Gene Knockout Protocols

Second Edition

Edited by

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Preface

Following the completion of the mouse and human genome sequences, a major challenge is the functional characterization of every mammalian gene and the deciphering of their molecular interaction network. The mouse offers many advantages for the use of genetics to study human biology and disease, unmatched among other mammals. Its development, body plan, physiology, behavior, and diseases have much in common, based on the fact that 99% of the human genes have a mouse ortholog. The investigation of gene function using mouse models is based on many years of technological development. In the two decades since gene targeting in murine embryonic stem (ES) cells was first described by Mario Capecchi and colleagues, more than 3000 predesigned mouse mutants have been developed. To date, a variety of mouse mutagenesis techniques, either gene- or phenotype-driven, are used as systematic approaches. The availability of the genome sequence supports gene-driven approaches such as gene-trap and targeted mutagenesis in ES cells, allowing efficient and precise gene disruption. In combination with the use of site-specific DNA recombinases, in particular the Cre/loxP system, gene disruption can be directed to specific cell types in conditional mouse mutants. Furthermore, chemical and transposon mutagenesis of the mouse genome enables us to perform phenotype-driven screens for the unbiased identification of phenotype–genotype correlations involved in models of human disease. Over the next several years, the mouse genome will be systematically altered, and the techniques for achieving predesigned manipulations will be constantly developed further and improved.

The second edition of Gene Knockout Protocols brings together distinguished contributors with extensive experience in the gene targeting and mouse genetics fields. In line with the successful format of Methods in Molecular Biology, the volume provides a comprehensive collection of step-by-step protocols of use not only for the beginner in the field but also for experienced scientists. The new edition particularly emphasizes the range of new mutagenesis techniques developed over the last seven years, but also covers the basic methods relevant to researchers performing classical gene targeting experiments. The 25 chapters of this volume are organized into four sections on gene modification in ES cells, stem cell manipulation, the generation of genetically engineered mice, and mutant phenotype analysis. The contents reflect the diversification of mutagenesis approaches that now include, besides classical gene targeting, gene modification by oligonucleotides, gene trap mutagenesis, RNAi-mediated knockdown, transposon, and ENU mutagenesis. Conditional gene inactivation through Cre/loxP recombination is covered by chapters on the construction of conditional vectors for gene targeting, gene trap, gene knockdown, and chromosome engineering, complemented by chapters on the generation of constitutive and inducible Cre transgenic mice and the Cre mouse strain database. While most of the chapters describe methods to generate new mutants or transgenic mice the content is completed by techniques relevant for the preservation and phenotyping of mutants. These
include sperm freezing, ES cell line establishment, ES cell in vitro differentiation, mouse pathology, mutant phenotyping, and the influence of genetic background on phenotypes.

We hope that this new edition of *Gene Knockout Protocols* that provides a unique collection of bench protocols written by experts will be a valuable resource for all scientists in the field and will further stimulate research on mouse genetics.

Ralf Kühn  
Wolfgang Wurst
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Color Plate 1  Protocol for mES cell-derived cardiac differentiation. Five-day EBs were plated onto gelatin- or laminin-coated plates and cultured in IMDM+20%FCS supplemented with L-glutamine, NEAA, and MTG for up to 24 days. Multilineage progenitors at the intermediate stage 2 co-express nestin and desmin, while terminally differentiated cardiac clusters (stage 3) show well-organized sarcomeric staining of Z-disk epitopes of titin. Beating frequency measured from a beating cluster (phase contrast) by the LUCIA HEART imaging system is shown at the right, bar = 50 μm (see discussion on p. 229)

Color Plate 2  Protocol for mES cell-derived neuronal differentiation. ES cells were cultured as EBs for 4 days. After plating onto gelatin, cells were cultured in B1 supplements and FCS-containing medium for 24 h (*). After medium change (at day 4+1), EB outgrowths were cultured until day 4+8 without FCS to select for neural progenitors. At day 4+8, EBs were dissociated and replated onto poly-L-ornithine/laminin until day 4+14, when differentiation of mature neurons was induced by “Neurobasal” medium, B27 supplement, and SPFs (“survival promoting factors”). The table shows the media, additives, and substrates used with this protocol. Differentiation led to nestin-positive neural progenitors (stage 2) followed by β-III-tubulin-expressing neuronal cells at stage 3 (4+14 d) and dopaminergic neurons expressing tyrosine hydroxylase at stage 4. A phase contrast picture shows the morphology of the ES cell-derived neurons at stage 4 (right) (see discussion on p. 230)

Color Plate 3  Protocol for mES cell-derived pancreatic differentiation. Scheme displays media, additives, and substrates used during the differentiation process. Five-day EBs were plated onto gelatin for spontaneous differentiation in IMDM containing 20% FCS, L-Glut, NEAA, and MTG. At day 5+9, EBs were dissociated and replated onto poly-L-ornithine/laminin and subjected to differentiation by adding the differentiation factors niacinamide (NA), laminin, insulin, sodium selenite, transferrin, progesterone, and putrescine (and FCS for 24 h after plating). After medium change (at day 5+10), differentiation was continued (without FCS) until day 5+28. During spontaneous differentiation, nestin/CK19 co-expressing multilineage progenitors were formed (stage 2). Directed differentiation resulted in C-peptide/nestin-positive committed progenitors (stage 3) and insulin/C-peptide co-expressing islet-like clusters (stage 4; images
Color Plate 4  Protocol for mES cell-derived hepatic differentiation. Scheme displays media, additives, and substrates used during the differentiation process. Five-day EBs were plated onto gelatin for spontaneous differentiation in IMDM containing 20% FCS, L-Glut, NEAA, and MTG. At day 5+9, differentiation into the hepatic lineage was induced by dissociation of the EBs and replating onto collagen I. Cells were cultured in differentiation medium (HCM) containing 10% FCS until day 5+9+30. Spontaneous differentiation led to nestin/AFP-positive multilineage progenitors (stage 2). Differentiation resulted in albumin/AFP co-expressing committed progenitors at stage 3, and albumin- and AAT-positive, partially binucleated hepatocyte-like cells (stage 4, images from (53)) with cuboidal morphology (phase contrast, right, from (77)) at day 5+9+30. Cell nuclei were visualized by Hoechst 33342 (blue). Bars = 20 μm (see discussion on p. 232)