**Pooled RBP CRISPR sgRNA plasmid library preparation**

*First PCR:* Prepare first PCR reaction using 0.5 ng plasmid library per replicate in 50 µL reactions using 25 µL 2X Q5 (NEB #M0492L), and 2.5 µL of each primer at a concentration of 20 µM for a 50 µL total volume.

PCR program: 98°C 30 sec, 98°C 15 sec, 68°C 1 min, 72°C 1 min, GOTO step2 6 times, 72°C 2 min, HOLD 4°C.

Primers:

Fwd: CCTACACGACGCTCTTCCGATCTTGTGGAAAGGACGAAACACCG

Rev: GTTCAGACGTGTGCTCTTCCGATCTCCACTTTTTCAAGTTGATAACGGACTAGCC

*Cleanup:* Cleanup first PCR with 1.8X AmpureXP beads (Beckman Coulter #A63881) according to manufacturer’s instructions and elute in 20 µL H2O for second PCR input.

*Second PCR:* Prepare second PCR reaction using 20 µL DNA elution from 1st bead cleanup, 25 µL 2X Q5, 2.5 µL each of 20 µM Illumina sequencing primers for a 50 µL total volume.

PCR program: 98°C 30 sec, 98°C 15 sec, 68°C 1 min, 72°C 1 min, GOTO step2 6 times, 72°C 2 min, HOLD 4°C.

Primers: Illumina dual indexed D50X, D70X adapters

*Cleanup:* Cleanup second PCR with 1.4X AmpureXP beads according to manufacturer’s instructions and elute in 20 µL H2O.

*Quantification*: Library size (~260 bp) and concentration were calculated using D1000 Tapestation (Agilent #5067-5582) and sequenced to 6M reads per library on the Hi-Seq4000 in single-end 75 bp mode.