



The ENZYME Company

DATA SHEET

PCR products cloning kits

End-polishing cloning kit contains all components for the 3'-end-polishing of amplicons (if the amplicons contain 3'-overhang) and blunt-end cloning of the resultant fragments. The kit is useful for blunt-end fragment preparation from fragments with 5' or 3'-overhangs of all types (obtained after restriction endonucleases digestion, after exonucleases treatment, after unspecific degradation etc.) with the possibility of the blunt-end cloning of the end-polished fragments.

The plasmid used for the cloning is Amp^r, the recombinant plasmid may be selected by Lac⁺/Lac⁻ phenotype detection (blue/white selection on the X-gal/IPTG - containing plates).

The kit contains (for 20 reactions)

- 10X Psp buffer (50 µl)
- dNTPs mix (8 mM of each) (40 µl)
- Psp DNA polymerase (20 µl)
- 4X ligase buffer (50 µl)
- ATP 5 mM (20µl)
- T4 DNA ligase (20µl)
- blunt-ended vector (20µl)
- PA (linear polyacrilamide) (120 µl)

Cloning of the PCR products in the blunt-end vector

Protocol

PCR products for the effective cloning should present in amount not less than 200ng after purification. The fragments should be purified from the primer-dimers with the help of Wizard purification system (Promega) or similar techniques. The amplicons can be also purified by the electrophoresis in agarose gel.

If PCR has been performed with the use of Taq DNA polymerase, the "polishing of the fragment ends" (removing dA overhangs from 3'-ends) is absolutely required before cloning.

1. End-polishing

Prepare the following mixture for end-polishing:

- Amplicon - more than 200ng
- 10x Psp buffer – 5 µl
- dNTP mix (8mM of each) – 2 µl
- Psp DNA polymerase – 1 µl
- Water – up to 50 µl

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Incubate 40 min at 72°C under the layer of mineral oil. Precipitate DNA by ethanol and dissolve in the minimal volume of H₂O (2-5µl).

2. Ligation with the vector

Prepare the mixture

4x ligase buffer	2,5µl
5 mM ATP	0,5 µl
vector (50ng/µl)	1µl
amplicon	150-300ng (not more then 5µl)
ligase (100u/µl)	1µl
H ₂ O (if the volume is less then 10 µl)	up to 10 µl

Ligation should be performed 12-14 hours at 14-16°C. Add 3-4 µl of linear polyacrylamide (LPA) and precipitate by ethanol. Dilute the precipitate in water. Transformation and plating out (in Amp-containing plates) as described elsewhere.

Select white recombinant colonies, proof the insert by mini-prep analysis.

Remarks

1. Vector is PBSII SK digested by Sma I. The detailed map of the vector is enclosed.
2. We recommend to plate-out cells after transformation onto the several plates to avoid high density of E.coli colonies on the plate.
3. DTT may form white flakes in the ligasex10 buffer. Dissolve the flakes completely by heating up before the use of the buffer.

Version 260407

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