

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	
	Extract DNA	6/3+10/69	13
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Jason Largent	Tim Van		Bio Chem

Title: Extraction & Characterization of Bacterial DNA

Reference: Experiment #13 A+B in modern Exp. Bio Chem, 3rd Slides on blackboard, Bacterial DNA

Synopsis: To extract DNA from the Bacteria *Bacillus subtilis* + then analyze it with Spectrophotometric methods, Using the absorbance, find the concentration of DNA + assess its purity. Then determine the hyperchromic effect.

Procedure

Suspended freeze-dried bacteria in 125 mL Saline-EDTA in 50 mL E-Flask

add 0.5 mL of 10 mg/mL lysozyme solution

incubate @ 37°C for 30 min

add 1.0 mL of 25% SDS ^{solution} + heat @ 60°C for 10 min + a microspatula tip full of solid SDS

Cool to room temp

add 4.5 mL of 5M Na perchlorate
Place in 50 mL Nalgene centrifuge tube

add 20.0 mL of 24:1 chloroform: iso amyl alcohol.

gently shake 20-30 min w/ venting

Observations

0.0992g of bacteria

Milky yellowish, not dissolved

Still cloudy, less yellow more off white

Cleared up a bit after heating but still some cloudiness, a bit more yellow again

turns to a milk like color.

needed venting 2-3 times.

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Procedure

Centrifuge @ 7800 RPM
for 5 min

Using a pipet, xfer top
aqueous layer w/ DNA into small
beaker

add 2x 5mL of ice cold ethyl
alcohol down side of beaker
w/ pipet

Use a glass stirring rod.
Gently swirl + spin gathering
DNA strands.

dissolve the strands in 5mL
of Tris Buffer

xfer 2mL of ↑ into a new
vial

add 10μL of Ribo nuclease A
(20mg/mL)

store in fridge

Observations

clear layers at top
& bottom with a milky
thin layer between

avoid the milky protein
layer, even if leaving some behind.

did this for 20 min.
- kept getting a thin string around
rod where the 2 layers meet.

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Procedure 2.7 mL

Untreated DNA Sample
analyzed w/UV Spec.

dilute to $\approx 0.400 A_{260}$

ribonuclease A
Treated w/RNA
analyzed

Standard DNA control
analyzed

The control was split into
2 - 2 mL portions, (~~both~~ heated)

The other 2 were divided
into 3 equal portions,

2 samples from each group were boiled for 5 min in a
water bath. 1 sample of each was then placed in
an ice bath. (quick cooled) while the other
was allowed to cool slowly to room temp (slow cooled).
The slow cooled sample was analyzed while still hot,
all samples were analyzed when back at room temp.

Observations

A_{260} 0.6864
 A_{280} 0.3747

not enough sample to get
to 10.0 mL, diluted to
4.63 mL
 $A_{260} = 0.4299$

A_{260} 0.6731
 A_{280} 0.3373

diluted to 5.5 mL
 A_{260} 0.3500
 A_{280} 0.1811

A_{260} : 0.4403
 A_{280} 0.2518

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The values obtained are given in the table below

	RT 1	RT 2	SC Hot	SC RT	QC RT
untreated	0.4299	0.457	0.7510	0.589 *	0.4957
treated	0.3500	0.378	0.4514	0.589 *	0.4104
Standard	0.4403	NA	0.6152	0.612	0.603

Table 1. A₂₆₀ Values for samples, RT = room temp, SC = slow cooled, QC = quick cooled.

* After measuring the untreated + treated hot samples, they were accidentally returned to the same vial, mixed.

Calculations:

untreated dilution.

$$\frac{\text{observed } A_{260}}{\text{desired } A_{260}} = \frac{0.6864}{0.400} = 1.716 \text{ titration factor}$$

titration factor x Volume = total Volume to dilute to.

$$1.716 \times 2.7 \text{ mL} = 4.63 \text{ mL}$$

treated

$$\frac{0.6731}{0.400} = 1.683 \times 2.9 \text{ mL} = 4.88 \text{ mL}$$

(note this was very low A₂₆₀, and 1 mL of the other untreated sample was added, that had same readings)

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Concentration of DNA isolated

untreated: $\frac{0.4299}{x} = \frac{1.00}{50 \frac{\mu\text{g}}{\text{mL}}}$ $x = 21,495 \mu\text{g/mL}$

$(x) \times (TF) = 21,495 \times 1.716 = 36.89 \mu\text{g/mL}$

$\times 3 \text{ mL original} = 110.66 \mu\text{g}$

~~treated~~

theoretical yield

1880 $\mu\text{g DNA}$

$\times 0.0992\text{g} = 746.0 \mu\text{g expected}$

0.250 g cells

$\frac{110.66}{746.0} \times 100 = 14.83\%$

Purity ratios

$\frac{A_{260}}{A_{280}} = \text{ratio}$

Pure DNA ≈ 1.90

untreated

$\frac{0.6864}{0.3747} = 1.832$

treated

$\frac{0.6731}{0.3373} = 1.996$

Standard

$\frac{0.4403}{0.2518} = 1.749$

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hyperchromic effect calcs,

Un treated ~~And~~ using RT#2 as comparison to account for any time impact

SC _{hot}	SC _{RT}	QC@RT
$\frac{0.7510}{0.457}$	$\frac{0.589}{0.457}$	$\frac{0.4957}{0.457}$
1.64	1.29	1.08

Treated

SC _{hot}	$\frac{.4514}{.378} = 1.19$
SC@RT	$\frac{.589}{.378} = 1.55$
QC@RT	$\frac{.4104}{.378} = 1.09$

Standard

SC _{hot}	$\frac{.6152}{.4403} = 1.39$
SCRT =	$\frac{.612}{.4403} = 1.39$
QC@RT =	$\frac{.603}{.4403} = 1.37$

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Conclusions/Discussion

During the preparation of the samples, several steps were taken to isolate the DNA. The freeze dried cells were placed in EDTA to help buffer it from changes in pH and ionic strength. Lysozyme was added to break the cell walls, along with heating. This helps release the DNA, which resulted in a thicker fluid. SDS detergent was then added to help dissociate the protein/lipid complexes within the cell membranes, and denatures proteins. Sodium perchlorate is then added which increases the salt content to help reduce ionic interactions with the DNA and cations. A solution of chloroform and isoamyl alcohol (24:1 ratio) was added to further denature the protein in the sample (done by the chloroform) and to help provide a more distinct barrier between the aqueous and organic layers (done by the alcohol).

After centrifuging, the top layer (aqueous) should not have any proteins left in it, and it was placed in a small beaker. Ice cold ethyl alcohol was added slowly, and the layer between the DNA solution and alcohol would help precipitate the DNA when stirred and spun with a glass rod. The DNA was removed and redissolved in a Tris buffer for storage and analysis. One sample had Ribonuclease A added to destroy any RNA, which could then be compared to the untreated sample and determine if there was any RNA contaminating the results.

Several analyses were performed on our samples of untreated DNA recovered from *Bacillus Subtilis* and one treated with Ribonuclease A. The same analyses were performed on a standardized sample of pure DNA. The major areas examined were looking at the purity ratio, looking at the hyperchromic effect and determining the original concentration of DNA in the sample collected.

Using the untreated sample A260 result and the known value that 1.00 A260 is roughly 50 ug/mL. The measured value of 0.4299 A260, using the dilution factors, estimated that we started with 110.66 ug. The theoretical yield, given that there is 1880 ug/.250 g of cells and we started with 0.0992g was 14.83%, which seems pretty low. We spun the DNA on the stirring rod for over half an hour, but we were still getting some small amounts. Perhaps we could have spent more time gathering DNA.

The purity ratios were found comparing the A260 to A280, which are the max absorbances for DNA and Protein. Pure DNA is known to have a ratio of 1.90. Our ratios for untreated = 1.832, treated = 1.996, and standard = 1.749. These results are somewhat confusing, as the pure standard was actually the lowest ratio. The smaller the ratio represents a higher amount of protein present generally. The treated was the highest, which may be due to the RNA being destroyed and not interfering with the results. These results do speak highly of the efficiency of the chloroform-isoamyl alcohol at removing contaminating proteins, as all of the ratios were relatively high. Given that the treated sample was the closest to "pure" DNA, it could be that the RNA left over in the untreated sample contaminated the results.

When looking at the results for the hyperchromic effect, it is expected that DNA heated then cooled back to room temperature should have roughly 40% higher absorbance than it did prior to heating. This is due to the fact that single stranded DNA has higher absorbance than double stranded DNA. When the DNA is heated, the hydrogen bonds between the strands are broken (denaturing the DNA). As the solution is allowed to cool, the strands can reform, but they do not always form back into a standard double stranded helix. It was expected that the quick cooled sample would have higher A260 increase than the slow cooled, but still less than DNA that is still hot. This is due to the quick cooling not having a chance to form double stranded bonds as readily as slowly cooled samples.

The results were inconsistent between the types of samples. For untreated, the A260 while boiling was 64% higher, but the quick cooled had the lowest (8%). The slow cooled sample was 29% higher. Untreated, boiling was 19% higher, slow cooled 55%, and quick cooled 9%. Standard was consistent across all 3 tests, 40% hot, 39% slow cooled and quick cool was 37%.

None of the samples met the expectations of boiling > quick cool > slow cooled. There was not a consistent pattern between the types of samples either. That the boiling sample wasn't quite as high as expected in some cases could be due to the lack of speed in getting to the spectrophotometer. The quick cool was consistently the lowest

