

## MAXAM-GILBERT SEQUENCING

A modified, abbreviated version of the Maxam-Gilbert sequencing is described. A complete description including the methods to prepare and isolate asymmetrically labeled fragments of DNA and to prepare and run sequencing gels can be found in *Methods in Enzymology* 65: 497-559.

### **Reagents:**

- Dimethylsulfate (DMS) Extremely dangerous.
- Hydrazine (HZ) Very dangerous.
- Formic acid
- Piperidine. Stock is 10 M. Dilute to 1.0 M just before use.
- 100% Ethanol
- dH<sub>2</sub>O
- Acetic Acid. Glacial is 17.4 M. Dilute to 1 M.
- 0.3 M Sodium acetate (pH 5.2)
- 5 M NaCl
- 10 N NaOH
- 500 mM EDTA
- tRNA. Stock solution is 1mg/ml in dH<sub>2</sub>O.
- Calf thymus DNA. Stock solution is 1 mg/ml in TE.

### Buffers:

#### DMS buffer

- 50 mM sodium cacodylate (pH 8.0)
- 1 mM EDTA

#### DMS stop

- 1.5 M sodium acetate (pH 7.0)
- 1.0 M mercaptoethanol
- 100 µg/ml tRNA

#### HZ Stop

- 0.3 M sodium acetate
- 0.1 M EDTA
- 25 µg/ml tRNA

#### Loading buffer

- 80% (v/v) formamide
- 50 mM Tris-borate (pH 8.3)
- 1 mM EDTA
- 0.1% (w/v) xylene cyanol
- 0.1% (w/v) bromophenol blue.

### Hints:

1. 200 cps per 5  $\mu$ l reaction or greater is recommended.
2. -70°C incubations can be done on powdered dry ice.
3. 30 minute 90C incubation should be done with a device which holds Eppendorfs closed.
4. During multiple ethanol precipitations monitor presence of pellet with Geiger counter.

#### Nucleotide sequencing gels:

All gels are pre-run at 1200 volts for 30' (do not go longer on 20% gels)

#### Nucleotides 1-50

Use a 20% gel,

Run BPB to 15cm for nucleotide 1 on bottom. Alternatively, for a better spread, run BPB to 20 cm for nucleotide 3 on bottom. To do this, run the gel at no more than 28 milliamps, 2000-2500 volts.

At 2000 volts, this takes 1 1/4 hours.

#### Nucleotides 40-150

Use an 8% gel.

Run XC to 27 cm. To do this, run the gel at no more than 28 milliamps. Voltage should start at about 1500 volts and drops during run as plates heat up.

The run should take 3 - 3 1/2 hours.

#### Nucleotides 140-?

Use an 8% gel.

Run XC to 2 gel lengths (72 cm). Re-load dyes when XC is at 36 cm. To do this, run the gel at no more than 28 milliamps. Voltage should start at about 1500 volts and drops during run.

The run should take 7 - 8 hours.