Journal of Clinical and Translational Pathology **2022** vol. 2(3) | 108–115
DOI: 10.14218/JCTP.2022.00014

Original Article

A Simple, Robust, and Cost-effective Method for Genotyping Small-scale Mutations

Liang Zheng[1,2#*], Jake Hill[1#], Lucy Zheng[1], M.A. Karim Rumi[1,2] and X. Long Zheng[1,2*]

[1]Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, Kansas City, KS, USA; [2]Institute of Reproductive and Developmental Sciences, The University of Kansas Medical Center, Kansas City, KS, USA

Received: June 8, 2022 | **Revised:** August 15, 2022 | **Accepted:** August 24, 2022 | **Published:** September 14, 2022

Abstract

Background and objectives: Genotyping is crucial for studying gene functions in animals and detecting genetic variants in humans. Traditional methods using agarose or polyacrylamide gel electrophoresis focus on detecting large-size differences (20–2,000 bp) between wild-type and mutant genes. While endonuclease digestion can identify heterozygous mutations, it fails to distinguish wild-type genes from homozygous mutants of similar size. This study aimed to develop a novel, simple, and reliable genotyping method for animals or cells following genetic modifications. ***Methods:*** We introduced an improved genotyping method utilizing 2% agarose gel electrophoresis after T7E1 or Surveyor endonuclease digestion to initially separate heterozygous mutations from wild-type and homozygous mutations. By adding a wild-type PCR product to potentially homozygous samples, forming heteroduplexes, we differentiated wild-type from homozygous mutations with nearly identical sizes or single base pair substitutions without relying on Sanger sequencing. ***Results:*** This method was validated in genotyping zebrafish mutants with 2-8 bp deletions or insertions and mouse mutants with 1- or 8-bp substitutions. Agarose gel clearly distinguished wild-type, heterozygous, and homozygous mutations ranging from 1–8 bp. Sanger sequencing confirmed the accuracy of our genotyping results. ***Conclusions:*** Our novel and improved genotyping method offers a rapid, economical approach for genotyping small deletions or single base pair substitutions. This technique has broad applications in clinical and research laboratories, especially in the era of gene editing and for detecting naturally occurring mutations.

Citation of this article: Zheng L, Hill J, Zheng L, Rumi MAK, Zheng XL. A Simple, Robust, and Cost-effective Method for Genotyping Small-scale Mutations. J Clin Transl Pathol 2022;2(3):108–115. doi: 10.14218/JCTP.2022.00014.

Keywords: Agarose gel electrophoresis; Polymerase chain reaction; T7E1 endonuclease; Genotyping; Gene editing.

Abbreviations: ADAMTS13, A disintegrin and metalloprotease with thrombospondin type 1 repeat, 13; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; T7E1, T7 endonuclease I; TAE, Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer.

[#]These authors contributed equally.

[*]**Correspondence to**: Liang Zheng, Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, United States. ORCID: https://orcid.org/0000-0003-0120-9343. Tel: +1-205-213-3847, Fax: +1-913-588-7073. E-mail. lzheng@kumc.edu; X. Long Zheng, Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, United States. ORCID: https://orcid.org/0000-0003-1680-5295. Tel: +1-913-588-7071, Fax: +1-913-588-7073, E-mail. xzheng2@kumc.edu

Introduction

Genotyping is an important and essential process for conducting the research of genes and gene variants associated with human diseases. Polymerase chain reaction (PCR) coupled with agarose[1–3] or SDS-polyacrylamide gel electrophoresis (PAGE)[4] has been extensively used for genotyping single genes with a large deletion or insertion of nucleotides ranging from 20 to 2,000 bp.[1–3] However, due to the limited resolution of the gel electrophoresis-based methods, it might be difficult to determine the genotype by simply relying on the size difference of the targeted PCR products between the wild-type and mutant genes if the area of change in the nucleotide is small.[5] As such, a high-resolution melting curve analysis would allow for discrimination between the wild-type and heterozygous genotypes based on the fluorescence changes during the DNA melting phase after the PCR.[6,7] This method requires a strict primer design as 100-bp amplicons are optimal for the detection of a nuclease-induced small insertion or deletion.[7,8] However, this method may not appear to be sensitive enough to differentiate between the wild-type and small-scale homozygous mutations given that the melting curves would be very close to each other.[8] For a single or two base pair substitution, deletion, and insertion, it is nearly impossible to know the genotype without sequencing. Therefore, Sanger sequencing is the gold standard method for identifying such small area mutations. Nevertheless, it would still require purification of high-quality DNA, which would be time-consuming and costly for a large number of samples. Thus, there is an unmet need for a simple, rapid, reliable, and low-cost method for genotyping animals in a routine research laboratory.

T7 endonuclease I (T7E1)[9–11] and Surveyor[12,13] endonuclease recognize and cleave heteroduplexes with more than two base pairs and single nucleotide mismatch, respectively. The introduction of the endonuclease digestion has allowed for separation of a heterozygous mutant from a wild-type or homozygous mutant genotype as a result of the heteroduplex formation in amplified PCR products after denaturation and reannealing. However, the endonuclease digestion alone does not distinguish a wild-type genotype from a homozy-

Table 1. Primers used for the PCR amplification

Gene name	Gene symbol	Primer sequences
Ankyrin repeat domain protein, 26	*ankrd26*	Forward: TTATTCTTAGAGAATGGGCG Reverse: CCTTATCCAGCTGGTTTAAA
A disintegrin-like metalloproteinase with thrombospondin motif type 1 member, 13	*adamts13*	Forward: AGATTCACTGAAACCTCAAG Reverse: CAGCTGCTCGCAACACATATC
von Willebrand factor	*vwf*	Forward: GCTTTGGTCTACACTAGATG Reverse: TAAATACAGCAAGATGTGCATG
Disruptor of telomeric silencing 1-like	*dot1l*	Forward: CTGTGGGGCAGTTTGTACCT Reverse: CTCCACAAGGGACAGCATGT
Complement factor H	*cfh*	Forward: ATTGACCAGCTACAGACAGTATCA Reverse: CATGCATGTGCCTTTCTAAACA

gous mutation because the amplified PCR product of either genotype does not form the heteroduplex that is required for endonuclease cleavage.

Here, we describe a new, simple, robust, and low-cost method using routine agarose gel electrophoresis of the PCR products following T7E1 or Surveyor nuclease digestion for genotyping animals with a small-scale insertion, deletion, or substitution in the targeted sequence, which is in many cases difficult to differentiate from the original wild-type sequence. Such a method may be widely used in many research laboratories for genotyping animals.

Materials and methods

Genomic DNA extraction and PCR

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (US). All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kansas Medical Center, Kansas City, KS, USA. Tissue samples were obtained from the animals to be genotyped and wild-type controls. Tail fin cuttings and tail clippings were obtained from zebrafish and mice, respectively. Genomic DNA was extracted using a cell lysis buffer containing protease plus (Biotool, Jupiter, FL, USA). PCR was performed using Invitrogen Platinum™ II Hot-Start Green PCR Master Mix (2x) for 20-μL reactions with gene specific primers (Table 1). The PCR program was as follows: A temperature of 95°C for 4 min for initial denaturation, then 35 cycles at 95°C for 30 s for denaturation, 57°C for 30 s for annealing, 72°C for 30 s for extension, and the final extension was conducted at 72°C for 10 min.

Heteroduplex formation and digestion

The PCR product (10 μL) of each sample was then denatured and reannealed with or without addition of the wild type PCR product using a PTC-200 Peltier Thermal Cycler following the heating cycle: 95°C for 2 min, −2°C/s to 85°C, and −0.1°C/s to 25°C. The resulting products were digested using T7E1 (New England BioLabs, Ipswich, MA, USA) for mutations ≥2bp or using a Surveyor (Integrated DNA Technologies, Coralville, IA, USA) for a single point mutation.

Agarose gel electrophoresis

After digestion, the samples were resolved via 2% agarose gel electrophoresis. Eight microliters of each sample was loaded into the gel and run in 1x Tris-acetate-EDTA (TAE) running buffer.

Sanger sequencing

Sanger sequencing was utilized to verify the genotyping method by sequencing the targeted PCR products. This process was performed at McLab (South San Francisco, CA, USA). Initially, the targeted PCR products were prepared and purified to eliminate any residual primers, nucleotides, or enzymes. These purified products were then mixed with a sequencing reaction mix, which included a sequencing primer, DNA polymerase, and a combination of normal dNTPs and fluorescently labeled ddNTPs. During thermal cycling, DNA strands were denatured, the sequencing primer annealed to the single-stranded DNA, and DNA polymerase synthesized new DNA strands. The random incorporation of ddNTPs resulted in DNA fragments of varying lengths, each terminating at a fluorescently labeled base. These fragments were separated by size using capillary electrophoresis. As fragments passed through the capillary, a laser excited the fluorescent dyes, and a detector recorded the emitted fluorescence. The resulting chromatogram displayed the DNA sequence as colored peaks corresponding to each base. This sequence data was then aligned with reference sequences to confirm the presence of wild-type, heterozygous, and homozygous mutations. The Sanger sequencing results corroborated the genotyping outcomes, thereby validating the accuracy and reliability of the novel genotyping method.

Results

The new assay design

Figure 1 outlines a general protocol for the new genotyping method. The first step was to amplify the targeted sequences using a routine PCR from genomic DNA isolated from animals. The PCR products were denatured and annealed in the absence or presence of a wild-type PCR product and digested with T7E1 or Surveyor endonuclease. The digested materials were then separated using 2% agarose gel stained with GelRed nucleic acid staining dye (Fisher Scientific, Hampton, NH< USA). As predicted, in the absence of an added wild-type PCR product, it was easy to identify the heterozygous animal from the wild-type and homozygous mutant animals because the heteroduplexes that formed in the PCR products of the heterozygous samples were efficiently cleaved by T7E1 or Surveyor endonuclease; in the presence of an added wild-type sequence, the homozygous mutant animals could be easily separated from the wild-type animals as the added wild-type sequence resulted in the formation of heteroduplexes with the homozygous mutant sequence, but not with its own wild-type sequence that would be sensitive to prote-

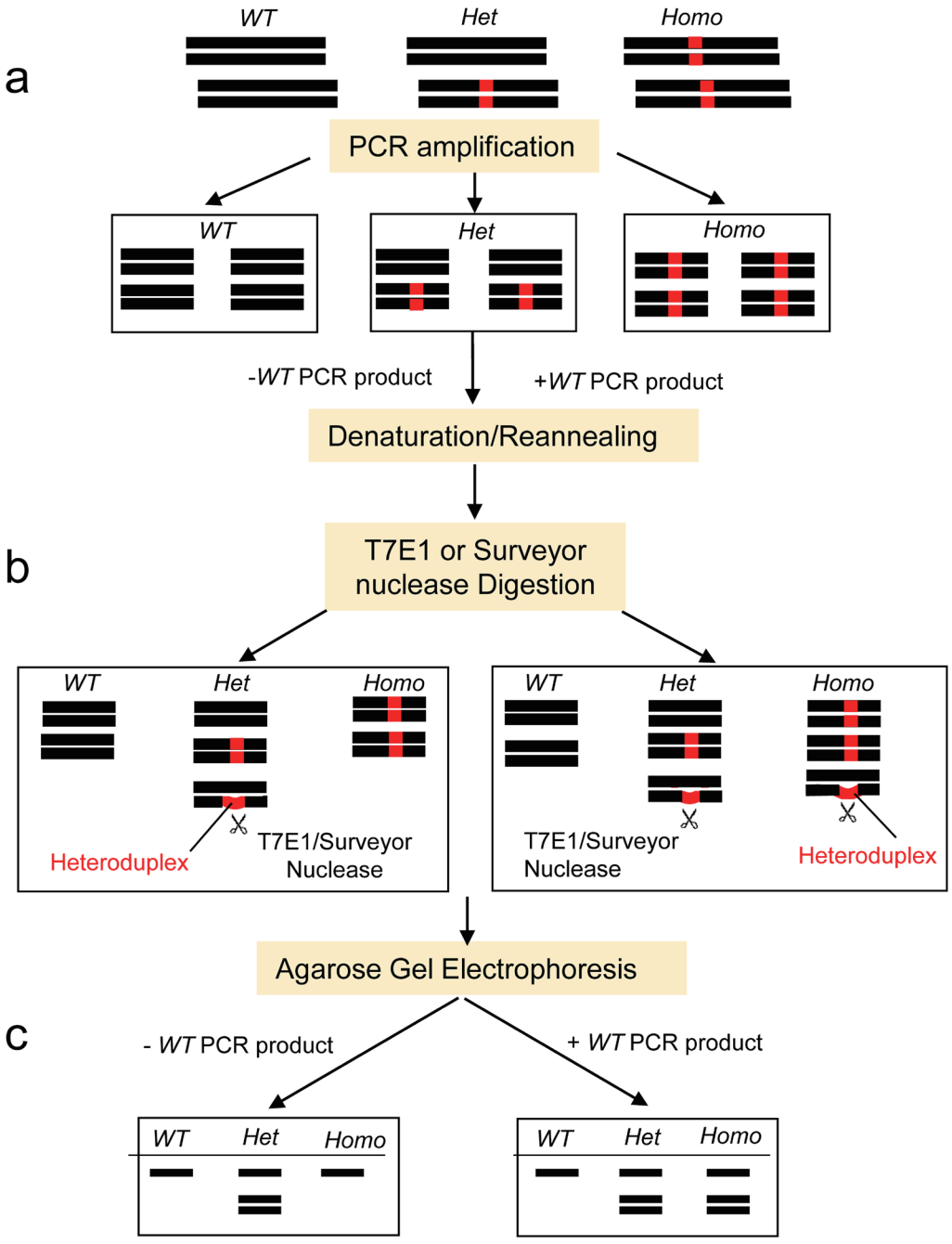

Fig. 1. An outlined protocol for the new genotyping method. Genomic DNA was released from the samples following tissue lysis. The cell lysate was then directly used for the PCR to amplify the target gene region. The PCR product was split into two aliquots. In one aliquot, a wild-type (*WT*) control PCR product was added; in another aliquot, no *WT* control PCR product was added. The PCR products were then denatured at 95°C and reannealed. Heteroduplexes of the DNA fragments were expected to form in the heterozygous (*Het*) mutant products in this step. However, when mixed with a *WT* control PCR product, the artificial heteroduplexes of DNA were also expected to form in the homozygous (*Homo*) mutant samples, but not in the wild-type samples. T7 endonuclease I (T7E1) recognized and cleaved the heteroduplexes with a mismatch ≥2 base pairs but the Surveyor nuclease could cleave a single base mismatch and indels up to at least 12 nucleotides. Thus, we could clearly differentiate the *WT*, *Het*, and *Homo* animals with various sizes of mutations based on the cleaved products revealed on 2% agarose gel electrophoresis.

olysis by endonuclease.

Identification of an 8-bp deletion mutation in adamts13 using agarose gel electrophoresis following T7E1 digestion

To begin assessing the utility of this new genotyping strategy, we began with genotyping zebrafish containing an 8-bp deletion mutation in zebrafish *adamts13 (*a disintegrin and metalloprotease with thrombospondin type 1 repeat, 13*)* (Fig. 2a).[8] Following the PCR amplification, T7E1 digestion with or without addition of a wild-type PCR product resulted in two cutting bands (~230 bp and ~310 bp) in the heterozygous

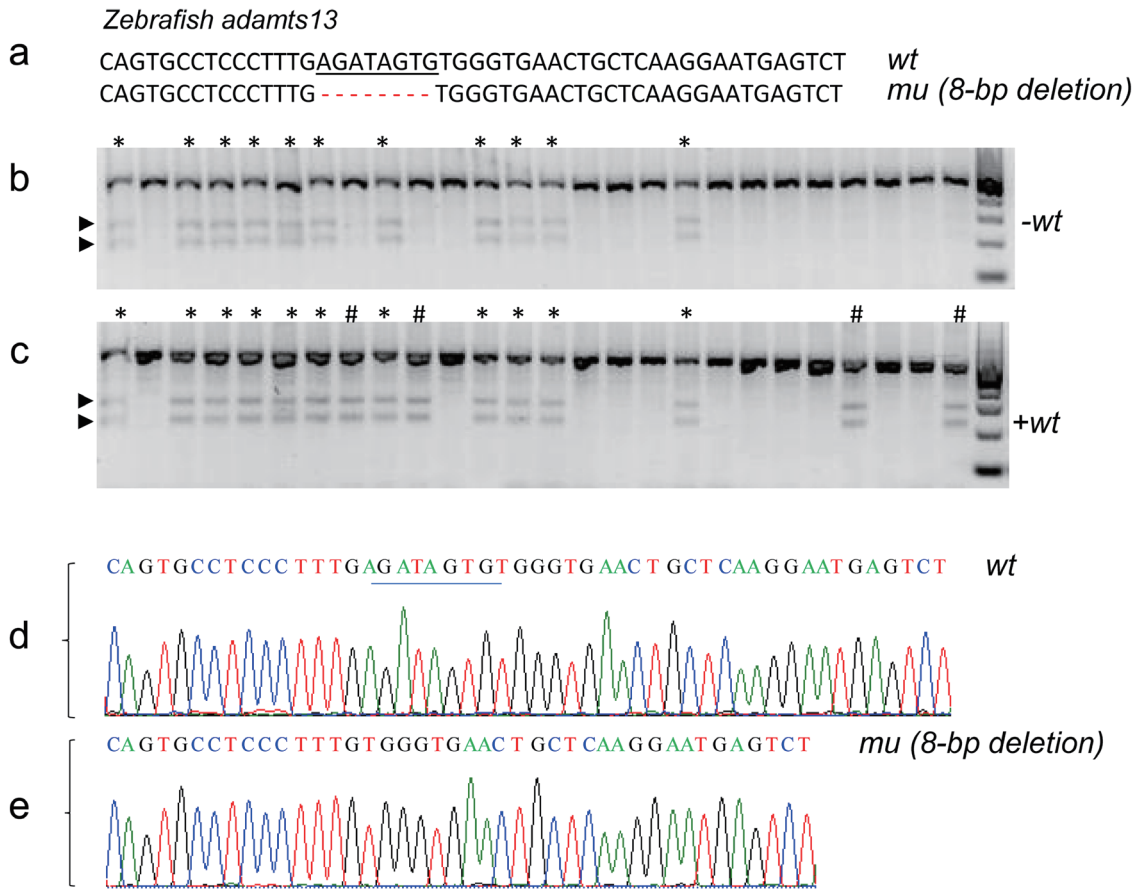

Fig. 2. Genotyping *wild-type*, heterozygous, and homozygous 8 bp-deletion mutation in zebrafish *adamts13*. (a) The expected wild type (*wt*) and mutant (*mu*) (8-bp deletion) sequences of zebrafish adamts13. (b–c) are the T7E1 digestion patterns of the PCR products in the absence (−*wt*) and presence (+*wt*) of the *wt* PCR product, respectively. Here, * and # indicate the presence of the cleavage bands (arrowheads) in the absence and presence of a *wt* sequence, respectively. (d–e) show the Sanger sequence tracings of *wt* and *mu* (−8 bp), respectively.

mutant products, but not in the wild-type and homozygous mutant products (Fig. 2b). The addition of a wild-type PCR product to the potential homozygous nutant sequence resulted in efficient cleavage of the artificial heteroduplexes formed between the added wild-type sequence and the homozygous mutant sequence (but not in the wild-type samples) (Fig. 2c). Sanger sequencing confirmed the accuracy of the wild-type (Fig. 2d) and mutant (Fig. 2e) sequences of zebrafish *adamts13*. These results demonstrated that the new method could quickly and accurately identify all three genotypes in animals with an eight-nucleotide deletion.

Identification of a 2-bp deletion mutation in ankrd26 using agarose gel electrophoresis following the T7E1 digestion

To further confirm the robustness of this new assay, we took advantage of another existing zebrafish line in the lab with a 2-bp deletion in a gene encoding ankyrin repeat domain-containing protein-26 (*ankrd26*) (Zheng *et al*., 2021a, unpublished) (Fig. 3a). Following the heterozygous-to-heterozygous breeding, we anticipated to generate wild-type, heterozygous, and homozygous mutant animals. As shown, the heterozygous animals were easily differentiated from the wild-type or homozygous mutant animals as there were two resulting cutting bands in the PCR products of the heterozygous animals following the T7E1 endonuclease digestion (Fig. 3b). However, there was no difference with regard to the size of the PCR products between the wild-type and homozygous mutant sequence and no resulting cutting band observed in those PCR samples following the T7E1 digestion. Again, we added a wild-type PCR product to either a prospective wild-type or homozygous mutant PCR product to create an artificial heterozygous PCR product. These artificial heteroduplexes became sensitive to digestion by the T7E1 endonuclease (Fig. 3c). This provided an easy and reliable method to separate the wild-type from the homozygous mutant animals. All the genotyping (delGC) results based on this new method were also confirmed by Sanger sequencing with 100% accuracy (Figs. 3d, e). These results suggest that the new method allows quick and accurate genotyping animals with two nucleotide deletion.

Identification of a 7-bp insertion mutation in vwf using agarose gel electrophoresis following the T7E1 digestion

We created *a* zebrafish line with a 7-bp insertion in *vwf* (Von Willebrand facor) (Fig. 4a*),* resulting in *vwf*$^{-/-}$ phenotype.[8] As shown, two cut bands (~210 bp and ~350 bp) were detected following the T7E1 digestion in the PCR products from the heterozygous animals with a 7-bp insertion in *vwf* (Fig. 4b). Again, addition of a wild-type PCR product to a potential homozygous mutant PCR product resulted in an expected cut

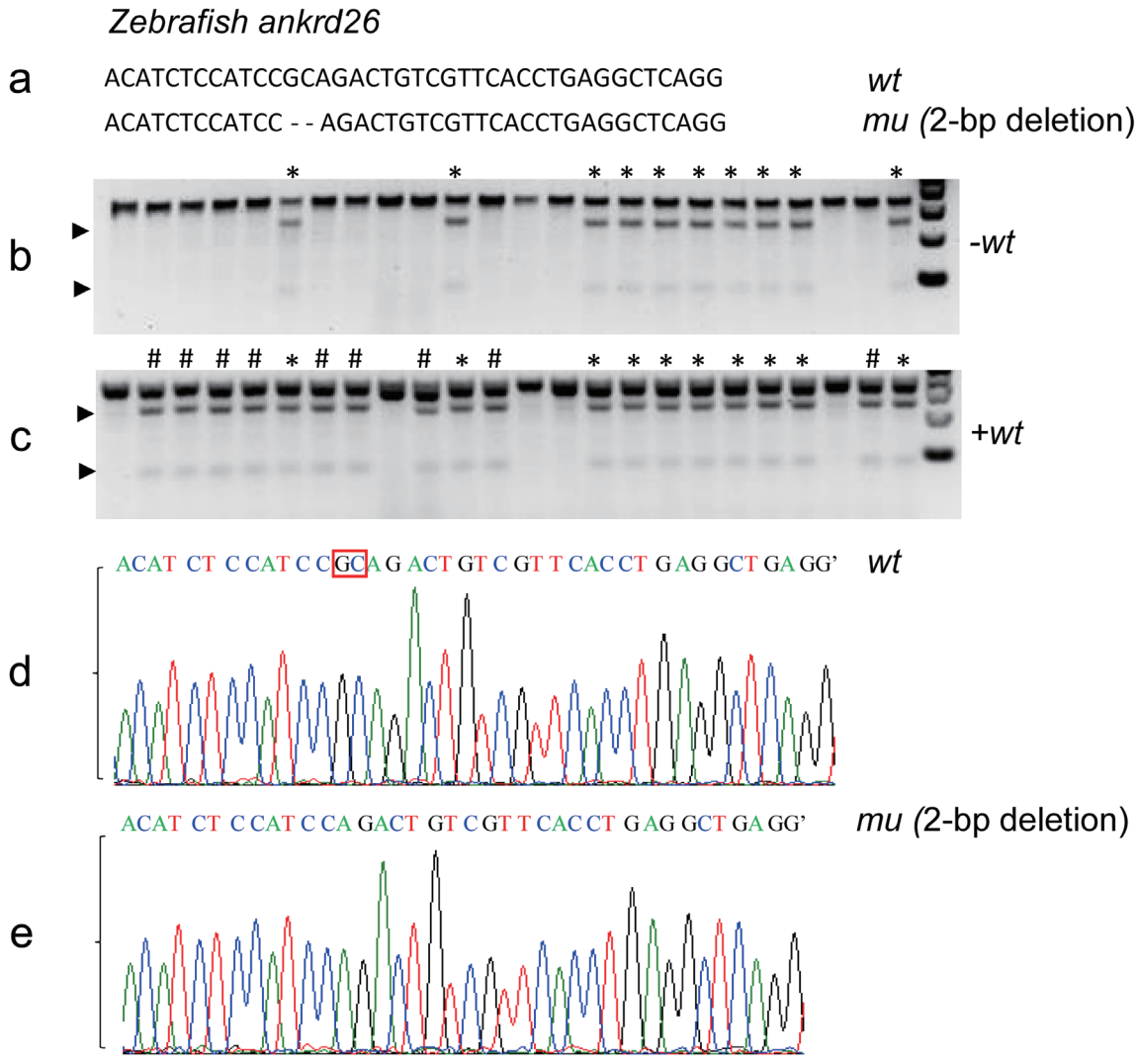

Fig. 3. Genotyping wild-type, heterozygous, and homozygous zebrafish with a 2 bp-deletion in *ankrd26*. (a) The expected DNA sequences of the wild-type (*wt*) and mutant (*mu*) (i.e., 2 bp-deletion) ankrd26. (b–c) The T7E1 digestion patterns of the PCR products in the absence and presence of a *wt* PCR product, respectively. Here, * and # indicate the presence of the cleavage bands (arrowheads) in the absence (−) and the presence (+) of a *wt* PCR sequence, respectively. (d–e) Sanger sequencing results confirmed the genotyping results (−2 bp) revealed by the agarose gel electrophoresis following T7E1 digestion.

pattern of the artificial heteroduplexes (Fig. 4c). Sanger sequencing confirmed 100% accuracy of the genotyping results with the new method (Fig. 4d, e). These results suggest that the new method is capable of discriminating between three genotypes in animals with a seven-nucleotide insertion.

Identification of the substitution mutations of dot1l using agarose gel following the T7E1 nuclease digestion

We further explored the possibility that this genotyping method could discriminate substitution mutations of the same length. A scattered 8-bp substitution mutation in a disruptor of telomeric silencing 1-like (*dot1l*) was created in a mouse (Fig. 5a) (Rumi *et al*., unpublished data). Following the T7E1 digestion of the targeted gene PCR products, we could clearly distinguish the heterozygous mutant from the wild-type and homozygous mutant samples based on two expected cutting bands (~205 bp and ~405 bp) in the heterozygous samples on the agarose gel (Fig. 5b). The same digestion pattern was found in the potential homozygous mutant samples following the addition of a wild-type PCR product (Fig. 5c). Again, Sanger sequencing confirmed the 8-bp scattered substitution in 100% of the samples genotyped for the targeted *Dot1l* mutation using our new method (Fig. 5d, e). These results demonstrated that the new method could easily identify all three genotypes in animals with the eight scattered nucleotide substitution.

Identification of a single base pair substitution mutation of cfh using agarose gel following the Surveyor nuclease digestion

Surveyor nuclease cleaves any site with a mismatched base pair with high efficiency.[12] To assess if the new method could genotype animals with a single nucleotide substitution, we used Surveyor nuclease as a digestion enzyme for the PCR product of the murine complement factor H (*cfh*) (Fig. 6a).[14,15] As shown, two faint, but distinct cut bands of ~200 bp and ~350 bp were detected after the Surveyor nuclease

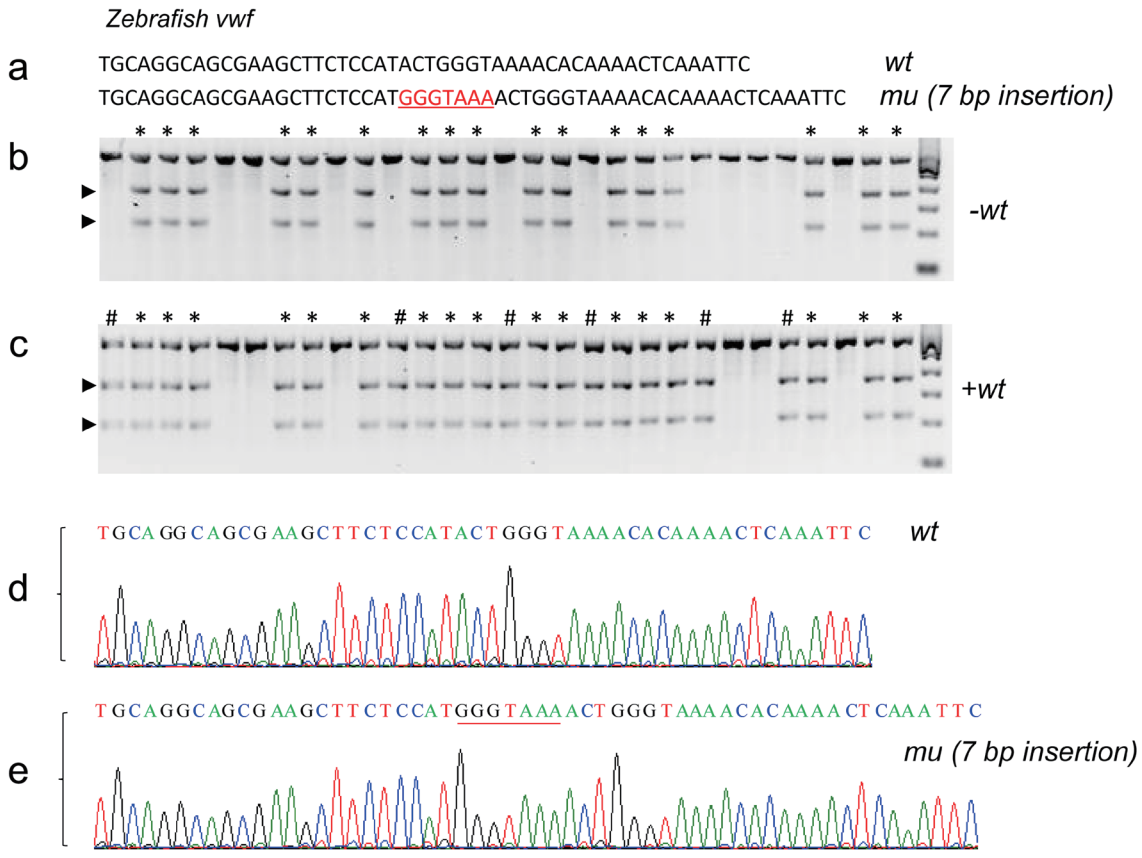

Fig. 4. Genotyping wild-type, heterozygous and homozygous zebrafish with 7 bp-insertion in *vwf*. (a) The expected DNA sequences of the wild-type (wt) and mutant (mu) *(i.e.,* 7 bp-insertion) in zebrafish von Willebrand factor *(vwf)*. (b–c) are the T7E1 digestion patterns of the PCR products in the absence (−) and the presence (+) of a wt PCR product, respectively. Here, * and # indicate the presence of the cleavage bands (arrowheads) in the absence and presence of a wt PCR sequence, respectively. (d–e) Sanger sequencing results confirmed the genotyping results (+7 bp-insertion) revealed by the agarose gel electrophoresis following the T7E1 digestion.

digestion in three PCR samples (Fig. 6b), thus indicating the heterozygous genotype of these animals. Similarly, addition of a wild type PCR product to a potentially homozygous *cfh* mutant resulted in the cleavage of the heteroduplexes by the Surveyor protease (Fig. 6c). Again, all samples with a specific mutation identified by our new method were verified by Sanger sequencing with 100% concordance (Fig. 6d, e). These results indicated that our new genotyping method was able to discriminate between all three genotypes in animals with a single nucleotide substitution without the need for Sanger sequencing.

Discussion

A variety of genotyping methods have been described in the past, including agarose gel electrophoresis, SDS-PAGE, melting curve analysis, enzyme digestion, and Sanger sequencing, etc.[9,15–17] These methods have intrinsic limitations in either lacking sensitivity or costing too much. Zhu *et al*. described a method using 15% polyacrylamide gel for detecting the digestion fragment by the T7E1 endonuclease.[18] Even with this high-resolution gel, this method could not differentiate the wild type from a homozygous mutant with a small deletion. Bhattacharya and Meir also described a method using 4–6% agarose (or high concentration) gel electrophoresis for genotyping mice with a deletion of greater than two base pairs following the T7E1 endonuclease digestion.[4] Their high concentration agarose gel electrophoresis took too long to run and generated excessive heat that resulted in the diffusion of the PCR products into the gel and inability to accurately genotype the animals with nucleotide substitutions.

Here, we describe a novel, robust, and cost-effective method for genotyping animals or cells using routine 2% agarose gel electrophoresis and T7E1 or Surveyor endonuclease digestion. We could detect the difference between the heterozygous from the wild-type and homozygous mice and zebrafish with a small deletion, insertion, or substitution. Upon spiking the wild-type PCR product, we could reliably differentiate the sequence between the wild-type and the homozygous mutant. The addition of the wild-type sequence to the homozygous mutant sequence to create artificial heteroduplex is sensitive to be cleaved by the T7E1 endonuclease.

However, the detection of a sequence with only one substitution remains to be a challenge. While Surveyor nuclease has shown to be able to cleave the heteroduplexes formed between a wild-type sequence and those with only one nucleotide substitution, its cleavage efficiency would still remain limited.[13] In our preliminary result, the cleavage efficiency of a single point mutation of *cfh* by Surveyor nuclease was much less than that with an indel mutation by T7E1 with only the faint cleavage band visible. Therefore, further optimization of Surveyor nuclease or experimental

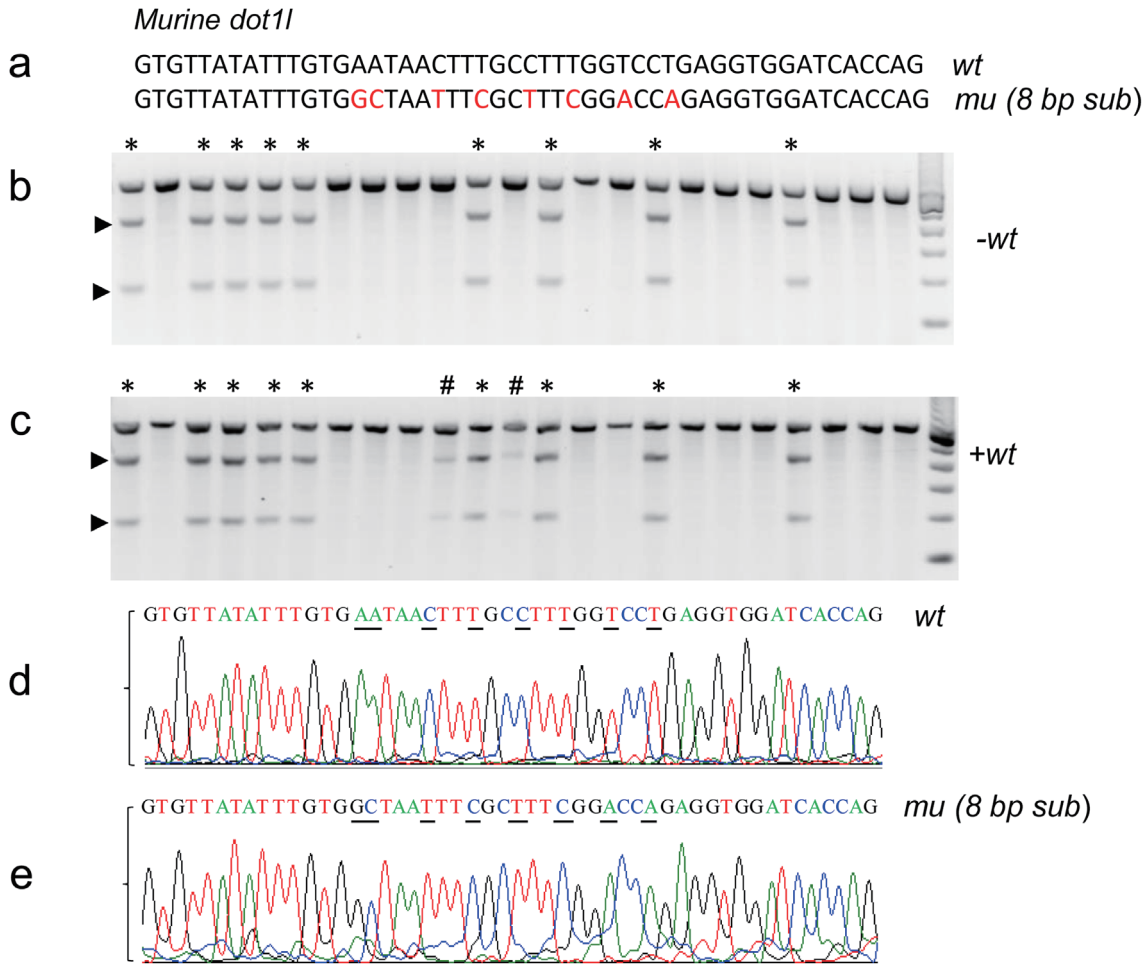

Fig. 5. Genotyping wild-type, heterozygous, and homozygous mice with 8 bp-substitution in *dot1l*. (a) The expected DNA sequences of the wild-type (*wt*) and mutant (*mu*) (i.e., 8 bp- substitution) in *dot1l*. (b–c) The T7E1 digestion patterns of the PCR products in the absence (−) and the presence (+) of a *wt* PCR product, respectively. Here, * and # indicate the presence of the cleavage bands (arrowheads) in the absence and presence of a *wt* PCR sequence, respectively. (d–e) Sanger sequencing of the PCR products confirmed the genotyping results (i.e., 8 bp- substitution) above.

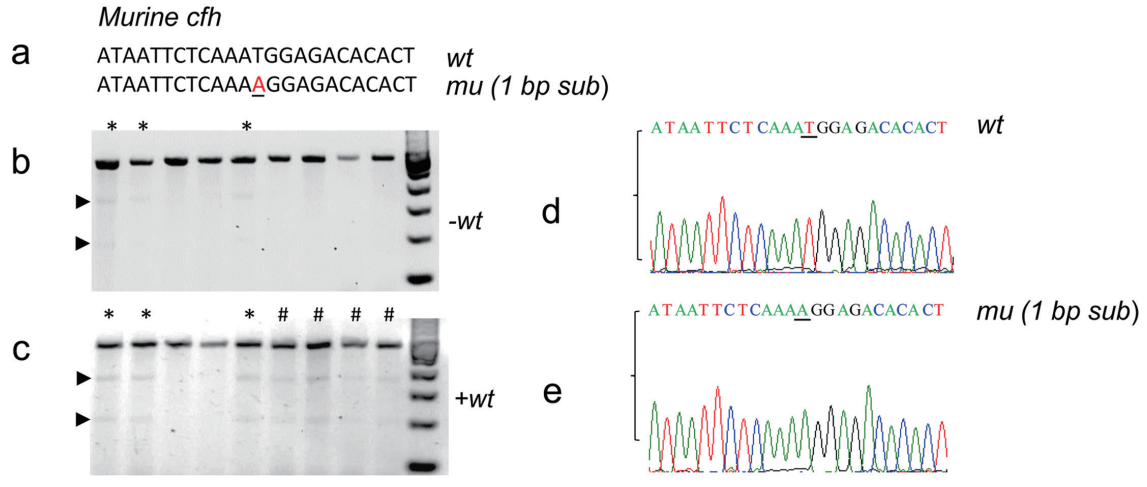

Fig. 6. Genotyping wild-type, heterozygous, and homozygous mice with a single nucleotide substitution in *cfh*. (a) The expected DNA sequences of the wild-type (*wt*) and mutant (*mu*) murine *cfh*. (b–c) The Surveyor nuclease digestion patterns of various PCR products in the absence (−) and the presence (+) of a *wt* sequence, respectively. Here, * and # indicate the presence of the cleavage bands (arrowheads) in the absence (i.e., heterozygous) and the presence (i.e., homozygous) of a *wt* PCR sequence, respectively. (d–e) Sanger sequencing confirmed the *wt* and *mu* (one nucleotide substitution) sequences revealed above.

conditions would be needed to develop more robust genotyping methods for detecting the difference of a gene with a single point mutation.

Conclusions

We developed a rapid, cost-effective, and highly accurate genotyping method using the normal agarose gel electrophoresis following the T7E1 or Surveyor nuclease digestion, with and without the added wild-type sequence. This new method would significantly accelerate the routine molecular biology laboratory to reliably genotype animals and cells with various gene mutations at a record speed at a minimal cost.

Acknowledgments

None.

Funding

This study was supported in part by a grant from the National Institutes of Health (R01HL144552 and R01HL157975-01A1 to X.L.Z.) and a grant from the National Blood Foundation (NBF 22-09R to L.Z.).

Conflict of interest

XLZ is a consultant for Alexion Pharmaceuticals, Takeda, Sanofi-Genzyme, and BioMedica. Additionally, XLZ is also the co-founder of Clotsolution. XLZ has been an associate editor of the *Journal of Clinical and Translational Pathology* since 2022. The authors have no other conflict of interests to disclose.

Author contributions

LiZ, JH, and XLZ conceived the research, analyzed the results, and wrote the manuscript. LiZ, JH, and LuZ performed the experiments and collected the data. MAKR provided critical reagents and mice. All authors revised and approved the final version of the manuscript for submission.

Ethical statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (US). All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kansas Medical Center.

Data sharing statement

The technical appendix, gene sequences, and dataset used in support of the findings of this study are included within the article.

References

[1] Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schömig A, Kastrati A. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clin Chem 2002;48(9):1377–1382. PMID:12194911.
[2] Day IN, Humphries SE. Electrophoresis for genotyping: microtiter array diagonal gel electrophoresis on horizontal polyacrylamide gels, hydrolink, or agarose. Anal Biochem 1994;222(2):389–395. doi:10.1006/abio.1994.1507, PMID:7864363.
[3] Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 1991;48(1):137–144. PMID:1670750.
[4] Bhattacharya D, Van Meir EG. A simple genotyping method to detect small CRISPR-Cas9 induced indels by agarose gel electrophoresis. Sci Rep 2019;9(1):4437. doi:10.1038/s41598-019-39950-4, PMID:30872606.
[5] Barril P, Nates S. Introduction to Agarose and Polyacrylamide Gel Electrophoresis Matrices with Respect to Their Detection Sensitivities. In: Magdeldin S. (ed). Gel Electrophoresis - Principles and Basics. London: IntechOpen; 2012. doi:10.5772/38573.
[6] Thomsen N, Ali RG, Ahmed JN, Arkell RM. High resolution melt analysis (HRMA); a viable alternative to agarose gel electrophoresis for mouse genotyping. PLoS One 2012;7(9):e45252. doi:10.1371/journal.pone.0045252, PMID:23028882.
[7] Thomas HR, Percival SM, Yoder BK, Parant JM. High-throughput genome editing and phenotyping facilitated by high resolution melting curve analysis. PLoS One 2014;9(12):e114632. doi:10.1371/journal.pone.0114632, PMID:25503746.
[8] Zheng L, Abdelgawwad MS, Zhang D, Xu L, Wei S, Cao W, *et al*. Histone-induced thrombotic thrombocytopenic purpura in *adamts13* $^{-/-}$ zebrafish depends on von Willebrand factor. Haematologica 2020;105(4):1107–1119. doi:10.3324/haematol.2019.237396, PMID:31753928.
[9] Zhu X, Xu Y, Yu S, Lu L, Ding M, Cheng J, *et al*. An efficient genotyping method for genome-modified animals and human cells generated with CRISPR/Cas9 system. Sci Rep 2014;4:6420. doi:10.1038/srep06420, PMID:25236476.
[10] Zheng X, Yang S, Zhang D, Zhong Z, Tang X, Deng K, *et al*. Effective screen of CRISPR/Cas9-induced mutants in rice by single-strand conformation polymorphism. Plant Cell Rep 2016;35(7):1545–1554. doi:10.1007/s00299-016-1967-1, PMID:27007717.
[11] Mashal RD, Koontz J, Sklar J. Detection of mutations by cleavage of DNA heteroduplexes with bacteriophage resolvases. Nat Genet 1995;9(2):177–183. doi:10.1038/ng0295-177, PMID:7719346.
[12] Qiu P, Shandilya H, D'Alessio JM, O'Connor K, Durocher J, Gerard GF. Mutation detection using Surveyor nuclease. Biotechniques 2004;36(4):702–707. doi:10.2144/04364PF01, PMID:15088388.
[13] Vouillot L, Thélie A, Pollet N. Comparison of T7E1 and surveyor mismatch cleavage assays to detect mutations triggered by engineered nucleases. G3 (Bethesda) 2015;5(3):407–415. doi:10.1534/g3.114.015834, PMID:25566793.
[14] Ueda Y, Mohammed I, Song D, Gullipalli D, Zhou L, Sato S, *et al*. Murine systemic thrombophilia and hemolytic uremic syndrome from a factor H point mutation. Blood 2017;129(9):1184–1196. doi:10.1182/blood-2016-07-728253, PMID:28057640.
[15] Zheng L, Zhang D, Cao W, Song WC, Zheng XL. Synergistic effects of ADAMTS13 deficiency and complement activation in pathogenesis of thrombotic microangiopathy. Blood 2019;134(13):1095–1105. doi:10.1182/blood.2019001040, PMID:31409673.
[16] Zivelin A, Rosenberg N, Peretz H, Amit Y, Kornbrot N, Seligsohn U. Improved method for genotyping apolipoprotein E polymorphisms by a PCR-based assay simultaneously utilizing two distinct restriction enzymes. Clin Chem 1997;43(9):1657–1659. PMID:9299950.
[17] Sharma-Kuinkel BK, Rude TH, Fowler VG Jr. Pulse Field Gel Electrophoresis. Methods Mol Biol 2016;1373:117–130. doi:10.1007/7651_2014_191, PMID:25682374.
[18] Zhu X, Xu Y, Yu S, Lu L, Ding M, Cheng J, *et al*. An efficient genotyping method for genome-modified animals and human cells generated with CRISPR/Cas9 system. Sci Rep 2014;4:6420. doi:10.1038/srep06420, PMID:25236476.