Application note

GVG Genetic Monitoring

Elimination of "off-target" sites in CRISPR/Cas9-generated animals using highly informative STR marker panels

CRISPR/Cas9 system is a convenient genome editing tool. However, due to its tendency to cut DNA not only in the target region but also in sequences similar to the target, "off-target" effects occur. The localization and subsequent removal of potential "off-targets" in transgenic mice is labour-intensive and time-consuming. By combining CRISPR/Cas9 technology with speed congenics using GVG GM's standard STR panel "off-targets" can be eliminated without prior knowledge of their localization.

Principle

Expample for a generation of a C57BL/6NCrl mouse with a CRISPR/Cas9 mutation

Step 1

- Generation of the desired CRISPR/Cas9 mutation in one of the C57BL/6J substrains
- Potential "off-targets" would have a "BL/6J" genetic background.

Step 2

- Backcrossing to substrain C57BL/6NCrl by speed congenics, positive selection for CRISPR/Cas9 mutation
- Since all genomic parts of "BL/6J" are replaced by C57BL/6NCrl, all "off-targets" are effectively removed from the genome.

Standard marker panel with 246 STR loci

GVG GM has developed a standard marker panel with 246 STR loci that is extremely flexible and can be applied to any combinations of inbred mouse strains or substrains.

- About 50 % can distinguish between different substrains of C57BL/6J and C57BL/6N
- About 85 % can distinguish between different inbred strains

Our service: Fast results, customer-friendly presentation of analysis data

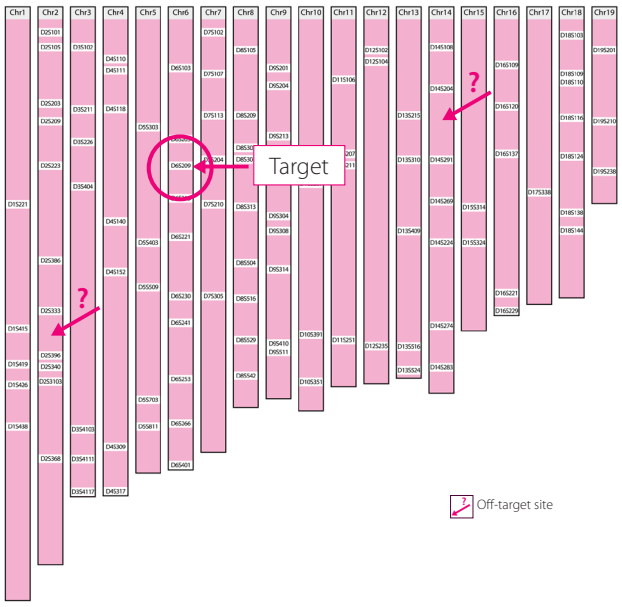
- Genotyping results within 10 working days
- Analysis data in customer-friendly tabular form and as an easy-to interpret karyogram (see examples overleaf)

We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

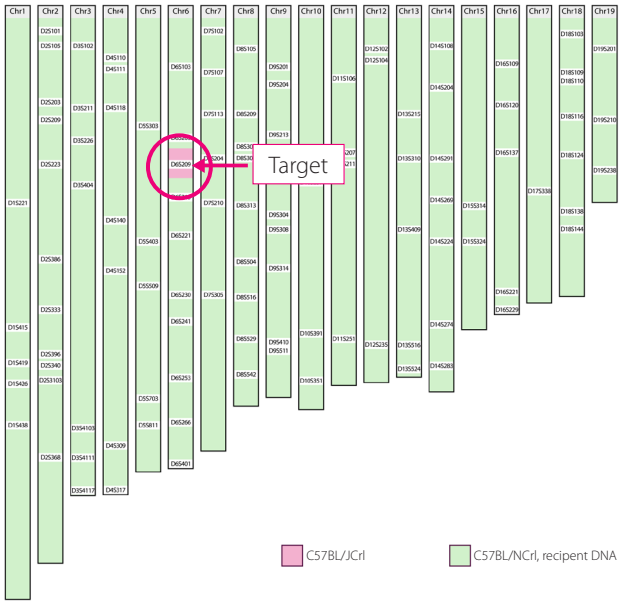
Ex.: Generation of a C57BL/6NCrl mouse with a CRISPR/Cas9 mutation

Figure 1: Karyogram of substrain C57BL/6JCrl



CRISPR/Cas9-mutated mouse of substrain C57BL/6JCrl with target region on chromosome 6 and two additional "off-target" regions on chromosomes 2 and 14

Figure 2: Karyogram of N5 generation, genetic background C57BL/6NCrl



CRISPR/Cas9-mutated mouse after five backcrossing generations. Thanks to the replacement of "BL/6J" genetic background by recipient DNA of substrain C57BL/6NCrl, all "off-target" sites have been eliminated.

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