Klenow Reaction and Cy Dye coupling for *P. falciparum* genomic DNA
9/29/2003

3 µg genomicDNA, sheared to between 500 bp-1.5 kb fragments (2 µg genomicDNA/hybe)
50 µg pdN9 primer
1x EcoPol buffer (NEB)
1x dNTP mix (75 µM dATP, 15 µM dTTP, 60 µM aa-dUTP, 15 µM dGTP, 15 µM dCTP)
50 U klenow/50 µl reaction

	Volume (1 rxn)	**Volume (5 rxns)**	**Volume (9 rxns)**	**Volume (11 rxns)**
200 ng/µl gDNA	15 µl			
10 mg/ml pdN9	0.25 µl			
Total volume	**15.25 µl**			
10x EcoPol buff	5 µl	25 µl	45 µl	55 µl
10x dNTP mix	5 µl	25 µl	45 µl	55 µl
Klenow	1 µl	5 µl	9 µl	11 µl
ddH_2O	23.75 µl	118.75 µl	213.75 µl	316.25 µl
Total volume	**34.75**	**173.75**	**312.75**	**437.25**
Final vol/rxn	**50 µl**	**50 µl**	**50 µl**	**50 µl**

1. Boil gDNA/pdN9 for 5 minutes at 99°C, then cool on ice for 5 minutes.
2. Add 2nd mixture. Mix well.
3. Incubate: 37°C, 2-3 hrs.
4. Add additional 1µl klenow, leave at 37 °C, 2-3 hrs.

20x dNTPs (1 dGTP:1 dCTP:5 dTTP/aa-dUTP:5 dATP):

100 mM stock solutions		*20x final conc.*
dATP	3.75 µl	1.5 mM
dTTP	0.75 µl	0.3 mM
aa-dUTP	3.00 µl	1.2 mM
dGTP	0.75 µl	0.3 mM
dCTP	0.75 µl	0.3 mM
ddH_2O	241 µl	
total vol.	**250 µl**	

10x dNTPs (1 dGTP:1 dCTP:5 dTTP/aa-dUTP:5 dATP):

100 mM stock solutions		*10x final conc.*
dATP	1.875 µl	0.75 mM
dTTP	0.375 µl	0.15 mM
aa-dUTP	1.50 µl	0.60 mM
dGTP	0.375 µl	0.15 mM
dCTP	0.375 µl	0.15 mM
ddH_2O	245.5 µl	
total vol.	**250 µl**	

Clean up Klenow reactions with Zymo columns (200 µl DNA binding buffer/50 µl rxn mix). Wash 2x with 200 µl wash buffer and elute with 12 µl ddH_2O. Add 1 µl 1M NaBicarb, pH 9.0 (final conc. 0.1 M NaBicarb). Couple DNA to appropriate Cy dye 2 hrs to O/N in the dark at RT. Clean up coupled Klenow reactions with Zymo columns (200 µl DNA binding buffer/10 µl mix as before, elute with 16 µl EB buffer (Qiagen)). Ready for hybridization to DNA microarrays.

40 µl hybe mix (for thick lifter slips):
Boil mixture 2 mins, 99°C.
Cool by spinning at RT.
Apply entire mix to array
Hybridize at 65°C, O/N

30 µl labeled DNA (15 of each-cy3 & cy5)
6 µl 20x SSC
2 µl 10% SDS
1 µl 1M Hepes, pH 7
40 µl total volume