

PCR Activity Guide: Part 2



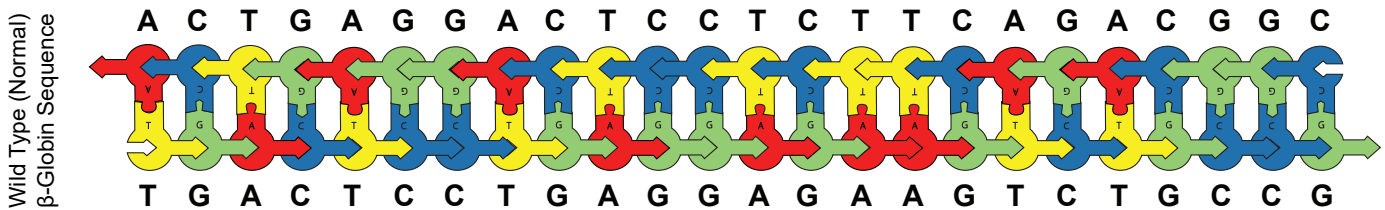
Polymerase Chain Reaction (PCR) as a Medical Tool Step-By-Step Instructions

Create two double-stranded template DNA sequences:

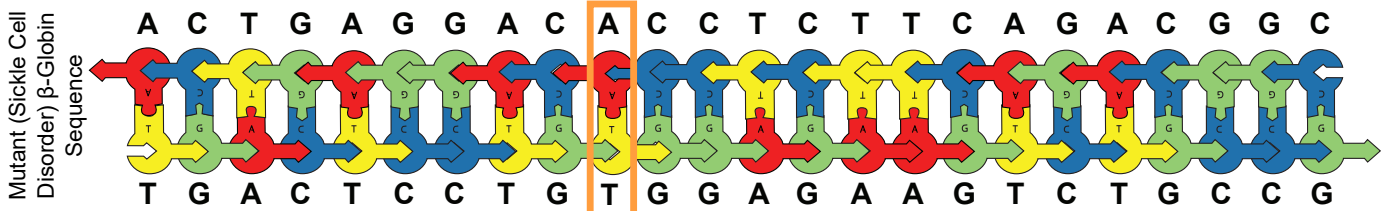
3' ACTGAGGACACCTCTTCAGACGGC 5'
5' TGA CTCTGTGGAGAAGTCTGCCG 3'

3' ACTGAGGACACCTCTTCAGACGGC 5'
5' TGA CTCTGTGGAGAAGTCTGCCG 3'

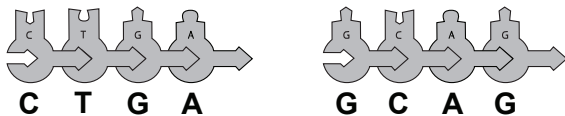
Assembly Guide



Site of point mutation
for sickle cell anemia



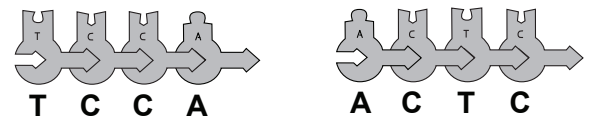
Wild Type (Normal) Primer Sequence



Normal Primer 1: 5' CTGA 3'
Normal Primer 2: 5' GCAG 3'

Normal Allele PCR Product: 16 bp

Mutant Primer Sequence



Mutant Primer 1: 5' ACTC 3'
Mutant Primer 2: 5' TCCA 3'

Mutant Allele PCR Product: 11 bp

Note: The above sequences are located within the **beta-globin gene**. The top sequence is for **wild-type** (normal) beta globin. The bottom sequence codes for **mutant** beta-globin. The point mutation at Position 10 (the "T" on the 3'-5' strand is replaced by an "A") results in an abnormal beta-globin protein that can cause sickle-cell anemia.

Below both of these template strands of dsDNA are four different primers. **Amplification-refractory mutation system (ARMS)** PCR is utilized to determine if an individual has a specific point mutation. Four different primers are utilized in this protocol, one set (Normal Primers 1 and 2) that will detect the wild-type form of the β -globin gene, and a second set (Normal Primers 1 and 2) that will detect the mutant allele. This diagnostic test can be run in a single reaction tube PCR.

PCR Activity Guide: Part 2



General Lesson Plan:

- Provide each lab group with an “unknown” patient DNA sequence, either the wild-type (normal) or mutant (sickle cell) sequence. Students will use the given DNA sequence to construct the dsDNA model using the colored foam nucleotides.
 - *[Option: To shorten this activity, provide students with the pre-assembled DNA templates.]*
- Provide the primer sequences for students. They will need to construct and use two pairs of primers (1 set of wild type/normal primers and another set of mutant primers). Use grey-colored nucleotides to represent the primers. For students to run 3 cycles of PCR for this simulation, they will need to have a total of 7 copies of each primer.
 - *[Option: To shorten this activity, provide students with pre-assembled primers.]*
 - *[Option: Create small flags, like are used in Part 1: Exploring PCR. Flags should label the primers: Wild-Type Primer 1, Wild-Type Primer 2, Mutant Primer 1, and Mutant Primer 2. Use dissecting pins to label the foam primer segments]*
 - **Suggestion:** *It is suggested to **not tell** students which primer corresponds to the mutant allele and which corresponds to the wild type. This will encourage them to then work through and model the PCR process again to determine the length of the short PCR product that is produced. It is suggested that you tell the students at the end of the activity the length of the short PCR product for both the mutant and the wild type and that the students use that to determine if the target sequence contains the mutant allele or not.*
- Students will run a simulated PCR activity using the primers provided. They must match the correct primers to their unknown DNA template sequence to determine whether their patient has the wild-type (normal) allele or the mutant (sickle cell anemia) allele. Once students determine whether the DNA has the normal or mutant allele, they will demonstrate the process of PCR through 3 cycles.
- Differentiated/Extended Option: Each student group can be given 2 unknown DNA sequences to represent the sequences on the “maternal” and “paternal” chromosomes. Their patient can either be homozygous normal (2 normal alleles), heterozygous for sickle cell trait (1 normal allele, 1 mutant allele), or homozygous for sickle cell anemia (2 mutant alleles).
- After the PCR reaction is complete, students can “run” the PCR products through a gel electrophoresis to show a difference between banding patterns of homozygous normal (one single “band” at 16 bp), heterozygous (two “bands”: one at 16 bp, the other at 11 bp), or homozygous mutant (one single “band” at 11 bp).

[Note that this is only a simulated activity to demonstrate a single reaction tube PCR genotyping test; the actual primers are 24-35 nucleotides in length and therefore will use a slightly higher annealing temperature of 60-65°C or higher. In addition, the PCR products must have at least a 100 bp difference to be able to clearly identify band separation using gel electrophoresis.]

PCR Activity Guide: Part 2



ARMS-PCR

ARMS-PCR (amplification refractory mutation system) can be used for genotyping specific single nucleotide polymorphisms (SNPs) or alleles. This technique is also called allele-specific amplification (ASA). It permits rapid analysis of any known mutations in genomic DNA.

The ARMS-PCR differs from traditional PCR in these ways:

1. Two different sets of primers are designed to identify the wild-type (normal) and mutant alleles.
2. A higher annealing temperature and limited number of cycles in ARMS-PCR will reduce false positives.

The ARMS-PCR technique requires the design of two different PCR primers that overlap with the mutation site. The single base at the 3' end of each primer is modified to base pair with either the normal allele or the mutant allele.

- Normal primer: the 3' end of the primer matches the normal DNA sequence
- Mutant primer: the 3' end of the primer matches the mutant DNA sequence

The “normal” form of the primer is refractory to (or resistant to) PCR on mutant template DNA. The “mutant” primer is refractory to PCR on the normal template DNA.

The ARMS-PCR genotyping test can be performed simultaneously in a single reaction tube with four primers. This requires the design of four different primers. A different set of forward and reverse primers are designed to target each specific allele. The length of the PCR product for the normal allele must be a different bp length than the PCR product for the mutant allele so that the two differently-sized PCR products can be distinguished on a gel. A gel electrophoresis is run on the PCR products to determine the patient genotype.

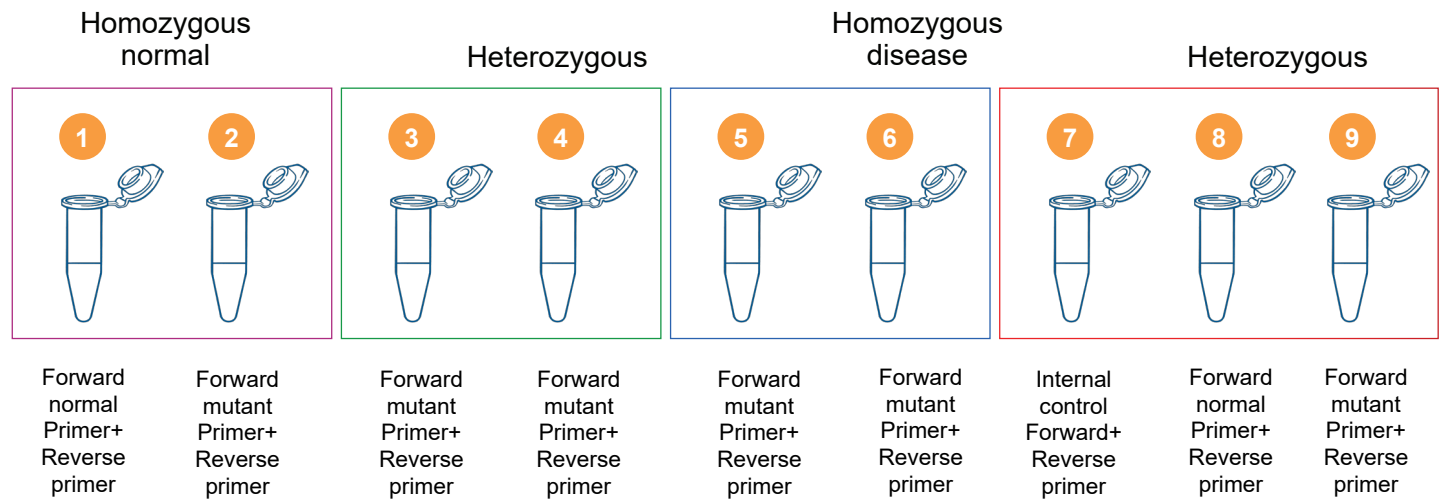
PCR Activity Guide: Part 2

Two options of ARMS-PCR:

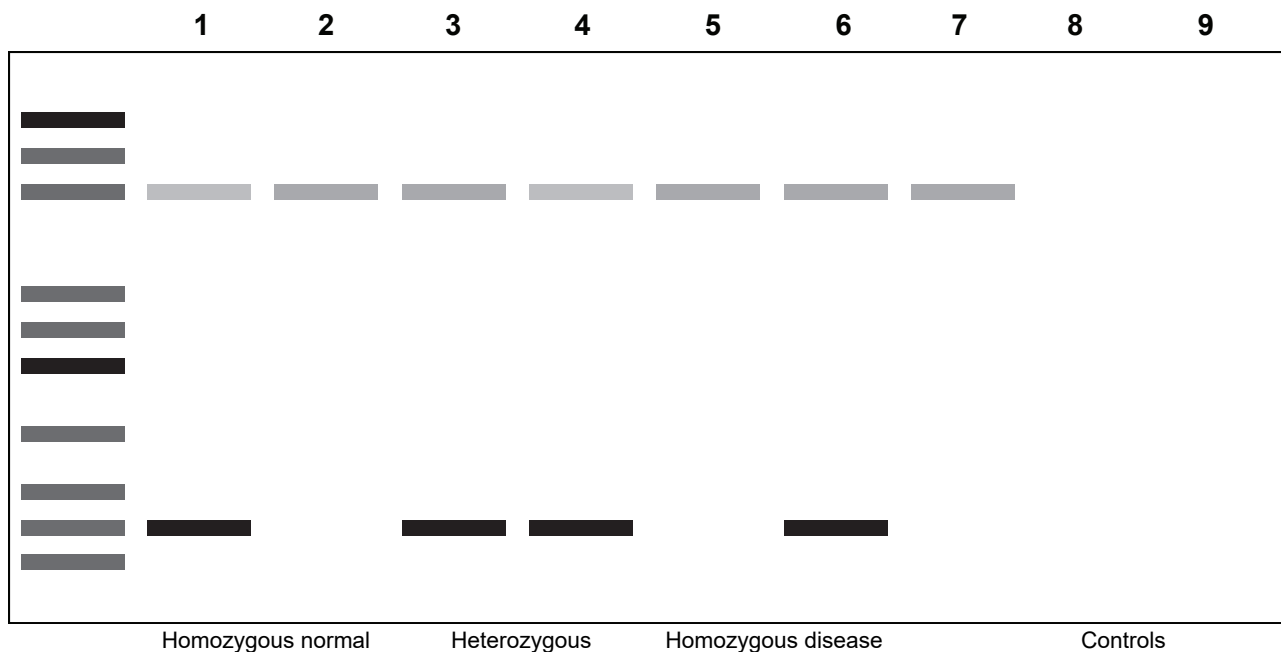
Option A - Run the genotyping test in 2 different reaction tubes

- Tube 1 = Normal Forward Primer + Reverse Primer
- Tube 2 = Mutant Forward Primer + Reverse Primer
- A gel electrophoresis is run on the PCR products to determine the patient genotype

Source: <http://geniticeducation.co.in/what-is-arms-pcr-or-allele-specific-pcr/>



Internal control Forward + reverse primer in tubes 1, 2, 3, 4, 5 and 6



PCR Activity Guide: Part 2

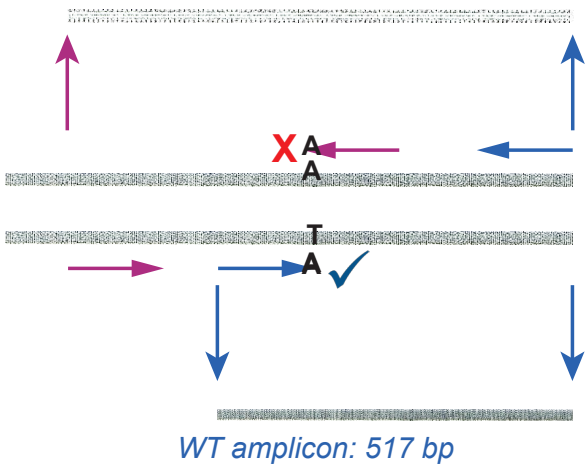


Option B - Run the genotyping test in 1 single reaction tube

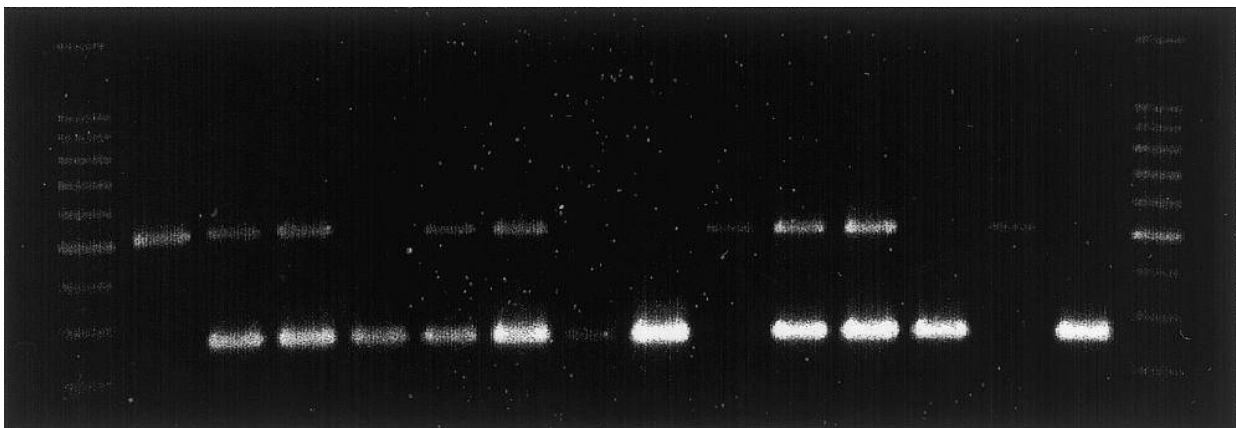
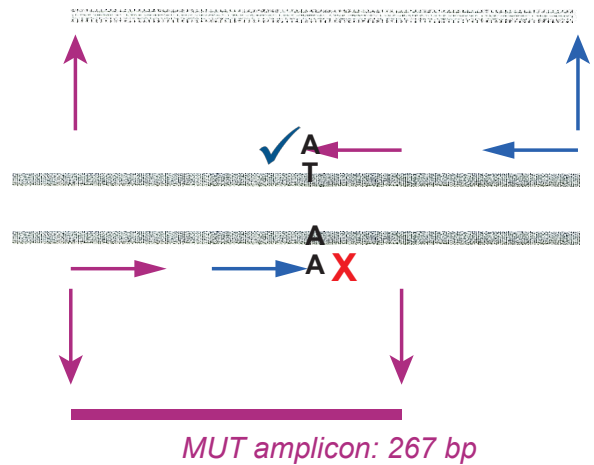
This requires the design of 4 different primers. A different set of forward and reverse primers are designed to target each specific allele. The length of the PCR product for the normal allele must be a different bp length than the PCR product for the mutant allele so that the two differently-sized PCR products can be distinguished on a gel. A gel electrophoresis is run on the PCR products to determine the patient genotype.

Source: Waterfall, C. M. and Cobb, B. D. (2001). Single tube genotyping of sickle cell anemia using PCR-based SNP analysis. *Nucleic Acid Res.* 29(23): e119.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC96713/>

Wild-Type (Normal) PCR product: 517 bp



Mutant PCR product: 267 bp



- Homozygous WT (wild-type): one upper band shown (517 bp)
- Heterozygous: two bands (267 bp, 517 bp)
- Homozygous MUT (mutant): one lower band shown (267 bp)

PCR Activity Guide: Part 2



Resources:

- <http://geneticeducation.co.in/what-is-arms-pcr-or-allele-specific-pcr/>
- Gen, Maj, and Suhaib Ahmed. "Amplification Refractory Mutation System (ARMS) ." *Genetics Resource Center* , 7 Oct. 2014, grcpk.com/wp-content/uploads/2014/10/7.-ARMS.pdf.
- Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C., & Markham, A. F. (1989). Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res.* 17 (7): 2503-2516.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC317639/>
- Singh, P. J., Shrivastava, A. C., & Shrikhande, A. V. (2015). Prenatal diagnosis of sickle cell disease by the technique of PCR. *Indian H Hematol Blood Transfus.* 31 (2): 233-241.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4375164/>