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Hybrid selection protocol using 10x Single-Cell RNA-Seq assay library

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

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Abstract

This protocol is for target enrichment of cDNA libraries generated with 10x Genomics single-cell RNA-seq assays. The protocol consists of parts of vendor provided protocols with minor modification. The hybridization and PCR part is based on "CG000059_DemonstratedProtocolExome_RevC" (10x Genomics) and the capture part is based on "SureSelectXT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library Protocol Version C3, September 2019" (Agilent).

Materials

Oligos

	A	B	C
	Oligo Name	Vendor	Sequence
	P5 primer	IDT	5'-AATGATACGGCGACCACCGA-3'
	P7 primer	IDT	5'-CAAGCAGAAGACGGCATACGA-3'

Reagents

	A	B	C
	Reagents	Vendor	Part Number
	xGen® Universal Blocking Oligo – TS-p5, 25 rxn	IDT	1016184
	xGen® Universal Blocking Oligo – TS-p7(8nt), 25 rxn	IDT	1016188
	SureSelectXT Reagent kit for 16 samples,includes library prep and enrichment reagents for post-capture processing on the HiSeq platform. Includes indexes 1 - 16.	Agilent	G9611A
	SureSelect Custom Tier3 3Mb-5.9Mb Probe (up to 180k oligos) sufficient for post-capture processing of 16 samples. With the following configuration: Design/ ELID # : S3333112	Agilent	5191-6910
	Dynabeads MyOne Streptavidin T1, 2ml	Thermo Fisher Scientific	65601
	Amp Mix	10x Genomics	220129
	Agencourt AMPure® XP SPRI beads, 60 ml	Beckman Coulter Genomics	A63881

Safety warnings

For hazard information and safety warnings, please refer to the MSDSs (Material Safety Data Sheets).

Sample preparation

30m

1 Use 750 ng of cDNA library generated with the 10x Chromium single-cell RNA-seq assay.

2 Add 1 µL each of TS-p5 and TS-p7 blocking oligos.

3 If the total volume exceeds 3.4 µL, use a speed-vac and set the temperature at 30 °C to reduce the volume to the desired volume of 3.4 µL.

20m

Buffer and reaction mix preparation

20m

4 Prepare the Hybridization Buffer with reagents from the SureSelectXT kit at Room temperature

10m

Prepare the volume for at least 5 reactions to ensure accurate pipetting.

	A	B	C
		1x (µl)	5x (µl)
	Hyb1 (orange)	6.63	33.15
	Hyb2 (red)	0.27	1.35
	Hyb3 (yellow)	2.65	13.25
	Hyb4 (black)	3.45	17.25
	Total	13	65

5 Prepare the block mix at 4 °C

10m

	A	B
		1x (µl)
	Indexing Block1 (green)	2.5
	Block 2 (blue)	2.5
	H2O	0.6
	Total	5.6

Sample and block denaturing

5m

6 Add 5.6 µL Block mix to the concentrated 3.4 µL sample, mix well and transfer to the 0.2ml PCR tube strip (Axygen), and place into a thermocycler following

Thermocycler Conditions: (Lid@ 105 °C , 100 µl)

95 °C , 00:05:00

65 °C , 00:00:00 hold

5m

Hybridization

1d

7 While step 6 mix is incubated at 65 °C for 00:05:00 , prepare the following at Room temperature

1d

	A	B
		1x (µl)
	Hybridization Buffer (Step 4)	13
	25% RNase Block solution (for >= 3Mb)	0.5 µl RNase Block (purple)+1.5 µl H2O=2 ul
	Capture library(>=3 Mb)	5
	Total	20

Mix well by high speed vortexing for 00:00:05 , spin down briefly, add into the sample and block mix at 65 °C , mix by pipetting 8-10x, use a new cap strip to seal the tubes

Incubate at 65 °C for 16:00:00 to 24:00:00

Library capture

2h

8 In a strip tube, use 50 µL MyOne Streptavidin T1 beads per sample, wash with 200 µL SureSelect Binding buffer 3 times, resuspend in 200 µL SureSelect Binding buffer.

15m

9 Bring the washed beads from step 8 near the thermocycler, add the 65 °C reaction mix (~ 27 µL left) right into the 200 µL beads, gentle pipetting mix, incubate at Room temperature for 00:30:00 , pipetting mix 6 times every 00:05:00

35m

10 Place on a magnet stand to pellet the beads and remove the supernatant once the solution appears clear. Resuspend the beads in 200 µL SureSelect Wash buffer 1, incubate at Room temperature for 00:15:00 — 15m

11 Meanwhile, pre-warm SureSelect Wash buffer 2 at 65 °C in strip tubes with 200 µL per well and 3 wells per reaction on a 96-well heating block (or a thermocycler with reaction volume capacity of 200 µL) — 5m

12 Place step 10 reaction mix on a magnet stand to pellet the beads and remove the supernatant once the solution appears clear.

13 Resuspend the beads in 200 µL pre-warmed SureSelect Wash buffer 2, incubate at 65 °C for 00:10:00 Place the reaction mix on a magnet stand to pellet the beads and remove the supernatant once the solution appears clear. — 15m

14 Repeat step 13 two more times and total three washes with pre-warmed SureSelect Wash buffer 2. And make sure all the wash buffer has been removed in the final wash. — 30m

15 Resuspend the beads in 30 µL H_2O and keep on ice. — 5m

Enrichment PCR — 2h

16 On ice, assemble the following mix — 30m

	A	B
	Amp Mix	50
	P5 primer (10µM)	2
	P7 primer (10µM)	2
	*cDNA containing beads	30
	H2O	16
	Total	100

Thermocycler Conditions: (Lid@ 105 °C , 100 µl)

98 °C , 00:00:45

then 9 cycles of

98 °C , 00:00:15
60 °C , 00:00:30
72 °C , 00:00:30
then
72 °C , 00:01:00
4 °C , 00:00:00 hold

17 Place the PCR reaction tube on a magnet stand, wait until it clears and recover 100 µL supernatant to a new PCR tube strip. 5m

18 Add 180 µL SPRI beads (1.8x) to the recovered PCR product, mix well by pipetting, pellet the beads on a magnet stand, after supernatant removal, wash with 300 µL 80% ethanol twice, elute with 20 µL buffer EB (10 mM Tris-HCl, pH 8.5). 15m

19 QC with Quant-it (Thermo Fisher Scientific) and BioAnalyzer DNA HS assay (Agilent). 1h

Protocol references

1. "CG000059_DemonstratedProtocolExome_RevC"
https://assets.ctfassets.net/an68im79xiti/Zm2u8VlFa8qGYW4SGKG6e/4bddcc3cd60201388f7b82d241547086/CG000059_DemonstratedProtocolExome_RevC.pdf

2. "SureSelectXT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library Protocol Version C3, September 2019"
newer version at https://www.agilent.com/cs/library/usermanuals/public/G7530-90000.pdf