Formic acid treatment of radiolabelled fragments

Procedure to generate "G+A" ladder. Used as a standard in DNase footprinting.

Both formic acid and piperidine are hazardous and should be handled in a fume hood. Piperidine-contaminated waste should be stored in the fume hood for 24 hours before disposal.

1. Set up a reaction containing: 12 µl DNA solution (usually dilute 3-4 µl DNA in H_2O)
   50 µl formic acid

   Incubate for 2.5 minutes at room temperature.

2. Stop by adding:200 µl 0.3 M NaAc pH 7.0 + 1µl glycogen
   700 µl ice-cold 100% ethanol
   Precipitate for 15 minutes at -70°C then centrifuge for 15 minutes at 4°C.

3. Wash 3 times with 1 ml 70% ethanol, each time centrifuging for 10 minutes at 4°C and discarding supernatant.

4. Dry pellet under vacuum.

5. Prepare (immediately before use) a 1 in 10 dilution of the stock 10M piperidine in H_2O (100 µl needed for each cleavage reaction).

6. Resuspend the dried pellet in 100 µl of 1M piperidine - will need to vortex.

7. Incubate at 90°C for 30 minutes.

8. Ethanol precipitate (add 10 µl 3M NaAc pH 7.0 and 300 µl ice-cold ethanol, +1µl glycogen and incubate at -70°C for 15 minutes - can leave overnight at this stage). Centrifuge for 15 minutes at 4°C and discard supernatant.

9. Wash pellet twice with 1ml 70% ethanol, centrifuging for 10 minutes and discarding supernatant.

10. Dry pellet under vacuum.

11. Resuspend pellet in 20 µl DNase I blue. Load 0.5-2 µl (~~50-100~~ cps) on a footprinting gel.