Biomek LIC and Transformation

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The LIC reaction inserts the Target gene into the vector plasmid. During the Transformation reaction, the plasmid is taken up by an E. coli cell, the cells multiply in the culture media, each forms a colony when plated on agar prepared with the antibiotic against which the plasmid confers resistance. See **T4 Reaction** protocol and **Vector Preparation** protocol.
Input: Insert plate from T4 Reaction.
Output: individual agar plate for each gene, mixed plasmid culture plate
Next methods: Picking & Growing Clones for Plasmid Purification, Plasmid miniprep.

Materials/Reagents/Equipment	Vendor/ Location
Disposables	
PCR plate & mat	E&K (Cat#: 489096 & 402096)
Breatheable sealing film	E &K (Cat#: 1896100-S)
Sterile reservoir	Matrix (Cat# 8096)
Sterile glass beads	Bench Rm. 318
Sterile 2 ml square-well culture plate	E &K (Cat#: 662000)
Reagents	
T4-treated insert plate	Freezer
Vector plate, conc. T4-treated Vector stock	Freezer
Competent DH5a cells	-80 Freezer
LB media	Bench
LB media w/ Ampicillin (.1 mg/ml)	Refrigerator
Agar plates w/ Ampicillin (.1 mg/ml)	Cold Room
Equipment	
Biomek 2000 w/ Thermablock & adapter	Beckmann Coulter
PCR machine	Applied Biosystems
Waterbath at 4^{o}	
2 ice buckets, foil, tray	

Description

1.The Biomek 2000 mixes 4 ul of each insert with 4 ul of Sma-cut, T4 treated vector in the LIC rxn plate. The LIC reaction takes place during the 5 minute incubation on the worksurface.
2.Competent cells are added manually to the LIC rxn plate which then incubates on the 4^{o} Thermablock for 30 minutes; it is then manually transferred to a PCR machine for a Heatshock which causes the plasmids to be taken up by the cells, i.e. to transform them.
3. The plate is then returned to the Thermablock. The Biomek transfers LB media the transformation rxns to a culture plate which incubates at 37^{o} for an hour.
4. Half of the culture is plated, the rest is returned to the Biomek which adds LB media with antibiotic. Both the agar plates and the culture plate are placed at 37^{o} overnight.

Procedure:

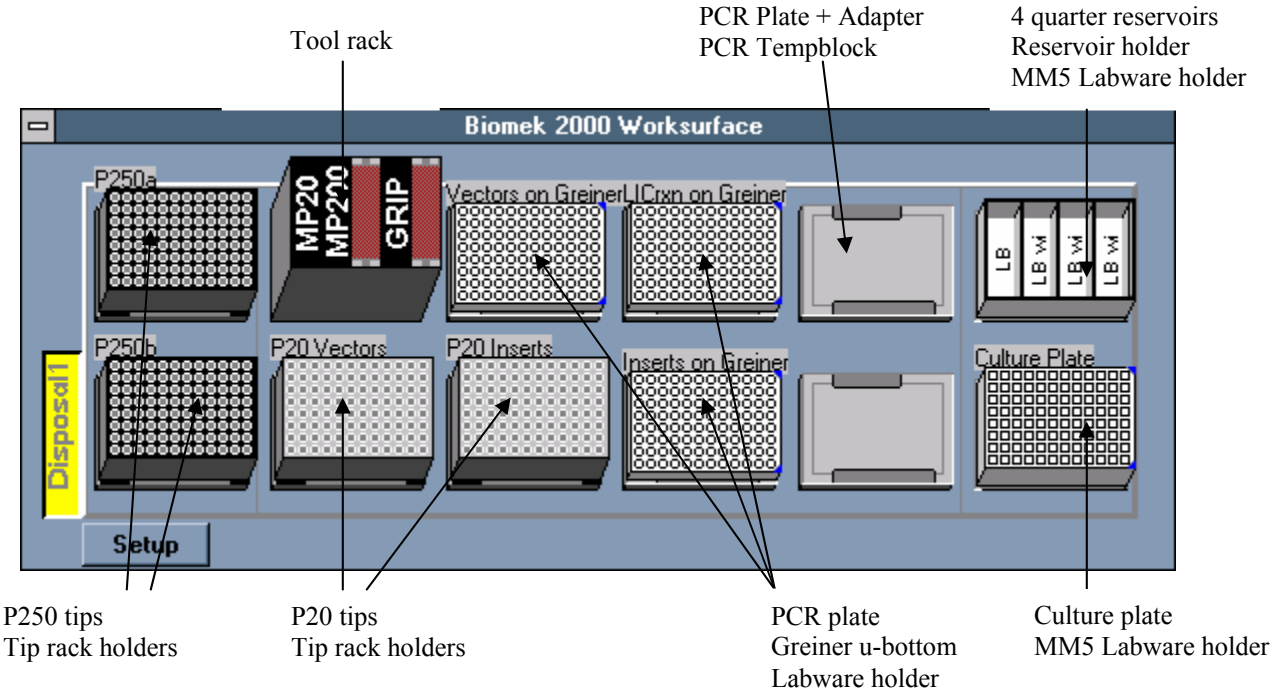
NOTE: TURN ON THE WATER BATH AT 4° IN ROOM # 324 AT LEAST 30 MINUTES BEFORE STARTING THE METHOD.

I. Preparing Vector plate

___1. Remove Vector plate and Vector stock and place on ice. On Vector plate, vector column # match the Vector #, i.e., pB3 is in column 3.
___2. Calculate total volume of 5 ng/ul vector needed: Volume needed for each well of the vector column: 4 ul x (# of samples per row) + 8 ul. Dilute up from concentrated stock of quantified, Sma-cut, T4 treated vector, if necessary.
___3. Dispense vector to the Vector plate, cover and keep plate on ice.

II. Setting up the worksurface

___1. Thaw Insert plate on ice.
___2. Do not fill reservoirs with media until later in method.
___3. Leave autoclaved culture plate covered in foil.
___4. Place Vector plate on support u-bottom Greiner plate at A3.
___5. Place Insert plate on support u-bottom Greiner plate at B4.
___6. Place clean labeled PCR plate for LIC rxn at A4.
Note: The Biomek will transfer 4 ul of the vector to the LIC rxn plate wells in columns matching the Insert plate pattern, then add 4 ul of the insert to those wells. It will pause for 5 minutes for the LIC rxn to take place.



III. Running Biomek method:

___1. Double click on Biomek Lab Book Manager icon on the desktop.
___2. Select the **LIC & Transformation** folder and click on **Set as Current Lab Book**.
___3. Click **Close.**

___4. Double click on **Biomek Edit** icon on the desktop.
___5. Click **Method → Open →** Select **LIC manual transf**.
___6. Click on **Edit → Patterns →** Select **LIC Inserts → Allow Changes →** Set the pattern to match the pattern on Insert plate→ Click OK to confirm the pattern.
___7. Click on **Edit → Patterns →** Select **LIC Vectors → Allow Changes →** Set the pattern to match the pattern on the Vector plate→ Click OK to confirm the pattern
___8. Click on **Edit → Patterns →** Select **LIC rxn → Allow Changes →** Set the pattern to match the pattern on the LIC rxn plate→ Click OK to confirm the pattern.
___9. Click on **Edit → Patterns →** Select **Culture Plate → Allow Changes →** Set the pattern to match the pattern on the Culture plate→ Click OK to confirm the pattern.
___10. Scroll down the method to **Making Mixed Plasmid** step and double-click on pipetting commands to open Pipette Transfer windows: check that the 2 Destination labware patterns add up together to the Culture Plate pattern.
Note: Quarter reservoirs only hold 32 ml
___11. Click on the running man button to start the method. (Note: Save all the settings and click **Accept All** to confirm the configuration.).

Note: Thaw competent cells x (# of targets x 50 ul + 400 ul) on ice, when starting Biomek method.

A. LIC Reaction.

The Biomek 2000 mixes 4 ul of each insert with 4 ul of Sma-cut, T4 treated vector in the LIC rxn plate. The LIC reaction takes place during the 5 minute incubation on the worksurface. An alarm announces the start of the incubation period. After 5 minutes or more, click OK to continue.The Gripper then moves the plate to the Thermablock.

B. Transformation

__1. Put disposable reservoir on ice.
__2. Dispense thawed cells with pipettor into reservoir.
__3. Using 250 ul 8-channel multi-pipettor and sterile tips, dispense cells to LIC rxn plate columns on Thermablock.
__4. Cover plate with mat.
__5. Let cells and plasmid incubate for 30 minutes at 4^{o}.
__6. Turn on PCR machine → select User (F5) → move highlight to Barb with arrow → select Accept (F1) → Select Run (F1) → highlight Heatshock → select Start (F1) → reaction volume 60 ul → select Start → lid will heat to 103^{o}, then display will show chart of run. When block reaches 4^{o}, Pause (F1) or place plate on block and close lid.
__7. Add LB media to left reservoir at A6.
__8. After Heatshock run is over, and plate has been at 4^{o} for at least 2 minutes, return plate to Thermablock on Biomek, remove mat, and click OK to continue.
__9. Turn off PCR machine by pressing Stop button twice, Exit (F5) and Power.
__10. After the Biomek has transferred LB and transformation to the culture plate, cover the culture plate with Breatheable seal, label and place on shelf in to warm room (37^{o}) for 1 hour or more. Discard LIC rxn plate and turn off Waterbath.

C. Plating

__1. Make map of culture plate in Excel from Insert map.
__2.Take culture plate, agar plates (# of targets) and glass beads to Hood in Rm. 314 and map of plate.
__3. Label a row of 8 agar plates: target ID,vector name, date, on the bottom, according to 1st column of map. Turn over.
__4. Dispense 130 ul of each transformation in 1st column with pipettor, add 4-5 glass beads by shaking beaker, cover and stack plates, shake vigorously to spread culture.
__5. Repeat with other columns.
__6. Make shallow container of foil, uncover and tap agar plates over foil to remove beads. Collect beads to return to "Dirty glass beads" beaker by sink in Rm. 318.
__7. Stack agar plates upside-down on tray and let incubate overnight in warm room.

D. Making Mixed Plasmid

__1. Return Culture plate with half of transformation to B6 on Biomek worksurface.
__2. Fill reservoirs with LB w/ antibiotic according to Step 10 of Running Biomek method above.
__3. Click OK for 1ml of media to be added to each well.
__4. Cover the culture plate with Breatheable seal and put on plate shaker (275rpm) in warm room overnight

E. After Method

__1. Clean up Biomek worksurface, discard used tips, empty and rinse reservoirs.
Tip usage: A1: # of columns of samples + 1
B1: 1 column
B2 and B3: # of columns of samples
__2. Exit method and log to return to Desktop.