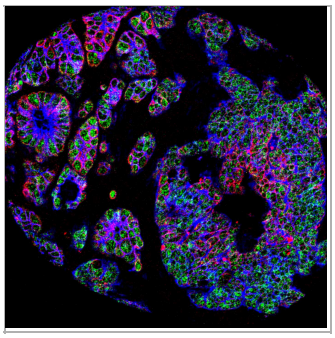

VERSION 2
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MANUSCRIPT CITATION:
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We use this protocol and it's working

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Oligonucleotide-polymer conjugation for imaging mass cytometry V.2

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ABSTRACT

This protocol describes the procedures to label 5'-thiol modified oligonucleotides with maleimide carrying polymers which are also chelated with metal-ions.

This is the basis to perform RNAscope with Imaging Mass Cytometry since therein we use metal readouts instead of fluorescence readouts.

This protocol has been used in the following publications:

https://doi.org/10.1016/j.cels.2018.04.004

and

https://doi.org/10.1126/sciimmunol.abk1692

For an example of an agarose gel of conjugated and unconjugated oligonucleotides see the attached image.

ATTACHMENTS

oligolabelling.png

MATERIALS TEXT

A	B
Oligos	5’ thiol labeled
TCEP (stock 0.5 M)	Bond-Breaker TCEP Solution, ThermoFisher
ddH20 (molecular biology grade)	
Ethanol (70% in ddH2O ice cold)	
MaxPar polymers	Fluidigm
Lanthanide stock solution	Fluidigm
C-Buffer (Max-Par Kit)	Fluidigm
L-Buffer (Max-Par Kit)	Fluidigm
Microcon30 spin columns	MRCF0R030 (Millipore)
Centrifugal Filter Unit: 3 kDa Amicon® Ultra500 µL V bottom	UFC500396 (Millipore)
Ethanol 100 % (-20°C)	
Ethanol 75% (4°C)	
table top centrifuge (12000 rcf)	
Eppendorf cooling centrifuge 5430R (or similar)	
Nanodrop (or other means of DNA concentration determination)	

ATTACHMENTS

oligolabelling.
png

Oligonucleotide resuspension and reduction

15m

1 Resuspend lyophilized oligos in ddH20 (RNAase and DNAse free water) to a final concentration of 250 µM in 1.5 mL eppendorf tubes

5m

2 Determine concentration at NanoDrop.
Note: If there is a big discrepancy between what you measured and what should be there then rather use concentrations from your measurements and continue with those.

10m

3 Oligonucleotide thiol reduction

35m

Note: for the conjugation with the maleimide-polymers 2 nmol of oligos are needed. However, there is a

purification step before so the starting material should be more. As little as 5 nmol starting material can work but it is recommended to start with 12.5-25 nmol and potentially use less when more experienced with the protocol.

Mix 50 µl of the 250 µM oligonucleotides with 5 µl or 0.5 M TCEP (final TCEP conc. 50 mM).
Mix by pipetting and incubate at room temperature for 30 min.

Move to the next step while incubating.

Polymer metal chelation

44m

4 Note: This part comes from the Fluidigm protocol "Lanthanide Labeling of antibodies – PRD002 Version 11" 3m

Spin the polymer tube for 10 seconds in a microfuge to ensure that the reagent is at the bottom of the tube.

5 Resuspend the polymer with 95 µL of L-Buffer and mix by pipetting 3m

6 Add 5 µL of lanthanide metal solution to the tube (final concentration: 2.5 mM in 100 µL) and mix by pipetting 3m

7 Incubate at 37 °C for 30–40 minutes in a water bath or heat block. 35m

Purification of reduced oligonucleotides

41m

8 Add 5.5 µl volume of 3M NaAc (final conc 0.3 M) to the oligos and briefly vortex. 3m

9 Add 150 µl (2.5 volumes) of 100% EtOH (-20°C) and vortex. and put it to -20°C for at least 30 min. 3m

10 Incubate mix at -20°C for at least 30 min or longer. 35m

Cool down an Eppendorf centrifuge 5430R (or similar) to 4°C

Purification of metal-chelated polymers

11 Add 100 µL of L-Buffer to a 3 kDa Amicon filter. 2m

12 Add the 100 µL metal-loaded polymer mixture that incubated at RT to the filter containing the 200 µL L-Buffer. 2m

13 Centrifuge at 12,000 x g for 25 minutes at RT in a table to centrifuge 25m

14 Repeat the wash by adding 400 µL of C-Buffer to the filter and centrifuge at 12,000 x g for 45 minutes at RT. 45m

Move to oligonucleotide purification.

Purification of oligonucleotides and polymers 40m

15 Remove oligos from -20°C. Spin down oligos for at least 30 min at max speed (~30'000 rcf). 30m
Note: Keep in mind the orientation of the tubes to find pellet which will be very small.

16 Remove tube from centrifuge and remove supernatant with a suction device or pipette carefully without disturbing the pellet. 5m

17 Add 500 µl ice-cold 70% EtOH. Do not disturb the pellet and insert tubes in the centrifuge in same direction as before and spin for 3 min at max speed. 5m

18 Remove tubes from centrifuge and remove supernatant with a suction device or pipette carefully without disturbing the pellet. Place tube in a rack on the bench, open the lid and briefly air-dry the pellet (1-3 min). 5m

19 Resuspend pellet in 50 µl C-buffer. 2m

20 Determine concentration at Nanodrop (ssDNA mode). Calculate the volume containing 2 nmol of the reduced oligo. 5m

21 Remove the polymers from the table top centrifuge. The volume should be 20-30 µl. 2m

Conjugation and purification

3h 25m

22 Mix 2 nmol of the reduced oligo with the 20-30 µl of the polymer in C-buffer. Fill to 200 µl with C-buffer and mix with pipette. 5m

23 Incubate for 2 h at RT. 2h

24 After 2 hours add TCEP to the reaction to a final concetration of 5 mM (1:100 from 0.5 M stock). Incubate another 30 min at RT. 30m

25 Transfer the reaction onto flat filter tube Microcon 30 and add 300 µl ddH_2O. Spin 12 min at 12'000 rcf. 15m

26 Discard flow through and repeat for a total of 3 washes with 250µl DEPC water each (each 10 min). 35m

27 After the final wahsing step the oligos are retained in the tiny residual volume on top of the filter! Resuspend oligos from the top of the filter by adding 50µl of ddH_2O water and transfer to new tube. Repeat with another 50 µl of ddH_2O water. 5m

28 Determine concentration at Nanodrop (ssDNA). 5m

Agarose gel and storage

29 Prepare a 4% agarose gel with TAE buffer with gelred (1x) or any other means available for nucleic acid detection.
Load the conjugated and unconjugated oligos (roughly 500 ng is well visible and gives you a good impression of your labeling efficiency) next to each other on the gel and run at 120 volts for

Note: Be careful, oligos are very small (~20 nucleotides) and run fast. No ladder required since the unconjugated oligos serve as a control.

30 Prepare a 10 or 1 µM mix of the labelled oligos with ddH_2O.
Store the oligos at 4°C for further use with e.g. RNAscope.

Note: The oligos are stable at 4°C for several months. However, one may also freeze an aliquot of the oligos for later usage. We have tried this in the past and thawed, conjugated oligos worked well. Avoid freeze-thaw cycles. For higher plexicity it is recommended to store the oligos at higher concentrations to avoid adding too much "water" to the hybridization reaction.