Azide-DNA Synthesis

1. Mix approximately 20 μl 1 mM amine-DNA (dissolved in deionized or DI water), 39 μl 60 mM azide-(PEG)4-NHS (dissolved in dimethylformamide or DMF), 40 μl DMF and 1 μl TEA (Triethylamine) and react for two hours.

2. Add approximately 200 μl ethanol and 7.5 μl 4 mM NaCl solution to the mixed solution and put in a refrigerator at 4°C for 30 minutes.

3. Centrifuge the solution at 20,000 g for 30 minutes.

4. Remove the supernatant and add 200 μl ethanol to resuspend the precipitant.

5. Centrifuge the solution at 20,000 g for 10 minutes. Remove the supernatant and dry the precipitant with a vacuum desiccator.

6. Resuspend the dried azide-DNA in 100 μl phosphate-buffered saline (PBS).

7. Determine the final concentration of the azide-DNA solution from optical absorption of DNA at 260 nm using a Perkin-Elmer Lambda-950 spectrophotometer.

DNA-lipid Conjugate

1. Dissolve DSPE-PEG(2000)-DBCO in ethanol to form a 1 mM solution.

2. Mix Tthe azide-DNA solution and DSPE-PEG(2000)-DBCO solution at a 1:5 molar ratio.

3. Store the mixed solution in dark at room temperature overnight.

4. Centrifuge the solution at 20,000 g for 30 minutes.

5. Remove the supernatant and add 200 μl ethanol to resuspend the precipitant.

6. Centrifuge the solution at 20,000 g for 10 minutes.

7. Repeat 4-6 for 3 times to thoroughly remove the unreacted DSPE-PEG(2000)-DBCO.

8. After washing, dry the precipitant with a vacuum chamber for 15 minutes.

9. Resuspend the dried DNA-lipid conjugate was resuspended in 100 μl PBS.

10. Determine the final concentration of the DNA-lipid solution from optical absorption of DNA at 260 nm using a Perkin-Elmer Lambda-950 spectrophotometer.

DBCO-RNA synthesis

1. Mix approximately 20 μl 1 mM amine modified RNA (dissolved in DI water), 39 μl 60 mM DBCO-NHS (dissolved in DMF), 40 μl DMF and 1 μl TEA and incubate for two hours.

2. Add 200 μl ethanol and 7.5 μl 4 mM NaCl solution to the mixture.

3. Incubate the solution at 4°C for 30 minutes and then centrifuge at 20,000 g for 30 minutes.

4. Remove the supernatant and add 200 μl ethanol was added to resuspend the precipitant.

5. Centrifuge the solution at 20,000 g for 10 minutes.

6. Remove the supernatant and dry the precipitant with a vacuum chamber.

7. Dissolve the dried DBCO-RNA in 100 μl 0.1% PBST (0.1 % Tween 20 in 1× PBS).

RNA-surface preparation

1. Etch glass coverslips in Piranha solution (3:1 v/v sulfuric acid: hydrogen peroxide) for 30 minutes.

2. Wash the coverslips were then washed with sufficient DI water and the wash with ethanol.

3. Dry the surfaces with filtered air stream.

4. Add approximately 100 μl 5% (v/v) 3-azidopropyltriethoxysilane in DMF on one surface. Put another coverslip on top forming a sandwich structure and incubate overnight.

5. Wash with ethanol to remove excess silane and dry in air stream.

6. Add approximately 100 μl 50 μM DBCO-RNA on the azide modified surface and put another surface on to form sandwich. The surfaces are incubated overnight for DBCO-RNA to click onto azidesilane.

7. Wash the RNA-decorated surface with DI water and store in -20°C.

Fabrication of microchannel

1. Bond the Inlet and outlet ports (LabSmith) to the slides using epoxy.

2. Use the electronic cutter (Silhoutte Curio) to cut 2-mm flow channels on the adhesive sheets.

3. Sandwich the channels between the slides and coverslips.