Genomic DNA Labeling Protocol_Version B

1. Prepare genomic DNA according to protocol in Guthrie & Fink

2. Digest 4 µg of genomic DNA with 20 units of a restriction enzyme that recognizes a 4-base sequence (e.g., Hae III) in a volume of 60 µl.

- **a. Incubate reaction at 37 degrees for 30-60 minutes.**
- **b. Clean up DNA using Qia-quick PCR purification kit(QIAGEN).**
- **c. Elute in 30 µl Buffer EB.**

Speedy Alternative: **(DeRisi style)**
Sonicate DNA to an average of 500bp with a Branson 450 Sonicator:
Resuspend DNA in 300µl (approx. 450 ng/µl).
Use the microtip at Power=2, 70% duty, for 10 seconds keeping DNA on ice throughout.

3. Add 10 µg random 6-mer (pdN_6 from Pharmacia) and water for volume of 39 µl.

- **a. Incubate 100 degrees for 5 min.**
- **b. Place tube on ice for 5-10 min.**
- **c. Centrifuge briefly at 4 degrees to bring down condensation.**

4. On ice, add:

5µl 10x Klenow buffer (provided with USB exo- Klenow)
2µl 3 mM aa-dUTP/dNTPs**
4µl Klenow exo- from USB (10 units/µl)
Reaction volume is now 50µl.

5. Incubate 37 degrees for 2-4 hours.

6. Add 5µl 0.5 M EDTA (pH 8.0) to stop reaction.

7.Clean-up reaction:

- **a. Add 440µl water to stopped reaction.**
- **b. Apply to Microcon-30 and spin for 8 min. at 13,000 rpm in microfuge.**
- **c. Discard flo-thru and add 500 µl water to filter, spin again.**
- **d. Elute DNA**
- **e. Dry eluate in speed vac**

8. Continue labeling by coupling amino allyl-dUTP to Cy dyes, as described in protocol for labeling RNA

****to get a 3 mM nucleotide stock:**

Dilute the "50X" stock described in RNA labeling protocol by 8.33-fold; this final mixture will contain 3 mM of dA, dG, dC; 1.2 mM of dT; and 1.8 mM of aa-dU.