**CRISPR library construction and screening**

A set of ~9,800-sgRNA oligos that target 1,000 human ubiquitin ligase genes and 546 epigenetic related genes were designed for array-based oligonucleotide synthesis (CustomArray). Unique binding of each sgRNA was verified by sequence blast against the whole human genome. In the sgRNA pooled library, six gRNAs against each of the 1,546 human human ubiquitin ligase genes and 546 epigenetic related genes were obtained from validated sgRNA libraries published previously. The synthesized oligo pool was amplified by PCR and cloned into LentiGuide-Puro backbone (#52963) by in-fusion assembly (Clontech #638909). The *HOXA9P2A-mCherry* reporter cell line was overexpressed with lentiviral Cas9 followed by infection of pooled sgRNA library at low M.O.I (~0.3). Infected cells were selected by blasticidine and puromycin and later sorted for mCherryHigh and mCherryLow populations between days 10-12. The sgRNA sequences were recovered by genomic PCR analysis and deep sequencing using MiSeq for single-end 150-bp read length (Illumina).

**Data analysis of CRISPR screening**

The raw FASTQ data were de-barcoded and mapped to the original reference sgRNA library. The differentially enriched sgRNAs were defined by comparing normalized counts between samples. Normalized counts for each sgRNA were extracted and used to identify differentially enriched sgRNA by DESeq2. The combined analysis of six sgRNAs against each human ubiquitin ligase genes and epigenetic related genes was conducted by using the MAGeCK algorithm.

|  |  |  |  |
| --- | --- | --- | --- |
| Recombinant DNA reagent | Lenti-Guide-Puro plasmid | Addgene | 52963 |
| Software, algorithm | MAGeCK | https://sourceforge.net/ p/mageck/wiki/Home/ | PMID: 25476604 |
| Software,  algorithm | DESeq2 | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |  |