LIC Vector Preparation Protocol I

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Summary: This protocol describes the standard procedure for preparing LIC Vector Stocks. Qiagen Kit purified vector, 5 ug, is digested with SmaI in 50 ul NEB#4. Digestion checked using 1 ul on agarose gel electrophoresis. T4 DNA polymerase exonuclease digestion in the presence of dATP yields LIC sticky ends. Gel purification of vector is not essential if SmaI digestion is complete, however if background is high, it might be necessary.

Materials/Reagents/Equipment	Vendor	Stock Number
Disposables		
PCR tubes	Marsh, Perkin-Elmer	
Reagents		
10x NEB4 Buffer, –20C	New England Biolabs	
Sma I restriction enzyme, 20 U/ul	New England Biolabs	
T4 DNA polymerase	New England Biolabs	
dATP 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 50 ul Reaction
Label PCR tube – **only on upper sides** (otherwise ink stains temp block). Setup PCR machine to run at 25° C, 3 x 60 minutes.
SmaI Digestion: mix 5 ug plasmid [___ul} with 5 ul 10xNEB4 buffer, and add water to 49 ul [___ul]. Add 1 ul [20U] SmaI. Incubate 25° C for 1-2 hr, then analyze [see below]. Continue incubation.
Analysis: run 1 ul of reaction mix on 1.2% agarose/TAE gel electrophoresis. Standards = undigested plasmid, 0.1 ug and marker, 1 Kb Plus ladder [4 ul]. If reaction is complete continue below; if incomplete, add another 1 ul of SmaI and repeat incubation.
Setup PCR machine to run 37 ° C for 30 min, then 70 ° C for 20 min, then 4 ° C termination.
T4 DNA Polymerase Treatment: Add 1 ul of dATP [100 mM], **0.5 ul of BSA** [10 mg/ml], mix and then add 1 ul [20U] T4 DNA Pol. Incubate in PCR machine cycle above.
Transfer treated plasmid to 1.5 ml microcentrifuge tube labeled with plamid name, treatment, date.
Usage: use 0.5 ul [50 ng plasmid] for each LIC reaction.

LIC Vector Preparation Datasheet

Name		Date	

Samples			