

## Removal of excess linkers/adapters

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### Equipment and reagents

- ◆ Disposable 5 ml or 10 ml plastic pipette
- ◆ Sterile glass wool
- ◆ Size-based separation matrix resin
- ◆ Column buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA)

### Method

- 1 Build the column in a sterile 5 ml or 10 ml plastic pipette. Plug the bottom of the pipette sufficiently with sterile glass wool in order to retain the matrix but allow the eluate to flow through the column.
- 2 Pre-swell the size-separation matrix resin in column buffer for 1 h at room temperature. Pour the resin into the pipette giving a bed volume of 5 ml. Wash the column with 50 ml column buffer. This removes any ligase inhibitor from the resin that may interfere with further cloning reactions.
- 3 Load the sample on to the column, wash with more column buffer (e.g. 5 ml) and collect between 20 and 40 100- $\mu$ l fractions.
- 4 Thorough washing of the column with more buffer allows reuse.
- 5 Analysis of the fractions can be carried out by various methods. Simple electrophoresis of a sample from each fraction may show which fractions contain the modified DNA. Alternatively trace amounts of radiolabel can be incorporated into the linkers/adapters and the fractions analysed using a scintillation counter. This should show two peaks of radioactivity: an earlier peak containing the modified DNA and a final large peak containing excess labelled linkers/adapters. The column may also be equilibrated before use with known DNA size markers. Analysis by gel electrophoresis will then determine which fraction should contain DNA of a particular length.