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MINI-REVIEW

Overview of thermostable DNA polymerases for classical PCR applications: from molecular and biochemical fundamentals to commercial systems

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Abstract During the genomics era, the use of thermostable DNA polymerases increased greatly. Many were identified and described—mainly of the genera *Thermus*, *Thermococcus* and *Pyrococcus*. Each polymerase has different features, resulting from origin and genetic modification. However, the rational choice of the adequate polymerase depends on the application itself. This review gives an overview of the most commonly used DNA polymerases used for PCR application: KOD, Pab (Isis™), Pfu, Pst (Deep Vent™), Pwo, Taq, Tbr, Tca, Tfi, Tfl, Tfu, Tgo, Tli (Vent™), Tma (UITma™), Tne, Tth and others.

Keywords Thermostable DNA polymerase · Polymerase chain reaction (PCR) · Extension rate · Error rate · Half-life time · Extension temperature

Introduction

It is a matter of fact that thermostable DNA polymerases with 5′→3′ amplification activity are one of the key enzymes in many molecular applications. Therefore, industrial business volume is much and there is financial pressure to find better polymerases than the established ones. Mainly A-type and B-type polymerases are in use (Table 1). The increasing microbiology genome projects in combination with new molecular technologies facilitate launching new thermostable DNA polymerases. However, marketing names like super, turbo, ultra, dream, gold and many more confuse the users and are not helpful. Published values of fidelity are mostly analysed at optimal conditions with λ-DNA. These error rates cannot transmit for any application. A PCR process must often be validated before becoming standard. Time and temperature of denaturing can deactivate the polymerase more or less depending on the used enzyme. Factors like DNA origin, primer and product length as well as guanine–cytosine content should have a direct influence on the choice of polymerase (Wu et al. 1991). Salt, magnesium and deoxyribonucleotide triphosphate (dNTP) concentrations can greatly affect the PCR (Ling et al. 1991; Owczarzy et al. 2008). Additives like BSA (Al-Soud and Rådström 2001), dimethylsulfoxide (Chester and Marshak 1993), formamide (Sarkar et al. 1990), betaine (Henke et al. 1997; Rees et al. 1993), ethylene glycol and 1,2-propanediol (Zhang et al. 2009), and others (Al-Soud and Rådström 2000; Varadaraj and Skinner 1994) are in use to optimize specificity and PCR amplification. Most of these important factors are recommended for calculations of the theoretical annealing temperature. Basis of this calculation is the melting temperature. Many formulas are used addicted in similar but not identical results (Table 2). The real optimal annealing temperature is very often determined by using a thermal cycler with temperature gradient. Additional, the heating and cooling rates of the thermal cycler, the thermal conductivity of the thermoblock material, the volume of the mixture (Chang and Lee 2005) and the thickness of the used plastic material have an important influence on the evaluation of an optimal PCR process (Table 3). The performance of the thermal cyclers as well as the transfer of protocols from one system to another are also important points which could be considered (Schoder et al. 2003, 2005). However, this review describes the common widely used thermostable DNA polymerases (Table 1) and their important features for PCR applications.

A-type polymerases from bacteria *Thermus*

The genus *Thermus* belongs to the bacteria and to the class of *Deinococci*. *Thermus* species grow optimal at 65–70 °C, and

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Table 1 Overview of the most common thermophilic DNA polymerases and their characteristics (n.p. = not published)

Name of DNA polymerase	Species origin	Optimal extension temperature (°C)	Extension rate [kbp/min]	Error rate = mutation frequency per bp per duplication [mf×bp^{-1}×d^{-1}]	5′→3′/ 3′→5′ exonuclease activity	Extra nucleotide overhang	Half-life time measured between 95 and 100 °C	References
Deep Vent™	*Pyrococcus* species GB-D	72 – 75	1.4	1.2×10^{-5}– 2.7×10^{-6} [exo^{-}: 2.0×10^{-4}]	No/yes [exo^{-}: no/no]	95 % blunt [exo^{-}: 70 % blunt]	95 °C/23 h 100 °C/8 h	Cline et al. (1996) Huang and Keohavong (1996)
KOD1	*Thermococcus kodacaraensis*	72 – 75	6.0 – 7.8	2.6×10^{-6}	No/yes	Blunt	95 °C/12 h 100 °C/3 h	Takagi et al. (1997)
Pab (Isis™)	*Pyrococcus abyssi*	70 – 80	n.p.	6.7×10^{-6}– 6.6×10^{-7}	No/yes	Blunt	100 °C/5 h	Dietrich et al. (2002)
Pfu	*Pyrococcus furiosus*	72 – 80	0.5 – 1.5	2.2×10^{-6}– 0.7×10^{-6} [exo^{-}: 6.0×10^{-5}– 2.0×10^{-5}]	No/yes [exo^{-}: no/no]	Blunt	95 °C/95 % after 1 h incubation	Cline et al. (1996) Kim et al. (2007)
Pwo	*Pyrococcus woesei*	72	n.p.	n.p.	No/yes	Blunt	100 °C/2 h	Dabrowski and Kur (1998)
Taq	*Thermus aquaticus*	68 – 80	1.0 – 4.8	1.8×10^{-4}–8.0×10^{-6}	Yes/no	95 % 3′A	97 °C/10 min 95 °C/40 min	Eckert and Kunkel (1990) Flaman et al. (1994) Lee et al. (2010)
Tbr (DyNAzyme™)	*Thermus brokianus*	72	n.p.	n.p.	Yes/no	3′A	96 °C/150 min	n.p.
Tca	*Thermus caldophilus*	70 – 80	1.0 – 2.3	n.p.	Yes/no	3′A	95 °C/70 min	Park et al. (1993)
Tfi	*Thermus filiformis*	70 –72	1.0	n.p.	Yes/no	3′A	94 °C/40 min	Choi et al. (1999) Zheng et al. (2008)
Tfl	*Thermus flavus*	70 –74	2.0 – 4.0	n.p.	Yes/no	3′A	97 °C/10 min 95 °C/40 min	Kaledin et al. (1981)
Tfu	*Thermococcus fumiculans*	72	0.32	5.3×10^{-5}–0.9×10^{-5}	No/yes	Blunt	95 °C/3.3 h 100 °C/2 h	Cambon-Bonavita et al. (2000)
Tgo	*Thermococcus gorgonarius*	72	1.5	5.6×10^{-6}– 3.5×10^{-6}	No/yes	Blunt	95 °C/2 h	Bonch-Osmolovskaya et al. (1996)
Tli (Vent™)	*Thermococcus litoralis*	72 – 80	1.0	4.5×10^{-5}– 2.8×10^{-6} [exo^{-}: 1.9×10^{-4}]	No/yes [exo^{-}: no/no]	Blunt 95 % [exo^{-}: blunt 70 %]	95 °C/6.7 h 100 °C/1.8 h	Cline et al. (1996) Mattila et al. (1991)
Tma (UlTma™)	*Thermotoga maritima*	65 – 75	n.p.	7.4×10^{-5}– 3.2×10^{-5}	No/yes	Blunt 95 %	97.5 °C/50 min	Diaz and Sabino (1998) Flaman et al. (1994)
TNA1_pol	*Thermococcus* sp. NA1	75	3.6	2.2×10^{-4}	No/yes	n.p.	95 °C/12.5 h 100 °C/3.5 h	Kim et al. (2007)
Tne	*Thermotoga neopolitana*	72 – 75	n.p.	n.p.	No/yes	n.p.	90 °C/1 h	Chatterjee et al. (2002)
Tpe	*Thermococcus peptonophilus*	75	2.0	3.4×10^{-6}	No/yes	90 % blunt	95 °C/4 h	Lee et al. (2010)
Tth	*Thermus thermophilus*	70 – 74	1.5 – 2.0	n.p.	Yes/no	3′A	95 °C/20 min	Carballeira et al. (1990)
Tzi (Pfx50™)	*Thermococcus zilligii*	68	0.5 – 1.0	2.0×10^{-6}	Yes/no	Blunt	n.p.	Griffiths et al. (2007)

Table 2 Formulas for calculation of the melting temperature (T_m) of oligonucleotides (summarized in von Ahsen et al. 2001). Empirical formulas based on GC content, length and the concentration of monovalent cations [Na^+]. PCR is typically performed in 0.05 M monovalent cation concentration [Na^+]. The number of nucleotides in the oligonucleotide is n. Mismatches and additives like detergents are not recommended. The empirical results calculated with the T_7 primer allow the deductive reasoning that optimal annealing conditions should be optimized using gradient function of thermal cyclers. However, next to the manual way for empirical calculation of the T_m, many programs are in use which consider additional sequence-specific stacking effects and thermodynamic data

Formulas for calculating the melting temperature (T_m) of oligonucleotides using the example of T_7 primer with the sequence TAATACGACTCACTATAGGG and 0.05 M monovalent cation concentration	T_m (°C)
$4\times(G+C)+2\times(A+T)$	56.0
$69.3+0.41\times(\%GC)-650/n$	53.2
$69.3+0.41\times(\%GC)-535/n$	59.0
$77.1+0.41\ (\%GC)+11.7\ (\log_{10}[Na^+])-528/n$	51.9
$77.8+0.41\ (\%GC)+11.7\ (\log_{10}[Na^+]\times(1.0+0.7\times[Na^+])^{-1}-528/n$	53.1
$80.4+0.345\ (\%GC)+\log_{10}[Na^+]\times[17.0-0.135(\%GC)]-550/n$	51.6
$81.5+0.41\ (\%GC)+16.6\ (\log_{10}[Na^+])-500/n$	51.3
$81.5+0.41\ (\%GC)+16.6\ \log_{10}([Na^+]\times(1.0+0.7\times[Na^+])^{-1})-500/n$	51.1

they survive temperatures between 50 and 80 °C. Their heat stable A-type DNA polymerase which belongs to the same family that includes the prokaryotic Pol I polymerases (Braithwaite and Ito 1993) has been analysed since many years (Chien et al. 1976), and ever since this time more and more polymerases from *Thermus* were characterized. The enzymes have a molecular mass of approximately 94–95 kDa (Choi et al. 1999; Lawyer et al. 1993; Park et al. 1993), and an intrinsic 5′→3′ exonuclease activity, but lack a 3′→5′ proofreading nuclease activity. The fidelity of all enzymes depends on the pH and on concentrations of substrates Mg^{2+} and dNTP which formed complexes with primers and templates. Fidelity is good at equimolar concentrations of total dNTPs to Mg^{2+} (Ling et al. 1993). *Thermus* polymerases add a 3′-adenine overhang to each end of the PCR product. Such PCR-amplified products are cloned into linearized vectors that have complementary 3′-thymine overhangs (Holton and Graham 1991; Trower and Elgar 1994). This cloning strategy is well known as TA cloning (Zhou and Gomez-Sanchez 2000). Commercialized kits with pre-prepared vectors and PCR reagents are greatly sold since the 1990s. Over the years, many DNA polymerases were isolated from different *Thermus* species and commercialized.

Table 3 Thermal conductivity of different materials. The thermal conductivity determines the necessary energy consumption thus also the time until the reaction reaches the programmed temperature. The thermal conductivity is the capability to transport thermal energy through heat conduction [W = watt, m = meter, K = kelvin]. Silver and aluminium blocks are used in thermal cyclers. PCR reaction mixtures contain natrium or potassium salts. Tubes and microtitre plates with different thickness are in use

Material	Thermal conductivity (W/mK)	Comments
Diamond	2,300	The best material but too expensive
Silver	429	The best metal used for themoblocks
Copper	401	Used for heated lids
Gold	310	Used as thin overlay to reduce the oxidizing of silver
Aluminium	237	The most common used material for thermoblocks and heated lids
Natrium	141	A component of PCR buffer
Potassium	135	A component of PCR buffer
Water	0.6	The volume of a solution have an influence on the thermal flow, in particular higher volumes, e.g. 100 µl
Plastic	0.2–0.4	The thickness has a direct influence of the thermal flow. Much more than a low volume solution, e.g. 10–20 µl
Air in thin layers	0.02	Inhibit the thermal flow and the PCR, e.g. plastic is not compatible with the block, or bubbles in the reaction mix

Taq

The most popular and well-known thermophilic DNA polymerase is Taq, isolated from the thermophilic eubacterium *Thermus aquaticus* (Kaledin et al. 1980). Taq was the first thermostable enzyme used for PCR (Saiki et al. 1988) and became one of the most important tools in commercialized molecular biology. Its optimal elongation temperature is 75–80 °C (Lawyer et al. 1993), but mainly lower temperatures (68–72 °C) are used and recommended by many suppliers, e.g. for AT-rich DNA, 68 °C is recommended. The native form produced in *Thermus aquaticus* as well as its recombinant form produced by *Escherichia coli* is available. For optimal elongation, divalent magnesium ions are necessary (Lawyer et al. 1993). Merchants recommend optimizing PCR conditions between 1 and 5 mM Mg^{2+}. The half-life time of

the Taq is short compared to other polymerases isolated from *Archaea*: only 40 min at 95 °C or 9 min at 97.5 °C (Lawyer et al. 1993). It is the best to use a denaturing time as short as possible (Yu and Pauls 1992). And, it is recommended to work with a short initial step using a relatively low denaturing temperature, e.g. 94 °C, or to put the enzyme into the reaction mixture after the initial denaturing. The reduced enzyme activity can be compensated by extending each following elongation step. Most thermal cyclers have this option by using time increments. Unwanted activity while increasing the temperature of the first denaturing step could be reduced by working with hot start Taq offered from different merchants. Different approaches for hot start activation in PCR are possible: blocked DNA polymerase activity by chemical modification (Birch et al. 1998; Moretti et al. 1998), specific antibodies (Kellog et al. 1994; Paul et al. 2010; Sharkey et al. 1994), or aptamers (Dang and Jayasena 1996) as well as reduced activity at lower temperatures by amino acid mutation (Kermekchiev et al. 2003). Another possibility for hot start is a separation of the mix and the Taq by a wax layer which melts between 60 and 80 °C (Chou et al. 1992).

In general, amplification efficiencies of Taq are round about 80 % at targets shorter than 1 kb with a CG content between 45 to 56 % (Arezi et al. 2003). Product yields generally decrease with increasing amplicon size above 1 kb. A novel strategy to enhance the processivity and to improve the performance of the Taq is to fuse the Taq with a thermostable DNA binding protein. Covalent linking of the polymerase domain with the Sso7d DNA binding protein from *Sulfolobus solfataricus* increases the processivity (Wang et al. 2004). A similar protein which is used in the same manner is Sac7d of *Sulfolobus acidocaldarius* (Priyakumar et al. 2010; Wang et al. 2012).

Taq is very often utilized in diagnostics. Amplification is totally inhibited in the presence of 0.004 volume percent blood in the PCR mixture (Al-Soud and Rådström 1998). It seems that contents of haemoglobin and lactoferrin of the plasma inhibit the amplification. BSA was the most efficient amplification facilitator to increase this tolerance (Al-Soud and Rådström 2001). Ca^{2+} has inhibitory effects, too. On the other hand, Taq tolerates higher amounts of collagen than Pwo (Kim et al. 2000).

Another discussed point in history was the fidelity of Taq. Of course, it has a 5′→3′ exonuclease activity and no 3′→5′ exonuclease (Tindall and Kunkel 1988). Therefore, its fidelity is not so good compared to Pfu or Vent™. However, low fidelity conditions were used in the past and published; the error rate was approximately $1–2\times10^{-4}$ $mf\times bp^{-1}\times d^{-1}$ [mutation frequency per base pair per duplication] (Eckert and Kunkel 1990; Keohavong and Thilly 1989). High fidelity conditions resulting in 1.2×10^{-5} to 3.3×10^{-6} $mf\times bp^{-1}\times d^{-1}$ (Eckert and Kunkel 1990; Flaman et al. 1994; Ling et al. 1991; Lee et al. 2010) were later published. Therefore, the fidelity of Taq is better than its reputation.

Tfi

Tfi DNA polymerase derived from *Thermus filiformis* (Choi et al. 1999) is also used in PCR applications. The enzyme can be used without paying any licensing fee. Like Taq, Tfi has 5′→3′ exonuclease activity. PCR performance is comparable to Taq in yield, specificity, fidelity, and robustness. Optimal extension temperature is 72 °C, but merchants recommend working with 68 °C. Tfi can be substituted for Taq DNA polymerase in virtually any application and is ideal for genomic, cDNA and plasmid targets up to 4 kb. In addition, Tfi is free of licensing restrictions that accompany Taq polymerase. Tfi can be considered for use as a component in a diagnostic application if qualified by the end user. A mutant of Tfi which should be much more thermostable is commercialized, too. This mutant is patented (Zheng et al. 2008).

Tfl and Tth

Tfl and Tth polymerases are commercialized enzymes but not so well known as Taq. Tfl was isolated from *Thermus flavus* (Kaledin et al. 1980; Harrell and Hart 1994), Tth from *Thermus thermophilis* HB8 (Carballeira et al. 1990; Rüttimann et al. 1985). Tfl has nearly the same characteristics as Taq (Table 1). This applies also to Tth, exception is the half-life time which is shorter. Tfl and Tth polymerases display optimal activity at temperatures between 70 and 74 °C. The extension rate of Tth is round about 1,500 nucleotides per minute, the rate of Tfl is not published. Both enzymes are more resistant against inhibition than Taq in the presence of vitreous eye fluid (Wiedbrauk et al. 1995). Tth tolerates higher concentrations of other inhibitory components than Taq (Al-Soud and Rådström 2001).

Tbr (DyNAzyme™), Tca and Hot Tub™

Tbr polymerase from *Thermus brockianus* is much better known under the brand DyNAzyme™. Tbr has a relatively long half-life time of 2.5 h at 96 °C compared to other polymerases isolated from *Thermus*. Optimal amplification conditions are at 72 °C with 1.5 mM Mg^{2+} in the reaction. Marketing material informed that amplification up to 6 kb of M13 DNA is possible but a science-based publication lacks.

Another but not commercialized polymerase is the Tca from *Thermophilus caldophilus* (Park et al. 1993). Its characteristics are nearly the same as Taq (Table 1) except its half-life time which is 70 min at 95 °C, much longer than Taq has.

Many DNA polymerases from *Thermus* are described but it seems that the market is saturated with such enzymes. This could be one of the reasons why the Hot Tub™ DNA polymerase from *Thermus ubiquitous* launched at the beginning of the 1990s was disconnected.

A-type polymerases from bacteria *Thermotoga*

The polymerases from *Thermotoga* which also belongs to the bacteria are not so well known. They differ in their characteristics compared to polymerases from *Thermus*. Only a few enzymes were described.

Tma (UlTma™) and Tne

The Tma polymerase from *Thermotoga maritima* is not so well known but well characterized. *Thermotoga maritima* belongs to the domain of bacteria and to the family of *Thermatogaceae*. It grows from 50 °C up to 90 °C and is the only eubacterium growing at these high temperatures (Huber et al. 1986); only *Archaea* grows under comparable conditions. The enzyme is available under the brand UlTma™ since the end of the 1990s. Tma polymerase has a size of 100 kDa (Chatterjee and Potomac 1999) and an inherent 3′→5′ exonuclease proofreading activity with no 5′→3′ nuclease activity (Diaz and Sabino 1998). A truncated form with 86 kDa is patented as well as a form with 70 kDa. Its half-life time is 50 min at 97.5 °C (Gelfand et al. 1995). Reaction conditions and fidelity are similar to Taq (Diaz and Sabino 1998) as well as the inhibitory effects of blood (Al-Soud and Rådström 2001). The enzyme is also similar to a few B-type polymerases from *Archaea*. This was an evidence for a lateral gene transfer between *Archaea* and *Bacteria* but a genetic check up between UlTma™ and B-type DNA polymerases could not confirm this hypothesis (Huang and Ito 1998).

Another similar polymerase compared to Tma is Tne. The enzyme was isolated from *Thermotoga neopolitana* located in African continental solfataric spring (Windberger et al. 1989). Tne has the same molecular weight and exonuclease activity than Tma (Yang et al. 2002). The half-life time was tested at very low conditions: 60 min at 90 °C (Chatterjee et al. 2002), optimal extension temperature is 72–75 °C. Although Tne was patented, the enzyme is not commercially available.

B-type DNA polymerases from archaea *Thermococcales*

The genera *Pyrococcus* and *Thermococcus* belong to the domain *Archaea*, the phylum *Euryarchaeota* and the order *Thermococcales*. The hypothermophilic *Archaea* and their enzymes are of interest for biotechnology application. Many B-type DNA polymerases were isolated, patented and launched.

Pyrococcus is a thermoautotrophic archaeon. Its optimal growth temperature is nearly 100 °C in the deep sea (Fiala and Stetter 1986; Zillig et al. 1987). The molecular mass of all isolated B-type DNA polymerases from *Pyrococcus* is approximately 90 kDa (Takagi et al. 1997). All enzymes have a 3′→5′ exonuclease activity which is able to eliminate misincorporated or mismatched bases but no terminal transferase activity. But they differ in half-life time, optimal reaction conditions, fidelities and elongation rates. Recombinant enzymes are mainly produced in *E. coli*, and many of them are commercially available. The KOD polymerase, previously isolated from the strain *Pyrococcus* sp. KOD1, belongs to the B-type DNA polymerases isolated from *Thermococcus* because phylogenetic analysis of 16S ribosomal DNA has shown its new phylogenetic position (Atomi et al. 2004).

Thermococcus is also a hyperthermophilic marine archebacterium. It belongs to the *Euryarchaeota* and grows optimally at temperatures of 80 °C or higher. Many thermostable B-type DNA polymerases are also isolated and commercialized. Their characteristics are similar to their closely related enzymes from *Pyrococcus*: 3′→5′ exonuclease activity, no 5′→3′ exonuclease activity, molecular weight round about 88–90 kDa, low error rate and long half-life time (Table 1). In the following, the major thermophilic B-type polymerases are described.

KOD

KOD polymerase was isolated from *Thermococcus kodakarensis* KOD1, previously *Pyrococcus* sp. KOD1 (Atomi et al. 2004). Its recombinant enzyme produced in *E. coli* has optimal activity at pH 6.5 at 75 °C (Takagi et al. 1997) but very often 68–72 °C are used for elongation. Mg^{2+} is optimal between 1.5 and 4.0 mM. Merchants recommended to validate in 0.25 mM steps using $MgSO_4$. The polymerase produces blunt-ended DNA products suitable for cloning in blunt end ligation kits. Thermostability is 12 h at 95 °C, resulting in the use of high denaturing temperatures, e.g. 98 °C. Its elongation rate is 6.0–7.8 kb per min; this is very fast in comparison to other described DNA polymerases (Takagi et al. 1997). Therefore, it could be important to adjust the extension time to 10–25 s/kb to avoid degradation of the PCR product. Validation should be done in 5 s/kb steps. The fidelity of 2.6×10^{-6} ($mf\times bp^{-1}\times d^{-1}$) is comparable to other B-type polymerases but much better than Taq. Long distance PCR over 5 kb is possible but resulted in a loss of product yield (Nishioka et al. 2001). KOD has 3′→5′ exonuclease activity. A mixture of KOD and $KOD^{(exo-)}$ is designed for reliable amplification of long, complex targets up to 15 kb with robust yield and accuracy. It can also be used for incorporation of derivative dNTPs (Masud et al. 2001; Nishioka et al. 2001; Sawai et al. 2002). KOD Hot Start DNA polymerase was optimized for the amplification of the most difficult targets, e.g. 90 % GC-rich (Rual et al. 2004); it is a mixture of KOD and two monoclonal antibodies. The antibodies inhibit the exonuclease and DNA polymerase activities at ambient temperatures (Mizuguchi et al. 1999), providing high template specificity by preventing primer degradation and mispriming events during reaction set-up. KOD was launched approximately 10 years later than Taq. Nevertheless, it was used

for many applications, e.g. construction of knock-out targeting vector (Kim et al. 2005), gene cloning (Herrin et al. 2005; Ikehara et al. 2004), genomic DNA cloning (Nisole et al. 2004), second-strand cDNA synthesis (Sasaki et al. 2004), synthetic gene synthesis (Wu et al. 2006) and much more. Furthermore, polymerases isolated from *Thermococcus kodakarensis* are also available under different brands, e.g. AccuPrime™ Pfx.

Tli (Vent™)

Tli DNA polymerase was isolated from *Thermococcus litoralis* (Neuner et al. 1990). The enzyme has a molecular weight of approximately 89 kDa and is commercialized and well known under the brand Vent™ polymerase. Both native and its recombinant form produced in *E. coli* have nearly the same activity and fidelity (Mattila et al. 1991; Kong et al. 1993). Vent™ contains a 3′→5′ proofreading nuclease activity resulting in high fidelity of 4.5×10^{-5}–2.8×10^{-6} mf× $bp^{-1}\times d^{-1}$ (Cariello et al. 1991; Mattila et al. 1991). Fidelity experiments were performed at 2 mM $MgSO_4$. The error rates up to 10 mM Mg^{2+} are in the same range (Ling et al. 1991); this applies also for the pH range between 7.0 and 8.5. Vent™ produces 95 % blunt end products and can be used for appropriate ligation. But the 3′→5′ exonuclease activity could be problematic for the ligation because oligonucleotides might be degraded at their 3′-termini. Phoshorothioate linkages at the 3′-end of oligonucleotides avoid this problem (de Noronha and Mullins 1992). These linkages enhance the priming specificity, too. The amplification rate with phoshorothioate protected primers and used Vent™ in comparison to Pfu was differentially discussed. On one hand, the amplification rate of Vent™ is lower (Skerra 1992), on the other hand better than Pfu (Vigneault and Drouin 2005). A mutant with 3′→5′ exonuclease negative activity is also commercialized: Vent™ $^{(exo-)}$. This mutant produces only 70 % blunt end products and 30 % single base overhang (unpublished data). Primers are not degraded but the lost nuclease activity results in a 40 times lower fidelity (Mattila et al. 1991). Nevertheless, Vent™ $^{(exo-)}$ is a useful tool for blunt end ligation. Vent™ has got compared to other polymerases a long half-life time which is nearly 2 h at 100 °C. This is much better than Taq and also better than Pfu (Kong et al. 1993). Therefore, Vent™ is especially useful for high temperature DNA synthesis reaction conditions (Mattila et al. 1991). The amplification by Vent™ showed a high tolerance against blood components (Al-Soud and Rådström 2001).

Pab (Isis™)

The archaeon *Pyrococcus abyssi* grows at anaerobic hyperthermophil conditions at 100 °C in the deep sea. Its Pab DNA polymerase is highly thermostable (Gueguen et al. 2001). Pab has a 3′→5′ exonuclease activity, which is responsible for correction of mismatched dNTPs. The half-life time is 5 h at 100 °C, much better than Pfu. Its recombinant form produced in *E. coli* was on the market under the brand Isis™ DNA polymerase. Optimal fidelity was measured at 40 μM dNTPs and 1.5 mM $MgSO_4$. The error rate is between 4.7×10^{-6} and 6.6×10^{-7} ($mf\times bp^{-1}\times d^{-1}$) depending on Mg^{2+} and dNTP concentration (Dietrich et al. 2002). This means that the probability of misincorporating a base is 1 per 1,600 molecules of 1.0-kb double-stranded DNA per cycle. The polymerase is attractive for applications requiring a strict limitation of errors in PCR (Dietrich et al. 2002). Pab compared to Taq, Pfu, Vent and KOD is not so thoroughly explored.

Pfu

In contrast to Pab, Pfu DNA polymerase is very well known. The enzyme was isolated from the hyperthermophilic marine archebacterium *Pyrococcus furiosus* (Lundberg et al. 1991). Both its native and recombinant forms are commercially available. Their activities are the same (Lu and Erickson 1997). The mechanistic architecture of crystallized Pfu was described in detail (Kennedy et al. 2009). One of the main characteristics is the high fidelity. Low error rates, approximately 1.0×10^{-6} ($mf\times bp^{-1}\times d^{-1}$), were observed during PCR amplification performed in the presence of 2–3 mM Mg^{2+}, 100–300 μM each dNTP (André et al. 1997; Cline et al. 1996; Flaman 1994; Lundberg et al. 1991). The pH range between 8.5 and 9.1 adjusted at 25 °C seems to be optimal. Temperature dependency of the buffer resulted in a real value round about pH 7.1 to 7.7 at 72 °C (Cline et al. 1996). Increasing Mg^{2+} concentrations from 2 to 10 mM has no influence on the fidelity but lower concentration than 2 mM have a negative effect. Hot spots of mutations are three transversions: GC→TA, AT→TA, AT→CG, and one transition: AT→GC (André et al. 1997). The proofreading 3′→5′ exonuclease activity removes mismatches which is one of the reasons for its high fidelity. But this advantage is also a disadvantage because its nuclease activity degrades free primers at the 3′-end. Therefore, it is recommended to work with nuclease-resistant phosphorothioate linkages at the 3′-end of oligonucleotides to avoid degradation (Skerra 1992). On the other hand, there is a $Pfu^{(exo-)}$ commercialized. Its 3′→5′ exonuclease activity is inactivated. Primers are not degraded but the lost nuclease activity results in a 40 times lower fidelity (Cline et al. 1996), approximately 4.7×10^{-5} ($mf\times bp^{-1}\times d^{-1}$). During the amplification, the Pfu and $Pfu^{(exo-)}$ do not generate an extra nucleotide overhang. Therefore, the products can be used for blunt ligation.

$Pfu^{(exo-)}$ seems to be an attractive alternative for a highly efficient ligation-mediated polymerase chain reaction (Angers et al. 2001). The amplification with a GC content higher than 70 % was successful using 20 mM NaCl instead of KCl and 20 % dimethylsulfoxide. Moreover, extremely GC-rich DNA sequences, e.g. CGG repeats, were also amplified by using Pfu (Chong et al. 1994). Especially the long half-life time (Table 1) can be used for high temperature denaturing conditions, e.g. 98 °C. In contrast to the A-type DNA polymerases from *Thermus*, the extension rate of 0.5–1.5 kb$\times$min^{-1} is low. Elongation steps should be longer. Amplification efficiencies at target lengths between 2 and 4 kb are much better than with Taq (Arezi et al. 2003). Processivity could be enhanced by fusing Pfu with thermostable Sso7d DNA binding protein from *Sulfolbus solfataricus* or Sac7d of *S. acidocaldarius* (Wang et al. 2004, 2012). Moreover, Pfu is commercially available mixed with an archaeal dUTPase. In contrast to Taq, Pfu utilizes dUTP more. The accumulation of dUTP was found during PCR through dCTP deamination, and the low incorporation of dUMP limited the efficiency of Pfu (Slupphaug et al. 1993). The dUTPase converted dUTP to dUMP and inorganic pyrophosphate. The combination of both enzymes resulted in the amplification of longer targets and higher yield (Dabrowski and Kiaer Ahring 2003; Hogrefe et al. 2002). In addition, kits with Pfu and monoclonal antibodies were launched for hot start resulting in an increase of fidelity, specificity and thoughput.

Pst (Deep Vent™)

Pst DNA polymerase was isolated from *Pyrococcus* species GB-D which grows in the deep sea at 2,000 m. It is able to grow at 104 °C (Jannasch et al. 1992). Its DNA polymerase which has a molecular weight of 89 kDa is on the market as Deep Vent™. The thermostability is very strong: 8 h at 100 °C and 23 h at 95 °C. The extension rate is 1.4 kbp$\times$min^{-1}. Deep Vent™ is generally produced in *E. coli.* The fidelity is 1.2×10^{-5}–2.7×10^{-6} (mf$\times$bp$^{-1}\times$d^{-1}) using 2 mM $MgSO_4$. Fidelity seems to be in a good range but it is discussed that its related enzymes Pfu, Pab or Pwo are more accurate (Cline et al. 1996; Flaman et al. 1994). Pst has no 5′→3′ proofreading exonuclease activity but 3′→5′ one resulted in a PCR product with 95 % blunt ends (Table 1). A mutant with inactivated 3′→5′ exonuclease activity is on the market (Huang and Keohavong 1996), which has a lower fidelity with 2.0×10^{-4} (mf$\times$bp$^{-1}\times$ d^{-1}). The ends of PCR product are a mix of blunt and single base 3′ overhang (Table 1). Merchants recommend to use Deep Vent™ for GC-rich and looped sequences as well as for primer extension. A special application is the primer extension with α-L-threofuranosyl thymidine-3′-triphosphate 1 (Chaput and Szostak 2003).

Pwo

Pwo DNA polymerase was isolated from *Pyrococcus woesei*. Its recombinant form is produced in *E. coli* (Dabrowski and Kur 1998; Ghasemi et al. 2011). The enzyme has got a molecular weight of 90 kDa and possesses, like all B-type polymerases, a high 3′→5′ exonuclease activity well known as proofreading activity. There is no detectable 5′→3′ exonuclease activity. The nucleotide concentration should be at least 200 μM for each dNTP. Lower concentrations might increase fidelity but may also lead to degradation of primers and products by elevated 5′→3′ exonuclease activity. To overcome slow degradation of primers, nuclease-resistant phosphorothionate protected primers could be used, or long primers with maximized GC content may be advantageous. Alternatively hot-start should reduce the problem of primer degradation. The half-life time of Pwo is 2 h at 100 °C. Merchants recommend validating optimal Mg^{2+} conditions which should be between 1 and 10 mM; standard is 2 mM. In contrast to Taq which requires optimal activity using $MgCl_2$, Pwo shows higher activity with $MgSO_4$. PCR products are blunt-ended and can therefore be directly used for blunt-end ligation without any pretreatment of the ends. When changing from Taq to Pwo, DNA polymerase problems have been observed. Merchants recommend readjusting the optimal annealing temperature. Amplification was inhibited by blood components (Al-Soud and Rådström 2001) and collagen (Kim et al. 2000).

Tgo

Tgo DNA polymerase is an enzyme isolated from *Thermococcus gorganarius* living in geothermal vents in New Zealand (Miroshnichenko et al. 1998). The enzyme has no detectable 5′–3′ exonuclease activity (Bonch-Osmolovskaya et al. 1996) but it has got a high 3′→5′ exonuclease activity, also known as proofreading activity. The inherent 3′→5′ proofreading activity of Tgo results in a very good fidelity up to 3.5×10^{-6} (mf$\times$bp$^{-1}\times$ d^{-1}). The nucleotide concentration should be at least 200 μM for each dNTP. Lower nucleotide concentrations can increase fidelity but can also enhance the degradation of primers and products. Furthermore, the 3′→5′ exonuclease activity of Tgo also acts on single-stranded oligonucleotides in the presence and absence of dNTPs. This activity does usually not interfere with PCR performance but it should be taken into consideration for primer design. Longer primers with phosphorothioate protection or with maximized GC content and focussed complementarity at the 5′-end can be advantageous. Fragments up to 3.5 kb can be amplified with the standard protocol containing 50 mM Tris/HCl pH 8.5, 12.5 mM $(NH_4)_2SO_4$ and 35 mM KCl. It is important to titrate the amount of Tgo. The optimal concentration of Tgo varies between 0.4 and 1.25 units per reaction, depending on the

amount of template which can differ from 5 ng up to 200 ng. Tgo exhibits increased thermal stability with a half-life of more than 2 h at 95 °C (Table 3). The standard concentration of Mg^{2+} is 1.25 mM. Variations have a low effect on the sensitivity (Bonch-Osmolovskaya et al. 1996). The products are blunt-ended and can therefore be directly used for blunt end ligation without any pretreatment of the ends. The enzyme accepts modified nucleotides such as digoxigenin-dUTP, biotin-dUTP and fluorescein-dUTP. For switching from Taq DNA polymerase to Tgo, it seems to be better to lower the annealing temperature by 2–3 °C since the 3′→5′ exonuclease activity will shorten the oligonucleotides during cycling, and to increase the elongation time.

Tfu, TNA1_pol, Tpe, Tzi and other B-type polymerases

Tfu DNA polymerase was isolated from *Thermococcus fumicolans* (Cambon-Bonavita et al. 2000). Polymerization activity is optimal at 72 °C, and its half-life time at 95 °C is 3.3 h. Measured fidelity is quite good with values between 0.9×10^{-5}–5.3×10^{-5} ($mf\times bp^{-1}\times d^{-1}$). But its elongation rate is 0.32 kb per min which is very low. Nevertheless, products up to 10 kb could be amplified. However, Tfu was commercialized.

TNA1_pol polymerase was isolated from *Thermococcus* sp. NA1 (Kim et al. 2007). Its characteristics are similar to Vent™: 90 kDa, 3′→5′ exonuclease activity, optimal extension temperature at 75 °C and half-life time of 3.5 h at 100 °C (Kim et al. 2007). However, the extension rate with 3.6 kb/min is much better. Amplification of a 15-kb-long fragment from λ DNA is described, and it was discussed that this amplification rate is better than performed by Vent™ or KOD having a limit at 8–9 kb (Kim et al. 2007; Mattila et al. 1991).

Tpe DNA polymerase was isolated from hyperthermophilic archaea *Thermococcus peptonophilus*. Optimal PCR reaction conditions are pH 7 at 75 °C, 2 mM Mg^{2+}, 80 mM KCl and 0.02 % Triton X (Lee et al. 2010). The described fidelity is 3.37×10^{-6} $mf\times bp^{-1}\times d^{-1}$. Tpe could amplify target DNA up to 4 kb. Ratios of 31:1 of Taq to Tpe DNA polymerase mixtures allowed PCR amplification of targets with high yield between 6 and 8 kb and at a maximum up to 15 kb (Lee et al. 2010).

Tzi DNA polymerase was isolated from *Thermococcus zilligii* (Griffiths et al. 2007). PCR products up to 1,600 bp DNA fragments was amplified. The recombinant form was launched under the brand Pfx50™ DNA polymerase. The enzyme was fused to an accessory protein. The highly thermostable polymerase has got a proofreading 3′→5′ exonuclease activity, while the accessory protein stabilizes primer–template complexes in PCR. In addition, the fusion enzyme has an intrinsic hot-start capability for room temperature reaction assembly.

Other polymerases from *Thermococcus* species were described more or less completely, but these enzymes are neither well known nor available: Tag (Böhlke et al. 2000), Tce (Kim et al. 2011), Tma (Bae et al. 2009), Tpa (Ppyun et al. 2012), Tthi (Marsic et al. 2008) and Twa (Cho et al. 2012). These enzymes are not described in this review.

Other thermostable DNA polymerases

In contrast to the polymerases from *Euryarchaeota*, where several enzymes are commercialized, a few from *Crenarchaeota* are also characterized, e.g. *Pyrobaculum islandicum* (Kähler and Antranikian 2000), *Pyrodictum occultum* (Uemori et al. 1995), *Aeropyrum pernix* (Cann et al. 1999), and *S. solfataricus* (Datukishvili et al. 1996), but all of them are without any commercial significance. An extremely thermostable DNA polymerase from the archaebacterium *Pyrolobus fumarius* retains full activity after incubation at 95 °C for 4 h (Callen and Mathur 1999). Merchants recommend this enzyme for GC-rich templates. A novel family are the thermostable Y-DNA polymerases. The enzymes are used for PCR amplification of damaged or ancient DNAs (McDonald et al. 2006).

Mixtures of A-type with B-type polymerases

The efficient amplification of longer targets than 5 kb with only one polymerase seems to be problematic (Barnes 1994). The processivity of polymerases could be enhanced by linking a DNA binding protein, e.g. Sac7d and Sso7d (Priyakumar et al. 2010; Wang et al. 2004, 2012). Another method of resolution to increase the processivity is the application of mixtures with A-type and B-type polymerases (Barnes 1994). Its use resulted in the effective amplification of longer targets. A small amount of 3′→5′ proof reading enzyme increases the amplification of longer targets: Up to 42 kb could be amplified with such mixtures (Cheng et al. 1994). Activity proportions from 10:1 to 200:1 of A-type to B-type are used and must be validated depending on target and enzymes (Cheng et al. 1994). Many mixtures are launched under different brands. Often, it seems to be not obvious what is behind the name, e.g. the Herculase™ is a mixture of Taq and Pfu, or the Expand™ is a mixture of Taq and Pwo.

Patent situation

Most of the described polymerases are patented, a few not. The question is: What is the key of innovation to patent a new thermostable DNA polymerase? Is the isolation from a new organism enough innovation? In general, most of the new patented enzymes work like the described and patented ones before. Considerably, there is no new creative mind, is not it?

In spite of this blemish, more and more similar enzymes were accredited as patents.

Conclusion

The conclusion of this review is that no thermostable DNA polymerase is the best one. Table 1 represents a compressed overview of the characteristics well-known thermostable DNA polymerases. The sections of the specific enzymes supply much more information, advantages and disadvantages. Therefore, this mini review should be a helpful tool for scientists working with PCR. However, the application itself reduces the choice of enzymes. Evaluation of optimal conditions will be often necessary for most PCR processes.

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