

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 H₂O
- 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 ³⁵S 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):

	<u>dGTP</u>	<u>dATP</u>	<u>dTTP</u>	<u>dCTP</u>	<u>10 x HIN</u>
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 96 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.