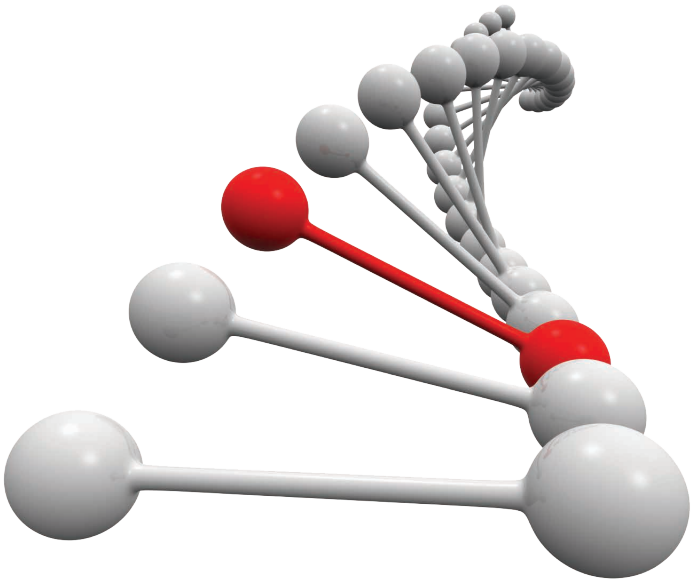
Detection of SNPs by fast, simple, and economical PCR

ARMS PCR with Platinum II *Taq* Hot-Start DNA Polymerase

Summary

Amplification refractory mutation system (ARMS) PCR using Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase is a rapid and reliable method to detect single-nucleotide polymorphism (SNP) alleles in genomic samples, without requiring special setup.



Introduction

SNPs are highly abundant genetic variations in organisms. SNPs are important genetic markers since they are associated with human population diversity, disease susceptibility, and drug responsiveness [1]. A variety of SNP detection methods have been developed, and their simplicity, reliability, and cost-effectiveness are critical for a basic research laboratory to perform SNP genotyping.

ARMS is a type of polymerase chain reaction (PCR) that allows genotyping of known SNPs—e.g., normal and mutant alleles. Allele-specific ARMS amplicons are detected by agarose gel electrophoresis. The system is fast, simple, and economical since no special lab setup or expensive equipment is required. ARMS can also distinguish heterozygotes from homozygotes for an allele of interest. This experimental system is called ARMS because the "normal" setup is refractory to PCR on mutant DNA and the "mutant" setup is refractory to PCR on normal DNA [2].

ARMS concept

ARMS is based on a single PCR with four primers under standard cycling conditions (Figure 1), and each allele-specific reaction is prepared for normal and mutant targets followed by gel electrophoresis. The alleles are differentiated by sequence-specific primer design, where the 3′ terminal base is complementary to each allele so that only the target allele is amplified. Allele differentiation is further improved by introduction of a mismatch at the 3′ penultimate base of the primer, since two mismatched bases at the 3′ end of the primer prevent PCR extension when the template contains a nontarget allele (Figure 2). In this way, the presence or absence of an allele-specific PCR amplicon is used for detection of SNPs in a sample [3].

There are two important prerequisites before setting up an ARMS experiment. First, the SNP sequence must be known in order to design allele-specific primers. Second, a non-proofreading DNA polymerase (i.e., without 3′–5′ exonuclease activity) must be chosen in order to maintain the 3′ mismatched bases in the primers during PCR.

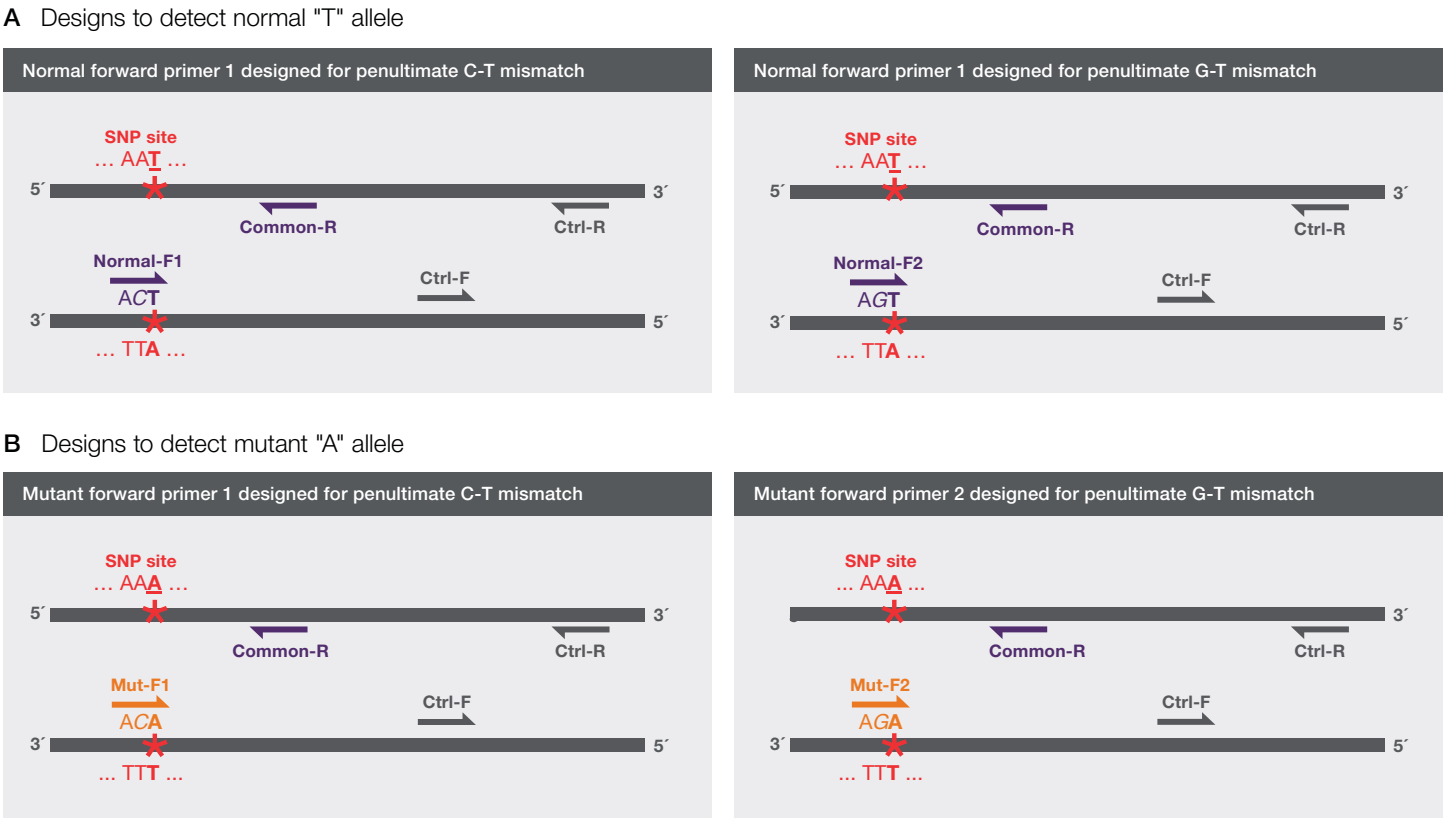


Figure 1. ARMS experimental designs. Two sets of reactions are required to detect **(A)** normal and **(B)** mutant alleles. The allele-specific primer contains a terminal base (bold) complementary to the target allele and a penultimate mismatch (italic) to improve PCR specificity. In this example, penultimate bases with two mismatches of different strength are evaluated for their effects on allele-specific PCR.

ARMS PCR with Platinum II *Taq* Hot-Start DNA Polymerase

To identify two alleles of a SNP in human genomic DNA (gDNA), ARMS experiments were set up with Platinum II *Taq* Hot-Start DNA Polymerase (**see the note on enzyme choice**). Human gDNA samples homozygous for the normal T allele or the mutant A allele were used as PCR templates.

Two sets of reactions were prepared, one for the normal T allele and the other for the mutant A allele. To examine the effect of the penultimate mismatched base, two sub-reactions were set up under the normal and mutant groups using the allele-specific primers with additional C-T and G-T mismatches, which exert strong and weak destabilization on primer binding, respectively [4]. As an internal control, a set of primers that amplifies a region outside the SNP site was included in all reactions (Figures 1–2, Tables 1–2).

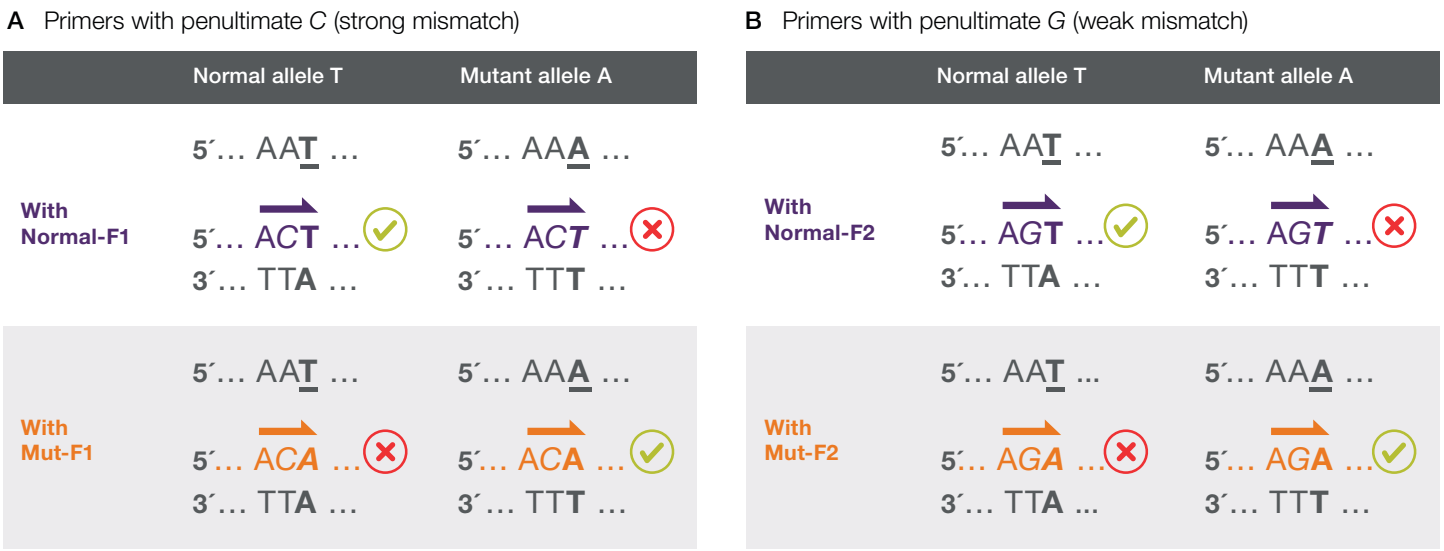


Figure 2. Allele-specific ARMS primers. Two forward primers with different 3′ terminal bases (bold) are used to differentiate SNP alleles (underlined and bold) according to their base complementarity. To further prevent amplification of nontarget alleles, a destabilizing mismatch (italic) is also introduced to the penultimate base at the primer's 3′ end: **(A)** C-T, providing a strong mismatch, and **(B)** G-T, providing a weak mismatch.

Table 1. Reagents for ARMS PCR.

Enzyme	Platinum II *Taq* Hot-Start DNA Polymerase (**Cat. No. 14966001**)
Template	Human gDNA samples with T/T or A/A genotype at the SNP site
Primers	
Common reverse primer	For both normal and mutant alleles Common-R: 5′ CTTAAGTATGCGATTAGATTCCAGTTAATTC 3′
Allele-specific forward primers	Two sets with a different destabilizing mismatch next to the 3′ end, to examine their effect on PCR With penultimate C-T (strong mismatch) for normal and mutant alleles: • Normal-F1: 5′ CTGGCTATAAGGAATTCGAATTTGGGAGAC***T*** 3′ • Mut-F1: 5′ CTGGCTATAAGGAATTCGAATTTGGGAGAC***A*** 3′ With penultimate G-T (weak mismatch) for normal and mutant alleles: • Normal-F2: 5′ CTGGCTATAAGGAATTCGAATTTGGGAGAG***T*** 3′ • Mut-F2: 5′ CTGGCTATAAGGAATTCGAATTTGGGAGAG***A*** 3′
Control forward and reverse primers	Detection of a sequence outside the SNP site: • Ctrl-F: 5′ TGGGAGTCCTGATAACCCAGGC 3′ • Ctrl-R: 5′ TGCACTTGGGATATACTGTGTTAGGG 3′

Table 2. Reaction conditions and reagent amounts for 20 µL ARMS PCR.

Component	For normal allele	For mutant allele
Human gDNA	20 ng	20 ng
Common-R primer	0.2 µM	0.2 µM
Normal-F1 or Normal-F2 primer	0.2 µM	–
Mut-F1 or Mut-F2 primer	–	0.2 µM
Ctrl-F primer	0.2 µM	0.2 µM
Ctrl-R primer	0.2 µM	0.2 µM
dNTP mix	0.2 mM	0.2 mM
Platinum II *Taq* Hot-Start DNA Polymerase	0.2 U	0.2 U
5X Platinum™ II PCR buffer	4 µL	4 µL
Water, nuclease-free	To 20 µL	To 20 µL
Optional: Platinum™ GC Enhancer (only for targets with >65% GC content)	4 µL	4 µL

Advantages of using Platinum II *Taq* Hot-Start DNA Polymerase in ARMS PCR

The selection of an enzyme is important for a successful ARMS PCR. The following features of Platinum II *Taq* Hot-Start DNA Polymerase make it an excellent choice for ARMS PCR:

- **Non-proofreading enzyme:** Deliberate mismatches in the primers remain intact during PCR for allele-specific detection.
- **Universal annealing buffer:** All four primers of the reaction can anneal at 60°C without requiring tedious optimization for primer annealing.
- **Hot-start modification:** PCR specificity is enhanced by helping to prevent amplification of misprimed sequences, since the enzyme becomes active only after the initial denaturation step. In high-throughput genotyping where assembled reactions may sit at room temperature for an extended period, hot start allows reaction stability without compromising yield and specificity for up to 24 hr.
- **High inhibitor tolerance:** gDNA of suboptimal purity can be used in ARMS with little concern over PCR inhibition.
- **Fast DNA synthesis:** With extension at 15 sec/kb, cycling can be completed in as little as 40 min.
- **GC enhancer included:** Target alleles with >65% GC content can be detected with minimal optimization.

The cycling protocol was followed as recommended for Platinum II *Taq* Hot-Start DNA Polymerase:

- Initial denaturation: 94°C for 2 min
- Denaturation: 94°C for 15 sec
- Annealing: 60°C for 15 sec
- Extension: 68°C for 15 sec
- Total number of PCR cycles: 30

After PCR, completed reactions were loaded onto an agarose gel and separated by gel electrophoresis.

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ARMS PCR results

The results show that ARMS PCR with Platinum II *Taq* Hot-Start DNA Polymerase reliably identifies SNP sequence variants in human gDNA. The allele-specific primers efficiently amplify the target allele while being refractory to the nontarget allele (Figure 3). Both C-T and G-T mismatches of the penultimate bases worked equally well in identifying SNPs in this case.

Conclusions

The fast, reliable, and cost-effective ARMS PCR method provides clear differentiation between SNP genotypes in a DNA sample. The benefits of the method are enhanced by using the robust Platinum II *Taq* Hot-Start DNA Polymerase and its special buffer formulated for a universal annealing temperature.

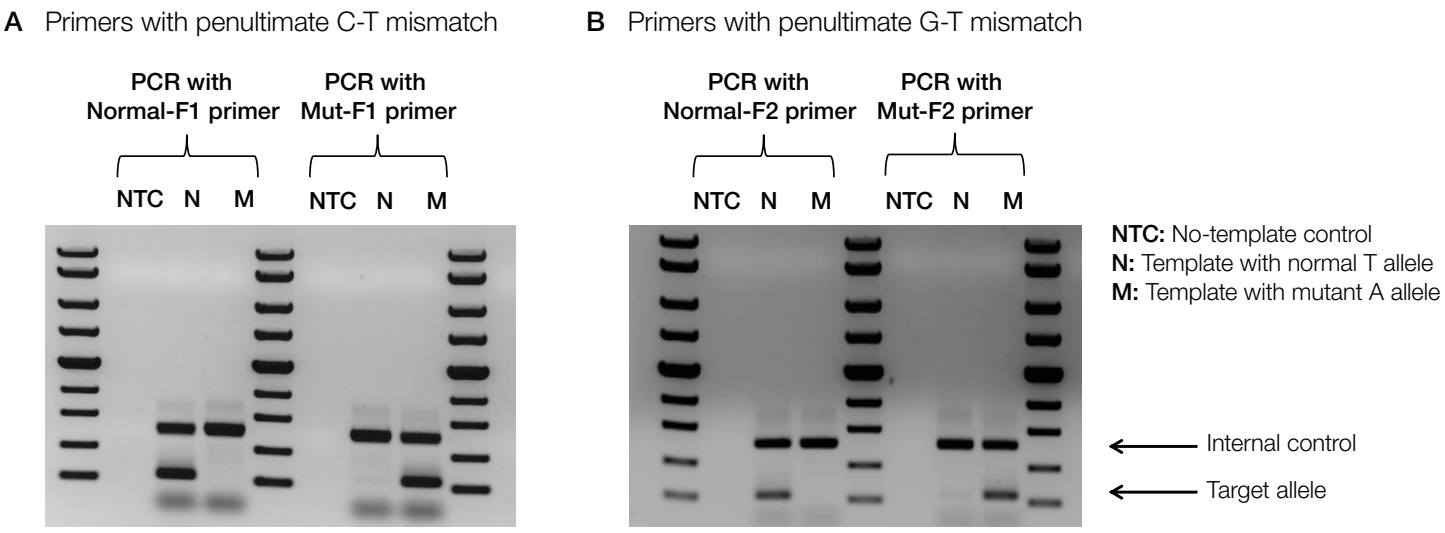

Figure 3. Reliable SNP detection. Human gDNA with homozygous SNPs (T/T or A/A) was amplified using Platinum II *Taq* Hot-Start DNA Polymerase. Each reaction contained either an A allele–specific or T allele–specific forward primer with a penultimate C-T mismatch **(A)** or G-T mismatch **(B)** and a common reverse primer, plus internal control primers. The A allele–specific primer amplified the target only from the normal gDNA with the A/A genotype, and the T allele–specific primer worked only on the mutant gDNA with the T/T genotype. The size marker was Thermo Scientific™ Zip Ruler™ DNA Ladder Express 2.

References

1. Shastry BS (2002) SNP alleles in human disease and evolution. *J Hum Genet* 47(11):561–566.
2. Newton CR, Graham A, Heptinstall LE et al. (1989) Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 17(7):2503–2516.
3. Gaudet M, Fara AG, Beritognolo I et al. (2009) Allele-specific PCR in SNP genotyping. *Methods Mol Biol* 578:415–424.
4. Little S (2001) Amplification-refractory mutation system (ARMS) analysis of point mutations. *Curr Protoc Hum Genet* Chapter 9: Unit 9.8.

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