Bacterial Culture Preparation for High Throughput Sequencing

(A) <u>PROCEDURE</u>:

(1) Prepare the 1X LB Freezing Buffer by adding the appropriate amount of each stock solution to a sterile container; e.g., an empty autoclaved bottle or a presterilized polypropylene blue cap tube. If the plasmid vector is ampicillin resistant, be sure that your medium has amp at the final concentration of 150μg/ml. If any stock solution is suspected of being contaminated, reautoclave it and/or autoclave the 1X working solution before use.

Steps 2-7 and 9 below are performed under sterile conditions in the laminar flow hood. Before working in the hood, spray down and wipe the working bench surface with 70% ethanol and turn on the laminar flow 10-15 minutes prior to actually working in the hood. Generally when working in the hood it is also a good idea to be wearing clean clothes; i.e., don't come from the field into the hood; make sure your hands are clean; initially and periodically spray your arms and hands with 70% ethanol (For this purpose only use 70% and not 95% because 70% is more bactericidal and 95% is **HIGHLY FLAMABLE**). Also make that any pipetting device; i.e., the multichannel L1000 and L200 are clean; if necessary disassemble and clean with 70% ethanol (ask how to do this).

(2) Transfer the necessary volume of 1X LB Freezing Buffer to a sterile 82mM polystyrene petri dish.

(3) Using the multichannel L1000 pipetteman, transfer 1.25ml (2 x 625μl) of 1X LB Freezing Buffer from the polystyrene petri dish to each column of wells in a clean, sterile 96-well growth block.

(4) Using either sterile toothpicks and forceps or your hands, or sterile L20 pipette tips and an L20 pipetteman, touch a single while colony on the transformant plate and inoculate the LB in well #A1 of the 96-well growth block by placing this toothpick into that well. Leave the toothpick/L20 tip in well #A1. Repeat the colony selection process with a second white colony and place this toothpick/L20 tip into well B1.

(5) Repeat this process until the entire column #1 is completed. Then start column #2.

(6) Remove the toothpicks/L20 tips from column #1. Continue doing this until the entire plate is completed. This systematic process prevents accidentally picking two different colonies into the same well. Also always start with column #1 because if only half a plate (48 samples) is to be submitted; i.e., columns 1- 6 are the ones that the sequencing facility will start their DNA preps with.

<u>Note</u>: For sequencing at Michigan State University only, leave wells A1 and H12 empty, which are for their sequencing controls. For sequencing at Amplicon Express and Columbia University, use all wells.

(7) Cover growth block with a sheet of sterile AirPore tape. **DO NOT** cover the growth block with the plastic lid provided with growth block. Aeration of the growth blocks is critically important to yield healthy cultures and AirPore tape "breathes," so cover with AirPore tape sheet only.

(8) Grow cultures at 37^{o}C, for 22h shaking vigorously at 250-300 rpm. (Use the wooden block in the shaker to hold the racks.)

(9) After growth is complete, take the 96-well growth block back the laminar flow hood. Using the multichannel L200 pipetteman, aliquot 100ul of the healthy overnight culture into a Greiner plate. Cover the Greiner plate with sterile foil tape. Also cover the 96-well growth block with sterile foil tape. The balance of the culture in the growth block; i.e., the remaining ~1ml is the Stockinger lab's

back-up culture. Keep this back-up bacterial culture in case an unforeseen event (the dry ice thaws out due to a late FedEx delivery) or technical difficulties arise at the sequencing facility.

(10) Freeze both the back-up culture and the Greiner plate at –80^{o}C.

(11) Fill out a sequencing submission form (if required) and mail the Greiner plate to the appropriate sequencing facility.

(B) PACKAGING AND SHIPPING FROZEN BACTERIAL CULTURES:

Package the Greiner plates containing the frozen bacterial cultures into a Styrofoam container with dry ice. Put the s Styrofoam container into a cardboard box (if it is not already in one) for FedEx shipping.

Bacterial cultures must be (FedEx) shipped overnight packed with enough dry ice to ensure the cultures stay frozen in the event an overnight delivery is not made.

For a small package (1-2 plates), 3lbs of dry ice are required. For larger packages containing more plates (5-6) 6-8lbs of dry ice are required. This will last 2-3 days.

Only mail packages on Monday, Tuesday or Wednesday; i.e., do not mail packages on Thursday or Friday!!!!

Purchase dry ice from:

Smith's Dairy
1381 Dairy Lane
Orrville, OH 44667
Tel: 330-683-8710
Hours: Monday – Friday; 8:00AM-5:00PM
$1.00/lb

(C) MATERIALS:

1. 96-well sterile growth block (Qiagen) Cat # 19573Square-Well Blocks (24)

   Wrap these in foil and autoclave prior to use

2. AirPore Tape Sheets (Qiagen)
   (1) Cat # 1957 $61.00 AirPore Tape Sheets (50)
   (2) Cat # 120001 $29.00 AirPore Tape Sheets (25)

   Wrap these in foil and autoclave prior to use

3. Greiner plates: Cellstar, suspension plate, 96 well with lid, Sterile No. 650185.

   Wrap these in foil and autoclave prior to use.

4. Foil Tape

   Wrap these in foil and autoclave prior to use

5. Sterile toothpicks or sterile L20 pipette tips.

LB Freezing Buffer Stocks:

10X Solution 1:

Stock	Quantity for 100ml	10X Stock Conc	Final Conc
$MgSO_4 \cdot 7H_2O$	0.097 g $MgSO_4$	4mM	

10X Solution 2:

Stock	Quantity for 100ml	10X Stock Conc	Final Conc
K_2HPO_4	6.27g	360mM	36mM
KH_2PO_4	1.80g	132mM	13.2mM
$(NH_4)_2SO_4$	0.899g	68mM	6.8mM
Na-Citrate (Dihydrate; FW 294.10)	0.499g	17mM	1.7mM

Correct pH to 7.5 using NaOH

10X Solution 3:

Stock	Volume for 100ml	10X Stock Conc	Final Conc
Glycerol	44ml	44%	4.4%
ddH_2O	56ml		

2X LB:

Stock	Volume for 500ml	10X Stock Conc	Final Conc
Bacto Tryptone	10g		
Yeast Extract	5 g		
NaCl	5 g		

Correct pH to 7.5 using NaOH (less accurate alternative = pH ~2 pellets per liter)

150mg/ml ampicillin stock solution:

Stock	Volume for 10ml	100X Stock Conc	Final Conc
Ampicillin	1.5g	44%	4.4%
ddH_2O	10ml		

Vortex to dissolve the ampicillin
Filter-sterilize the amp by passing it through a 0.2μm filter fitted onto a syringe
Collect the amp into a sterile test tube
Dispense 1.0 to 1.5ml aliquots into sterile microfuge tubes
Store ampicillin frozen at –20°C

1X LB Freezing Buffer:

Stock	Volume for 100ml

2X LB	50 ml
10X Solution 1	10 ml
10X Solution 2	10 ml
10X Solution 3	10 ml
Sterile dH_20	20 ml
150mg/ml Amp	0.15 ml

Prepare the 10X stock solutions, the 2X LB and the Amp. Autoclave these. Store at RT under sterile conditions. Prepare the 1X LB Freezing Buffer immediately before picking the overnight cultures. Prepare only as much 1X LB Freezing Buffer as will be used at any one given time. This reduces the risk of contamination. In addition to the normal components usually found in regular LB medium, LB Freezing Buffer growth medium has potassium phosphate buffer (PB) and glycerol. PB helps keep the medium at physiological pH during growth and glycerol minimizes damage to E. coli cells resulting from freezing and thawing of the cultures at $-80^{o}C$.