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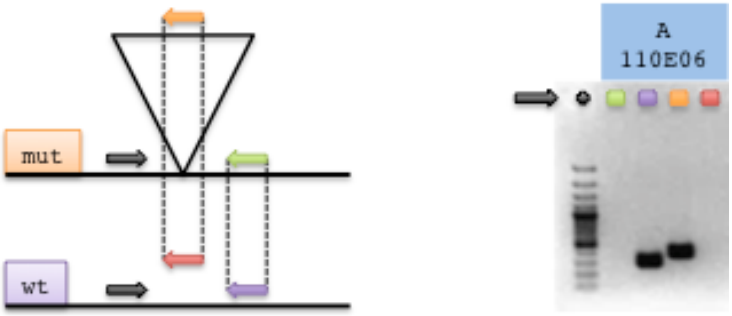
Integration PCR: PCR Strategy to Confirm the Genomic Integration Site of Gene Trap Cassettes

An alternative, more direct way to confirm the correct genomic integration site of any ES cell line in our collection is to use the sequence which we initially retrieved by iPCR and used to map the exact genomic location of the mutagen. By extracting the whole genomic sequence/locus for a particular gene from e.g. MGI, you can easily design primers* surrounding the "mapped sequence" and integration site, respectively. By doing so, you can run 4 PCR reactions on gDNA extracted from wildtype/unmodified and mutated ESCs in the following way: by using the primers flanking the potential genomic integration site on wildtype gDNA (wt, black and purple arrows; purple lane in A), you should obtain a single PCR band, confirming that the primers in fact work. Using the same primer pair on gDNA from the respective clone (mut, black and green arrows; green lane in A), you will not obtain a PCR band as the mutagen is too large for PCR amplification. Conversely, under the assumption that the genomic location of the mutagen was mapped correctly, you should obtain a PCR product with a mutagen-specific primer (mut, black and orange arrows; orange lane in A), but not on wt gDNA (wt, black and red arrows; red lane in A). This is graphically summarized below and a typcial example shown (A):



Should you observe any deviation from the expected pattern, (e.g. if you receive a PCR product in the green lane, or no PCR product in the orange lane), you should be cautious and compare the results also to the bcPCR
Possible explanations are (i) the retrieved sequence of the INT site was apparently mapped to the wrong chromosomal location, (ii) the clone is a mixed clone, i.e. it contains another clone, which could have been introduced during the production process, or (iii) the clone was initially not targeted homozygously.
Please make sure that you choose the right combination of primers in dependence of the orientation of the mutagen and gene locus, respectively (see below, preferred primer combinations are marked in red).

Mutagen-specific PCR primer sequences for

- Retro, Lenti, Tol2-GT:

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RV-INT-fwd	GCCAGAACCAGAAGGAACTTGAC
RV-INT-rev	TACAGACGCAGGCGCATAACATC
LV-INT-rev	AGAGCTCCTCTGGTTTCCCTTTC
T2-INT-fwd	GAGCCAGAACCAGAAGGAACTTG

- Tol2-pA:

T2-INT-rev	CCGGGCAATGGATTGATATTGCG
T2-INT-fwd	GAGCCAGAACCAGAAGGAACTTG

Integration site-specific PCR primer sequences
Int-F, custom, ~23 bps
Int-R, custom, ~23 bps
designed by Clone Manager 9 Software (Sci-Ed Software)

Primer combinations in dependence of gene trap insertion:

INT-PCR reaction (4 PCR rxns w/ respective primer combinations on gDNA from wt and mut)

crude ES cell lysate	5 µl	
FWD primer 5 µM	2 µl	
REV primer 5 µM	2 µl	
10 mM dNTP mix		1 µl
10x Klentaq buffer	5 µl	

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20x Klentaq polymerase	2 µl
dH2O	33 µl
	50 µl

Biorad C1000 cycle parameters …

95deg, 3'
95deg, 15'|
58deg, 20'| 35x
72deg, 32'|
72deg, 5'

analyze 20 ul on an agarose gel according to the scheme from above.

* Please be aware that you have to invert the extracted genomic sequence if the strand of integration for your clone is negative. You can find the ''(mapped) sequence'' and the information for the strand of integration for each clone on its ''cell information''-page. We recommend to design the primers in a way that you get a (wildtype-int) PCR product with a length of 200-500bp. And be aware that the primer sites shouldn't be or reach into the mapped sequence otherwise you miss the genetrap (depending on mutagen, see FAQs).