LIC Reaction/Transformation Protocol, Manual

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Summary: This protocol describes the standard procedure for LIC reactions of prepared vector and Target Insert and transformation into competent TOP10 cells. The transformation is split between plating and liquid culture. The plating gives individual colonies for clone isolation. The liquid culture yields a "mixed" miniprep of plasmid for characterizaton of the reaction/transformation and potential isolation of insert containing vectors.

Materials/Reagents/Equipment	Vendor	Stock Number
Disposables		
PCR 96 well plate	Marsh, Perkin-Elmer	
Reagents		
Prepared LIC vector	BSGC	
Prepared LIC Target Insert	BSGC	
TOP10 cells, chem.. competent	Invitrogen	
Water, double deionized, autoclaved, RT		
LB-agar plates, Amp	BSGC	
Glass beads, sterile		
Equipment		
PCR Machine 2400 or 9600	Perkin-Elmer	
Pipetman		
Water bath at 42 C		

Procedure for one Reaction
Label microcentrifuge tube; thaw chemical competent TOP10 cells [each tube contains 50 ul; use for 4-5 transformations].
Add 0.5 ul prepared lic-vector on side of tube. Add 1.0 prepared lic-target gene directly to the drop of vector, mix gently. Incubate room for 5-30 min, then place in ice.
Add 10-12 ul competent TOP10 cells; ice for 5-30 min. Heat shock at 42 C for 30-45 sec. Return to ice for 2 min.
Add 200 ul of LB medium without antibiotics. Incubate without shaking at 37 C for 30 min to 1 hr.
One-half of the transformation [100 ul] plate onto LB + Amp plate.
One-half of the transformation [100 ul] add to 2.5 ml LB + Amp liquid medium. Grow overnight with shaking at 37 C.
Next day
Plate: count colonies; pick 4-8 colonies into LB or TB plus Amp. If many colonies to pick, do in a 96 well growth plate.
Liquid: perform crude miniprep plasmid. Save as Mixed Transformation Plasmid. Analyze on agarose gel electrophoresis to indicate rate of insertion.
Next day
Perform crude miniprep plasmid on overnight cultures.