**Identification and characterization of essential genes in the human genome**

The optimized genome-wide Cas9/sgRNA-based screening system described in Park et al enables large-scale loss-of function genetic screening in cultured human cells. The library targets 18,543 human genes with approximately 10 sgRNAs each and includes a number of non-targeting controls.

1) Derive a cell line expressing Cas9-GFP

 a) Generate Cas9-GFP lentivirus ([https://www.addgene.org/tools/protocols/plko/#E](https://www.addgene.org/tools/protocols/plko/%22%20%5Cl%20%22E)) using #86145 pLenti-Cas9-GFP.

 b) Transduce target cells.

 c) After 72 hours, FACS GFP+ cells to isolate either single-cell clones or GFP-high population.

2) Package lentivirus as described here: http://www.addgene.org/tools/protocols/plko/#E. Depending on the amount of virus desired, 4 to 12 T-175 flasks should be seeded for transfection with DNA amount and transfection reagent volume adjusted proportionally.

3) Transduce target cells according to the guidelines here:

http://www.addgene.org/tools/protocols/plko/#F. A spin-infection may boost the effective viral titer for some cell lines. Enough cells and virus should be used such that each sgRNA integrates into 100-1,000 cells. (100 may be sufficient for positive selection-based screens but for negative selection a 1,000-fold representation of the library is recommended)

4) 48 hours after transduction, select target cells with puromycin for 2-3 days. Performing a kill curve on unmodified cells is highly recommended.

5) After selection, harvest an initial population of cells and freeze at -20oC.

6) If appropriate, a selection agent (ie. drug) can be applied approximately one week after infection to allow sufficient passage of time for knockouts to be generated. Performing a kill curve on

unmodified cells is highly recommended.

7) Passage cells for the duration of the experiment being sure to maintain the minimal representation of the library.

8) At the end of the experiment, harvest cells and freeze at -20oC.

9) Amplify the sgRNA barcodes from the initial and final cell population according to the PCR protocol.