Amplification Refractory Mutation System (ARMS)

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The Amplification Refractory Mutation System (ARMS) is an application of PCR in which DNA is amplified by allele specific primers. In PCR mismatch at the 3' end of the primer can dramatically reduce the annealing and hence the amplification. This is due to the absence of 3' to 5' exonuclease proofreading activity of Taq polymerase. High fidelity DNA polymerases, that have this activity, cannot be used in ARMS. It is an extremely useful method for identification of point mutations or polymorphisms.

Since the ARMS PCR is mostly done to identify a mutation or a polymorphism it is also important that it should be able to identify whether the change in DNA is heterozygous or homozygous. A heterozygote or homozygote is differentiated by using ARMS primers for the mutant/polymorphic and the normal (wild type) alleles. The reactions for the mutant and the normal alleles are usually carried out in separate tubes. But these may be done in the same tube after labeling the two primers with different fluorescent dyes.

ARMS primer design

General principles of designing a PCR primer as discussed in chapter 3 also apply to the ARMS primers. The ARMS PCR requires a pair of primers including a common and an ARMS primer. The common primer is like any other PCR primer. But the ARMS primer has the following special features:

1. The primer is usually 30 bases in length.
2. The nucleotide at the 3' end of the primer should be complementary to the target nucleotide i.e. G for C or C for G and T for A or A for T. Mismatch at this position can dramatically reduce the amplification. A:G, G:A, and C:C mismatches have the worst effect whereas the other mismatches have varying degrees of effect. For example in a mutation with A-T substitution the ARMS primer for the mutant allele should have the last nucleotide complementary to the nucleotide T i.e. it should have A. The primer for the normal allele at the same position should be complementary to the nucleotide A i.e. it should have T (Fig. 7.1).
3. An additional mismatch at one of the last five nucleotides of the ARMS primer further increases its specificity.
4. It is customary to include an internal PCR control in ARMS reactions. A pair of primers is designed to amplify a region of the gene of interest that usually is free of mutations. An amplification of the internal control region and no amplification by the ARMS primer indicate a true negative. In a false negative result neither the ARMS primer nor the internal control shows any amplification. There could be several reasons for the false negative result e.g. too little or too much DNA, poor

quality of DNA template, failure to add primer, Taq, or other reagents and presence of PCR inhibitors.

5. The sensitivity and specificity of an ARMS reaction can be controlled by stringent reaction conditions. Good primer design, higher annealing temperature and limited number of cycles are important in avoiding false results. The number of cycles should be just enough to give a clear positive result. Increasing the number of cycles un-necessarily can cause false positives. The usual length of ARMS primer is 30 bases. Primers of this length have a high Tm and annealing temperature and are therefore more specific.

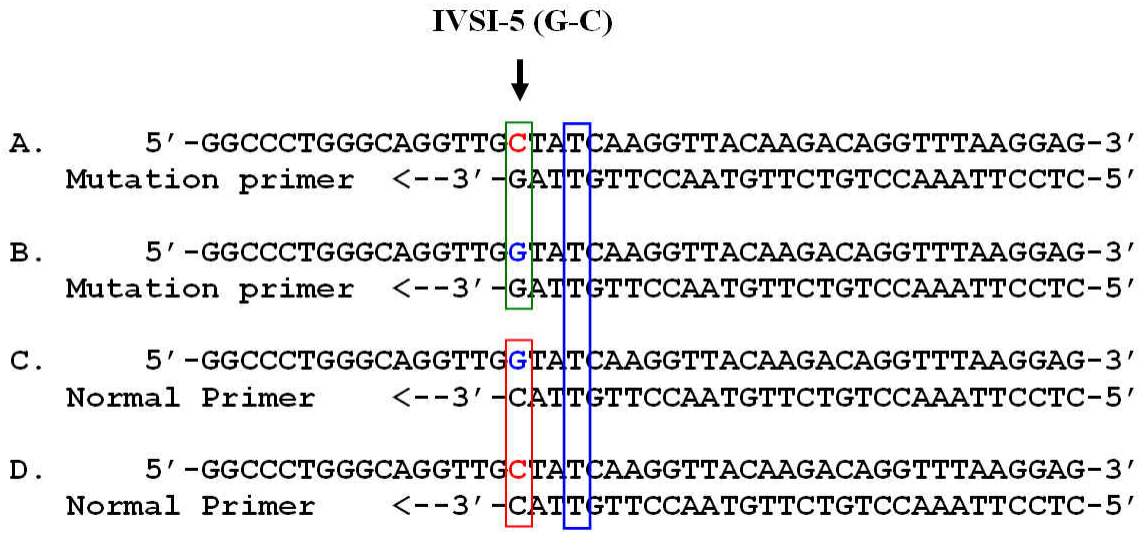

Fig. 7.1 ARMS primers for the normal and the mutant allele of a point mutation, IVSI-5 (G-C), in the β-globin gene. Segment A shows the matched ARMS primer for the mutation (C), segment B shows the mismatch between the mutant ARMS primer and the normal sequence (G), segment C shows the matched ARMS primer for the normal sequence (G), segment D shows the mismatch between the normal ARMS primer and the mutation (C). A deliberate mismatch (T:T) is also added at position enclosed by blue box.

PCR conditions for ARMS

Locus: β-globin gene

GenBank accession NG_000007.3

Allele: β-thalassaemia mutation IVSI-5 (G-C)

- Forward primer: 5’-ACCTCACCCTGTGGAGCCAC
- Reverse primer (ARMS)

5’-CTCCTTAAACCTGTCTTGTAACCTTGTTAG

- Amplified product 285 bp
- Control primer (forward):
  5'-CAATGTATCATGCCTCTTTGCACC
- Control primer (reverse):
  5'-GAGTCAAGGCTGAGAGATGCAGGA
- Amplified product: 861 bp

- Reaction volume: 25µl
- PCR mix: 22µl
- Primer concentration: 1µl (5 pmol each/µl) (Chapter 3)
- Taq polymerase: 0.5 units (0.1µl)
- Template DNA: 2µl (~300ng)

Thermal cycling:

- Initial denaturation: 1 minute at 94°C
- No. of cycles: 25
- Denaturation: 1 minute at 94°C
- Annealing: 1 minute at 65°C
- Extension: 1 minute 30 seconds at 72°C
- Final extension: 3 minute at 72°C

Electrophoresis: 10 X 10 cm 6% polyacrylamide, 40 minutes at 150 volts.
Staining: 0.1% silver nitrate
Result: Fig. 7.2

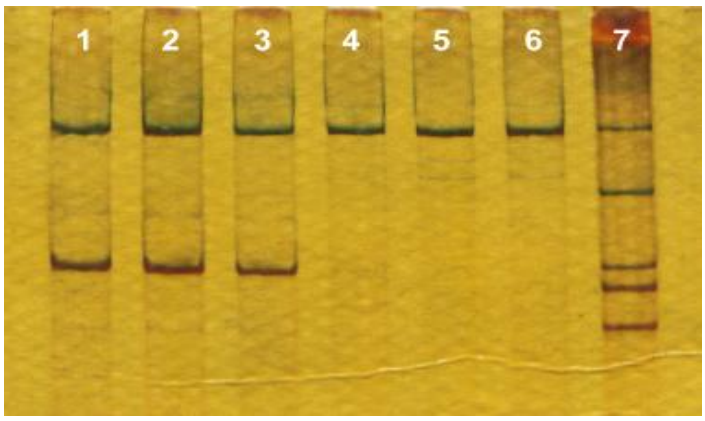

Fig. 7.2. Silver stained polyacrylamide gel electrophoresis after ARMS PCR. All lanes show 861bp internal control fragment. Lanes 1-3 shows 285bp fragment of IVS1-5 mutation. Lanes 4-5 are negative for the same mutations. Lane 7 shows allelic ladder for various thalassaemia mutations.

Bibliography

1. Kwok S, Kellogg DE, McKinney N, Spasic D, Goda L, Levenson C, Sininsky JJ (1989) Effect of primer template mismatches on the polymerase chain reaction: Human immunedeficiency virus type 1 model studies. *Nucleic Acids Res* 18: 999-1005.
2. Newton CP, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, Smith JC, Markham AF (1989) Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 17: 2503-2516.
3. Old JM, Varawalla NY, Weatherall DJ (1990) Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet 336: 834-837.