# SNICR barcode library generation

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*In Development*

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ABSTRACT

SNICR barcodes contain a 10x Capture Sequence (Capture Sequence 2) at the 3' end of the transcript. However, the barcodes are located 3' of a full-length H2B-GFP transcript, and are therefore much longer than typical Capture Sequence expressing gRNAs. The 10x protocol can be followed for the first step to perform cDNA amplification from the capture sequence, but afterwards must follow a custom protocol to recover the long transcripts and sub-amplify the barcodes.

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## 1 Overview

SNICR barcodes contain a 10x Capture Sequence (Capture Sequence 2) at the 3' end of the

transcript. However, the barcodes are located 3' of a full-length H2B-GFP transcript, and are therefore much longer than typical Capture Sequence expressing gRNAs. The 10x protocol can be followed for the first step to perform cDNA amplification from the capture sequence, but afterwards must follow a custom protocol to recover the long transcripts and sub-amplify the barcodes.

10x steps

2 Follow 10x Single Cell 3' v3.1 (Dual Index) with Feautre Barcode technology for CRISPR Screening up through Step 2.2, making sure to use Feature cDNA Primers 1 (2000096), which contains primers against both mRNA from oligo dT beads (Truseq Read 1) and against RNA from capture sequence beads (Nextera Read 1).

3 DO NOT follow step 2.3B; the SNICR barcodes amplified from Nextera Read 1 are with the rest of the 40uL transcriptomic fraction in 2.3A due to their length.

Subamplification of SNICR barcodes 1m 50s

4 Prepare PCR mix to subamplify SNICR barcodes and add Truseq R2 and Nextera R1-P5 handles

- **10 µL** cDNA
- **2.5 µL** Primer 1 (BFP-perturb + Truseq R2)
- **2.5 µL** Primer 2 (Nextera R1 + P5)
- **25 µL** Kapa HiFi 2x Master Mix (Roche, KK2601)
- **10 µL** UltraPure H2O

2m 50s

5 Run two-step PCR

1. **98 °C** **00:01:00**
2. **98 °C** **00:00:20**
3. **72 °C** **00:00:30**
4. repeat steps 2-3 13x total
5. **72 °C** **00:01:00**
6. **4 °C** hold

6 Perform 0.7X-1.2X SPRI cleanup. Always thoroughly vortex SPRI beads before adding and always wait for beads to fully settle to the magnet before proceeding.

6.1 Add **35 µL** SPRI beads (0.7X) to bind large fragments. Pipette mix 15x and



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place on magnet HIGH until beads separate.

6.2 Aspirate **85 µL** of supernatant and SAVE in new tube strip. This fraction has the PCR product.

6.3 Add **25 µL** SPRI beads (1.2X) to bind DNA. Pipette mix 15x and place on magnet HIGH until beads separate.

6.4 Remove and discard supernatant.

6.5 Add **200 µL** of fresh 80% EtOH and let sit for 30 seconds. Remove EtOH.

6.6 Add **200 µL** of fresh 80% EtOH and let sit for 30 seconds for a second wash. Remove EtOH.

6.7 Spin down tubes briefly, and place on magnet LOW. Use a P20 to remove any remaining EtOH trace without disturbing beads.

6.8 Remove from magnet and add **20 µL** of EB to beads to elute. Pipette mix 15x and wait for 2 minutes.

6.9 Place tube strip back on magnet and transfer eluted DNA to new tube strip. This will serve as input for the following round of PCR amplification.

7 Prepare PCR mix to further amplify barcodes and add P7 handle.

**20 µL** cDNA

**2.5 µL** Primer 1 (Truseq R2 + P7)

**2.5 µL** Primer 2 (Nextera R1 + P5)

**25 µL** Kapa HiFi 2x Master Mix (Roche, KK2601)



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8 Run two-step PCR

2m 50s

1. **98 °C** **00:01:00**
2. **98 °C** **00:00:20**
3. **72 °C** **00:00:30**
4. repeat steps 2-3 13x total
5. **72 °C** **00:01:00**
6. **4 °C** hold

9 Perform 1.2X SPRI cleanup to clean and concentrate final PCR product.

9.1 Add **50 µL** SPRI beads (1X) to bind barcodes. Pipette mix 15x and place on magnet HIGH until beads separate.

9.2 Remove and discard supernatant.

9.3 Add **200 µL** of fresh 80% EtOH and let sit for 30 seconds. Remove EtOH.

9.4 Add **200 µL** of fresh 80% EtOH and let sit for 30 seconds for a second wash. Remove EtOH.

9.5 Spin down tubes briefly, and place on magnet LOW. Use a P20 to remove any remaining EtOH trace without disturbing beads.

9.6 Remove from magnet and add **20 µL** of EB to beads to elute. Pipette mix 15x and wait for 2 minutes.

9.7 Place tube strip back on magnet and transfer eluted DNA to final tube strip..



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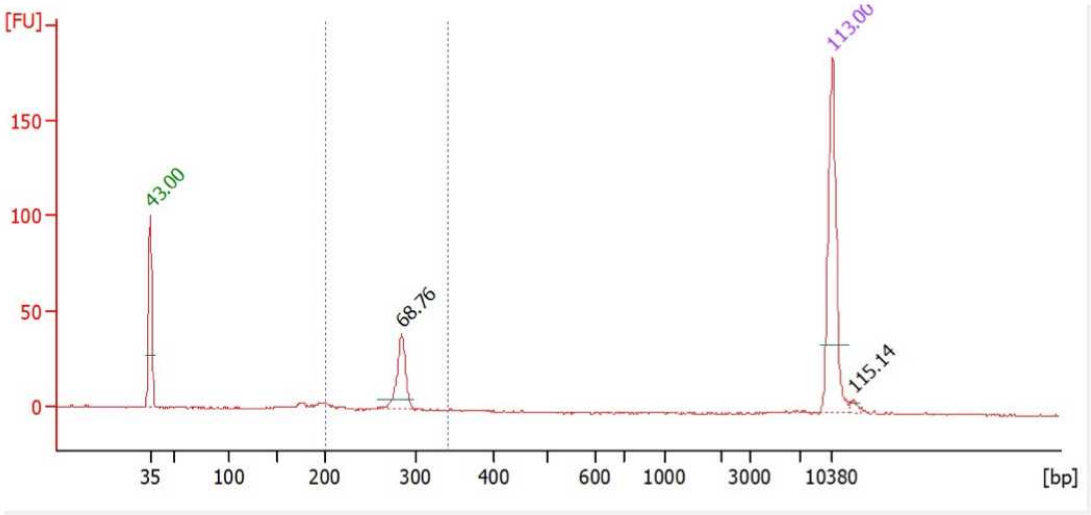
10 Check barcode quality by running BioAnalyzer on 1:10 diluted barcodes.




| From [bp] | To [bp] | Corr. Area | % of Total | Average Size [bp] | Size distribution in CV [%] | Conc. [pg/µl] | Molarity [pmol/l] | Color |
|---|---|---|---|---|---|---|---|---|
| 200 | 341 | 68.3 | 67 | 278 | 7.6 | 52.36 | 285.5 | |



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