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STICR Barcode Library Amplification Protocol

Forked from STICR Barcode Library Amplification Protocol

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ABSTRACT

Barcode amplification protocol for SNICR libraries based on:
Delgado, R.N., Allen, D.E., Keefe, M.G. *et al.* Individual human cortical progenitors can produce excitatory and inhibitory neurons. *Nature* **601,** 397–403 (2022). https://doi.org/10.1038/s41586-021-04230-7

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FORK NOTE

FORK FROM

Forked from STICR Barcode Library Amplification Protocol, Julia Schroeder

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PCR

1 Following cDNA amplification in 10X workflow, bead purify as directed in instructions and set aside 10ul of cDNA to use in the following reaction. This should leave you with **30 µL** of bead purified cDNA to complete the rest of the standard 10X whole transcriptome library with.

Primers: Reverse primer (P5-Read1) plus a forward primer (P7-i7 index-Read2-Upstream_Barcode sequence). If you plan to sequence multiple barcode libraries on the same lane, you will need to use different reverse primers as they will need to have different i7 indexes.

2 PCR Reaction Mix

Amplify library with standard NEB Protocol for Q5 Hot Start High-Fidelity 2X Master Mix in **50 µL** reaction:

1. **25 µL** Q5 High-fidelity 2X Master Mix
2. **2.5 µL** **10 micromolar (µM)** i7_indexed Reverse Primer (283-290)
3. **2.5 µL** **10 micromolar (µM)** i5_indexed Forward Primer (291-298)
4. **10 µL** molecular grade H_20
5. **10 µL** 10X cDNA

3 PCR program:

1. **98 °C** for **00:00:30** seconds
2. **98 °C** for **00:00:10** seconds
3. **62 °C** for **00:00:20** seconds
4. **72 °C** for **00:00:10** seconds
5. Repeat steps 2 through 4 ~15X (if unsure, run tests with 2uL starting cDNA at a range of cycles, but do not exceed 20 cycles for final preparation)
6. **72 °C** for **00:02:00** minutes
7. **4 °C** Hold

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Post PCR cleanup

4 Perform dual-sided SPRI selection

5 Add **30 µL** of SPRI beads to **50 µL** of PCR reaction, mix by pipetting 15 times.

6 Incubate at RT for **00:05:00** minutes

7 ⚠

Place on 10X magnet on High for **00:03:00** minutes. **DO NOT discard supernatant.**

8 Transfer supernatant to new PCR tube.

9 Add **10 µL** of SPRI beads, mix by pipetting 15 times.

10 Incubate at RT for **00:05:00** minutes

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11 Place on 10X magnet on High for **00:03:00** minutes

12 Carefully remove and discard supernatant (do not disrupt beads).

13 Wash beads with **200 µL** of freshly prepared 80% EtOH.

14 Let stand **00:00:30** seconds, remove EtOH.

15 Wash 1 additional time with 80% EtOH for **00:00:30** seconds

16 Remove EtOH with pipette, briefly centrifuge and return PCR tube to 10X magnet in low position. Remove any residual EtOH with pipette.

17 Add **22 µL** of Buffer EB, mix by pipetting 15 times.

18 Let stand **00:05:00** minutes RT.

19 Place on 10X magnet on low for **00:03:00** minutes

20 Remove supernatant to new PCR tube. This is your barcode library.

21 You can confirm library prep with Agilent BioA/Tapestation.Trace should look like:

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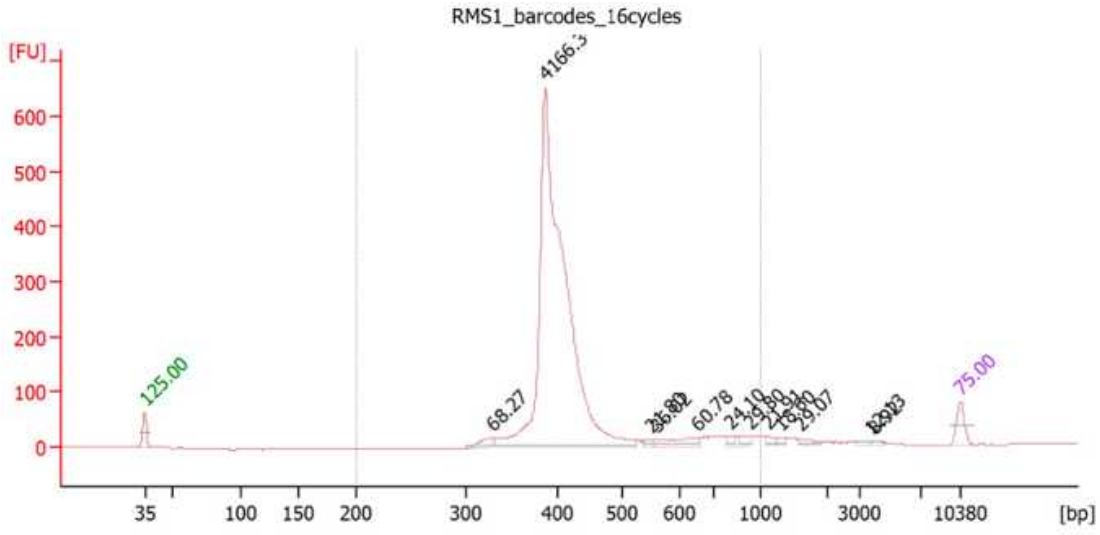

Region table for sample 2 : **RMS1_barcodes_16cycles**

Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Co From lor [bp]	To [bp]
2,240.8	95	427	23.0	4,302.73	15,864.0	200	1,000

22 Ensure that separate libraries were prepared with different antibodies before pooling and submit for sequencing ~30 million reads per library.

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