DelBank: a mouse ES-cell resource for generating deletions

Chromosomal deletions are valuable reagents for identifying and mapping genetic function. To simplify the creation of deletions in mice, we developed a collection of embryonic stem (ES) cell clones called DelBank. DelBank is based upon a technology in which radiation-induced deletions of herpes simplex virus thymidine kinase (tk) cassettes, inserted by homologous recombination into F1 hybrid ES cells, are selected with the antiviral drug 1-2'-deoxy-2'-fluoro-β-D-arabinofuranosyl-5-iodouracil (FIAU)1,2. The deletions range in size from less than 1 cM to over 20 cM. Cells of the F1 generation retain germline competence following irradiation, and the heterozygosity enables the molecular mapping of deletion endpoints.

DelBank consists of more than 90 F1 hybrid ES cell clones, each containing a randomly integrated tk-containing vector. We generated clones using either a plasmid vector (pBanTKcass3; Fig. 1a) or modified retroviral poly(A) trap vector1 (Fig. 1b). These were introduced into v6.4 (C57BL/6j×129S4/SvJae) or v17.2 (BALB/cJ×129SvEvBom) ES cells1 by electroporation (plasmid) or retroviral infection (gene trap), followed by selection for

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**Fig. 1** DelBank vector strategies. **a**, Plasmid rescue. DNA from transformed ES cell clones was cleaved with BglII, circularized and transformed into bacteria. The sequence of the 'rescued' mouse DNA was determined to generate amplifiers that identify SCPPs. **b**, Gene trapping. PA, poly(A) signal; SD, splice donor. Shown is a productive gene trap, where transcripts from an endogenous gene (left) undergo splicing into a vector splice acceptor (SA) and are terminated at vector PA, while the neo gene acquires a host PA. Arrowheads indicate LoxP sites.
transfectants in G418. We recovered DNA flanking the integration sites of single-copy insert pBanTKасс clones by plasmid rescue (Fig. 1). We sequenced the inserts, designed PCR primers to identify single-strand conformation polymorphisms (SSCPs) between Mus spretus and C57BL/6j, and then mapped the insertion sites on the BSS interspecific backcross mapping panel (www.jax.org/resources/documents/cmdatal). DelBank integration sites are distributed throughout the genome, with at least one per mouse chromosome (Fig. 2). We include some insertions that were targeted by homologous recombination to chromosomes 5 and 17 (refs. 1, 2). Several DelBank integrations lie near regions syntenic between Mus musculus and Mus spretus (refs. 1, 2). Several DelBank insertions lie near regions syntenic to those deleted in human contiguous gene syndromes.

As DelBank clones are useful only if they retain germline colonizing capability, 10 parental lines were evaluated by us and DelBank users, and at least eight produced germline chimeras. To explore the extent of DelBank’s utility, we successfully induced deletions and produced germline chimeras with a clone containing an insertion [Tg(kneo)20Jcs] near the mouse counterpart of the Prader–Willi syndrome (PWS) critical region on chromosome 15. We are expanding DelBank to reach a goal of over 200 clones. Many DelBank clones remain unmapped, but the insertion sites will become known as genome data become available. Assuming an average maximum viable deletion size in mice of approximately 5 cM (refs. 2, 5, 6), each clone could potentially yield deletions covering 10 cM, for a DelBank total of 2,000 cM, slightly more than the mouse genome size of approximately 1,500 cM. We maintain a searchable DelBank website (http://lena.jax.org/∼jcis/delbank.html) containing sequence and map positions of insertion sites and technical information on the construction and use of DelBank. Clones are freely available to academic researchers.

The ability to rapidly create deletion complexes along chromosomes will facilitate systematic functional analyses of the mammalian genome. Deletions can be used for multiple applications, including region-directed N-ethyl-N-nitrosourea saturation mutagenesis, modeling human deletion syndromes7,8, mapping existing mutations9 or defining quantitative trait loci10.

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Neal C. Goodwin1, Yasumasa Ishida2, Suzanne Hartford3, Cate Wnek3, Rebecca A. Bergstrom1, Philip Leder3 & John C. Schimenti1
1The Jackson Laboratory, Bar Harbor, Maine 04609, USA. 2Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan. 3Department of Genetics, Harvard Medical School, Howard Hughes Medical Institute, Boston, Massachusetts, USA. Correspondence should be addressed to J.C.S. (e-mail: jcs@jax.org).