For life science research only.
Not for use in diagnostic procedures.

Pwo Master

Cat. No. 03 789 403 001

100 reactions

Version 07
Content version: May 2019

Store at +2 to +8°C

1. Product overview

Content

Vial		Content
1	Pwo Master	• 10 vials of 2× conc. master mix, each vial for 10 reactions • Each vial contains 25 U Pwo$^{SUPER\ YIELD}$ DNA Polymerase, reaction buffer with 4 mM $MgCl_2$ and PCR-grade dNTP´s (dATP, dCTP, dGTP, dTTP, each 0.4 mM) in a total volume of 250 µl
2	PCR-grade Water	• 4 vials (1 ml each)

Pwo Master is a premixed 2× conc. master mix containing Pwo$^{SUPER\ YIELD}$ DNA Polymerase, an optimized buffer system and PCR-grade deoxynucleotides. After adding the template DNA, primers and adjusting the volume to a final volume of 50 µl with PCR-grade water, the reaction mix directly can be applied to the cycler.

This ready-to-use mix offers a convenient solution for the high fidelity amplification of fragments up to 3 kb and eliminates individual adjustment of the reagent compositions.

As an additional advantage, the kit can be stored at +2 to +8°C and eliminates thawing of the reaction mix.

The core component of the Pwo Master is the proofreading polymerase Pwo DNA Polymerase.

Pwo DNA polymerase was originally isolated from the hyperthermophilic archeon Pyrococcus woesei. It is a highly processive 5´–3´ DNA polymerase and possesses a 3'–5' exonuclease activity, also known as proofreading activity. The enzyme has no detectable 5´–3´ exonuclease activity. The inherent 3´–5´ exonuclease proofreading activity of Pwo results in an approximately 18-fold increased fidelity of DNA synthesis compared to Taq DNA polymerase.

Pwo$^{SUPER\ YIELD}$ DNA Polymerase combines Pwo DNA Polymerase with a new optimized buffer system. This new buffer system enhances the enzymatic properties of the enzyme, resulting in higher yields of the amplification reaction without changing the fidelity of DNA synthesis.

Fidelity of *in vitro* DNA polymerization is one of the most important subjects in PCR. For many applications, where a homogenous DNA population is analyzed (i.e. direct sequencing or restriction endonuclease digestion), the mutations that are induced by the polymerase during PCR are of little concern. However, if only a small amount of template DNA is used as starting material and if after PCR, single DNA molecules are analyzed, PCR artifacts can be a significant problem. Therefore, fidelity of DNA polymerization is of particular importance for the:

- Cloning of PCR products
- Study of allelic polymorphism in individual RNA transcripts (3, 4)
- Characterization of the allelic stage of single cells (5) or single DNA molecules (6, 7)
- Characterization of rare mutations in tissue (8)
- Characterization of a population of cells in culture

The PCR products generated with Pwo$^{SUPER\ YIELD}$ DNA polymerase are blunt-ended and can be used directly for blunt-end ligation without any pretreatment of the ends.

Enzyme properties

Volume activity	0.1 U/µl
Standard enzyme concentration	2.5 U per 50 µl reaction
Proofreading activity	Yes
Error rate*	Pwo$^{SUPER\ YIELD}$ DNA Polymerase has approx. an 18-fold higher fidelity of DNA synthesis, compared to Taq.
Optimal elongation temperature	72°C
Final Mg^{2+} concentration	2 mM
PCR product size	up to 3 kb
PCR cloning	Blunt cloning
Incorporation of modified nucleotides	no
Storage/stability	Stable at +2 to +8°C until the expiry date printed on the label.

* Fidelity determined with the lacI assay (1).

Quality control

Each lot of Pwo Master is function tested in PCR. Routinely, quality control is performed using human genomic DNA and specific primers for tPA to get a 1.7 kb PCR product

2. Standard protocol for DNA amplification

General

The optimal conditions (incubation times and temperatures, concentrations of template DNA) depend on the system used and have to be determined individually (2).

As a starting point, use the following guidelines:

- The Pwo Master is formulated as such that the final reaction mix contains 2 mM $MgCl_2$ and 0.2 mM dNTPs. By using these conditions, the ability to amplify different fragments of human genomic DNA, ranging from 1.1 to 3 kb was demonstrated. All fragments were obtained with high yield and specificity.
- Primer design: The 3´–5´ exonuclease activity of the enzyme acts also on single stranded DNA (e.g. primers) in the absence and presence of dNTP. This activity does usually not interact with the PCR performance, but it can be taken into consideration for primer design. To overcome slow degradation of primers, also nuclease resistant dNTPs can be used for primer synthesis. Additionally, the use of longer primers with maximized GC content and focussed complementarity at the 5´-end can be of advantage.
- Upstream and downstream primer: it is recommended to start with 0.4 µM (final concentration) of each primer. For optimization, the concentration can vary between 0.1 and 0.6 µM.
- The optimal buffer for the template DNA is either sterile double-distilled water, PCR-grade water (see related products) or 5–10 mM Tris (pH 7-8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.
- Amount of template DNA:
  - Genomic DNA: 5–500 ng
  - Plasmid DNA: 100 pg–10 ng

Preparation of reaction mixes

Step 1

Set up **Master Mix** in a sterile thin-walled PCR tube on ice

Component	Volume	Final conc.
Downstream primer	x µl	400 nM
Upstream primer	x µl	400 nM
Template DNA	x µl	5–500 ng*
PCR-grade water	add up to 25 µl	
Final volume	25 µl	

*up to 500 ng genomic or 10 ng plasmid DNA

Mix the reagents and centrifuge briefly to collect the sample at the bottom of the tube.

Step 2

Component	Volume	Final conc.
Pwo Master	25 µl	2.5 Units per reaction

Note: The preparation of a separate Master Mix avoids that the enzyme interacts with primers or template which could lead to a partial degradation of primer and template by the 3´-5´exonuclease activity of the enzyme.

Pipet the Pwo Master to the Master Mix prepared in step 1, mix well, and overlay with 30 µl mineral oil if necessary (depending on cycler used).

Thermal cycling

Step	Action
1	Place samples in the thermal cycler, and start cycling using the thermal profiles mentioned below.
2	Thermal cycling profile

	Temperature	Time	Number of cycles
Initial denaturation	94°C (92°C-95°C)	2 min	1×
Denaturation	94°C (92–95°C)	30 s	20–30×
Annealing	50–68°C[a]	30 s	
Elongation	72°C	30 s–4 min[b]	
Final elongation	72°C	5 min	1×

Step	Action
3	After cycling, the samples may be frozen for later use. Possible further procedures: Check the PCR product on an agarose gel for size and specificity using an appropriate size marker.

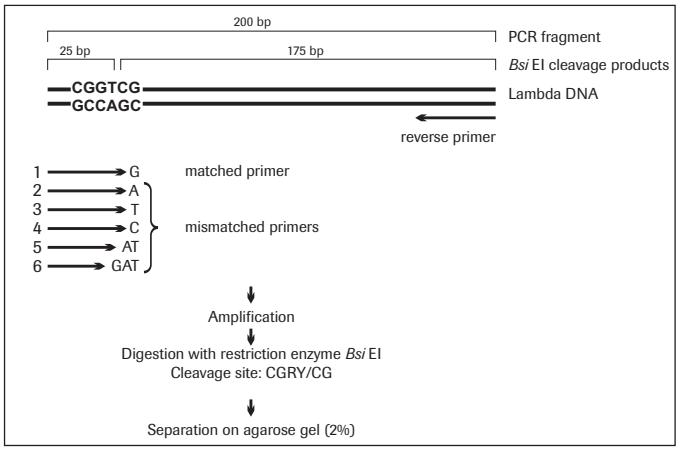
[a] Optimal annealing temperature depends on the melting temperature of the primers and the system used.

[b] Elongation time depends upon length of the product to be amplified (~1 min per kb).

3. Typical results

3´-mismatched primer correction assay

1a



1b

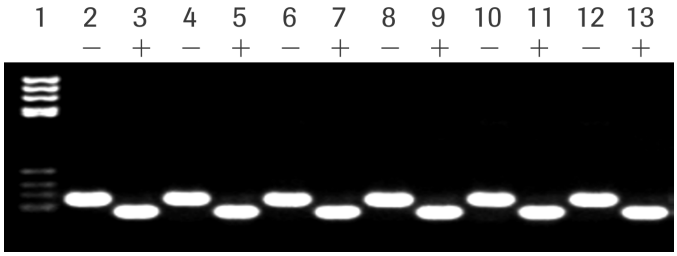


Fig. 1:
a: Flowchart for 3´ mismatched and matched primers: *Bsi* EI recognizes CGRYCG.
b: PCR products of a 200 bp target from lambda DNA using perfectly matched and partially mismatched primers and Pwo DNA polymerase.

lane 1:	DNA molecular weight marker V
lane 2,3:	Primer I (G:C match)
lane 4,5:	Primer II (A:C mismatch)
lane 6,7:	Primer III (T:C mismatch)
lane 8,9:	Primer IV (C:C mismatch)
lane 10,11:	Primer V (AT:CG mismatch)
lane 12,13:	Primer VI (GAT:AGC mismatch)
lane 2,4,6,8,10,12 (−):	without restriction enzyme digestion (200 bp fragment)
lane 3,5,7,9,11,13 (+):	restriction enzyme digestion with *Bsi* EI (175 + 25 bp fragment)

Amplification of different targets from human genomic DNA

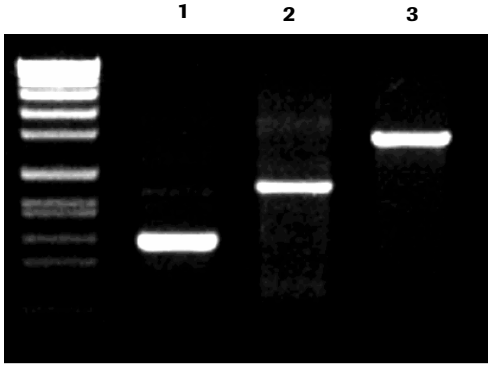


Fig. 2: The ability of the Pwo Master to amplify different targets without individual adjustments of the reagent compositions is demonstrated – the results show that fragments up to 3 kb can be obtained with high yield and specificity

Lane 1: 1.1 kb Collagen fragment
Lane 2: 1.7 kb tPA fragment
Lane 3: 2.8 kb p53 fragment

4. Troubleshooting

Problem	Possible cause	Recommendation
Little or no PCR product	Pipetting errors	• Check all concentrations and storage conditions of reagents.
	Difficult template e.g. GC-rich templates	• Add DMSO (titrate up to 8%). However, adding DMSO may negatively influence the accuracy of the reaction. • Use the GC-RICH PCR System (see related products)..
	Primer problems:	
	primer design not optimal	• Design alternative primers.
	primer concentration not optimal	• Both primers must have the same concentration. • Titrate primer concentration (0.1–0.6 µM).
	annealing temperature too high	• Reduce annealing temperature • Determine the optimal annealing temperature by touch-down PCR.
	primer specificity not optimal	• Perform nested PCR with nested primers.
	quality or storage problems	• If you use an established primer pair, check performance on an established PCR system (control template). • Make sure that the primers are not degraded. • Always store primers at –15 to –25°C.
	Formation of primer dimers	• Reduce concentration of primers • Use FastStart Taq DNA Polymerase (see related products).
	DNA template problems	Check quality and concentration of template: • Analyze an aliquot on an agarose gel to check for possible degradation. • Make a control reaction on template with an established primer pair or PCR system. • Check or repeat purification of template.
	Cycle conditions not optimal	• Decrease annealing temperature. • Increase cycle number. • Make sure that the final elongation step was carried out.
Multiple bands or background smear	Annealing temperature too low	• Increase annealing temperature according to the primer length.
	Primer design or concentration not optimal	• Review primer design. • Titrate primer concentration (0.1–0.6 µM). • Both primers must have the same concentration and similar annealing temperatures. • Perform nested PCR with nested primers.
	Difficult template *e.g.*, GC- rich template	• Use the GC-RICH PCR System (see related products).
	DNA template problems	• Use serial titration/dilution of template to avoid the influence of potential PCR inhibitors.
PCR products in negative control experiments	Carry-over contamination	• Exchange all reagents, especially water. • Use aerosol-resistant pipette tips. • Set up PCR reactions in an area separate from that used for PCR product analysis.

5. References

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5 Li, H. et al. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4580–4584.
6 Jeffreys, A. J. et al. (1990) *Cell* **60**, 473–485.
7 Ruano G. et al. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6296–6300.
8 Cha, R. S. et al. (1992) *PCR Methods Applic.* **2**, 14–20.

6. Ordering Information

PCR Product Selection Guide:

Needs	Size	Prevention of Carry over contamination*	Accuracy compared to Taq DNA Pol.	Use this RAS Science product	Pack size	Cat. No.
Standard PCR	up to 3 kb	Yes	1	Taq DNA Polymerase	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
			1	PCR Core Kit^{PLUS}	1 kit	11 585 541 001
		No	1	PCR Core Kit	1 kit	11 578 553 001
			1	PCR Master	1 kit	11 636 103 001
	up to 5 kb	Yes	6	Expand High Fidelity^{PLUS} PCR System	125 U 2× 250 U 10× 250 U	03 300 242 001 03 300 226 001 03 300 234 001
		No	3	Expand High Fidelity PCR System	100 U 2× 250 U 10× 250 U	11 732 641 001 11 732 650 001 11 759 078 001
			3	High Fidelity PCR Master	1 kit	12 140 314 001
Maximum specificity	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
High Fidelity PCR	up to 3 kb	No	18	Pwo DNA Polymerase	100 U 2× 250 U	11 644 947 001 11 644 955 001
			18	Pwo Master	1 kit	03 789 403 001
	up to 5 kb	No	3	Expand High Fidelity PCR System	100 U 2× 250 U 10× 250 U	11 732 641 001 11 732 650 001 11 759 078 001
			3	High Fidelity PCR Master	1 kit	12 140 314 001
		Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
			6	Expand High Fidelity^{PLUS} PCR System	125 U 2× 250 U 10× 250 U	03 300 242 001 03 300 226 001 03 300 234 001
Long Template PCR	5–20 kb	No	3	Expand Long Template PCR System	150 U 2× 360 U 10× 360 U	11 681 834 001 11 681 842 001 11 759 060 001
	> 20 kb	No	2	Expand 20 kb^{PLUS} PCR System	200 U	11 811 002 001
Difficult templates & challenging assays	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	50 U 100 U 500 U 4× 250 U 10× 250 U 20× 250 U	12 158 264 001 12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
		No	3	GC-RICH PCR System	100 U	12 140 306 001
	5–20 kb	No	3	Expand Long Template PCR System	150 U 2× 360 U 10× 360 U	11 681 834 001 11 681 842 001 11 759 060 001

* incorporation of dUTP

PCR Nucleotide Selection

Product	Description	Pack size	Cat. No.
Set of Deoxy-Nucleotides, PCR Grade	Separate vials of dATP, dCTP, dGTP, and dTTP. 100 mM each	4× 25 µmol (4× 250 µl)	11 969 064 001
		4× 125 µmol (4× 1250 µl)	03 622 614 001
PCR Nucleotide Mix	Premixed ready-to-use solution of PCR Grade dATP, dCTP, dGTP, and dTTP. 10 mM each.	100 reactions 1000 reactions	11 581 295 001 11 814 362 001

Additional Reagents

Product	Description	Pack size	Cat. No.
Water, PCR Grade	Specially purified water for use in PCR	25 ml (25 vials of 1 ml)	03 315 932 001
		25 ml (1 vial of 25 ml)	03 315 959 001
		100 ml (4 vials of 25 ml)	03 315 843 001

Changes to previous version

Editorial changes.

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