CDK tiling base editing mutagenesis screen deconvolution
10/30/24

Description: This is a protocol for the (1) gDNA extraction of samples, (2) PCR1, (3) PCR2 for the generation of the sensor libraries in illumina format for NGS.

1. **gDNA extraction**

- Samples are pelletted into 1000X cell pellets (2.75-3 million cells/pellet).
- Extraction of the entire pellet is performed using the QIAGEN DNeasy Blood & Tissue Kit, following the manufacturer's protocol.
    - Full protocol here: Genomic DNA extraction from cells (QIAGEN DNeasy Blood & Tissue …
- The gDNA is eluted in 100 uL of Buffer AE (to increase concentration)
- **Note: Do not use vacuum manifold for gDNA extractions. Use normal centrifugation protocol.** (viscosity of solutions is inconsistent and can lead to reduced yields)
- Information about DNA extraction yields and PCR yields is stored here: CDK_deconvolution

2. **PCR1 Reaction**

- Following Ondine's sensor prep protocol here: Preparing sgRNA-sensor construct for NGS.docx

Name	PCR #	Read Type	Barcode Sequence	Sequence (5' to 3')	Tm (NEB)	Tm (IDT OligoAnalyzer)
Sensor_PCR_F_1	1 (universal)	F	None	CGCTCTTCCGATCTCTAGCGTTCGAGTTAGGAATT	74	64
Sensor_PCR_R_1	1 (universal)	R	None	CTGAACCGCTCTTCCGATCTTTGTGGAAAGGACGAAACACC	78	67.3
Sensor_PCR_F_2	2 (universal)	F	None	AATGATACGGCGACCACCGAGATCTACACCGCTCTTCCGATCTCTAGCGT	83	70.6
Sensor_PCR_Barcode_X	2 (custom)	R	NNNNNNNN	CAAGCAGAAGACGGCATACGAGATNNNNNNNNNCCTGCTGAACCGCTCTTCCGATCT		

- Performing 4x PCR1 reactions for each sample with 20 uL of gDNA (leaves ~20 uL of leftover gDNA)
- Perform PCR reactions using Q5 High Fidelity 2X Master Mix (NEB #M0429S).

Component	50uL Reaction	Master Mix (X120) X130
Q5 High-Fidelity 2X Master Mix	25 uL	3,250 uL
10 uM Forward Primer	2.5 uL	325 uL
10 uM Reverse Primer	2.5 uL	325 uL
Template DNA	20 uL	20 uL each
H2O	-	
Total	**50 uL**	**1200 uL**

For master mix: pipette 30uL to each PCR tube

- **Include water-only control reactions!!! (to assess primer dimers)**

- Cycle conditions (25x CYCLES)!!!:

Temperature (°C)	cycle no.
98	30 s
98	10 s
64	30 s
72	30 s
72	2 min
4	keep

Named “Sensor-PCR1” under Grace’s folder in thermocycler 1.

After PCRs are completed:

- Pool up to 4 PCR reactions and purify using PCR purification kit (QIAquick PCR Purification Kit) according to manufacturer’s protocol and elute in 50uL of EB buffer.
- Add 10uL of 6X loading dye to each sample eluted.
- Run samples on a 1% agarose gel alongside a 100bp ladder.
- Gel purify the correctly sized band using (Qiagen Gel Extraction Kit) and elute in 30uL of EB buffer.
    - **Expected PCR1 amplicon = 245 bp**
    - **Expected PCR2 amplicon = 310 bp**
- Measure DNA concentration using the NanoDrop 2000 (ThermoFisher).

Plasmid Pool PCR1 reaction:

- Lower cycle count (8 cycles)
- 2X PCRs

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3. **PCR2 Reaction**

- Performing 1x PCR2 reaction for each sample with 10 ng of template DNA
- Doing the same for plasmid library, but decreasing the template amount to 1 ng
- Modifying from 2.5 uL of each primer to 1 uL of each primer (to reduce primer dimers)

- Using barcoded PCR primers that match up with sample number (i.e. sample #2 = barcode #2)

Primer	**Sequence**
Sensor_PCR2_F	AATGATACGGCGACCACCGAGATCTACACCGCTCTTCCGATCTCTAGCGT
Sensor_PCR2_R_BARCODE	CAAGCAGAAGACGGCATACGAGATNNNNNNNNCCTGCTGAACCGCTCTTCCGATCT

Component	**50uL Reaction**	**Master Mix (X40)**
Q5 High-Fidelity 2X Master Mix	25 uL	1000 uL
10 uM Forward Primer	1 uL	40 uL
10 uM Reverse BARCODED Primer	1 uL	40 uL
Template DNA (1ng/uL)	10 uL	-
H2O	13 uL	520 uL
Total	**50 uL**	**250 uL**

Aliquot 40 uL/PCR tube and add template DNA

- Cycle conditions (10x CYCLES)!!!:

Temperature (°C)	cycle no.
98	2 min
98	10 s
70	30 s
72	30 s
72	2 min
4	keep

- **After completion, gel extract samples and Qubit for pooling**

- **Notes:**
    - Redoing samples **2, 3, 4, 5, 6, 7, 9, 10, 13, 15, 16, 17** because of lack of yield
    - Starting from gDNA extraction (had duplicate cell pellets for each sample)
    - Also redoing PCR2 for the plasmid pool, with 10 ng/uL of template DNA