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

The optimized genome-wide Cas9/sgRNA-based screening system described in Wang et al enables large-scale loss-of function genetic screening in cultured human cells. The library targets 18,166 human genes with approximately 10 sgRNAs each and includes a number of non-targeting controls. The full list of targets in the library can be found here: <http://www.sciencemag.org/content/suppl/2015/10/14/science.aac7041.DC1/aac7041_SM_Table_S1.xlsx>. As the backbone of the library contains both Cas9 and the sgRNA, no modification of the target cell line is required prior to screening.

1) 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.

**NOTE:** Due to the large size of the vector, it may be challenging to achieve sufficient viral titers for conducting genome-wide screens. It is recommended to confirm the size of the library using gel electrophoresis as recombination may occur. In addition, one must ensure that the library and packaging plasmids are free of endotoxin, that the packaging HEK-293T cells are healthy prior to transfection and that the transfection is efficient. Finally, it may be necessary to concentrate the virus prior to transducing targeting cells.

2) 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)

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

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

5) 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.

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

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

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