LIC Insert Preparation Manual

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Summary: This protocol describes the standard procedure for preparing LIC Insert Stocks. Qiagen Kit purified Target PCR, 1 ug, is digested with T4 DNA polymerase exonuclease digestion in the presence of dTTP yields LIC sticky ends

Materials/Reagents/Equipment	Vendor	Stock Number
Disposables		
PCR tubes	Marsh, Perkin-Elmer	
Reagents		
T4 DNA polymerase	New England Biolabs	
dTTP mixture, 100 mM, –20C	Pharmacia, Promega	
Water, double deionized, autoclaved, RT		
Agarose	Amresco,	
TAE Buffer	BSGC	
I Kb Plus DNA ladder	Invitrogen	
Equipment		
PCR Machine 2400 or 9600	Perkin-Elmer	
Pipetman		
Agarose Gel Electrophoresis	Invitrogen	

Procedure for 20 ul Reaction
Label PCR tube – **only on upper sides** (otherwise ink stains temp block).
Setup PCR machine to run 37 ^{o} C for 30 min, then 70 ^{o} C for 20 min, then 4 ^{o} C termination.
T4 DNA Polymerase Treatment: Add 0.5 ul of dTTP [100 mM], **0.5 ul of BSA** [10 mg/ml], mix and then add 0.5 ul [20U] T4 DNA Pol. Incubate in PCR machine cycle above.
Usage: use 1.0 ul [20 ng Insert DNA] for each LIC reaction.

LIC Insert Preparation Datasheet

Name		Date	

No.	Sample		