Genomic DNA Labeling For Malaria Using Klenow Polymerase

Sonication

Start with ~4 µg starting material

Dilute DNA in 200 µl volume water in 1.5 ml Eppendorf tube
Sonicate: 30 pulses power level 1, 1 second on/1 second off

Re-concentrate with Zymo columns

Follow protocol with kit
Elute with water
Spec to determine actual amount
Run 2 µl on gel to see size distribution (1-5 kb works well)

Label with Klenow enzyme in 50 ul reaction

Prime 3 µg of sheared gDNA (2-5 µg works fine) with 50 µg pdN9

Add 5 µl of pdN9 (10 mg/ml)

Boil reaction mixture at 99˚ C for 5 minutes then cool on ice for 5 minutes

Combine reagents:
5 µl NEB 10x NEBuffer 2 (or 10x EcoPol buff)
5 µl 15 x dNTP mix (use 1/5 dilution of RT mix)

3 mM dATP
1.5 mM dTTP
1.5 mM dCTP
1.5 mM dGTP

1 µl Klenow (or 4 µl NEB enzyme equivalent to 20~30 NEB units)
Add water up to 50 µl
1.5 µl dUTP labeled Cy dye

Incubate at 37˚ C for 2-3 hours then add 1 µl Klenow and leave at 37˚ C for 2-3 hours.

Cleanup and Concentration (Use Zymo columns)

Raise volume to 200 µl with water
Add reaction mixture to 350 µl of DNA binding buffer
Add 550 µl DNA of the mix to the column—spin 9.3 rcf x 1000
Reload the column with flow through—spin
Wash twice with 200 µl wash buffer 16.1 rcf x 1000
Spin dry for 30 seconds
Elute with 13 µl of 10mM Tris pH 8.5 into a new tube—let sit for a few minutes then spin again.