Thermophilic Polymerases

A Users Guide

Traditional Enzymes & Engineered Variants

- Polymerase Pedigree
- Polymerase Selection Guide
- Convenience versus Flexibility
- Taq Polymerase
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- Proofreading Polymerases

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Jena Bioscience

Jena Bioscience GmbH was founded in 1998 by a team of scientists from the Max-Planck-Institute for Molecular Physiology in Dortmund. 25+ years of academic know how were condensed into the company in order to develop innovative reagents and technologies for the life science market.
Since the start up, the company has evolved into an established global reagent supplier with more than 5500 products on stock and > 3000 customers in 50+ countries. Jena Bioscience serves three major client groups:

- **Research laboratories at universities, industry, government, hospitals and medical schools**
- **Pharmaceutical industry in the process from lead discovery through to pre-clinical stages**
- **Laboratory & diagnostic reagent kit producers and re-sellers**

Our company premises are located in the city of Jena / Germany with a subsidiary in Teltow, in the vicinity of the German capital Berlin.

Jena Bioscience's products include nucleosides, nucleotides and their non-natural analogs, recombinant proteins & protein production systems, reagents for the crystallization of biological macromolecules and tailor-made solutions for molecular biology and biochemistry.
In our chemistry division, we have hundreds of natural and modified nucleotides available on stock. In addition, with our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from mg to kg scale.
In the field of recombinant protein production, Jena Bioscience has developed its proprietary LEXSY technology. LEXSY (Leishmania Expression System) is based on a S1-classified unicellular organism that combines easy handling with a full eukaryotic protein folding and modification machinery including mammalian-like glycosylation. LEXSY is primarily used for the expression of proteins that are expressed at low yields or are inactive in the established systems, and expression levels of up to 500 mg/L of culture were achieved.

For the crystallization of biological macromolecules – which is the bottle-neck in determining the 3D-structure of most proteins – we offer reagents and tools for crystal screening, crystal optimization and phasing that can reduce the time for obtaining a high resolution protein structure from several years to a few days.
Our specialized reagents are complemented with a large selection of products for any molecular biology & biochemistry laboratory such as kits for Standard PCR and Real-Time PCR, fluorescent probes, oligonucleotides, cloning enzymes, mutagenesis technologies, and many more…
We combine highest quality standards for all our products (certified according to DIN EN ISO 9001) with individualized customer support. We establish direct lines of communication from clients to our in-house scientists, resulting in productive interactions among people with similar research interests who speak the same language. Furthermore, we offer support programs and attractive discount schemes for young scientists establishing their own labs. If you wish to receive more information, just send an e-mail to **info**@stratech.co.uk.

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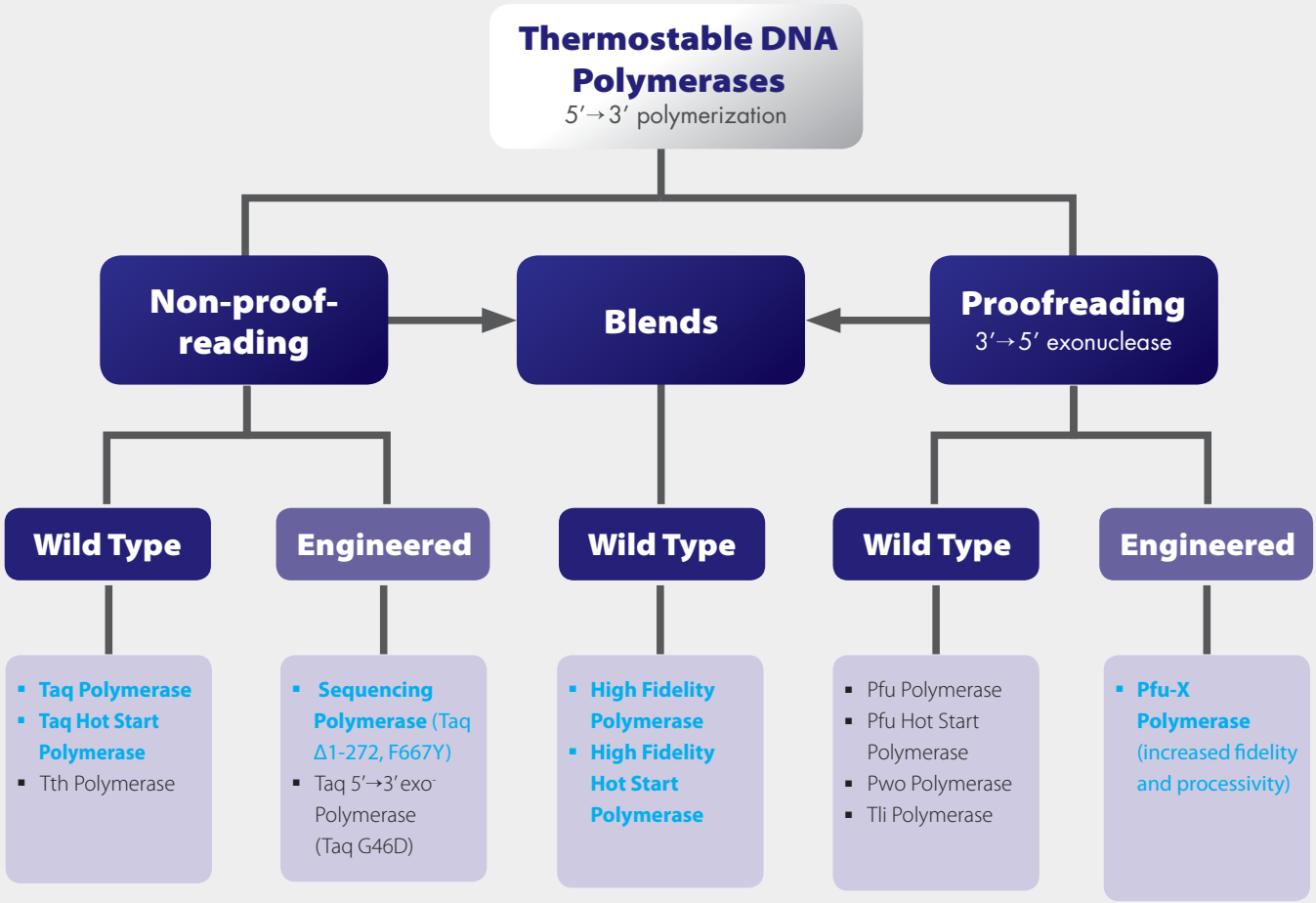
Polymerase Pedigree

Thermostable DNA polymerases are heat-resistant, template-dependent enzymes that add free nucleotides to the 3'-end of a newly synthesized complementary DNA strand. They can be divided into proofreading enzymes (with inherent 3'-5' exonuclease activity) and non-proofreading enzymes that lack exonuclease activity. 3'-5' exonuclease activity occurs upon incorporation of a mismatched base: The polymerase reverses its direction by one base pair, excises the mismatch, re-inserts the correct base and continues replication. DNA polymerases are commercialized in various forms including engineered mutants and blends of polymerases to achieve optimal results in a large variety of DNA synthesis reactions (Fig. 1).

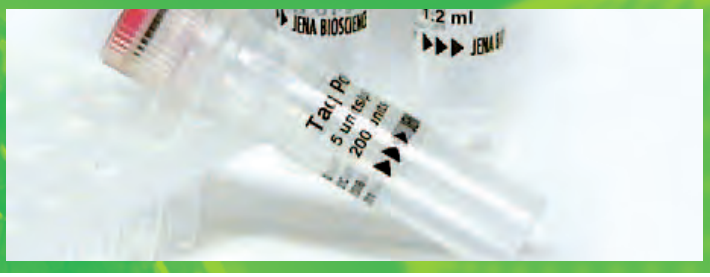


Taq: Thermus aquaticus, Tth: Thermus thermophilus, Pwo: Pyrococcus woesei, Pfu: Pyrococcus furiosus, Tli: Thermococcus litoralis.

Enzymes available from Jena Bioscience

Figure 1

Proofreading and non-proofreading enzymes are marketed as wild type, engineered mutants, and blends thereof covering a very broad range of applications.



Polymerase Selection Guide – What enzyme do I need?

The available portfolio of our polymerases (Fig. 1) allows choosing the most appropriate enzyme for a particular application. In most cases it is desired that a PCR yields large amounts of DNA with high specificity (no by-product DNA) and high fidelity (minimum number of mutations).

Since these requirements sometimes may be contradictory – and also depend on the buffer system and the cycling regime – Jena Bioscience offers the polymerases Taq Pol & Taq Pol / high yield, Taq Hot Start & High Fidelity Hot Start, High Fidelity, and Pfu-X that cover the entire range of applications (Tab. 1).

Table 1

Selection guide for choosing the most appropriate polymerase.

Enzyme	Efficiency / Yield	Specificity	Fidelity / Error rate [1], [2]	Application
Taq Polymerase	++	++	10^{-5}	▪ Standard PCR / optimized for minimal by-product formation ▪ Routine and plate based PCR, automated pipetting
Taq Polymerase / high yield	+++	+	10^{-5}	▪ Standard PCR / optimized for high efficiency in a broad range of reaction conditions ▪ Incorporation of modified nucleotides
Taq Hot Start Polymerase	++	+++	10^{-5}	▪ High specificity PCR / high sensitivity PCR ▪ Plate based PCR and automated pipetting
High Fidelity Polymerase	+++	++	2×10^{-6}	▪ High fidelity PCR / long range PCR (> 30 kb) ▪ Amplification of GC-rich and other difficult templates
High Fidelity Hot Start Polymerase	+++	+++	2×10^{-6}	▪ High fidelity PCR with highest specificity and sensitivity ▪ Long range PCR, amplification of difficult templates and of small template amounts
Pfu-X Polymerase	+++	+++	2×10^{-7}	▪ Amplification with highest fidelity ▪ High speed amplification of difficult and long templates

References:

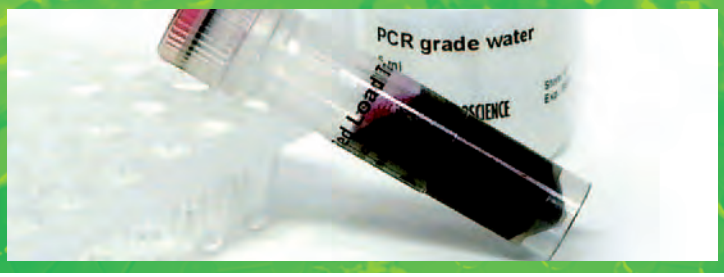
[1] The error rate of a polymerase is calculated as number of mutations per number of base pairs per DNA doublings (PCR cycles).
ER = MF / (bp · d)
ER: Error rate
MF: number of mutations (mutation frequency)
bp: number of base pairs (fragment length)
d: DNA doublings (number of PCR cycles)

[2] Jena Bioscience, 2011

Convenience versus Flexibility

A PCR reaction requires DNA-template, primers, dNTPs, and DNA polymerase in an appropriate buffer. All these components may be set up individually which allows high flexibility but – on the other hand – requires inconvenient multiple pipetting steps. In order to allow the user to compromise between convenience and flexibility, Jena Bioscience offers its polymerases in five types of formulations (Fig. 2).

Figure 2
Five types of formulation ranging from ready-to-use room-temperature-stable lyophilisates and mastermixes (that require a minimum of pipetting) to polymerase kits that allow adaptation for any PCR. Lyophilisates and Ready-to-Use Mixes with primers are available upon request.



Taq Polymerase

Taq polymerase is the traditional work horse for PCR. Our enzyme is the product of the wild type *Thermus aquaticus* DNA polymerase gene expressed in *E. coli*. Each lot of our Taq polymerase is subjected to a set of quality control assays, including a low copy 4 kb PCR (Fig. 3), a qPCR-based contamination test for bacterial and human DNA (Fig. 10), a FRET assay to verify absence of contamination with DNases, RNases and nicking enzymes, and a colorimetric assay to test for proteolytic activities.

Taq polymerase comes with complete optimized reaction buffer (including $MgCl_2$) and with additional buffer (without magnesium but with a separate vial of $MgCl_2$) allowing optimization of Mg^{2+}-concentration for individual assays [3], [4], [5].

The enzyme works for most PCR reactions and primer-template combinations without the need for optimization. The very robust enzyme-buffer system is particularly suitable for plate based PCR and automated pipetting.

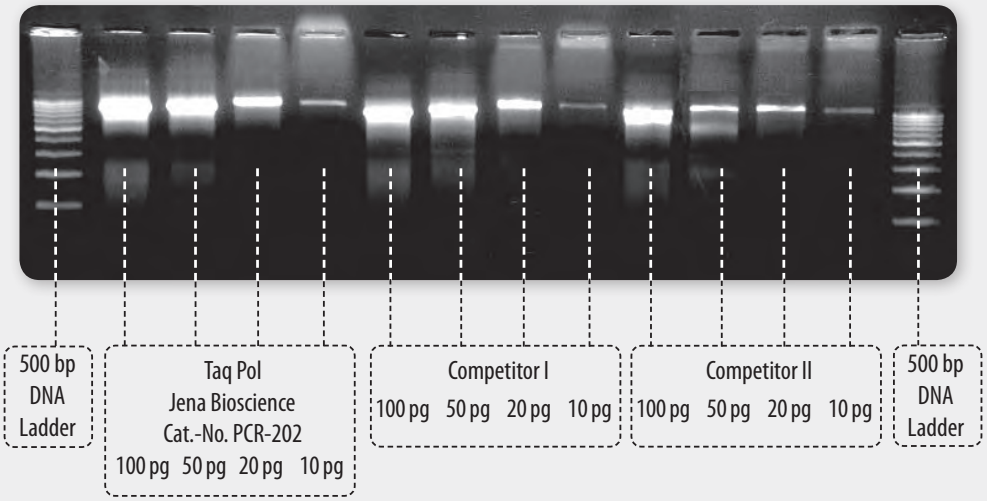


Figure 3

One important parameter that separates high-quality polymerases from others is amplification of long fragments from minimum amounts of template. The gel shows Jena Bioscience's Taq Pol routine QC assay – amplifying a 4 kb fragment from lambda DNA in a dilution series from 100 pg down to 10 pg of template – compared to other Taq polymerases. Its high amplification efficiency – especially at lowest template amounts – and minimal formation of by-products distinguish Taq Pol from competitor enzymes. Assay: Amplification of lambda DNA (template dilution series), 4 kb fragment, 1.25 units Taq Pol / reaction. 95°C, 2 min; 30x (95°C, 10 s; 61°C, 20 s; 72°C, 4 min); 72°C, 4 min.

References:

[3] Mullis *et al.* (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymol.* **155:**335.

[4] Saiki *et al.* (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnostics of sickle cell anemia. *Science.* **230:**1350.

[5] Gelfand (1992) Taq DNA Polymerase. In: *PCR Technology* (Erlich). Oxford University Press, New York. 17.

Apart from the ability of Taq polymerase to amplify low amounts of template, the speed of amplification is an important criterion when using a polymerase since it greatly affects the overall running time of the PCR. Specialized mutant enzymes with increased processivity polymerize up to several thousands of nucleotides per minute while common wild type Taq polymerase preparations are considered to form approximately 1.000 new base pairs per minute.

The Jena Bioscience Taq polymerase was systematically investigated for its processivity, and all lots released allow amplification of up to 3 kb of DNA per minute without significant loss of yield (Fig. 4).

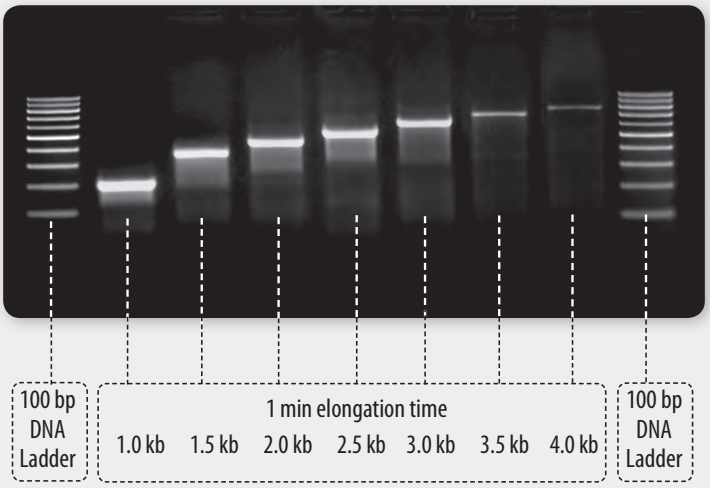


Figure 4

Jena Bioscience Taq Pol is capable of incorporating up to 3 kb per min without significant loss of yield. Assay: Amplification of lambda DNA, 100 pg, various fragment lengths, 1.25 units Taq Pol / reaction, 95°C, 2 min; 30 x (95°C, 10 s; 59°C, 20 s; 72°C, 1 min); 72°C, 1 min.

In addition, the polymerase's thermostability is an important parameter. During a conventional PCR the temperature exposure accumulates to roughly 10-15 min at 95°C and additional 30-60 min at 72°C. This significant heat stress can only be withstood by high quality enzyme preparations. The thermostability of Jena Bioscience Taq Pol is shown in Fig. 5. The enzyme is stable at 95°C for up to 30 min without significant loss of activity.

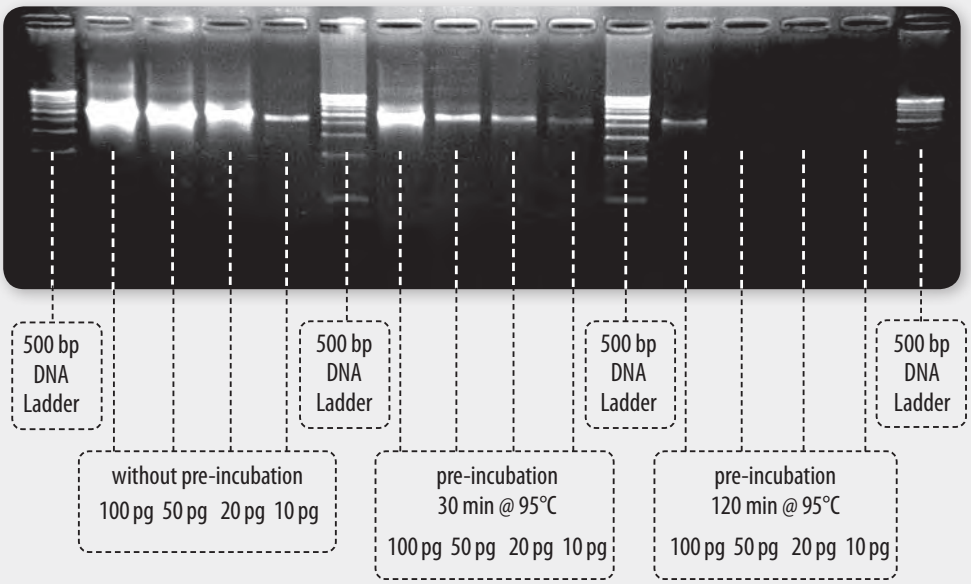


Figure 5

Exposure of Jena Bioscience Taq Pol to heat stress (30 min @ 95°C) results in a minor decrease of activity only and thereby ensures functionality of the enzyme even for prolonged PCR programs. Significant decrease of activity is only observed after excessive heat stress (120 min @ 95°C) which is way beyond the requirements in PCR. Assay: Amplification of lambda DNA (template dilution series), 4 kb fragment, 1.25 units Taq Pol / reaction, 95°C, 0 / 30 / 120 min; 95°C, 2 min; 30x (95°C, 10 s; 61°C, 20 s; 72°C, 4 min); 72°C, 4 min.

Jena Bioscience Taq polymerase comes in five formulations ranging from lyophilized, room temperature stable beads in preloaded PCR tubes over convenient and cost-effective mastermixes and core kits to individual Taq Pol for flexible adaption to customized assays (Tab. 2).

Table 2
Summary of Jena Bioscience Taq Pol formulations.

Lyophilisate				
Taq Master Lyophilisate	preloaded 8-tube strips or 96-well plates, PCR-grade water	S pack	PCR-152S-8TS	12 strips (96 reactions)
		L pack	PCR-152S-8TL	60 strips (480 reactions)
Ready-to-Use Mix / direct gel loading				
Red Load Taq Master	5x conc. Mastermix, PCR-grade water	S pack	PCR-108S	100 reactions
		L pack	PCR-108L	500 reactions
Ready-to-Use Mix				
Taq Master	5x conc. Mastermix, PCR-grade water	S pack	PCR-102S	100 reactions
		L pack	PCR-102L	500 reactions
Core Kit				
Taq Core Kit	Taq Pol, dNTP Mix, Reaction Buffer complete, Reaction Buffer without $MgCl_2$, $MgCl_2$ stock solution	S pack	PCR-232S	200 units
		L pack	PCR-232L	1,000 units
Polymerase Kit				
Taq Pol	Taq Pol, Reaction Buffer complete, Reaction Buffer without $MgCl_2$, $MgCl_2$ stock solution	S pack	PCR-202S	200 units
		L pack	PCR-202L	1,000 units

PCR Enhancers and High Yield Buffer system

Alongside the pivotal ingredients of PCR – template, primers, polymerase, and dNTPs – a variety of additives has been identified that shows strong effects upon yield, specificity and consistency of PCR (Tab. 3). Each of these may have a beneficial effect on individual amplifications but it is still largely impossible to predict their effects a priori. Therefore, an individual assay optimization may be essential for cumbersome template-primer combinations.

Table 3
Selected additives known to affect PCR.

Enhancer	Recommended concentration	References
DMSO (dimethyl sulfoxide)	2 – 10 %	[6], [7], [8]
Betaine	1 – 1.5 M	[9], [10], [11], [12]
Formamide	1 – 5 %	[13]
Non-ionic detergents (e.g. Triton X-100, Tween 20 or Nonidet P-40)	0.1 – 1 %	[14], [15]
TMAC (tetramethylammonium chloride)	15 – 100 mM	[16], [17]
7-deaza-2'-deoxyguanosine (dC^7GTP)	3:1 (dC^7GTP : dGTP)	[18]
BSA (bovine serum albumin)	0.1 – 0.5 µg/µl	[19], [20], [21]
SSB (single-stranded DNA binding protein)	0.2 – 5 ng/µl	[22], [23]

References:

[6] Smith *et al.* (1990) Using cosolvents to enhance PCR amplification. *Amplifications* **5**:16.

[7] Kulandaiappan *et al.* (1994) Denaturants or cosolvents improve the specificity of PCR amplification of a G+C-rich DNA using genetically engineered DNA polymerases. *Gene* **140**:1.

[8] Hung *et al.* (1990) A specificity enhancer for polymerase chain reaction. *Nucleic Acids Research* **18**:1666.

[9] Rees *et al.* (1993) Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry* **32**: 137.

[10] Weissensteiner *et al.* (1996) Strategy for Controlling Preferential Amplification and Avoiding False Negatives in PCR Typing. *BioTechniques* **21**: 1102.

[11] Baskaran *et al.* (1996) Uniform amplification of a mixture of deoxyribonucleic acids with varying GC content. *Genome Research* **6**: 633.

[12] Henke *et al.* (1997) Betaine Improves the PCR Amplification of GC-Rich DNA Sequences. *Nucleic Acids Research* **25**: 3957.

[13] Sarkar *et al.* (1990) Formamide can dramatically improve the specificity of PCR. *Nucleic Acids Research* **18**: 7465.

[14] Demeke *et al.* (1992) The Effects of Plant Polysaccharides and Buffer Additives on PCR. *Bio Techniques* **12**: 332.

[15] Bachmann *et al.* (1990) Improvement of PCR amplified DNA sequencing with the aid of detergents. *Nucleic Acids Research* **18**: 1309.

[16] Hung *et al.* (1990) A specificity enhancer for polymerase chain reaction. *Nucleic Acids Research* **18**: 4953.

[17] Chevet *et al.* (1995) Low concentrations of tetramethylammonium chloride increase yield and specificity of PCR. *Nucleic Acids Research* **23**: 3343.

[18] McConlogue, L. *et al.* (1988) Structure-independent DNA amplification by PCR using 7-deaza-2'-deoxyguanosine. *Nucl. Acids Res.* **16(20)**: 9869.

[19] Kreader *et al.* (1996) Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and environmental microbiology* **62**: 1102.

[20] Henegariu *et al.* (1997) Multiplex PCR: Critical Parameters and Step-by-Step Protocol. *Bio Techniques* **23**: 504.

[21] Giambernardi *et al.* (1998) Bovine Serum Albumin Reverses Inhibition of RT-PCR by Melanin. *Bio Techniques* **25**:564.

[22] Schwarz *et al.* (1990) Improved yields of long PCR products using gene 32 protein. *Nucleic Acids Research* **18**: 1079.

[23] Barski *et al.* (1996) Rapid assay for detection of methicillin-resistant Staphylococcus aureus using multiplex PCR. *Mol Cell Probes* **10**: 471.

In order to avoid such tedious optimizations, we developed High Yield Buffer that contains a balanced composition of PCR enhancers for maximum efficiency. High Yield Buffer decreases the melting temperature of dsDNA and primer/ssDNA complexes and thus achieves superior amplification results especially for difficult template-primer combinations. It also facilitates incorporation of labeled/modified nucleotides into the newly synthesized DNA strand. However, in complex assays the increased yield may come at the cost of reduced specificity, i.e. formation of unspecific DNA by-products. Taq Pol / High Yield Buffer is recommended for applications that require high yields (Fig. 6) and for PCR-mediated DNA labeling.

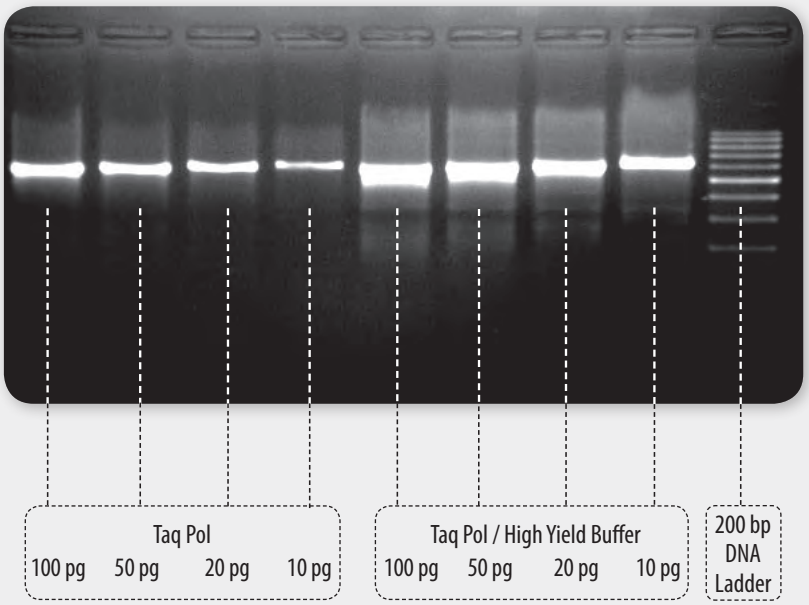


Figure 6
High Yield Buffer yields 3-10fold more PCR product compared to the conventional Taq Pol buffer system, but in difficult assay set-ups the increased yield may come at the cost of reduced specificity. Assay: Amplification of lambda DNA (template dilution series), 1 kb fragment, 1.25 units Taq Pol / reaction, 95°C, 2 min; 30 x (95°C, 10 sec; 59°C, 20 sec; 72°C, 1 min); 72°C, 1 min.

All Taq Pol formulations (Tab. 2) are alternatively available with High Yield Buffer at no additional costs.

Hot Start Polymerase

Our Hot Start polymerase is Taq Pol with inhibited enzymatic activity at ambient temperatures. This inactivation prevents extension of non-specifically annealed primers and primer-dimers during PCR setup [24], [25], [26], [27], [28] and results in decreased formation of undesired by-products. During the initial denaturation step of the cycling program the inhibitor quickly dissociates and the polymerase becomes fully active.

Hot Start Pol thus provides improved sensitivity and specificity when prolonged room-temperature exposure during PCR set up is inevitable and/or when low-copy-number templates are amplified from a complex matrix. This was analyzed for detection of genetically engineered soy in a soybean flour matrix using Taq Pol and Hot Start Pol. While both polymerases generate the characteristic signal, the formation of undesired non-specific by-product DNA is greatly reduced by Hot Start Pol (Fig. 7).

In addition, every production lot of our Hot Start Pol is analyzed for amplification of a 345 bp fragment of the Insulin receptor gene from low amounts of human genomic DNA (Fig. 8).

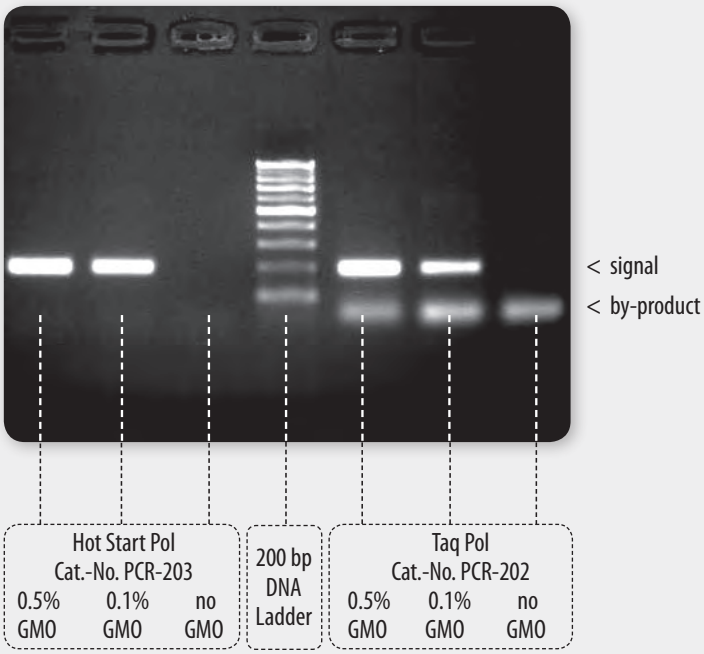


Figure 7

Detection of genetically modified Roundup Ready® soy (Monsanto) down to 0.1% in soybean flour was compared for Hot Start Pol and conventional Taq Pol. Formation of by-product and associated risk of false positive detection is greatly reduced by Hot Start Pol.

Assay: Amplification of genomic DNA, 210 bp fragment of soy transgene, 150 ng DNA / reaction, 1.25 units Hot Start Pol / reaction, 95°C, 2 min; 35x (95°C, 30 sec; 56°C, 30 sec; 72°C, 30 sec); 72°C, 4 min.

References:

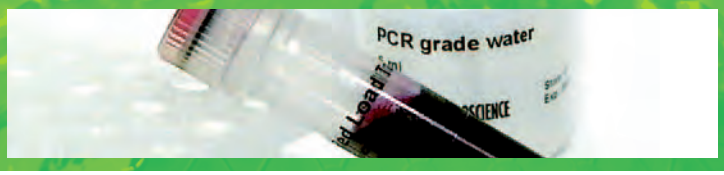
[24] Birch *et al.* (1996) Simplified hot start PCR. *Nature* **381**: 445.

[25] Agüero *et al.* (2003) Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. *J Clin Microbiol.* **41**: 4431.

[26] Ailenberg *et al.* (2000) Controlled hot start and improved specificity in carrying out PCR utilizing touch-up and loop incorporated primers (TULIPS). *Biotechniques.* **29**: 1018.

[27] Chou *et al.* (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res.* **20**: 1717.

[28] Kellogg *et al.* (1994) TaqStart Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *Biotechniques.* **16**: 1134.



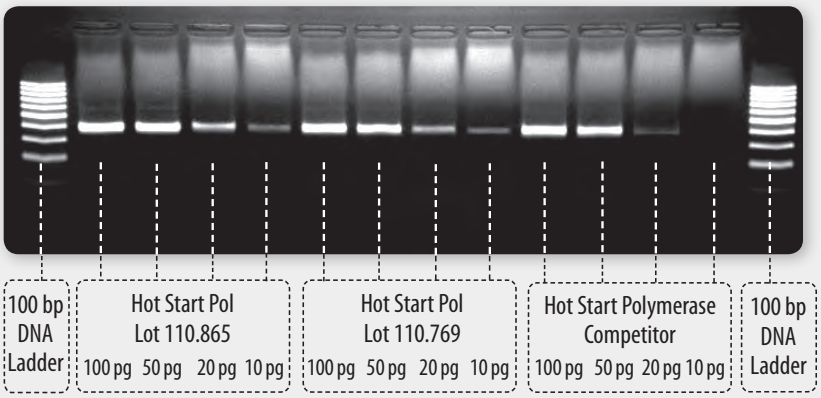


Figure 8

Two production lots of Jena Bioscience Hot Start Pol and a hot start enzyme from a competitor were assayed for amplification of a 345 bp fragment of the Insulin receptor gene from human genomic DNA in a template dilution series. Our Hot Start Pol yields a reproducible signal down to 10 pg of template corresponding to approximately 3 copies of human genomic DNA. Assay: Amplification of human genomic DNA, 345 bp fragment of Insulin receptor gene, 1.25 units Hot Start Pol / reaction, 95°C, 2 min; 35x (95°C, 10 sec; 50°C, 20 sec; 72°C, 1 min); 72°C, 1 min.

Jena Bioscience Hot Start Pol comes in five formulations ranging from lyophilized, room temperature stable beads in preloaded PCR tubes over convenient and cost-effective mastermixes and core kits to individual Taq Pol for flexible adaption to customized assays (Tab. 4).

Table 4

Summary of Jena Bioscience Hot Start polymerase formulations.

Lyophilisate				
Hot Start Master Lyophilisate	preloaded 8-tube strips or 96-well plates, PCR-grade-water	S pack	PCR-153S-8TS	12 strips (96 reactions)
		L pack	PCR-153S-8TL	60 strips (480 reactions)
Ready-to-Use Mix / direct gel loading				
Red Load Hot Start Master	5x conc. Mastermix, PCR-grade water	S pack	PCR-109S	100 reactions
		L pack	PCR-109L	500 reactions
Ready-to-Use Mix				
Hot Start Master	5x conc. Mastermix, PCR-grade water	S pack	PCR-103S	100 reactions
		L pack	PCR-103L	500 reactions
Core Kit				
Hot Start Core Kit	Hot Start Pol, dNTP Mix, Reaction Buffer complete, Reaction Buffer without $MgCl_2$, $MgCl_2$ stock solution	S pack	PCR-233S	200 units
		L pack	PCR-233L	1,000 units
Polymerase Kit				
Hot Start Pol	Hot Start Pol, Reaction Buffer complete, Reaction Buffer without $MgCl_2$, $MgCl_2$ stock solution	S pack	PCR-203S	200 units
		L pack	PCR-203L	1,000 units

Proofreading Polymerases

Proofreading polymerases (also see Fig. 1) are characterized by an inherent 3'–5' exonuclease activity that corrects mismatch errors during DNA replication and thereby greatly reduces the frequency of mutations. While Taq polymerase as typical non-proofreading enzyme incorporates approximately 1 mismatch per 10^4–10^5 nucleotides, the fidelity of proofreading enzymes is higher by a factor of 20–50 (Tab. 5) [29], [30], [31]. Therefore, for a number of applications such as cloning, sequencing, amplification of long DNA fragments and mutational analyses the use of proofreading enzymes is highly recommended [32], [33], [34], [35].

Table 5
Fidelity (error rates) of Jena Bioscience polymerases.

Enzyme	Error Rate [36], [37]	Fidelity compared to Taq Polymerase
Taq Polymerase / Hot Start Polymerase	10^{-5}	NA
High Fidelity Polymerase / High Fidelity Hot Start Polymerase	2×10^{-6}	5 x
Pfu Polymerase	5×10^{-7}	20 x
Pfu-X Polymerase	2×10^{-7}	50 x

References:

[29] Lundberg *et al.* (1991) High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus. *Gene.* **108**: 1.

[30] Tindall *et al.* (1988) Fidelity of DNA synthesis by the Thermus aquaticus DNA polymerase. *Biochemistry.* **27(16)**: 6008-13.

[31] Cline *et al.* (1996) PCR Fidelity of Pfu DNA Polymerase and Other Thermostable DNA Polymerases. *Nucleic Acids Res.* **24-18**: 3546.

[32] Barnes (1994) PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. *Proc Natl Acad Sci USA.* **91**: 2216.

[33] Cheng *et al.* (1994) Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc. Natl. Acad. Sci. (USA)* **91**: 5695.

[34] Cline *et al*. (1996) PCR fidelity of pfu DNA polymerase and other thermostable DNA polymerases. *Nucleic Acids Res.* **24**: 3546.

[35] Kim *et al.* (2008) Crystal structure of Pfu, the high fidelity DNA polymerase from Pyrococcus furiosus. *Int J Biol Macromol.* **42**: 356.

[36] ER = MF / (bp · d)

ER: Error rate; MF: number of mutations (mutation frequency); bp: number of base pairs (fragment length);
d: DNA doublings (number of PCR cycles)

[37] in-house data

High Fidelity Polymerases

High Fidelity Polymerase is a blend of Taq Pol with a proofreading enzyme in order to combine the speed of Taq polymerase with enhanced fidelity for highly accurate and efficient PCR. It shows excellent results with very long (30+ kb), GC-rich, and other "difficult" templates. A hot start version of High Fidelity Pol is also available and recommended when maximum specificity is additionally required.

We have established a sensitive assay to evaluate performance of High Fidelity Pol that is based on amplification of a dilution series of an 18 kb template. The assay is very sensitive to even minor lot-to-lot variations, and every production lot is routinely tested for yielding a signal with as little as 0.05 – 0.1 ng of template (Fig. 9).

High Fidelity Pol and High Fidelity Hot Start Pol are available as core kits (including enzyme, dNTP mix and reaction buffer) and as single polymerase kits (enzyme and reaction buffer only, Tab. 6).

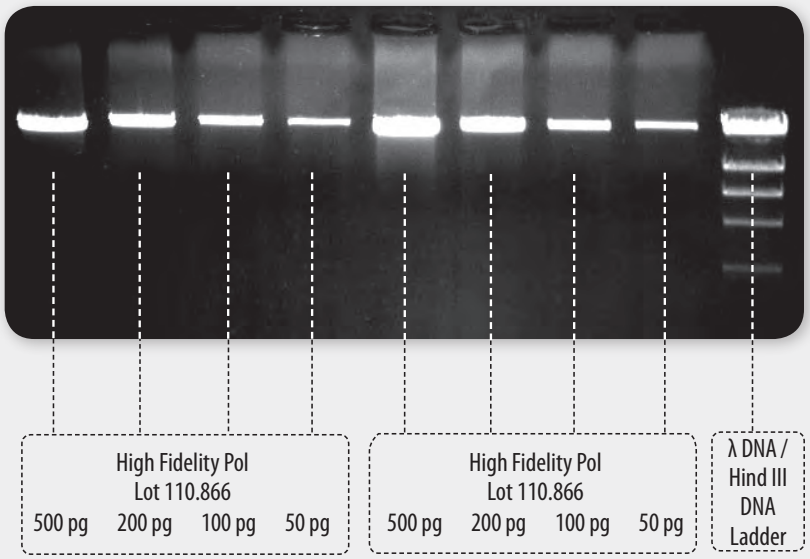


Figure 9

Every production lot of High Fidelity polymerase is assayed for amplification of a dilution series of an 18 kb fragment down to 50 pg of template. Assay: Amplification of lambda DNA (template dilution series), 18 kb fragment, 1.25 units High Fidelity Pol / reaction, 95°C, 2 min; 20x (95°C, 10 sec; 59°C, 20 sec; 68°C, 18 min).

Table 6

Formulations of High Fidelity Pol and High Fidelity Hot Start Pol.

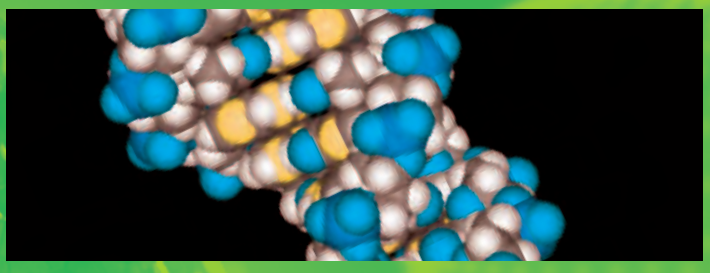
Core Kits				
High Fidelity Core Kit	High Fidelity Pol, dNTP Mix, High Fidelity Buffer	S pack	PCR-234S	100 units
		L pack	PCR-234L	500 units
High Fidelity Hot Start Core Kit	High Fidelity Hot Start Pol, dNTP Mix, High Fidelity Buffer	S pack	PCR-235S	100 units
		L pack	PCR-235L	500 units
Polymerase Kits				
High Fidelity Pol	High Fidelity Pol, High Fidelity Buffer	S pack	PCR-204S	100 units
		L pack	PCR-204L	500 units
High Fidelity Hot Start Pol	High Fidelity Hot Start Pol, High Fidelity Buffer	S pack	PCR-205S	100 units
		L pack	PCR-205L	500 units

Pfu-X Polymerase

Pfu-X polymerase is an engineered variant of Pfu DNA polymerase (the gene product of the DNA polymerase of *Pyrococcus furiosus*), showing a 2-3x higher accuracy and a 4-5x increased processivity than its wild type.
The enzyme catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction and possesses a very stringent inherent 3'→5' exonuclease proofreading activity that results in a greatly increased fidelity. Compared to Taq, Pfu-X incorporates approximately 50x fewer mismatches during replication, and compared to High Fidelity Pol its fidelity is still superior by more than one order of magnitude (Tab. 5). In addition, it shows a high processivity allowing shorter elongation times and more rapid PCR protocols.
Pfu-X is the gold standard of DNA polymerases and is recommended for all PCRs that require fidelity and speed.
Pfu-X polymerase is available as core kit (including enzyme, dNTP mix and reaction buffer) and as single polymerase (enzyme and reaction buffer only, Tab. 7).

Table 7
Formulations of Pfu-X.

Core Kit				
Pfu-X Core Kit	Pfu-X Polymerase, dNTP Mix, Pfu-X Buffer	S pack	PCR-237S	100 units
		L pack	PCR-237L	500 units
Polymerase Kit				
Pfu-X Polymerase	Pfu-X Polymerase, Pfu-X Buffer	S pack	PCR-207S	100 units
		L pack	PCR-207L	500 units



Quality Control

In addition to the functional testing of our polymerases outlined in this paper, all our PCR reagents are assayed for the most critical contaminants that may interfere with PCR analyses: bacterial and human DNA.

Since the presence of only a few copies of such DNA may result in false positive signals during later application, Jena Bioscience analyzes each lot at the very end of the production process by a highly sensitive qPCR for absence of contaminating DNA (Fig. 10).

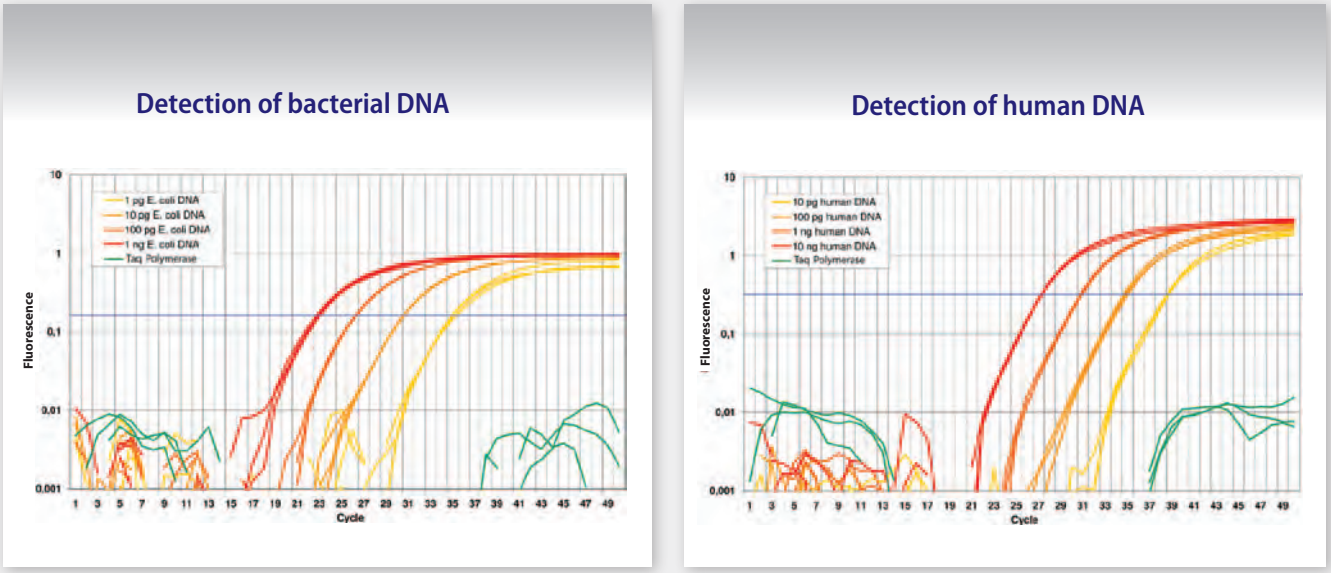


Figure 10

Absence of contaminating bacterial and human DNA is tested by a multiplex qPCR assay. Bacterial DNA is amplified using primers and probes for the 16S rRNA gene, human DNA is detected by amplification of a beta-actin gene fragment. Traces (as few as singles copies) of contaminating DNA are typically detected at ct-values in between 35 and 45. All polymerase lots are verified free of bacterial and human DNA.

Quality control of our polymerases as well as that of all other reagents is performed according to our DIN EN ISO 9001 certified quality management system. We commit ourselves to work according to international standards and continuously improve our quality management system and our in house processes to meet highest quality standards.

Other Jena Biosciences Products

Product Categories

Nucleotides

- Nucleotides for Applications on DNA
- Nucleotides for Applications on RNA
- Nucleotides for applications on Proteins/Enzymes
- Nucleotides in Cell Biology
- Nucleotides in Signal Transduction
- Nucleotides as 19F-NMR probes
- Nucleotides for Drug Discovery
- Nucleotides for Click Chemistry
- Nucleotides for Assays

Nucleosides

- Cancer and Proliferation Marker Nucleosides
- Nucleosides for Applications on Proteins/Enzymes - Sulfonyl Fluoride Probes

Nucleotides Kits

- Non-hydrolyzable Nucleotide Kits

Other Nucleotides

- Immobilized Nucleotides for Affinity Chromatography

Proteins

- Recombinant Proteins
- Native Proteins
- Detection & Analysis
- Purification

LEXSY Expression

- LEXSY Configurations
- LEXSY Background
- LEXSY Cultivation

Crystallography & Cryo-EM

- Get started
- Screening
- Screening Membrane Proteins
- Optimization
- Data Collection
- Phasing
- Cryo-EM

Molecular Biology

- Endpoint PCR
- Real-Time PCR / qPCR
- Reverse Transcription / RT-PCR
- Isothermal DNA Amplification & LAMP
- in vitro Transcription
- RNA/DNA Preparation
- DNA Cleanup
- DNA Ladders
- Protein MW Marker
- Buffers and Reagents
- Food Control
- MSI Analysis
- Enzymes
- Cloning and Mutagenesis
- Contamination Control

Probes & Epigenetics

- Reactive Components
- DNA/cDNA Labeling
- RNA/cRNA Labeling
- Protein Labeling
- Cell Labeling
- Epigenetics

RNA Technologies

- RNA Synthesis
- RNA Labeling & Modification
- RNA Analysis & Detection
- RNA-guided gene targeting

Click Chemistry

- Click Reagents by Application
- Click Reagents by Chemistry

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