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Tersus PCR kit

Cat: 108405 100 rcs

Reagent Tersus PCR kit is designed only for researches and/or professionally trained users.

User Manual

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I. Kit Components and storage conditions

Material provided	Quantity
50X Tersus Polymerase mixture	100 µl
10X Tersus buffer	600 µl
50X dNTP mixture (10 mM of each)	120 µl
Control DNA template	10 µl
Primer mixture for the control reaction (5 µM of each)	10 µl
Sterile water for PCR	4.5 ml

Store all components at -20°C

II. Product description

Reagent kit "Tersus PCR kit" includes all necessary components for 100 standard PCR amplifications (volume 50 µl).

A. Tersus Polymerase features:

1. High precision DNA synthesis;

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2. 5'-3' polymerase activity;
3. Absence of 5'-3' exonuclease activity;
4. 3'-5' exonuclease (proofreading) activity;
5. Hot Start capability;
6. High amplification specificity;

Mixture of the thermo stable DNA polymerases Tersus (further Tersus polymerase) is recommended for application when strict requirements to amplification accuracy are specified: DNA fragments re-cloning, site-directed mutagenesis, DNA fragments amplification for following sequence analysis.

High precision of DNA synthesis is a major feature of the Tersus polymerase. Using of the Tersus polymerase for DNA fragments re-cloning enables to reduce expenses for further sequence to ensure the absence of point mutation in the cloned fragment.

Due to **high specificity** of Tersus polymerase it can be applied for operations with complex templates, for instance high homological repeats amplification, genome DNA or cDNA amplification (Fig.1).

Tersus polymerase has **Hot start capability**. Polymerase is active only when reaction mixture is heated during the first PCR cycle.

The length of products depends on properties of a DNA template (origin, GC content, structure). When simple templates are amplified (plasmid DNA, phage λ DNA, preliminarily amplified DNA fragments), Tersus polymerase allows to get fragments 15-20 kbp (Fig.2). However, the best results are obtained when fragments up to 2-2.5 kbp are amplified. For the amplification of longer fragments additional optimization of the PCR conditions are required.

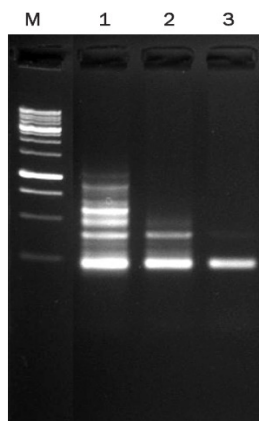


Fig. 1 Amplification of L1Hs repeat fragment with 5 ng human genome DNA during 28 PCR cycles

Line 1 – Taq polymerase; 2 – Encyclo polymerase; 3 – Tersus polymerase; M – 1 kbp DNA marker; 1.2% agarose

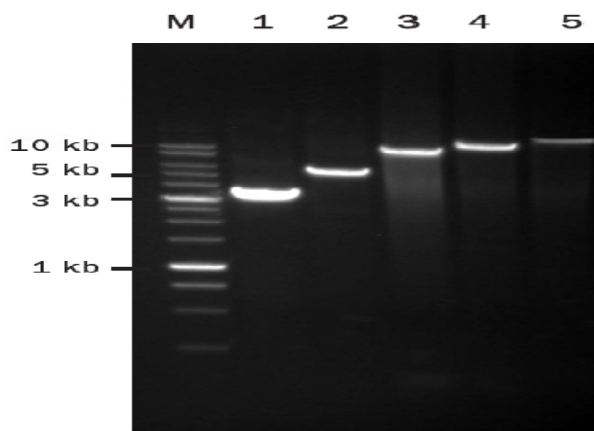


Fig.2 Amplification of phage DNA fragments with different length

Amplification of fragments was carried out with 35ng DNA template during 15 (line 1 and 2) and 19 (lines 3-5) PCR cycles

1 – 3kb fragment, 2 – 5 kb fragment, 3 – 8 kb fragment, 4 – 10 kb fragment, 5 – 15 kb fragment; M – 1 kb DNA marker; 1.2% agarose.

B. 10X Tersus buffer:

Reagent kit "Tersus PCR kit" includes 10X reaction buffer (10XTersus buffer) providing optimal condition for Tersus polymerase. Using of buffer solutions optimized for other commercially available polymerases can essentially reduce reaction quality. 1X Tersus buffer contains 3 mM Mg²⁺.

C. dNTP mixture:

dNTP mixture included in the kit represents enzymatic synthesized highly purified triphosphates in concentration 10 mM of each.

Application of highly purified dNTPs increases polymerase turnover number, reduces mistake probability.

D. Product Use Limitations:

Reagent kit "Tersus PCR kit" is designed only for researches and/or professionally trained users. The kit cannot be used for:

1. TaqMan PCR assay due to the absence of 5'>3' exonuclease activity;
2. SNP detection with allele specific PCR due to 3'>5' exonuclease activity (proofreading).

III. PCR protocol

A. Important Comments and Recommendations

1. Required control reactions:

Negative control

Even small amount of contaminating DNA can lead to the formation of unspecific product during PCR.

We recommend:

- (1) mix reagents for PCR in a zone separated from the places for DNA isolation and PCR products analysis;
- (2) use tips with aerosol filters;



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(3) to control possible contamination level perform negative control PCR together with other PCR tests (add sterile water instead of DNA template into the reaction mix).

Positive control

Positive control is required to check function of all reaction components. Control PCR conditions are described in the section III D.

2. Reaction mixture for PCR:

When several simultaneous PCRs are carried out preparation of the total reaction mixture containing components common to all reactions is recommended. Components that vary from reaction to reaction are added after aliquoting reaction mixture. Use of the total reaction mixture allows to reduce variations of the different components amount from tube to tube. Before aliquoting of the reaction mixture it is important to stir it carefully avoiding foaming and using centrifugation to remove drops from the tube walls.

3. Requirements to DNA template:

1. We recommend to dilute DNA template in water or in low saline buffer (pH 7.5-8.5). Concentration of EDTA (more than 1 mM) can reduce PCR efficiency.
2. Reamplification of the individual DNA fragments usually does not require additional condition selection for the reaction, minimal amount of template to start is 1-100 pg DNA depending on the DNA fragment length.
3. To amplify unique non-repetitious sequence from the complex templates (genomic DNA, cDNA first strand) 10-200 ng DNA is required. Warm template up to 65°C during 1-2 min and mix thoroughly by pipeting before adding to the reaction.
4. We recommended to add DMSO to PCR mix at concentration 3-5% the annealing temperature should be reduced 2-3 degrees when GC-rich template is amplified.

B. PCR Protocol

1. Prepare reaction mixture for PCR; add reagents in the order according to **Table 1**.
2. Mix components thoroughly, use a microcentrifuge to remove drops from the tube walls.
3. Divide reactive mixture into required number of the 0.2 ml or 0.5 ml sterile PCR tubes.

Note: We recommend using thin walls PCR tubes.

4. Add reagents that vary from reaction to reaction.
5. Add a drop of the mineral oil to each tube if thermal cycler is not equipped with heat lid.



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Table 1. Preparation of the reaction mixture for PCR

Component	25µl	50 µl	Final concentration
Sterile water	to 25 µl	to 50 µl	
10X Tersus buffer	2.5 µl	5 µl	
50X dNTP mixture	0.5 µl	1 µl	1X (0.2 mM of each)
PCR primer 1			0.2-0.5µM
PCR primer 2			0.2-0.5µM
DNA template			1ng-200ng/50µl
50X Tersus polymerase	0.5 µl	1 µl	1X
Total volume	25 µl	50 µl	

Final concentrations of the reagents in this protocol are optimal for amplification of DNA template in size up to 3 kbp. Otherwise additional optimization of the reaction conditions is required.

6. Perform amplification as described in the section III.C.

7. After amplification is completed analyze PCR products with gel electrophoresis with 1.0-3.0% agarose.

PCR products can be stored at -20°C.

C. PCR parameters

Use parameters from the Table 2 as an instruction for PCR program creation.

Optimal conditions for amplification, such as temperature, incubation time, PCR cycle number can vary depending on many factors (PCR cycler characteristics, sample volume, template properties, primers structure).

Final optimization should be performed by the user individually for each experiment

Table 2. PCR parameters

PCR step	Number of cycles	Temperature	Incubation time
Pre-denaturation	1	92-95°C	1-5 min
Denaturation	10-38	92-95°C	5 s-1 min
Annealing		T _m	5 s-1 min
Elongation		72°C	1 min/ 1-1.5 kbp

*T_m – annealing temperature of a primer

Denaturation:

Longer pre-denaturation is recommended for plasmid DNA (3-5 min) and complex genome DNA (2 min).

In other cases time of the preliminary denaturation can be decreased to 1 min and less. Use denaturation time as short as possible (for instance 15-20 sec) for further cycles to minimize DNA template degradation.



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Annealing:

Optimal temperature of the annealing depends on the primers structure and usually varies from 55°C to 72°C. Using primers with low annealing temperature (less than 50°C) can essentially increase amount of the unspecific products. To calculate apparently annealing temperature (T_m) use next equation:

$$T_m = 2^\circ\text{C} \times (A+T) + 4^\circ\text{C} \times (G+C).$$

However the optimal annealing temperature can differ from the calculated temperature. In some cases annealing temperature increased on five degrees ($T_m +5^\circ\text{C}$) allows to improve essentially PCR specificity. To get high specificity of PCR it we recommend to use primers with high annealing temperature (e.g. 65-68°C). To optimize PCR try to use pairs of the primers with similar T_m . Choose the lower temperature for primer with different T_m .

Elongation:

The elongation temperature in the majority of reactions is 72°C. Elongation time depends on the length of DNA template (1 min for each 1 kb).

Number of PCR cycles:

Required number of PCR cycles depends on the specific DNA template concentration at amplification starting point. We recommend to minimize number of PCR cycles since their abundant number can lead to unspecific PCR product formation. In the case of insufficient number of PCR cycles it is possible to put tube with the rest of reaction mixture back to the cycler and carry out additional cycles.

Dependence of the PCR product yield of the beta-actin gene (838 bp) at the initial concentration of the DNA template (human genome DNA, 27 PCR cycles) is shown on the Fig.3

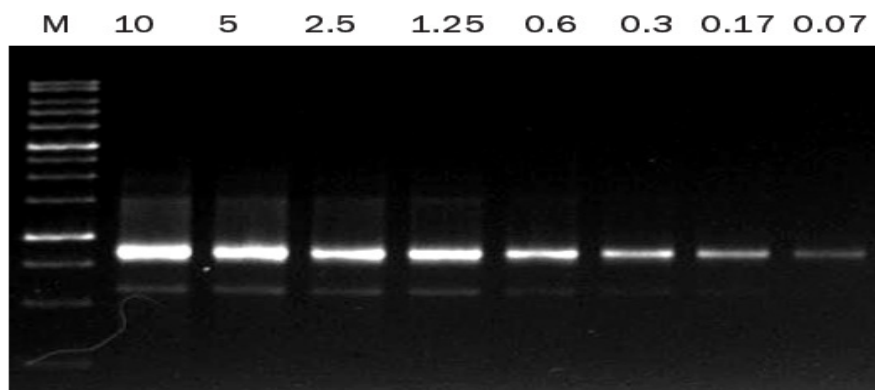


Fig.3 Amplification of beta-actin fragment from genome DNA at different dilutions

Amount of the DNA template at the PCR start pointed above lines (ng per 25 μl reaction). M – 1 kbp DNA marker; 1.2% Agarose

D. Positive control

1. Prepare reaction mixture for a positive PCR control in a sterile tube, use control DNA template and primers included in the kit:

- 20 μl sterile water
- 2.5 μl 10X Tersus buffer
- 0.5 μl 50X dNTP mixture
- 1 μl primer mixture for the control reaction (5 μM of each)
- 0.5 μl control DNA template
- 0.5 μl 50X Tersus polymerase

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25 µl total volume

2. Mix components of the reaction mixture accurately, use a microcentrifuge to remove drops from the tube walls.
3. Drop the mineral oil to each tube if thermal cycler is not equipped with heated lid.
4. Amplify control sample using following conditions*:

Preliminary denaturation 95°C, 1 min

18 PCR cycles

denaturation: 95°C, 15 s

annealing: 60°C, 15 s

elongation: 72°C, 2 s

***Reaction conditions are optimized for MJ Research PTC 200 DNA Thermal Cycler. They can be different for other cyclers**

5. When amplification is completed analyze PCR products with gel electrophoresis with 1.5 % agarose. Amplification of the control DNA template results in PCR product in length 1600 kbp.

IV. Troubleshooting

Following recommendations can be used for problem solving for the most PCR applications. However we are not able to ensure that they are usable for all PCR applications of the "Tersus PCR kit".

Problem	Possible solutions	Reason
A. No PCR product or lower PCR product yield than expected	Any component of the reaction was not added or was degraded	Check accuracy of the reaction mixture preparation. Make sure that Tersus buffer is used. Check function of the components with a control PCR. Try to optimize PCR conditions.
	Insufficient number of PCR cycles	Increase PCR cycle number adding 3-5 cycles every time
	Suboptimal annealing temperature of the primers and annealing time	Carry out several reactions with different annealing temperature or use temperature gradient with step in 1-2°C. Increase annealing time in 5-15 s. If calculated annealing temperature lower than 50°C use other primers
	Too short elongation time	Increase gradually elongation time in the rate of 1 min per 1kb
	Unsuccessful primer structure	Check matching of the primer structure and DNA template sequence
	Too low concentration or lack of the DNA template in a sample	Analyze DNA with electrophoresis to estimate concentration. Made serial dilutions of the template

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Problem	Possible solutions	Reason
	Low quality of the DNA template	Check the DNA template quality with agarose electrophoresis. Change the template
	DNA template contains inhibiting PCR additives	Repurify the DNA template
	Too complex for PCR GC rich DNA template	Add DMSO at concentration 2-6% in a reaction mixture
	Insufficient amount of the polymerase	In some cases PCR product yield can be increased by increasing polymerase concentration in the reaction mixture. However polymerase concentration increase more than twice can lead to significant background amplification
	Buffer in which DNA is diluted has too high concentration of EDTA	Concentration more than 5mM of EDTA in a sample can have adverse effect on PCR efficiency because of binding Mg ²⁺ ions in the reaction buffer. Change a buffer for DNA dilution
B PCR products contains multiple band or appears as a smear when analyzed with agarose gel electrophoresis	Too many PCR cycles	Repeat reaction controlling product formation with less cycles
	Too low annealing temperature	Increase annealing temperature at 2-3°C. Use temperature gradient with a step 1-2°C
	Too high DNA template concentration	Analyze the DNA template with gel electrophoresis to estimate the concentration. Make a serial dilution of the template
	Contamination with a foreign template	Control the contamination level of pipettes and tubes with a negative control (sterile water instead of DNA template). In the case of detection of contamination change the reagent, clean rooms and pipettes.
	Excess of polymerase activity	If optimization of PCR parameters was not successful try to reduce twice concentration of Tersus polymerase

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Problem	Possible solutions	Reason
	Unsuccessful primer structure	Check matching of the primer structure and DNA template sequence

Catalog #	Description	Pack size
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