

nanoCAGE – DAY 3

Quantified pool(s) of cDNA PCR products:

Before start checklist:

- Pipettes 1000 µL, 200 µL, 10 µL, 2 µL
- Filtered Tips Low-bind
- 0.2 mL PCR tubes low-bind + caps
- 1.5 mL microcentrifuge tubes low-bind
- 96-well PCR plate low-bind
- 384-well microplate for fluorescence assays
- Ultra-Pure Water
- Magnet for beads separation
- AMPure XP beads, resuspended and at room temperature
- Freshly prepared 70% EtOH, at room temperature
- Lambda DNA standard stock solution (1 ng/µL), on ice
- Quant-iT PicoGreen Reagent (200x), in fridge until use
- TE buffer (1x), at room temperature
- Bioanalyzer DNA High Sensitivity DNA kit, at room temperature 30 min before use
- Tagment DNA Buffer / NT Buffer, on ice
- Amplicon Tagment Mix / Nextera PCR Mastermix, in freezer until needed
- 12 Nextera N-series Index primers / nanoCAGE custom S-series primer (10 µM), on ice
- NaOH 5 M, pH ≥ 13/ Qiagen EB buffer, at room temperature
- Illumina sequencing kit (e.g., MiSeq Reagent Kit v2) / PhiX Sequencing Control v3 / nanoCAGE Sequencing primer Read 1 (100 µM), in freezer until needed
- Quantified pool(s) of purified cDNA PCR products, on ice

WORKFLOW

INSTRUCTIONS

NOTES/OBSERVATIONS

TIME/DATE

Quantified pool of purified cDNA PCR products



- Quantified pool(s) of purified cDNA PCR products prepared on Day 2
- Prepare ≥ 2.5 µL of a dilution of the pool at 0.1 – 0.2 ng/µL

- Either one pool containing all cDNA PCR products samples or several pools containing cDNA PCR products samples grouped by experimental condition

nanoCAGE library



- Add in PCR tube low-bind:
 - 5 µL of Tagment DNA Buffer
 - 2.5 µL of the dilution of the pool of cDNA PCR products (0.25 – 0.5 ng)
 - 2.5 µL of Amplicon Tagment Mix
- Incubate at 50°C for 10 min, Hold at 10°C
- Add 2.5 µL of NT buffer as soon as the temperature reaches 10°C
- Mix by pipetting 10x carefully and incubate 5 min at RT
- Add:
 - 2.5 µL of a Nextera XT N-series Index primer
 - 2.5 µL of nanoCAGE custom S-series primer (10 µM)
 - 7.5 µL of Nextera PCR Mastermix
- Mix by pipetting carefully, spin down briefly
- Incubate at 72°C for 3 min, 95°C for 30 s, then perform 12 PCR cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 1 min, and incubate at 72°C for 5 min, hold at 10°C

- Mix NT buffer carefully in order to avoid the formation of bubbles
- If processing several pools of cDNA PCR products, choose one Nextera N-series Index primer per pool when performing the Reduced-Cycle PCR Amplification
- If processing a single pool of cDNA PCR products, perform the PCR reaction using a single Nextera N-series Index primer and do not sequence the index.
- Alternatively, perform the PCR using 2.5 µL of an equimolar mix of the 12 Nextera N-series Index primers (i.e., mix 2 µL of each primer) and sequence the index

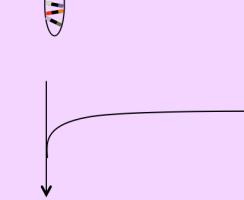
Purified nanoCAGE library



- Add 22.5 µL of AMPure XP beads at RT
- Mix by pipetting 10x slowly and incubate for 5 min
- Pellet on magnet and pipette off the supernatant
- Keep on magnet, wash 3x with 200 µL fresh 70% EtOH, do not disturb the pellet
- Resuspend pellet in 20 µL water, incubate at RT for 5 min
- Pellet beads on magnet, elute purified cDNA PCR products and transfer to a new PCR tube low-bind

- Aspirate supernatant carefully
- In order to avoid losing the sample, be sure not to aspirate beads in the pipette tip together with the solution

Quantified purified nanoCAGE library



- Prepare ≥ 20 µL of 1:10 and 1:20 dilutions of the purified nanoCAGE library
- Use 3 × 1 µL of purified nanoCAGE library and 3 × 1 µL of the 1:10 and 1:20 dilutions to visualize the size profile of the nanoCAGE library and confirm the final concentration on an Agilent Bioanalyzer High Sensitivity DNA chip
- (optional) Use 3 µL of the dilutions 1:10 and 1:20 of the purified nanoCAGE library for quantification by Quant-iT PicoGreen dsDNA assay

- If processing several nanoCAGE libraries prepared from different groups of samples, first quantify each library individually, then prepare a mix by adding equal quantities of each library and quantify dilutions of the pool as indicated
- Quantify the nanoCAGE library by selecting nanoCAGE fragments in the 150 bp – 1,500 bp range
- In general, sequence the nanoCAGE library at a concentration of 6 – 12 pM
- Add 10% of PhiX Sequencing Control library to the final nanoCAGE sequencing sample
- Output sequencing data as FASTQ files for bioinformatics analysis and demultiplexing based on barcode, index, and UMI informations

Sequence the nanoCAGE library on an Illumina sequencing system