

# Direct-seq: employing programmed gRNA scaffold for streamlined scRNA-seq in CRISPR screen

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## Method Article

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#### tRNA\_Read2 primer:

5'- GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGACTCTGAATCCAGCGAT -3'

#### P5\_read1 primer:

5'- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC -3'

#### P7\_Read2\_Index2 primer:

5'- CAAGCAGAAGACGGCATACGAGATGGAGCTACGTGACTGGAGTTCAGACGTGT -3'

## Equipment

Countess II (Invitrogen)

Chromium Controller (10x Genomics, PN-110203)

Chromium Accessory Kit (10x Genomics, PN-110204)

Vortex Genie 2 (Scientific Industries, 200-SI-0236)

Proflex PCR system (Thermo Fisher, 4484073)

Qubit 4 Fluorometer (Thermo Fisher, Q33238)

Fragment Analyzer-12 / 96 (Agilent, GENE-QC006)

0.22µM Filter (Merck Millipore, SLGV033RB)

0.2ml Thin-walled tubes (Thermo Fisher, AB-0620)

MicroAmp optical 8-Tube Strip (Thermo Fisher, A30588)

1.5mL Microcentrifuge Tubes low retention (Thermo Fisher, 3451)

Precision pipette tips 10µl (Mettler Toledo, 30389226)

Precision pipette tips 200µl (Mettler Toledo, 30389240)

Precision pipette tips 200µl wide-O (Mettler Toledo, 30389241)

1250µl Pipette Tip (Thermo Fisher, TFLR1121000-Q)

## Procedure

### Day1

#### 1. Single cell capture and cDNA amplification

At the end of CRISPR screening, cells were prepared as single cell suspension and loaded into the Chromium Single Cell B Chip. The cDNA was generated, purified and amplified (the 1<sup>st</sup> PCR) following the manual of Chromium Single-Cell 3' Reagent Kits v3 User Guide.

#### 2.cDNA size selection

cDNA was separated into two parts according to the size.

(1) Long cDNA fragments, which mostly represented mRNA, were size-selected by 0.6x AMPure XP beads. The cDNA binding to the beads was called "**long cDNA**", and will subject to mRNA library preparation. The "long cDNA" was then eluted in nuclease-free H<sub>2</sub>O following the 10x protocol.

(2) Short cDNA fragments, which mostly represented the index gRNA, were collected from the supernatant of the 0.6x beads selection, and then followed by a 1.2x size selection. The cDNA binding to the beads was called "**short cDNA**". The "short cDNA" was then eluted in 25 uL nuclease-free H<sub>2</sub>O.

### Day2

#### 3. 3' Gene Expression Library construction

The "long cDNA" was used for mRNA library preparation by following the manual of Chromium Single-Cell 3' Reagent Kits v3 User Guide.

## 4. Index gRNA Library construction

The "short cDNA" was used for the index gRNA library preparation by nested PCR.

### 4.1. Index gRNA sequence enrichment

(1) In total of eight index gRNA enrichment PCR (the 2<sup>nd</sup> PCR) reactions were conducted, with each reaction including 3 uL template, 25 uL NEBNext Ultra II Q5 Master Mix (NEB #M0544S), 2.5 uL tRNA\_Read2 primer (10uM), 2.5 uL P5\_read1 primer (10uM) and nuclease free water up to 50 uL.

(2) The PCR program was set as: (1) 98 °C for 30 s, (2) 14 cycles of 98 °C for 10 s, 60 °C for 10 s, then 72 for 10 s and (3) 72 °C for 2 min.

(3) The PCR products were combined and purified by 0.7–1.0x double-sided size selection (collect the supernatant from the 0.7x beads size selection, and followed by 1.0x beads selection, then collect elution from the beads) and eluted in 80µl of nuclease free water.

### 4.2. gRNA library construction

(1) In total of five library preparation PCR (the 3

rd PCR) was conducted, with each reaction including 10 uL template, 25 uL NEBNext Ultra II Q5 Master Mix, 2.5 uL P7\_Read2\_Index2 primer (10µM) and 2.5 uL P5\_read1 primer (10µM).

(2) The PCR program was set as: (1) 98 °C for 30 s, (2) 5 cycles of 98 °C for 10 s, then 54 °C for 15 s, then 65°C for 20 s and (3) 72 °C for 2 min.

(3) The PCR product was cleaned up and size selected with 0.7–1.0x AMPure XP beads.

(4) All eluted DNA was combined, and quantified. An aliquot was sent for NGS sequencing using the Illumina platform.

## Troubleshooting

(1) 2ng of the mRNA library and the index gRNA library were loaded into Fragment Analyzer to check the library quality. The typical library sizes were as below (Blue: mRNA library; Black: index library). (Figure 2)

(2) During index gRNA enrichment procedure, the cDNA size was shown as Figure 3

## Time Taken

About two days. See "Procedure".

## Anticipated Results

The mRNA library is constructed in very standard procedure, and the 10x manual should be strictly followed.

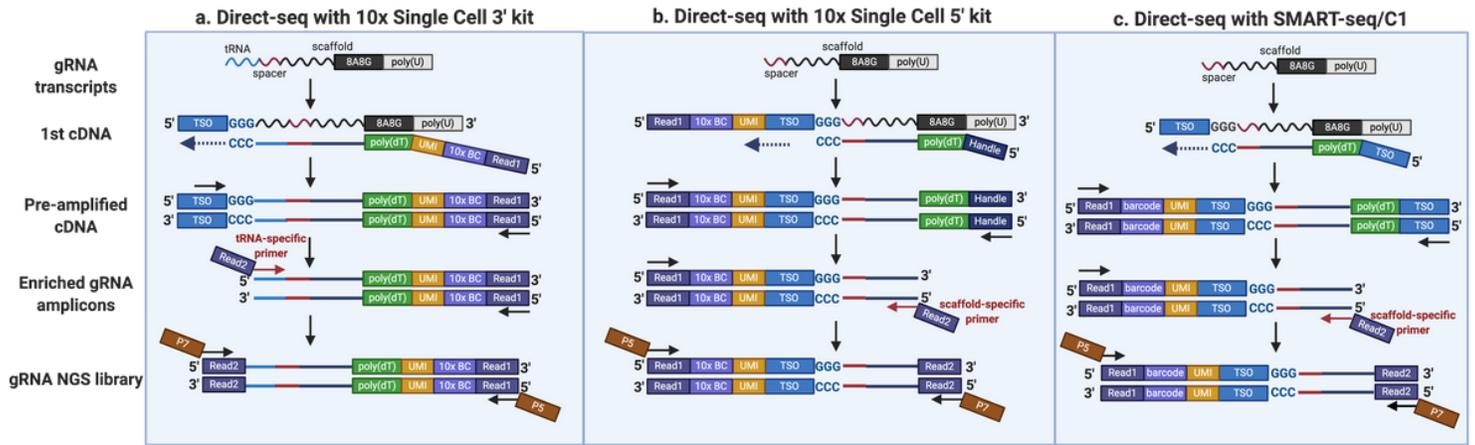
For the index gRNA library, the cDNA from gRNA transcripts were progressively enriched, which can be clearly reflected by the shift of fragment size as shown in the Figure3. The size-shift meets that expectation indicates efficient enrichment of the index gRNA.

## References

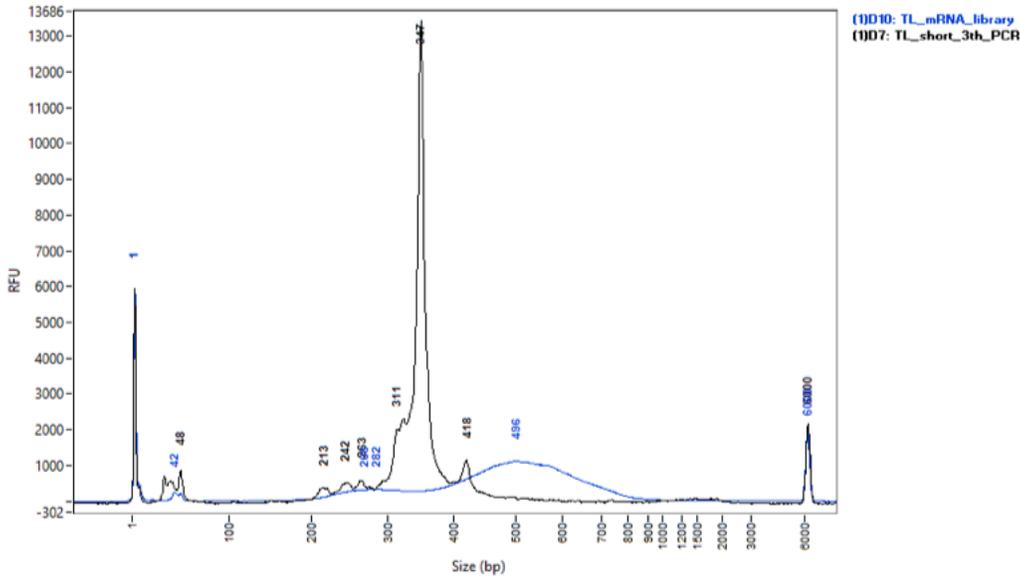
Song, Q. et al, Direct-seq: employing programmed gRNA scaffold for streamlined scRNA-seq in CRISPR screen. Genome Biology, 2020. DOI : 10.1186/s13059-020-02044-w

## Acknowledgements

## Figures



**Figure 1**  
 Workflows of the Direct-seq in different single cell RNA-seq platforms. (a) The suggested procedure to use with the 10x 3' kit; (b) The suggested procedure to use with the 10x 5' kit. The standard protocol should be followed until the pre-amplification of cDNA. The supernatant from size-selection contains gRNA sequences and could be specifically enriched by the nested PCR. The 1st step could use a scaffold-specific primer, and 2nd step could add the P5 and P7 sequencing adapters; (c) When work with the SMART-seq protocol, the cell barcode and UMI are suggested to be included in the template switch oligos (TSO) to enable sample pooling and eliminate PCR artifacts. The nested PCR is also suggested to make the gRNA library.



**Figure 2**  
 2ng of mRNA library and index gRNA library were loaded into Fragment Analyzer to check the library quality. The typical library sizes were as below (Blue: mRNA library; Black: index library).

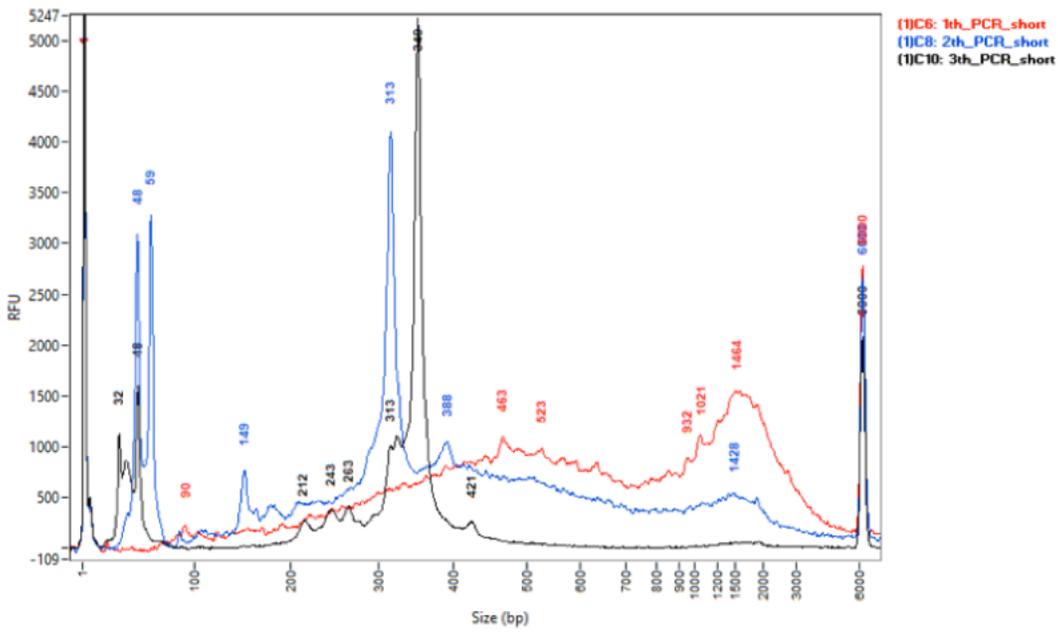


Figure 3

During index gRNA enrichment procedure, cDNA size that amplified from each was shown as below.