**Pooled RBP CRISPR sgRNA plasmid amplification**

\*Protocol adapted from genome-scale CRISPR Knock-Out pooled library plasmid amplification (Shalem *et al.,* Science. 2014)

*Library transformation*. Add 2 µL of 50 ng/µL to 25µL of electrocompetent cells (Lucigen Endura #60242). Electroporate according to manufacturer’s instructions/suggested parameters. Recover in 975 µL recovery media (provided). Add an addition 1 mL of recovery medium and shake at 37°C for 1 hour.

*Plate transformations.* Pool 8mL of electroporated cells. To determine the transformation efficiency, plate 20 µL of a 40,000-fold dilution onto a pre-warmed, ampicillin 10cm petri dish. Plate the rest of the electroporated cells (4mL per pre-warmed, amplicillin 24.5 cm2 bioassay plate, or alternatively, 400 µL per pre-warmed, ampicillin, 10 cm petri dish). Grow plates inverted at 32°C for 14 hours.

*Transformation efficiency.* Count the number of colonies on the dilution plate and multiply by 40,000. The total number of colonies should be approximately 300X the total library (between 3.5-4 x106). **Do not proceed if efficiency is less than 50X.**

*Harvest and maxiprep*. Rinse plates with 10 mL of LB per 24.5 cm2 plate (or 500 µL per 10 cm petri dish). Scrape all the colonies into the LB with a spreader and pipette off the liquid with scraped colonies. Repeat this step with an additional 5-10 mL of LB. Spin down and remove supernatant. Weigh the bacterial pellet and proceed with maxiprep (Invitrogen #K210017) according to manufacturer’s instructions using one maxi column per 0.45 g of bacterial pellet.

Proceed to library preparation to verify representation and pool complexity by deep sequencing.