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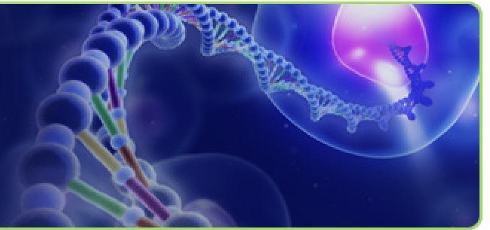

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A conditional knockout resource for the genome-wide study of mouse gene function

The parallel construction of conditional targeting vectors by serial 96-well BAC recombineering and high-throughput gene targeting in C57BL/6 ES cells was designed and presented in a recent publication by Skarnes et. al. Ongoing large-scale production of targeted ES cell lines demonstrates rates of homologous recombination in C57BL/6 ES cells well above the historical average. The generation of thousands of lacZ-tagged conditional alleles has been a major contributor to the international mouse knockout programs. accomplished due to this newly designed technology.

ECM 630 High Throughput Electroporation System

High-throughput ES cell production:
Electroporation conditions were optimized for C57BL/6N ES cells16 in multi-well cuvettes. The goal was to minimize the number of cells and amount of plasmid DNA required to obtain sufficient drug-resistant colonies for screening (Table 1). After selection, expansion and freezing, most (65%) ES cell clones retained their ability to colonize the germ line of mice. The final targeting constructs were prepared for ES cell electroporation. Before electroporation, vectors were linearized and examined by gel electrophoresis. JM8 mouse ES cell lines derived from the C57BL/6N strain were grown either on a feeder layer of SNL6/7 fibroblasts (neomycin and/or puromycin resistant) or on gelatinized tissue culture plates.

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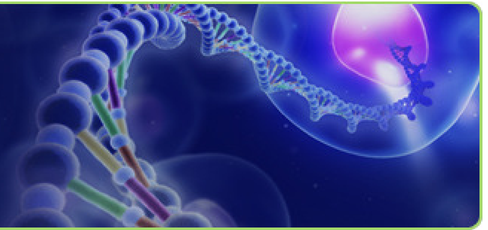

Electroporations of ES cells were carried out in a 25-well cuvette using the ***ECM 630 96-Well Electroporator /HT-200*** automatic plate handler set at 700 V, 400V, 25µF. Immediately before electroporation, cell suspensions of ~1 x 10^7 cells and, 2 µg of linearized targeting vector DNA were mixed in a final volume of 120 µl PBS. Cells were seeded onto a 10-cm dish (with feeders or gelatin) and colonies were picked after 10 d of selection in 100 µg (active) per ml Geneticin (Invitrogen). To expand cells into duplicate wells for archiving and preparation of genomic DNA, confluent cultures of JM8 ES cells grown on feeder cells were washed twice with pre-warmed PBS and trypsinized for 15 min at 37 ºC. Five volumes of pre-warmed media were added and the cells were gently dispersed by tituration and passed at a dilution of 1:4 into new plates containing feeder cells. Passage of cells grown on gelatinized plates was carried out in a similar manner except that the cells were trypsinized for 10 min and passed at a dilution of 1:6 into freshly gelatin-coated plates (0.1% gelatin, Sigma G1393). Culture medium was replaced daily and cells reached confluence 2 days after passage.

Results:
Half of all genes are expressed at a sufficient level in ES cells to support a targeted trapping strategy; a promoter-driven cassette for positive selection for non-expressed genes combined with negative DTA selection to select against random insertions. Different positive–negative targeting cassettes were electroporated and from the analysis of approximately 30 ES cell clones per unique construct, the authors recovered targeted events for 80% of genes with an average targeting efficiency of 35% (Table 1). A combination of factors probably contributed to their high targeting efficiencies, including the use of isogenic DNA, relatively long recombineered homology arms and DTA negative selection.

Table 1 | Targeting efficiency using promoterless and promoter-driven cassettes

Vector type	Number of unique targeting vectors	Number of successful electroporations	Number of colonies*	Number of genes targeted	Genes targeted (%)	Targeting efficiency (%)	Number of colonies screened*	Number of targeted clones*	Number of targeted clones with 3′ *loxP* site*
Promoterless	1,285	778	224	621	48	51	24	12	6
Promoter	1,811	1,671	348	1,440	80	35	29	10	3.5
Promoter (−DTA)	87	87	729	49	56	12	34	4	1

*Average values.

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