HiDi® DNA Polymerase: Applications from mutation detection to genome editing

A frequently used method for the analysis of single nucleotide variations in DNA is allele-specific PCR (asPCR). asPCR poses major challenges for the DNA polymerase used for this purpose, e.g. avoiding the generation of false results due to the formation of by-products. Several independently conducted studies show that HiDi® DNA polymerase is ideally suited for use in asPCR in numerous research areas ranging from mutation detection to genome editing.

Keywords: asPCR, allele-specific PCR, mutation detection, SNP detection, CRISPR/Cas, TALEN

The polymerase chain reaction (PCR) has become indispensable in many areas of biology and medicine. A special field of application is the detection of single nucleotide variations in DNA, such as mutations or single nucleotide polymorphisms (SNPs). For this purpose, systems are used whose signaling varies between single nucleotide variations (e.g. replacement of C by an A). A frequently used method for the analysis of single nucleotide variations is allele-specific PCR (asPCR). It is based on the distinction between canonically paired and mismatched primer/template complexes in PCR. At best, PCR proceeds from canonically paired primer/template complexes, whereas it is suppressed from mismatched complexes. Noteworthy, asPCR, unlike other methods, does not require prior amplification of the target material. Modern approaches also combine amplification and detection in a single reaction and measure the increase in amplified DNA during the reaction in real time by the increase in a fluorescence signal coupled to the progress of DNA synthesis catalyzed by the DNA polymerase in the PCR. Clearly, the ability of a DNA polymerase to differentiate between canonical and mismatched primer/template complexes is instrumental in the success of asPCR. However, the propensity of DNA polymerases to form template-dependent and -independent byproducts problematizes this approach.

Drum et al. reported the generation of improved DNA polymerases for asPCR by mutagenesis of a truncated variant of Thermus aquaticus (Taq) DNA polymerase [1]. Based on these results, **HiDi® DNA polymerase** was developed by myPOLS Biotec, where HiDi stands for High Discrimination. **HiDi® DNA polymerase** is increasingly and successfully used for asPCR. One application example is described in detail below, before other recently reported applications are described.

Application – An Example

Some people love it in their food, others so not at all: coriander. A SNP has been reported to be involved in the difference in taste perception, as it is located near a set of genes encoding odorant receptors [2]. Here, we detect this SNP (rs72921001) in the genomic DNA of HeLa cells as an example.

For primer design, we chose two primers that form either a canonical or a mismatched primer end (Fig. 1A). The asPCRs using **HiDi® DNA polymerase** were performed starting from 1 ng/µl gDNA isolated from HeLa cells in the presence of a dye indicating real-time DNA product formation. The experiment indicates amplification starting from the primer specific for the C allele most efficiently. The A allele-specific primer is discriminated and almost no amplicon is observed even with PCR of up to 50 cycles (Fig. 1B), which was also confirmed by downstream melting point analysis (Fig. 1C). The PCR products were then additionally analyzed by agarose gel electrophoresis. The specific product formed is alternatively visualized by ethidium bromide staining at the amplicon length of 109 bp (Fig. 1D).

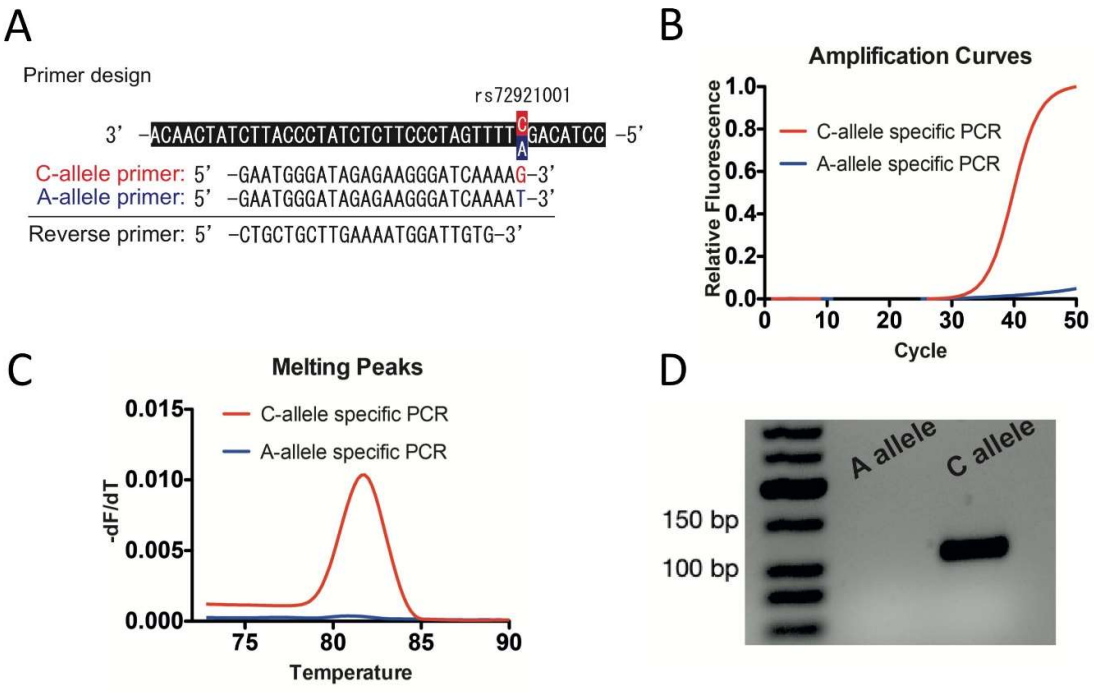

Fig. 1: Application example for the detection of a single nucleotide variation by asPCR. **A**, Primer design. Shown in black is the gene sequence of interest. Below are shown the primer sequences specific for the C and A allele, respectively. **B**, PCR curves for the C- and A-allele specific primers, respectively. **C**, melting point analysis of the two PCRs. **D**, Analysis of the PCR products by agarose gel electrophoresis.

The excellent properties of **HiDi® DNA polymerase** in asPCR have also been demonstrated in recently published and partly comparative studies by different research groups.

For example, Z. Yang *et al.* investigated the tendency of DNA polymerases in PCR to form non-specific by-products such as primer dimers and ways to avoid these undesirable side reactions [3]. They found that **HiDi® DNA polymerase** gave excellent results and confirmed that this enzyme efficiently discriminates between single mismatches at the primer end.

Application in Cancer and Pathogen Research

In addition to these basic studies, applications in cancer or pathogen research, among others, have been described.

- The rapid and aggressive spread of artemisinin-resistant *Plasmodium falciparum* variants is a growing threat to the elimination of malaria. O. Miotto *et al.* in a global research consortium used **HiDi® DNA polymerase** in an asPCR approach to detect a mutation found in Papua New Guinea and responsible for artemisinin resistance [4].
- Bovine viral diarrhea virus (BVDV) threatens livestock worldwide. M. Shiokawa *et al.* use **HiDi® DNA polymerase** to distinguish viral BVDV populations that vary in only one nucleotide [5].
- Y. Tsukumo *et al.* employ **HiDi® DNA polymerase** to study the effect of EGFR-activating mutations on a lung cancer cell line [6].
- Kubosaki *et al.* use **HiDi® DNA polymerase** for the analysis of a fungus producing a carcinogen [7].

Applications in Genome Editing

Modern genome editing techniques, such as CRISPR/Cas- or TALEN-based approaches, have revolutionized the possibilities for genome editing and will continue to solidify this development in the long term. Both CRISPR/Cas- or TALEN-based approaches allow the substitution of single nucleotides in the genome. The substitution of a single nucleotide after CRISPR/Cas or TALEN experiments must be demonstrated to prove a successful experiment. Compared to more complex DNA sequencing, asPCR is a faster, more direct approach for this and thus **HiDi® DNA polymerase** logically finds numerous applications, some of which are described below.

- M. Serif *et al.* and J. M. Buck *et al.* describe genome editing in the diatom Phaeodactylum tricornutum and use **HiDi® DNA polymerase** for verification of genome editing by asPCR [8-10].

- H. Morisaka *et al.* confirmed single nucleotide substitutions mediated by CRISPR/Cas3 and CRISPR/Cas9 genome-wide in human cells by "HiDi PCR", an asPCR based on **HiDi® DNA polymerase** [11].
- Also, in a "one-SHOT" genome editing in human cells described by Y. Yokouchi *et al.*, **HiDi® DNA polymerase** is used to characterize edited loci [12].
- H. Matsunari *et al.* induced new phenotypes in pig fetuses by genome editing of cloned pigs and demonstrate successful editing by using **HiDi® DNA polymerase** [13].
- **HiDi® DNA polymerase** also finds application in the methodological advancement of genome editing. For example, "Bindel PCR" was recently described as a new and rapid method for identifying CRISPR/Cas9-induced biallelic mutants by modified PCR with **HiDi® DNA polymerase** by T. Sakurai *et al.* [14].

Conclusion

asPCR poses major challenges for the DNA polymerase used for this purpose. Several independently conducted studies show that **HiDi® DNA polymerase** is ideally suited for use in asPCR in numerous research areas.

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