

Addition of linkers/adaptors to blunt-end DNA fragments

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Equipment and reagents

- ◆ T4 DNA ligase and 10 × ligation buffer (0.66 M Tris-HCl, pH 7.6, 50 mM MgCl₂, 50 mM DTT, 10 mM ATP)
- ◆ Size-based separation matrix resin poured in a column (e.g. a 10 ml pipette)
- ◆ Restriction endonuclease plus appropriate reaction buffer
- ◆ DNA linkers/adaptors
- ◆ Water-bath or heating-block

Method

- 1 Prepare a ligation mixture containing DNA and linkers/adaptors in a 50 µl volume (see [DNA ligation](#)). Keep a 1:1 ratio of linkers/adaptors to DNA (e.g. 1 µg DNA to 1 µg linkers/adaptors) as this will ensure a large molar excess of linker/adaptor molecules to the longer DNA fragments.
- 2 Add 5 units T4 DNA ligase (i.e. a sufficient quantity to promote blunt-end ligation) and incubate for 16 h at 15 °C.
- 3 Stop the reaction by heating at 70 °C for 10 min.
- 4 Increase the volume of the ligation mixture to 100 µl by adding 10 µl of the appropriate 10 × restriction enzyme buffer and 40 µl water.
- 5 Add 100 units of the appropriate restriction endonuclease and incubate at 37 °C for 12 h.
- 6 Heat at 70 °C for 10 min to inactivate the restriction endonuclease.
- 7 Separate the larger DNA fragments (now with linkers/adaptors ligated to their termini) from the shorter digested linkers/adaptors by size fractionation on the appropriate size-separation-based column (see [Removal of excess linkers/adaptors](#)).
- 8 Purify and concentrate the modified DNA fragments by ethanol precipitation (see [Purification of DNA by phenol extraction and ethanol precipitation](#)).

Notes

The modified DNA fragments may contain internal recognition sites for the restriction endonuclease used to cleave the ligated linkers/adaptors, and therefore these sites must first be blocked by treatment with an appropriate DNA methylase before restriction endonuclease digestion. Set up a 20 μ l reaction containing the DNA, the relevant methylase, the appropriate buffer (obtained from the supplier of the methylase) and 80 μ M *S*-adenosylmethionine. Incubate at 37 °C (for most methylases) for 1 h then recover the DNA by phenol extraction and ethanol precipitation (see [Purification of DNA by phenol extraction and ethanol precipitation](#)).