

## Insert Preparation Datasheet T4 Polymerase Digest

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Input: microplate of normalized clean PCR product

Output: microplate of T4-treated Inserts

Next method: LIC reaction

Materials/Reagents/Equipment	Vendor
<b>Disposables</b>	
15 ml conical tube	
25 ml divided reservoir	
<b>Reagents</b>	
microplate of normalized clean PCR product	
NEB buffer #4	
BSA	
dTTPs	
T4 polymerase	
<b>Equipment</b>	
250ul multichannel pipettor	
ice bucket	
PCR thermocycler	MJ Research

1. Thaw all reagents except T4 polymerase on ice.
2. Make mix for (#targets + 4) in 15 ml conical tube on ice:

	<b>1 reaction</b>	<b>(# of samples + 4) reactions</b>
Buffer #4	10 ul	
dTTPs	2 ul	
BSA	2 ul	
T4 polymerase	1 ul	
H2O	35 ul	
Total volume	50 ul	

50 ul of mix is added to the 50 ul normalized cleaned-up PCR product for a total volume of 100 ul.

3. Using multichannel pipettor and reservoir on ice, dispense 50 ul of mix to each sample of clean PCR product. Mix by aspirating and dispensing. Cover with mat.
4. Turn on PCR thermocycler, select **Run** => **T4**. The program incubates the plate at 37 degrees for 30 minutes, then denatures the polymerase with 20 minutes at 70 degrees.
5. Label the plate I+date and T4 on right-end, and store in Insert rack in Freezer.

6. Update Storage Inventory file: enter plate ID in CompII Cloning/Targets Data/Storage Inventory/Inserts/rack map.