T-A cloning Vectors

A method for direct cloning a PCR product, by the T-vector technique. This protocol works on the basis of the fact that given an equimolar amount of dNTPs in a PCR reaction, Taq DNA polymerase I adds an extra (template-independent) A, so most PCR products have an extra A at the 3' end.

If the enzyme is supplied with only dTTP, then an extra T will be added, so this is a cheap and easy way to make the T-vectors for cloning PCR products with A 3' overhangs.

Ref: Marchuk, D., *et al*. (1991) *Nucleic Acids Res*. **19:** 1154

Making the T-vector:

- Digest 5 ug pBluescript II with EcoRV (blunt cutter).
- Heat kill the enzyme or gel purify using Qiagen column elute in 50ul EB.
- Add 10 ul of 10X PCR buffer, 2 ul of 100 mM dTTP, 37.0ul of distilled water and 1.0 ul of Taq DNA polymerase. Incubate at 72 C for 2 hours.
- Purify the T-vector by phenol/chloroform extraction and ethanol precipitation or purify over a Qiagen column. Resuspend/elute the prepared T-vector in 100 ul of water or TE. We use about 2- 5ul in a ligation reaction.

Cloning the PCR product:

- Purify the PCR product over a Qiagen column (Gel purify if necessary). Elute in 50 ul EB. Use 5-10 ul of this PCR product in a ligation reaction.
  For example:

PCR insert	10ul
T-Vector	5ul
10X Ligase buffer	2ul
Water	2ul
T4 Ligase	1ul

- Ligate 15min RT(rapid ligation kit) to overnight (15C).
- Transform into E. coli (XL1Blue).
- Plate on Amp (75ug/ml) X-gal (spread 60ul of 2% solution on plate) IPTG (0.1-1mM)
- Pick white colonies to prep.