Mouse messenger-RBP sgRNA library protocols

**Amplification**

The library can be amplified in high efficiency electrocompetent bacteria such as Stbl4 (Thermo Scientific #11635018) or Endura (Lucigen #60242) following the manufacturer’s instructions. Electroporated bacteria should be plated on LB-AMP (50-100µM) across several large plates (Corning #CLS431272-16EA). Coverage of the library will require around 1-5x106 bacterial colonies. After overnight incubation, the bacteria can be harvested with help of LB broth media and a razor blade. Then, the plasmid DNA can be isolated with a maxiprep kit (Qiagen®: 12162) following the manufacture’s protocol.

**NGS sequencing**

We recommend that genomic DNA (gDNA) is isolated as previously described1. Then, next generation sequencing (NGS) libraries are generated in one round by PCR from 2.5µg gDNA in 22 cycles with Q5 polymerase following the manufacturer’s protocol with published primers2. Then, PCR products are concentrated (Zymo Research: D4031), size selected by gel electrophoresis and purified (Zymo Research: D4005).

We recommend multiplexed NGS libraries are sequenced with an Illumina™ HiSeq with a 100bp single end read. For our analysis, the start of the iCRISPR scaffold sequence (GTTTAAGAGCTAT) within each read was identified, and the reads trimmed to encompass the 19 bases immediately preceding this sequence. Bowtie3 was used to map these sequences with zero mismatches to a custom genome comprising the sgRNA sequences, and Seqmonk used to quantify the abundance of each sgRNA (<https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/>). Analysis of our genetic screens was performed with the MAGeCK software4.

1. Chen, S. *et al.* Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. *Cell* (2015) doi:10.1016/j.cell.2015.02.038.

2. Joung, J. *et al.* Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. *Nat. Protoc.* (2017) doi:10.1038/nprot.2017.016.

3. Anders, S. & Huber, W. Differential expression analysis for sequence count data. *Genome Biol.* (2010) doi:10.1186/gb-2010-11-10-r106.

4. Li, W. *et al.* MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* (2014) doi:10.1186/s13059-014-0554-4.