

A-TAILING BLUNT PCR PRODUCTS

A rapid method for cloning blunt ended PCR products into T-overhanging cloning vectors (After Promega[®] pGEM-EASY manual).

1. Isolate PCR product from gel or PCR reaction using appropriate Qiagen kit. Normally this will be in a volume of ~50 μ l.
2. Set up a reaction:
 - 25 μ l PCR product
 - 5 μ l 10 X Taq reaction buffer
 - 5 μ l $MgCl_2$
 - 5 μ l dNTP (10mM stock)
 - 1 μ l Taq polymerase
 - 9 μ l H_2O

Quantity of DNA can vary but probably should not be more than ~1 μ g.

3. Incubate at 70°C for ~ 30'.
4. Run some reaction on a gel to quantify.
5. Set up ligation. No need to clean up reaction and obviously it's hard to heat-inactivate Taq!