Methods for validating CRISPR-Cas9 editing efficiency:

- Targeted deep sequencing:
  - Extremely sensitive, high-throughput and comprehensive but expensive and labor intensive.
- Quick and Dirty methods: High Resolution Melt Analysis or T7 endonuclease assay.
  - Both rely on PCR amplification followed by heteroduplex formation. These methods can be used to identify the presence of CRISPR-generated mutations, but are only semi-quantitative.
- TIDE (Tracking of Indels by DEcomposition) sequencing:
  - This method is quantitative, relatively inexpensive, easy to perform and accurately reports the percentage of alleles with CRISPR induced mutations (small insertions or deletions (INDELs)),

TIDE sequencing basic protocol:

1. PCR amplify 400-600bp region centered on CRISPR target site with HIFI DNA polymerase using genomic DNA isolated from a pool of cells treated with CRISPR and a pool of untreated cells.

2. Purify PCR amplicons with a DNA column purification kit.

3. Submit purified samples for Sanger sequencing with a sequencing primer 10-50bp inside the amplicon. Primer must be >150bp upstream or downstream of target site.

4. Input control and CRISPR treated ab1 traces into online web tools to decompose mixed sequence traces (indicative of CRISPR induced INDEL formation) and estimate the percent of reads that have INDELs.

Web tools:

https://tide.deskgen.com

https://ice.synthego.com/#/

Citations:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5772360/pdf/41598_2018_Article_19441.pdf

https://academic.oup.com/nar/article/42/22/e168/2411890