

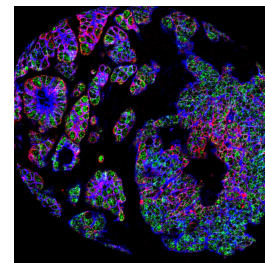
Jan 05, 2023

Version 2

Oligonucleotide-polymer conjugation for imaging mass cytometry V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.dm6gpjz91gzp/v2>



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DOI: <https://dx.doi.org/10.17504/protocols.io.dm6gpjz91gzp/v2>

Protocol Citation: Danielschulz 2023. Oligonucleotide-polymer conjugation for imaging mass cytometry. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.dm6gpjz91gzp/v2> Version created by [Danielschulz](#)

Manuscript citation:

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

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Protocol status: Working

We use this protocol and it's working

Created: January 05, 2023

Last Modified: January 05, 2023

Protocol Integer ID: 74783

Keywords: RNAscope, Imaging Mass Cytometry, multiplexed imaging, rnascope with imaging mass cytometry, unconjugated oligonucleotide, oligonucleotide, imaging mass cytometry, mass cytometry, cytometry, polymer conjugation, rnascope, agarose gel, example of an agarose gel, fluorescence readout, agarose

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](#)

644KB



Materials

A	B
Oligos	5' thiol labeled
TCEP (stock 0.5 M)	Bond-Breaker TCEP Solution, ThermoFisher
ddH ₂ O (molecular biology grade)	
Ethanol (70% in ddH ₂ O 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)	



Oligonucleotide resuspension and reduction

15m

- 1 Resuspend lyophilized oligos in ddH₂O (RNAase and DNAse free water) to a final concentration of 250 μ M 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
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. 35m

Mix 50 μ L of the 250 μ M oligonucleotides with 5 μ L of 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 μ L of L-Buffer and mix by pipetting 3m
- 6 Add 5 μ L of lanthanide metal solution to the tube (final concentration: 2.5 mM in 100 μ L) 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 μ L volume of 3M NaAc (final conc 0.3 M) to the oligos and briefly vortex. 3m



9 Add 150 μ l (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 μ L of L-Buffer to a 3 kDa Amicon filter.

2m

12 Add the 100 μ L metal-loaded polymer mixture that incubated at RT to the filter containing the 200 μ L 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 μ L 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 ($\sim 30'000$ 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 μ l 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 μ l 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 μ l.

2m

Conjugation and purification

3h 25m

22 Mix 2 nmol of the reduced oligo with the 20–30 μ l of the polymer in C-buffer. Fill to 200 μ l 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 concentration 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 μ l ddH₂O. Spin 12 min at 12'000 rcf.

15m

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

35m

27 After the final washing 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 50 μ l of ddH₂O water and transfer to new tube. Repeat with another 50 μ l of ddH₂O 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 μ M mix of the labelled oligos with ddH₂O.
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.