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BICCN_DART-FISH

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ABSTRACT

This protocol documents DART-FISH procedures used to generate spatial transcriptomic data from human brain section for BICCN.

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PROTOCOL CITATION

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MATERIALS TEXT

Reagents

A	B	C
material	vendor	catalog number
UltraPure™ DEPC-treated Water 1L	ThermoFisher Scientific	750023

Pierce™ 16% Formaldehyde (w/v), Methanol-free	ThermoFisher Scientific	28908
PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free	ThermoFisher Scientific	AM9624
Ethyl alcohol, Pure	Sigma-Aldrich	E7023
Triton™ X-100 solution	Sigma-Aldrich	93443
pepsin	Sigma-Aldrich	10108057001
SuperScript™ IV Reverse Transcriptase	ThermoFisher Scientific	18090010
Advantage® UltraPure dNTP Combination Kit	ClonTech	639132
RNase inhibitor	Enzymatics	Y9240L
Aminoallyl dUTP, 4 mM in TE buffer *UltraPure Grade*, Anaspec Inc	Anaspec (VWR)	AS-83203
BS(PEG)9	ThermoFisher Scientific	21582
UltraPure™ DNase/RNase-Free Distilled Water	TheroFischer Scientific	10977023
Ribonuclease; RNAse H; Conc. 5,000 U/mL; 5,000 U; incl. 10X buffer	Fisher Scientific	50305945
RNase Cocktail™ Enzyme Mix	Invitrogen	AM2288
Ampligase® Enzyme and Buffer	VWR	76081-598
SSC (20X), RNase-free	ThermoFisher Scientific	AM9763
Formamide (Deionized)	ThermoFisher Scientific	AM9342
BSA, Molecular Biology Grade	NEB	B9000S
Phi29 DNA polymerase (10U/uL)	ThermoFisher Scientific	EP0094
Acryloyl-X, SE in DMSO	ThermoFisher Scientific	A20770
Acrylamide/Bis-acrylamide, BioReagent, for molecular biology, 37:1 (ratio)	Sigma-Aldrich	A6050-100ML
Ammonium persulfate	Sigma-Aldrich	A3678
TEMED	Fisher Scientific	17919
gel slick solution	Lonza	50640
TrueBlack lipofuscin autofluorescence quencher	Biotium	23007
Silicone Isolators JTR20R-2.5 20mm DIA x 2.4 mm Depth 25 x 25mm OD No PSA	Grace Bio-Labs	664304
Coverslips, Glass, 18mm dia.	Ted Pella	260369
Azer Scientific cover glass, No. 1.5, 24 x 60 mm	Neta Scientific	Azer-1152460

Probes

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A	B	C
oligo name	vendor	sequence
5N_dc10-Cy5_N9	IDT	/5AmMC12/CCGATAGTCACGATCTGTGGNNNNNNNN*N
dT20_dc7-488	IDT	CATGGATTCGCGGAGGATCATTTTTTTTTTTTTTTTTT*T
FISSEQ_ppRCA primer	IDT	GATATCGGGAAGCTGA*A*G
DARTFISH_anchor_Cy3	IDT	/5Cy3/CTTCAGCTTCCCGATATCCG
dcProbe7-AF488	IDT	/5Alex488N/TGATCCTCCGCGAATCCATG
dcProbe10-ATTO647N	IDT	/5ATTO647NN/CCACAGATCGTGACTATCGG
dcProbe0-AF488	IDT	/5Alex488N/TGTATCGCGCTCGATTGGCA
dcProbe0-Cy3	IDT	/5Cy3/CGTATCGGTAGTCGCAACGC
dcProbe0-ATTO647N	IDT	/5ATTO647NN/ACGCTACGGAGTACGCCACT
dcProbe1-AF488	IDT	/5Alex488N/TCTTGCGTGCGATACGGAGT
dcProbe1-Cy3	IDT	/5Cy3/AACGGTATTCGGTCGTCATC
dcProbe1-ATTO647N	IDT	/5ATTO647NN/CTGGTTCGGGCGTACCTAAC
dcProbe2-AF488	IDT	/5Alex488N/AGAACTTGCGCGGATACACG
dcProbe2-Cy3	IDT	/5Cy3/CTACTTCGTCGCGTCAGACC
dcProbe2-ATTO647N	IDT	GACGAACGGTCGAGATTTAC/3ATTO647NN/
dcProbe3-AF488	IDT	/5Alex488N/GAATTGTCCGCGCTCTACGA
dcProbe3-Cy3_2	IDT	/5Cy3/TCGTACTTCGACGGCACTCA
dcProbe3-ATTO647N	IDT	/5ATTO647NN/AACTGCGACCGTCGGCTTAC
dcProbe4-AF488	IDT	/5Alex488N/CGGAATACGTCGTTGACTGC
dcProbe4-Cy3	IDT	/5Cy3/TACCATTCGCGTGCGATTCC
dcProbe4-ATTO647N_2	IDT	/5ATTO647NN/ACTCTACCGGCAATCGCGTC
dcProbe5-AF488	IDT	/5Alex488N/GAGTGTCGCGCAACTTAGCG
dcProbe5-Cy3	IDT	/5Cy3/ACGTCTGCGTACCGGCTTAG
dcProbe5-ATTO647N	IDT	/5ATTO647NN/CATGCGATTAACCGCGACTG
dcProbe6-AF488_2	IDT	/5Alex488N/CTTGCGGCGACAGTCGAACA
dcProbe6-Cy3	IDT	/5Cy3/TCGTAACCCGTGCGAAGTGC
dcProbe6-ATTO647N	IDT	/5ATTO647NN/CTCTCGTAGCGTGCGATGAG
dcProbe7-AF488_2	IDT	/5Alex488N/TTAGGTCGCCTACCGACTGC
dcProbe7-Cy3	IDT	/5Cy3/GCCACATCGACTCGGTCTAT
dcProbe7-ATTO647N	IDT	GCTCAGCCGGACGAGTAGAT/3ATTO647NN/

preparation 10m

1 Make sure that the padlock probes have 5' phosphate. In case they do not, run T4 PNK reaction and clean up the product using Zymo ssDNA/RNA clean up columns.

2 Wash, dry and UV the silicone isolators. UV the EasyDip jars. Move away unused stuff from the

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bench and clean and undust the working area. Set the HybEZ oven 37C.

permeabilization

3 Prepare 75ml of 4% formaldehyde in 1x PBS. Requires two vials of 16% formaldehyde.

A	B
component	volume (mL)
DEPC-water	52
16% PFA	20
10X PBS	8

4 Take the sections that are on 25mm*60mm coverslips out of -80C on dry ice, insert them into the slide holder, submerge the slide holder in the staining jar containing 4% PFA in PBS. Fix for 60mins at 4C. 1h

01:00:00 4C

5 Remove the DEPC-PSBT jar and the 4% PFA jar containing the samples from 4C. Insert the sample holder into the cold 1x PBST jar. Incubate for 3 minutes. Then insert the sample holder in the room temperature 1x PBST jar. Incubate for 3 minutes. 6m

00:03:00 in 4C DEPC-PBST with occasional agitation

00:03:00 in RT DEPC-PBST with occasional agitation

dehydration and mounting 20m

6 Prepare jars of 50%, 70% and two 100% ethanol. Dehydrate Section with 20m

00:05:00 50% EtOH at room temperature

00:05:00 70% EtOH at room temperature

00:05:00 100% EtOH at room temperature

00:05:00 100% EtOH at room temperature

7 Take the coverslips out of the sample holder. 5m

00:05:00 Air drying at room temperature.

In the meantime, put 20mm diameter Press-To-Seal silicone isolators on a kipwipe on a flat surface. Carefully put the coverslips on silicone isolators, with the tissue sample in the hole. Gently press on the back of the coverslip to seal completely. You may want to stack a 20mm diameter isolator on top of the already mounted 20mm isolator to increase the volume.

permeabilization 10m

8 Permeabilize sample with **0.25% Triton X-100** in **DEPC-1X PBS** for 10m

00:10:00 at Room temperature

875 µL DEPC-H2O **100 µL 10x PBS** **25 µL 10% Triton-X 100**

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A	B
component	x1 volume (uL)
DEPC-water	875
10X PBS	100
10% Triton X-100	25

9 Wash thrice with DEPC-1X PBS and DEPC-Water

1 mL DEPC-1X PBS quick wash

1 mL DEPC-1X PBS quick wash

1 mL DEPC-water quick wash

pepsin digestion 10m

10 Digest with 0.01% Pepsin in 0.1N HCl. Pre-warm the pepsin to 1m 30s

37 °C for at least 5 minutes before use.

100 µL 0.01% Pepsin in 0.1N HCl for **00:01:30** at **37 °C**

A	B
component	x1 volume (uL)
1% pepsin	1
0.1N HCl	99

11 Wash twice with DEPC-1X PBS

1 mL DEPC-1X PBS quick wash

1 mL DEPC-1X PBS quick wash

reverse transcription 15m

12 Prepare Reverse Transcription Mix on Ice. 15m

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A	B
component	x1 volume (uL)
DEPC-water	90.75
5X SSIV buffer	30
10mM dNTP	3.75
100uM N5_dc10-Cy5_N9	3.75
100uM dT20_dc7-488	3.75
0.1M DTT	7.5
4mM aminoallyl-dUTP	1.5
RNase inhibitor (40U/uL)	1.5
SuperScript IV Reverse Transcriptase	7.5
total	150

Incubate human brain sections with the Reverse Transcription Mix

150 µL Reverse Transcription Mix for **00:10:00** at **4 °C** then **Overnight** at **37 °C**

13 Wash with 1X PBS twice.

1 mL 1X PBS quick wash

1 mL 1X PBS quick wash

cDNA crosslinking 1h 30m

14 Add the crosslinking mix to crosslink cDNAs with BS(PEG)9. Prepare 5mM BS(PEG)9 in PBS. 1h 30m

A	B
component	x1 volume (uL)
250mM BS(PEG)9	10
10X PBS	50
ultrapure water	440

Crosslink cDNA with BS(PEG)9

500 µL 5 mM BS(PEG)9 in PBS for **01:00:00** at **Room temperature**

Wash with PBS twice

1 mL 1X PBS quick wash

1 mL 1X PBS quick wash

Quench unreacted crosslinker with 1M Tris, pH 8.0

1 mL 1M Tris pH8.0 for **00:30:00** at **Room temperature**

Wash with PBS twice

1 mL 1X PBS quick wash

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1 mL 1X PBS quick wash

RNase digestion 1h

15 Prepare RNase Digestion Mix 1h

A	B
component	x1 volume (uL)
ultrapure water	168
10X RNase H buffer	20
RNase H (5U/uL)	10
RNase Cocktail	2

Add **200 µL RNase Digestion Mix**

Incubate at **37 °C** for **01:00:00** . Cover the silicone wells.

16 Wash samples with 1X PBS twice.

1 mL 1X PBS quick wash

1 mL 1X PBS quick wash

padlock probe hybridization 33m

17 Prepare the 484-gene-Hybridization Mix according to the table below. Preheat the probe-water mix to **85 °C** for **00:03:00** and immediately move them to a cold block or on ice. Then complete the Hybridization Mix. 3m

484-gene-Hybridization Mix

A	B
component	x1 volume (uL)
ultrapure water	98.2
10X Ampligase buffer	15
HB_Feb2022 probes (27.2 ng/uL)	25.3
100nM PLP1 oligos	1.5
Ampligase (5U/uL)	10

add **150 µL 484-gene-Hybridization mix** to human section

18 Incubate samples in the Hybridization Mix at **37 °C** for **00:30:00** , then at **55 °C** 30m

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Overnight .

For the overnight incubation, first set the Ez hyb oven to **60 °C** and then change it to **55 °C** as you put the samples in. Cover the sample well.

19 Wash samples with 1x PBS twice.

1 mL 1X PBS quick wash

1 mL 1X PBS quick wash

RCA 6h

20 Prepare RCA Primer Mix 1h

A	B
component	x1 volume (uL)
ultrapure water	119
20X SSC	20
formamide	60
100 uM FISSEQ_ppRCA primer	1

add **200 µL RCA Primer Mix** to each sample and incubate at **37 °C** for **01:00:00**

21 Wash samples with 2xSSC.

1 mL 2X SSC quick wash

1 mL 2X SSC quick wash

22 Prepare RCA Enzyme Mix on ice. 5h

A	B
component	x1 volume (uL)
ultrapure water	119.25
10X Phi29 polymerase buffer	15
10mM dNTP	3.75
4mM aminoallyl-dUTP	1.5
NEB BSA (20mg/mL)	7.5
ThermoFisher Phi29 polymerase	3
total	150

Add **150 µL RCA Enzyme Mix** to each sample

Incubate samples in RCA Enzyme Mix at **30 °C** for **05:00:00**

23 Wash samples with 1x PBS twice.

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1 mL 1X PBS quick wash

1 mL 1X PBS quick wash

rolony crosslinking 1h 30m

24 Crosslink with Acryloyl-X Mix and embed the sample in gel. 1h 42m

Prepare Acryloyl-X Mix

A	B
Component	x1 volume (uL)
10X PBS	50
10mg/mL Acryloyl-X, SE in DMSO	10
ultrapure water	440

add **500 µL Acryloyl-X Mix** to the sample and incubate for **00:30:00** at **Room temperature**

quick wash with 1xPBS.

1 mL 1X PBS quick wash

Incubate the sample with **300 µL Acrylamide Solution** for **00:30:00** at **Room temperature** .

Prepare Acrylamide Solution.

A	B
Component	x1 volume (uL)
10X PBS	50
40% Acrylamide/Bis (37:1)	50
ultrapure water	400

Aspirate the Acrylamide Solution. Do not wash the samples!!!

Prepare Polymerization Mix.

Prepare 5% TEMED: **5 µL TEMED** in **95 µL ultrapure water**

Prepare 4% APS: **10 mg Ammonium Persulfate** in **250 µL ultrapure water**

RNaseZap and UV 18mm coverslips and treat them with Gel-Slick.

A	B
component	x1 volume (uL)
Acrylamide solution	138
4% APS	6
5% TEMED	6

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Add **30 µL Polymerization Mix** to the sample and cover with Gel-Slick-treated coverslip for **00:30:00** at **Room temperature** in an Argon tank.

wash with 1x PBST for 3min twice

1 mL 1X PBST for **00:03:00**

1 mL 1X PBST for **00:03:00**

Carefully remove the Gel-Slick-treated coverslip on the samples

wash with 1xPBST for 3min twice

1 mL 1X PBST for **00:03:00**

1 mL 1X PBST for **00:03:00**

imaging 11m

25 8m

stain the sample with Probe Hybridization Mix with decoding probes (Take dcProbe0_AF488, dcProbe0_Cy3, dcProbe0_ATTO647N probes as an example).

Prepare Probe Hybridization Mix

A	B
component	x1 volume (uL)
100 uM dcProbe0_AF488	1
100 uM dcProbe0_Cy3	1
100 uM dcProbe0_ATTO647	1
100% formamide	60
20X SSC	20
ultrapure water	117

add **200 µL Probe Hybridization Mix** to each sample. Incubate for **00:08:00** at **Room temperature**

26 6m

Wash with10% formamide in 2X SSC twice. Then, image.

1 mL 10% formamide in 2X SSC for **00:03:00**

1 mL 10% formamide in 2X SSC for **00:03:00**

27 5m

Strip with **1 mL 80% formamide in 2X SSC** for **00:05:00** .

Wash with **1 mL 2X SSC buffer** once.

Repeat the decoding imaging with the next decoding probes (dcProbe1, dcProbe2, dcProbe3,

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dcProbe4, dcProbe5, dcProbe6, and dcProbe7).

28 After images of samples stained with dcProbe0, 1, 2, 3, 4, 5, 6, 7 were taken, take the nuclei staining images with Draq5 staining. 9m

add **500 µL Draq5 solution** to the sample. Incubate for **00:05:00** at **Room temperature**

wash the sample with 1x PBS twice. Then, image.

1 mL 1X PBS for **00:02:00**

1 mL 1X PBS for **00:02:00**

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