DNA end biotinylation for optical tweezers experiment

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Principle:
Filling in the recessed 3' ends with biotinylated dNTPs using klenow polymerase.

Materials:
1. Lambda phage DNA
From New England Biolabs
Alternatively, for nick-free lambda DNA, E.coli with phage can be grown and then DNA purified (Bensimon).

2. Klenow fragment
From Promega

3. dNTP (dATP, dGTP, dTTP)
From any PCR kit

4. Biotinylated dCTP
From Sigma

5. Polymerase buffer
10 × buffer: 500mM Tris-HCl (pH 7.2 at 25°C), 100mM $MgSO_4$, 1mM DTT.

6. 3M Sodium Acetate buffer, pH 5.2 (store at 4 °C)

7. 100% Ethanol (-20°C)

8. 70% Ethanol in sterile dH2O (-20°C)

9. Microcentrifuge (normal microcentrifuge in cold room works fine). All centrifugations should be on "soft" (no brake) setting.

Steps:
1. Place ~10 uG lambda-DNA in 50 uL of 1X polymerase buffer and heat to 65 degrees for 7 minutes to break apart sticky ends (lambda ladders).

2. Add 100X molar excess of dATP, dGTP, dTTP, and biolinylated dCTP.

3. Add 1 unit (~1 uL) of Klenow enzyme, mix well, and wait 30 min at room temperature.

4. Stop reaction with excess of EDTA (e.g., 10 uL of 100 mM)

5. Dialize over 0.025 um millipore filter or else spin several times in centricon-100 tube to remove excess biotin from DNA
Alternatively, precipitate DNA by the following steps: Transfer DNA to a container where it fills one fourth the total volume. Add one tenth volume of Sodium Acetate buffer to equalize ion concentrations. Add at least two volumes of cold 100% ethanol; let stand in -20°C freezer for 20 min. Centrifuge sample for 15 minutes at highest speed in a 4°C microcentrifuge.

Remove the supernatant. Add 200 μL of cold 70% ethanol; centrifuge for 5 minutes in a 4 °C centrifuge. Remove supernatant; evaporate remaining ethanol in a 37 °C water bath. Resuspend pellet in desired volume of water or TE (10 mM Tris, 1 mM EDTA, pH 8 for) buffer.

6. Store in 10 mM Tris, 1 mM EDTA, pH 8 for up to a year.