

$$42 \frac{\mu\text{g}}{\text{ml}} \rightarrow 1 \text{ AU}$$

$$(0.175 \text{ AU}) \left(42 \frac{\mu\text{g}}{\text{ml} \cdot \text{AU}} \right) = 7.35 \frac{\mu\text{g}}{\text{ml}}$$

↓ × 10

$$73.5 \frac{\mu\text{g}}{\text{ml}} = \text{conc. of 12-7-2}$$

$$183.75 \frac{\mu\text{g}}{\text{ml}} = \text{conc. 12-7-1}$$

← × 25

1.84 mg DNA in each sample in 25 ml RB flask

Subject

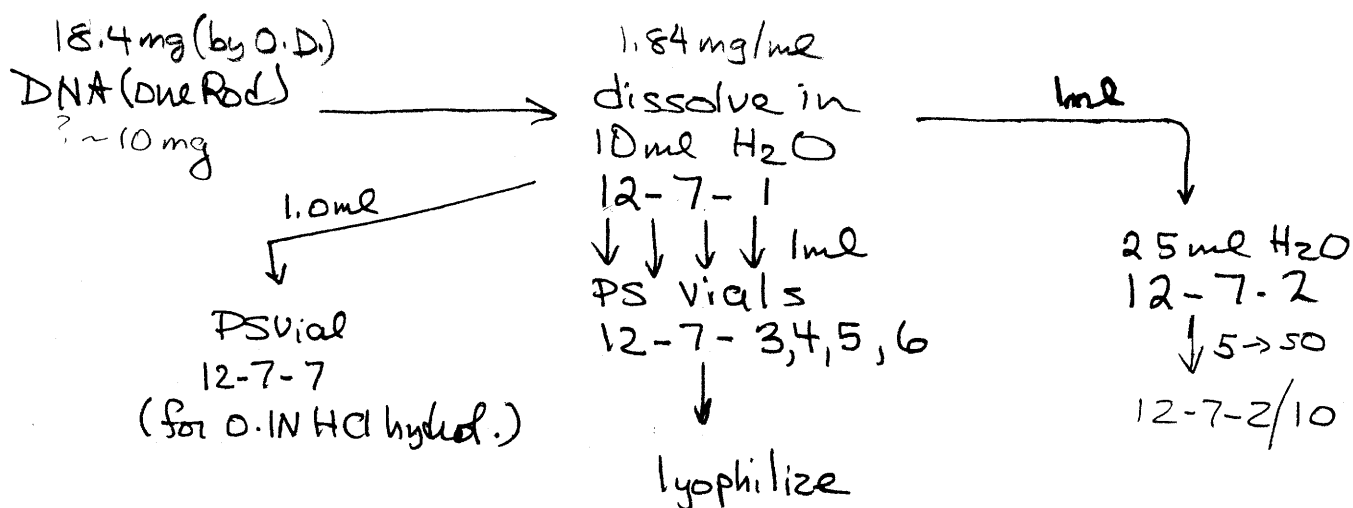
Instructor's Name

12-7-76

Quantitative Study on Release of Adduct With Formic Acid

Purpose: We believe that 790% of the radioactivity incorporated into DNA can be released as adduct with room temperature HCOOH hydrolysis.

This experiment was designed to test that figure and its precision,
Experiment



- each of the samples in PS vials should represent an excess of 1 mg.

Spectrophotometric Measurement of DNA Concentration

sample 12-7-2 → 1.75 AU
12-7-2/10 → 0.175 AU

So, samples 12-7-2 → 7 have 1.84 mg DNA.

12-75

1.84 mg DNA dis in 5.0 ml 10% MeOH \rightarrow 0.37 mg/ml

\Rightarrow 0.37 μ g DNA // μ l

50 μ l inj \Rightarrow 18.4 μ g DNA equivalent

now, 1:60 bases is mod

18.4 μ g DNA $\xrightarrow{\times 35}$ 6.44 μ g bases

\downarrow 1/60

0.11 μ g adduct = 110 ng

12-8-76

Hydrolysis:

- Each sample had lyophilized into a cone of DNA. (Sample 7 stored in freezer)
- add 1.0 ml HCOOH to samples 3 → 6
- stir on mag. stirrer for 1 h (not stirred ~5 min) at room temp
- cool
- add 2 x 2 ml of H₂O (total spl. vol = 5 ml)
- freeze all four samples - store 5, 6
- lyophilize 3 and 4. in standard way.

12-9-76

The two samples had lifted out of the ice/salt mixture and had thawed. There was no evidence that ~~the~~ bumping had occurred. However, the system may have been at P_{atm}.

12-12-76

After fixing the oil pump, I re lyophilized sample 7 (had lot of liquid) at ~~the~~ ice/salt temperature.

12-13-76

- by morning, the sample was dry
 Obs. - I don't know whether this sample is strictly quantitative - there was only a very small amount of DNA left in flask.

~~12-14-76~~

12-13-76

- add 5.0 ml 10% MeOH - formed a clear (blue-tinged) solution (no solubility problem) after heating with tap water.