Fast screening of mouse mutants generated after homologous recombination

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The screening of mouse mutants is usually time consuming and normally requires DNA analysis of tail tips either by conventional Southern blot analysis (1) or by Polymerase Chain Reaction (2). It would therefore be very useful if mice could be screened rapidly for the presence of the mutation with another method. This method would be especially advantageous when the homozygous mouse line cannot be maintained due to lethality or sterility before reaching adult age, and heterozygous mice have to be routinely tested to conserve this mutation.

A screening procedure based on the coat colour has been developed (3—5). Here we describe a test independent of the mouse genetic background based on the expression of LacZ in the skin of the ear of heterozygous mice.

We have generated mouse null mutants for the cell adhesion molecule E-cadherin (wvo-locus) and β-catenin. We designed the constructs so that the expression of LacZ reflects the expression of these two proteins; the lacZ gene was placed under the control of either the E-cadherin or the β-catenin promoter. This was accomplished by inserting the lacZ gene between the transcription start site and the translation start site for the E-cadherin gene (6) or replacing exon 2 of the β-catenin gene with the lacZ gene (H.H., L.L. and Rolf Kemler, in preparation). Normally these two genes are expressed in the skin. If the protein of interest is not expressed in the skin, then a promoter which is active in the skin can be placed in front of the lacZ gene in the construct. This promoter can be either ubiquitous or skin-specific. The expression of the LacZ marker can be followed in the ear clips of heterozygous mice.

Chimeric mice were obtained from homologously recombined ES cells and wild-type embryos; the chimeric males were crossed with females possessing the same background as the ES cells to generate congenic lines. Generally, ES cell lines are derived from 129/Sv. These ES cells are wild-type at the c-locus and white bellied agouti (A"/A"). A" is dominant to A, the wild-type allele of the agouti locus, and all lower alleles (7). The classical mice (C57Bl/6, Balb/c or CBA) which are used to produce the host embryos do not allow recognition of recombinant offspring by coat colour when the male chimeric mice are crossed with 129/Sv females. At weaning age, mice are classically numbered by ear clipping. Ear clips were collected in 24-well-plates, fixed for 40 min with 0.4% glutaraldehyde (v/v) in PBS at room temperature, stained without washing at 30°C, and left overnight in the X-gal solution [PBS containing 5 mM K-ferrocyanide, 5 mM K-ferricyanide, 2 mM MgCl2, 0.02% NP40 (v/v), 0.01% Na-deoxycholate (w/v) and 0.04% X-Gal (w/v)]. Using this method, we could screen over 200 mice efficiently at the same time in 2 active working hours without any problem. An example of such result is shown in Figure 1A. Over 300 mice were tested by LacZ staining and by Southern blot analysis (Fig. 1B) and a full agreement between these two techniques was observed. This assay has many advantages: it is quick (two working hours are required for 200 tested mice), easy, reliable (no false positive discovered) and cheap (compared to Southern blot analysis or PCR). Moreover, the number of mice is not limiting (200 mice can be treated at the same time), it is independent of the coat colour (black, agouti or albino mice can be clearly stained), β-

![Figure 1. Mouse genotyping by ear clip LacZ staining. Panel A: Ear clip staining of mice derived from a white heterozygous male (uvo" lacZ"/uvo" lacZ") with a white wild-type female (uvo" lacZ"/uvo" lacZ"). Ear clips were removed from 3 week old mice, fixed in glutaraldehyde and stained in the presence of X-Gal. LacZ positive ear clips are able to hydrolyze the non-coloured substrate to produce a blue colour that appears black on the picture. Panel B: Southern blot analysis of tail DNA isolated from the corresponding mice. DNA was digested with HindIII, separated on an agarose gel, blotted and hybridised with the b probe (6). Heterozygous mice present a polymorphism at the uvo-locus, so that the probe b hybridized to a 6.5 kb fragment of the wild-type allele and the 4.8 kb fragment of the mutant allele. Lanes 1 and 6 correspond to heterozygous mice (blue ears or two bands) and lanes 2—5 correspond to wild-type mice (white ears or one band).](https://example.com/figure1.png)

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galactosidase is stable (ear clips can be kept non-fixed at 4°C overnight), and finally it is especially useful when the mutation is homozygous lethal before adult age because mice have to be tested routinely.

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