**DIdeoxy Sequencing Reactions**

This is the original M13 sequencing protocol. All reagents must be prepared and calibrated before use. Recipes are provided at the end.

1) Annealing Sequencing Primer

| | | |
|---|---|---|
| A) | 5 ul DNA | single stranded approx. 1ug |
| B) | 1 ul Primer | (17 bp)(NEB and BRL make suitable primers) |
| C) | 1 ul 10xHin buffer | |
| D) | 5.5 ul | $H_2O$ |
| | 12.5 ul Total Reaction | |

Heat to at least 65° for 5 min. (Not greater than 80° )
Slow cool to room temp.

2) Add to each tube:

| | |
|---|---|
| 1 ul DTT | 0.1 M |
| 1 ul $^{35}$S dATP | |
| 1 ul Klenow Pol I | ( 1 unit per ul ) |
| 15.5 ul Total | |

3) Dilutions of deoxy nuclotides.

First Dilutions:

| STOCK | DILUTION |
|---|---|
| dGTP (10mM) | 4 --> 80 |
| dATP (10mM) | 4 --> 80 |
| dTTP (10mM) | 4 --> 80 |
| dCTP (10mM) | 4 --> 80 |
| | |
| ddGTP (10mM) | 1 --> 40 |
| ddATP (10mM) | 1 --> 160 |
| ddTTP (10mM) | 1 --> 10 |
| ddCTP (10mM) | 1 -->80 |

Second Dilutions ( mixes ):

| | dGTP | dATP | dTTP | dCTP | 10 x HIN |
|---|---|---|---|---|---|
| G° | 1-2 | - | 20 | 20 | 20 |

| | | | | | |
|---|---|---|---|---|---|
| $A^o$ | 20 | - | 20 | 20 | 20 |
| $T^o$ | 20 | - | 1 | 20 | 20 |
| $C^o$ | 20 | - | 20 | 1 | 20 |
| Chase | 20 | 20 | 20 | 20 | 20 |

4) Sequencing Reactions:

Set up 90 well conical bottom microtiter plate as follows;

| (samples) 1 2 3...n | | add 1ul | add 1 ul diluted |
|---|---|---|---|
| | G | $G^o$ | ddGTP |
| | A | $A^o$ | ddATP |
| | T | $T^o$ | ddTTP |
| | C | $C^o$ | ddCTP |

add 3 ul of mix from step 2 to proper sample well.
add nucleotides to rows with corresponding names.

Incubate at 32$^o$ for 30 minutes. (This reaction can be run in wide range of temperatures from room temp. to 37$^o$. There is also a protocol for sequencing DNA with G-C rich regions that uses a temp. of 42$^o$.)

5) Chase reaction:

a) add 1 ul chase (GATC)
b) 32$^o$ for 15 minutes.

6) Stop reaction.

After chase add 5 ul gel dye and boil for 1 minute.

Ready to load on gel or freeze for later use.

DIDEOXY SEQUENCING BUFFERS

TM BUFFER

| | | |
|---|---|---|
| 100mM TRIS HCl pH8.1 | 1M Tris HCl Ph8.1 | 100ul |
| 100mM MgCl2 | 1M MgCl2 | 100ul |
| | H2O | 800ul |

| | |
|---|---|
| Total | 1000ul |

10x HIN BUFFER

| | | |
|---|---|---|
| 70mM TRIS HCl pH7.6 | 2M Tris pH7.6 | 35ul |
| 70mM MgCl2 | 1M MgCl2 | 70ul |
| 500mM NaCl | 5M NaCl | 100ul |
| | H2O | 795ul |
| | Total | 1000ul |

Note: Either buffer may be used in the priming reaction of M13 sequencing but TM seems to work slightly better.

Dideoxy or Maxam & Gilbert Sequencing Sample Dye Stop Buffer

0.5ml Formamide [deionized]

20ul 10% Bromphenol Blue

20ul 10% Xylene Cyanol

10ul 0.5ml EDTA

10xSTBE

| | 1 liter | 2 Liter | 4 Liter | 6 Liter |
|---|---|---|---|---|
| Tris | 108gm | 216g | 432gm | 648gm |
| Boric Acid | 55gm | 110gm | 220gm | 330gm |
| EDTA | 9.3gm | 18.6gm | 37.2g | 55.8mg |

Note: 10xSTBE-Sanger TBE used in gradient sequencing gels. This recipe is about twice the concentration of normal TBE.