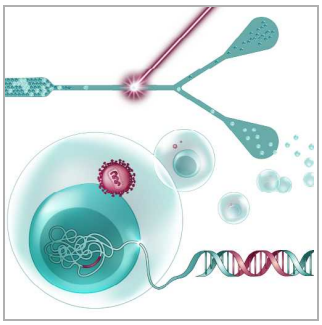


NOV 11, 2022

SHARE

WORKS FOR ME 3

FIND-seq protocol v1.0

DOI
dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

Iain C. Clark[1,2,3], Michael A. Wheeler[1,4], Hong-Gyun Lee[1], Liliana M. Sanmarco[1], Zhaorong Li[1,4], Shravan Thaploo[1], Carolina M. Polonio[1], Seung Won Shin[3], Giulia Scalisi[1], Joseph M. Rone[1], Federico Giovannoni[1], Stephanie E. J. Zandee[5], Alexandre Prat[5], Daniel C. Douek[6], Eli A. Boritz[6], Francisco J. Quintana[1,4], Adam R. Abate[2]

[1]Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA;
[2]Department of Bioengineering and Therapeutic Sciences, School of Pharmacy, University of California San Francisco, San Francisco, CA 94158, USA;
[3]Department of Bioengineering, College of Engineering, California Institute for Quantitative Biosciences, QB3, University of California Berkeley, Berkeley, CA 94720, USA;
[4]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA;
[5]Neuroimmunology Research Lab, Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC H2X 0A9, Canada;
[6]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Clark Lab

Seung Won Shin

COMMENTS 0

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

This protocol is a detailed description of FIND-seq, a single cell method for sorting cells based on RNA or DNA biomarkers. The protocol contains step-by-step instructions, key checkpoints, and troubleshooting guidelines.

DOI

dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

PROTOCOL CITATION

Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate 2022. FIND-seq protocol v1.0. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

FUNDERS ACKNOWLEDGEMENT

National Institutes of Health
Grant ID: 1U01AI129206

National Institutes of Health
Grant ID: UM1AI126611

National Institutes of Health
Grant ID: NS087867

National Institutes of Health
Grant ID: ES025530

National Institutes of Health
Grant ID: ES032323

National Institutes of Health
Grant ID: AI126880

National Institutes of Health
Grant ID: AI149699

Chan Zuckerberg Biohub
Grant ID:

NMSS and Progressive MS Alliance
Grant ID:

National Institutes of Health
Grant ID: K22AI152644

National Institutes of Health
Grant ID: 1K99NS114111

National Institutes of Health
Grant ID: F32NS101790

Dana-Farber Cancer Institute
Grant ID: T32CA207201

Basic Science Research Program, National Research Foundation of Korea
Grant ID: 2021R1A6A3A14039088

Fellowship, FAPESP BEPE
Grant ID: #2019/13731-0

KEYWORDS

Single cell sequencing, RNA sequencing, droplet microfluidics, droplet cytometry, nucleic acid cytometry

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 08, 2022

LAST MODIFIED

Nov 11, 2022

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

PROTOCOL INTEGER ID

68379

GUIDELINES

Equipment setup for microfluidic device operation

Detailed product information of the equipment used is given in the Materials tab.

1. **Bright-field inverted microscope** to visualize microfluidic device, droplet generation, re-injection, droplet sorting etc.
2. **Four syringe pumps** or equivalent pumps and **Air-line** with pressure gauge
3. **Microtubing (PE2/PE5)** to connect syringes to the device
4. **Needles** (23 gauge and 27.5 gauge), **Dark green blunt tip needles** (14 gauge, 2" tip, luer lock connection)
5. **Sterile 1 mL syringes**
6. **Microfluidic devices**; CAD files of the devices are provided on the Materials tab or on the Clark lab website (https://clarklab.berkeley.edu/protocols/) used for all experiments in the paper.

MATERIALS TEXT

Microfluidic devices CAD files

1. Device for Bubble Trigger Bubble_trigger_final.dwg
2. Device for Reinjection Coflow_reinjection.dwg
3. Device for Sorting Sorter_final.dwg

Chemistry

1. Perfluorooctanol **Sigma Aldrich Catalog #370533** (*Caution: Wear appropriate laboratory clothing and equipment and avoid contact with skin when handling this reagent. Use it only in a fume hood.)*
2. HFE-7500 3M Novec Engineered fluid **Fluorochem Catalog #051243** (*Caution: Avoid direct contact with this liquid, as it may cause respiratory, skin and eye irritation. Wear appropriate laboratory clothing and equipment when handling it.)*
3. Aquapel (*Caution: This material is toxic and moisture sensitive. Work in a fume hood and wear appropriate protective clothing and equipment when handling it.)*
4. Automated Droplet Generation Oil for EvaGreen **Bio-rad Laboratories Catalog #1864112**
5. Automated Droplet Generation Oil for Probes **BioRad Sciences Catalog #1864110**
6. DTT **Sigma Aldrich Catalog #43816-10ML**
7. EDTA **VWR international Ltd Catalog #E177**
8. Fluorinert FC-40 Oil **Sigma Aldrich Catalog # F9755** (*Caution: Avoid direct contact with this liquid, as it may cause respiratory, skin and eye irritation.Wear appropriate laboratory clothing and equipment.*)
9. n-Hexane **Alfa Aesar Catalog #43263-K2** (*Caution: This liquid is highly flammable and toxic. Use a fume hood and wear appropriate protective clothing and equipment when handling it.*)
10. SYLGARD™ 184 Silicone Elastomer Kit **Dow Corning Catalog #04019862**
11. Span 80 **Sigma Aldrich Catalog #S6760**
12. Ultra-low melt Agarose IX-A **Sigma Aldrich Catalog #A2576**

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

Molecular Biology

1. Ammonium Persulfate **Promega Catalog #V3131**
2. TaqPath™ qPCR Master Mix, CG **Thermo Fisher Catalog #A15297**
3. AmpureXP beads **Beckman Coulter Catalog #A63880**
4. Betaine BioUltra ≥99.0% (NT) **Sigma Aldrich Catalog #61962**
5. Bioanalyzer chips and reagents (DNA High Sensitivity and small RNA kit) **Agilent Technologies Catalog #5067-1548**
6. Dead Cell Removal Kit **Miltenyi Biotec Catalog #130-090-101**
7. dNTP Mix (25 mM each) **Thermo Fisher Catalog #R1121**
8. HBSS (1x) **Gibco - Thermo Fisher Catalog #14175-095**
9. KAPA HiFi Hotstart PCR kit **Roche Catalog #KK2502**
10. Lithium Chloride **Sigma Aldrich Catalog #L7026**
11. Magnesium Chloride **Sigma Aldrich Catalog #M1028**
12. Maxima™ H Minus Reverse Transcriptase **Thermo Fisher Scientific Catalog #EP0753**
13. Maxima H- RT Buffer **Thermo Fisher Scientific Catalog #EP0753**
14. NxGen® RNAse Inhibitor **Lucigen Catalog #30281-2**
15. OptiPrep™ Density Gradient Medium **Sigma Aldrich Catalog #D1556)**
16. PEG-8000 **Promega Catalog #V3011**
17. PEG-6000 **Alfa Aesar Catalog #A17541**
18. Pluronic F-68 **Gibco - Thermo Fischer Catalog #24040-032**
19. Pluronic F-127 **Anaspec Catalog #AS-84040**
20. Potassium Chloride **Santa Cruz Biotechnology Catalog #sc-301585**
21. Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #P8107S**
22. Qubit dsDNA HS Assay kit **Thermo Fisher Scientific Catalog #Q32854**
23. Qubit™ ssDNA Assay Kit **Thermo Fisher Scientific Catalog #Q10212**
24. RNAzol RT **Sigma Aldrich Catalog #R4533**
25. 5M NaCl solution **Thermo Fisher Scientific Catalog #AM9759**
26. SYBR™ Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO **Thermo Fisher Catalog #S7563**
27. TEMED **Invitrogen - Thermo Fisher Catalog #15524-010**
28. Tris-HCl pH 7.5 **Teknova Catalog #T5075**
29. Tris-HCl pH 8.3 **Teknova Catalog #T1083**
30. Tween-20 **Sigma Aldrich Catalog #P9416**

Oligonucleotide

1. Template switch oligonucleotide (TSO): AAGCAGTGGTATCAACGCAGAGTGAATrGrGrG
2. Acrydited oligo dT primer:
   /5Acryd/TTTTTTAAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN
3. Smart PCR primer: AAGCAGTGGTATCAACGCAGAGT

EQUIPMENT

1. Bright-field inverted microscope (e.g., Nikon, Olympus, Zeiss, Leica)

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

2. Spin coater (Laurell, model WS-650MZ-23NPP)
3. Oxygen plasma cleaner (GaLa Instrumente, Plasma Prep 2)
4. UV light source (OAI, model LS30/5)
5. Ceramic hot plates (VWR, cat. no. 97042)
6. Four syringe pumps (Harvard Apparatus, PHD 2000/2200, cat. no. 702001) or equivalent pumps such as those from KD Scientific, Chemyx, Cetoni, New Era, and so on
7. Centrifugal mixer (Thinky, Planetary Centrifugal 'Thinky' mixer, cat. no. ARE-310)
8. Benchtop centrifuge
9. Vortex mixer
10. Biosafety cabinet
11. Vacuum desiccator
12. Laser safety goggles (Thorlabs, cat. no. LG3 Orange Lens)
13. LabVIEW software (LabVIEW Core and LabVIEW field-programmable gate array (FPGA); National Instruments)
14. AutoCAD software (Autodesk)
15. Silicon wafers (3-inch diameter, Type-P, 1S polished; University Wafer, cat. no. S3P01SP)
16. Indium tin oxide (ITO) glass (50 × 75 × 0.7 mm; Delta Technologies,cat. no. CG-81IN-S207)
17. Cover glass (24 × 60 mm, No. 1.5; Corning, cat. no. 2980-246)
18. Glass slides (75 × 50 mm; Corning, cat. no. 2947-75X50)
19. Low-melting-temperature solder wire (composition 32.5 Bi, 16.5 Sn,diameter 0.020 mm; The Indium Corporation of America,cat. no. wirebn-53307)
20. Cutting mat (6 × 8 inches; Ted Pella, Harris cutting mat, cat. no. 15097)
21. Biopsy punches (0.5 and 0.75 mm diameter; Ted Pella, Harris Uni-Core,cat. no. 15071 and 15072)
22. Millipore filter, 0.22 µm (PVDF and polyethersulfonate type)
23. Needles (23 gauge and 27.5 gauge; Terumo Neolus, cat. no. NN2325R)
24. Dark green blunt tip needles (14 gauge, 2" tip, luer lock connection, McMaster Carr 75165A245)
25. Sterile 1 mL syringes (Braun Omnifix, cat. no. 9204512)
26. PTFE microtubing (0.56 × 1.07 mm; Fisher Scientific, cat. no. W39241)
27. PE-2 tubing (Intramedic)
28. PE-5 tubing (Intramedic)
29. Hemocytometer (Hausser Scientific, cat. no. 1490)
30. Adjustable 10, 200 and 1,000 µl pipettes and sterile pipette tips
31. Pipettes (5, 10, 25 and 50 ml; BD Falcon, cat. no. 357543, 357771, 357550 and 357600)
32. Sterile microcentrifuge tubes (0.5 ml and 1.5 ml)
33. Sterile conical tubes (15 ml and 50 ml; BD Falcon, cat. no. 352196 and 352070)
34. 100-micron cell strainer (VWR Cat. 21008-950)
35. 40-micron cell strainer (VWR Cat. 21008-949)
36. Scalpel (Becton Dickinson, No. 11, cat. no. 371611)
37. Diamond pen (VWR, cat. no. 201-0392)
38. Petri dishes (100 mm diameter × 15 mm; BD Falcon, cat. no. 351029)
39. Powder-free gloves
40. Wafer-handling tweezers
41. Sharp tweezers
42. Crystallizing dishes (Corning, cat. no. 3140-100)
43. Disposable mixing cups
44. Frosted Scotch tape
45. Aluminum foil
46. Qubit fluorometer (Thermo Fisher Scientific, model. no. Q32857)
47. Razor blades (0.009 inch; Thermo Fisher Scientific, cat. no. 940115)
48. Freezer (−20 °C; Panasonic, model. no. BZ10145190)
49. Freezer (−80 °C; Thermo Fisher Scientific, model. no. 989)

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

50. Countess II Automated Cell Counter (ThermoFisher Scientific Cat. AMQAX1000)
51. BD 3 mL syringe adapter for centrifuge

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

REAGENT SETUP

1 **20% (vol/vol) 1H-1H-2H-2H-Perfluoro-1-Octanol solution** (Use this reagent immediately after preparation.)

A	B
Reagent	Vol.
PFO	10 mL
HFE-7500	40 mL
Total	50 mL

0.1% SPAN-80 in Hexane (Use this reagent immediately after preparation.)

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Span-80 (in hexane)	20% (w/v)	0.1 % (w/v)	0.25 mL
n-hexane	-	-	50 mL
Total			50.25 mL

Cell Resuspension Buffer (Solution is stable for several days at 4 °C .)

A	B	C	D

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
HBSS buffer	1X	-	7.2 mL
Pluronic F-68	10% (v/v)	1% (v/v)	1 mL
Opti-prep	-	18% (v/v)	1.8 mL
Total			10 mL

Lysis Buffer (This solution is stable for several months at -20 °C and should be stored in small aliquots. When needed for experiment, add Proteinase K (2 µg/µL) to an aliquot and use immediately after preparation.)

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Tris-HCl pH 7.5	1000 mM	20 mM	0.2 mL
LiCl	8000 mM	1000 mM	1.25 mL
LiDS	10 % (v/v)	1 % (v/v)	1 mL
EDTA	500 mM	10 mM	0.2 mL
DTT	1000 mM	10 mM	0.1 mL
Proteinase K	20 µg/µL	2 µg/µL	1 mL
Nuclease free water	-	-	6.25 mL
Total			10 mL

Wash 1 Buffer (This solution is stable for several months at 4 °C.)

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Tris-HCl pH 7.5	1000 mM	20 mM	10 mL
LiCl	8000 mM	500 mM	31.25 mL
LiDS	10% (v/v)	0.1% (v/v)	5 mL
EDTA	500 mM	0.1 mM	0.1 mL
Nuclease free water	-	-	453.65 mL
Total			500 mL

Wash 2 Buffer (This solution is stable for several months at 4 °C.)

A	B	C	D
Reagent	Reagent conc	Final conc.	Vol.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B	C	D
Tris-HCl pH 7.5	1000 mM	20 mM	10 mL
NaCl	5000 mM	500 mM	50 mL
Nuclease free water	-	-	440 mL
Total			500 mL

5X RT Buffer (This solution is stable for several months at 4 °C.)

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Tris-HCl pH 8.3	1000 mM	250 mM	50 mL
KCl	1000 mM	375 mM	75 mL
MgCl2	1000 mM	15 mM	3 mL
DTT	1000 mM	50 mM	10 mL
Nuclease free water	-	-	62 mL
Total			200 mL

Tween Wash Buffer (This solution is stable for several months at room temperature.)

A	B	C
Reagent	Final conc.	Vol.
Tween-20	0.1% (v/v)	0.5 mL
Nuclease free water	-	500 mL
Total		500.5 mL

Conjugation Buffer (Use this reagent immediately after preparation.)

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Tris-HCl pH 8.3	1000 mM	375 mM	7.5 mL
Nuclease free water	-	-	12.5 mL

10% (wt/vol) APS (Use this reagent immediately after preparation.)

A	B	C
Reagent	Final conc.	Amount

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B	C
APS	10% (w/v)	0.1 g
Conjugation buffer	-	upto 1 mL
Total		1 mL

10% (vol/vol) TEMED (Use this reagent immediately after preparation.)

A	B	C
Reagent	Final conc.	Vol.
TEMED	10% (v/v)	100 µL
Conjugation buffer	-	900 µL
Total		1000 µL

(*Caution: TEMED is toxic. Addition of TEMED should be done under a chemical fume hood. Wear appropriate protective clothing and equipment when handling it.*)

AGAROSE CONJUGATION

2 Prepare reagents for agarose conjugation. Resuspend Acrydite-T5-Smart-dT Primer in Conjugation Buffer to a concentration of [M] 1000 micromolar (µM) ([M] 1 millimolar (mM)). Resuspend SFR Allyl Agarose in Conjugation Buffer in a 15 mL falcon to a final concentration of [M] 0.5 Mass / % volume . Prepare [M] 10 Mass / % volume APS and [M] 10 % (v/v) TEMED solutions.

2h

3 Heat SFR Agarose suspended in buffer to 95 °C for 02:00:00 , or until completely molten. Vortex over time to ensure homogenization. While vortexing, it is good to flip the tube and vortex on head/cap as well. This ensures agarose near the top of the falcon does not cool and harden.

4 Once homogenized, cool agarose to 45 °C . Temporarily placing heat block in ice is used to accomplish this faster.

30m

5 Place agarose under vacuum for 00:30:00 . Ensure the agarose does not boil. If boiling is seen, agarose is not cool enough.

6 Remove vacuum. Add reagents in the following order:
a. Add resuspended primer so final concentration of primer is [M] 50 micromolar (µM) .
b. Add [M] 10 Mass / % volume APS solution so the final concentration is [M] 0.1 Mass / % volume . Vortex tube thoroughly.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

c. Add [M] 10 % (v/v) TEMED solution so the final concentration is [M] 0.1 % (v/v). Volume of APS and TEMED added should be the same. Vortex tube thoroughly.

4h

7 Place tube back under vacuum for 04:00:00. **PAUSE POINT**

8 Remove tube from vacuum. Add the same volume of APS and TEMED as added in step 7. Final concentrations should now be [M] 0.2 Mass / % volume APS and [M] 0.2 % (v/v) TEMED. Vortex agarose and place under vacuum overnight at 45 °C. **PAUSE POINT**

9 Remove agarose from vacuum. Heat to 95 °C and vortex until completely molten and homogenized. Try to break polymer strands floating around by heating and vortexing as much as you can. Some strands may remain. While vortexing, it is good to flip the tube and vortex on the head/cap as well. This ensures agarose near the top of the tube does not cool and harden.

10 While molten, pour agarose in a 10 mL syringe with a 0.45 µm syringe filter attached. Filter agarose into another 15 mL falcon tube to remove unwanted polymer strands formed during the reaction.

11 Add ultra-low gelling temperature agarose to reach a final concentration of [M] 2 Mass / % volume agarose (SFR and Ultra-low gelling together).

12 After addition, heat tube to 95 °C and vortex to ensure homogenization, flipping tube on head occasionally.

1h

13 Once completely molten and homogenized, briefly centrifuge agarose to get all the agarose at the bottom of tube. Cool agarose to 4 °C by placing in ice bucket for at least 01:00:00. Allow agarose to harden.

14 Using a hypodermic needle, carefully pierce the bottom of the falcon tube. This should dislodge the hardened agarose from the bottom of the tube. Wash by transferring hard agarose gel to a 500 mL bottle of nuclease free distilled water and allow to sit overnight. **PAUSE POINT**

15 Repeat wash by re-transferring after the next night. Transfer agarose from wash bottle to a clean, dry 15 mL falcon tube. Ensure no water is transferred.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

Agarose Normalization

16 Quantify the oligonucleotide concentration

16.1 Melt agarose at 95 °C. Vortex and homogenize. Once agarose is molten, cool down to 70 °C or until viscosity is amenable to pipetting. Use ice to cool agarose tube down.

16.2 Dilute agarose in nuclease free water (1/80 dilution). Vortex to ensure agarose added is dissolved and evenly distribute. Triplicates are ready for measurement.

16.3 In triplicate, measure conjugated oligo-dT via the QuBit ssDNA Assay kit.

16.4 Calculate the average µM concentration of conjugate oligonucleotide. Use the reported molecular weight on the IDT datasheet for the oligo-dT primer. Remember to consider the 80-fold dilution.

1. e.g. If I get 5.52 ng/µL Qubit reading (1/80 dilution) for a 30 base ssDNA primer:
2. Molecular concentration (µM) = Qubit reading (ng/µL) * Dilution factor (80) * 1000 / Molecular weight of total primer (g/mol)
3. Molecular concentration (µM) = 5.52 ng/ul * 80 * 1000 / 9378.2 g/mol = 39 µM

17 Normalize the agarose concentration

17.1 Normalize the conjugated oligonucleotide concentration by addition of [M] 2 Mass / % volume Ultra-low melt Agarose. Prepare [M] 2 Mass / % volume Ultra-low melt Agarose by adding 0.08 g of agarose to 4 mL of distilled nuclease free water. Vortex and heat to 95 °C. Keep vortexing until completely dissolved.

17.2 Dilute conjugated agarose in [M] 2 Mass / % volume Ultra-low melt Agarose to reach a final concentration of [M] 8 micromolar (µM) conjugated oligonucleotide.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

17.3 Measure final conjugated oligonucleotide concentration with QuBit ssDNA kit using the same method as above. The final concentration should be [M] 8 micromolar (µM) .

1d

DAY 1: CELL ENCAPSULATION AND LYSIS

1h

18 Heat 3 mL of conjugated agarose-Oligo dT to 95 °C for at least 01:00:00 . Vortex repeatedly to ensure homogenization and no solid clumps. **CRITICAL STEP**: Agarose must remain molten while running the device. Allowing agarose to cool down will clog the tubing/microfluidic channels.

19 **Prepare cells for encapsulation. It is recommended to start with at least 25-30 million cells.**

3m

19.1 **Preparing cells from cell lines in culture**

Wash cells 1x in HBSS 400 x g, 00:03:00 . Resuspend in 15 mL HBSS for each wash. Filter through a 70-micron strainer.

1h 10m

19.2 **Preparing PMBCs**

a. Place 25 mL of RPMI with [M] 10 % volume FBS in 37 °C for 01:00:00 .

b. Remove PBMC vial from freezer on dry ice. (*CAUTION: Wear protective PPE, including a face shield, while defrosting PMBC vials.*)

c. Submerge in 37 °C water bath until only a small amount of ice is visible

d. Pipette cells into media that was pre-warmed in 37 °C

e. Centrifuge cells for 300 x g, 00:10:00 .

30m

20 Count cells using Trypan blue stain. For cell lines, you can use an automated cell counter (Countess II Automated Cell counter). For PBMCs, we recommend a manual hemocytometer.

5m

21 Based on the cell concentration, spin cells and resuspend in Cell Resuspension Buffer to $6.11x10^6$ cells/mL. We recommend resuspending in smaller volume of Opti-Prep than necessary to obtain a higher concentration, and then diluting down to the required concentration.

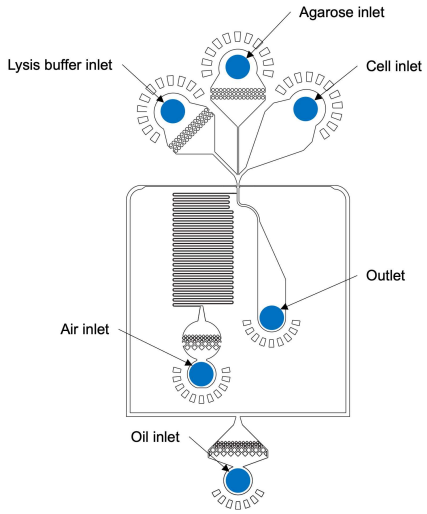
***Troubleshooting**

22 **Setup the drop making station**

Keep the agarose molten and place the bubble triggered co-flow microfluidic device on the stage. Connect the syringe tubing to the microfluidic device.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**



23 Encapsulate cells in molten agarose using the bubble triggered co-flow microfluidic device at the following flow rates.

A	B	C
Syringe Size	Reagent	Final Flow Rate
10 mL	Droplet Generation Oil for Probes (BioRad).	5000 µL/hr
3 mL	Cells filtered and resuspended in Cell Resuspension Buff	600 µL/hr
3 mL	Lysis Buffer with Proteinase K	600 µL/hr
3 mL	Oligo-dT conjugated Agarose	1200 µL/hr
-	Pressured air	20 psi

24 Collect drops in 15 mL tubes in the heat block at 55 °C.

25 Incubate for 02:00:00 at 55 °C. Cool on ice or at 4 °C for at least 01:00:00 or overnight. 3h
PAUSE POINT

DAY 2: BREAKING AND REVERSE TRANSCRIPTION 1d

26 **Remove oil and wash with hexane** (*Caution: Hexane and PFO are highly flammable substances.*)
Hardened agarose is henceforth referred to as beads. Remove oil at bottom of tube. Discard oil in appropriate waste bottle.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

27 Add 2x-5x volume [M] 20 % volume HFE/PFO solution to drop emulsion. Mix slowly by inverting tube by hand to adequately break emulsion.

28 Centrifuge at 2000 x g, 00:03:00. Oil will be at the bottom. Remove oil. Discard oil in waste bottle. 3m

29 Add > 25 mL of Hexane/SPAN-80 solution to the beads. Shake tubes gently until beads are not clumpy.
***Troubleshooting**

30 Centrifuge at 2000 x g, 00:03:00. Remove hexane with a pipette and discard in a hexane waste container. 3m
(*CAUTION: Hexane damages plastics and should not be aspirated. Hexane must be disposed of in a glass waste bottle.*)
CRITICAL STEP: You must proceed through the reverse transcription reaction. Beads must be kept on ice at 4 °C throughout washes. Warming the beads will allow mRNA to dissociate from oligo-dT resulting in loss of mRNA and single cell resolution.

31 **Water Washes** 5m
Add up to 50 mL of 4 °C Wash 1 Buffer. Resuspend by rotating by hand, flicking the bottom of the tube if necessary. Mix thoroughly and allow to sit on ice for 00:05:00.
CRITICAL STEP: Residual hexane will be on top after first wash. Be careful to aspirate it and not let it mix in with the rest of the solution.

32 Centrifuge for 4500 x g, 00:05:00. Aspirate buffer, not beads. The largest source of bead loss is getting too close to the water-agarose interface. 5m

33 Add up to 50 mL Wash 2 Buffer. Resuspend by rotating by hand, flicking the bottom of the tube if necessary. Mix thoroughly and allow to sit on ice for 00:05:00. 5m

34 Pre-weigh a new 50 ml tube. Filter beads through 100 micron strainer into pre-weighted tube.
***Troubleshooting**
CRITICAL STEP: Pre-weighing the tube now is necessary for correctly setting up the reverse transcriptase reaction later in the protocol. Pre-weighed tube is used to calculate weight, and subsequently volume, of agarose beads left after washes.

35 Centrifuge for 4500 x g, 00:05:00. Aspirate buffer, not beads. 5m

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

36 Add up to 50 mL Wash 2 Buffer. Resuspend by rotating by hand, flicking the bottom of the tube if necessary. Mix thoroughly and allow to sit on ice for 00:05:00. 5m

37 Centrifuge for 4500 x g, 00:05:00. Aspirate buffer, not beads 5m

38 Add up to 50 mL 5X RT Buffer. Resuspend by rotating by hand, flicking the bottom of the tube if necessary. Mix thoroughly and allow to sit on ice for 00:05:00. 5m

39 Repeat wash (steps 36 to 38) for a total of 2 RT Buffer washes.

40 Reweigh tube after final aspiration to obtain weight of agarose beads.

41 **Reverse Transcription Reaction**

Beads should be in 5X RT Buffer on ice. Adding enzyme last, prepare reverse transcription reagents in a separate 50 mL falcon tube on ice as follows. Based on volume of washed beads, prepare reverse transcription mix.

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
dNTP	25 mM	1 mM	1.2 mL
TSO	100 µM	2 µM	0.6 mL
MgCl2	1000 mM	6 mM	0.18 mL
Betaine	5 M	1 M	6 mL
PEG-8000	30% (w/v)	7.5% (w/v)	7.5 mL
Maxima H minus Rev	200 U/µL	2 U/µL	0.3 mL
NxGen Rnase inhibito	20 U/µL	0.5 U/µL	0.75 mL
Nuclease free water	-	-	13.47 mL

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B	C	D
Total			30 mL

2h

42 Mix RT reaction using rotator for 00:30:00 at room temperature and then 01:30:00 at 42 °C on a rotator.

10m

43 Take out of incubator, add 200 µL EDTA per 10 mL of reaction. Cool on ice for 00:10:00.
PAUSE POINT

43.1 Alternatively, you can immediately begin bead washes with Tween Wash Buffer (next step).

44 Wash beads 5x in Tween Wash Buffer. On the last wash, filter with 100 micron strainer into a pre-weighed 50 mL Falcon tube.

45 After the final wash with Tween Wash Buffer, weigh to determine the final mass of beads.

46 Spin down beads for bead counting and take a 50 µL aliquot. **PAUSE POINT**

47 **Bead counting**
Add 50 µL of beads to 150 µL of distilled nuclease free water to create a diluted bead stock.

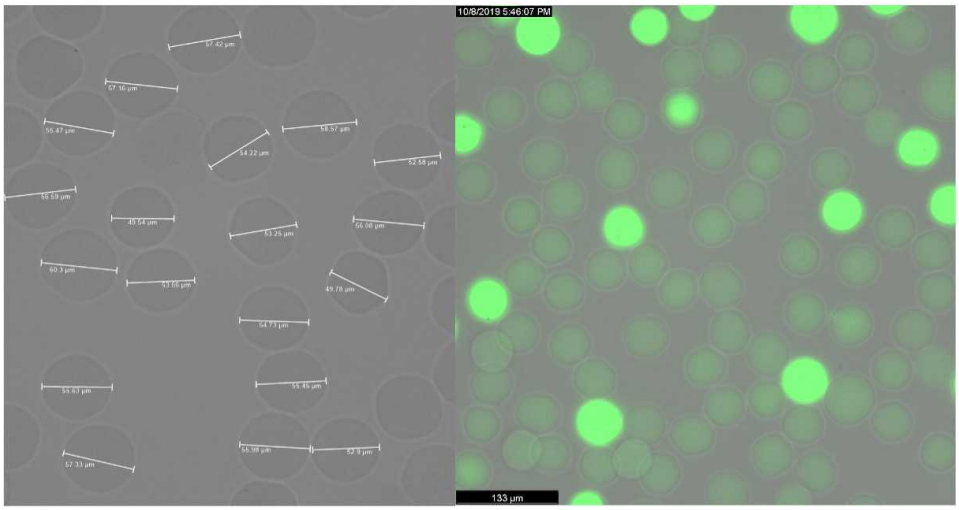
30m

48 In a separate tube, mix a small aliquot of beads from the diluted bead stock with SYBR green (10x final concentration of dye). Let sit for 00:30:00 in the dark.

49 Using hemocytometer, image beads fluorescence microscope. Take pictures of bead size, bead counts, and pictures at 20x magnification of bead lysis success.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**



Right: Bright field image of agarose beads and sizes. Left: Fluorescence images of stained genomes.

50 Quantify the number of beads/µL and genomes/µL. The ratio of genomes/bead should be ~1/10.

51 **Whole Transcriptome Amplification**

Set up a 25 µL PCR reaction as follows, thermocycling using three different cycle numbers (14, 16, 18).

PAUSE POINT

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol.
Kapa HiFi Master Mix	2 X	1 X	12.5 µL
SMART PCR Primer	10 µM	0.4 µM	1 µL
Beads		30 genomes/µL	X µL
Nuclease free water	-	-	11.5 -X µL
Total			25 µL

A	B	C	D	E
Thermocycling				
Step	Stage	Temperature	Time	Cycles
1	Initial denatura	95˚C	3 minutes	1x
	Denaturation	98˚C	15 seconds	

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

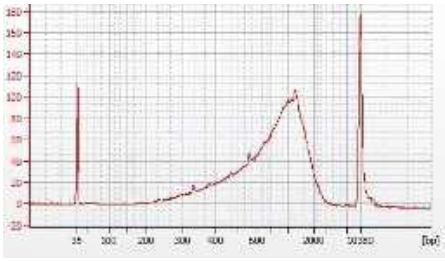
This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B	C	D	E
2				14,16,18x
	Annealing	67˚C	20 seconds	
	Elongation	68˚C	4 minutes	
3	Final elongatio	72˚C	5 minutes	1x
4	Hold	4˚C	∞	Hold

52 Use Ampure XP 2x beads for DNA clean-up. It is recommended to complete final elution in 20 µL - 30 µL of distilled nuclease free water. **CRITICAL STEP**: Over-drying of Ampure beads before elution step will result in loss of material.

53 Measure DNA concentration using Qubit dsDNA kit. Based on Qubit results, take aliquots of sample and resuspend them to 1 ng/µL final concentration. Run these aliquots on a Bioanalyzer chip to confirm the size of WTA product. ***Troubleshooting**

A good WTA product trace from Bioanalyzer looks like so:



1d

DAY 3: BEAD REINJECTION

54 Prepare a 2x PCR master mix.
Mix detection PCR reagents and beads in a 15mL falcon tube so that the final concentrations are:

A	B	C	D
Reagent	Reagent conc.	Final conc.	Vol µL
TaqMan Assay (900 nM primers, 250 nM prob	20 X	1 X	50
TaqPATH 2x Master Mix	2 X	1 X	500
Tween-20	10% (v/v)	2.5% (v/v)	50
PEG-6000	20% (w/v)	2.5% (w/v)	50
Beads			350
Total			1 mL

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

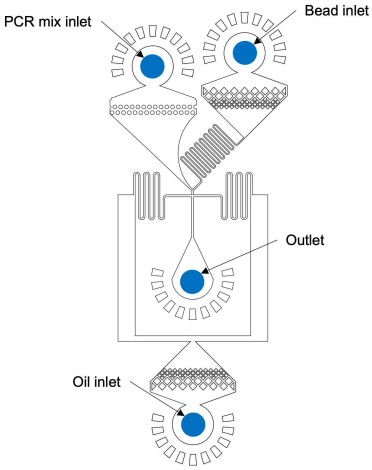
55 Soak for 01:00:00 on shaker in the dark at room temperature. 1h

56 Spin 15ml tubes at 4500 x g, 00:05:00 to separate PCR mix and beads. Separate beads and PCR mix. Add PCR mix (20% of bead volume) to beads, vortex briefly. This prevents beads from aggregating in the device and makes reinjection stable. 5m

57 **Load syringes**

- Load PCR mix into a 3 mL syringe with a HFE oil backing (no fluorosurfactant)
- Load beads into a 3 mL syringe
- Load Droplet generation oil for Evagreen into a 3 mL syringe

58 Place PCR mix, beads, and oil into the syringe pumps and connect the tubing to the reinjection device.



59 Start the reinjection device with the following flow rates, to create ~70 µm diameter droplets:

A	B
Channel	Flow rate
PCR mix	600 µL/hr
Beads	400 µL/hr
Evagreen oil	1800 µL/hr

60 Begin reinjection, collecting 30 µL aliquots into PCR strips. Occasionally during reinjection, verify that bead loading into droplets is close to 100% (or at least above 70%) by capturing videos.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

61 After collection, thermocycle strips as follows with ramp rate set to 1.0 C/s

A	B	C	D
Thermocycling			
Stage	Temperature	Time	Cycles
1	88˚C	10 minutes	1x
2	88˚C	30 seconds	55x
	60˚C	1 minute	
3	4˚C	∞	Hold

4h 40m

DAY 4: SORTING DROPLETS

62 See previously published paper of Mazutis et. al (Nat. Protoc. 2013 May;8(5):870-91.) for details on droplet sorting procedure.

63 **Load syringes**

- Load thermocycled emulsion into a 3 mL syringe
- Load Droplet generation oil for Evagreen into a 3 mL syringes (Oil 1)
- Load HFE-7500 into a 3 mL syringes (Oil 2)
- Load HFE-7500 into a 3 mL syringes (Oil 3)

64 Place emulsion, and oil into the syringe pumps and connect the tubing to the sorting device. Fill the saltwater electrode and moat channels with 2 M NaCl solution. Connect saltwater electrode to voltage amplifier.

65 Flow rates for reinjecting into a detection/sorting device:

A	B
Channel	Flow rate
Emulsion	100 µL/hr
Spacer oil	400 µL/hr
Oil 1	2000 µL/hr

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

A	B
Oil 2	3000 µL/hr
Air	2-5 psi

66 **Determining cycle number for Amplification of sorted material**
Cycling number is determined by performing WTA on a known number of sorted drops and calculating the number of cycles needed to obtain a desirable yield for positive drop sorts.

66.1 Sort 6-9 aliquots of the number of cells (# cells) that you will be collecting in the real experiment.

66.2 Perform WTA and bioanalyzer using 3 different cycle numbers in duplicate (or triplicate)

66.3 Plot the data and select the number of cycles that gives at least 2 ng/µL in 20 µL elution volume, or 40 ng of DNA, determined by qubit or bioanalyzer.

67 **For Single drop sorts**
Collect single drops into PCR strips.

67.1 After collection, overlay each tube with 37 µL of water.

67.2 Spin tubes at 20000 rpm, 00:05:00 . 5m

67.3 Freeze tubes at -80 °C for at least 02:00:00 or overnight. **PAUSE POINT** 2h

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

67.4 Take tubes out of freezer and heat tubes to 60 °C for 00:10:00 . 10m

67.5 Using the table below, prepare a Master mix.

A	B	C
Reagent	Reagent conc.	Vol. (for single tube
KAPA buffer	5 X	10 µL
dNTP	10 mM	1.5 µL
PCR primer	100 µM	0.2 µL
Kapa polymerase		1 µL
Nuclease free wate	-	0.3 µL
Total		13 µL

67.6 Add 13 µL of PCR reagents for 50 µL total reaction volume.

67.7 Flick the tube to mix, spin, and thermocycle.

67.8 Thermocycle according to previously determined cycle number (Step 53).

67.9 Cleanup WTA with 1.2X Ampure XP.
Add 60 µL Ampure XP beads to 50 µL WTA reaction.

68 **For 100 drop sorts**
Collect drops in Eppendorf tubes.

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

68.1 Remove oil from Eppendorf tubes, leaving only a small amount to ensure that no unbroken drops are removed.

68.2 Add a 50 µL aqueous overlay (distilled nuclease-free water).

2h 5m

68.3 Spin tubes at 2000 rpm, 00:05:00. Then freeze tubes at -80 °C for at least 02:00:00 or overnight. **PAUSE POINT**

10m

68.4 Take tubes out of freezer. Heat tubes to 60 °C for 00:10:00. Remove samples from 60 °C and carefully mix only the aqueous layer by pipet.

68.5 Carefully transfer the aqueous layer in tubes to PCR strips. It is better to transfer some oil than to not transfer all aqueous.

68.6 Set up the PCR master mix as follows:

A	B	C
Reagent	Reagent conc.	Vol. (for single tube)
KAPA buffer	5 X	10 µL
dNTP	10 mM	1.5 µL
PCR primer	100 µM	0.2 µL
Kapa polymerase		1 µL
Nuclease free water		4.3 µL
Total		17 µL

68.7 Add 17 µL of PCR master mix to 50 µL of aqueous layer

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**

68.8 Carefully mix by flicking the PCR tubes. Do not form emulsions. After mixing, spin tubes to separate aqueous and oil layers.

68.9 Thermocycle according to previously determined cycle number (Step 53).

68.10 Cleanup WTA with 1.2X Ampure.
Add 80.4 µL Ampure XP beads to 67 µL WTA reaction.

Troubleshooting

69

A	B	C	D
Step	Problem	Possible Reason	Solution
21	Cells are all dead or	Automated Cell counter may r	Mix cell resuspension thoroughly and
29	1) Solution seems t	1) Issue with agarose concent	Restart Experiment
34	1) Beads not passin	1) Beads are larger than norm	Split original tube from which beads
53	Yield of DNA materi	Low/zero yield may be due to	Restart Experiment

Citation: Iain C. Clark, Michael A. Wheeler, Hong-Gyun Lee, Liliana M. Sanmarco, Zhaorong Li, Shravan Thaploo, Carolina M. Polonio, Seung Won Shin, Giulia Scalisi, Joseph M. Rone, Federico Giovannoni, Stephanie E. J. Zandee, Alexandre Prat, Daniel C. Douek, Eli A. Boritz, Francisco J. Quintana, Adam R. Abate FIND-seq protocol v1.0 https://dx.doi.org/10.17504/protocols.io.q26g74o78gwz/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**