# Allele-specific PCR – a timeline

Allele-specific PCR (AS-PCR) is a widely used molecular biology technique that enables the identification of specific alleles within a mixed population of DNA. The method was first described in 1989 by Sommer et al., who used PCR primers with 3' single base mismatches to discriminate between different alleles of a gene associated with Phenylketonuria (PKU) in an affected family. Since then, various modifications and improvements have been made to the technique, including the development of increasingly user-friendly allele-specific detection systems. All allele-specific detection systems available on the market today are, by design, highly sensitive and specific, making them the ideal technology for detecting single nucleotide polymorphisms (SNPs) and other genomic variations. They require sequence information flanking the variant of interest and a fluorescent plate reader for endpoint measurement or a qPCR machine.

**Commercial allele-specific detection systems**

One of the first and most widely used allele-specific detection systems is TaqMan™. This technology uses fluorogenic probes that are designed to anneal to a specific sequence within the amplified PCR product. The probes are labelled with a fluorescent dye and a quencher molecule, which prevents the fluorescence from being detected until the probe is cleaved by the Taq polymerase during PCR amplification. A new labelled probe must be designed for each new assay, creating a cost-issue for studies where assay number or turnover is high. Moving on from the first, probe-based for detection methods developed, PACE®, Amplifluor™ and KASP™ genotyping all use PCR-based detection systems.

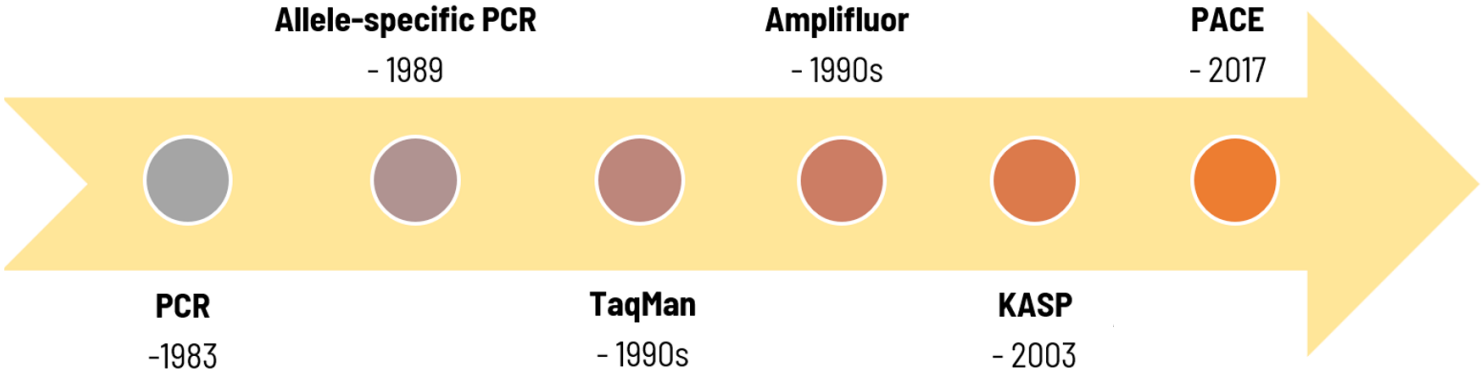


**Figure 1:** Allele-specific PCR – a timeline

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In each case, the target sequence is amplified by unlabelled allele-specific primers, two forward primers that are identical apart from the mismatched target base at the 5′ end, and a common reverse primer specific to downstream flanking sequence. The two allele-specific primers compete to anneal to the target DNA during PCR amplification, with the level of amplification determined by the ratio of the two alleles present in the samples. The fluorescent dye and quencher molecule are provided in the PCR master mix on a universal reporter cassette. The main difference between the three technologies is the construction of this reporter.

During PCR, short universal tag sequences (one for each allele) are added to the amplicons by the forward primers. The tags are then recognised by the reporter constructs (one labelled with a different fluorescent molecular for each allele), and a fluorescent signal generated as the target sequences are amplified. The reporter cassettes can be added in bulk to the master mix because, cleverly, they are identical for every assay. The result is a universal reporting master mix that offers game changing cost benefits to genotyping projects, regardless of size.

PACE®, designed by the developers of KASP™, is the latest iteration of allele-specific PCR that has emerged as a new generation PCR-genotyping technology with wider adaptability to applications such as marker-assisted selection.

## PACE v KASP applications

| | PACE | KASP |
|---|---|---|
| Patented chemistry | ✓ | ✓ |
| SNP and Indel detection | ✓ | ✓ |
| High performance on crude DNA samples | ✓ | × |
| Multiplex genotyping | ✓ | × |
| Real-time detection | ✓ | × |
| One-step RNA genotyping | ✓ | × |

**Figure 2:** A table comparing the applications of two of the primary commercial allele-specific detection systems.

PACE genotyping chemistry offers superior performance with crude DNA samples, plus multiplexing capabilities, the option to observe real-time curves, and one-step genotyping direct from RNA. Importantly, in addition to its improved applicability, PACE genotyping reagents retain full compatibility with existing KASP and Amplifluor assays/markers.

In conclusion, allele-specific PCR has come a long way since its first description in 1989. With the development of new and improved allele-specific detection systems, the latest of which is PACE genotyping chemistry, researchers now have affordable, accessible tools at their disposal for analysis of polymorphisms within DNA and RNA samples using any qPCR machine or thermal cycler and a fluorescent plate reader.

**Reference:** Sommer, Steve S., et al. "A novel method for detecting point mutations or polymorphisms and its application to population screening for carriers of phenylketonuria." *Mayo Clinic Proceedings.* Vol. 64. No. 11. Elsevier, 1989.