Tailing a PCR product

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Tailing is an enzymatic method for adding a non-templated nucleotide to the 3' end of a blunt, double-stranded DNA molecule. While Taq polymerase adds a A-tail to most PCR products, this is not the case for high-fidelity polymerases or proofreading polymerase mixes; these eithere do not add, or remove, the A overhang.

Protocol:

1. Purify PCR product using column (purification essential!)

2. Mix together:

12.5 µl	PCR product (no more than 1 µg; add more water if needed)
2.5 µl	10X Taq reaction buffer
0.375	0.1 M MgCl2
0.5 µl	10 mM dATP (final concentration 0.2 mM)
1 µl	Taq DNA polymerase (5 units/µl)
6.63 µl	water
(25 µl total volume)	

Incubate 70°C for 30-60 min.

Set up ligation (there is no need to purify the DNA from the reaction); typically add 1 µl to the pGEMT-Easy ligation reaction.