PROTOCOL FOR SEQUENCING BY THE MAXAM-GILBERT TECHNIQUE

Below, we describe in abbreviated form the chemical reactions developed by A. Maxam and W. Gilbert for the base-specific modification and cleavage of DNA. A more-detailed description, together with a thorough discussion of the methods used to isolate asymmetrically labeled fragments of DNA and to prepare and run sequencing gels has been published by Maxam and Gilbert in *Methods in Enzymology* (1980) **65** (part 1): 497–559.

Reagents

Dimethylsulfate (DMS) (Aldrich Chemical Co.).

Hydrazine (HZ) (Eastman Kodak).

Formic acid.

Piperidine (Fisher Scientific). Stock solution is 10 M. Dilute to 1.0 M just before use.

95% Ethanol.

70% Ethanol.

Distilled H_2O.

1 M Acetic acid.

0.3 M Sodium acetate (pH 5.2).

5 M NaCl.

1.2 N NaOH.

1 mM EDTA.

tRNA. Stock solution is 1 mg/ml in distilled H_2O.

Buffers

DMS buffer

50 mM sodium cacodylate (pH 8.0)
1 mM EDTA

It is usually unnecessary to adjust the pH.

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 mM EDTA
25 μg/ml tRNA

Loading buffer

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

Note

Filtration of the above buffers is usually not necessary. However, if a solution is turbid, filter it through a 0.45-μm nitrocellulose filter.

TABLE A.6. SUMMARY OF BASE-SPECIFIC REACTIONS FOR SEQUENCING END-LABELED DNA

	G	G & A	T & C	C	A>C
Mix	200 μl DMS buffer	10 μl H_2O	10 μl H_2O	15 μl 5 M NaCl	100 μl { 2 N NaOH / 1 mM EDTA
	5 μl [^{32}P]DNA	10 μl [^{32}P]DNA	10 μl [^{32}P]DNA	5 μl [^{32}P]DNA	5 μl [^{32}P]DNA
Chill to	0°C	0°C	0°C	0°C	*Heat to* 90°C, 6 min.
Add	1 μl DMS	50 μl formic acid	30 μl HZ	30 μl HZ	150 μl 1 N acetic acid 5 μl tRNA (1 mg/ml) 750 μl 95% ethanol
Incubate	20°C, 3-4 min.	20°C, 5 min.	20°C, 5 min.	20°C, 5 min.	
Add	50 μl DMS stop 750 μl ethanol	180 μl HZ stop 750 μl ethanol	200 μl HZ stop 750 μl ethanol	200 μl HZ stop 750 μl ethanol	
Store	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.
Centrifuge	10 min.	10 min.	10 min.	10 min.	10 min.
To pellet add	250 μl 0.3 M NaAc 750 μl ethanol	250 μl 0.3 M NaAc 750 μl ethanol	250 μl 0.3 M NaAc 750 μl ethanol	250 μl 0.3 M NaAc 750 μl ethanol	250 μl 0.3 M NaAc 750 μl ethanol
Store	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.	-70°C, 10-15 min.
Centrifuge	10 min.	10 min.	10 min.	10 min.	10 min.
Rinse pellet with *Vacuum dry*	70% ethanol	70% ethanol	70% ethanol	70% ethanol	70% ethanol
To pellet add	100 μl 1.0 M piperidine	100 μl 1.0 M piperidine	100 μl 1.0 M piperidine	100 μl 1.0 M piperidine	100 μl 1.0 M piperidine
Heat to *Lyophilize*	90°C, 30 min.	90°C, 30 min.	90°C, 30 min.	90°C, 30 min.	90°C, 30 min.
Add *Lyophilize*	10 μl H_2O	10 μl H_2O	10 μl H_2O	10 μl H_2O	10 μl H_2O
Add *Lyophilize*	10 μl H_2O	10 μl H_2O	10 μl H_2O	10 μl H_2O	10 μl H_2O
Add *Vortex*	10 μl loading buffer	10 μl loading buffer	10 μl loading buffer	10 μl loading buffer	10 μl loading buffer
Heat to *Chill in ice* *Load onto gel*	90°C, 1 min.	90°C, 1 min.	90°C, 1 min.	90°C, 1 min.	90°C, 1 min.

Reactions should be carried out in siliconized Eppendorf tubes.

Sequencing Gels

20% Acrylamide

acrylamide	96.5 g
methylene-bis-acrylamide	3.35 g
ultra-pure urea	233.5 g
5× TBE	100 ml
H_2O	to 500 ml

Urea mix

urea	233.5 g
5× TBE	100 ml
H_2O	to 500 ml

5× TBE

Tris base	54 g
boric acid	27.5 g
0.5 M EDTA (pH 8.0)	20 ml

To make an 8% sequencing gel, mix in a small flask:

20% acrylamide	20 ml
urea mix	30 ml
10% ammonium persulfate (freshly dissolved in water)	0.4 ml

Pour the solution into the barrel of a 50-ml syringe and add 50 μl of TEMED. Mix rapidly and inject the contents of the syringe (no needle should be used) into a preformed, sequencing-gel mold.