Application Note

Rapid and effective genotyping of Cre transgenic mice

Norman Moullan

Laboratory of Integrative Systems Physiology (LISP), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Sizes of Cre gene-specific DNA fragments from the Cre gene were unambiguously identified using the QIAxcel® system. The accuracy of the system allowed rapid and reliable identification of Cre transgenic mice.

Introduction

The Cre-lox system, which is not naturally present in the mouse genome, is a molecular tool for genome manipulation and has been successfully used to generate mouse mutants (1, 2). Initially, transgenic mouse lines are produced: one expressing the Cre recombinase and one carrying 2 *loxP* sites (34 bp sequences). Upon crossing Cre and loxP strains, the Cre recombinase cuts specifically at the *loxP* sites in tissues where Cre transgene is expressed (3, 4). Subsequent recombination of *loxP* sequences leads to rearrangements of the genome. Depending on the orientation of the *loxP* sites, deletions, inversions, or chromosomal translocations are generated. By targeting Cre recombinase to tissues of interest, conditional knockout mutants can be generated (2).

To test the successful insertion of the Cre transgene into the genome of transgenic mice, PCR-amplified Cre gene sequences were identified using QIAxcel system.

Materials and methods

Rapid genomic DNA extraction from mouse ear tissue was performed by incubating samples in 0.1N NaOH at 70–95°C for up to 10 minutes. Samples were placed on ice for 5 minutes and 30 µl 170 mM Tris·HCl (pH 8.0) was added. Samples were vortexed and centrifuged for 4 minutes at maximum speed, and 3 µl of the supernatant was used for PCR amplification.

Primers specific for the Cre transgene (sense: 5′- GAACC TGATG GACAT GTTCA GG -3′; anti-sense: 5′- AGTGC GTTCG AACGC TAGAG CCTGT -3′) were used to amplify a 320 bp fragment. Primers specific for the myogenin gene (sense 5′- TTACG TCCAT CGTGG ACAGC -3′ and anti-sense 5′- TGGGC TGGGT GTTAG CCTTA -3′) were included in the reaction to amplify a 250 bp control fragment. ▷

Standard amplification reactions (25 µl) were prepared using 0.4 µM of each primer. After initial denaturation at 95°C for 5 minutes, reactions were subjected to 35 cycles of 95°C, 62°C, and 72°C for 30 seconds each. Reactions were incubated for a final elongation at 72°C for 5 minutes.

Samples were analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit and the AL320 method. The QX Alignment Marker 15 bp/1 kb and the QX DNA Size Marker 50–800 bp were included in the analysis.

Results and Discussion

The QIAxcel system was used to detect the presence of the Cre transgene in putative transgenic mice. Figure 1 shows the results of genotyping in which the Cre-specific DNA fragment (320 bp, present in lanes 2, 6, 11, and 12) as well as the control fragment from the myogenin gene (250 bp, present in all lanes) were unambiguously identified.

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. Using the BioCalculator Software, the presence of specific DNA fragments as well as their sizes were accurately determined.

Cre transgenic mice will be used in breeding for knockout functional analysis.

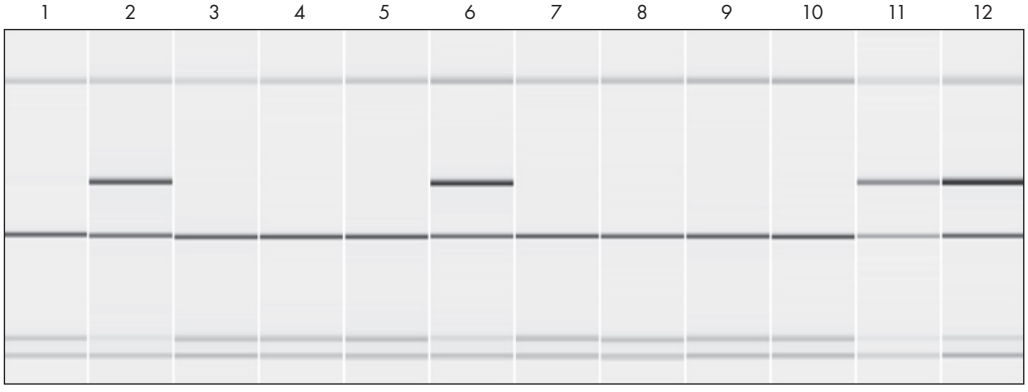

Figure 1. Detection of the Cre transgene in mice. DNA was isolated from mouse ear tissue and subjected to PCR amplification using primers for the Cre transgene as well as for a myogenin control. PCR products were analyzed on the QIAxcel system using the QIAxcel DNA Screening Kit. The 250 bp control fragment amplified from the myogenin gene is present in all lanes, indicating that amplification reactions were successful. The 320 bp fragment present in lanes 2, 6, 11, and 12 indicates the presence of the Cre transgene in these mice.

Conclusions

- The fragments amplified from the Cre transgene and from the myogenin control (320 bp and 250 bp, respectively) were easily distinguished using the QIAxcel system, allowing reliable identification of transgenic mice. The sizing accuracy and sensitivity obtained using the QIAxcel system is superior to conventional methods, such as agarose gel electrophoresis (data not shown).
- Due to automated electrophoresis analysis as well as automated data acquisition, the QIAxcel system ensures safe and reliable results.
- Because the QIAxcel system enables short running times and analysis of up to 96 samples in a single run, it is ideally suited for medium- to high-throughput mouse genotyping, significantly saving time.
- QIAxcel analysis results are fully reproducible due to controlled running conditions and automated data acquisition.
- Since QIAxcel capillary electrophoresis uses only minute quantities of DNA for electrokinetic injection, the samples are retained for downstream procedures, such as sequencing.

References

1. Araki, K., Imaizumi, T., Okuyama, K., Oike, Y., and Yamamura, K. (1997) Efficiency of recombination by Cre transient expression in embryonic stem cells: comparison of various promoters. J. Biochem. **122**, 977.
2. Nagy, A. (2000) Cre recombinase: the universal reagent for genome tailoring. Genesis **26**, 99.
3. Dali-Youcef, N. et al. (2007) Adipose tissue-specific inactivation of the retinoblastoma protein protects against diabesity because of increased energy expenditure. Proc. Natl. Acad. Sci. **104**, 10703.
4. Mataki, C. et al. (2007) Compromised intestinal lipid absorption in mice with a liver-specific deficiency of liver receptor homolog 1. Mol. Cell. Biol. **27**, 8330.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com/cre-transgenic**s and find out how automated gel electrophoresis can benefit your lab!