SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27	28	29	30	31	July 02
	9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality					
July 03	04	05	06	07	08	09
	Holiday (Independence Day)	8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	Free Day	Classes begin! 8:30-11am: BIOL4905 INTRODUCTION 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 DNA PREPARATION 8-10:20pm: Afternoon course	
10	11	12	13	14	15	16
	8:30-11am:BIOL4905 PROTEOMICS I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS III 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 RNA PREPARATION 8-10:20pm: Afternoon course	Virtual Independence Day Activity	
17	18	19	20	21	22	23
	8:30-11am:BIOL4905 qPCR / ROBOTS 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 DNA Sequence Analysis 8-10:20pm: Afternoon course	Midterm Break	8:30-11am:BIOL4905 Next Gen. Sequencing 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Automated Microscopy /AFM	
24	25	26	27	28	29	30
	8:30-11am:BIOL4905 Microarray I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Microarray II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Nanostring 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Flow Cytometry 8-10:20pm: Afternoon course	FINALS	
31	August 01	02	03			
	9:00-10:00am: Closing Reception		Grades available in PAWS			

Legend:
Orange: Courses Blue: Activities

Gel Electrophoresis Preparation

1. Prepare **200 ml 1x TBE buffer** from 10x stock (20 ml 10x TBE + 180 ml diH_2O)

2. Prepare **60 ml of 1% agarose** with 1x TBE buffer (0.5g agarose + 50 ml 1x TBE). Microwave for 40 seconds two times (not 80 seconds). Use gloves!

3. Once the side of the beaker is able to be touched **pour into casting tray** and insert comb.

4. While this solidifies move onto DNA preparation, but don't forget to come back to this **once solidified** and insert the tray into the gel running tank and **cover with 1x TBE** until gel is slightly covered. When placing the tray, ensure the comb side is aligned with the negative electrode side (black).

Gel Electrophoresis

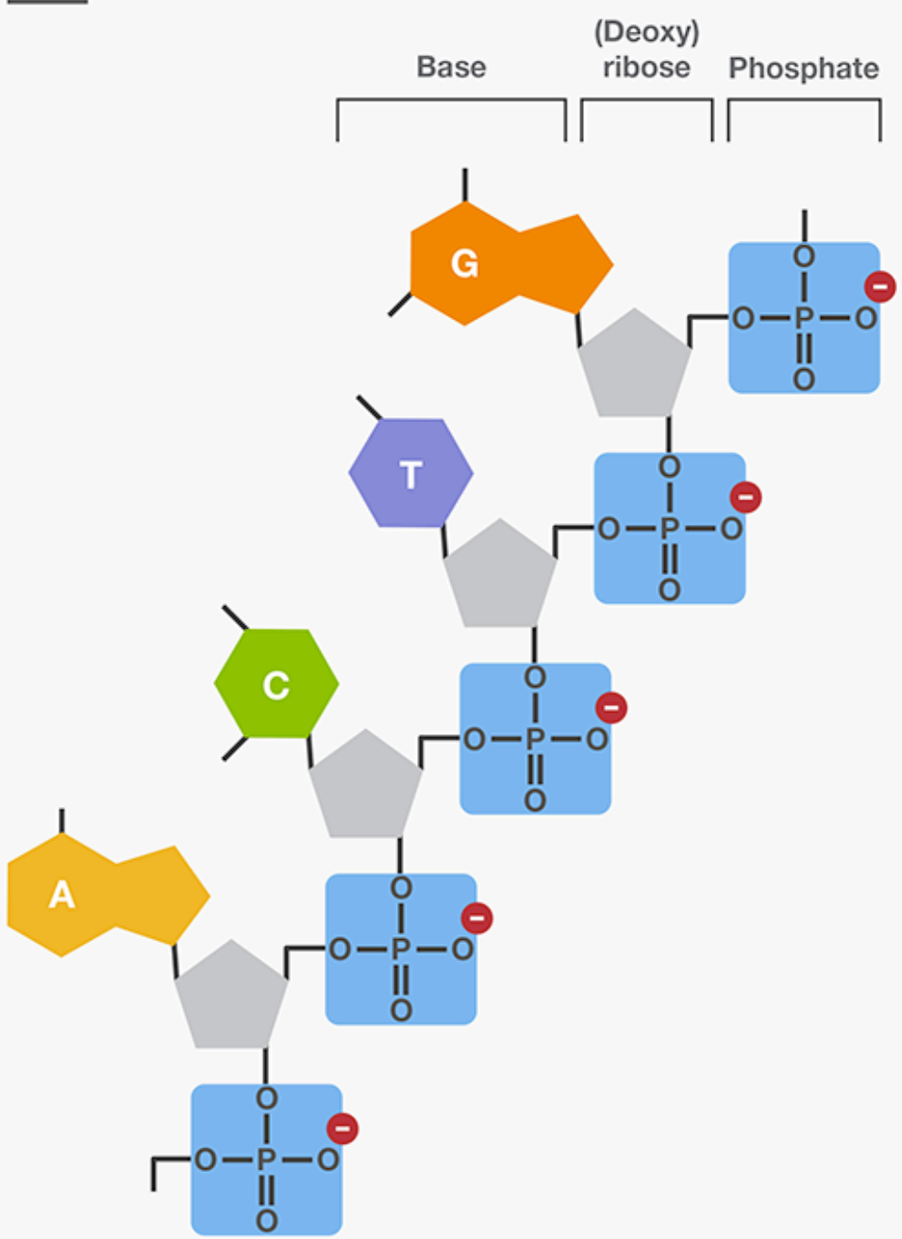
1. Take **5 ul of your DNA** sample and add it to a new microcentrifuge tube. Add **1 ul 5x DNA loading buffer**.

2. Load **5 ul of DNA ladder** to the first lane and **your sample** to the lane beside it.

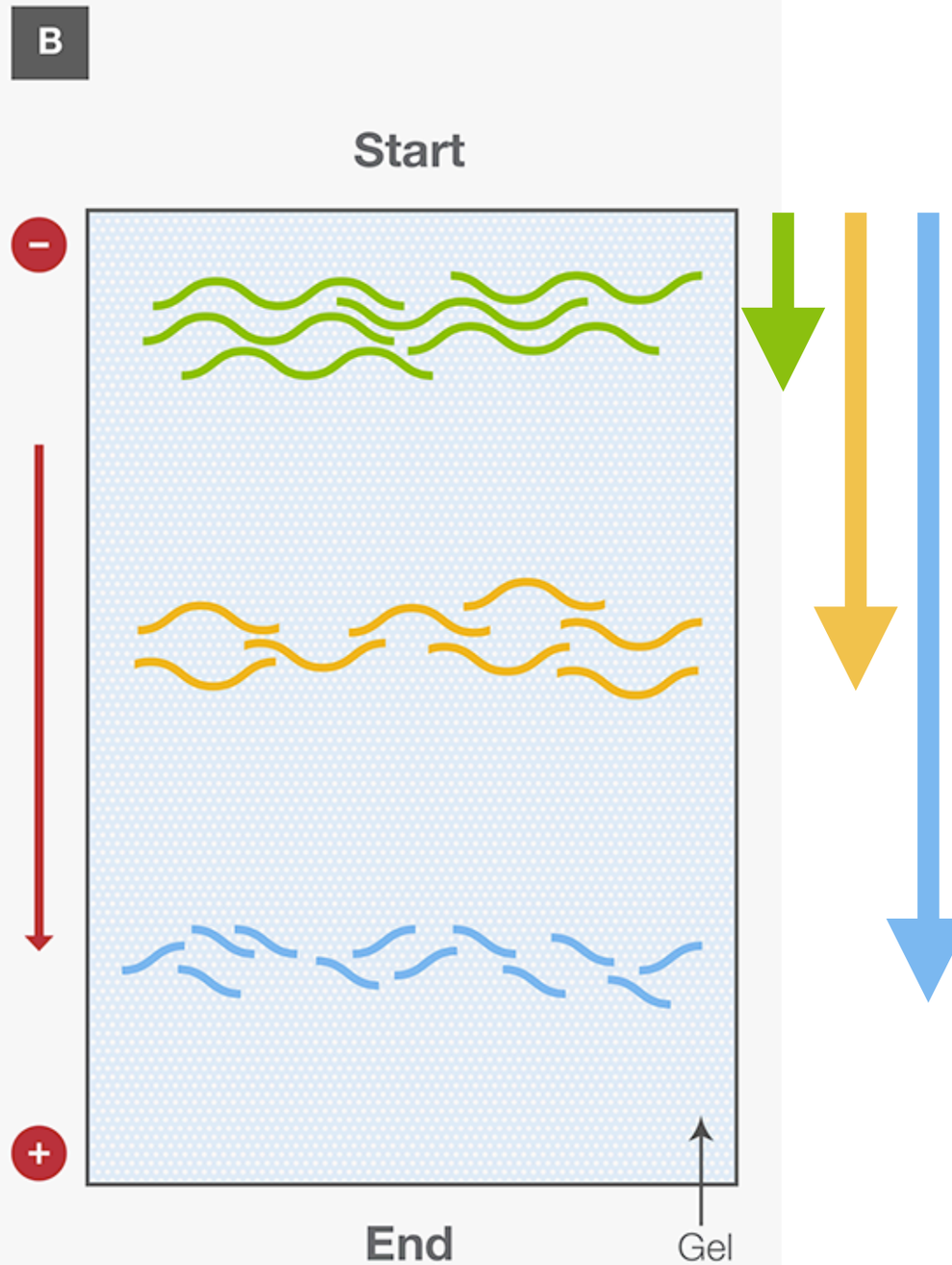
3. Start electrophoresis at **80V for 30 minutes** and power up to **100V**. Stop when the front running dye reaches ¼ from end. DNA will migrate from negative electrode to positive.

4. While your gel is running, check your DNA concentration using the **spectrophotometer**.

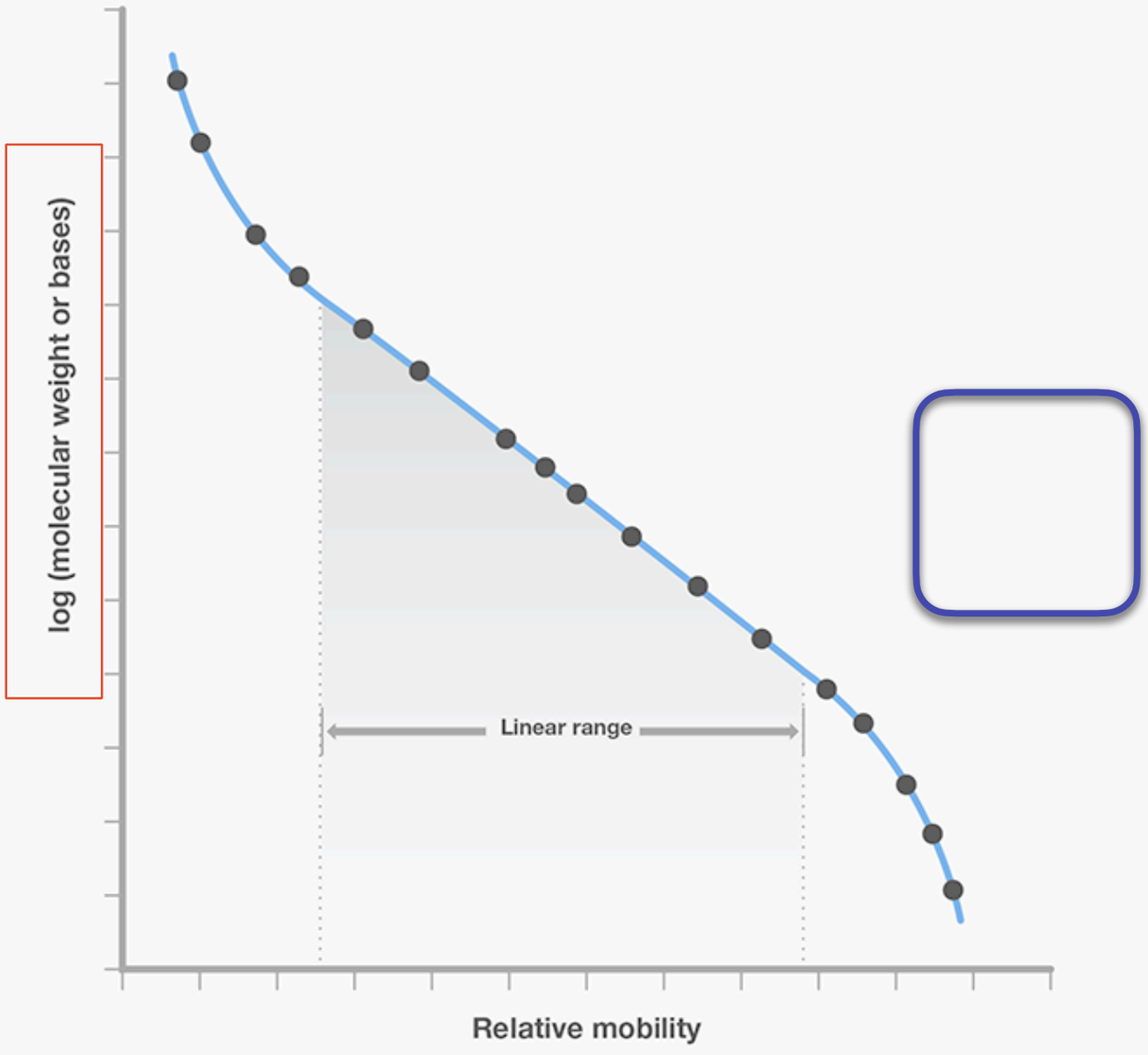
A



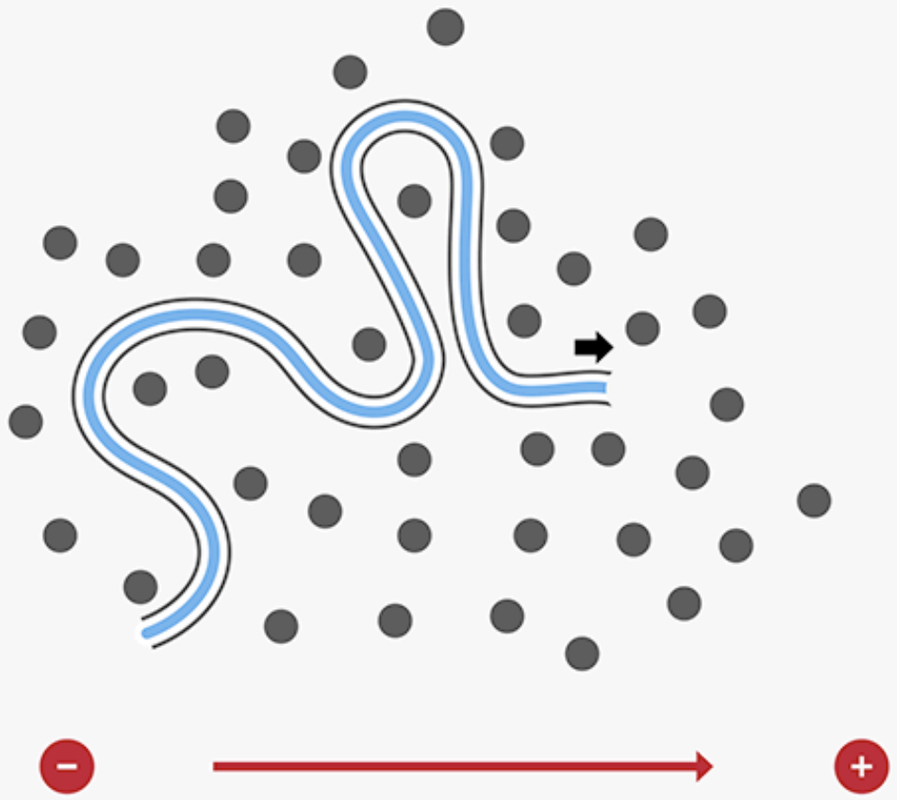
B



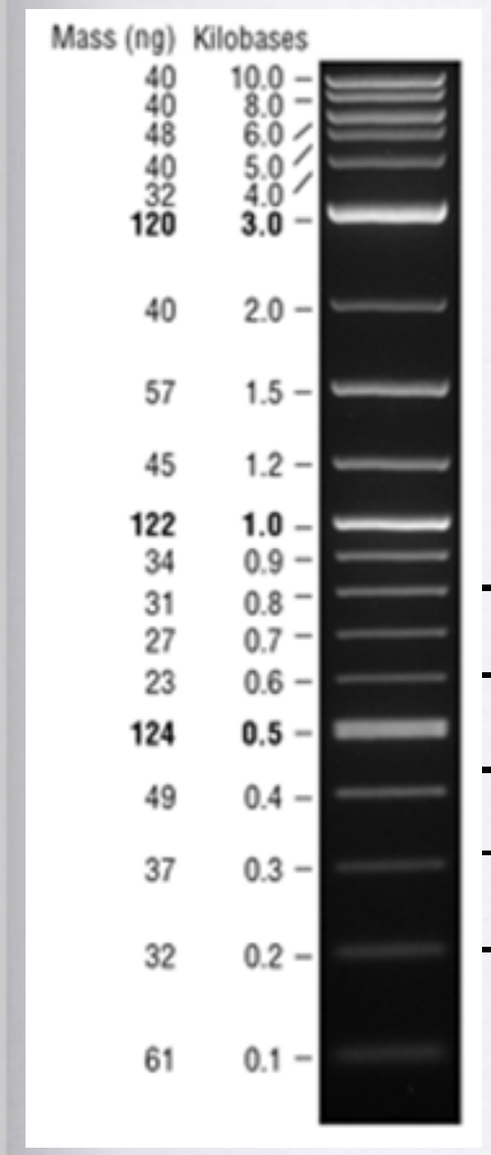
A

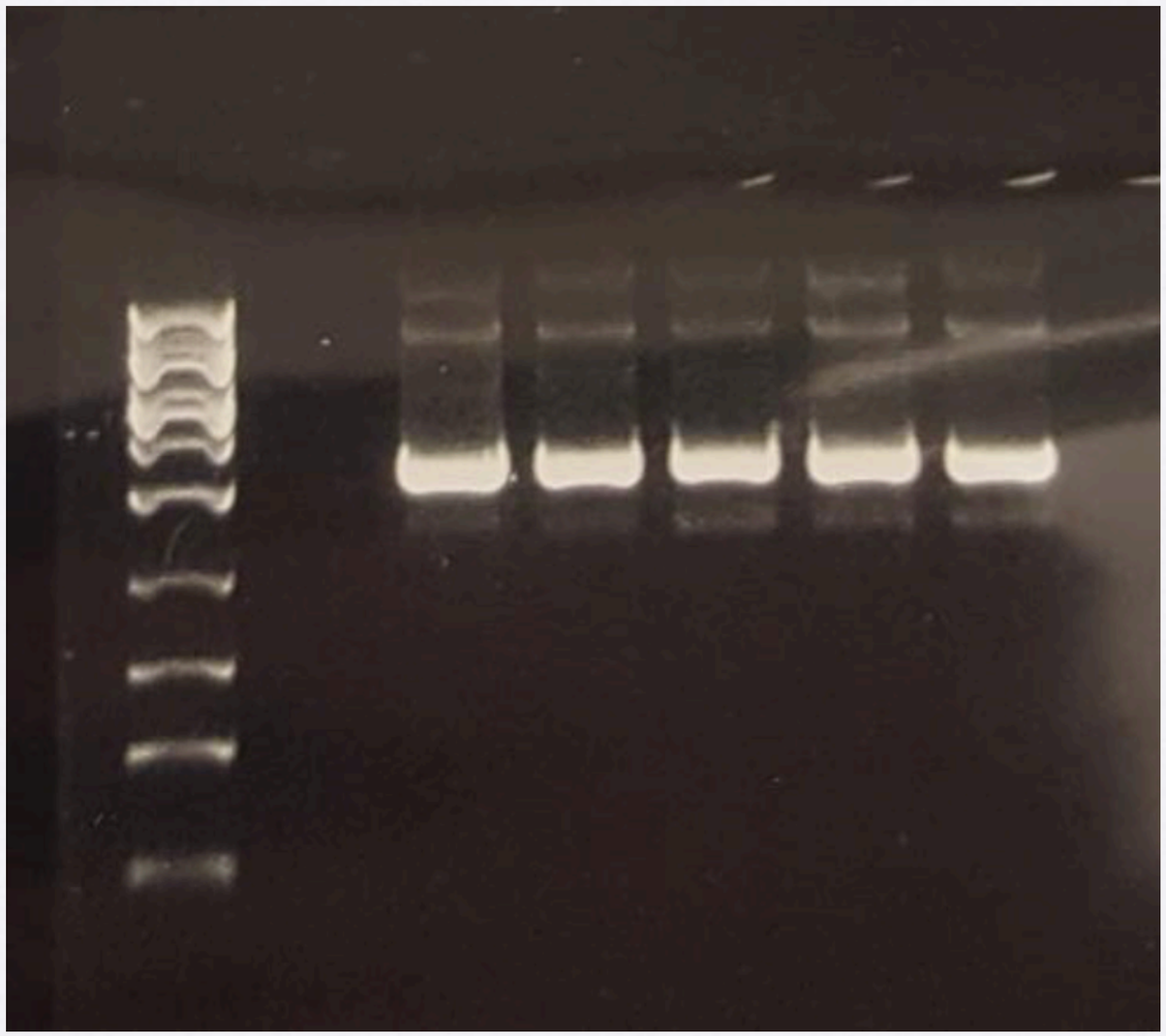

log (molecular weight or bases)
Linear range
Relative mobility

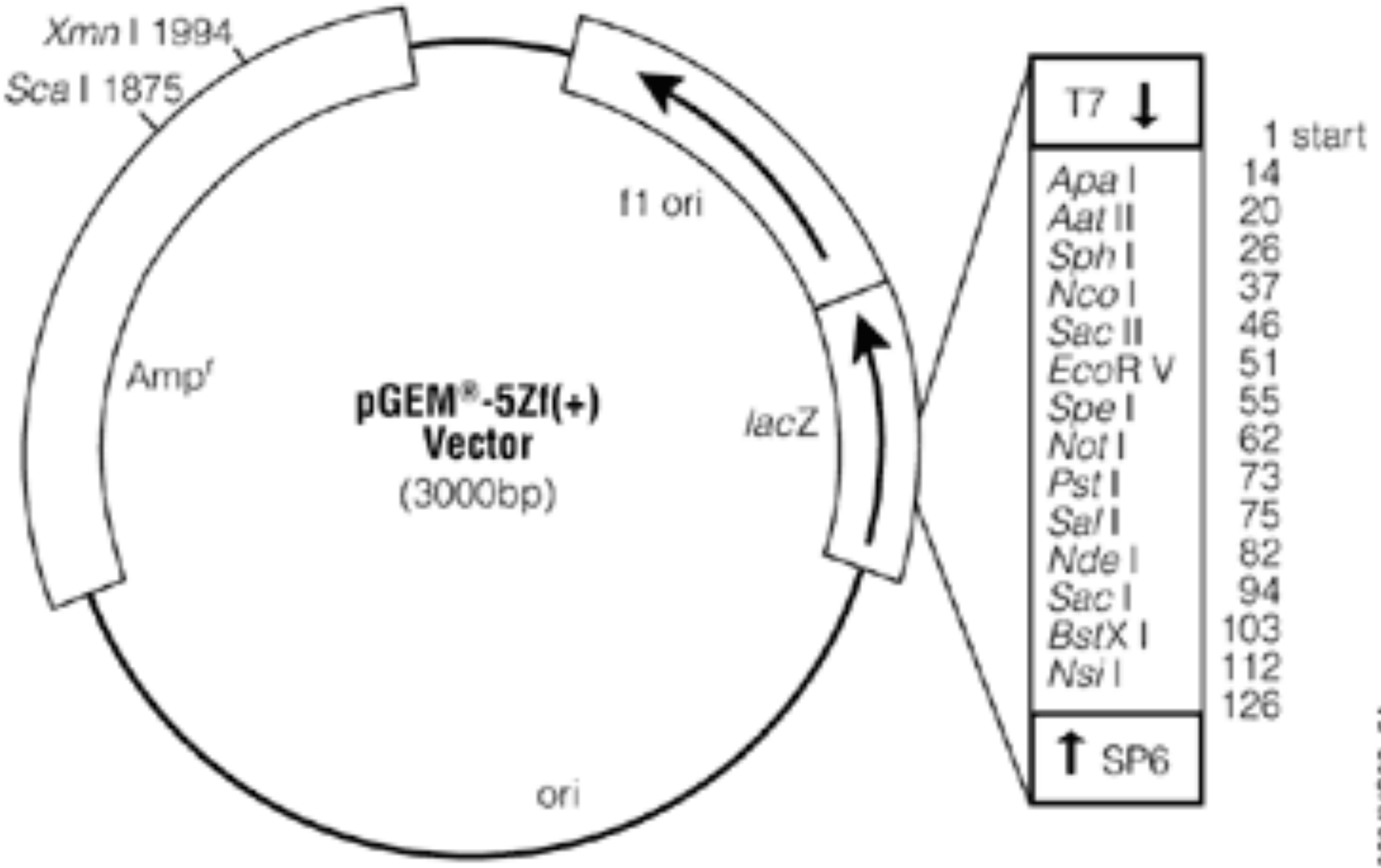
B

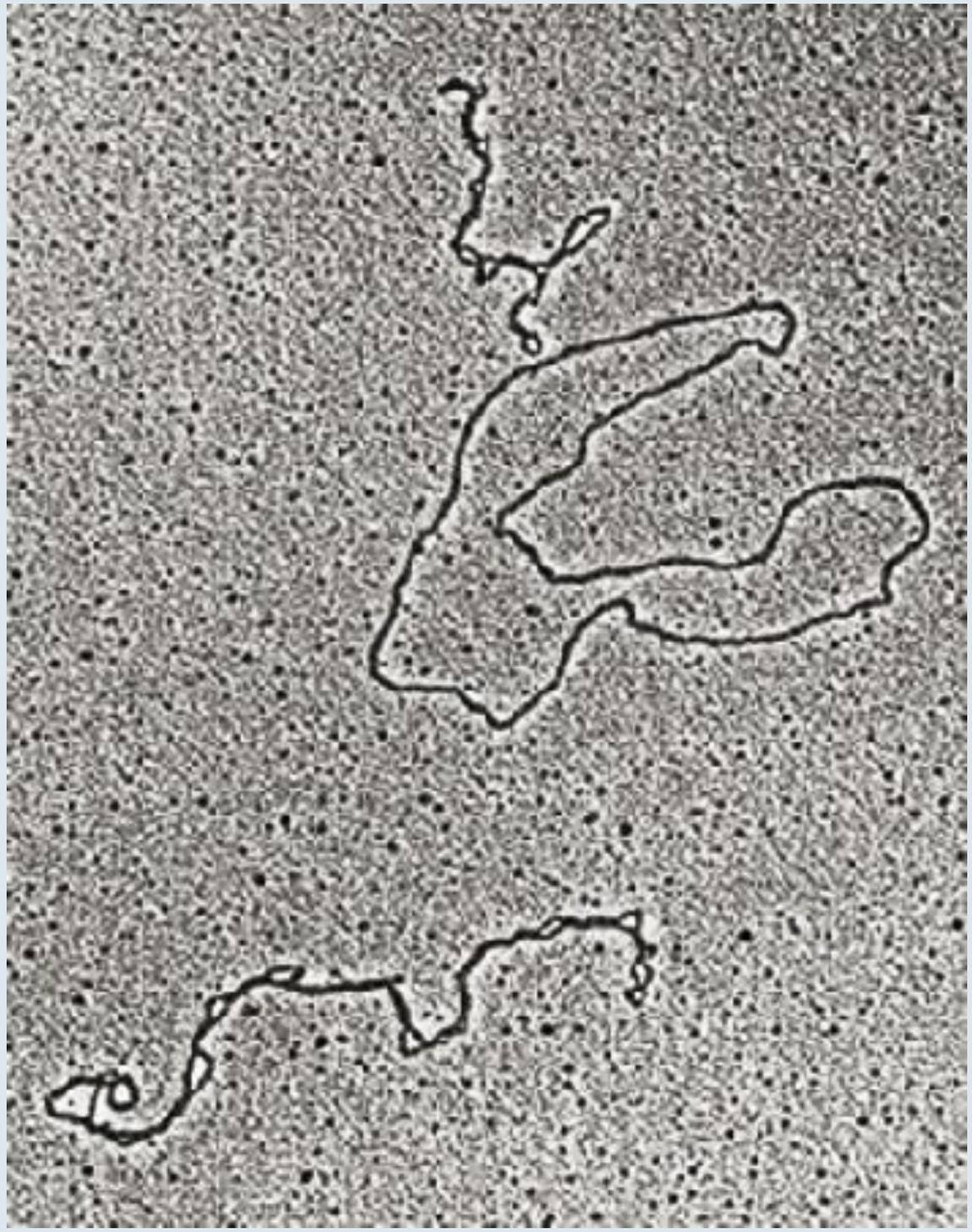


Control 6 7 8 9 10

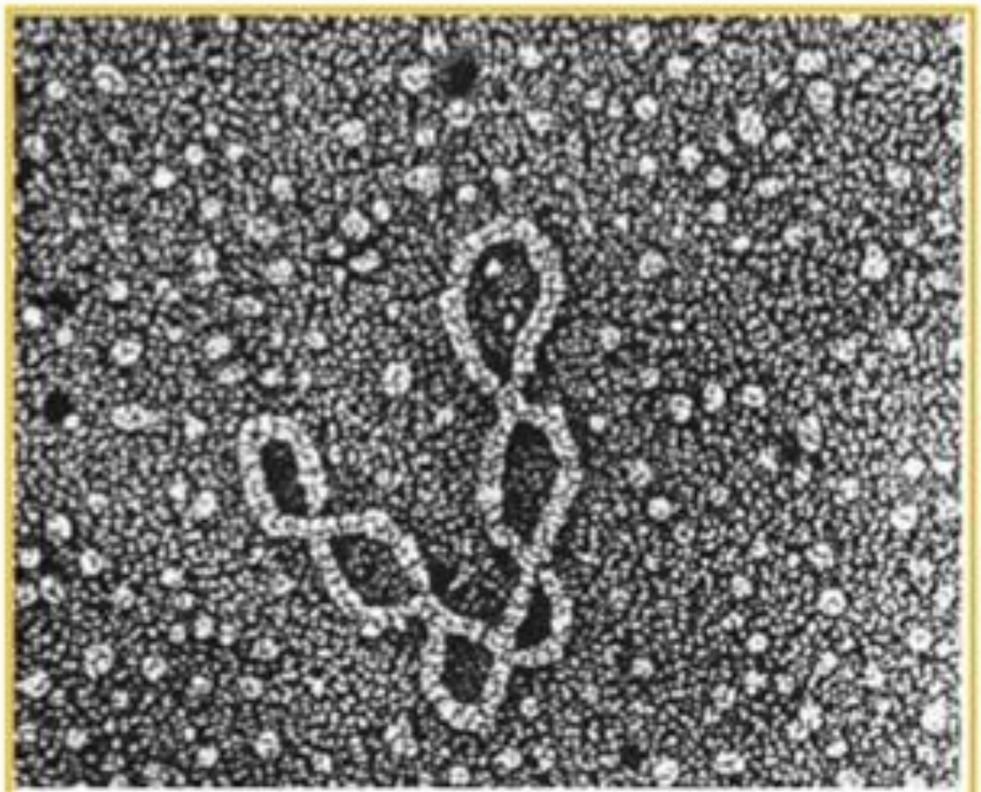





Xmn I 1994
Sca I 1875
Amp^r
f1 ori
pGEM®-5Zf(+)
Vector
(3000bp)
lacZ
ori
T7
Apa I 14
Aat II 20
Sph I 26
Nco I 37
Sac II 46
EcoR V 51
Spe I 55
Not I 62
Pst I 73
Sal I 75
Nde I 82
Sac I 94
BstX I 103
Nsi I 112
126
SP6
1 start
0284VC02_5A

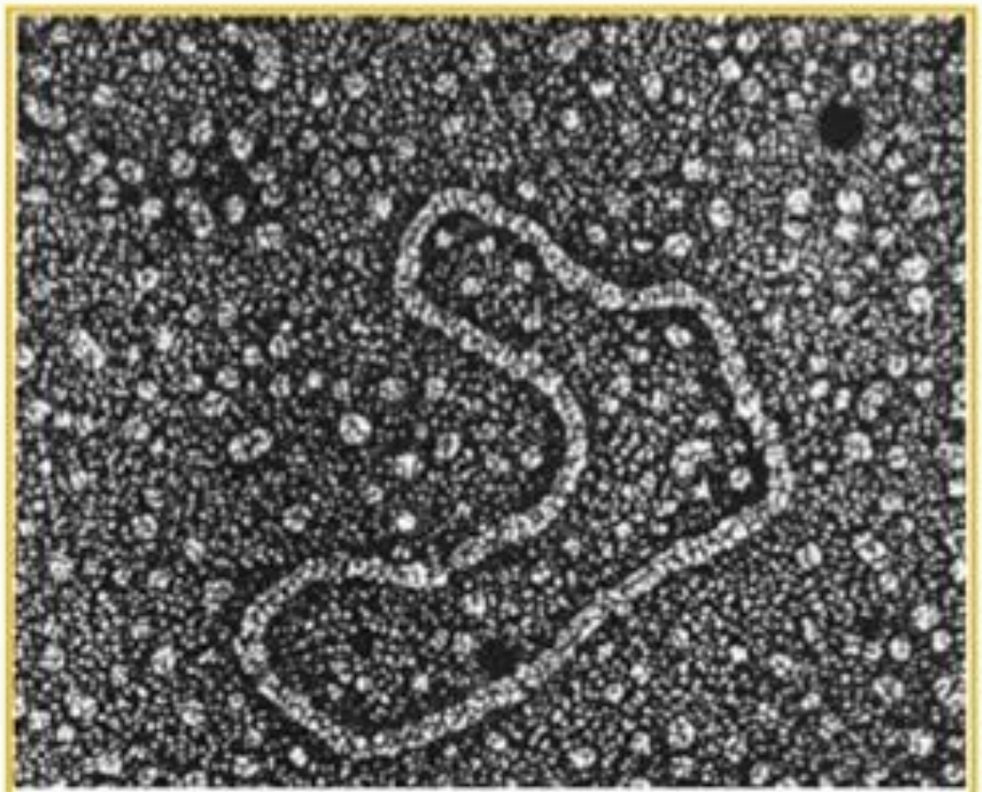


(a) Supercoiled

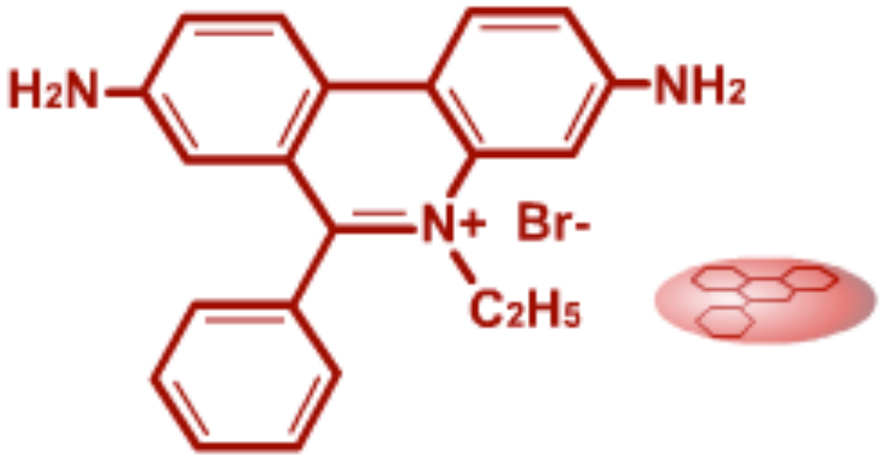


https://www.youtube.com/watch?v=az2c6UbEdug

(b) Relaxed circle

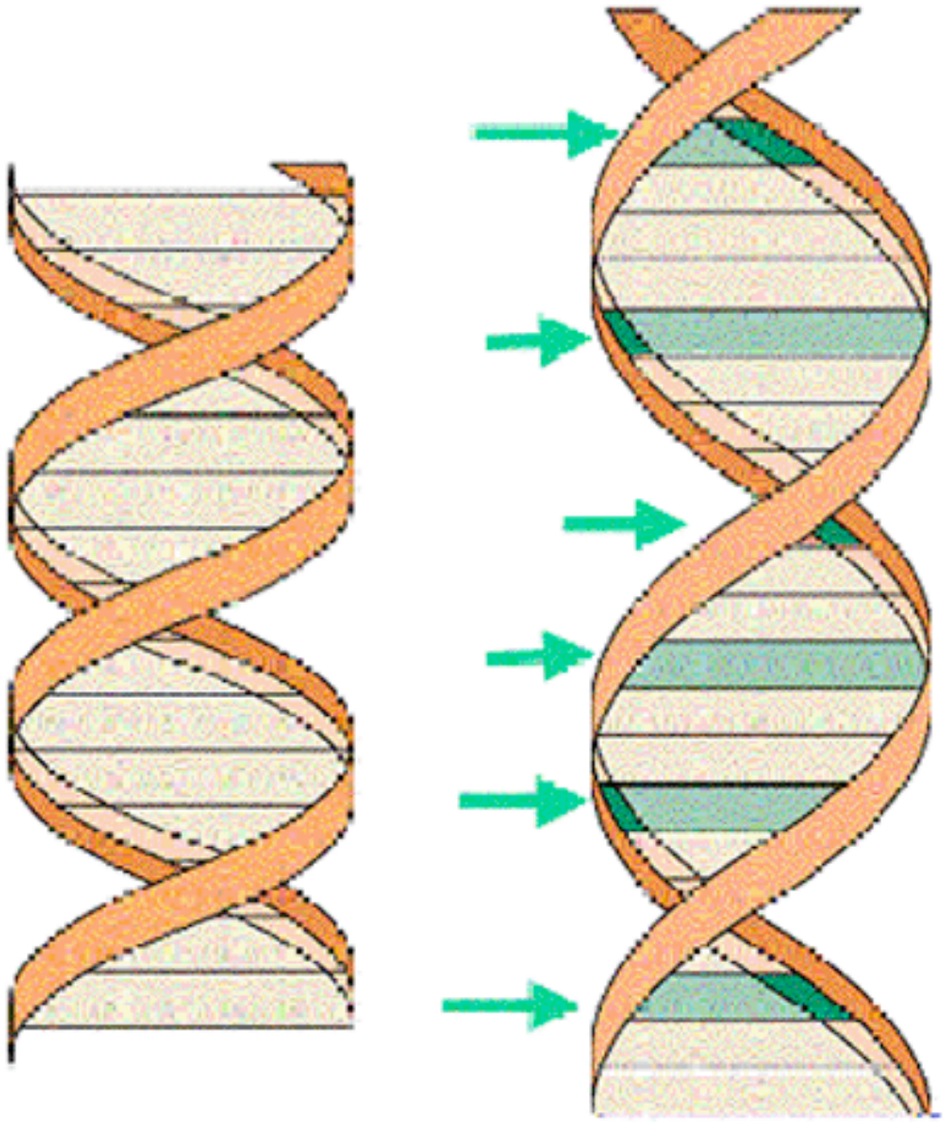


Ethidium Bromide



Ethidium Bromide (EtBr) is a planar compound which intercalates between base pairs in the DNA double helix. Each molecule of EtBr which intercalates causes the double helix to unwind, decreasing Tw and increasing Wr.

credits & copyright



Original DNA

DNA stretched by intercalated ligands

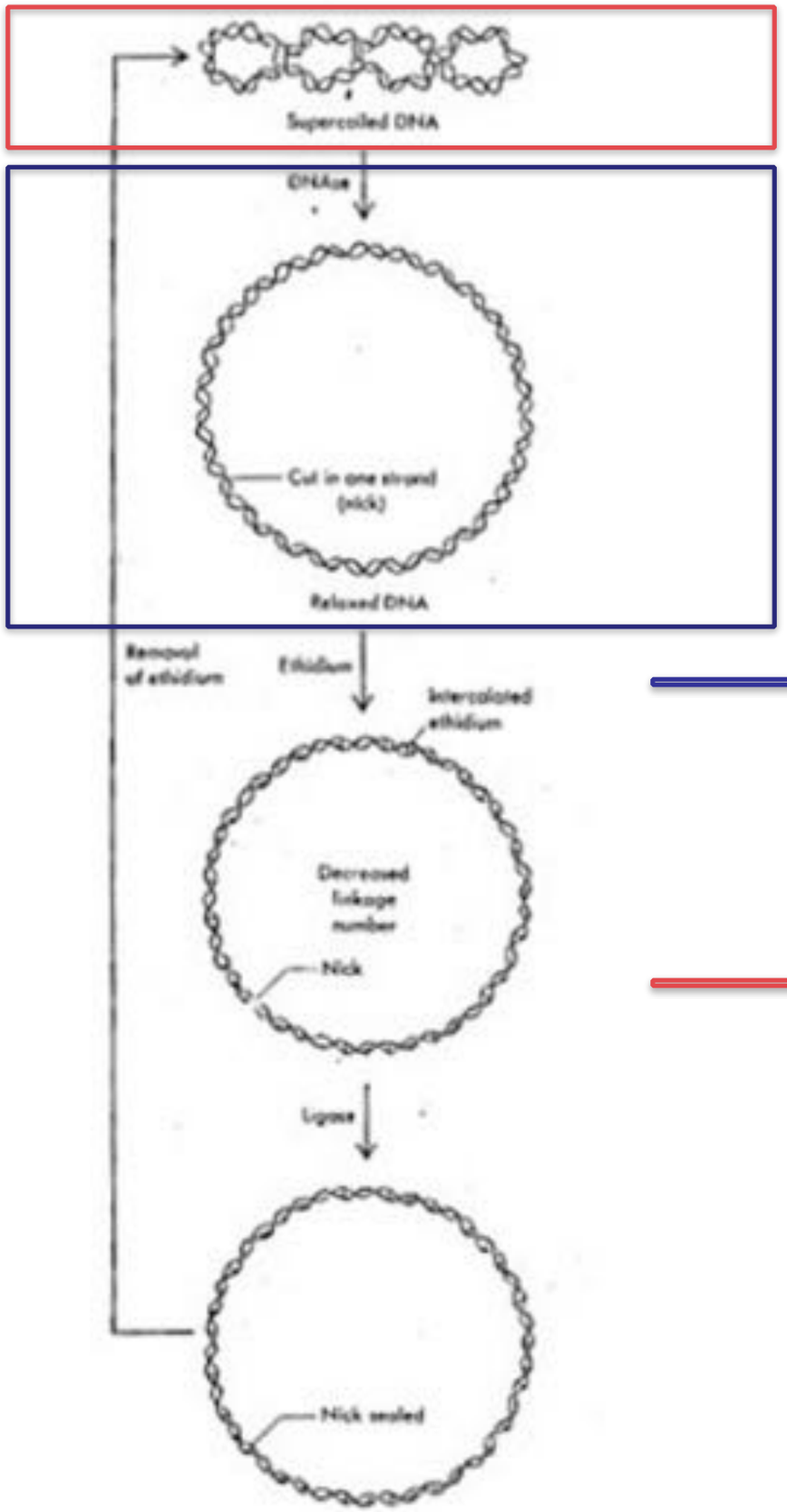
Stretching changes the frame needed by DNA Polymerase during replication.

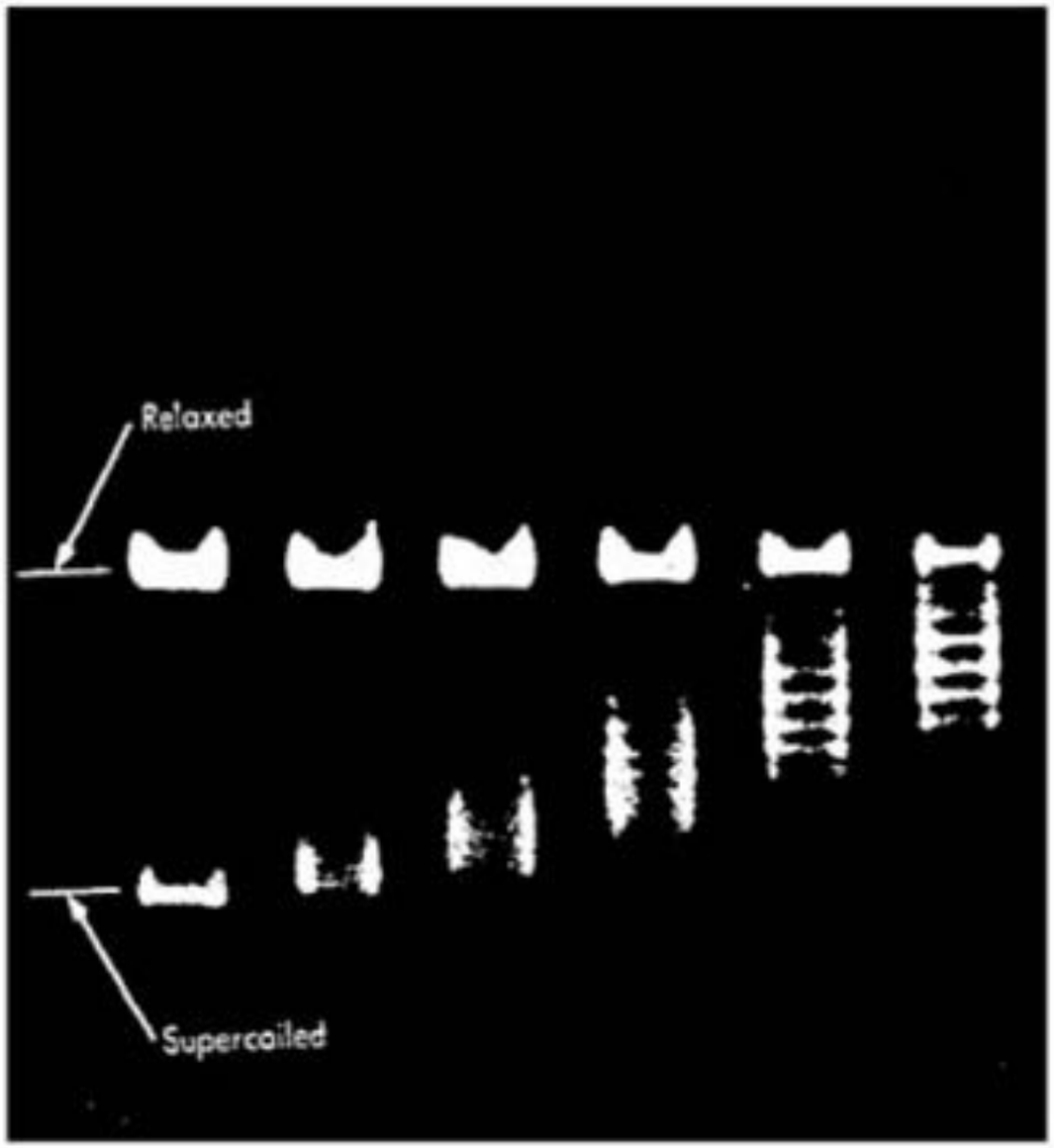
Extra nucleotides are added during replication

The DNA reading frame for RNA synthesis is changed:

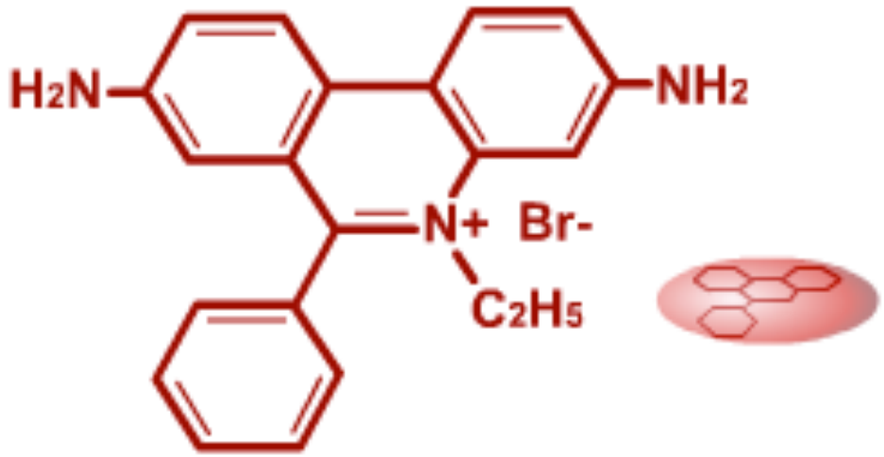
amino acid changes

altered protein


Supercoiled DNA
DNAse
Cut in one strand (nick)
Relaxed DNA
Removal of ethidium
Ethidium
Intercalated ethidium
Decreased linkage number
Nick
Ligase
Nick sealed


Relaxed
Supercoiled

Ethidium Bromide



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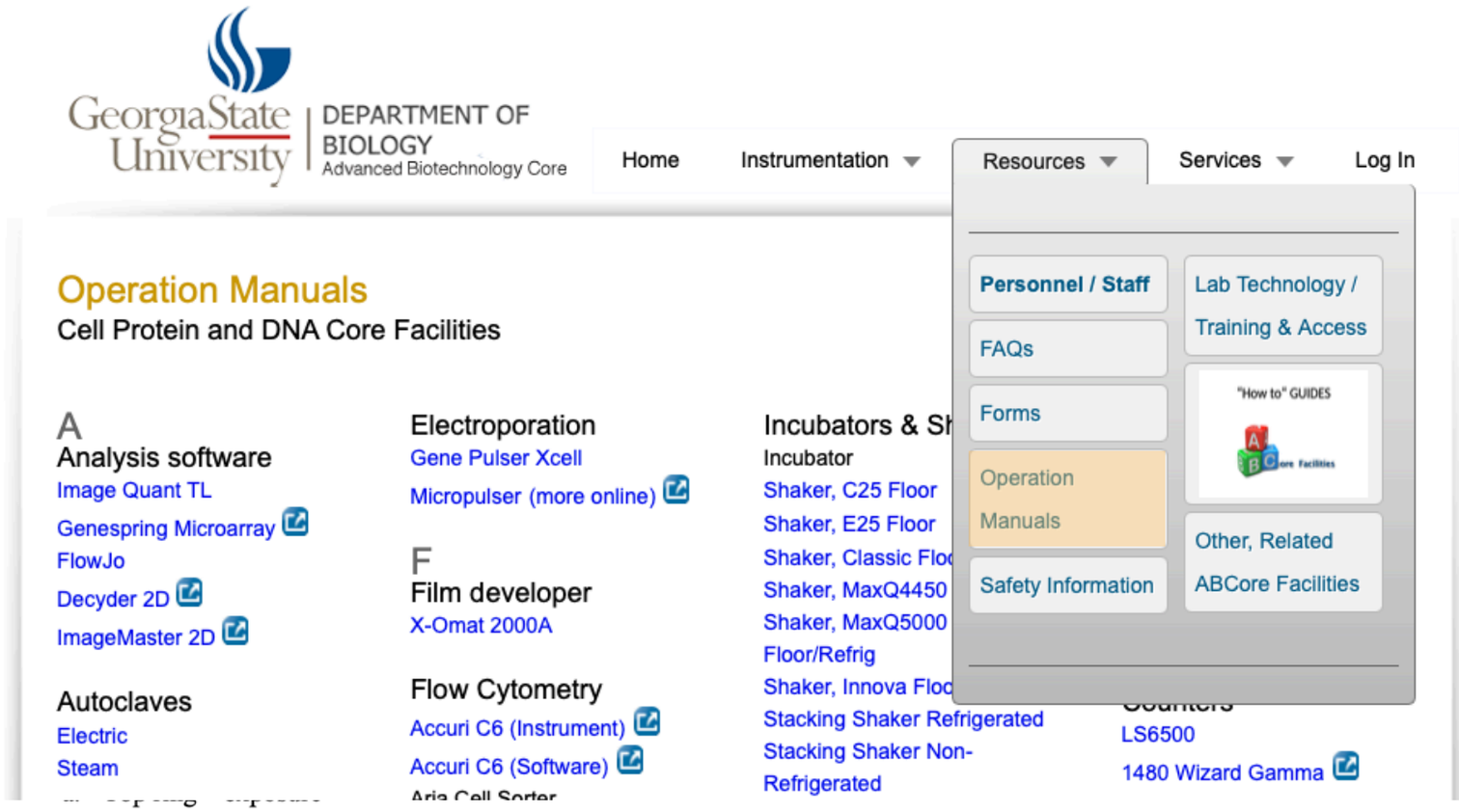
credits & copyright



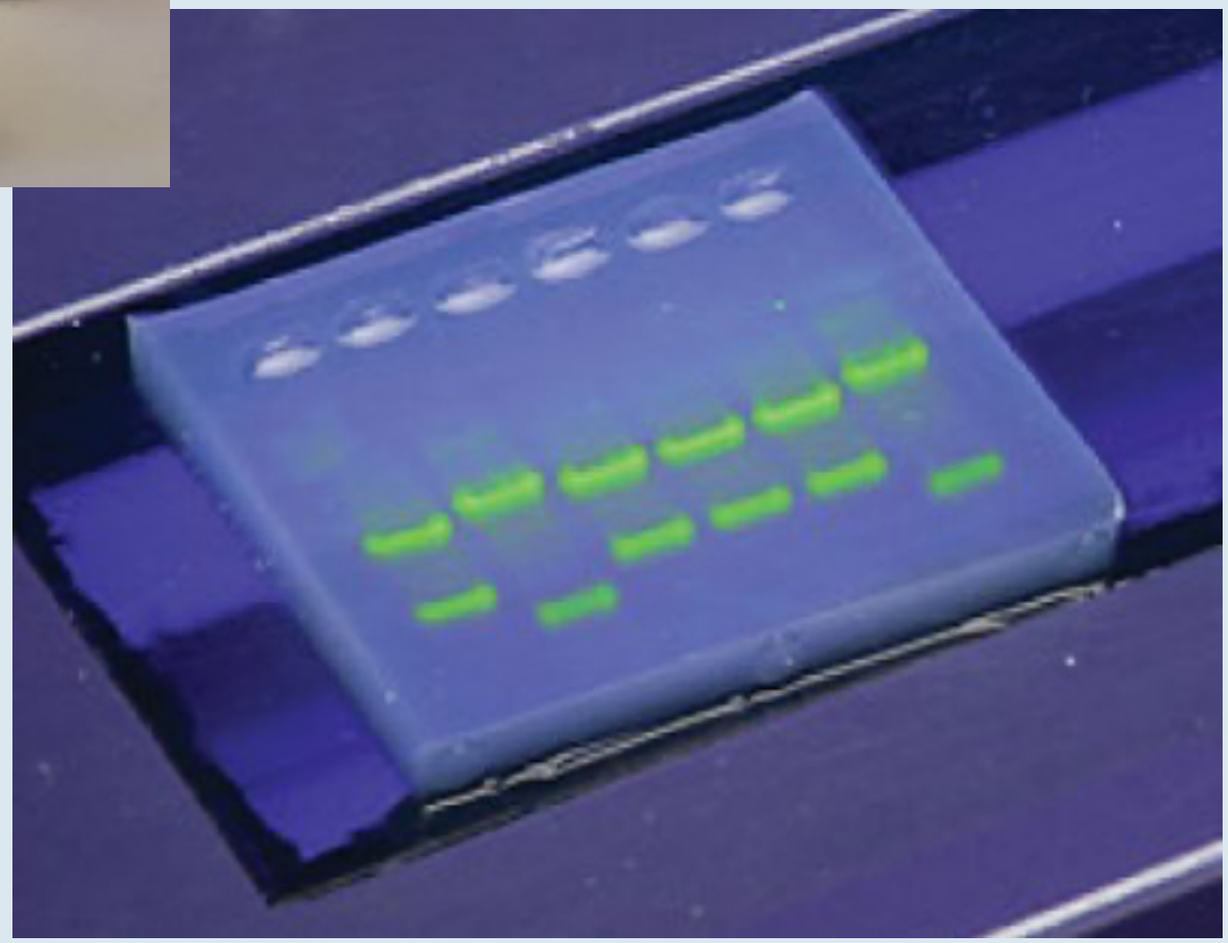
b. Middle ring – zoom
c. Bottom ring – focus

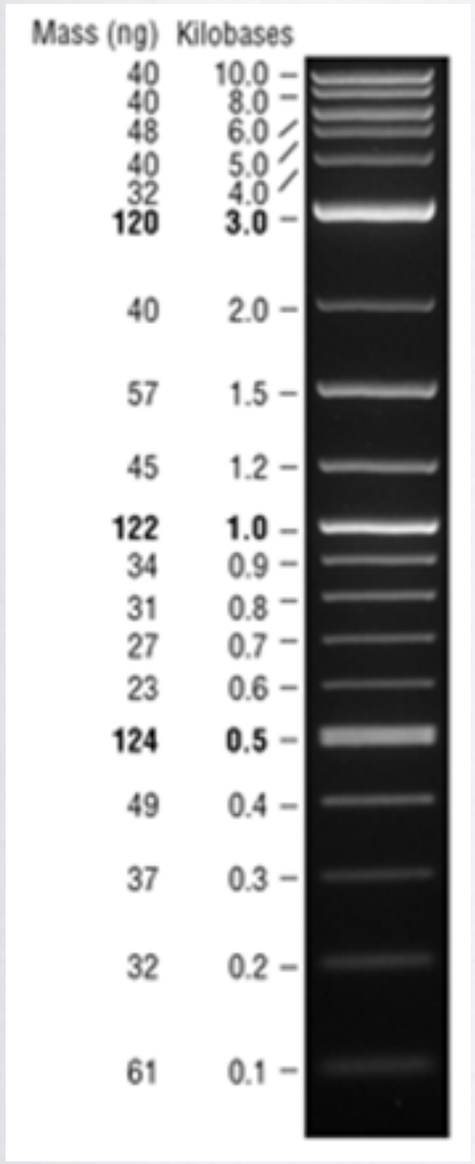
8. Press SNAP
9. To adjust image (invert, turn image), go back to Live. Select Preferences. Select PostProcessing tab (near the top).
10. Rotate image – use arrow to select; Invert – will change the image from black to white. OK.
11. Snap and now will see results of Preferences selection.
12. Press PRINT to print your image.
13. To save your image.
    a. Put a USB drive into USB port.
    b. Go to Preferences. Select Saving tab.
    c. Save images to: select usb
    d. Other selections do not change: autoprint – never; autosave – off; do not save images to computer or desktop
    e. OK
    f. Select save button on screen
14. Turn off ALL 3 – software, camera and transilluminator
15. Clean Transilluminator with H2O.

UVP GelDoc

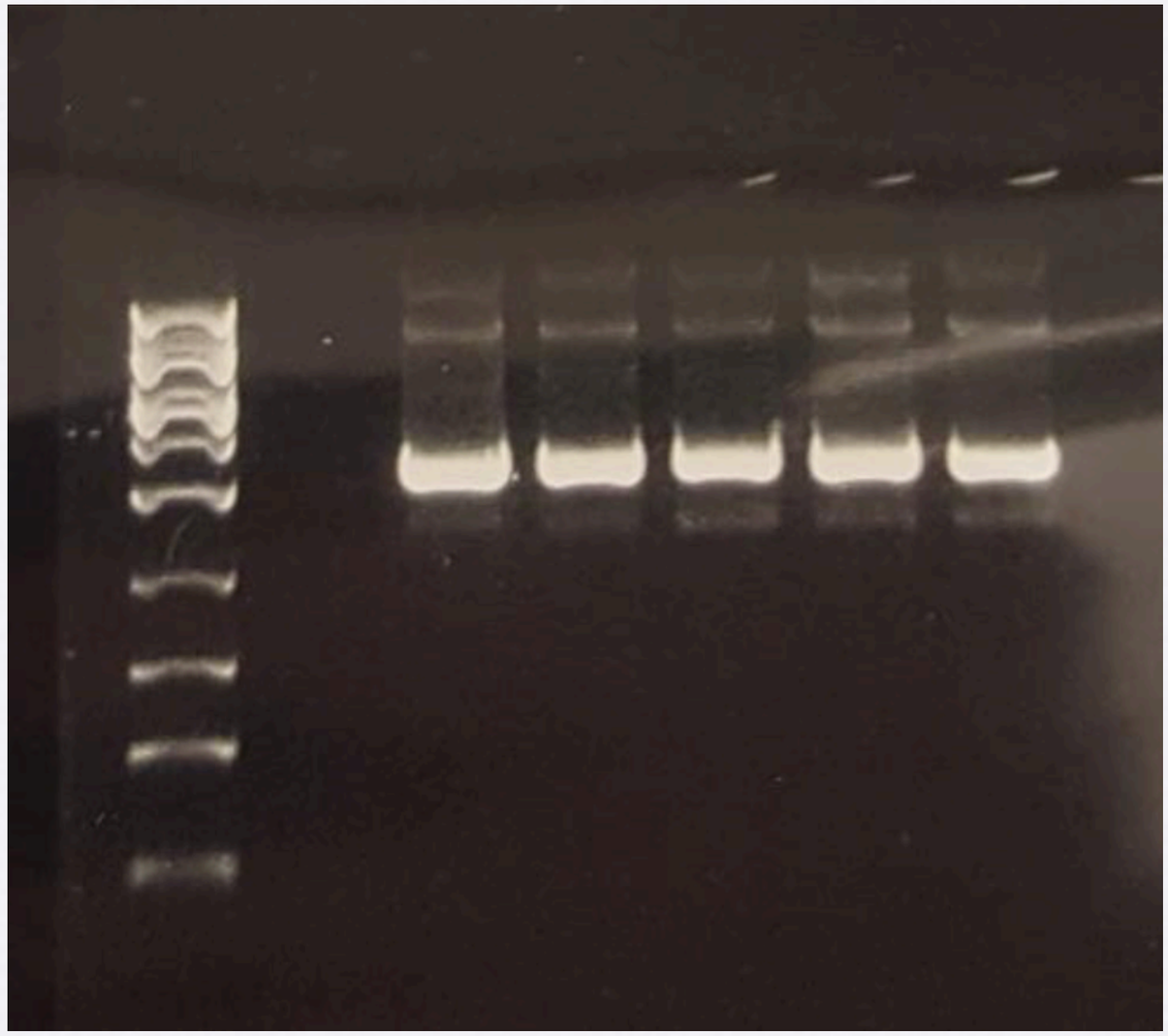
Gel documentation imaging s

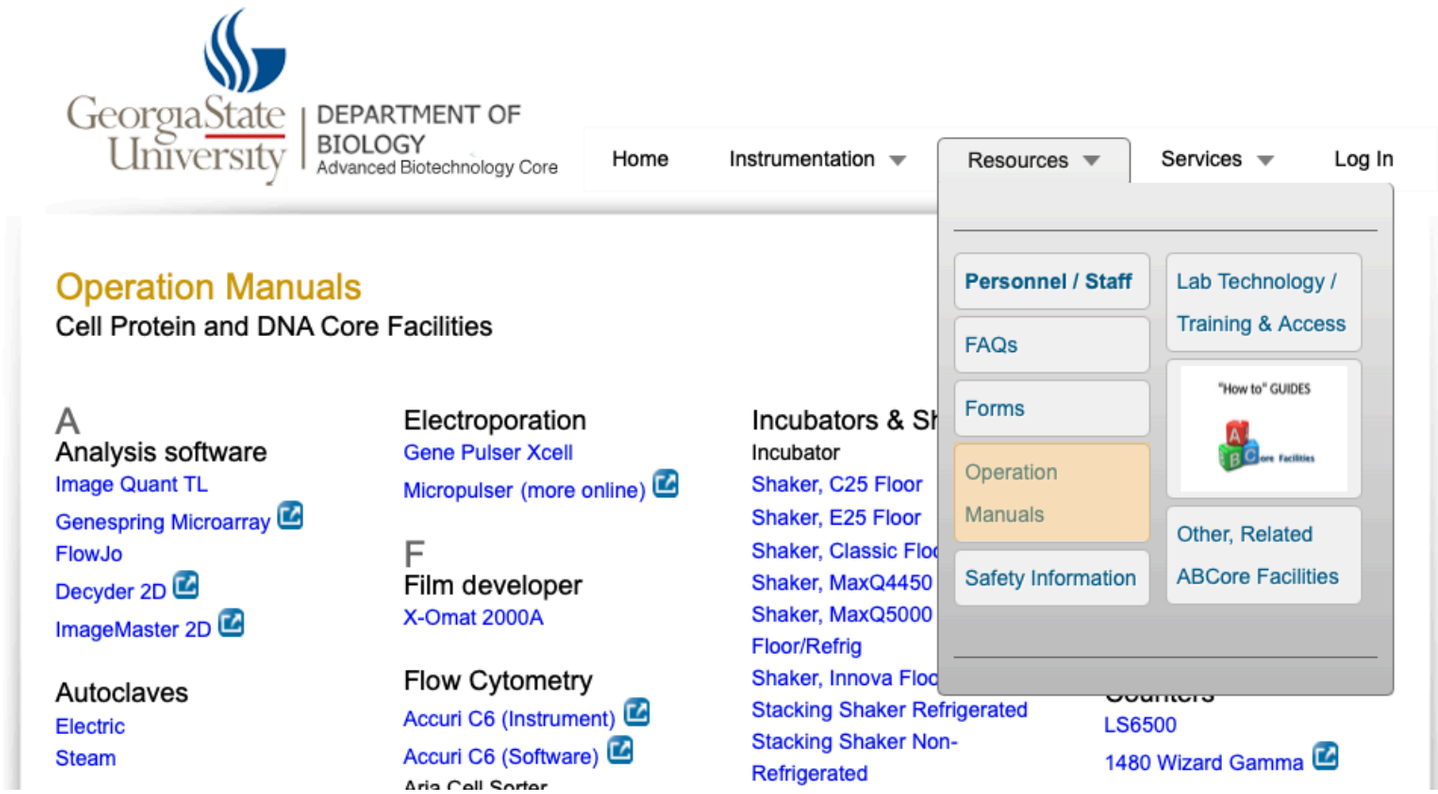

DNA
invitrogen
REF S33102
Store at: 15 °C / 30 °C
SYBR® Safe DNA gel stain
10,000X concentrate in DMSO
Protect from light *DO NOT FREEZE*
Qty: 400 µL
LOT 1691992
For Research Use Only
For Research Use Only.
Not for use in diagnostic procedures.





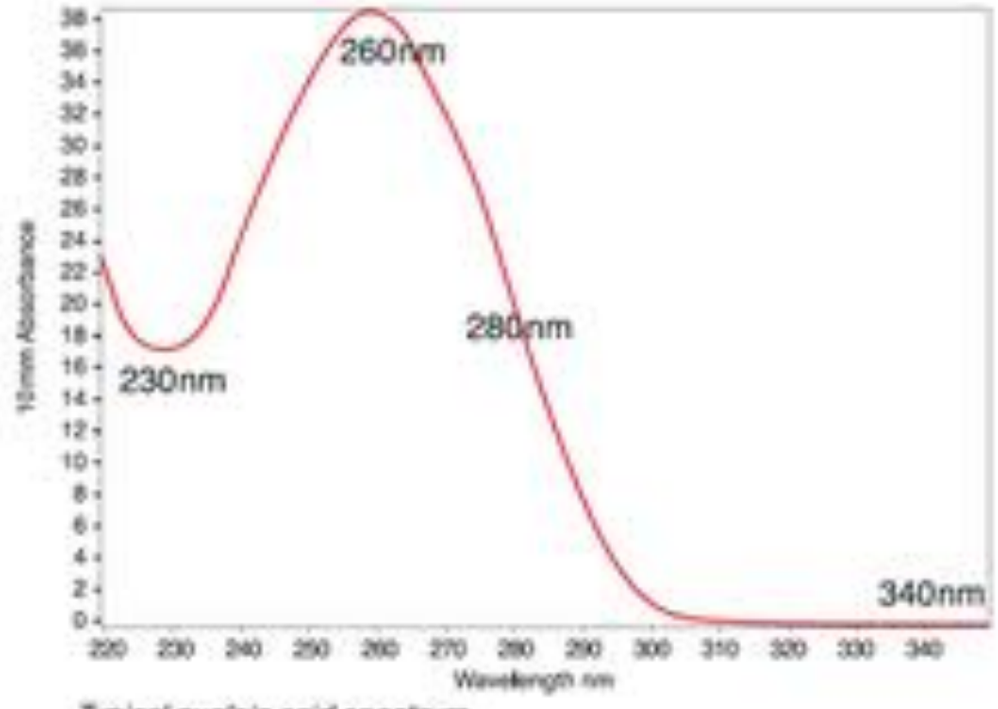
Control 6 7 8 9 10





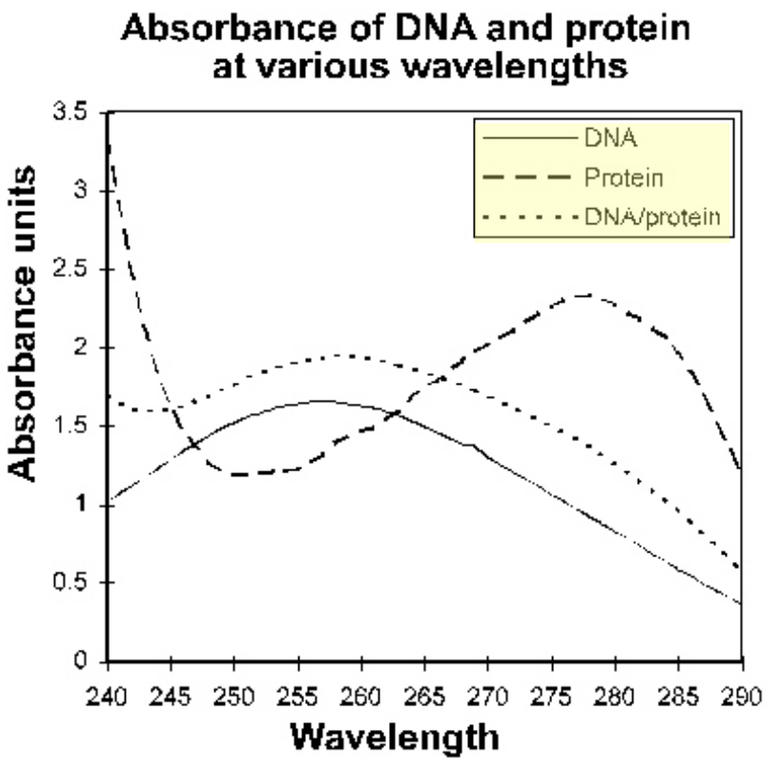
1. Warm up not needed
2. Turn on instrument, back of instrument, just above power cord.
3. Press button for what you need to do. DNA, RNA, Protein, O.D. 600, etc.
4. Using arrows and enter buttons, scroll to whatever submenu you want. DNA/RNA – select type of DNA or RNA; Proteins – select assay type, Absorbance – select wavelength you want.
5. Slide blue cover back.
6. Hold cuvette by grooved sides not clear sides. After sample loaded (at least 50 ul) in cuvette, place cuvette in slot with clear sides lined up with the arrow (direction of light path). Push firmly. Check to make sure no bubbles.
7. First blank with water or buffer. Select Blank button.
8. Enter dilutuon. Select Parameter/Dilution button. Cuvette size – Enter; Unit – select unit using arrows, Enter; Factor – leave as is, Enter.
9. Load sample into cuvette. Place in cuvette holder. Press Sample button.
10. For DNA samples, the window will show 230, 260, 280, 320 and ratios 260/280 (1:2) and 260/230.
11. Turn instrument off.
12. Put dust cover back on cuvette slot or slide cover over slot for Plus

Biophotometer D 30
Eppendendorf



Typical nucleic acid spectrum

Nucleic acids absorb ultraviolet light in a specific pattern. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.

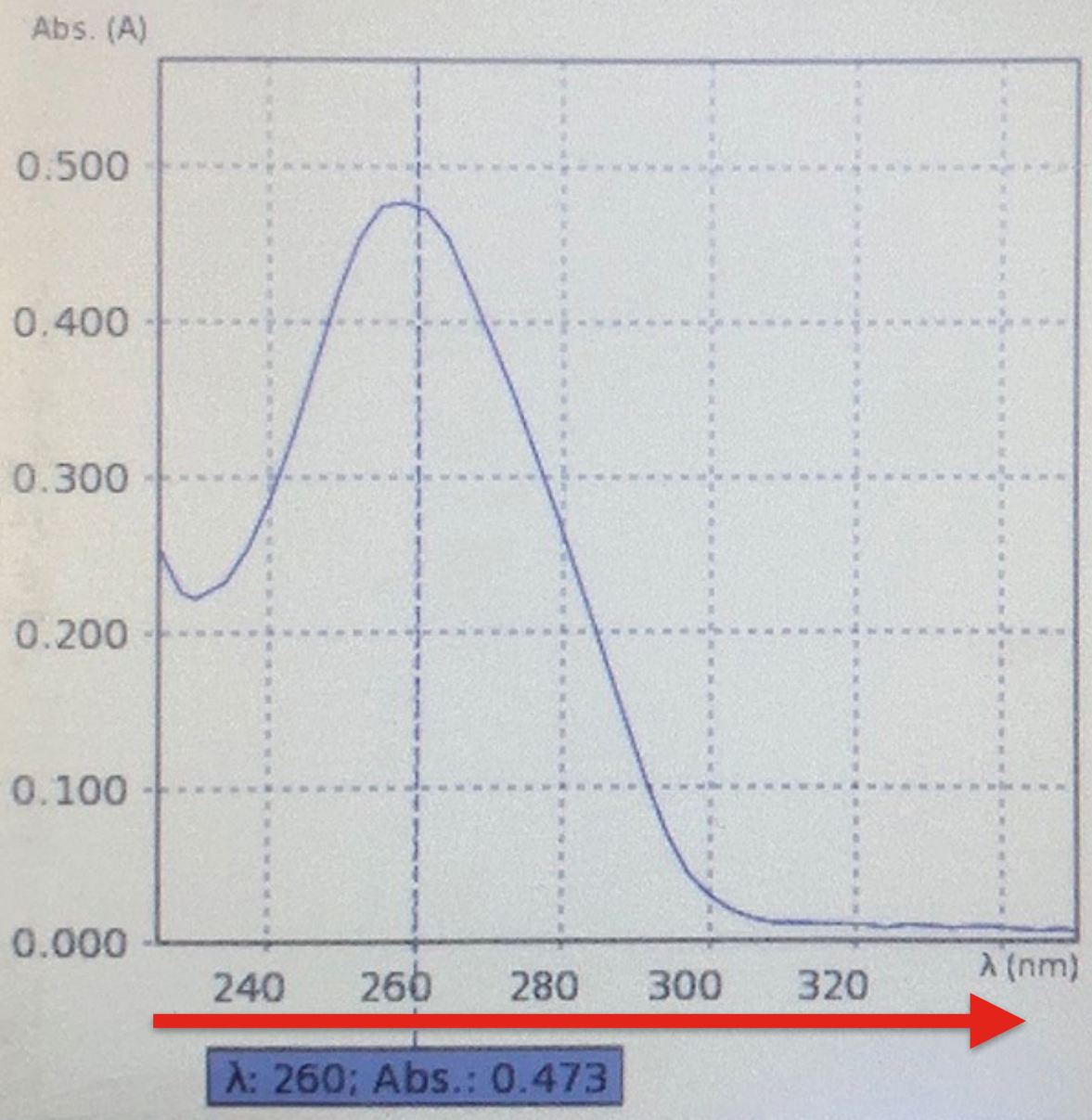


The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA.

A ratio of **~1.8** is generally accepted as “pure” for DNA;

dsDNA 1mm: check parameters | measure samples | process results | ...

dsDNA 1mm 2019-07-08 18:27:45



# 02	
ID:	

231.2 µg/mL

0.473 $A_{260/1\ mm}$

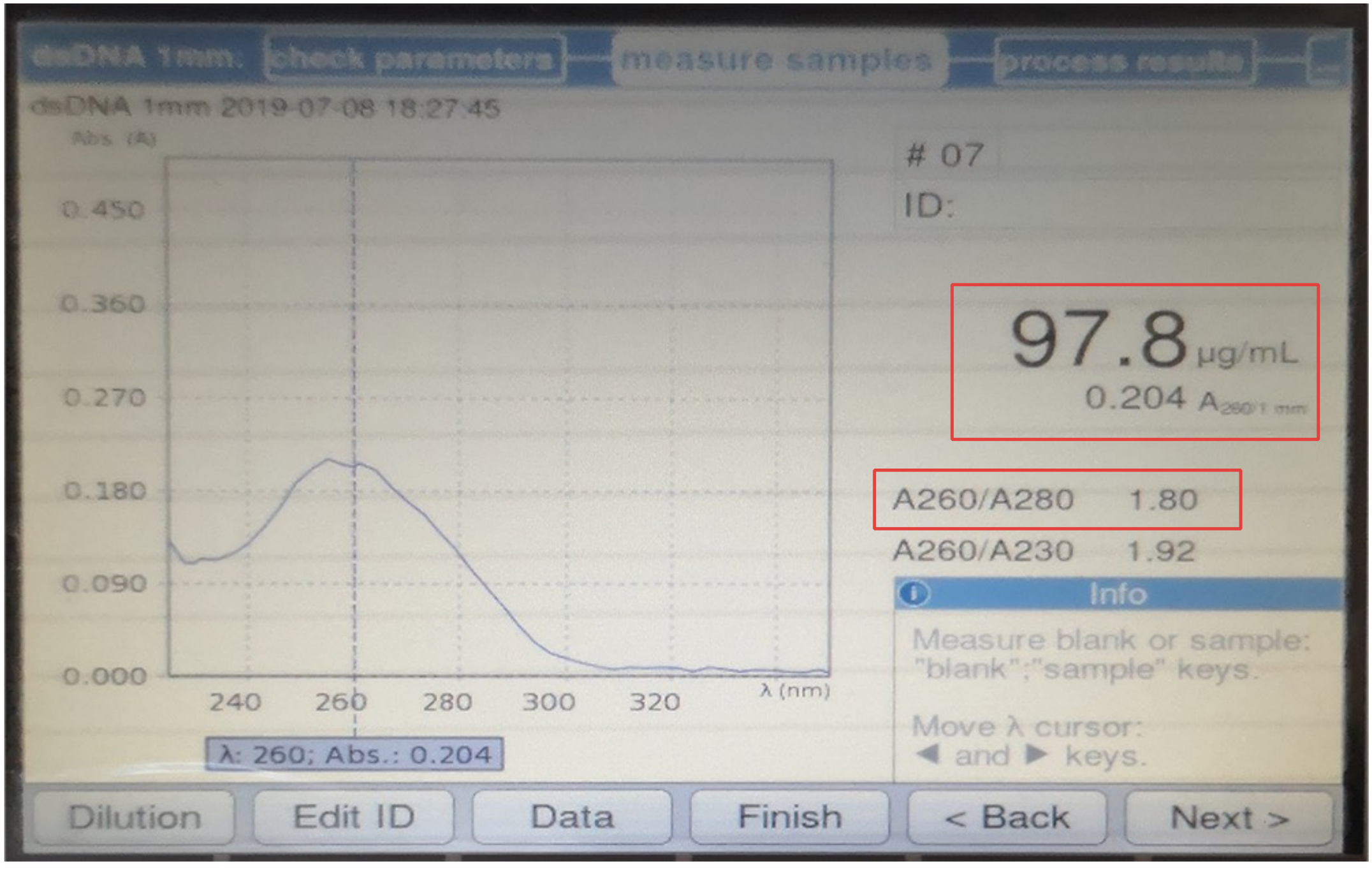
A260/A280	1.87
A260/A230	2.20

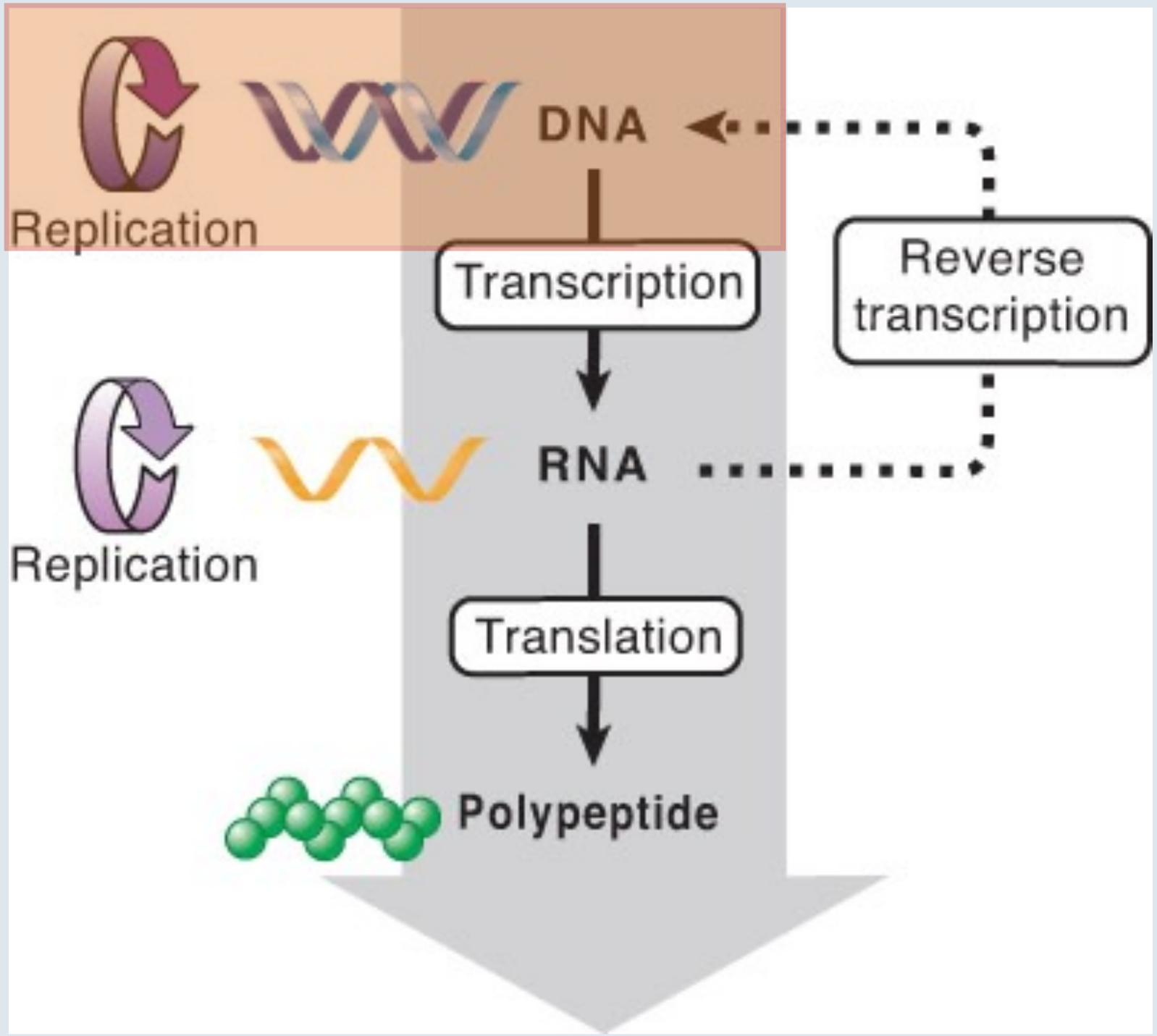
Info

Measure blank or sample: "blank";"sample" keys.

Move λ cursor: ◀ and ▶ keys.

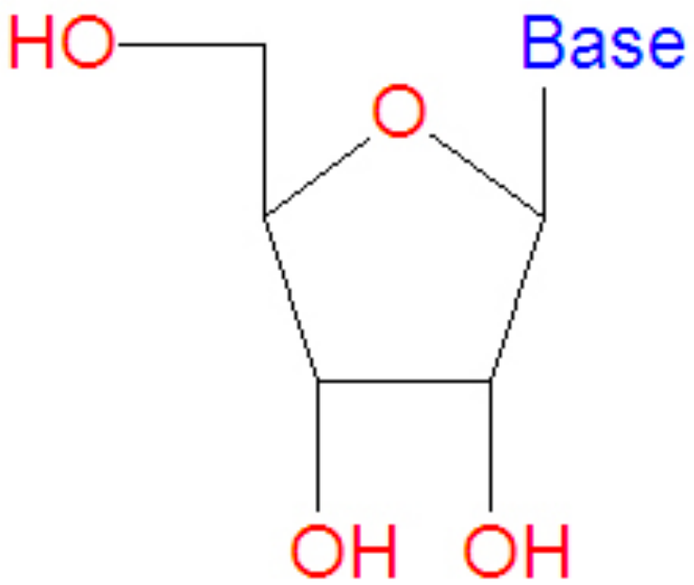
Dilution | Edit ID | Data | Finish | < Back | Next >


dsDNA 1mm:
check parameters
measure samples
process results
dsDNA 1mm 2019-07-08 18:27:45
Abs (A)
0.450
0.360
0.270
0.180
0.090
0.000
240
260
280
300
320
λ (nm)
λ: 260; Abs.: 0.204
07
ID:
97.8 µg/mL
0.204 A260@1 mm
A260/A280 1.80
A260/A230 1.92
Info
Measure blank or sample: "blank";"sample" keys.
Move λ cursor: ◄ and ► keys.
Dilution
Edit ID
Data
Finish
< Back
Next >

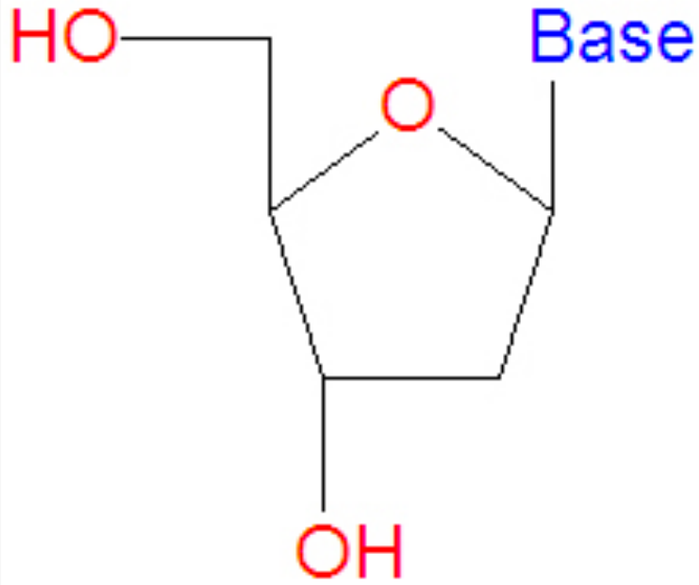


Information in nucleic acid can be perpetuated or transferred, but the transfer of information into a polypeptide is irreversible.

RNA Preparation

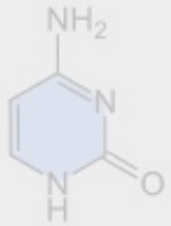


Ribonucleic acid (RNA)
Base= A, C, G or T

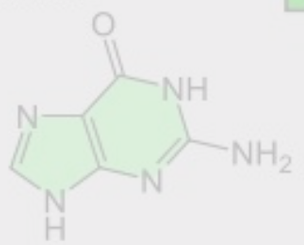


Deoxyribonucleic acid (DNA)
Base = A, C, G or U

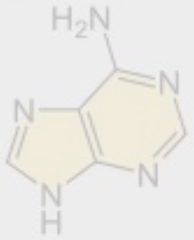
Cytosine C



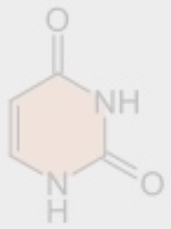
Guanine G



Adenine A



Uracil U



Nucleobases of RNA

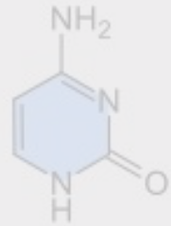
RNA

Ribonucleic acid

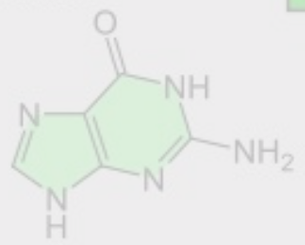
DNA

Deoxyribonucleic acid

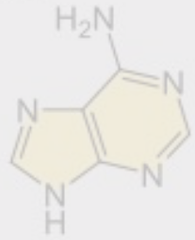
Cytosine C



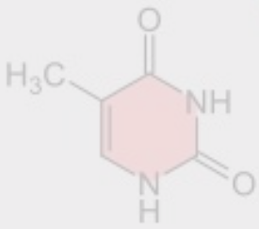
Guanine G



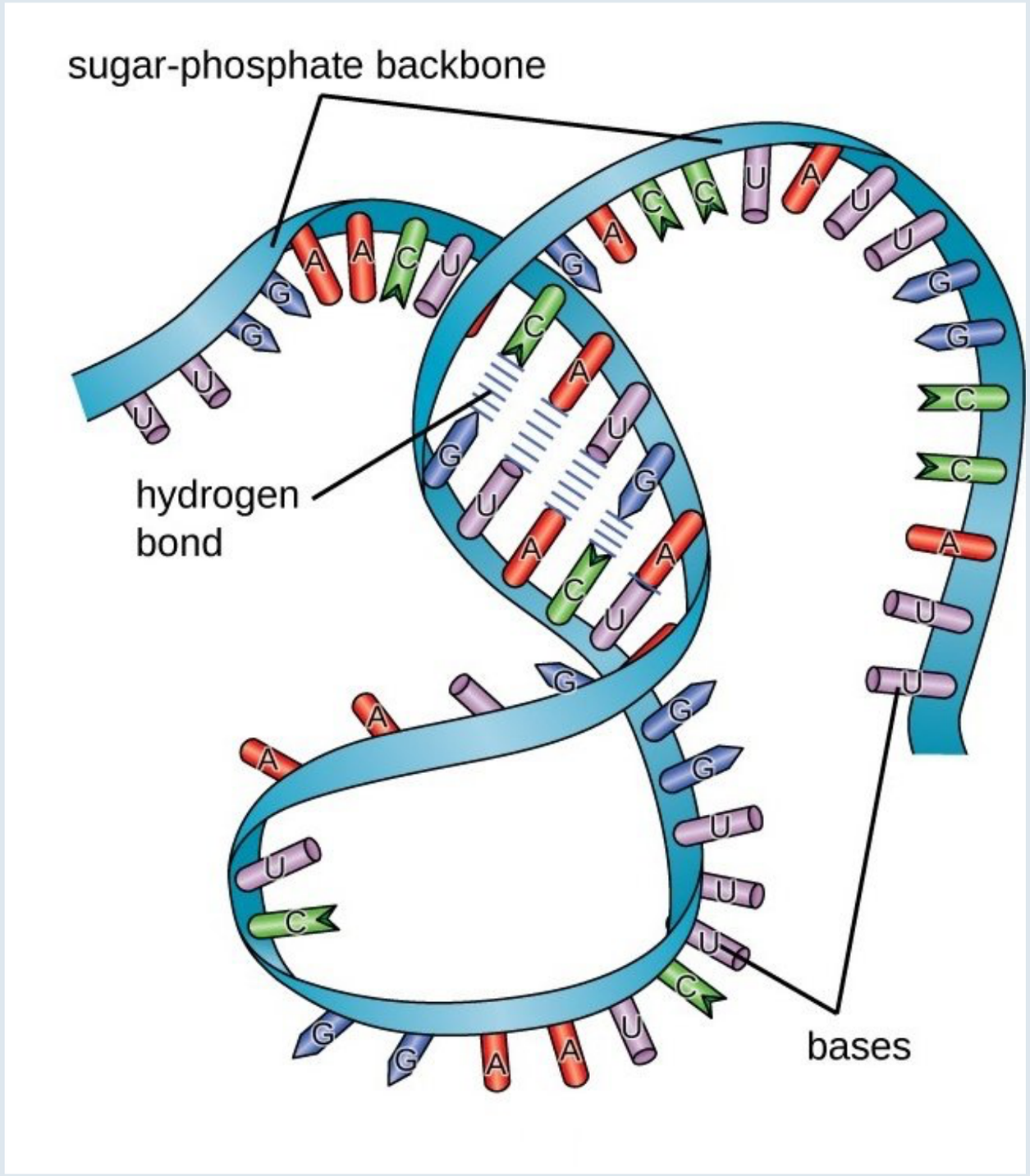
Adenine A

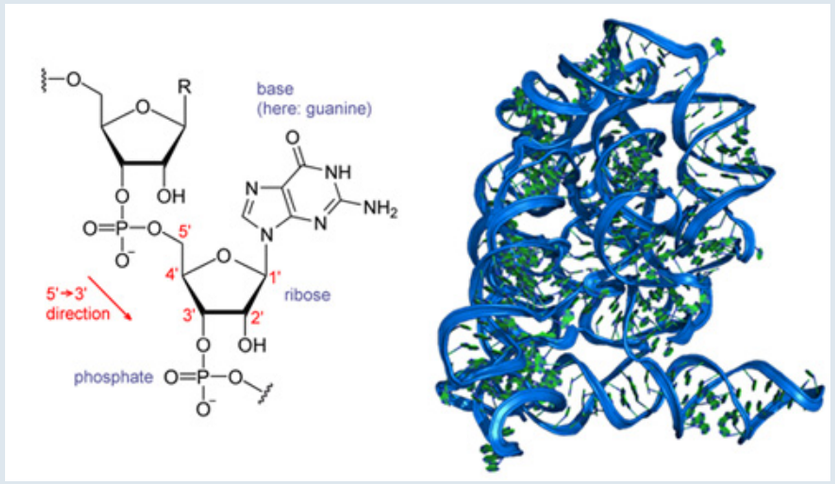


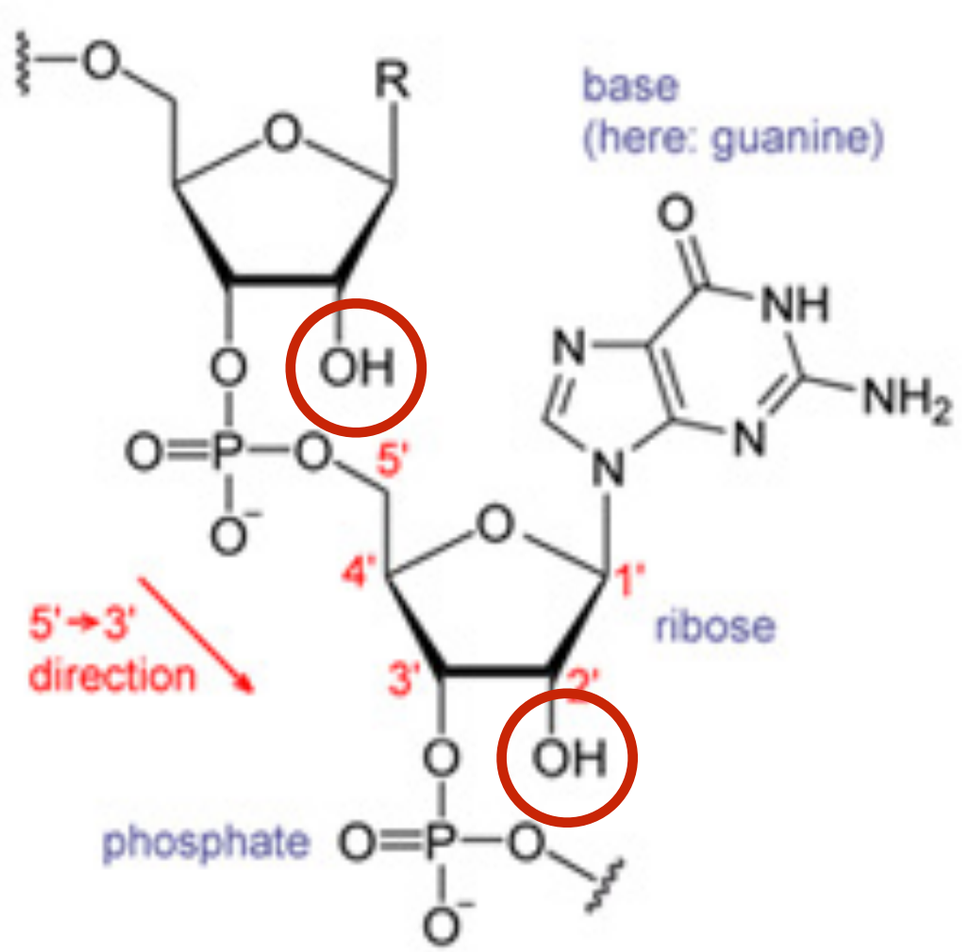
Thymine T

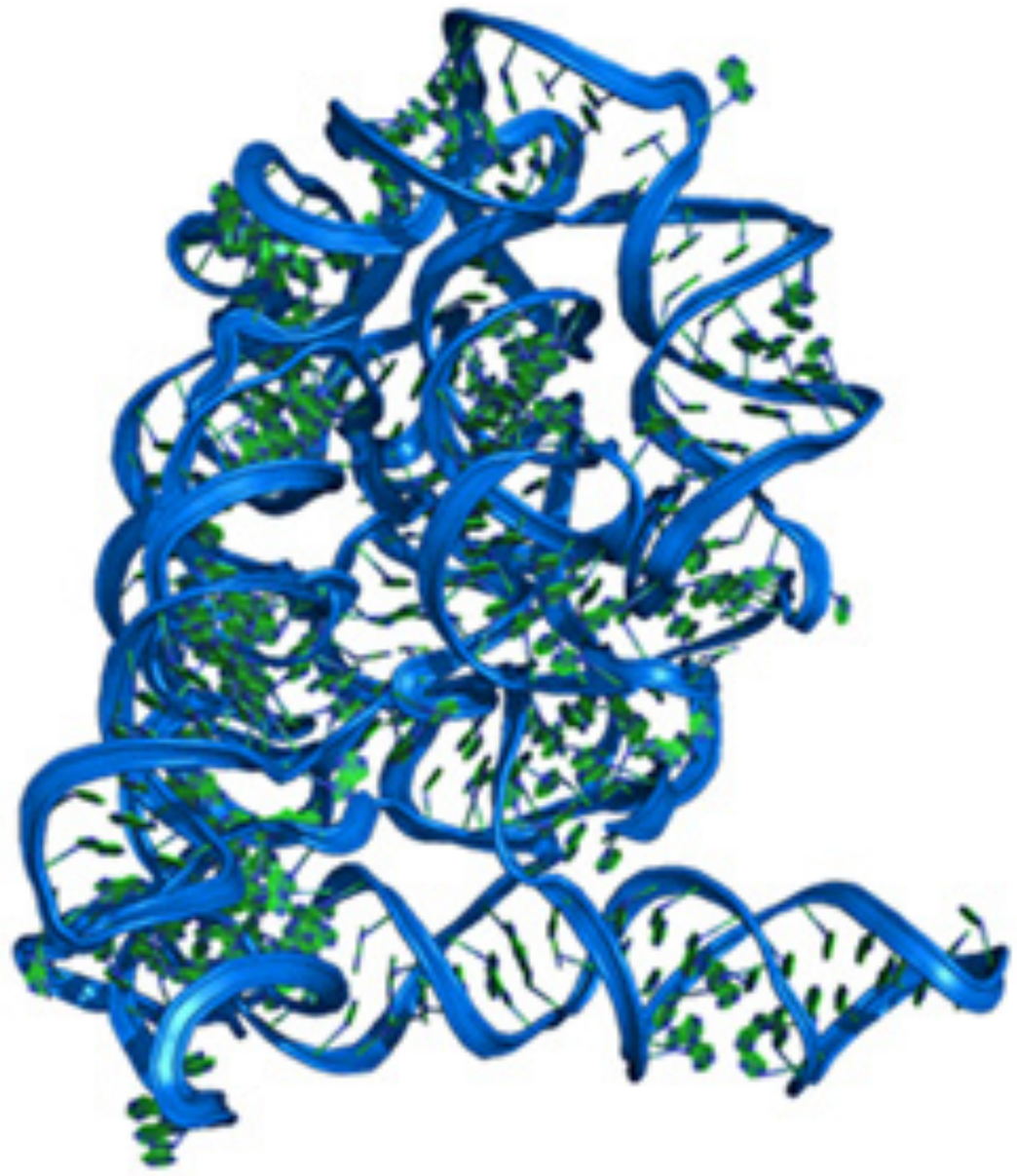


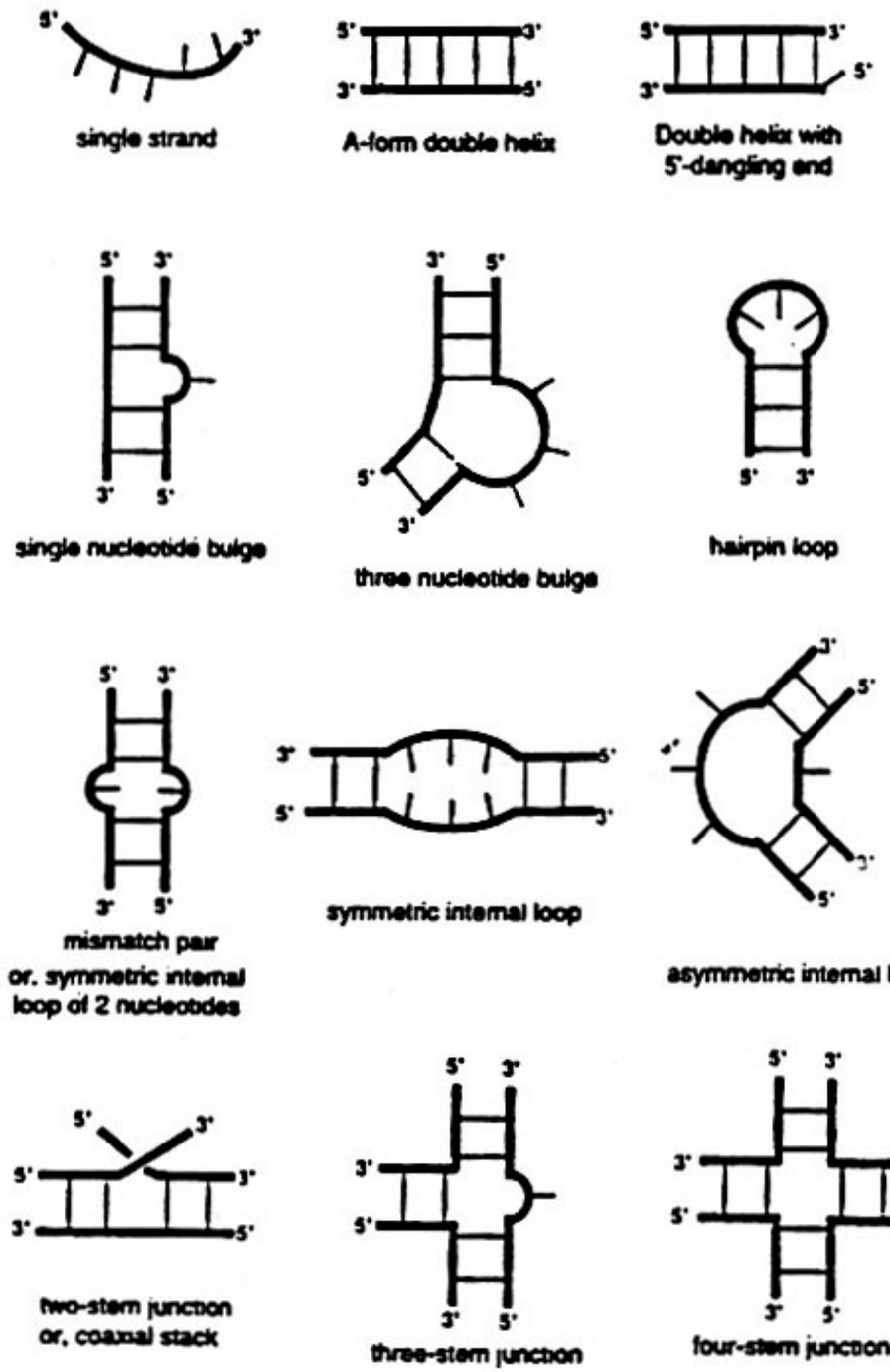
Nucleobases of DNA


sugar-phosphate backbone
hydrogen bond
bases

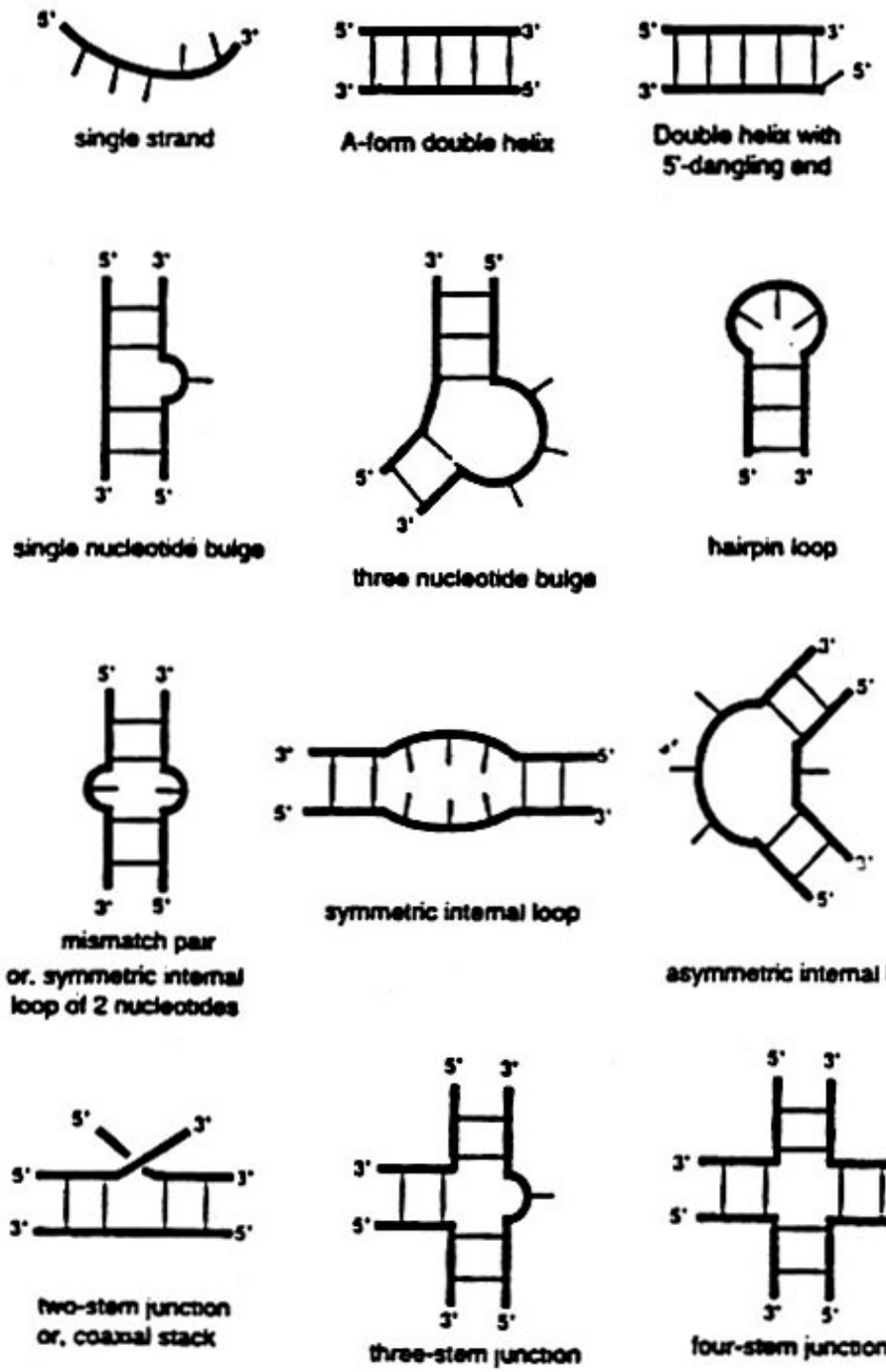

base
(here: guanine)
ribose
5'→3' direction
phosphate


base
(here: guanine)
O
NH
N
NH2
N
N
O
R
O
OH
O=P—O
O⁻
5'
4'
1'
ribose
5'→3'
direction
3'
2'
O
OH
phosphate
O=P—O
O⁻





Secondary Structures



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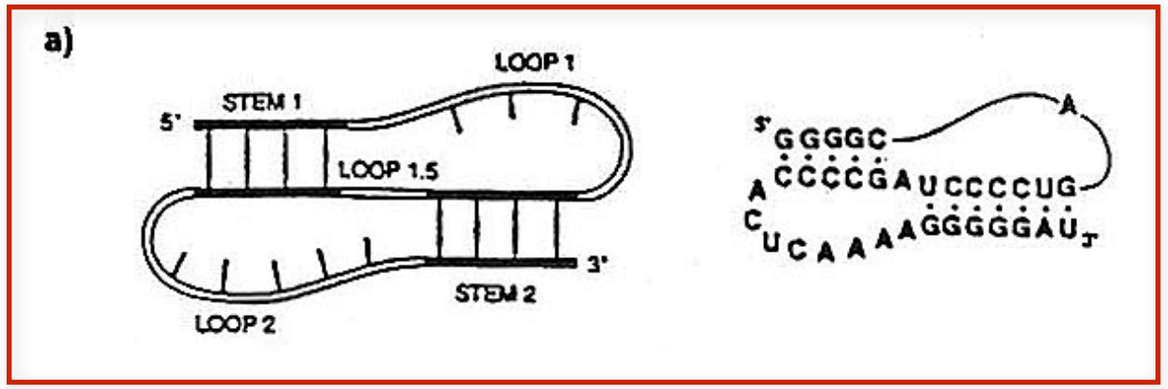

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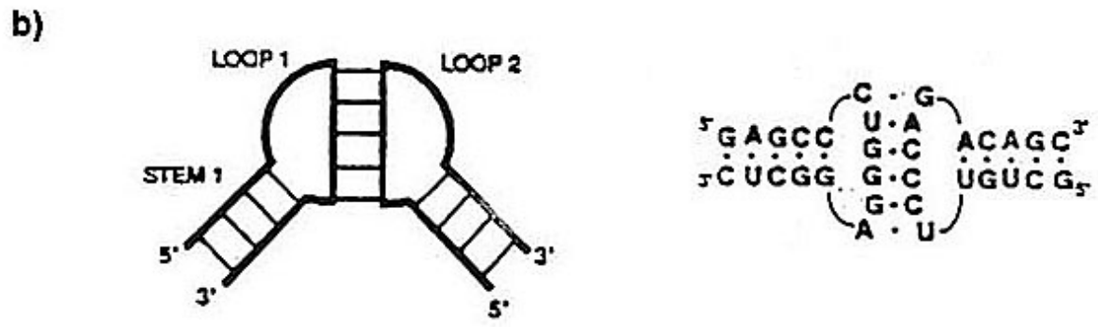
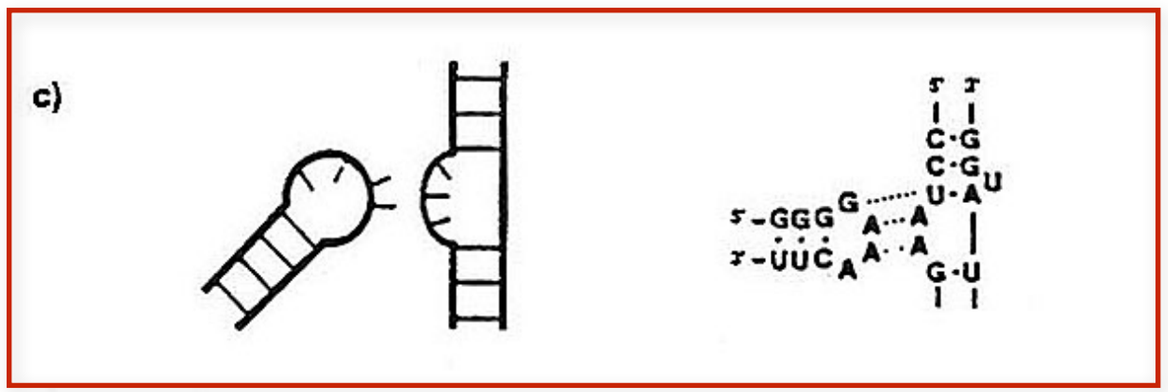
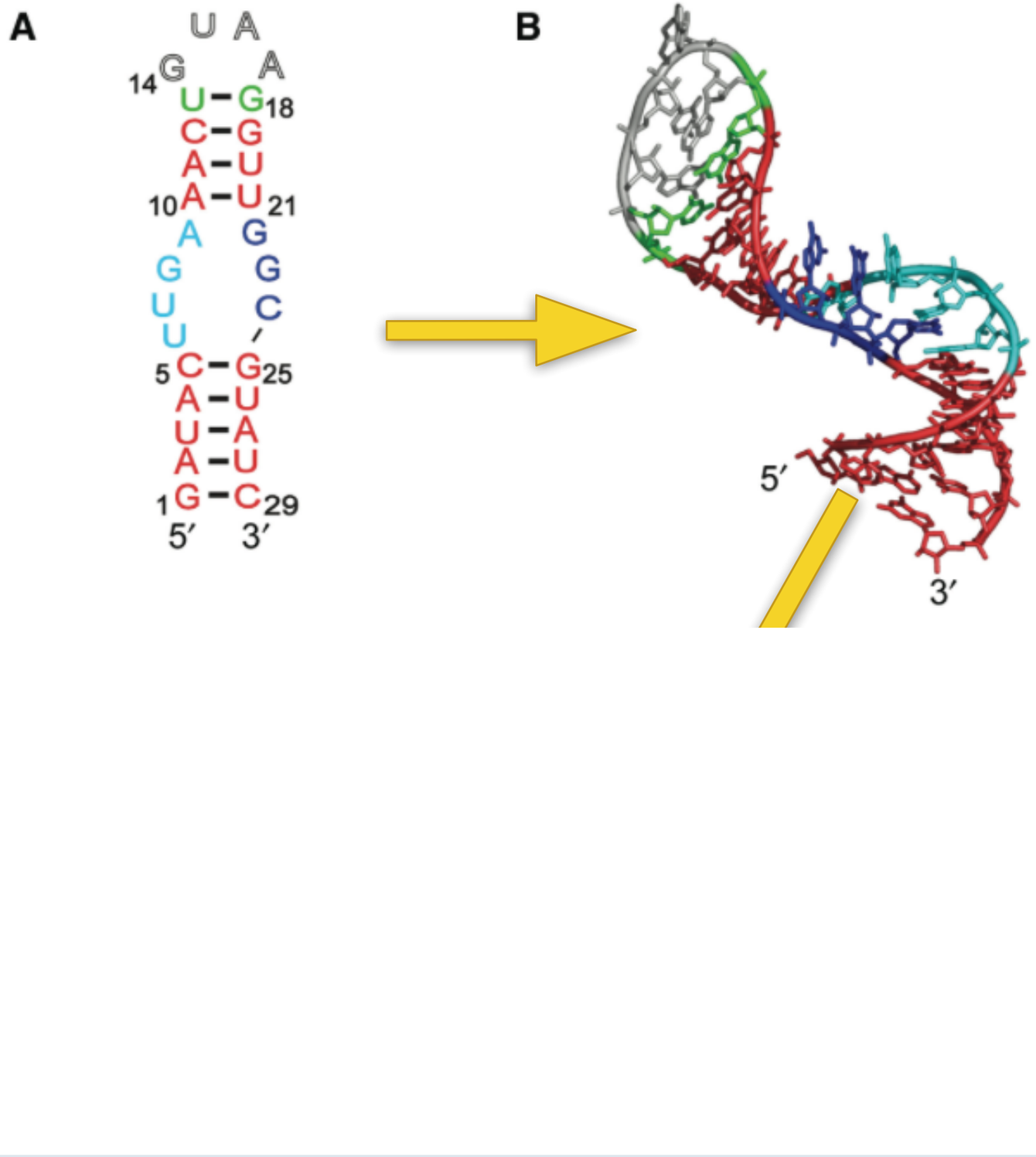


Kissing hairpins

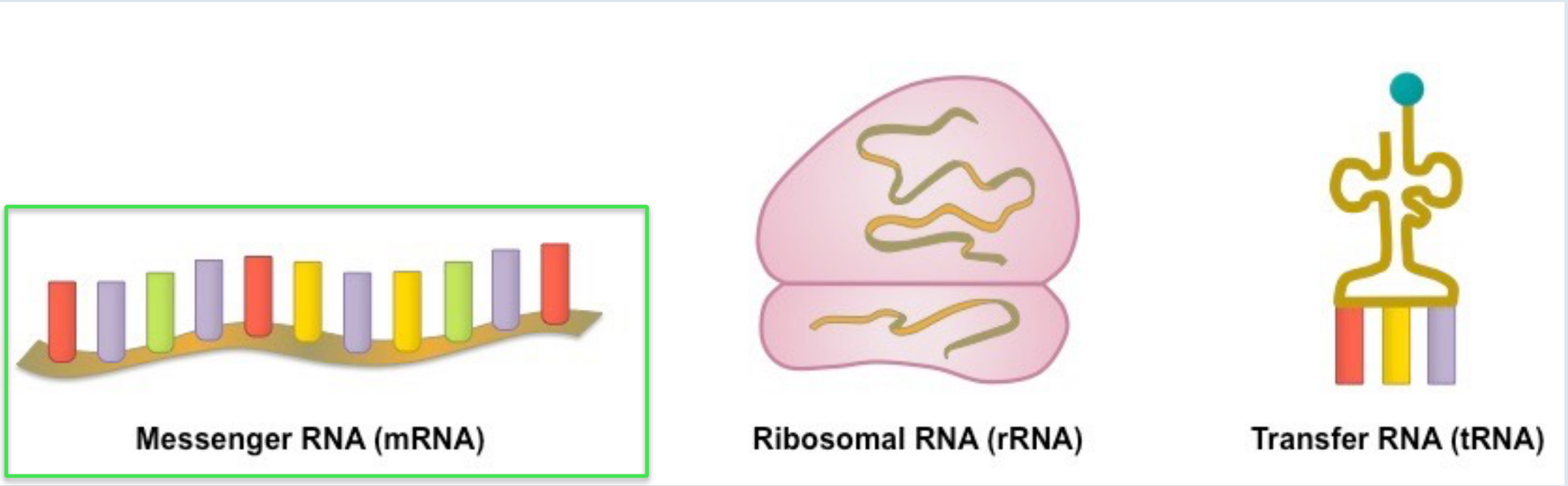


Hairpin loop - bulge contact

Tertiary Structures



DNA mimicry by a high-affinity anti-NF-{kappa}B **RNA aptamer**
Nucleic Acids Research react-text: 55 36(4):1227-36 April 2008



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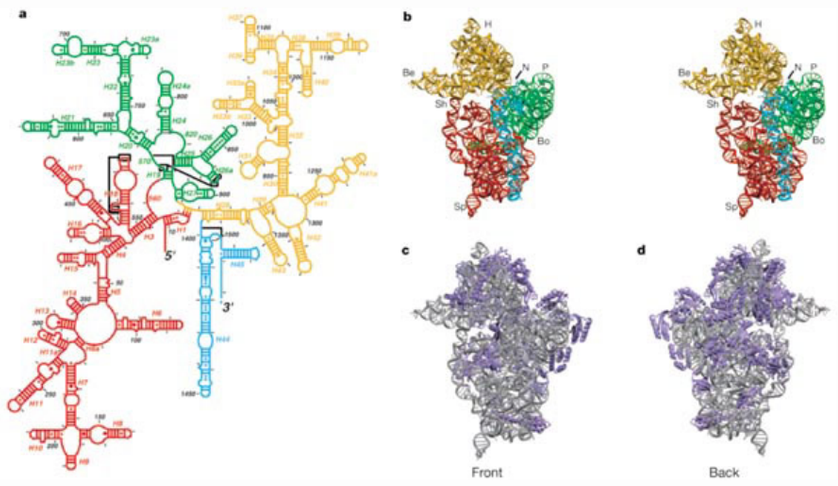

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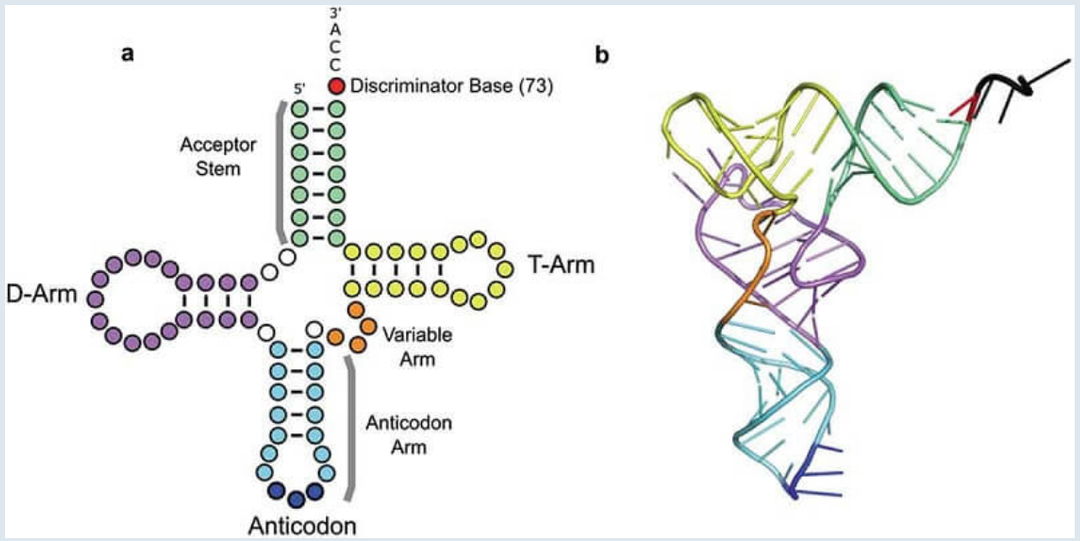
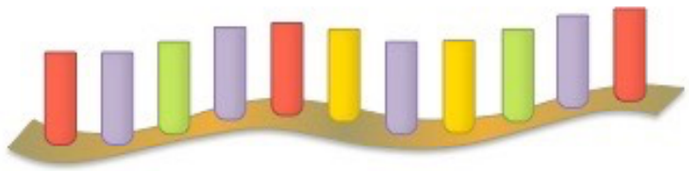
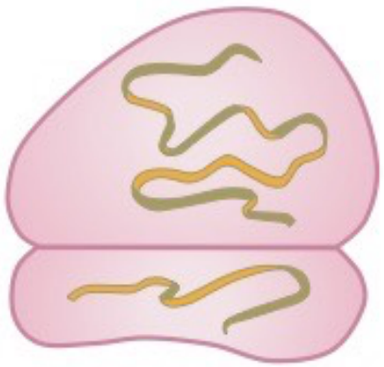




Messenger RNA (mRNA)



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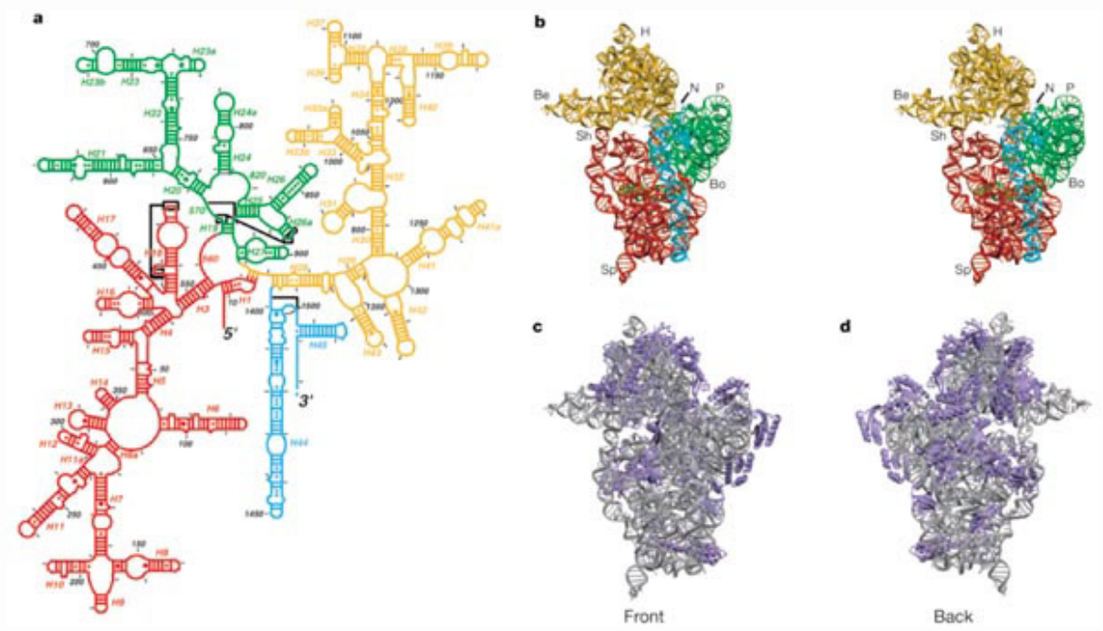

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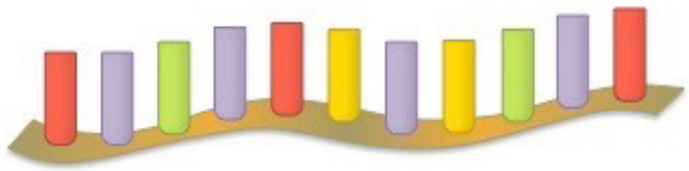

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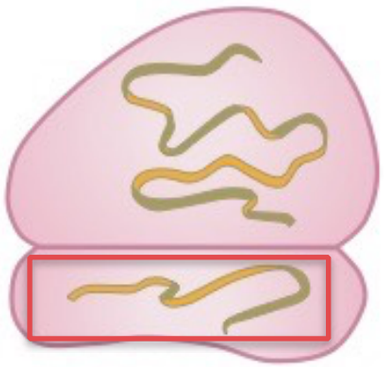

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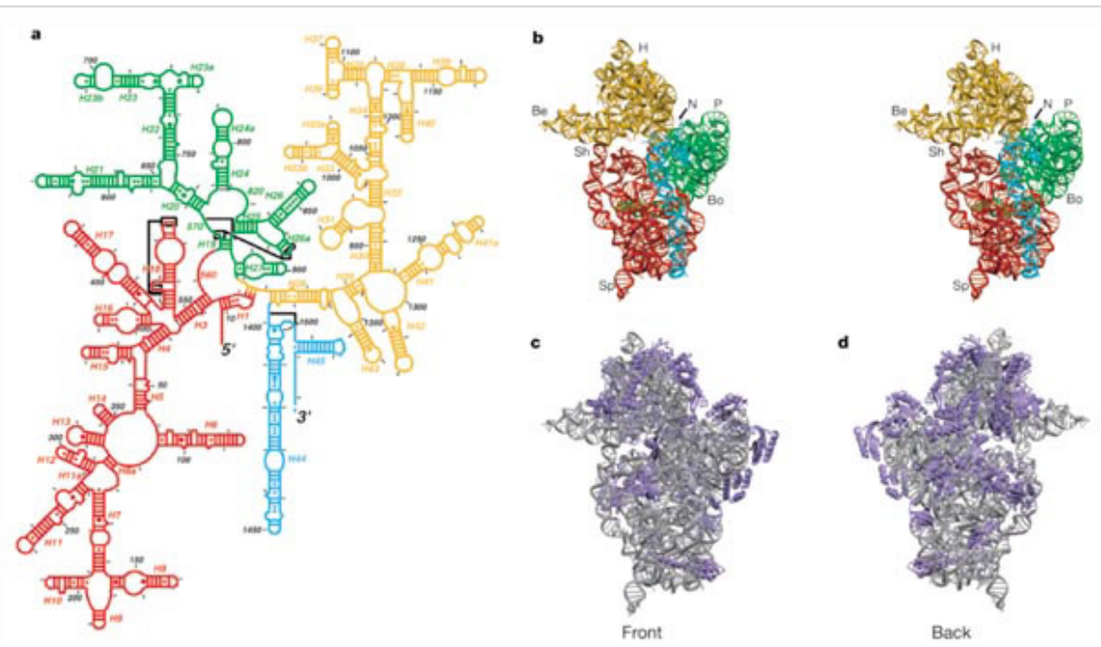

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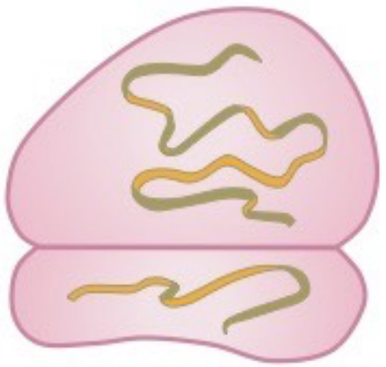

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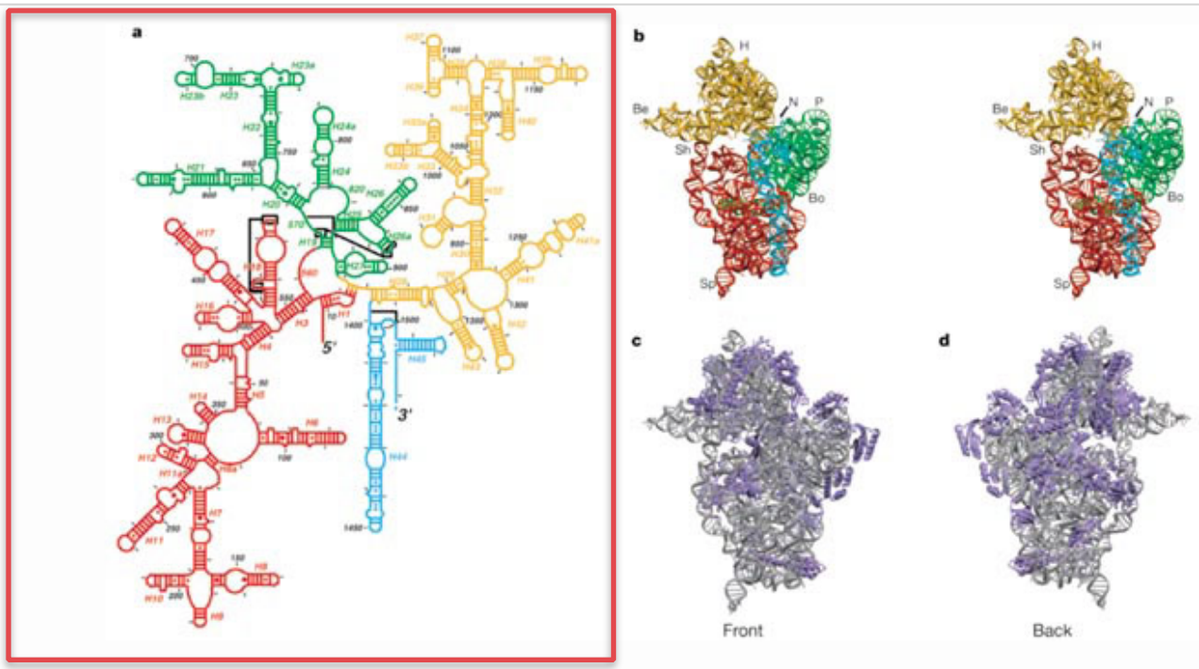

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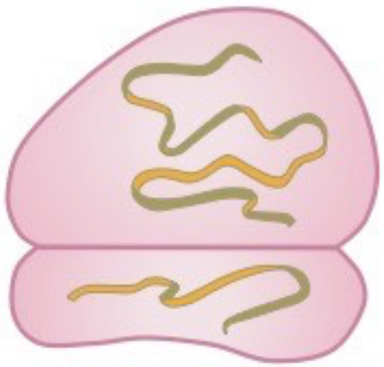

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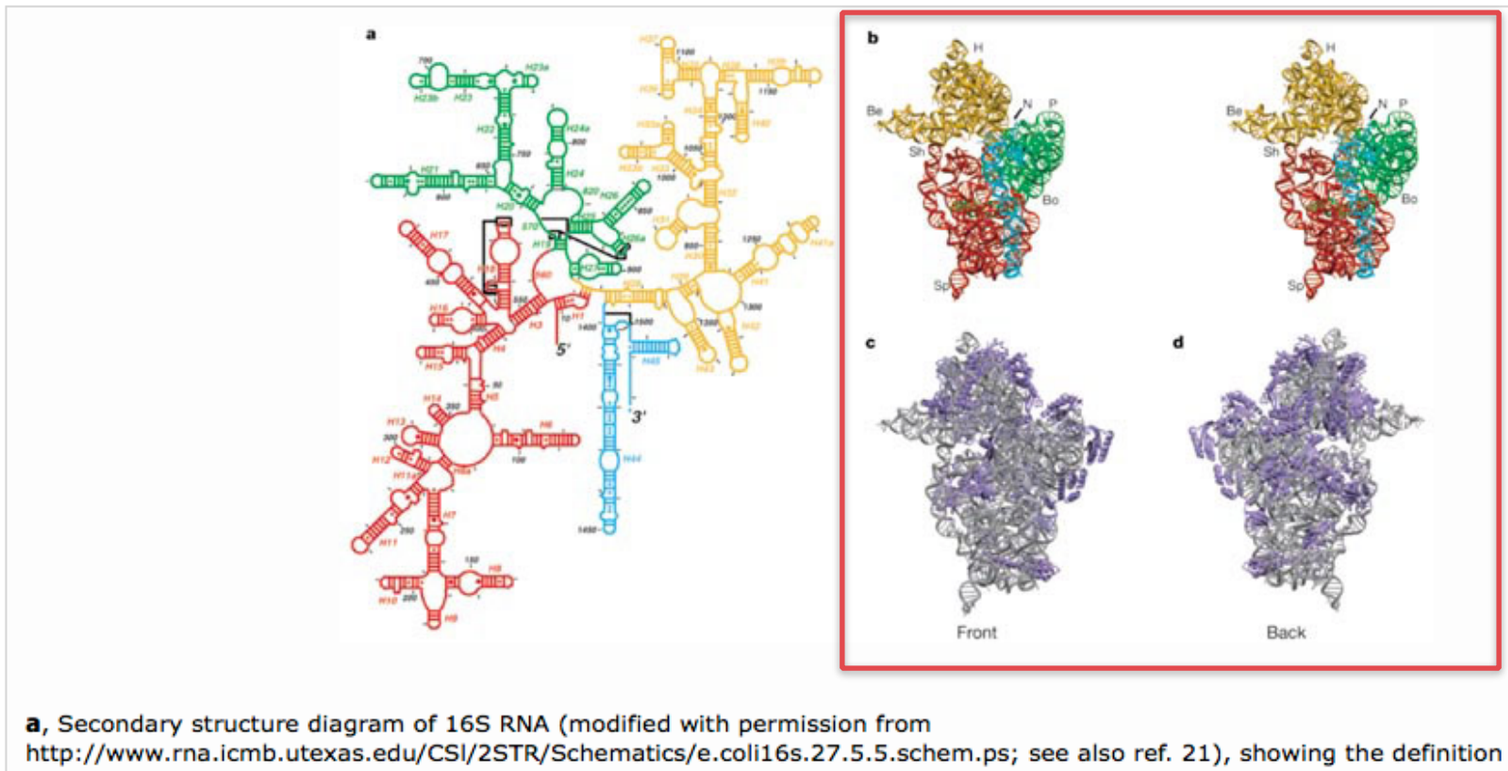

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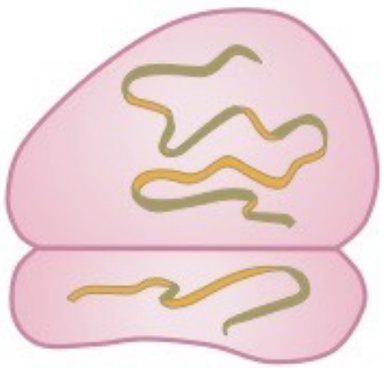

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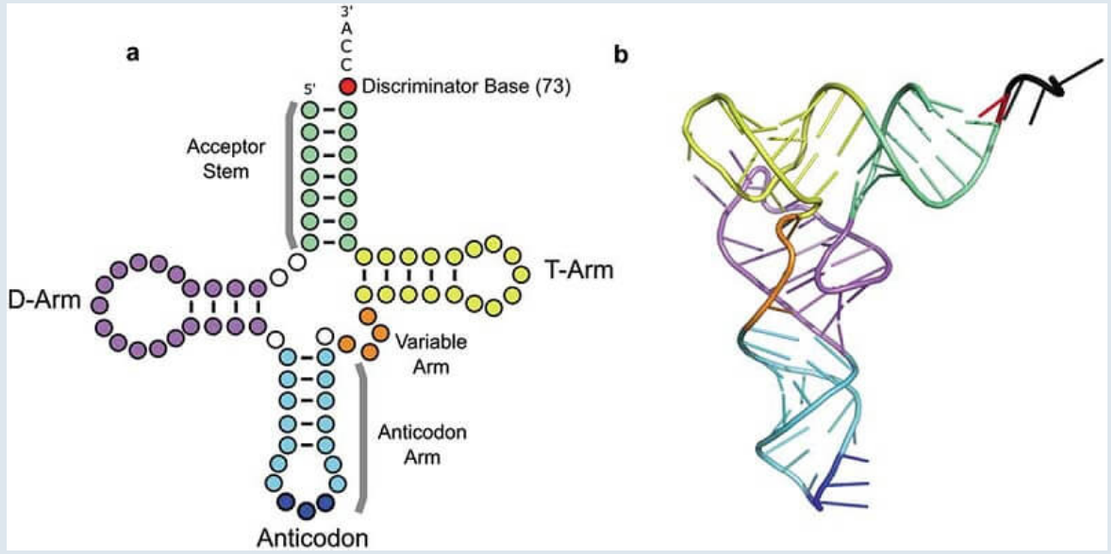

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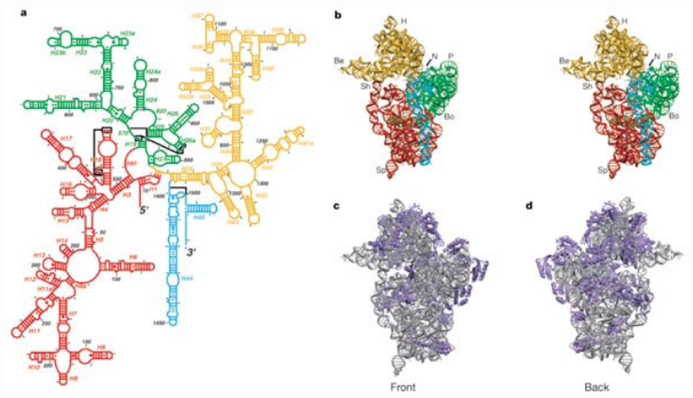

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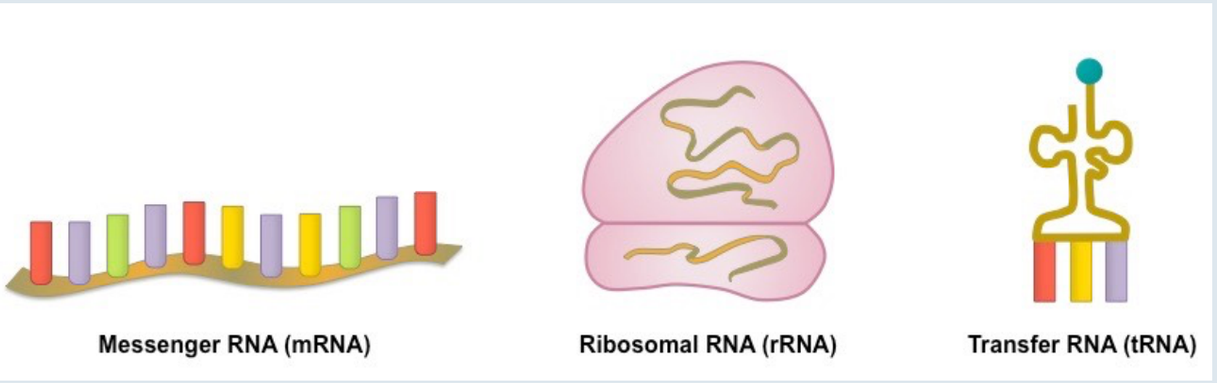
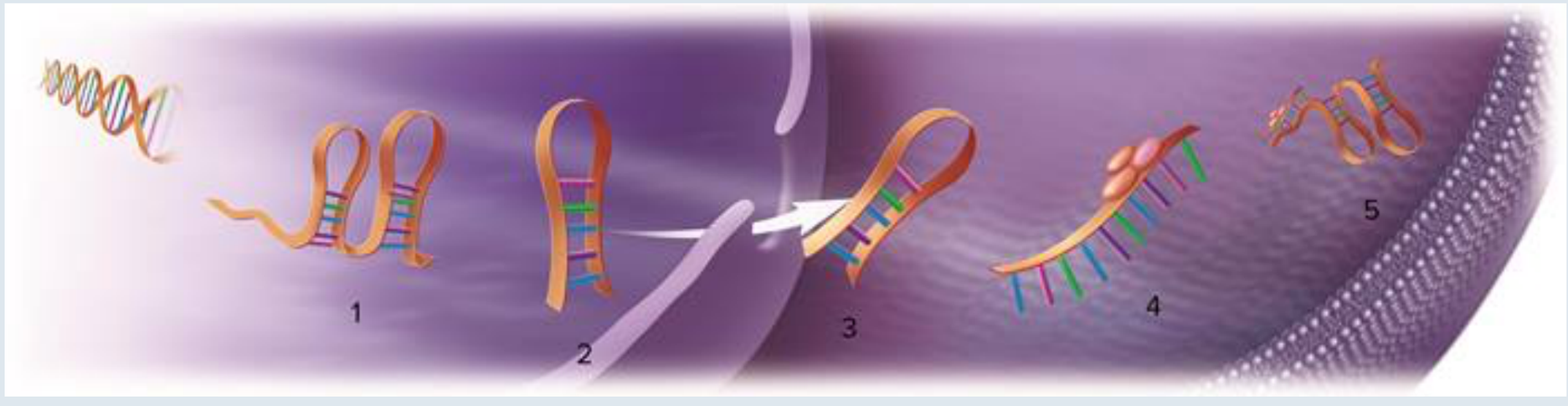
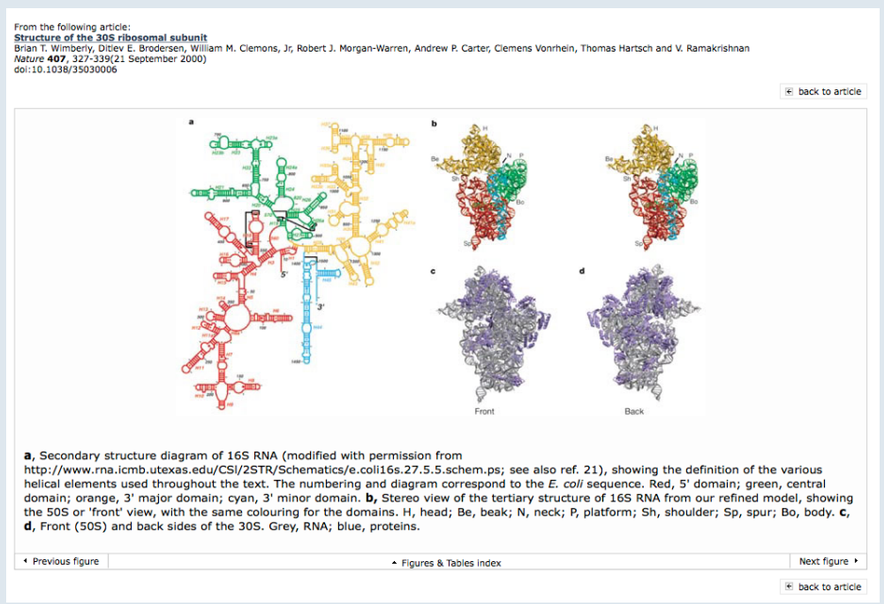




miRNA/siRNA

Mature **microRNAs** (**miRNAs**) are a class of naturally occurring, small non-coding **RNA** molecules, about **21–25 nucleotides in length.**



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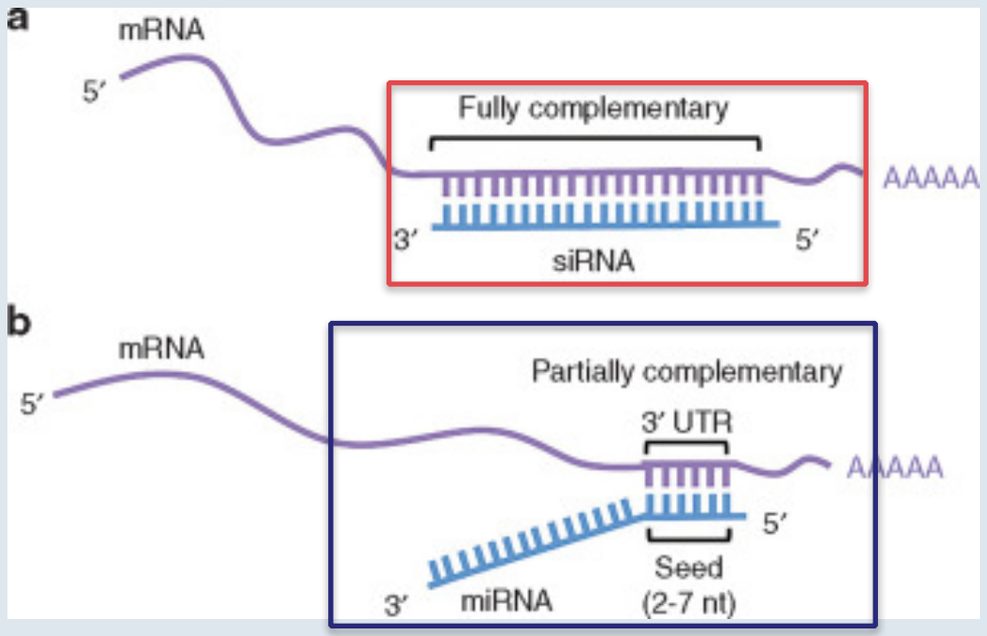


GSU Biology Core Facility

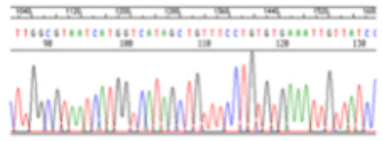
Supporting Life Sciences at GSU

http://biotech.gsu.edu/core_facility/index.html

Georgia State University

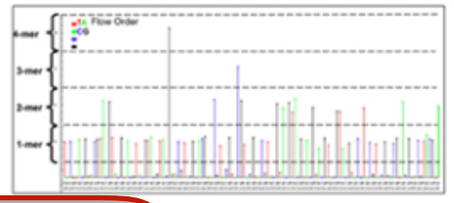
DNA Sequence Analysis:

Profiling DNA



Sanger Sequencing –
>800 base pairs/run

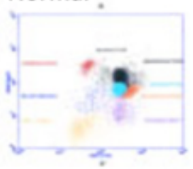
High Throughput Genomic Sequencing –
100,000 base pairs/run



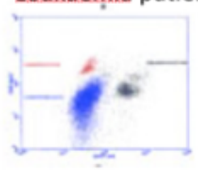
Flow Cytometry

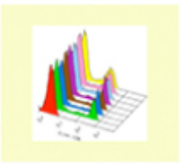
Profiling Cells

Normal



Leukaemia patient

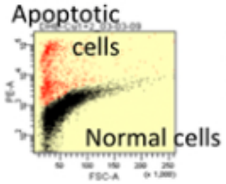




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

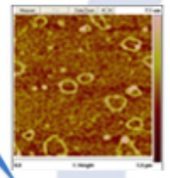


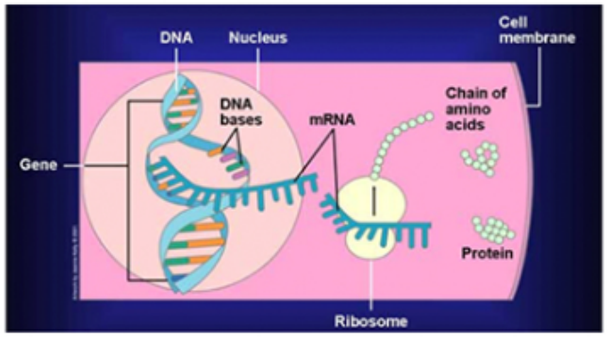
Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis





DNA

Replication

RNA

Expression

Protein

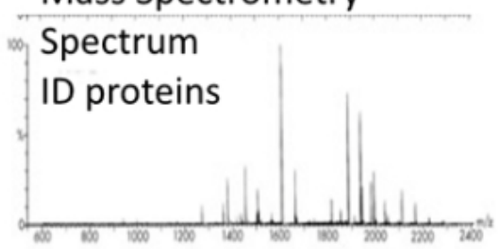
Expression

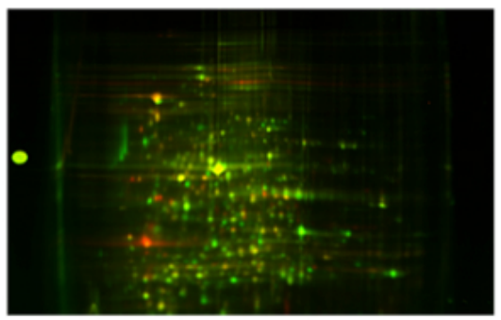
Cellular Functions

Mass Spectrometry

Spectrum

ID proteins





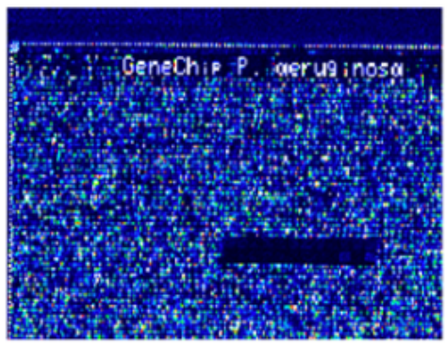
Proteomics

Profiling Proteins

2D Protein gel
Protein separation using
Electric charge and molecular weight

Microarray:Analysis

Profiling mRNA



Colour of pin-point dots demonstrates the presence / absence of gene sequences

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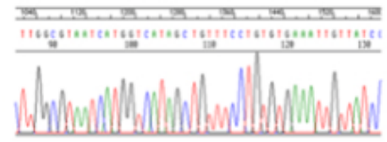

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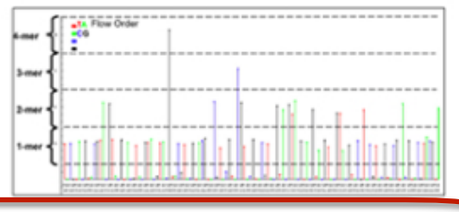

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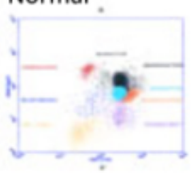
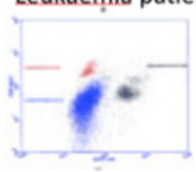
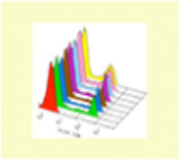
Profiling Cells

Normal



Leukaemia patient



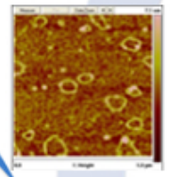


See effects of different drugs on Cell cycle

Atomic Force Microscopy

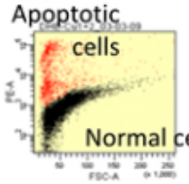
Imaging at the Ångström level

Protein structure analysis

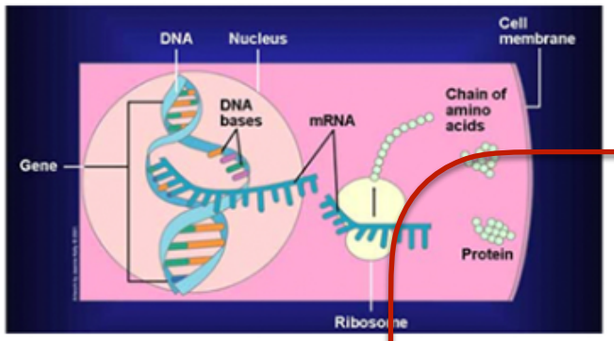


Apoptotic cells

Normal cells



Apoptosis -programmed cell death

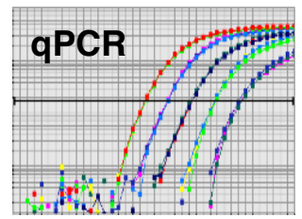


DNA

Replication

Cellular Functions

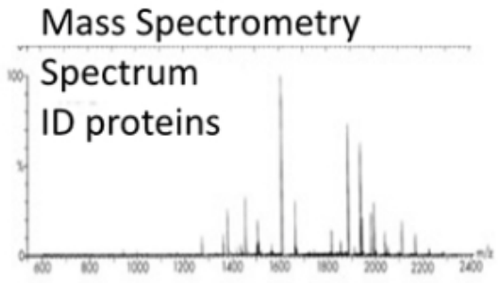
RNA Expression

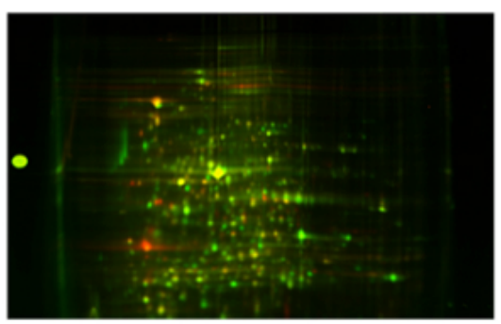


Mass Spectrometry

Spectrum

ID proteins





Protein Expression

Proteomics

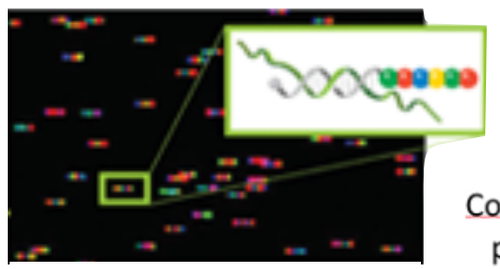
Profiling Proteins

2D Protein gel
Protein separation using
Electric charge and molecular weight

Microarray:Analysis

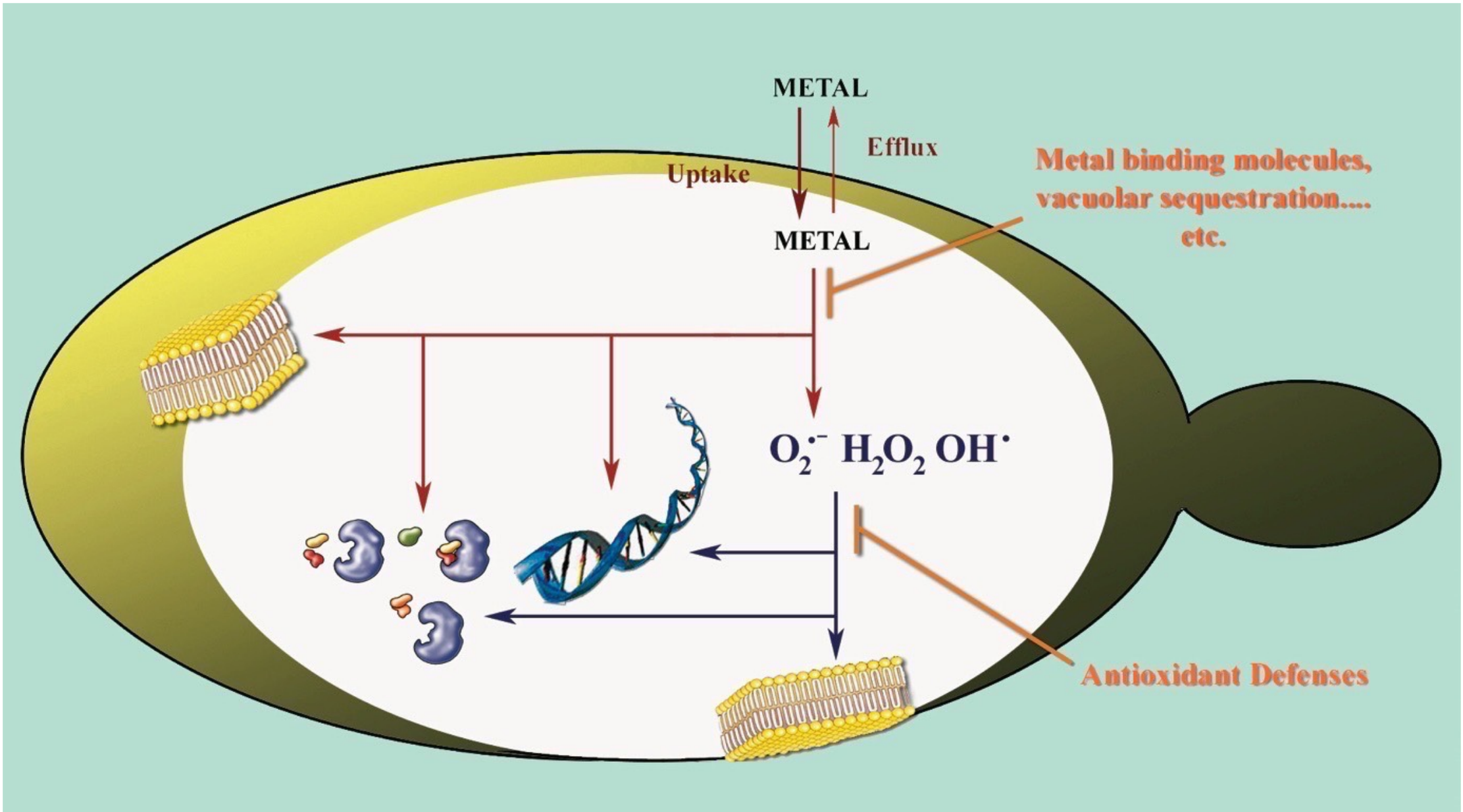
Profiling mRNA

Colour of pin-point dots demonstrates the presence / absence of gene sequences



Nanostring

Metals generate ROS and cause oxidative stress



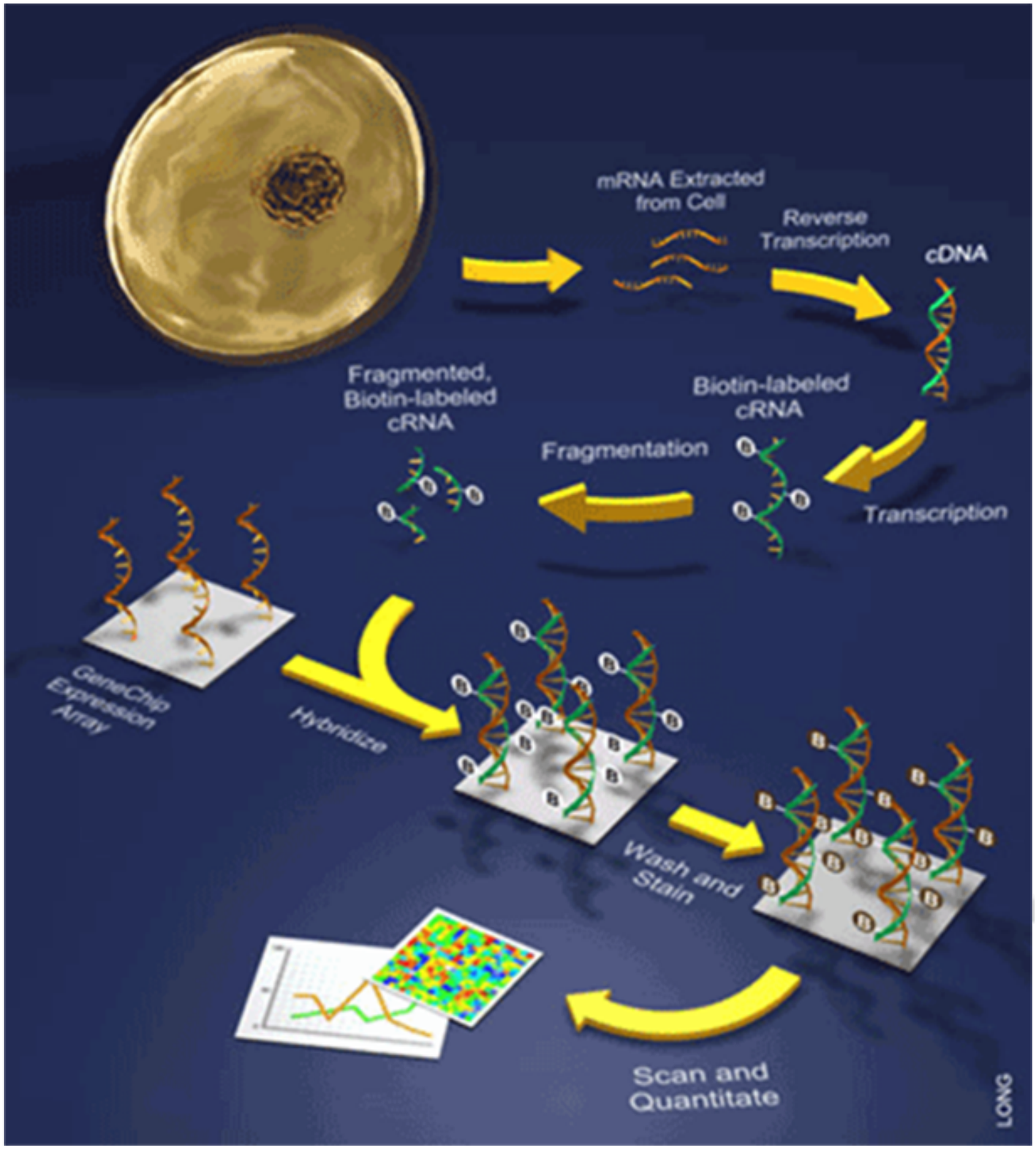
WT = Wild Type Strain

Δ = Strain with deletion in Yca1 ("caspase" gene)

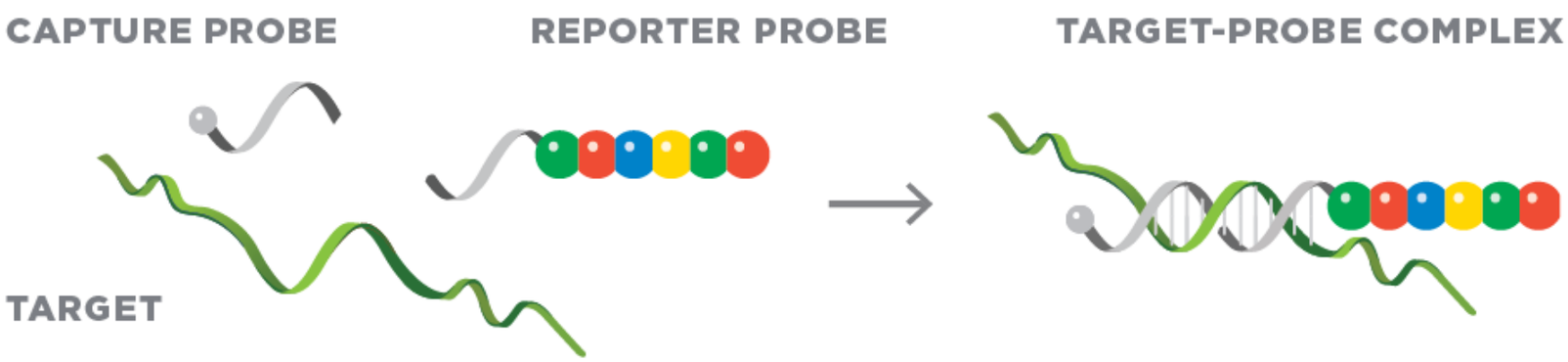
Cd = Cells Exposed to Cadmium (Heavy Metal)

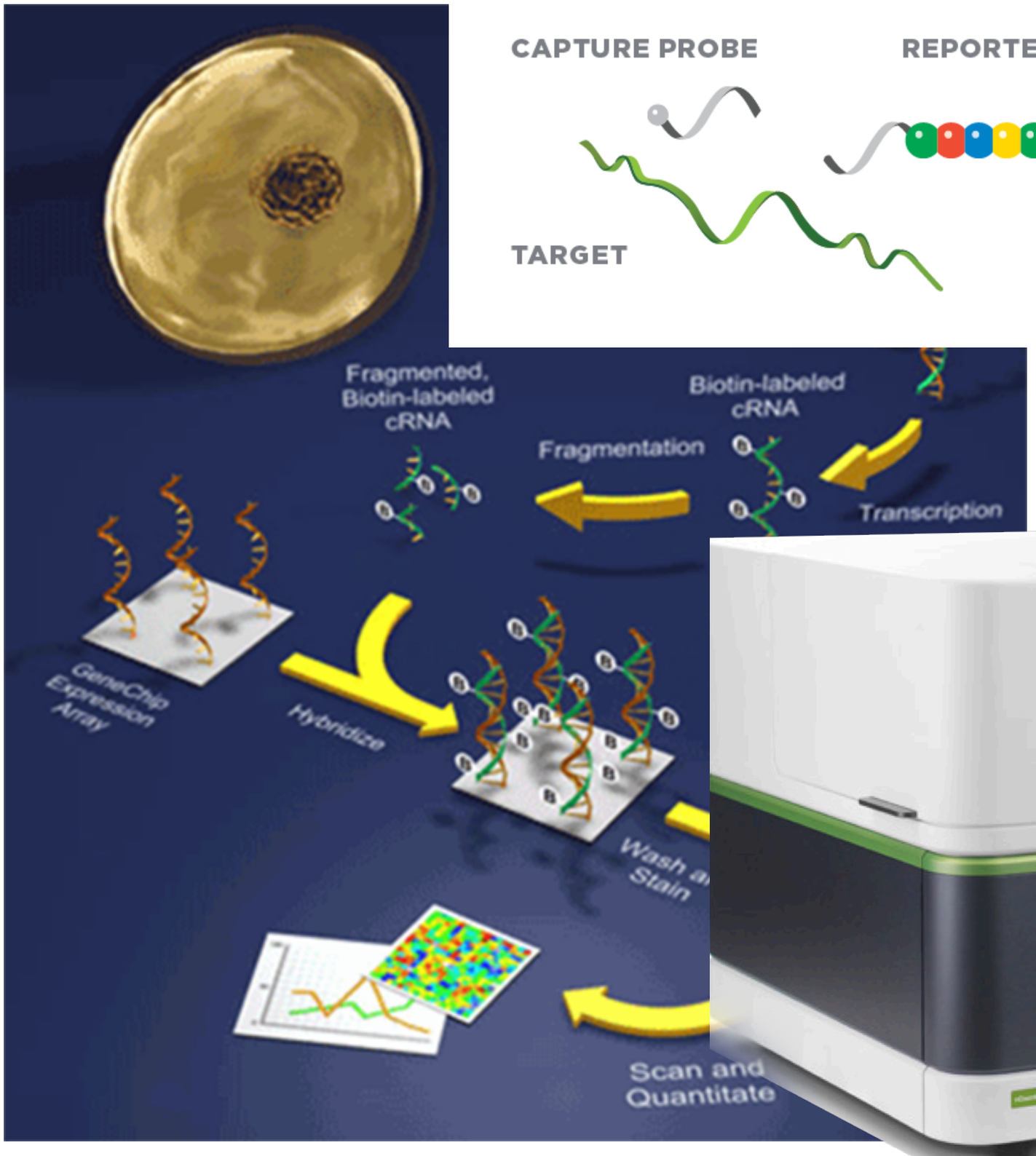
Cu = Cells Exposed to Copper (Heavy Metal)

Microarray technology at GSU



Nanostring technology at GSU






RNeasy® Mini Kit (50)
Cat. No. 74104
Store at room temperature (15-25°C)
QIAGEN
Tissue disruption in RNAwiz™
Add chloroform (RT, 5 min)
Centrifuge (10 min)
Isopropanol precipitate (RT, 5 min)
Centrifuge (10 min)
Drain, resuspend pellet
Total RNA
Northern Analysis, Nuclease Protection, RT-PCR, Translation

Principle and procedure

RNA purification using RNeasy technology

The RNeasy procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl water.

With the RNeasy procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently. Protocols for purification of small RNA using RNeasy Kits are available at www.qiagen.com/goto/microRNAprotocols.

In this handbook, different protocols are provided for different starting materials. The protocols differ primarily in the lysis and homogenization of the sample and in the adjustment of the conditions for binding RNA to the RNeasy membrane. Once the sample is bound to the membrane, the protocols are similar (see flowchart, next page).

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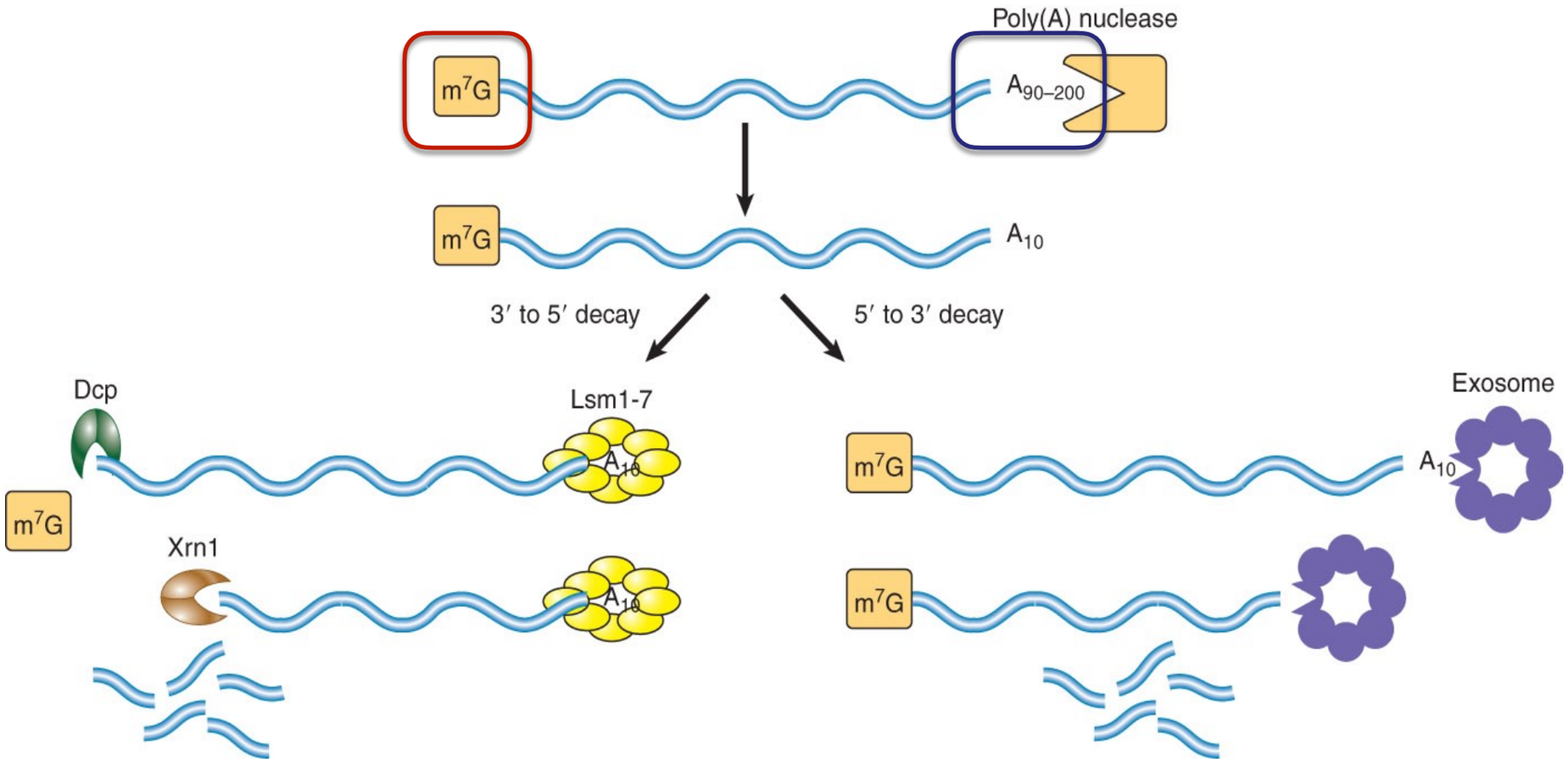
Most Eukaryotic mRNA is Degraded *via* Two Deadenylation-Dependent Pathways

- The two major mRNA decay pathways are initiated by deadenylation catalyzed by **poly(A) nucleases**.
- Deadenylation may be followed either by decapping and 5′ to 3′ exonuclease digestion, or by 3′ to 5′ exonuclease digestion.



The major deadenylation-dependent decay pathways in eukaryotes

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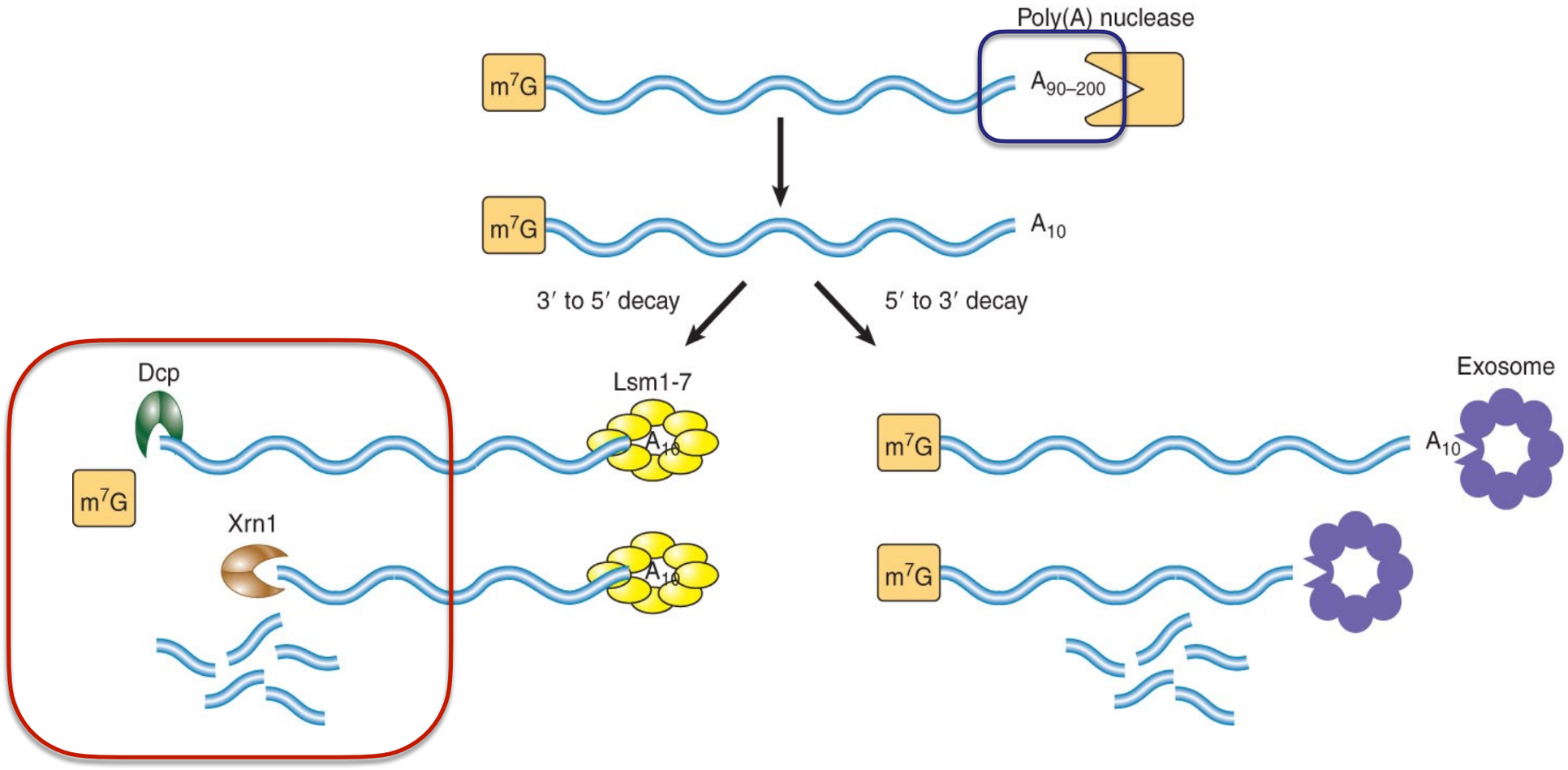

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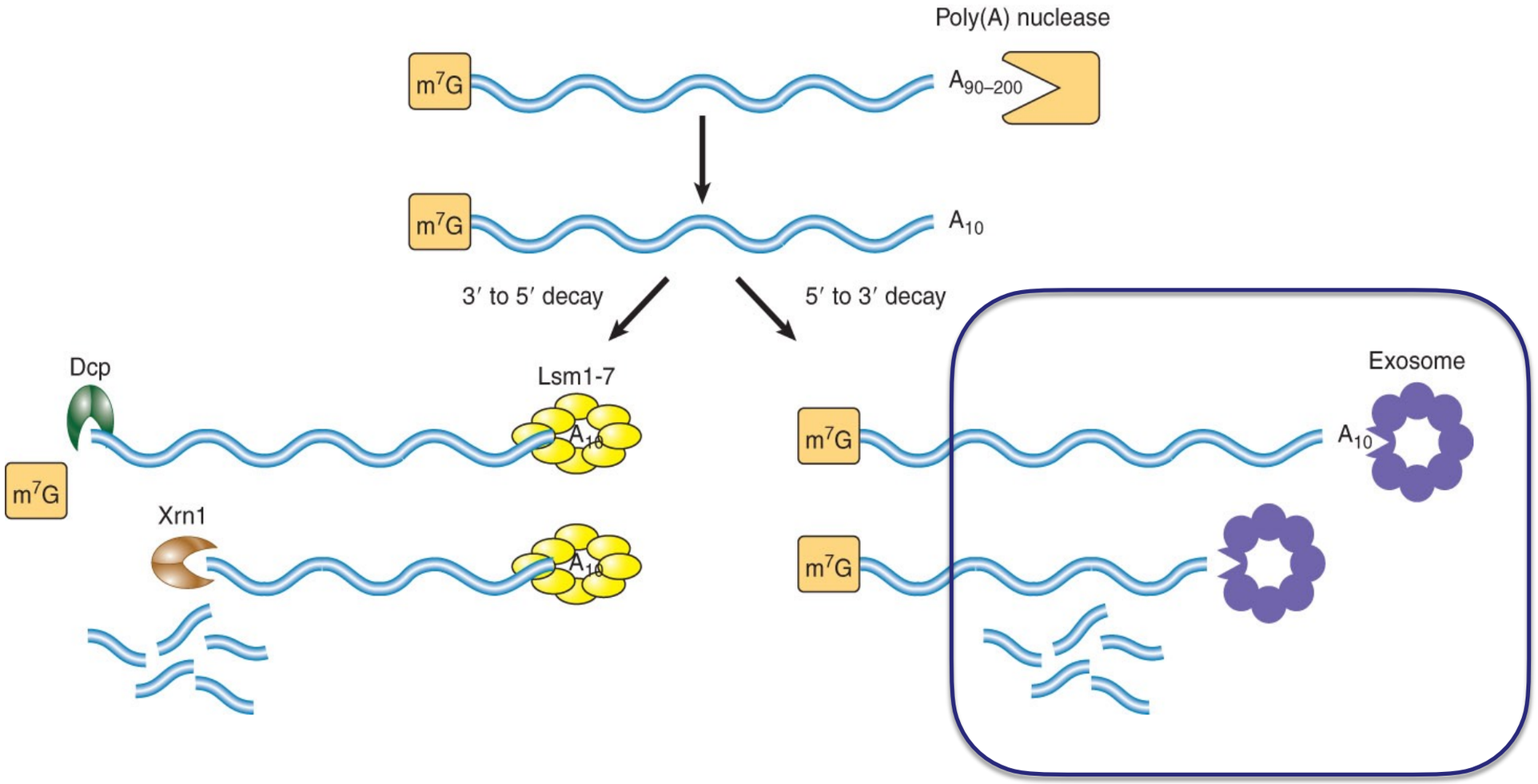
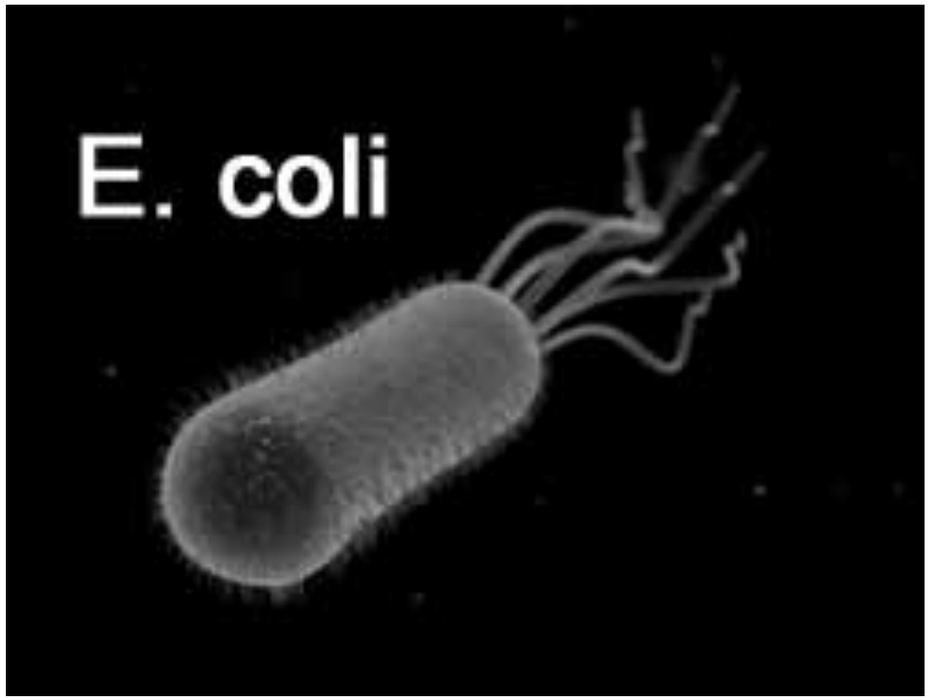


The major deadenylation-dependent decay pathways in eukaryotes



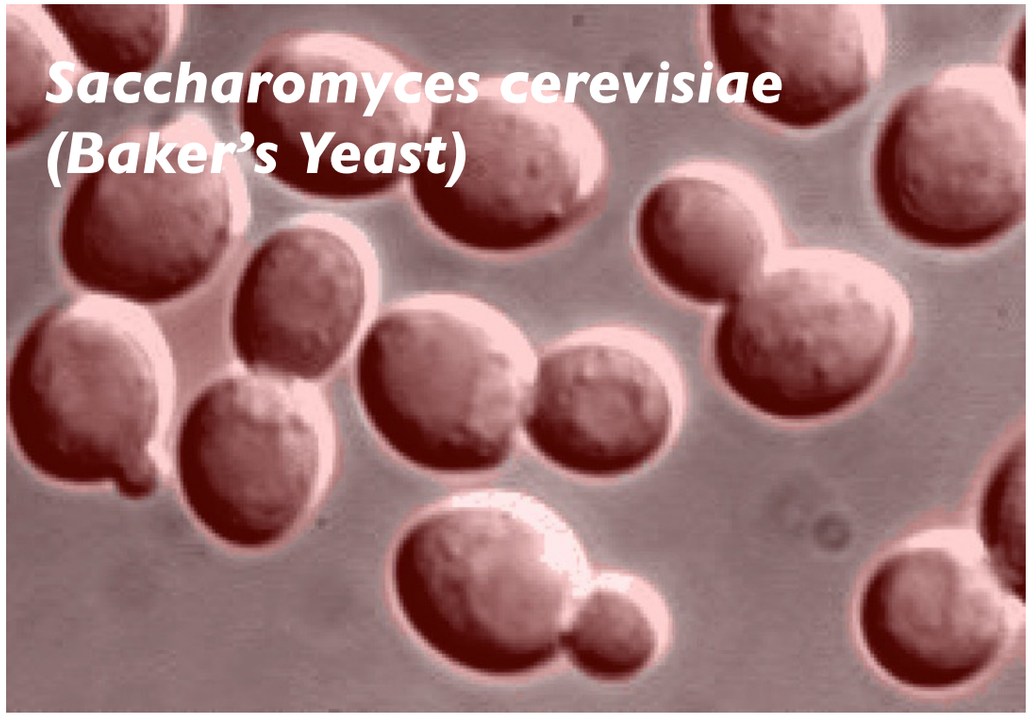
Bacteria thrive on many different types of food. But most yeasts can live only on sugars and starches. From these, they produce carbon dioxide gas and alcohol. Thus, they have been useful to man for centuries in the production of certain foods and beverages. They are responsible for the rising of bread dough and the fermentation of wines, whiskey, brandy and beer. They also play the initial role in the production of vinegar.

~ 1.5 - 3 μm

Prokaryote

DNA is Circular

Cell membrane



Yeasts are small, ***single-celled plants***. They are members of the family ***fungi*** (singular, ***fungus***), which also includes mushrooms. Fungi differ from other plants in that they have no chlorophyl.

~ 50 μm

Eukaryote

DNA is linear

Cell wall

Standard Operating Protocol: RNA Extraction

I. **Mechanical Lysis: -ALL Centrifugation steps will be at undertaken @ 4 ºC**

a. Centrifuge **~5ml yeast cell sample** (**3 min at 8,000 x g**) -and remove all media from cell pellet.

b. Add **600 µl Buffer RLT** to **sample cells** -**resuspend** cells and transfer to the screwcap microtube (these will be provided) containing acid-washed glass beads.

c. Beat samples in the bead-beater for 30 seconds and place in ICE for 30 seconds. Repeat this step **(stepC) for a total of 4 cycles.**

“Bead” Cell Homogenizer

Standard Operating Protocol: RNA Extraction

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c. Beat samples in the bead-beater for 30 seconds and place in ICE for 30 seconds. Repeat this step **(stepC) for a total of 4 cycles.**

II. Crude RNA Extraction:

a. Take 350 µl of the bead-beaten sample and pipette into a fresh microcentrifuge tube. **Add 350 µl of 70% ethanol** and mix well by pipetting.

b. Transfer total sample to a **clean RNeasy Spin column** and centrifuge for **30 seconds at 8,000 x g. Discard the flow-through.**

c. Add 700 µl Buffer RW1 and centrifuge for 30 seconds at 8,000 x g. **Discard the flow-through.**

d. Add 500 µl Buffer RPE to column and centrifuge for 30 seconds at 8,000 x g. **Discard the flow-through.**

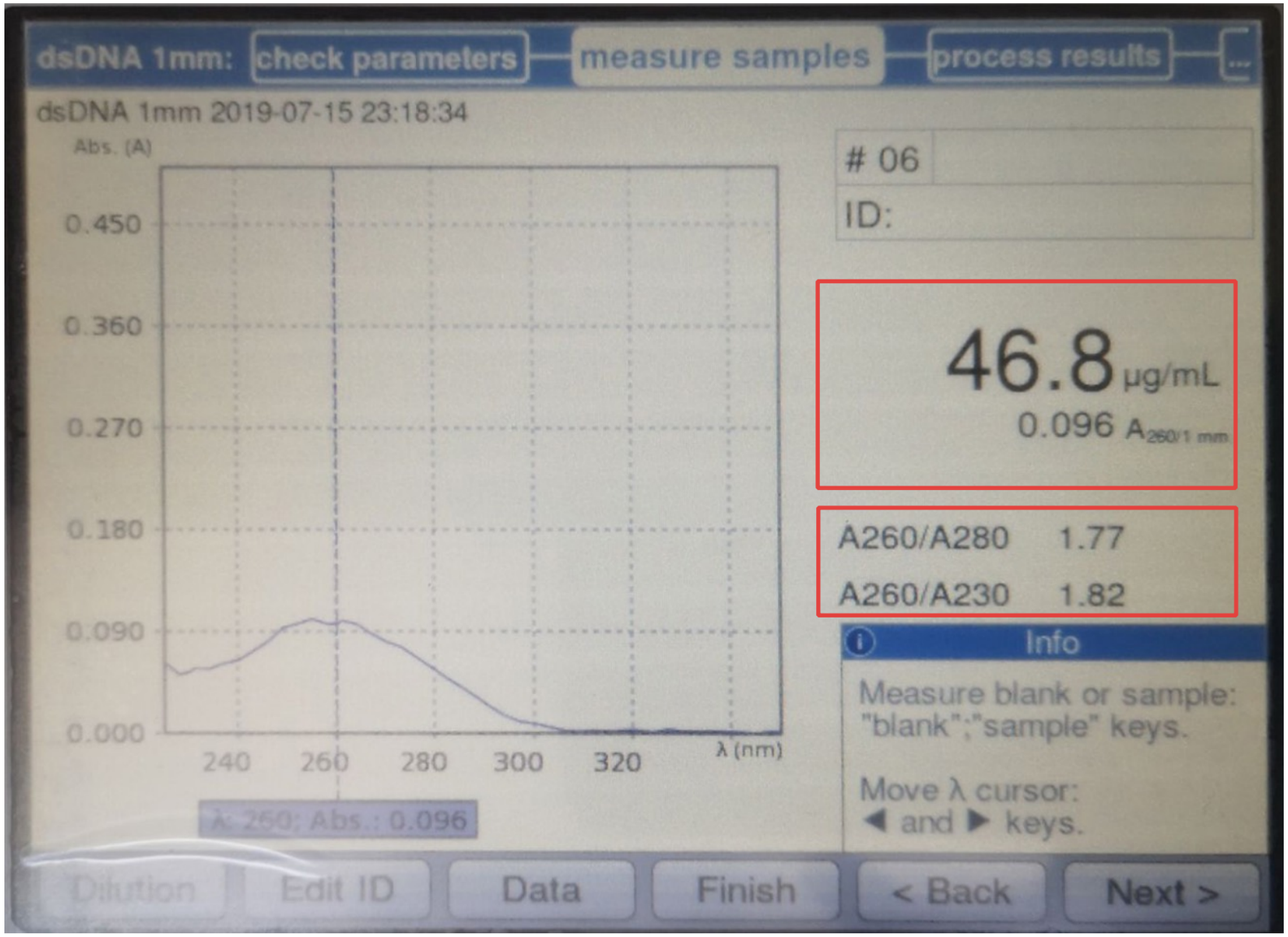
e. Repeat (d), -this time **centrifuging for 2 min at 8,000 x g**.

f. Replace collection tube with a NEW ONE and centrifuge for 1 min.

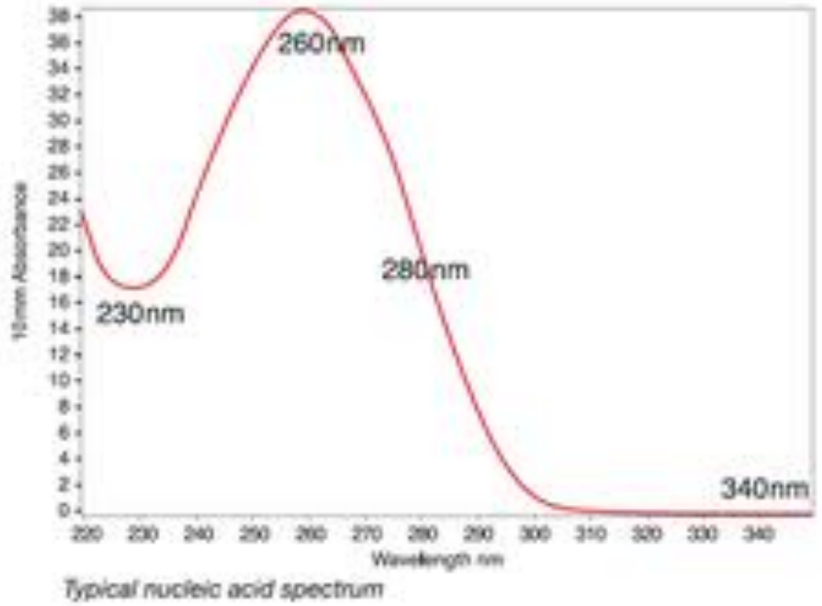
g. Place spin column in microcentrifuge tube and **add 30-50 µl of RNase free H_2O**. **Centrifuge for 1 min at 8,000 x g** to elute the RNA.

h. Repeat (g)

i. Measure RNA concentration using the Spectrophotometer.

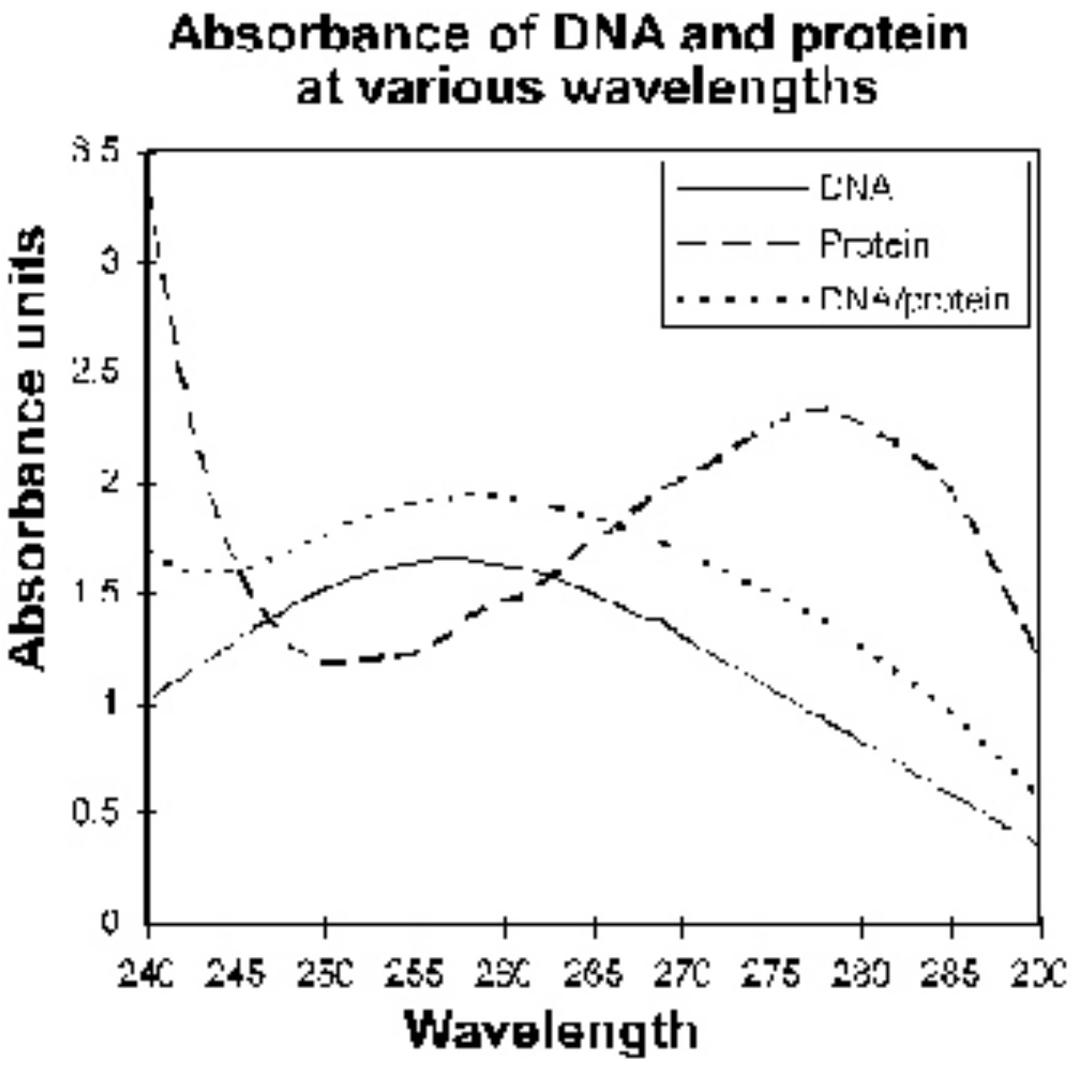


Biophotometer D 30
Eppendendorf



Typical nucleic acid spectrum

Nucleic acids absorb ultraviolet light in a specific pattern. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.



The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA.

A ratio of ~1.8 is generally accepted as “pure” for DNA;

a ratio of ~2.0 is generally accepted as “pure” for RNA.

Evaluating Concentration and Purity of RNA

Quantitation of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer. To ensure significance, readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. This relation is valid only for measurements in water. Therefore, if it is necessary to dilute the RNA sample, this should be done in water. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with RNaseZAP® followed by washing with RNase-free water. Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantitation is shown below:

Volume of RNA sample = 1.6 ml

Dilution = 10 µl of RNA sample + 490 µl distilled water (1/50 dilution).

Measured absorbance of diluted sample in a 1 ml cuvette (RNase-free): A260 = 0.75

Concentration of RNA sample = 40 x A260 x dilution factor
= 40 x 0.75 x 50
= 1500 µg ml^{-1}

Total yield = concentration x volume of sample in milliliters
= 1500 µg ml^{-1} x 1.6 ml
= 2400 µg = 2.4 mg RNA

http://www.cbil.upenn.edu/downloads_orig/EPConDB/download/Protocols/RNA%20-%20Evaluating%20Concentration%20and%20Purity.pdf