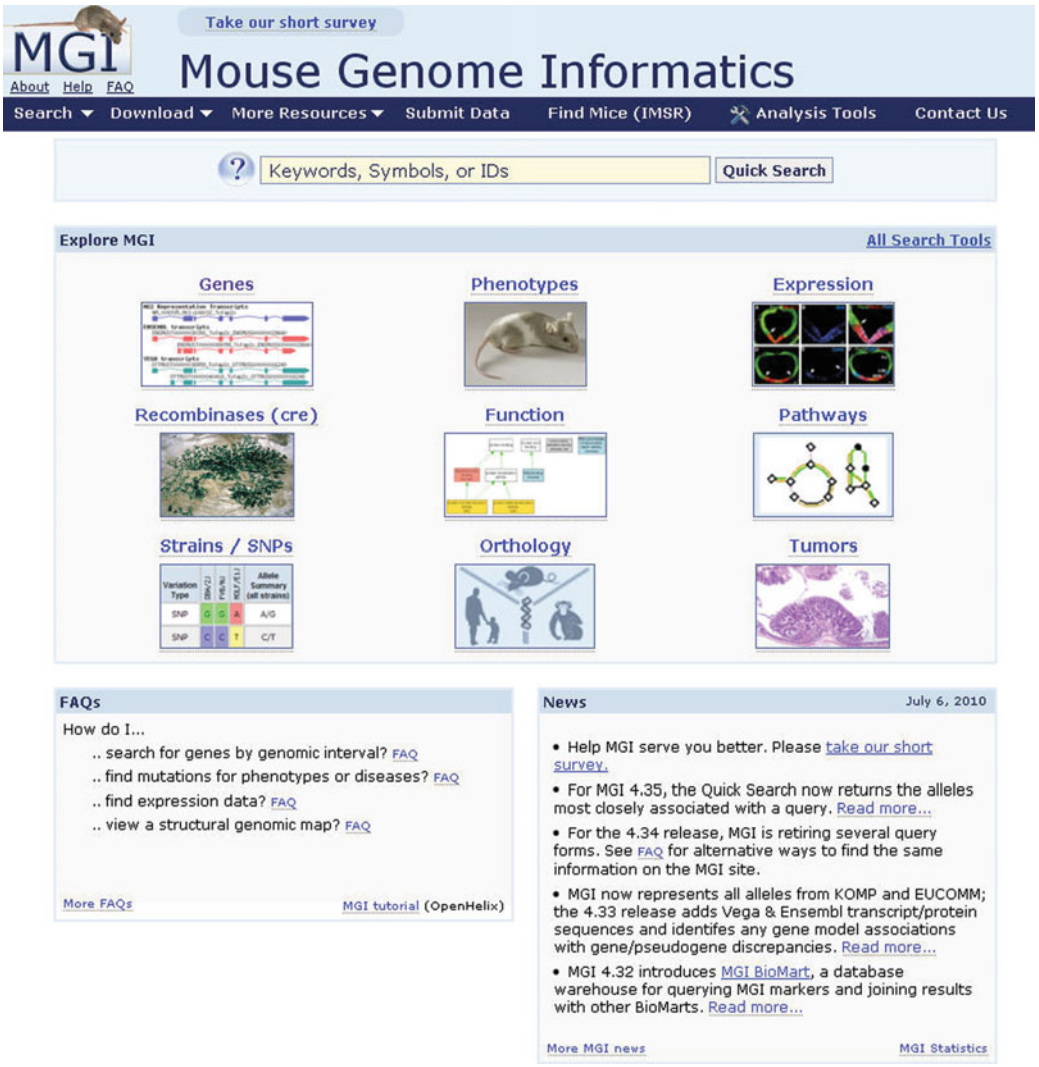

Fig. 2.5 The *Mouse Genome Informatics* (MGI) web site (http://www.informatics.jax.org) at The Jackson Laboratory. Main menu of the MGI web pages leading to various sections with different, but linked, types of information. If you are interested in statistics and would like to see the progress of mouse 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

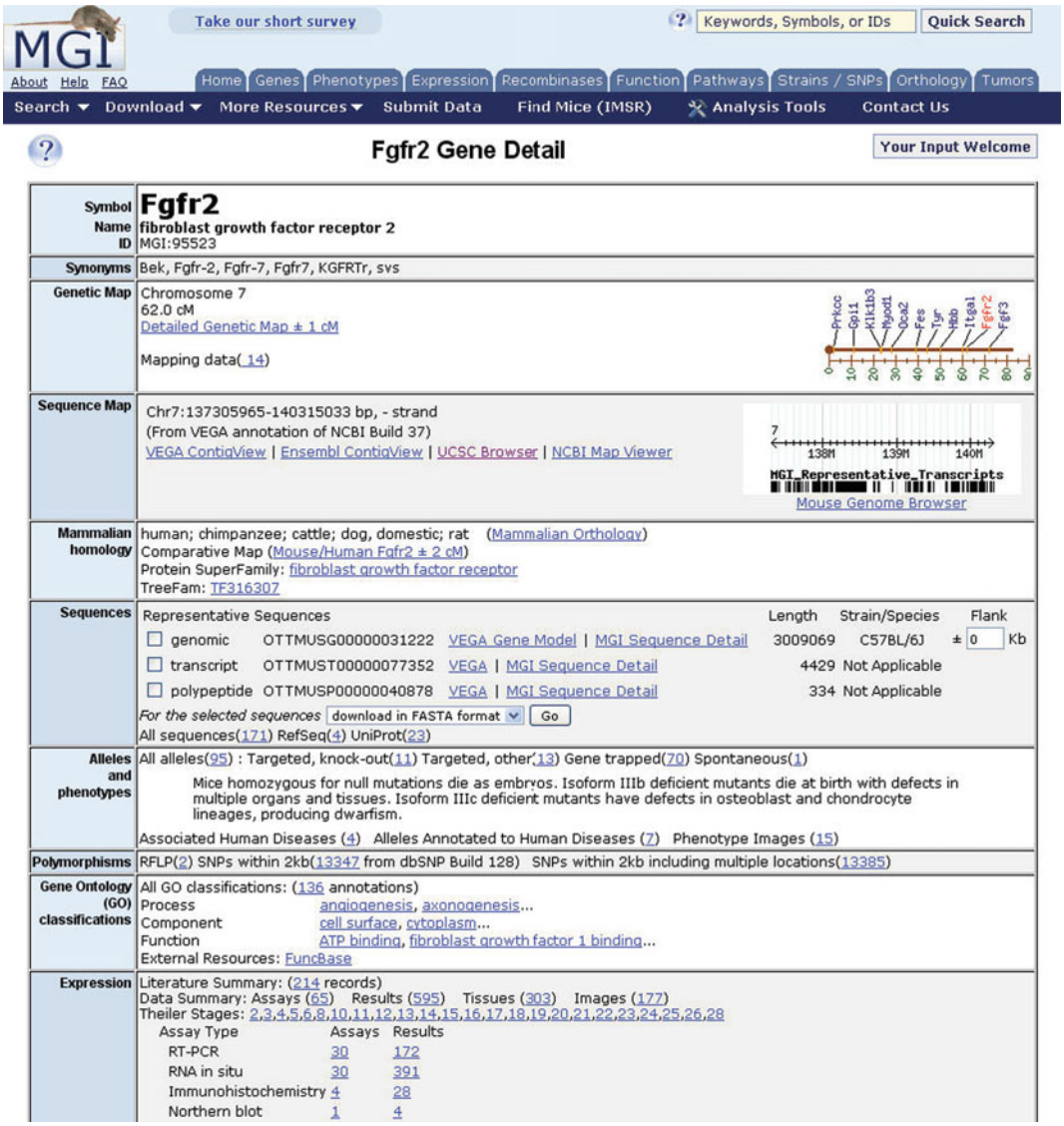

Fig. 2.6 Typical *example of MGI* search results regarding available information on the *Fgfr2* gene (list of topics covered is longer than shown, but it has been truncated for illustrative purposes). Each section is linked to additional sources of information.

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.

MGI 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.transtechsociety.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.europhenome.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_rerio/ 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/).

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