Student's Name Date

Subject Formic Acid Hydrol. DNA Instructor's Name

10-21-75

Preparation of DNA Standard

Weight : 26.6 mg
Vol. : 25ml water
Conc : 1.06 mg/ml

I have not quant. this solution spectrophotometrically yet.

10-21-75 - DNA

10-23-75 Lyophilization of DNA Samples

- Transfer 100 µl (106 µg DNA) of 10-21-75 into PS vials via syringe.
- freeze
- lyophilize overnight.

10-24-75

Samples:

- 10-23-1, -2, -3, -4 } to be hydrolyzed at appox 50, 60, 80, 100°C

They looked like small inverted cones of DNA after lyoph.

Store in freezer

10-25-75 Remove from freezer and let them come to room T.

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10-25-75 Formic Acid Hydrolysis of DNA

Sample 10-23-1 (106 μg DNA)
- add 0.20 ml HCOOH
- heat at 54°C in oil bath for one hour (variac = 18V)
- evaporate HCOOH away with gentle stream of helium

Observation: There was a white powder residue after ~30 min of evaporation - good.

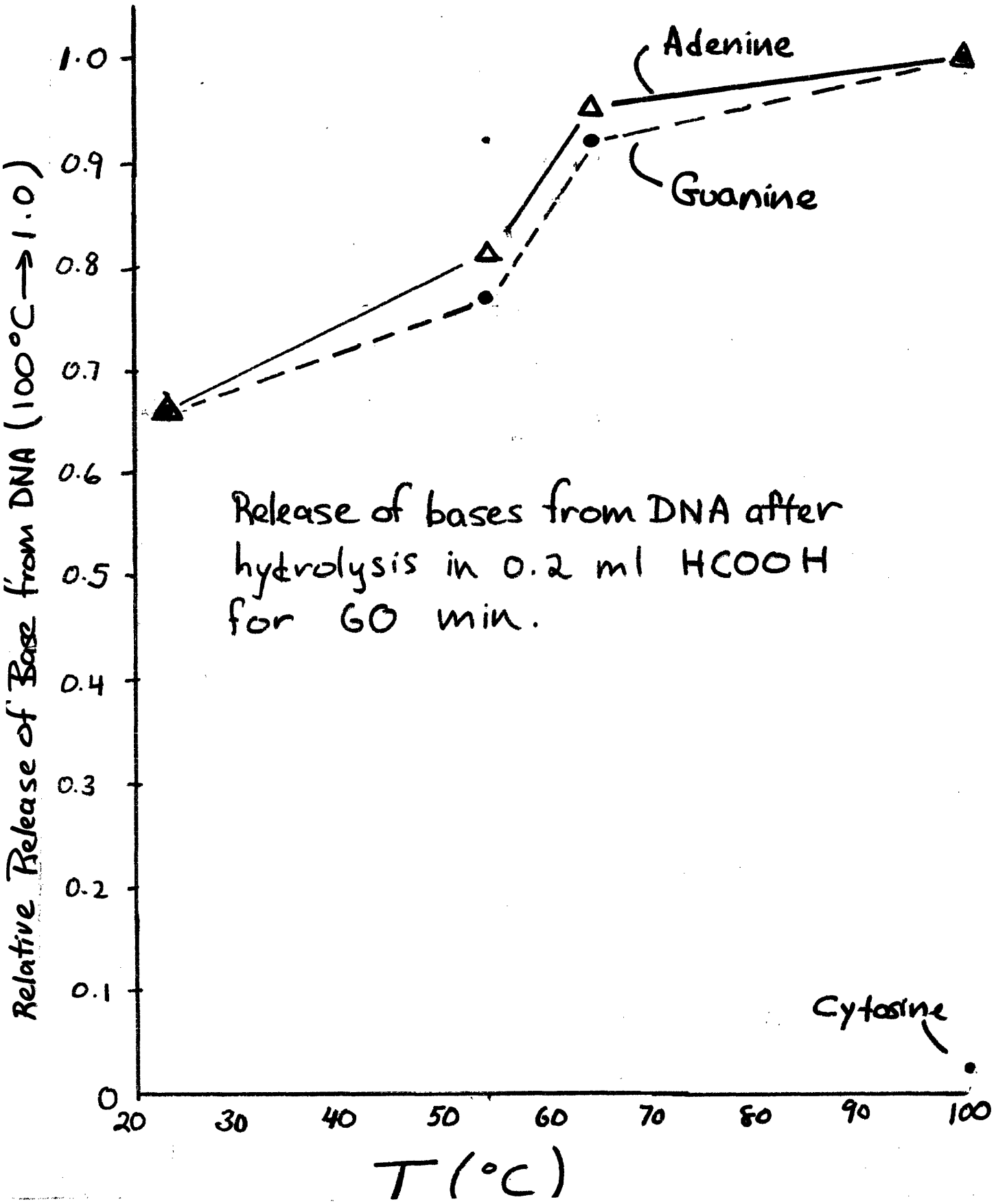
Sample 10-23-2 (106 μg DNA)
- add 0.20 ml HCOOH
- heat at 64°C (finally 69°C) for 60 min (variac = 22V)
- evaporate acid
- add 0.5 ml buffer

Sample 10-23-3 (106 μg DNA)
- add 0.20 ml HCOOH
- ~~heat~~ let stand at room T (23°C) for one hour
- remove acid
- add 0.5 ml buffer

Sample 10-23-4 (106 μg DNA)
- add 0.20 ml HCOOH
- heat to 100-105°C for 1 hr
- remove acid
- add 0.5 ml buffer.

10-25-75 Chromatographic Conditions:

0.1 M AF pH 5.38, T = 55, S = 20, P = 800/600
Col = 25 x 0.22 DCIA (old)


Adenine
Guanine
Cytosine
Release of bases from DNA after hydrolysis in 0.2 ml HCOOH for 60 min.
Relative Release of Base from DNA (100°C → 1.0)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
30
40
50
60
70
80
90
100
T (°C)

Student's Name

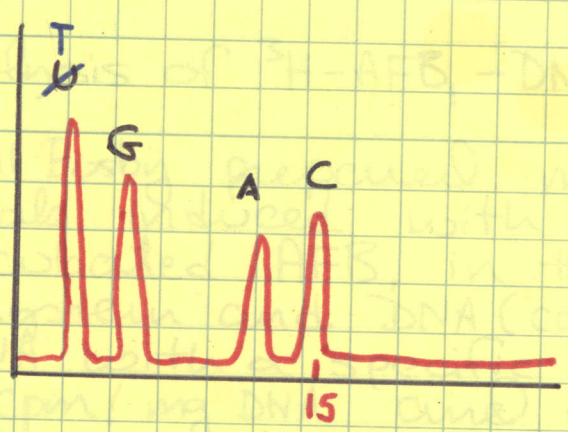
Date

Subject

Instructor's Name

10-25-75 Formic Acid Hydrolysis - Results

Things went extreemly well. The analysis conditions permitted 15 minute VGCA analysis - good sensitivity and sharp peaks.



Data:	x1.0 (U)	x0.4 G	x0.4 A	x0.4 C
7-23-1 (54°c)	152 (130%)	92 (77%)	135 (81%)	0
7-23-2 (64°c)	135 (115%)	110 (92%)	158 (95%)	0
7-23-3 (23°c)	172 (147%	78 (66%)	110 (66%)	0
7-23-4 (100-105°c)	117	119	167	5