DNA functionalization on oxide

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ABSTRACT

DNA functionalization

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Glass slide cleaning 1h 30m

1 1h 30m

Clean glass slides in acetone, IPA by sonicating (2 times each) and put in water and dry under N2.
Acetone, IPA in cabinet under fumehood.

After cleaning, put under UV for 60 mins. Check the marked level to be consistent every time.

Silanization 17h

2 17h

Put glass slides in a dessicator. Put APTMS (3-Aminopropyl)trimethoxysilane on one glass slide and in dessicator. Leave it for 16 h. Take it out and put it in oven for 1 h at 100 C.

After this step, the glass slides can be kept sealed in freezer for a long time if cannot perform next step immediately.

APTMS on the shelve (row 5). syringes between row 1 and 2 cabinet. needles row 4 middle part.

DNA attachment 2h 30m

3 2h 30m

Prepare controlled and test samples on glass slides.

EDC and DNAs in freezer, MES in fridge.
EDC is in powder format. Make 100mg/mL of EDC in 0.1M MES.
NOTE: once EDC is dissolved in 0.1M MES, do everything very quickly because EDC lifetime is short once dissolves in water.

(100uM) polyT: 4.5 uL polyT + 6.2 uL 0.1M MES + 4.3 uL EDC
(300uM) HLA: 1.5 uL HLA + 9.2 uL 0.1 MES + 4.3 uL EDC

On glass slides, put PDMS and wells for flowing above solns onto desired spots.
Flow above solns and leave them in ice for 2 h.

Obtain ice from across the room.

Passivation 2h 15m

4 Remove all the liq inside. Wash with DI for 3-4 times and leave it in DI for 5 mins. 2h 15m
Remove all the PDMS. and put new black chamber + PDMS.
Make 40% PEG: 100/68*weight of PEG powder= volume(uL) of DI need to be added.

11 ul NaHCO3 + 89 uL 40 % PEG
Flow the solution into the chamber and leave it for another 2 h in dark, RT.

NaHCO3 (use stock conc. 0.89M) on shelves where chlorides solutions are and PEG (mPEG-SVA) in freezer.

Capping 1h

5 Remove all the liq. Wash again as before. 1h
Make sulfo-NHS-acetate solution: 20mg sulfo-NHS-acetate + 80 ul of 0.1M NaHCO3.

Flow capping soln (Sulfo-NHS-acetate) 100 uL and leave it for 1 h in dark, RT.
Sulfo-NHS-acetate in freezer.

Cleaning

6 Remove all liq. Wash as before.
Fill the chamber with 1 mM MgCl2 soln in 1x PBS.
Leave it in 4 C when not in use.

MgCl2 on the shelve row 5.

Testing

7 **NOTE:** Do not leave the chamber in air. Always flow new solution to replace old one instead of emptying out the chamber.
Use buffer (1 mM MgCl2 in 1x PBS) for imaging.
Flow target solution (100 nM for test) into the chamber and incubate 15 mins.
Then flow in buffer to remove target solution and image.

To remove target bound to probes, wash with DI and leave it in DI for 5 mins.