Prepare Samples for Illumina Sequencing after library amplification

Illumina sequencing of the resulting plasmids is highly recommended to ensure the resulting plasmid libraries from this protocol are correct, have a large fraction of sgRNAs that match perfectly to the expected library, and the relative fractions of each sgRNA are tightly distributed. You only need ~100 sequencing reads per sgRNA in the library to be able to validate the library (ex. 20M reads for the entire CRISPRi genome-scale library), so you can include your samples as a <5% spike-in to other sequencing runs.

To sequence your libraries, follow Step 3 of the sequencing sample prep protocol with the following modifications:

- If you are spiking into a sequencing sample with high diversity, such as an RNA-seq experiment, you can omit the Set A/B strategy and just sequence with Set A primer pairs and sequencing primer
- For the PCR, perform one 100uL PCR per library. If you have cloned multiple sublibraries, PCR each individually with a different sample index to allow you to detect any cross-contamination or sample mix-ups (although be careful to not introduce this during the PCR!).
- Substitute the genomic DNA input into the PCR with 100ng library plasmid.
- Only perform 15 cycles of PCR.
- Primers for use with single or paired end sequencing. Compatible with both Hiseq 2500 and Hiseq 4000.

Crispri TSS (Index) paired end 5'

aatgatacggcgaccaccgaGATCTACACGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCCAAT**gcacaaaaggaaactcaccct

purple= adapter; blue= illumina primer site for sequencing; **black**= barcode; green= complements sg casette for enrichment

Crispr i TSS Common paired end 3'

caagcagaagacggcatacgaGATCGACTCGGTGCCACTTTTTC

purple= illumina adapter; black= complements sg for enrichment

a) PCR with 100ng of library and 15 cycles only

	100uL Rxn
• **Library**	**(~100 ng)**
• **5X Phusion HF Buffer**	**20 uL**

- **DMSO (100%)** **3 uL**
- **dNTPs (10 mM)** **1 uL**
- **Primer (Index) 100uM** **0.25 uL**
- **Primer (Common) 100uM** **0.25 uL**
- **HF Phusion** **1 uL**
- **Water** **Fill to 100uL**

PCR conditions

- **98ºC, 30s**
- **15 cycles**
  - **98ºC, 15s**
  - **56ºC, 15s**
  - **72ºC, 15s**
- **72ºC, 10min**
- **7ºC hold**

After PCR is complete combine reactions in a 1.5 mL nonstick tube. Proceed with SPRIbead purification (or appropriate column purification)

b) SPRIbead purification

Following the index PCR the library is ~264 bp. For purifying with SPRIbeads you will need 1.5 mL nonstick tubes, DynaMag 2 magnet (life tech), 80% EtOH (make fresh), and Qiagen EB.

- *Add 0.65X reaction volume SPRI beads (65 uL)*
  - *Pipette mix 15X*
  - *Incubate RT for 5 min*
  - *Place on magnet stand 5 min or until supernatant becomes clear*
    - *>300 bp on beads, < 300 bp in supernatant*
  - *Transfer supernatant to new tube. Keep this fraction as your library is here.*
- *Rebind. Add 1.0X your initial reaction volume SPRI*
  - *Pipette mix 15X*
  - *Incubate at RT for 5 min*
  - *Place on magnet stand for 5 min or until clear*
    - *150-300 bp on beads*
  - *Keep beads. Your library is here.*
  - *Wash beads. Add 1mL 80% EtOH. Incubate at RT 2min. Remove EtOH. Repeat for a total of two washes.*
  - *Air Dry. Beads will change from a glossy texture to matte (non-glossy) and will look dry. This can take from 5 to 15 min.*
  - *Elute 50 uL of Qiagen EB*