Linker Ligation

This protocol is for the Linker Ligation

Double stranded oligonucleotide linkers are often used to generate compatible overhangs not found in the insert. Linkers normally contain restriction enzyme recognition sequences and are digested after ligation to generate overhangs compatible with cloning vectors. Alternatively, linkers may have overhangs which are ready for ligation with a cloning vector and do not require further manipulation following ligation.

1. Prepare the following reaction mixture:

Linear DNA	100-500 ng
Phosphorylated linkers	1-2 µg
10X T4 DNA Ligase buffer	2 µl
50% PEG 4000 solution	2 µl
T4 DNA Ligase	2 u
Water, nuclease-free	to 20 µl
Total volume	**to 20 µl**

2. Mix thoroughly, spin briefly and incubate for 1 hour at 22°C.
3. Heat inactivate at 65°C for 10 min or at 70°C for 5 min.

Note
T4 DNA Ligase is active in PCR and restriction digestion buffers (see table below). Therefore, linker ligation reactions can be performed in the restriction enzyme buffer optimal for the subsequent digestion. In this case, the ligation reaction should be supplemented with ATP to a final concentration of 0.5 mM. After inactivation of the T4 DNA Ligase, add the restriction enzyme directly to the reaction mixture and incubate according to the digestion protocol.

Activity in PCR and restriction digestion buffers		
Buffers		**Activity*, %**
PCR and RT buffers		75
Restriction buffers for restriction enzymes	FastDigest®, 1X / 2X Tango™, B, G, O, R, KpnI, BamHI, EcoRI	75-100
	EcI136II, SacI	50

** activity of T4 DNA Ligase in variuos buffers supplemented with 0.5 mM ATP.*

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