Outline of somatic cells CRISPR gene knock out

1. Collect CRISPR target gene information including genomic DNA and protein sequences.
2. Design short guide RNA oligoes , digest CRISPR vector for cloning, anneal oligoes and ligate into CRISPR vector, DNA sequencing to screen positive clones.
3. Prepare DNA plasmid for customer. Two pairs CRISPR constructs are minimum.
4. Transfect CRISPR constructs to cells.
5. Treat with selection markers such as puromycin.
6. Do cell pool analysis by CEL1assay and mismatch assay or Tide analysis to determine CRISPR activity.
7. Sort cells into 96 well plate by series dilution or GFP enrich by Flow cytometry.
8. Expand cells from 96 well plate → 48 well plate → 24 well plate → 6 well plate → 60mm dish → 100mm dish.
9. Make cell stocks.
10. Harvest cells and make cell extracts and do Western blot analysis for confirmation.
11. Write detailed report.