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title: DNA curtains standard protocol
catagory:assay
pdf: /static/img/protocols/flowcell_protocol.pdf
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Lipids (Rinse) Buffer:

	50 mL	Final Concentration
1M Tris, pH7.5	500uL	10mM
5M NaCl	1mL	100mM

BSA Buffer:

Note: BSA buffer usually is the basis for your experimental buffer, so the salt conditions can and usually do vary. The following conditions are common.

	50 mL	Final Concentration
1M Tris, pH7.5	2mL	40mM
1M KCl	1mL	20mM
1M MgCl2	50uL	1mM
1M DTT	50uL	1mM
BSA	10mg	0.2 mg/mL

common imaging Buffer:

BSA buffer plus the following.

	50 mL	Final Concentration
40% Glucose	20uL/mL	0.8%
Gloxy	2uL/mL	?
1uM YOYO-1	?	?

Step 1: Cleaning

1. Clean out out two adapter tubes with ~3mL of 100% ethanol two times, followed by two rinses of ~3mL of ddH2O.
2. Attach adapters to flowcell and rinse twice with ddH2O, once in each direction.

3. Rinse flowcell with lipids buffer.
    a. ***At the end of this step, all air needs to be removed from the flowcell, introduction of air bubbles past this point ruins the experiment.***

Step 2: Making a lipid bilayer

1. Briefly vortex lipids prior to pipetting
2. Add 40uL of lipids to 960uL of lipids buffer and vortex to mix
3. Inject lipids into flowcell in 3 short injections (~300uL each) waiting 5-10min in between injections.
4. Rinse with ~2.5mL of lipids buffer and incubate for 30min. This step can be extended (1-1.5 hrs total keeping in mind total bilayer time)
5. *Note: These steps can be repeated for a slightly better bilayer.*

Step 3: (if double tethering, otherwise go to step 4)

1. Add 20uL of 1mg/mL anti-dig to 500uL of lipids buffer and vortex to mix
2. Inject anti-dig into flowcell in 2 short injections (~200uL each) waiting at least 15min in between injections. This step can be extended (1-1.5 hrs total keeping in mind total bilayer time)

Step 4: BSA wash/streptavidin

1. Wash flowcell with BSA buffer and let sit for at least 5min This step can be extended (0.5 hrs total keeping in mind total bilayer time).
2. Mix 15uL 1mg/mL streptavidin with 500uL BSA buffer. ***Do not ever vortex BSA buffer... Bubbles!***
3. Inject streptavidin into flowcell in 2 short injections (~200uL each) waiting at least 7min in between injections.
4. Wash flowcell with ~3mL BSA buffer

Step 5: DNA

1. Check flowcell direction!
2. Mix appropriate amount of DNA with ~1mL BSA buffer
3. Inject DNA into flowcell in 3 or more short injections (~300uL each) waiting 5-10min in between injections. **Important: Leave back-pressure syringe in place!**
4. Go to the scope