**Moffat Barcode library Amplification**

1. Dilute the plasmid DNA to 50 ng/μL in TE.
2. Electroporate the library using ElectroMAX™ Stbl4 Competent Cells (Invitrogen). Consult the manufacturer’s protocol for extra details. Set up a total of 4 electroporations as follows:
	1. Add 4 μL of 50 ng/μL BCL library to 25 μL of thawed Stbl4 electrocompetent cells to pre-chilled cuvettes (1.0 mm) on ice;
	2. Electroporate according to the manufacturer’s suggested conditions and protocol;
	3. Within 10 seconds of the pulse, add 975 μL of Recovery Medium (or SOC medium) to the cuvette;
	4. Transfer cells to a culture tube with an additional 1 mL of Recovery Medium;
	5. Place tubes in a shaking incubator at 250 rpm for 1 hour at 37°C.
3. Set up a dilution plate to titer the library and estimate transformation efficiency
	1. Pool all 8 mL of recovered cells and mix well.
	2. Transfer 10 μL of the pooled cells to 990 μL of Recovery Medium for an 800-fold dilution and mix well. Plate 20 μL of the dilution onto a pre-warmed 10-cm LB + carbenicillin (100 ug/mL) agar plate. This results in a 40,000-fold dilution of the full transformation that will be used to calculate the transformation efficiency.
4. Calculate transformation efficiency.
	1. Count the number of colonies on the 40,000-fold dilution plate (set up in step 4).
	2. Multiply the number of colonies by 40,000 to obtain the total number of colonies plated.
	3. Proceed if the total number of colonies represents a library coverage desired. Aim for 4-10 x 10^6 colonies. Obtaining sufficient number of colonies will ensure full library representation is preserved.
5. Plate the rest of library as needed by spreading the recovered cells on pre-warmed 15-cm LB agar plates (number of plates determined by transformation efficiency) containing carbenicillin (100 ug/mL). Spread 400 μL of recovered cells evenly on each plate
6. Incubate the plates for 14-16 hours at 30°C. Growth at this lower temperature minimizes recombination between long-terminal repeats (LTR).
7. Harvest 4-10 x 10^6 colonies
	1. Transfer 7 mL of LB + carbenicillin (100 g/mL) medium to one 15-cm plate.
	2. Scrape colonies off with a cell spreader.
	3. Transfer the scraped cells into a sterile 1 L Erlenmeyer flask or bottle using a 10-mL pipet.
	4. Rinse the scraped plate with an additional 5 mL of LB + carbenicillin medium and transfer to the bottle.
	5. Repeat steps (a)-(d) for all plates. Pool all scraped cells from 20 plates to a sterile bottle.
	6. Mix collected cells with a stir bar for 1 hour at room temperature to break up cell clumps.
	7. Transfer cells to pre-weighed centrifuge bottles.
	8. Centrifuge at 7,000 x *g* to pellet bacteria, then discard media.
	9. Weigh the wet cell pellet and subtract the weight of the centrifuge bottle to determine the final weight of the wet pellet.
8. Purify the library plasmid pool.
	1. Purify plasmid DNA using a maxi- or mega-scale plasmid purification kit.
	2. Perform multiple maxi or mega preps according to column capacity. Typically, a maxi column can process 1 g of wet cell pellet, and a mega column can process 2.5 g of wet cell pellet.