DNA Sample Preparation

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1. DNA: Lambda *Bst*E II Digest (Sigma Aldrich Catalog # D-9793)
2. Storage Buffer: 10mM Tris-HCl (pH 8.0), 1 mM EDTA
3. Adsorption Buffer: 40 mM HEPES (pH 6.8), 10 mM $NiCl_2$

NOTE: Nickel Chloride ($NiCl_2$) can be a skin irritant in both solid and solution form. Please wear gloves when handling any $NiCl_2$ containing substances and wash hands when finished. Please, dispose of any $NiCl_2$ containing solutions in the appropriate labeled waste container – on lab bench in Biology Lab. Do NOT dispose in sink or in trash bins.

Storage Buffer
(10mM Tris-HCl (pH 8.0), 1 mM EDTA)

10 mM Tris-HCl (MW = 157.60 g/mol)
1 mM EDTA (MW = 372.24 g/mol)
50 mL buffer volume

For Tris-HCl,
m = (10 mmol/L)(0.050 L) (157.60 mg/mL) = 0.0788 g

For EDTA,
m = (1 mmol/L)(0.050 L) (372.24 g/mol) = 0.0186 g

a. Weigh out 0.0788 g of TRis-HCl and 0.0186 g of EDTA.
b. Dissolve in 50 mL of Milli-Q water
c. Adjust pH to 8.0 (using 1 M NaOH)
d. Store buffer in fridge when not in use.
e. Note: Buffer must be filtered (using 0.45µm syringe filter) before used to dilute DNA.

Adsorption Buffer
(40 mM HEPES (pH 6.8), 10 mM $NiCl_2$)

40 mM HEPES (MW = 238.3 g/mol)
1 mM $NiCl_2$ (MW = 237.7 g/mol)
50 mL buffer volume

For HEPES,
m = (40 mmol/L)(0.050 L)(157.60 mg/mL) = 0.4766 g

For $NiCl_2$,
m = (10 mmol/L)(0.050 L)(372.24 g/mol) = 0.1188 g

a. Weigh out 0.4766 g of HEPES and 0.1188 g of $NiCl_2$.
b. Dissolve in 50 mL of Milli-Q water
c. Adjust pH to 6.8 (using 1 M NaOH)
d. Store buffer in fridge when not in use.
e. Note: Buffer must be filtered (using 0.45μm syringe filter) before used to dilute DNA.

DNA Preparation

a. DNA Solution comes as 863 μg/mL solution (~400-500 μL volume)
b. Mix solution by finger tapping the tube of solution (Do NOT vortex the solution.)
c. Aliquot solution into eppendorf tubes as 10 μL / tube.
d. Store the eppendorf tubes in the freezer.

DNA Dilution (863 μg/mL → 3 μg/mL):

a. Take one 10 μL aliquot of stock DNA and thaw to room temperature.
b. Remove Storage Buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) from fridge and allow to come to room temperature.
c. Add 2.87 mL of Storage Buffer to the 10 μL of DNA for a final concentration of 3 μg/mL.

v = [(863 μg/mL)(0.010 mL)] / (3 μg/mL) = 2.88 mL
(10 μL DNA) + (2.87 mL Storage Buffer) = 2.88 mL total volume

d. Mix the solution thoroughly by finger tapping the tube.
e. Aliquot the 3 μg/mL of DNA solution into eppendorf tubes as 100 μL / tube.
f. Store the eppendorf tubes in the freezer until ready to use in experiment.

On Day of Experiment...

DNA Dilution (3 μg/mL → 0.5 μg/mL):

a. Remove 1 eppendorf tube of 3 μg/mL DNA solution from the freezer and thaw to room temperature.
b. Add 500 μL of Adsorption Buffer (40 mM HEPES (pH 6.8), 10 mM $NiCl_2$) to the tube of 100 μL of DNA for a final concentration of 0.5 μg/mL. (Make sure buffer has been filtered: See buffer section, Step e)

$$v = [(3\ \mu g/mL)(0.100\ mL)] / (0.5\ \mu g/mL) = 600\ \mu L$$
(100 μL DNA) + (500 μL Adsorption Buffer) = 600 μL total volume

c. Mix the solution thoroughly by repeatedly pipetting the solution in and out of the same pipette tip.
d. Pipette 100 μL of solution onto a freshly cleaved mica substrate (mica + Teflon disk glued to a magnetic puck/glass slide).
e. Allow the DNA to adsorb onto the mica surface for 5 minutes.
f. Rinse the mica with Adsorption Buffer to remove DNA that has not adsorbed to the surface.
g. Add fresh Adsorption Buffer to form a sufficient size droplet of fluid and begin imaging.

This procedure should give you ~20-30% surface coverage of DNA over a 1 μm scan area.

Place remaining solution in fridge and use with 3 days. Discard after 3 days and use fresh DNA from freezer (3 μg/mL) and dilute.