# PCR Polishing Kit

## INSTRUCTION MANUAL

Catalog #200409
Revision A

**For In Vitro Use Only**
200409-12

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# PCR Polishing Kit

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# PCR Polishing Kit

## MATERIALS PROVIDED

| Materials provided[a] | Quantity |
|---|---|
| Cloned *Pfu* DNA polymerase[b,c] | 100 U (2.5 U/µl) |
| 10x cloned *Pfu* DNA polymerase buffer[d,e] | 1.0 ml |
| Control DNA (pUC19)[f] | 50 µl (10 ng/µl) |
| 10 mM dNTP mix[c,d] | 50 µl (2.5 mM each) |

[a] The PCR Polishing Kit contains enough reagents to perform 40 reactions.

[b] Stratagene Catalog #600153 (100 U), #600154 (500 U), #600159 (1000 U), and #600160 (5000 U).

[c] Do not store the cloned *Pfu* DNA polymerase or the 10 mM dNTP mix in a frost-free freezer.

[d] We recommend aliquoting the 10x cloned *Pfu* DNA polymerase buffer and 10 mM dNTP mix into smaller volumes following initial thawing. Avoid multiple freeze–thaw cycles to achieve maximum levels of incorporation.

[e] See *Preparation of Media and Reagents*.

[f] The control DNA is linearized with 3´-end extensions (pUC19, *Hin*d II-digested and *Taq* DNA polymerase treated).

## STORAGE CONDITIONS

**Cloned Pfu DNA Polymerase:** –20°C
**10× Cloned Pfu DNA Polymerase Buffer:** –20°C
**Control DNA:** –20°C
**10 mM dNTP mix:** –20°C (for up to 3 months); –80°C (for long-term storage)

## ADDITIONAL MATERIALS REQUIRED

Sterile microcentrifuge tubes (0.5 ml)
Mineral oil
Sterile distilled water ($dH_2O$)

Revision A

## INTRODUCTION

The PCR Polishing Kit is designed to increase the blunt-ended cloning efficiencies associated with polymerase chain reaction (PCR)-generated fragments.[1,2] Recent studies have shown that many species of DNA polymerases (e.g., T7, modified T7, *Taq*, Vent$_R$® and Klenow fragment) exhibit terminal deoxynucleotidyltransferase-like activity (extendase).[3,4] It has been found that the 3´-end nucleotide extension of PCR products by DNA polymerases is both nucleotide and polymerase specific. Each DNA polymerase, including the Klenow fragment, has characteristic terminal extendase activity with no consistent pattern of 3´-end modification. Therefore, it cannot be assumed that all DNA polymerases create blunt-ended fragments. Fortunately, *Pfu* DNA polymerase*, does not exhibit any DNA extendase and can be used to create blunt-ended fragments following PCR.[5–7] Due to the unique 3´ → 5´ exonuclease (proofreading) activity of the *Pfu* DNA polymerase, *Taq* DNA polymerase-generated PCR products can be polished with *Pfu* DNA polymerase following temperature cycling to create blunt-ended DNA fragments for use in cloning, mutagenesis and cDNA construction.

The PCR Polishing Kit is designed to polish the ends of the 3´-overhang extensions of polymerase-generated DNA fragments directly from a PCR amplification reaction. The PCR Polishing Kit can also be used to perform complete fill-in of 5´ overhangs to generate blunt ends. Dramatic increase in the population of blunt-ended DNA fragments following polishing treatment results in a drastic increase in overall experimental efficiency associated with procedures utilizing blunt-ended ligation.

## PRECAUTIONARY NOTES

- Before PCR polishing, it may be advantageous to verify the PCR products by agarose gel analysis in order to estimate the approximate concentration of PCR products and to ensure that the correct PCR products have been created following thermal amplification. PCR polishing is conducted using an aliquot of the PCR amplification reaction and will therefore polish the ends of all DNA fragments present. In a typical 100-µl PCR amplification reaction that has been cycled ~30 rounds exhibiting a strong band following agarose gel analysis, 5 µl of product can be used for PCR polishing.

- Before performing a 5´-fill-in reaction, the sample DNA should be extracted with phenol–chloroform and ethanol precipitated (see *Optional Purification Protocols*).

* U.S. Patent Nos. 6,489,150, 5,948,663, 5,866,395 and 5,545,552 and patents pending.

# PROTOCOL

## PCR Polishing Reaction

For polishing PCR-generated DNA fragments, transfer an aliquot of PCR product directly from the reaction tube into a sterile 0.5-ml microcentrifuge tube. For 5´-fill-in reactions, aliquot purified DNA (10–500 ng) into a sterile 0.5-ml microcentrifuge tube.

1. Add the following components in order to the sterile 0.5-ml microcentrifuge tubes:

| Control Reaction | Sample Reaction |
|---|---|
| 5.0 µl (50 ng) of control DNA | 5.0 µl (10–500 ng) of PCR DNA |
| 1.0 µl of 10× cloned *Pfu* DNA polymerase buffer | 1.0 µl of 10× cloned *Pfu* DNA polymerase buffer |
| 1.0 µl of dNTP mix | 1.0 µl of dNTP mix |
| 1.0 µl of cloned *Pfu* DNA polymerase (2.5 U/µl) | 1.0 µl of cloned *Pfu* DNA polymerase (2.5 U/µl) |
| $dH_2O$ to a total of 10 µl | $dH_2O$ to a total of 10 µl |

   Gently mix the components of both microcentrifuge tubes and add a mineral oil overlay.

2. Incubate the control and sample reactions at 72°C for 30 minutes. After the 30-minute incubation, remove the reactions to ice.

3. End-polished DNA fragments may be added directly to ligation reactions.

   ***Note*** Pfu *DNA polymerase has very little activity when used in ligation reactions (25°C). Therefore, PCR-polished DNA fragments need not be further purified for use in ligation reactions. However, an optional purification protocol has been outlined in the following section (see* Optional Purification Protocols*).*

## Optional Purification Protocols

The PCR-polished DNA fragments may be further purified to remove the *Pfu* DNA polymerase and excess dNTPs or for buffer exchange. The StrataPrep PCR purification kit [Stratagene Catalog #400771 (50 preps) and #400773 (250 preps)] can be used in the place of phenol–chloroform extraction procedures. Conventional phenol–chloroform extraction is outlined below:

1. Aliquot the PCR-polished reaction mixture, avoiding the mineral oil overlay, to a sterile 0.5-ml microcentrifuge tube. Adjust the final volume to 100 µl with TE buffer.[§]

2. Add an equal volume of phenol, vortex and remove the top aqueous phase to a sterile 0.5-ml microcentrifuge tube.

3. Add an equal volume of chloroform, vortex and remove the top aqueous phase to a sterile 0.5-ml microcentrifuge tube.

4. Precipitate the DNA using ammonium acetate as follows:

    a. Add 1/10 volume of 10× STE buffer.[§]

    b. Add an equal volume of 4 M ammonium acetate to the sample.

    c. Add 2.5 volumes of room temperature 100% (v/v) ethanol.

    d. Immediately spin in a microcentrifuge at room temperature for 20 minutes at 10,000 × *g* to pellet the DNA.

    e. Carefully remove and discard the supernatant.

    f. Wash the DNA pellet with 200 µl of 70% (v/v) ethanol.

    g. Spin in a microcentrifuge at room temperature for 10 minutes at 10,000 × *g*. **Carefully** remove the ethanol with a pipet.

    h. Dry the DNA pellet under vacuum.

    i. Dilute the DNA into TE buffer or into a preferred diluent prior to ligation.

[§] See *Preparation of Media and Reagents*.

## Standard Blunt-End Ligation of Control DNA

The unpolished control DNA (pUC19) contains 3´-end nucleotide extensions and will ligate at an extremely reduced efficiency in the presence of T4 DNA ligase. PCR polishing removes 3´-end nucleotide extensions created by DNA polymerases and will produce blunt-ended DNA molecules which will religate at high efficiency in the presence of T4 DNA ligase. For the efficient cloning of blunt-ended PCR products, we recommend using the PCR-Script Amp cloning kit (Stratagene Catalog 211190).[8,9] In the presence of rATP and T4 DNA ligase, ligations of control DNA can be performed as follows:

| Unpolished Control DNA | PCR-Polished Control DNA |
|---|---|
| 1.0 µl (10 ng) of unpolished control DNA (pUC19) | 2.0 µl (10 ng) of PCR-polished control DNA (pUC19) |
| 1.0 µl of 10× cloned *Pfu* DNA polymerase buffer | 1.0 µl of 10× cloned *Pfu* DNA polymerase buffer |
| 0.5 µl of rATP (10 mM stock) | 0.5 µl of rATP (10 mM stock) |
| 1.0 µl of T4 DNA ligase (4 Weiss U) | 1.0 µl of T4 DNA ligase (4 Weiss U) |
| 6.5 µl of $dH_2O$ | 5.5 µl of $dH_2O$ |

1. Incubate the ligations at room temperature (22°C) for 1 hour.

2. Transform 1–2 µl of the ligated DNA into competent *E. coli* cells. The control DNA (pUC19) provided is an ampicillin-resistance encoding plasmid and should be spread onto LB–ampicillin agar plates (see *Preparation of Media and Reagents*) following transformation.

3. Following the overnight incubation at 37°C, the transformation plates containing PCR-polished control DNA should exhibit a >20-fold increase in colony forming units (cfu) when compared to unpolished control DNA transformants.

## PREPARATION OF MEDIA AND REAGENTS

### 10× Cloned *Pfu* DNA Polymerase Buffer

200 mM Tris-HCl (pH 8.75)
100 mM KCl
100 mM $(NH_4)_2SO_4$
20 mM $MgSO_4$
1 mg/ml of bovine serum albumin (BSA)
1% Triton® X-100

### 10× STE Buffer

1 M NaCl
200 mM Tris-HCl (pH 7.5)
100 mM EDTA

### TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

### LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized $H_2O$ to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

### LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

## REFERENCES


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7. Costa, G. L. and Weiner, M. P. (1994) *Nucleic Acids Res* 22(12):2423.
8. Bauer, J. C., Deely, D., Braman, J., Viola, J. and Weiner, M. P. (1992) *Strategies* 5(3):62-64.
9. Costa, G. L., Sanchez, T. and Weiner, M. P. (1994) *Strategies* 7(2):52.


## ENDNOTES



## MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

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