PREPARATION OF DIDEOXY WORKING SOLUTIONS

(to sequence with radioactive dATP)

1. Prepare 0.5 mM Stocks of dCTP, dGTP, dTTP and 10 mM stocks of ddATP, ddTTP, ddCTP, ddGTP
   (These keep at -70°C indefinitely).

2. Prepare Zero Solutions.
   From 100 mM stock solutions, make 0.5 mM stocks (5 µl in 99.5 µl dH_2O).

	A	C	G	T
0.5 mM dCTP	20 µl	1 µl	20 µl	20 µl
0.5 mM dGTP	20 µl	20 µl	1 µl	20 µl
0.5 mM dTT	20 µl	20 µl	20 µl	1 µl
1XTE	20 µl	20 µl	20 µl	20 µl

   (These keep at -20°C about 2 months)

3. Prepare dideoxy nucleotide solutions

   ddATP = 0.1 mM — 1 µl of 5 mM ddATP in 99 µl dH_2O
   ddCTP = 0.1 mM — 1 µl of 5 mM ddCTP in 99 µl dH_2O
   ddGTP = 0.3 mM — 6 µl of 5 mM ddGTP in 94 µl dH_2O
   ddTTP = 0.5 mM — 10 µl of 5 mM ddTTP in 90 µl dH_2O

   (These concentrations can be varied to give you optimal results for the sequence of interest)
   (The solutions keep at -20°C about 2 months)

4. Combine ddNTP and dNTP
   Mix ddNTPs and dNTPs in a ratio of:
   1:1 for longer products
   2:1 for shorter products
   (For sequencing, you will need 3 µl of each mix per clone sequenced.)

dsDNA System Nucleotide Mix formulations

component	C Nucleotide Mix	A Nucleotide Mix	T Nucleotide Mix	G Nucleotide Mix	Chase
ddCTP	66 µM	---	---	---	---
ddATP	---	300 µM	---	---	---
ddTTP	---	---	---117 µM	---	--
ddGTP	---	---	---	66 µM	---
dCTP	1.66 µM	33 µM	33 µM	33 µM	2 mM
dATP	---	---	---	---	2 mM
dTTP	33 µM	33 µM	1.66 µM	33 µM	2 mM
dGTP	33 µM	33 µM	33 µM	1.66 µM	2 mM
NaCl	50 mM	50 mM	50 mM	50 mM	50 mM
Tris-HClPH 7.5	10 mM	10 mM	10 mM	10 mM	---
Tris-HCl pH 8.3	---	---	---	---	34 mM
$MgCl_2$	10 mM	10 mM	10 mM	10 mM	6 mM
dithiothreital	1 mM	1 mM	1 mM	1 mM	5 mM

SEQUENCING SOLUTIONS

Denaturing solution

500 µl: 100 µl 10 N NaOH

2 µl 0.5 M EDTA
398 µl dH_2O

Chase solution

1 ml: 20 µl 100 mM dATP
20 µl 100 mM dCTP
20 µl 100 mM dGTP
20 µl 100 mM dTTP
920 µl dH_2O

10x Hybridization buffer

1 ml: 200 µl 1 M Tris pH 7.5
2 µl 0.5 M EDTA
125 µl 4 M NaCl
100 µl 1 M $MgCl_2$
20 µl 0.5 M DTT
553 µl dH_2O

6x proteinase K buffer

500 ml: 30 µl 1 M Tris pH 7.6
60 µl 0.5 M EDTA
25.43 g LiCl
30 ml 20% SDS

SEALING GEL

10 ml: 8 ml dH_2O
2 ml 40% Acrylamide
--10 mg APS
--30 µl TEMED

SEQUENCING GEL

	8% Acrylamide gel	6% Acrylamide gel
50 ml:	10 ml 40% Acrylamide	7.5 ml 40% Acrylamide
	10 ml dH_2O	12.5 ml dH_2O
	25 g urea	25 g urea
	10 ml 5xTBE	10 ml 5xTBE
	--50 mg APS	--50 mg APS
	--22 µl TEMED	--22 µl TEMED