

DNA SEQUENCING

- **Maxam - Gilbert Direct Chemical Sequencing**
- **SANGER AND COLUNSON**

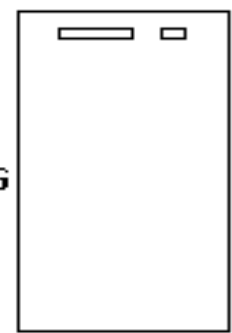
3- Chemical Sequencing of DNA

Reaction	Cleavage	Base Modification	Base Displacement	Strand Scission
R1	G>A	Dimethylsulfate	Heat at pH 7	NaOH
R2	A>G	Dimethylsulfate	acid	NaOH
* R3	C+T	Hydrazine	piperidine	piperidine
* R4	C	Hydrazine + Salt	piperidine	piperidine
* R5	G	Dimethylsulfate	piperidine	piperidine
* R6	G+A	Acid	acid	piperidine
R7	A>C	NaOH	piperidine	piperidine
R8	G	Methylene Blue	piperidine	piperidine
R9	T	Osmium Tetroxide	piperidine	piperidine

2-Single Stranded DNA Preparation

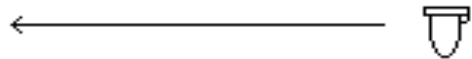
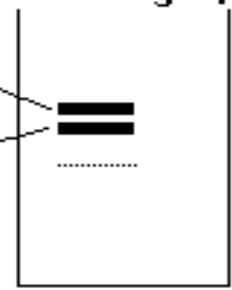


5% PAG
(1:50)



autoradiograph

Crush Gel
and
Elute DNA

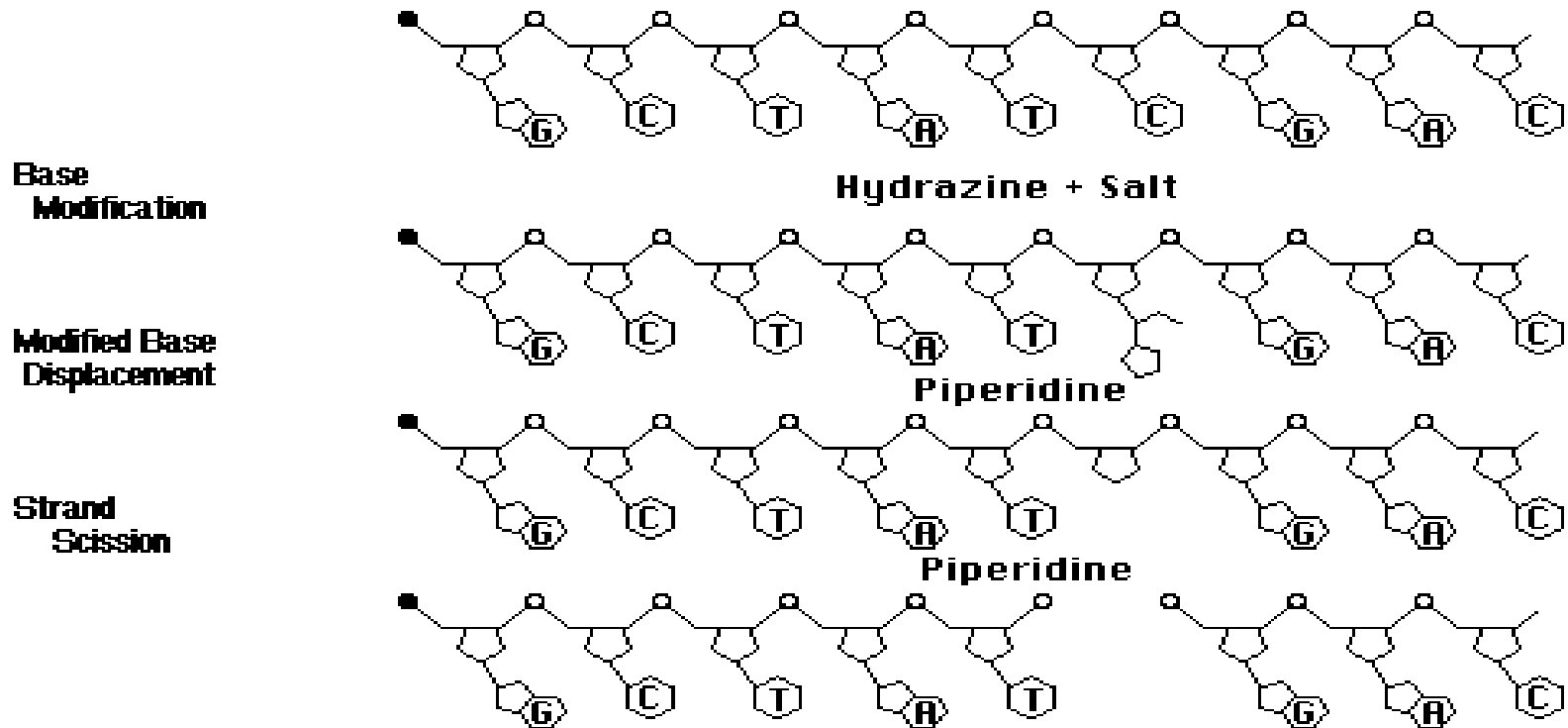


Sequence

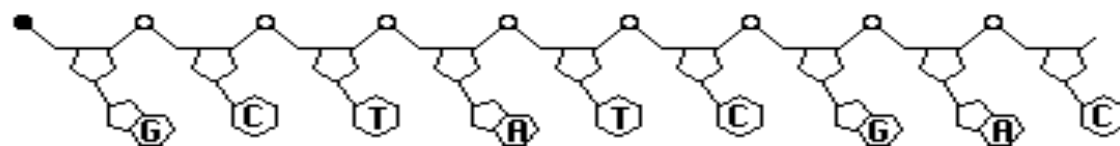
Purify,
Concentrate

Separation based on secondary structure formation.

5-The C Reaction

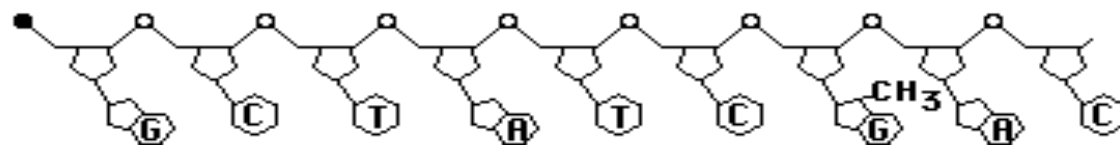


4-The G Reaction



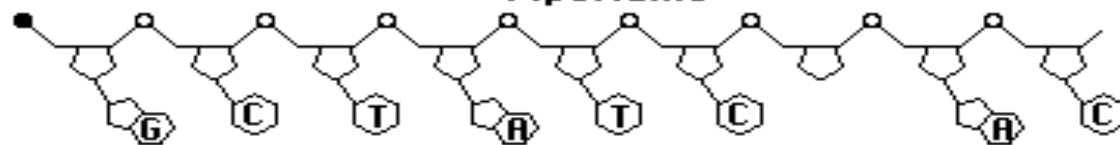
Dimethyl Sulfate

**Base
Modification**



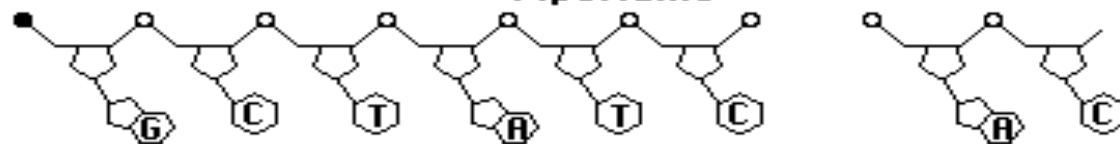
**Modified Base
Displacement**

Piperidine



**Strand
Scission**

Piperidine

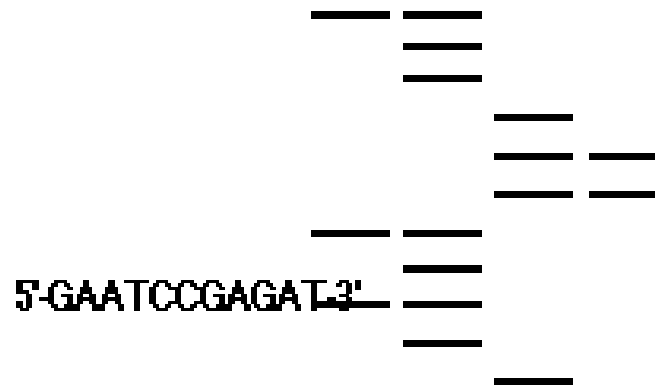
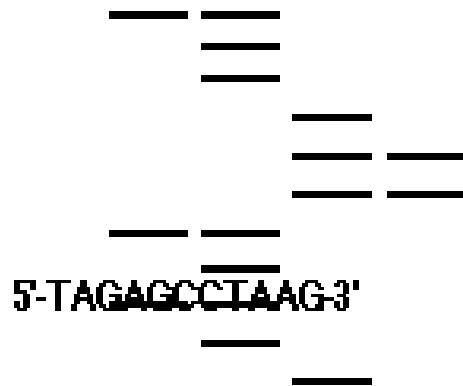


6-Reading Maxam & Gilbert Sequences

Reading chemical sequencing gels is dependent on which end of the DNA the label was attached to:

5' end labeled
G A+G T+C C

3' end labeled
G A+G T+C C



Direct Chemical Sequencing Of Labeled RNA

10-Direct RNA Sequencing - Chemical

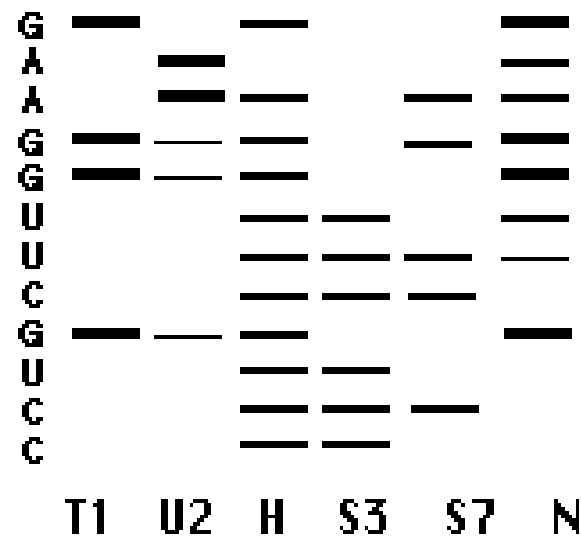
<u>Base</u>	<u>Modification Reaction</u>	<u>Cleavage</u>
G	Dimethyl Sulfate	Analine
A>G	Diethyl pyrocarbonate	Analine
C>U	Hydrazine, NaCl	Analine
U	Hydrazine	Analine
Random	6M urea, 50 mM NaOH	NaOH

Direct Enzymatic Sequencing Of Labeled RNA

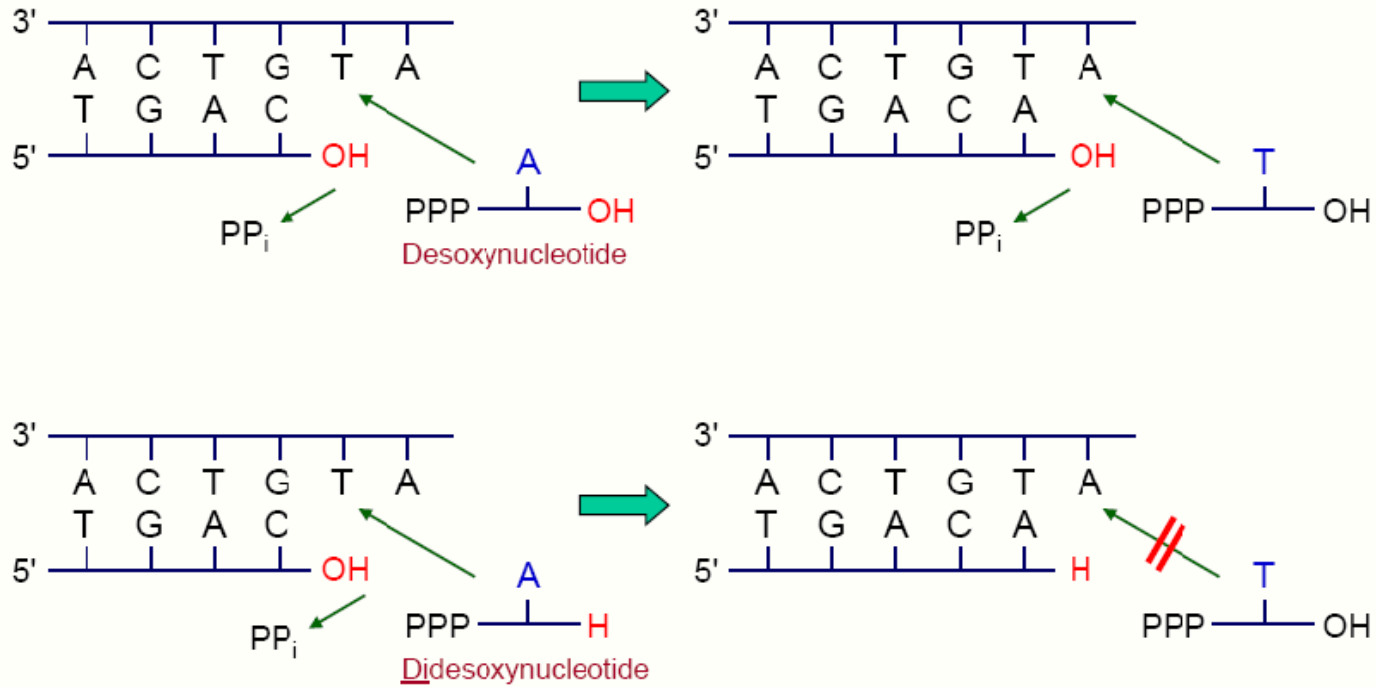
8- Direct RNA sequencing-Enzymatic

<u>Enzyme</u>	<u>Reaction</u>	<u>Phosphodiester bond cleaved</u>
RNase T1	20 mM sodium citrate pH 3.5	G-p-N
RNase U2	8M urea, 20 mM Tris-Cl pH 7.5	A-p-N (G-p-N)
RNase CL3	8M urea, 20mM Na Citrate pH 3.5	C-p-N (A-p-N)
S3	8M urea, 20 mM Tris-Cl pH 7.5 10mM CaCl ₂	Py-p-N Py-p-A
S7	8M urea, 20 mM Tris-Cl pH 7.5 10mM CaCl ₂	N-p-U N-p-A
Endonuclease from <i>Norassa</i> (N)	8M urea, 20 mM Tris-Cl pH 7.5	³² P-G-p-N U-p-N A-p-N
NaOH (H)	6M urea, 50 mM NaOH	Random

9- Reading RNA Sequencing Gels - Enzymatic



Sanger Di-Deoxy DNA sequencing (1964)



Primer 5' — 3'
Template 3' — 5'

+dNTPs + Polymerase

+ddATP
— GCTCTCA

+ddCTP
— GC
— GCTC
— GCTCTC

+ddGTP
— G
— GCTCTCAG

+ddTTP
— GCT
— GCTCT

