**Determination of edits in CRISPR-edited cell lines by sequencing**

Thanh Ngoc Nguyen (Laboratory of Michael Lazarou, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia) (nguyen.tha@wehi.edu.au)

**Buffers and reagents:**

* Zymo genomic DNA isolation kit (D3025)
* Qiagen PCR cleanup kit (Qiagen, #28104)
* T4 DNA ligase (NEB, M0202S)
* BamHI-HF (NEB, R3136S) and HindIII-HF (NEB, R3104S)
* NEB 5-alpha Competent E. coli (NEB #C2987)

**Procedures:**

1. Harvest the CRISPR-edited cells that need to be sequenced and the control parental cells.
2. Isolate genomic DNA using according to manufacturer’s instructions.
3. Amplify the region of interest (CRISPR-target region) via PCR using primers obtained when designing CRISPR construct (see “Generation of CRISPR constructs” protocol).
4. Run a 1 % DNA agarose gel to check if the PCR has worked.
5. If PCR products are present, clean them up with Qiagen PCR cleanup kit.
6. Send the cleaned-up PCR products to sequencing service with a sequencing primer. I normally choose a 15 bp DNA sequence (less than 60 % of GC content) within the region of interest at least 100 bp away from CRIPR target site as the sequencing primer.
7. Analyze the sequencing data using this website <https://ice.synthego.com/#/>
8. Sometimes, if the sequencing service provider(s) have trouble sequence the PCR products, it might be worth trying to clone these PCR products into a small non-expression plasmid such as pGEM4Z prior to sequencing:
* Incorporate BamHI site (GCGCGGATCC; BamHI site is highlighted in grey, the rest is overhang) and HindIII site (GCGCAAGCTT; HindIII site is highlighted in green, the rest is overhang) into the primers mentioned in step 3.
* Amplify the region of interest from genomic DNA isolated from the CRISPR-edited cells with these primers via PCR.
* Cut the amplified PCR products and pGEM4Z with BamHI and HindIII.
* Clean up the cut PCR products and pGEM4Z with Qiagen PCR cleanup kit.
* Ligate the PCR products and pGEM4Z together using T4 DNA ligase.
* Transform the ligated product mix into E. coli competent cells and plate on an Ampicillin agar plate.
* Screen for colonies with pGEM4Z ligated with the PCR products.
* Send them for sequencing with M13 forward or reverse primer.
* Align the sequencing data with the reference sequence to determine the edits.