T/A-based subcloning of PCR amplified DNA fragments

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Reagents

- *Taq* DNA polymerase
- 10 × *Taq* DNA polymerase buffer (200 mM Tris-HCl, pH 8.5, 15 mM $MgCl_2$, 500 mM KCl)
- Stock solution (e.g. 20 mM) of dTTP
- T4 DNA ligase and 10 × ligation buffer (0.66 M Tris-HCl, pH 7.6, 50 mM $MgCl_2$, 50 mM DTT, 10 mM ATP)

Method

1. Prepare a 50 μl reaction mixture containing 1.5 μg blunt-end plasmid DNA, 1 × *Taq* DNA polymerase buffer and 20 μM dTTP.
2. Add 1 unit of *Taq* DNA polymerase and incubate at 70 °C for 1 h. This will promote the addition of a single dTTP residue as a 3' extension to both termini of the plasmid DNA. This will allow subsequent cohesive ligation of the majority of PCR amplified DNA fragments which have a 3' dATP extension.
3. Purify the plasmid DNA by phenol extraction and ethanol precipitation (see Purification of DNA by phenol extraction and ethanol precipitation) and resuspend the DNA at a concentration of 50 ng/μl in sterile water.
4. Prepare a ligation mixture containing 0.25 units of T4 DNA ligase and an equimolar ratio of the plasmid DNA and PCR products (see DNA ligation). Incubate at 15 °C for 1–16 h.
5. The ligation is now ready for further analysis including transformation of *E. coli* cells.