Jan 20, 2026

Sanger sequencing of barcoded beads

Nature Communications

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8xpzdv2w/v1

Koen Theunis[1,2,3,4]

[1]Laboratory of Computational Biology, VIB Center for AI & Computational Biology, Leuven, Belgium;
[2]VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium;
[3]Department of Human Genetics, KU Leuven, Leuven, Belgium;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, United States

Aertslab

Koen Theunis
Laboratory of Computational Biology, VIB Center for AI & Com...

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl8xpzdv2w/v1

External link: https://doi.org/10.1038/s41467-026-68742-4

Protocol Citation: Koen Theunis 2026. Sanger sequencing of barcoded beads. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8xpzdv2w/v1

Manuscript citation:
Dickmänken H, Wojno M, Mahieu L, Theunis K, Ekşi EC, Christiaens V, Kempynck N, Rop FVD, Roels N, Spanier KI, Vandepoel R, Hulselmans G, Poovathingal S, Aerts S (2026) Evaluating single-cell ATAC-seq atlasing technologies using sequence-to-function modeling. Nature Communications 17(). doi: 10.1038/s41467-026-68742-4

Protocol status: Working
We use this protocol and it's working

Created: January 19, 2026

Last Modified: January 20, 2026

Protocol Integer ID: 238928

Keywords: ASAPCRN, hydrogel bead barcode qc, barcoded hydrogel bead, barcoded bead, hydrogel bead, beads this protocol

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000430
Aligning Science Across Parkinson's
Grant ID: ASAP-025179

Abstract

This protocol is used to test the purity of barcoded hydrogel beads like: https://www.protocols.io/view/hydrop-v2-bead-generation-amp-ligation-barcoding-8epv5n11dv1b/v2

Materials

Oligo's for HyDropATAC **v2** Sanger QC

	PCR	Name	Sequence (5'>3')	Purification
	first	ATAC-QC oligo	GTCTCGTGGGCTCGGCTGTCTCTTATACACATCTGACGCTGCCGACGA	Standard desalted
	second	P7 indexing primer	CAAGCAGAAGACGGCATACGAGATXXXXXXXXXXGTCTCGTGGGCTCGGAGATGTG	Standard desalted
	second	P5-Partial	AATGATACGGCGACCACCGA	Standard desalted

Oligo's for HyDropATAC **v1** Sanger QC

	PCR	Name	Sequence (5'>3')	Purification
	first	ATAC-QC oligo	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCGAGCCCACGAGAC	Standard desalted
	second	P7 indexing primer	CAAGCAGAAGACGGCATACGAGATXXXXXXXXXXGTGACTGGAGTTCAGACGTGTG	Standard desalted
	second	Dropseq-P5-oligo	AATGATACGGCGACCACCGAGATCTACACGCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGT*A*C	Standard desalted

Wash finalized barcoded beads

1 Take finalized barcoded beads from storage (−80 °C)

2 Add 5 µL beads to a 1.5mL microcentrifuge tube containing 500 µL of 0.04% BSA in PBS.

3 Spin 1 minute at 500 x g and remove supernatant but leave about 20 µL.
From this supernatant a negative control can be taken in order to investigate oligo bleeding from the beads.

4 Repeat these washes for a total of 3 washes

Pick individual beads

5 Prepare the **lid** of a petri dish (Falcon 351008) with plenty of 5 µL PBS/BSA drops as washing medium for picked beads later. Use 20-30 µL drops in the petri dish for washing and priming of the 75 µm capillaries (MXL3-STR and MXL3-75, CooperSurgical). Adjust the Stripper® pipette to 0.5 µL and prime with PBS/BSA.

6 Dilute beads by pipetting in 10-20 µL drops of PBS/BSA on a petri dish **lid** under a stereo microscope until individual beads are distinguishable from each other.

7 Now pick beads with Stripper® pipette and 75 µm capillaries (MXL3-STR and MXL3-75, CooperSurgical) and transfer them over three wash drops in order to verify that 1 bead was picked. The tip can be rinsed and primed from large drops of PBS/BSA in between the bead transfers.

8 Transfer a single bead to a PCR tube containing 1.5 µL PBS/BSA.

9 Wash the capillary 3 times in a large volume of PBS/BSA. Now additional beads can be picked.

10 A blank can be taken from an empty droplet that was used for washing.

11 At the end of the experiment, a few multi-droplet controls (n = 2, n = 5, n = 10) can be picked. Don't do this before picking single beads because beads can stay in the dead

volume of the capillary.

First PCR amplification of the barcoded bead oligo

12 Add 18 µL PCR master mix 1 to the PCR tubes containing beads and/or blanks.
Final concentrations: 1x KAPA HiFi hot start ready mix (Roche Cat. No. KK2602), 30mM DTT, 1 µM ATAC-QC oligo.

13

	A	B	C
	72 °C	3 m	
	98 °C	30 s	
	98 °C	10 s	|
	59 °C	30 s	| 22 cycles
	72 °C	30 s	|
	4-15 °C	hold	

14 Perform the purification according to the manufacturers recommendation with 1.5X of Ampure beads (add 30 µL). Elute in 10 µL EB buffer and transfer 8 µL to the next PCR reaction.

Second PCR reaction

15 Add 12 µL PCR master mix 2 to 8 µL sample.
Final concentrations: 1X KAPA HiFi hot start ready mix, 1 µM P7 indexing primer, 1 µM P5-Partial primer.

16

	A	B	C
	95 °C	3 m	
	98 °C	20 s	|
	67 °C	30 s	| 22 cycles
	72 °C	20 s	|
	72 °C	1 m	

	A	B	C
	4-15 °C	hold	

17 Perform the purification according to the manufacturers recommendation with 1.5X of Ampure beads (add 30 µL). Elute beads in 17 µL EB buffer and transfer 15 µL to a fresh tube.

Sanger sequencing

18 Send for Sanger sequencing (eg LGC Genomics) using a P7 primer (for HyDropV2) or P5 primer (for HyDropV1).

Expected result

19