APPLICATION NOTE

LabChip® GX Touch™ Nucleic Acid Analyzer

Authors

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Fast Genotyping of Genome-Edited Animals using Heteroduplex Mobility Assay and the LabChip® GX Touch™ Instrument

Introduction

In the few past years, engineered endonucleases (Meganucleases, ZFN, TALENS) and CRISPR/Cas9 technology have revolutionized genome editing possibilities. Genome editing can now be used in a wide range of species to generate gene inactivation (knockout) or small nucleotide changes (knockin). These endonucleases initiate double-stranded DNA breaks which are repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). The error-prone NHEJ pathway leads to nucleotide insertion (ins) or deletion (del), termed indels, which disrupt the targeted open reading frame, causing gene inactivation. Single-stranded donor oligonucleotides (ssODN) with single nucleotide or codon changes at the target site are used to make knockin edits via the HDR pathway.

CRISPR/Cas9, the easiest and least costly gene editing system, is widely used in mutation generation in various species. With increasing throughput, an effective mutation detection method is needed. Using on-target gene editing PCR in combination with the LabChip GX Touch instrument reveals both the amplicon of the target site and the heteroduplex (HD) signature, indicating whether NHEJ has occurred, even if it is a single base pair indel. For F_0 and F_1 generations, PCR sequencing should be performed to confirm the exact nucleotide mutation. For further generation genotyping analysis, the HD pattern alone can be used to determine whether NHEJ has occurred.

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Materials and Methods

Genomic DNA samples (from tails or ears, with or without purification) obtained from gene-edited animals were first amplified with on-target PCR. These PCR products, without any further processing, were analyzed on the LabChip GX Touch instrument. Due to the presence of indels in samples, heteroduplexes formed by non-fully complementary PCR-amplified DNA strands have different migration properties than homoduplexes when separated using capillary electrophoresis. The open, single-stranded configuration surrounding the mismatched region causes the mutation-specific HD to migrate more slowly than fully homologous PCR products (WT or homozygous).

Results

Capillary electrophoresis analysis of on-target PCR products performed with the LabChip DNA 5K assay showed an obvious distinction between a non-mutated (+/+) sample and a mutated homozygous (-/-) sample. The presence of HD denotes a heterozygous (+/-) sample (Figure 1). For a given mutation in each run, HD bands function as specific mutation signatures. An F_1 sample carrying a (+/-) genotype was used as a control. On an established line with a known mutation, starting with the F_2 generation and going forward, only HD patterns were used to determine offspring's genotypes.

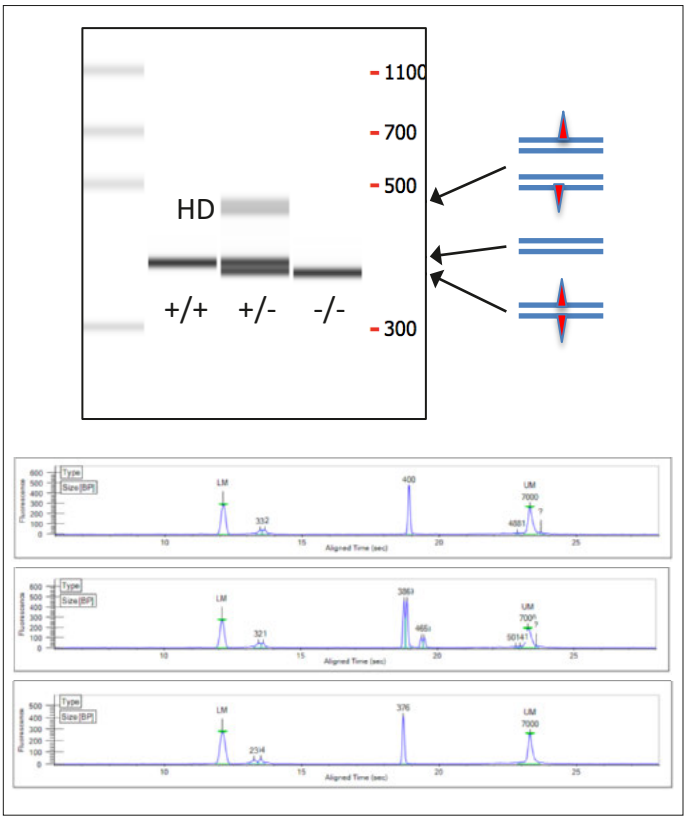

Figure 1. Typical genotyping results. Genomic DNA samples were on-target PCR amplified and electrophoretically separated. Wild Type allele (WT) and homozygous mutated allele (Ho Del 11 pb) were clearly separated. The presence of heteroduplexes (HD) demonstrated the heterozygous status.

On a new genome-editing project, the on-target PCR from the first F_0 tested animals showed a different HD pattern (samples C, D, E, Figure 2), indicating that NHEJ occurred in these animals. All PCR products from F_0 animals were sequenced to determine the exact nature of the mutation. After sequencing analysis, the animals with frame-shift mutations were crossed with a wild type (WT) specimen to generate F_1 animals and establish a mutated knockout line.

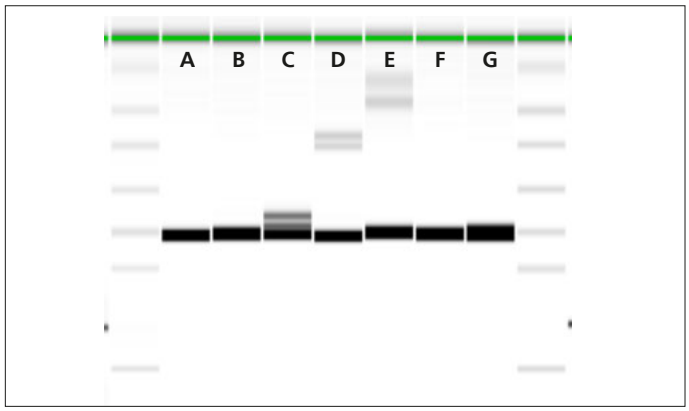

Figure 2. F_0 PCR samples. F_0 genomic DNA samples were on-target PCR amplified and electrophoretically separated. On this first run, samples C, D, and E showed HD, illustrating that NHEJ had occurred at the DNA level. Samples A, B, F, G appeared to be WT for all samples. DNA sequencing specified the exact mutation.

When differences between homozygous WT and homozygous mutated samples (A, C, D, E) were not obvious (Figure 3), HD was used as a reference for differentiation. This technique, called the WT test, is less expensive than the T7 Endonuclease I assay. After mixing the unknown PCR product with an equal quantity of WT PCR product, followed by a denaturation-renaturation cycle (15'), unknown PCR products were electrophoretically separated. If the unknown sample was WT, the sample band appeared to be like a WT band (D, E). If the unknown sample was homozygous, then HD appeared (A, C).

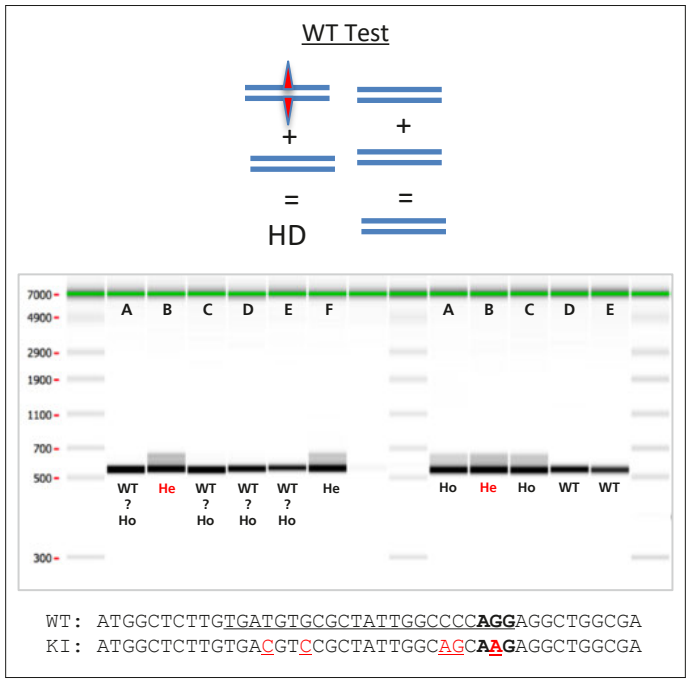

Figure 3. F_2 PCR samples (FLNA) + WT test. F_2 genomic DNA samples were on-target PCR amplified and electrophoretically separated. In this case, KI with 5 changing bases, WT samples, or Ho mutated samples were identical. The samples were clearly identified by the presence of the HD. To specify the genotype of the unknown samples (A, C, D, E), each PCR product was mixed with a WT PCR product, followed by a denaturation-slow annealing cycle. Following completion of the run, if the sample keeps its WT (D, E) signature, then it is WT. If the sample shows HD (A, C) then the sample is mutated homozygous. Sample B is a heterozygous sample as HD is the control.

Conclusion

Here we have demonstrated a rapid and efficient method to genotype samples from genome-edited animals. This method requires very simple manipulation of the samples (PCR and capillary electrophoresis) and can be used for high-throughput analysis. When genome editing lines are established, the use of sequencing is not necessary because the HD signature can be used, dramatically reducing analytical cost.

Reference

1. Chenouard V, Brusselle L, Heslan JM, Remy S, Ménoret S, Usal C, Ouisse LH, NGuyen TH, Anegon I, Tesson L. A Rapid and Cost-Effective Method for Genotyping Genome-Edited Animals: A Heteroduplex Mobility Assay Using Microfluidic Capillary Electrophoresis. J Genet Genomics. 2016 May 4. (43):341-48.

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