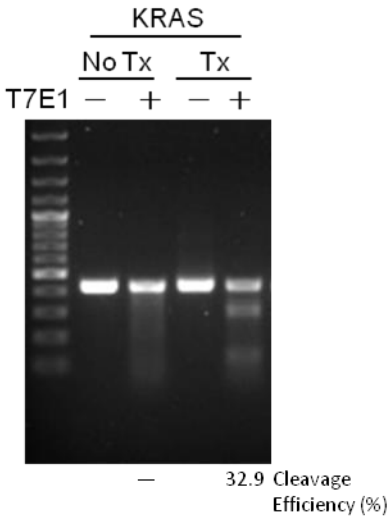
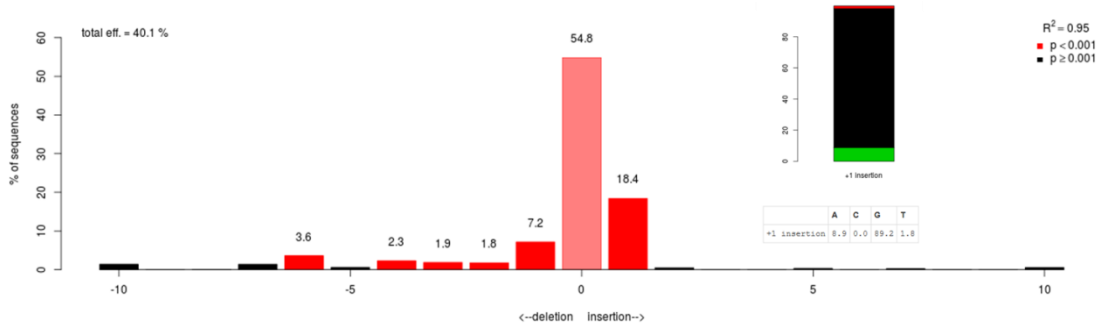
C6-18

Step 4-2: Cas9/ sgRNA Delivery

For pooled cells, the high levels of GFP expressing cells or the surviving puromycin-resistant cells were collected and assayed with T7E1 to detect the target cleavage effect or PCR-based sequence trace decomposition to identify gene editing (Brinkman et al., 2014). The software (https://tide-calculator.nki.nl/) quantifies the editing efficacy and simultaneously identifies the predominant types of insertions and deletions (indels) in the targeted pool of cells.





Step 4-3: Primer design and T7E1 analysis

A PCR amplicon of the genomic region is made using primers that flank the CRISPR target cleavage site. The primers are positioned 100 bases (or more) up- and down-stream surrounding the CRISPR target site. You may generate a region surrounding the CRISPR target site of the gene at NCBI website. The sequence is then used as the input of Primer3 program (http://frodo.wi.mit.edu/primer3/) to design checking primers. The reaction of T7 Endonuclease I (T7EI) was performed according to protocols of Transgenic Core Facility (Institute of Molecular Biology, Academia Sinica)(http://www.imb.sinica.edu.tw/TCF/pdfs/TALEN%20&%20CRISPR/(P)%20T7E1_digestion.pdf) and indel frequency was calculated according to (Ran FA et al., 2013).

Step 4-4: Single clone generation

For single-clone isolation, sort or dilute cells into 96-well plated after enrichment by puromycin or FACS sorting. Some adherent cell lines that DO NOT growth well by single cell dilution in 96-well plates can be seeded at lower dilution in a larger cell culture dish, such as 10- or 15-cm dish. The method allow cells that do not growth well on their own in a well to benefit by growth factors secreted into the culture medium from the mixture different clones.

Step 4-5: Single clone expansion

Expand single colonies into 24- or 12-well plates until the cells were dense enough. For method of limiting dilution into 10- or 15-cm dish, pick the single-cell clones by using pipette tips and subsequently transfer into 24- or 12-well plates.