

## Preparation of blunt-end DNA fragments

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### Equipment and reagents

- ◆ Klenow DNA polymerase
- ◆ 10× Klenow reaction buffer (500 mM Tris-HCl, pH 7.5, 100 mM MgCl<sub>2</sub>, 10 mM DTT)
- ◆ Stock solutions (e.g. 20 mM) of each dNTP (i.e. dATP/dCTP/dGTP/dTTP)
- ◆ T4 DNA polymerase
- ◆ 10× T4 pol reaction buffer (500 mM Tris-HCl, pH 8.8, 50 mM MgCl<sub>2</sub>, 50 mM DTT)
- ◆ Water-bath or heating-block
- ◆ Gel electrophoresis apparatus

### A. Filling in 5'-overhangs with Klenow DNA polymerase

- 1 Prepare a reaction mixture containing DNA, 1 × Klenow reaction buffer and 20 μM of each dNTP.
- 2 Add 1 unit of Klenow DNA polymerase and incubate at 15–37 °C for 15–30 min.
- 3 Stop the reaction by heating at 75 °C for 10 min.
- 4 The efficacy of the reaction can be monitored by a simple ligation experiment followed by gel electrophoresis. DNA that originally had complementary termini should not show no ligation ability when using low amounts of T4 ligase suitable only for cohesive-end ligation (see [DNA ligation](#)). An approximate ten-fold increase in the amount of ligase should now be required to achieve the necessary blunt-end ligation.

### B. Removal of 3'-overhangs by the exonuclease reaction of T4 DNA polymerase

- 1 Prepare a reaction mixture containing DNA, 1 × T4 DNA pol reaction buffer and 20 μM of each dNTP.
- 2 Add 1 unit of T4 DNA polymerase and incubate at 15 °C for 15–30 min.
- 3 Stop the reaction by heating at 75 °C for 10 min.

- 4 Analyse the efficacy of the reaction by ligation of a sample as described in step A.4 above.