SUMMER INSTITUTE CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
						July 02
						Early Arrival Airport Arrivals and Check-in
July 03	04	05	06	07	08	09
Early Arrival Airport Arrivals and Check-in	Airport Arrivals and Check-in 6:00pm: 4th of July Celebrations	9:30am-12pm: Campus tour, Panther ID & ISSS Check-in 12-2pm Lunch 2:00-6:00pm, Shuttle to local grocery store	9:30am-11:30am ISSS, OII, & Housing Orientation & Presentation **2:30-4:30pm:-Welcome Reception and Buddy Meet & Greet Event**	**Classes begin!** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 INTRO - TRAINING**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA PREPARATION**	Free Day
10	11	12	13	14	15	16
12:00-4:00pm: The World Coca-Cola and Georgia Aquarium	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS I**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS II**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm:BIOL4905 PROTEOMICS III** 6:00-10:00pm: Atlantic Station Shopping & Movie (Sign-up)	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS IV ?**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 RNA PREPARATION**	6:00-9:00pm: Dinner in America (Sign-up)
17	18	19	20	21	22	23
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 qPCR & AUTOMATION**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA SEQUENCING**	MINI BREAK	9-11:20am: Morning course **CDC TRIP** **1:30-4:30pm: BIOL4905 MICROSCOPY / AFM**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30 - 4:30pm: BIOL4905 NEXT GEN SEQ.** 5:30-7:30pm: Meet & Greet BBQ event @ The Commons	9:00am - 6:00pm: Outlet Mall
24	25	26	27	28	29	30
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 MICROARRAY I**	9-11:20am: Morning course 12:30 - 1:30pm: Lunch and LearnGrad School Info Session **2:00 - 5:00pm: BIOL4905 MICROARRAY II**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 NANOSTRING**	**Last day of classes** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 FLOW CYTOMETRY**	**FINALS**	Free Day
31	August 01	02	03	04		
Free Day	Activity Day at the Recreation Center (Sign-up)	Free Day	9:30-11:00am: Georgia Capitol Tour (Sign-up) 2:00-4:00pm: Closing Reception	Departures (check-out at 12:00pm)		

Note: Students may arrive prior to the program date with an extra charge of $35 per night. Earliest day to check-in to University Commons is July 2.

Legend:

Orange: Courses **Blue: Lunch Break.** **Red: Sign-up events**

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

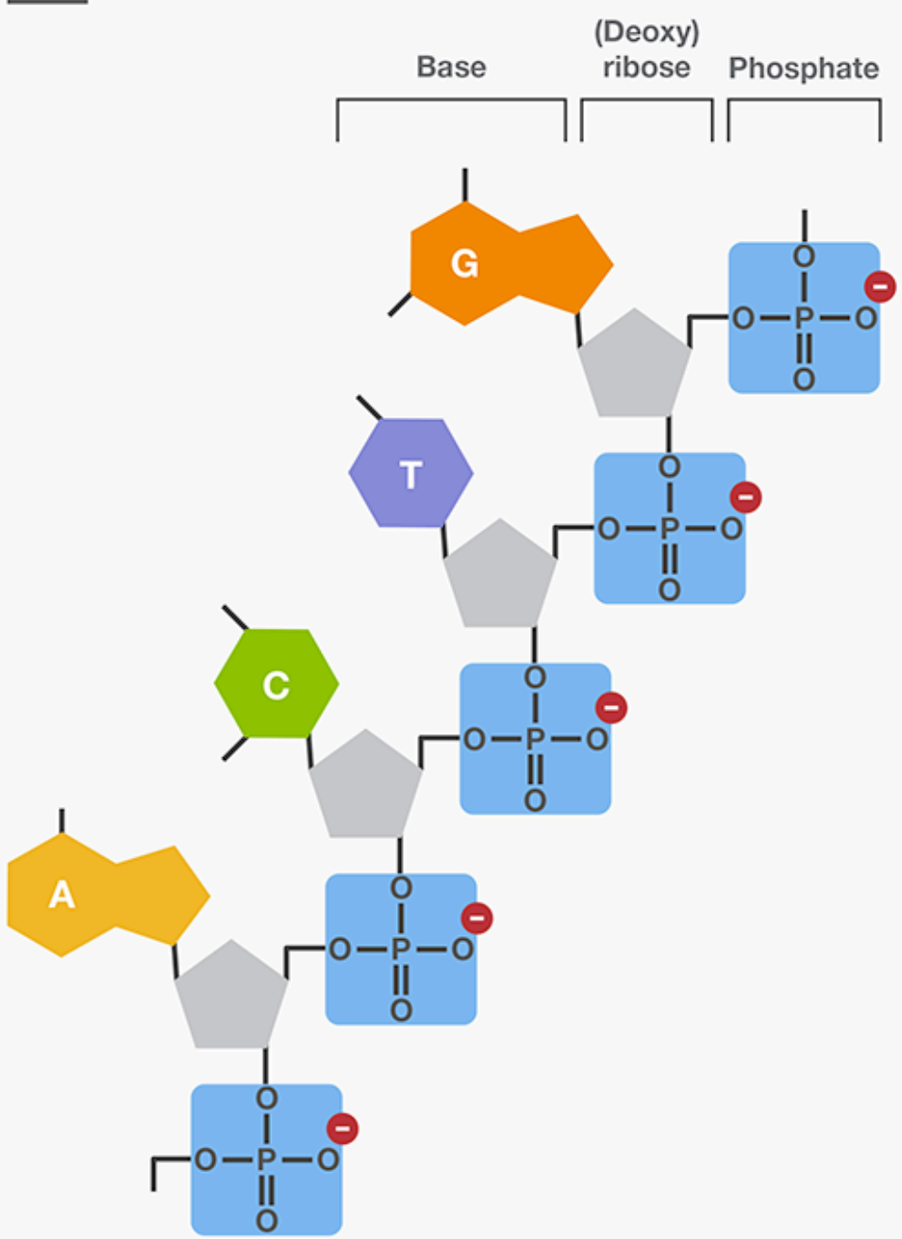
1. Take **5 ul of your DNA** sample and add it to a new microcentrifuge tube. Add **1 ul 5x DNA loading buffer**.

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

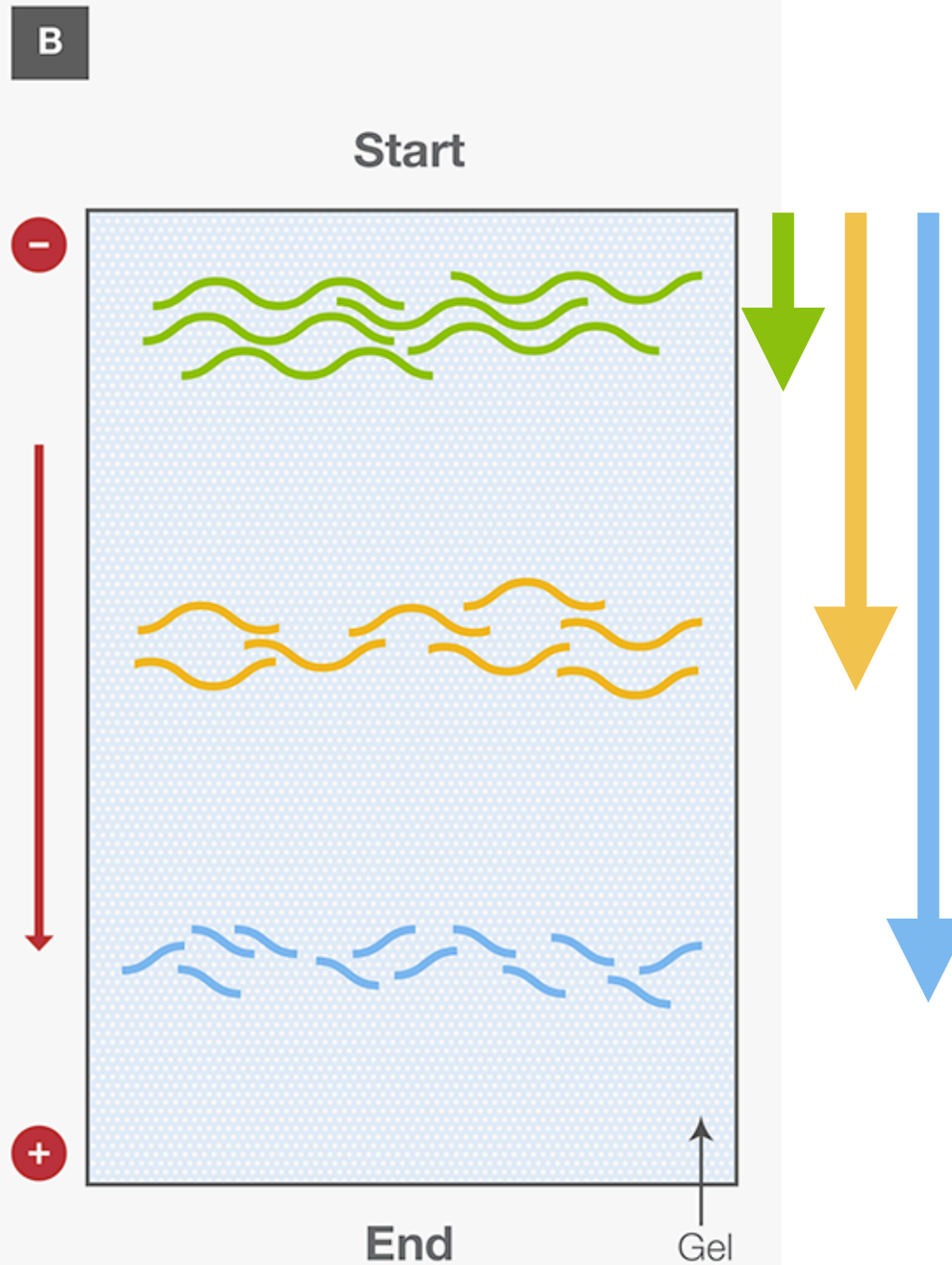
3. Start electrophoresis at **80V for 30 minutes** and power up to **100V**. Stop when the front running dye reaches ¼ from end. DNA will migrate from negative electrode to positive.

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

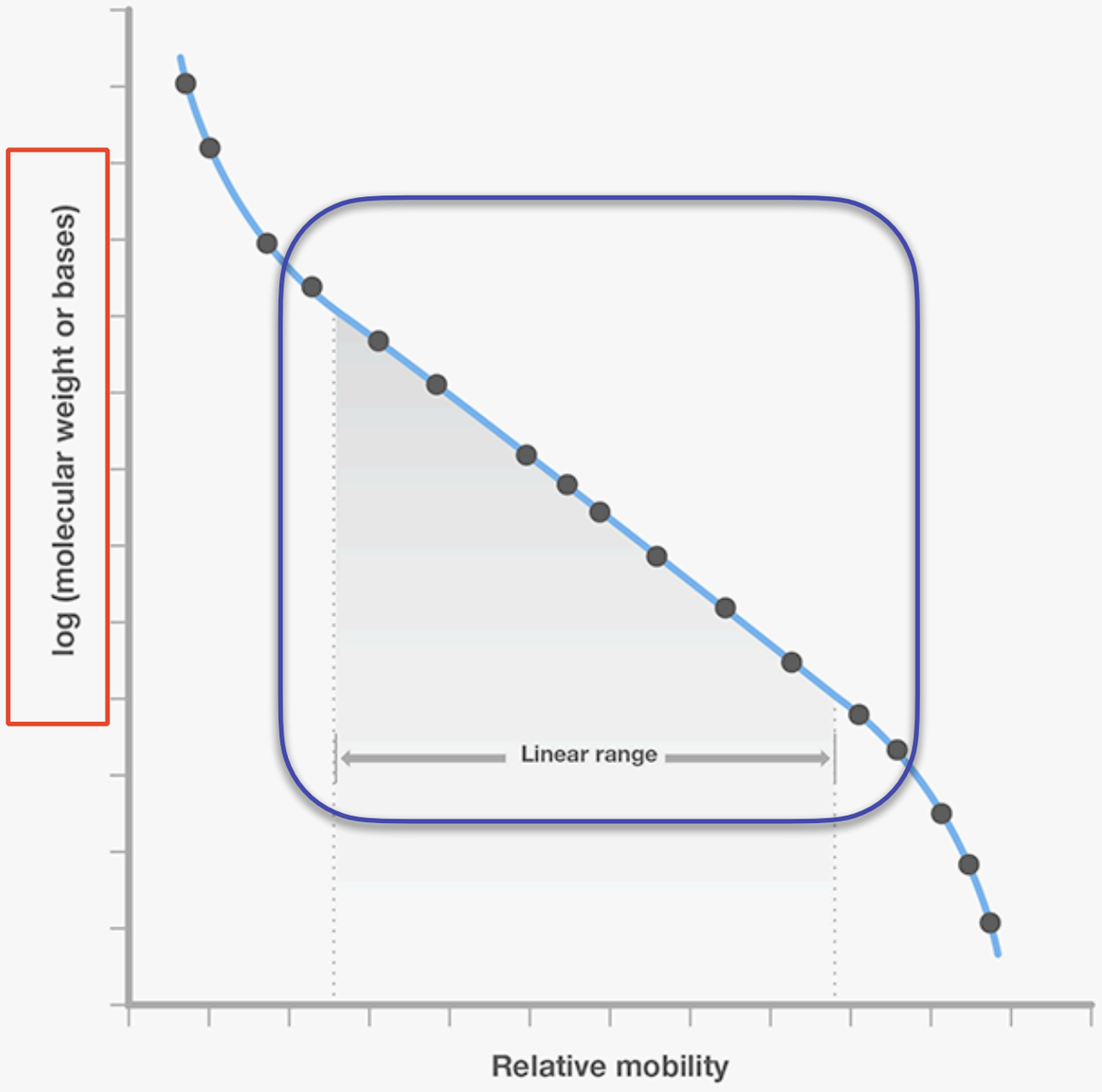
A



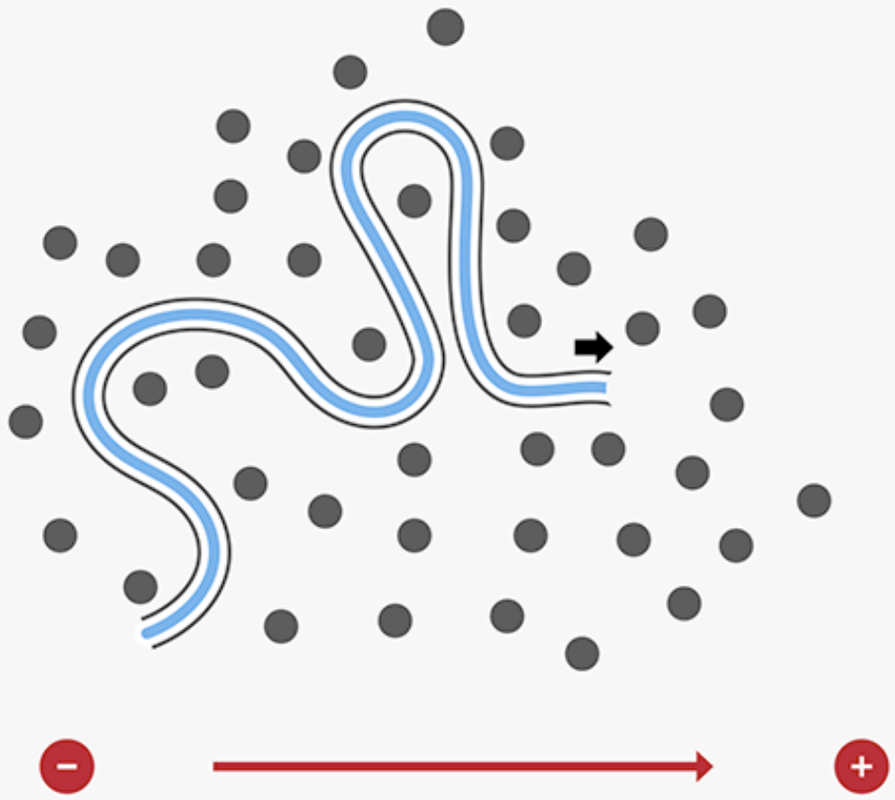
B



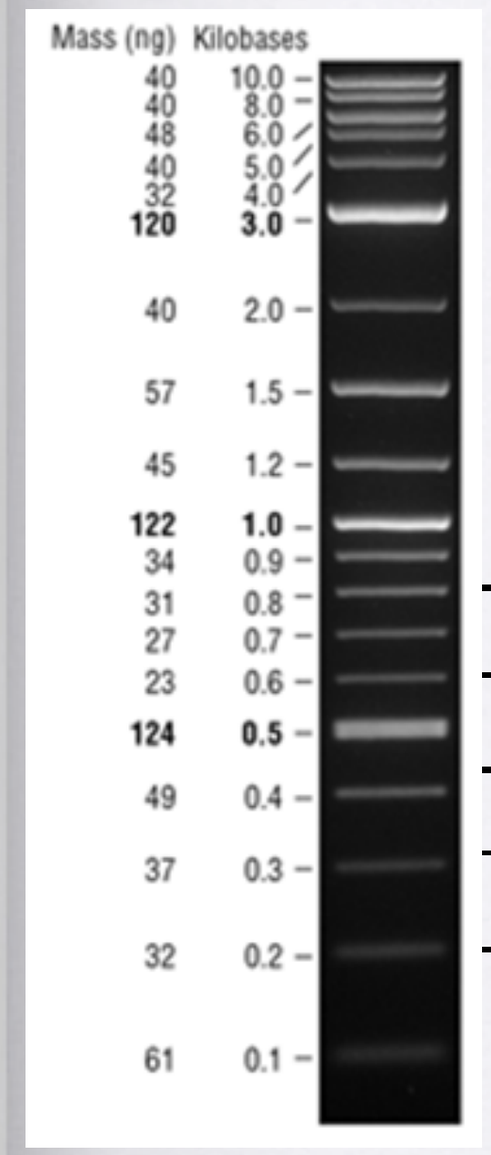
A

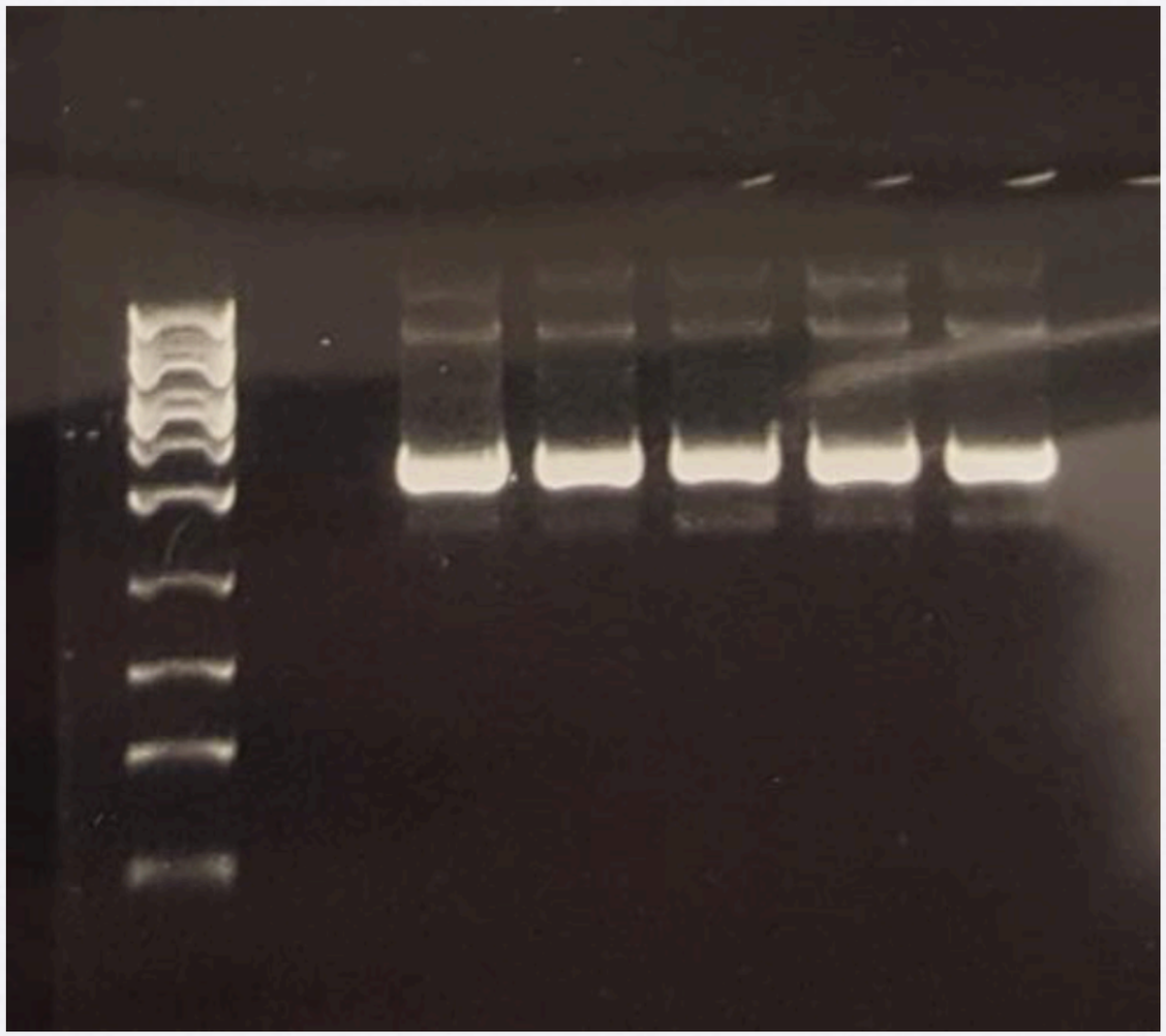

log (molecular weight or bases)
Linear range
Relative mobility

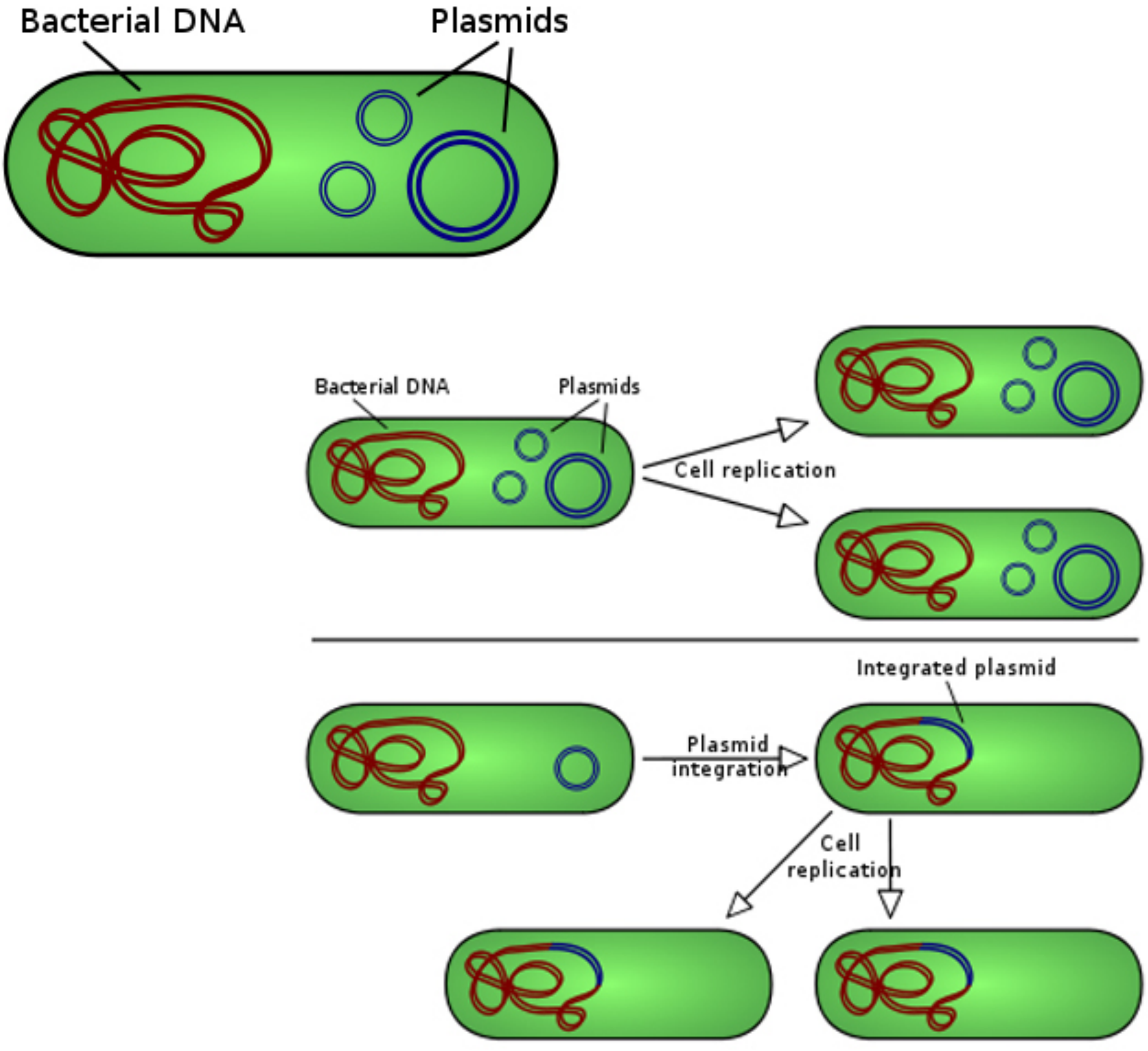
B

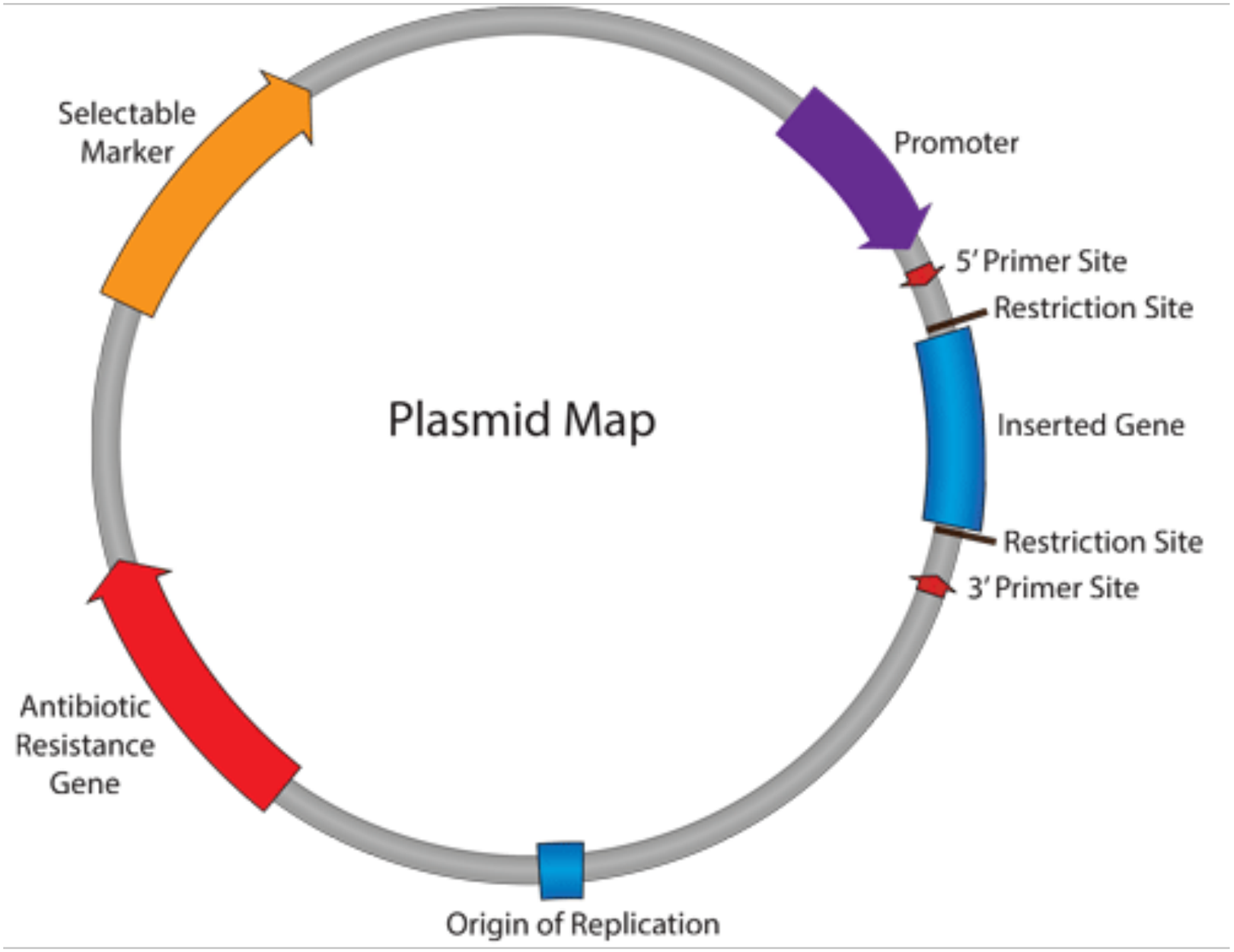

−
+

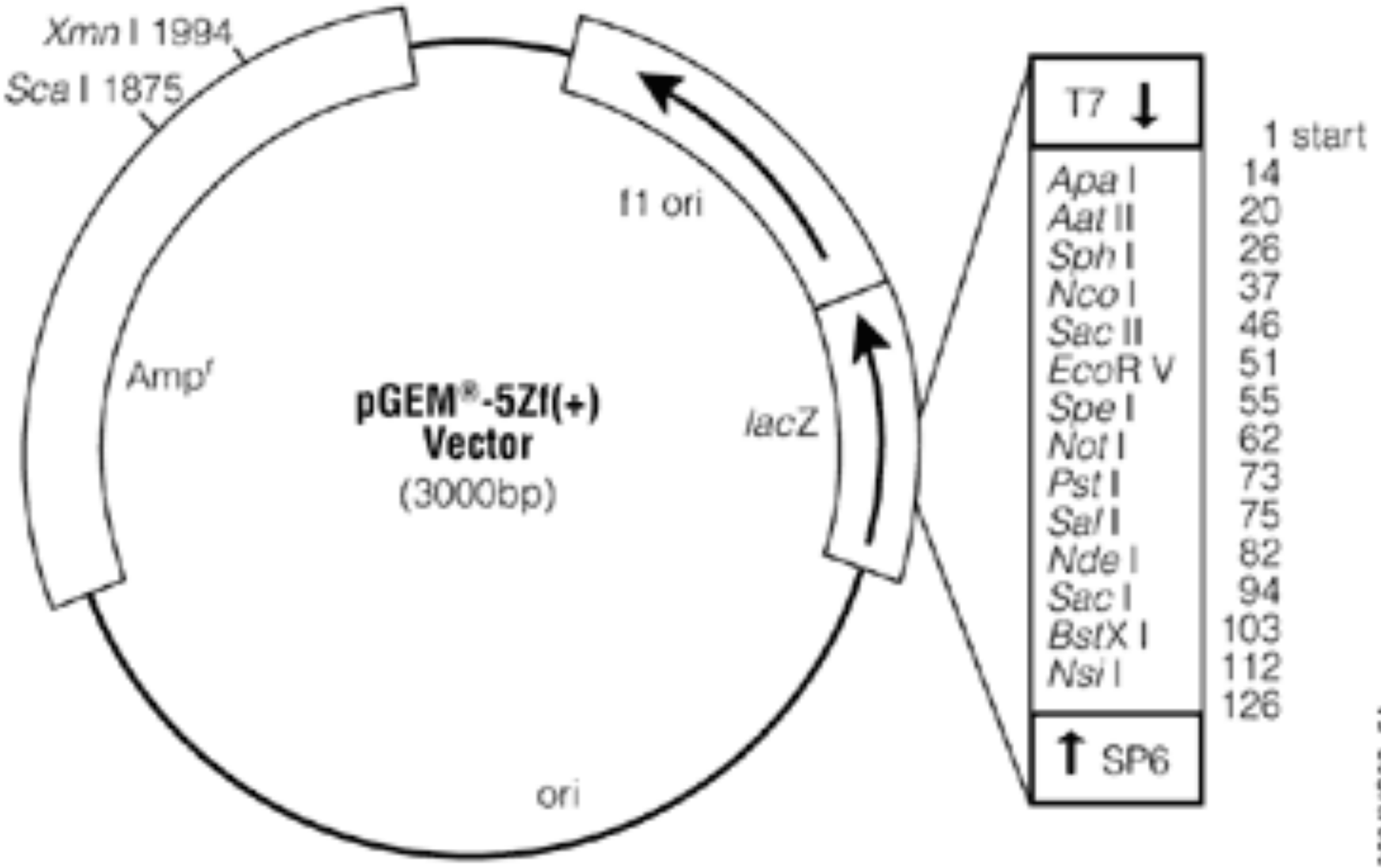
Control 6 7 8 9 10

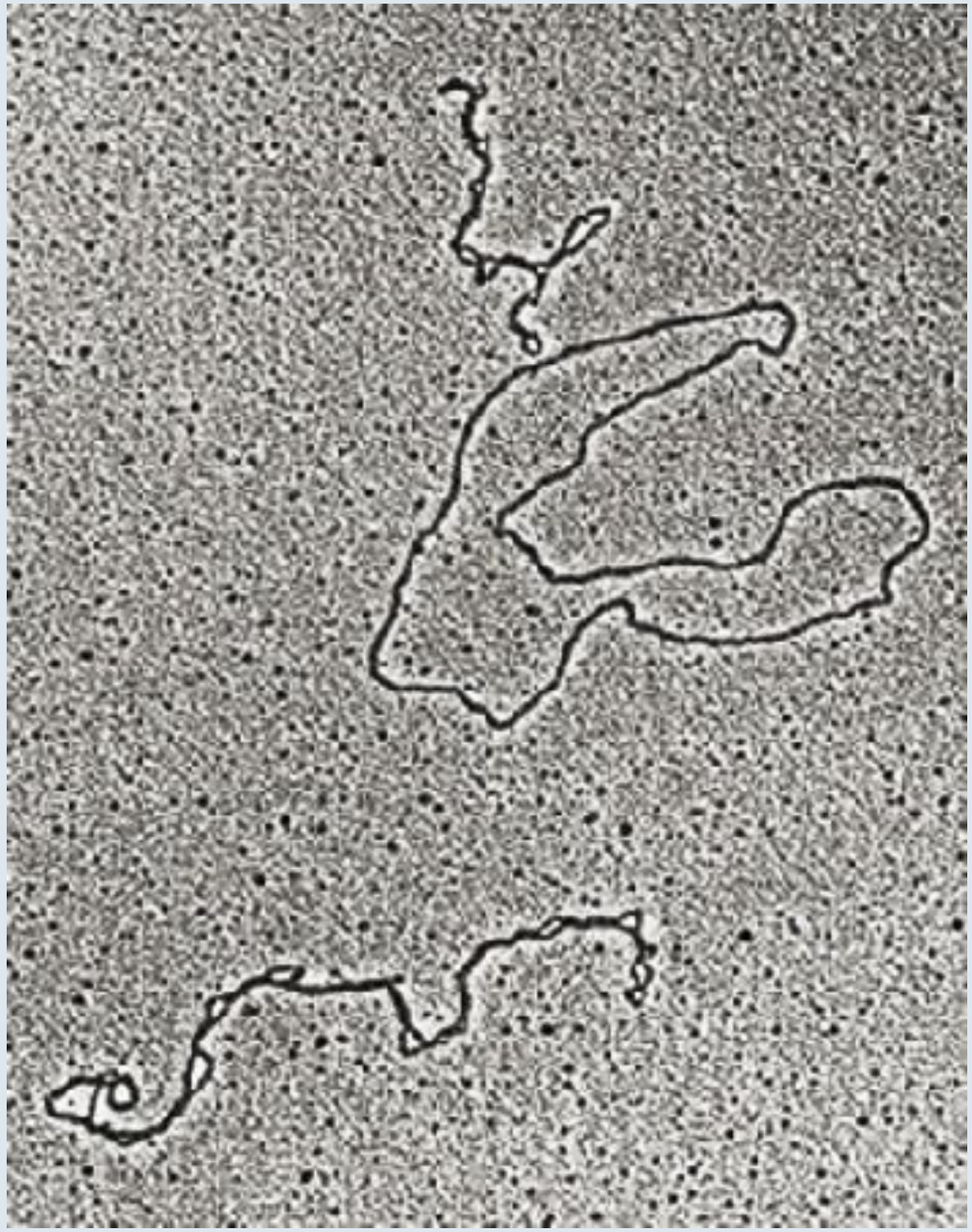




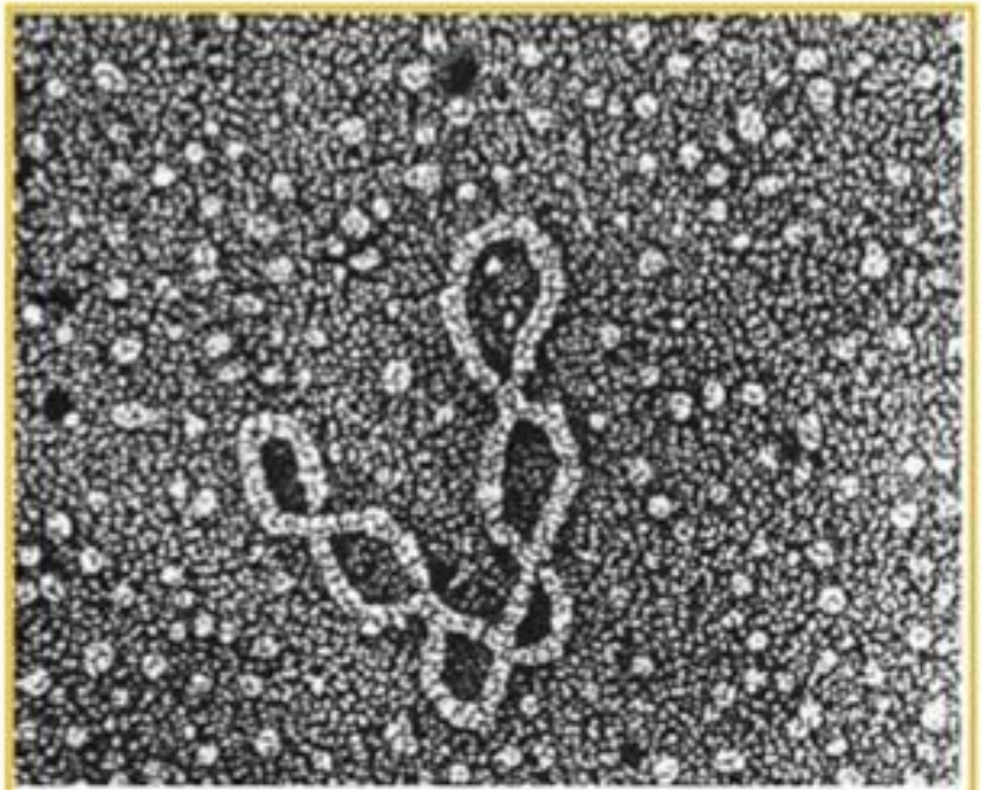

Bacterial DNA
Plasmids
Bacterial DNA
Plasmids
Cell replication
Integrated plasmid
Plasmid integration
Cell replication


Plasmid Map
Selectable Marker
Promoter
5' Primer Site
Restriction Site
Inserted Gene
Restriction Site
3' Primer Site
Antibiotic Resistance Gene
Origin of Replication


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

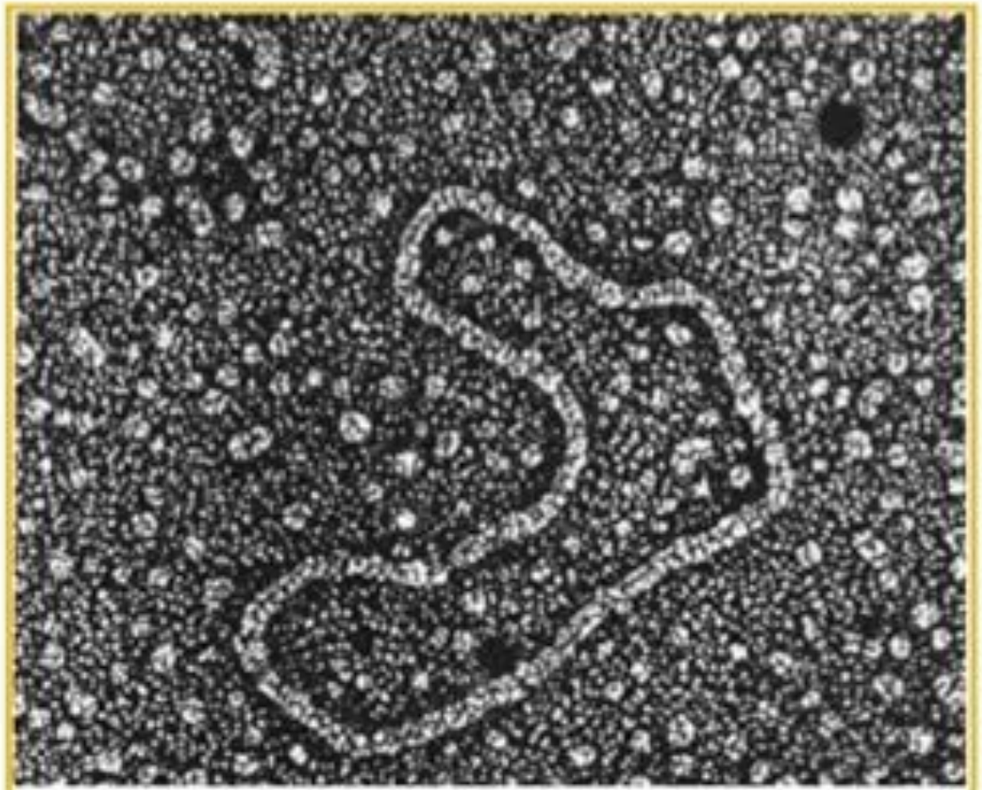


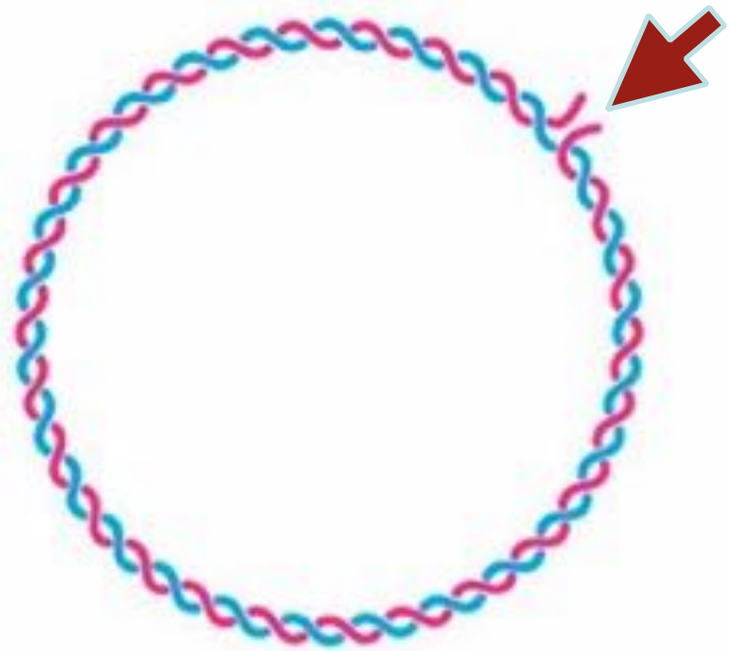
(a) Supercoiled



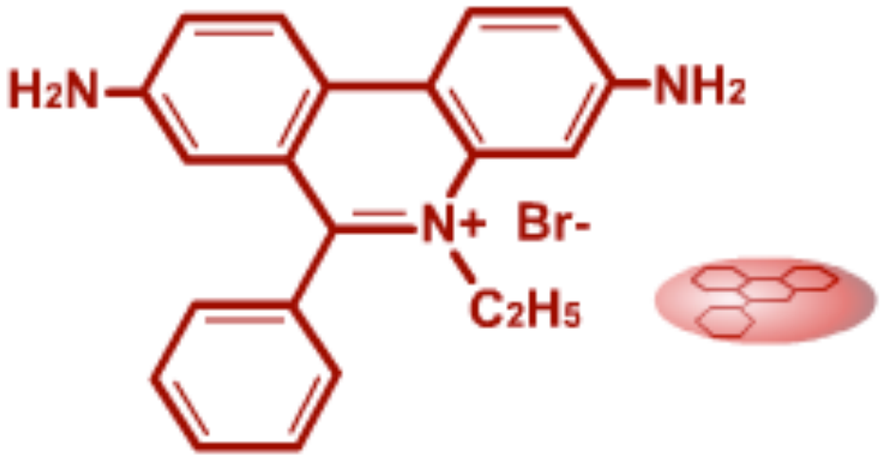
https://www.youtube.com/watch?v=az2c6UbEdug

(b) Relaxed circle



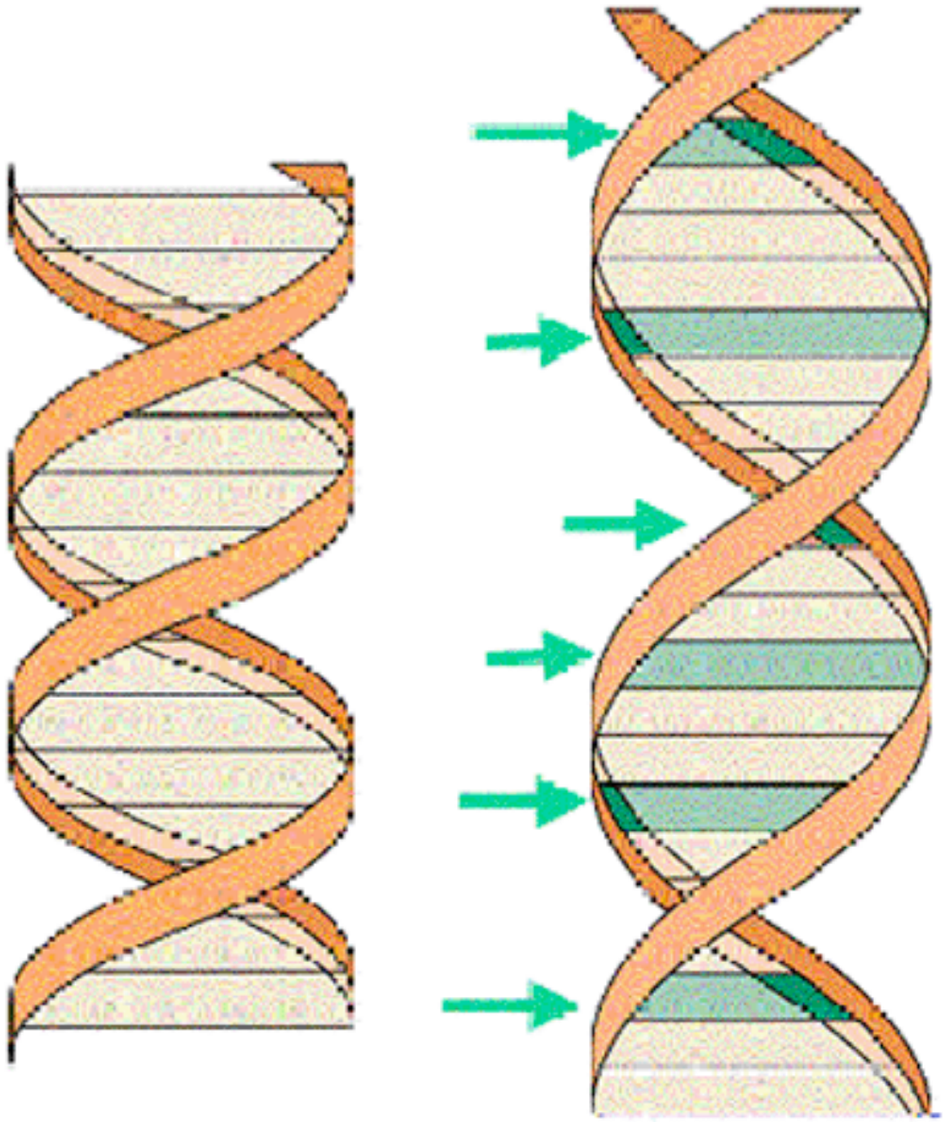


Ethidium Bromide



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.

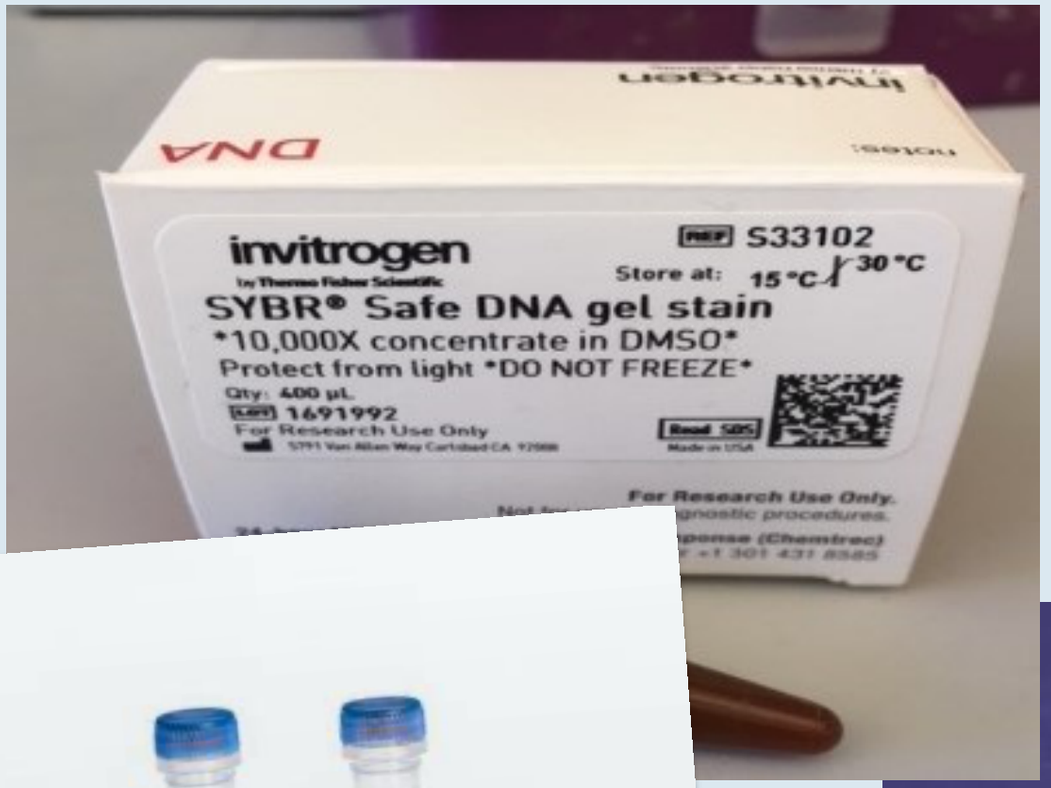
Extra nucleotides are added during replication

The DNA reading frame for RNA synthesis is changed:

amino acid changes

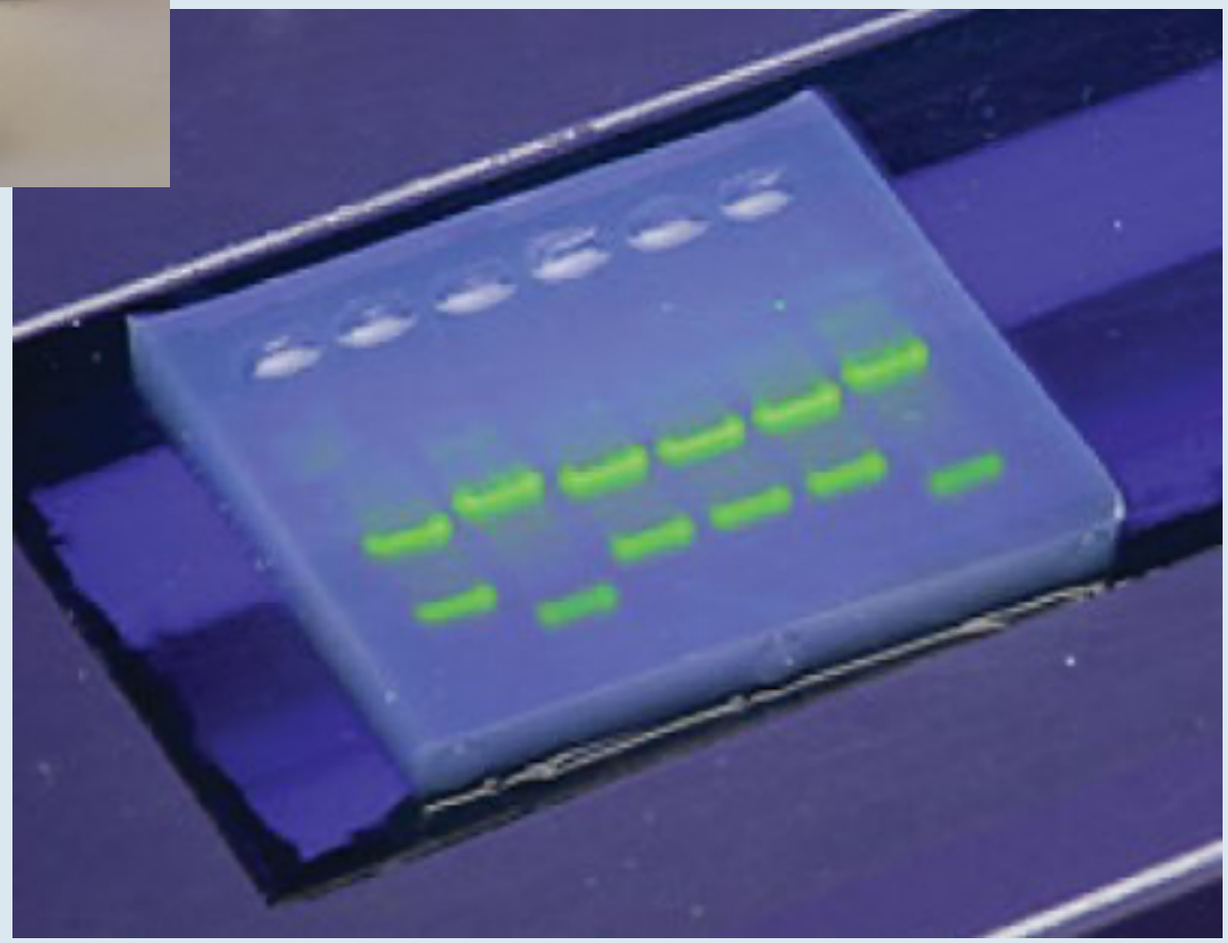
altered protein

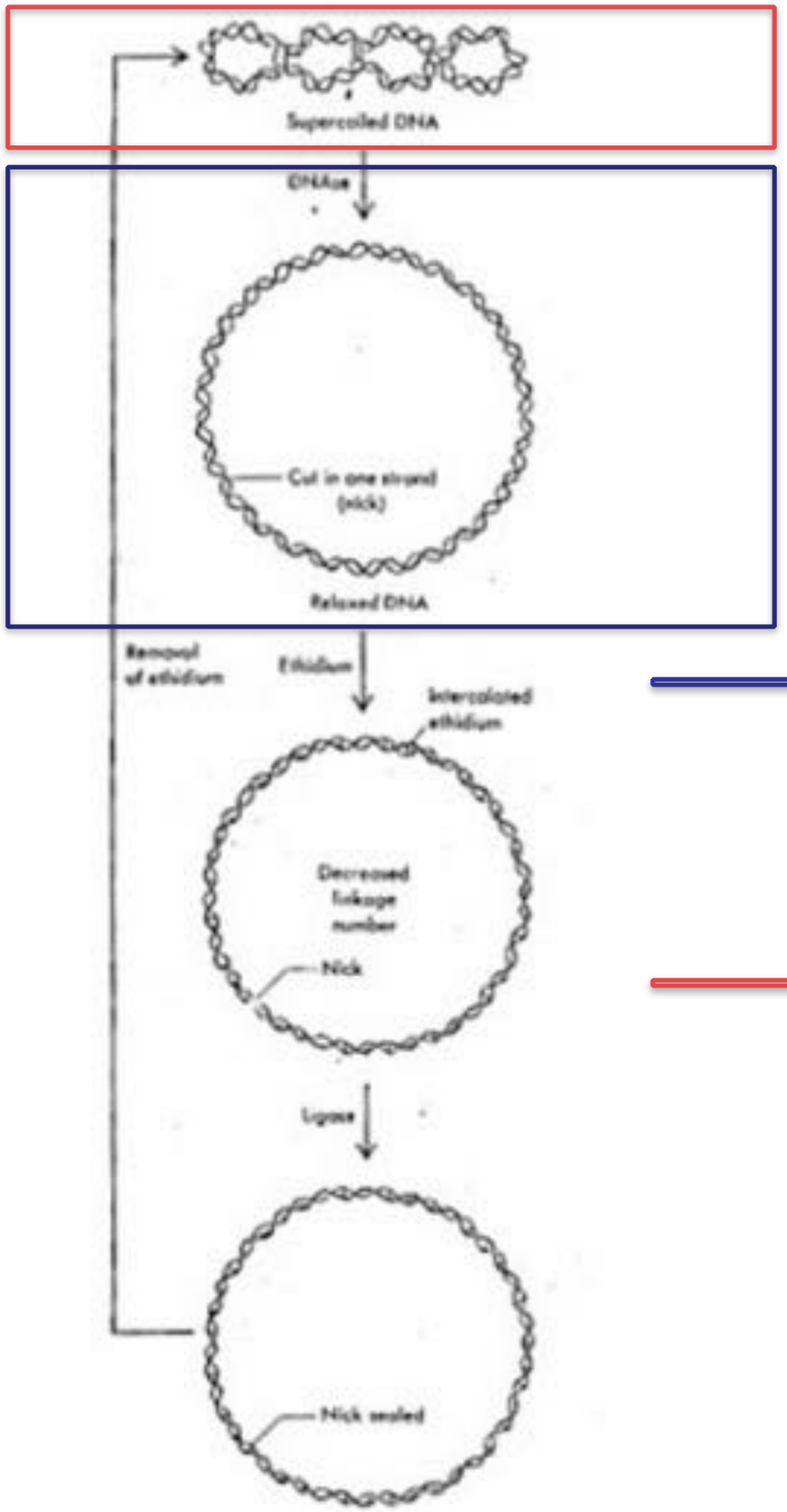
Intercalate

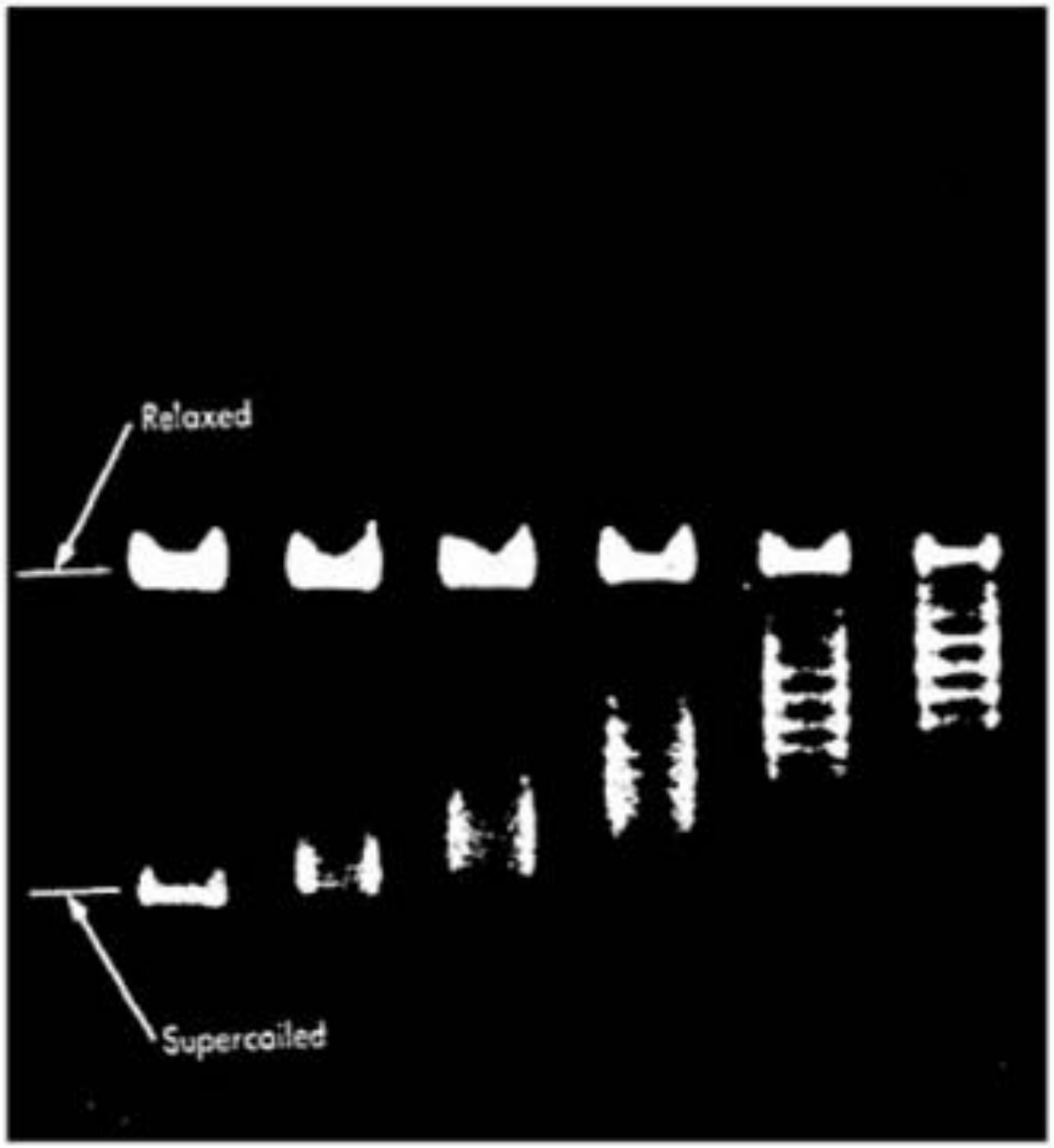

DNA
invitrogen
REF S33102
Store at: 15 °C / 30 °C
SYBR® Safe DNA gel stain
10,000X concentrate in DMSO
Protect from light *DO NOT FREEZE*
Qty: 400 µL
LOT 1691992
For Research Use Only
Read SDS
Made in USA
For Research Use Only.


Lonza
Lonza

GelStar™




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

Operation Manuals

Cell Protein and DNA Core Facilities

A

Analysis software

Image Quant TL
Genespring Microarray
FlowJo
Decyder 2D
ImageMaster 2D

Autoclaves

Electric
Steam

Electroporation

Gene Pulser Xcell
Micropulser (more online)

F

Film developer

X-Omat 2000A

Flow Cytometry

Accuri C6 (Instrument)
Accuri C6 (Software)
Aria Cell Sorter

Incubators & Sh

Incubator
Shaker, C25 Floor
Shaker, E25 Floor
Shaker, Classic Floo
Shaker, MaxQ4450
Shaker, MaxQ5000 Floor/Refrig
Shaker, Innova Floo
Stacking Shaker Refrigerated
Stacking Shaker Non-Refrigerated

Counters

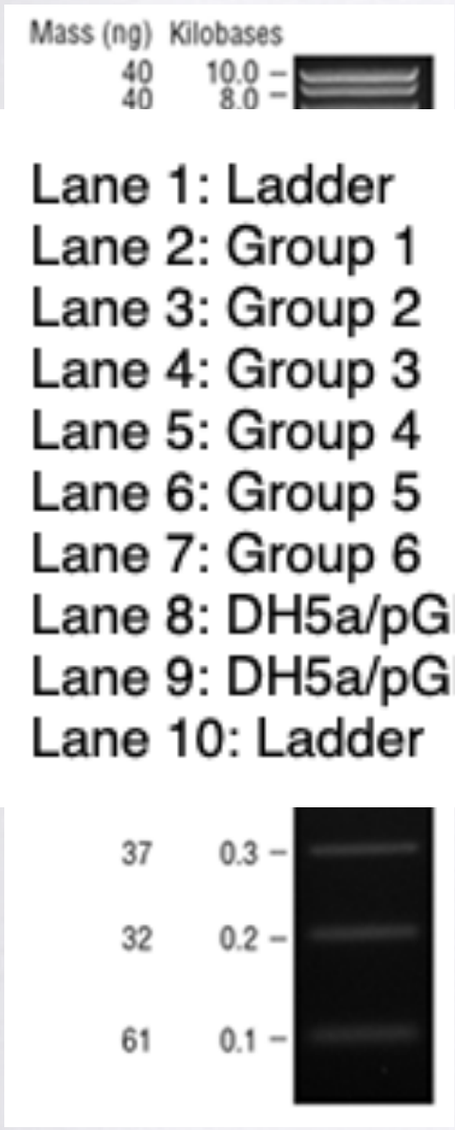
LS6500
1480 Wizard Gamma

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

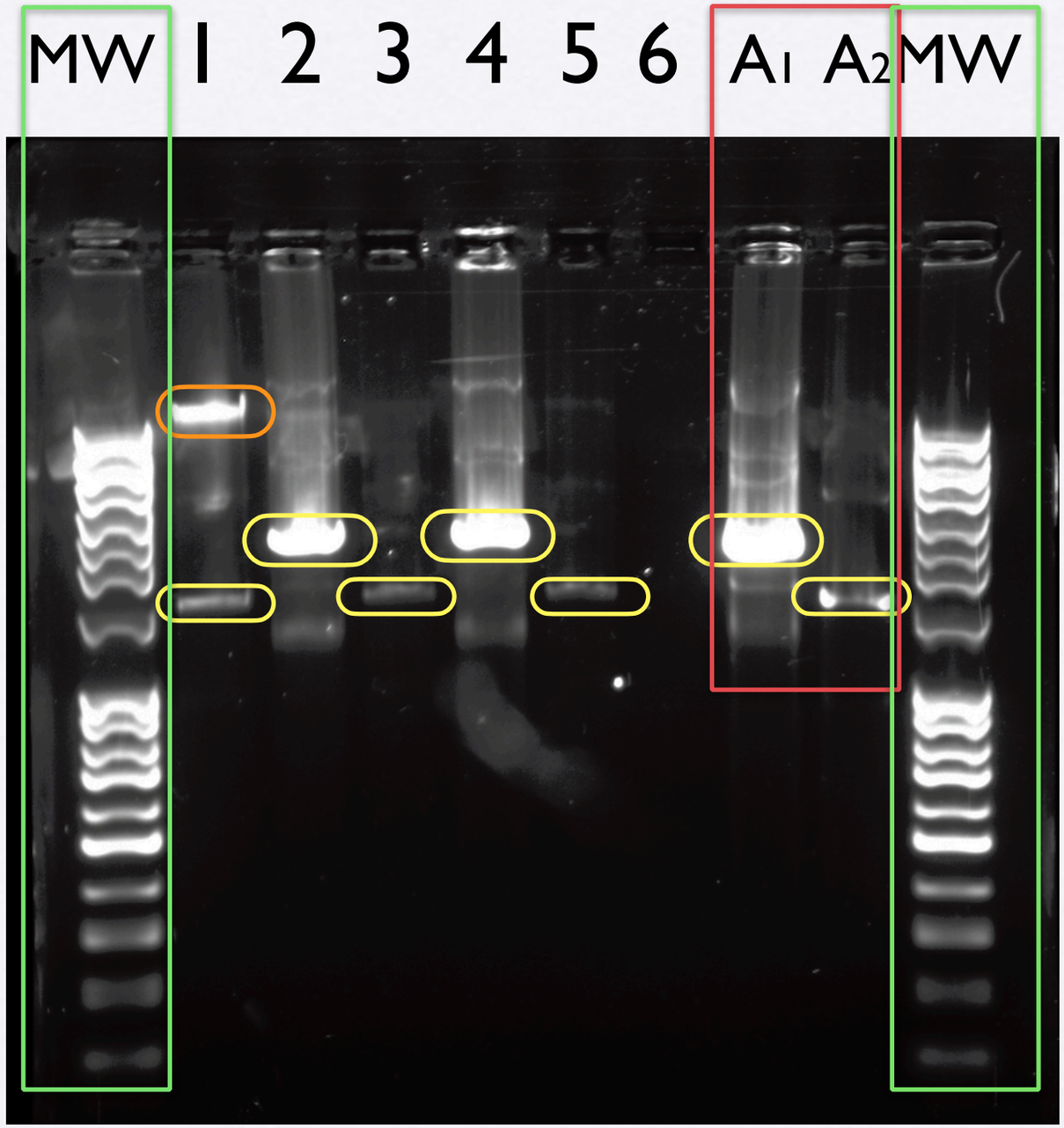
8. Press SNAP
9. To adjust image (invert, turn image), go back to Live. Select Preferences. Select PostProcessing tab (near the top).
10. Rotate image – use arrow to select; Invert – will change the image from black to white. OK.
11. Snap and now will see results of Preferences selection.
12. Press PRINT 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



Lane 1: Ladder
Lane 2: Group 1
Lane 3: Group 2
Lane 4: Group 3
Lane 5: Group 4
Lane 6: Group 5
Lane 7: Group 6
Lane 8: DH5a/pGEM17
Lane 9: DH5a/pGEM3Z
Lane 10: Ladder



Nanodrop 2000
Thermofisher

Spectrophotometers

ThermoFisher NanoDrop Plus (PSC 533)

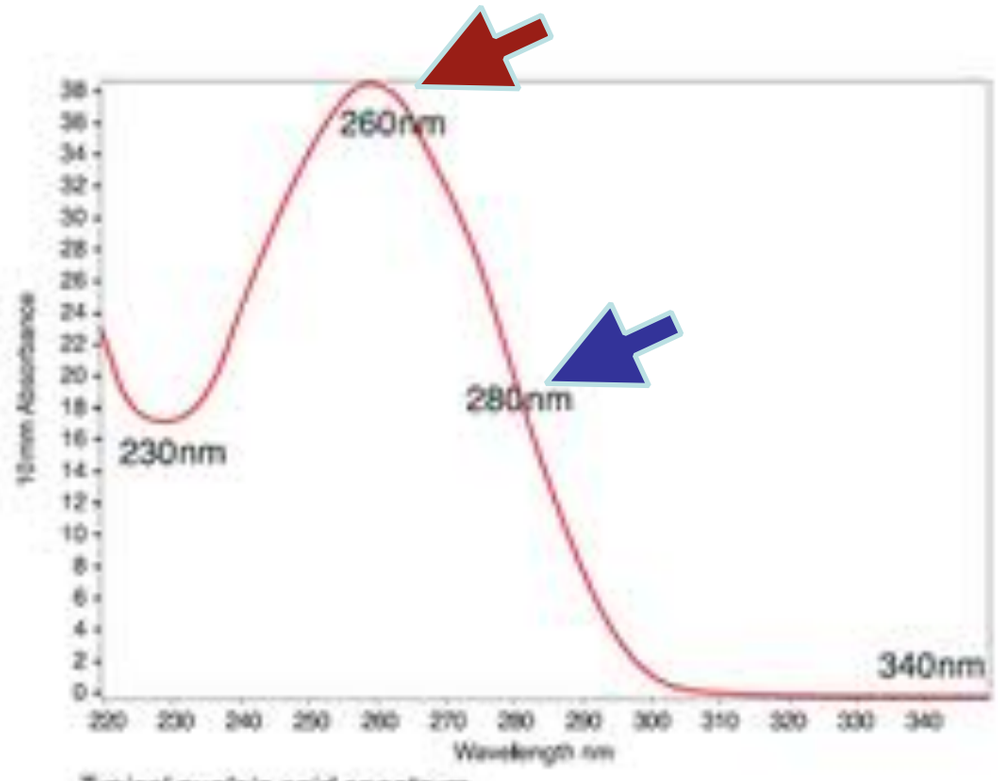
Every time you use the Spectrophotometers, you MUST sign the log book.

Contact: Ping Jiang (404) 413-5370; pjiang@gsu.edu for help with the Nanodrop

Nanodrop 2000/2000c

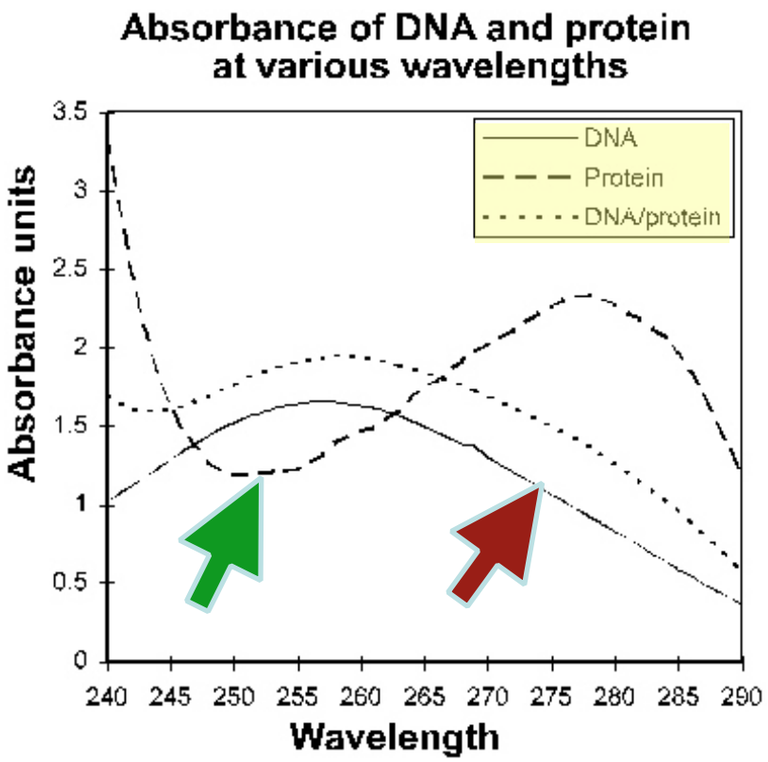
Field experience indicates that the following volumes are sufficient to ensure reproducibility:

Aqueous solutions of nucleic acids: 1 µL
Purified protein: 2 µL
Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2 µL
Microbial cell suspensions: 2 µL
It is best to use a precision pipettor (0-2 µL) with precision tips to ensure that sufficient sample (1-2 µL) is delivered.
Lower precision pipettors (0-10 µL and larger) are not as good at delivering 1 µL volumes to the measurement pedestal.
If the user is unsure about the sample characteristics or pipettor accuracy, a 2 µL sample volume is recommended.



Typical nucleic acid spectrum

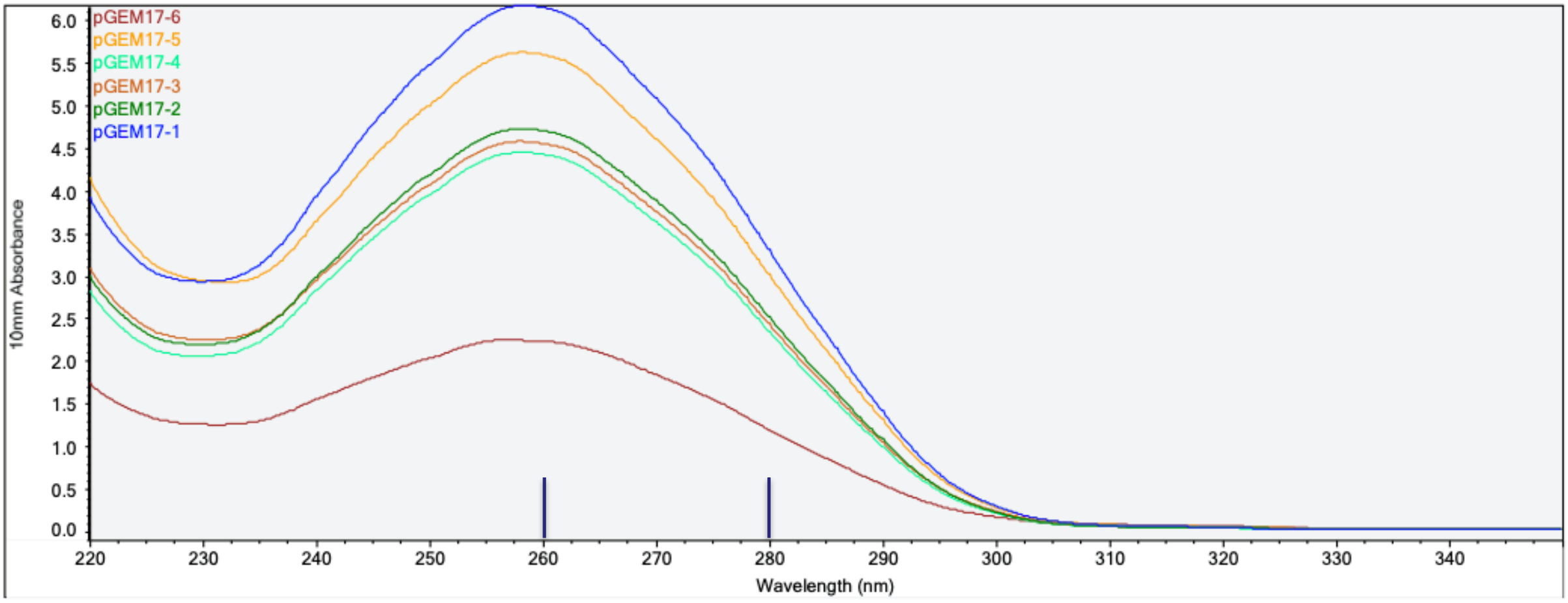
Nucleic acids absorb ultraviolet light in a specific pattern. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.



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;

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	pGEM17-1	aleach5	7/12/2022 2:59:42 PM	306.0	ng/µl	6.119	3.274	1.87	2.11	DNA	50.00
2	pGEM17-2	aleach5	7/12/2022 3:00:36 PM	233.7	ng/µl	4.675	2.489	1.88	2.16	DNA	50.00
3	pGEM17-3	aleach5	7/12/2022 3:01:18 PM	226.1	ng/µl	4.522	2.405	1.88	2.04	DNA	50.00
4	pGEM17-4	aleach5	7/12/2022 3:02:05 PM	219.8	ng/µl	4.397	2.319	1.90	2.17	DNA	50.00
5	pGEM17-5	aleach5	7/12/2022 3:02:58 PM	278.3	ng/µl	5.567	2.987	1.86	1.91	DNA	50.00
6	pGEM17-6	aleach5	7/12/2022 3:03:45 PM	110.0	ng/µl	2.199	1.164	1.89	1.80	DNA	50.00



Tools: OD_{260} Units of Nucleic Acid to Concentration

OD_{260} units =

Nucleic acid:
- (•) DNA
- ( ) RNA
- ( ) ssDNA
- ( ) Single-Stranded Oligo

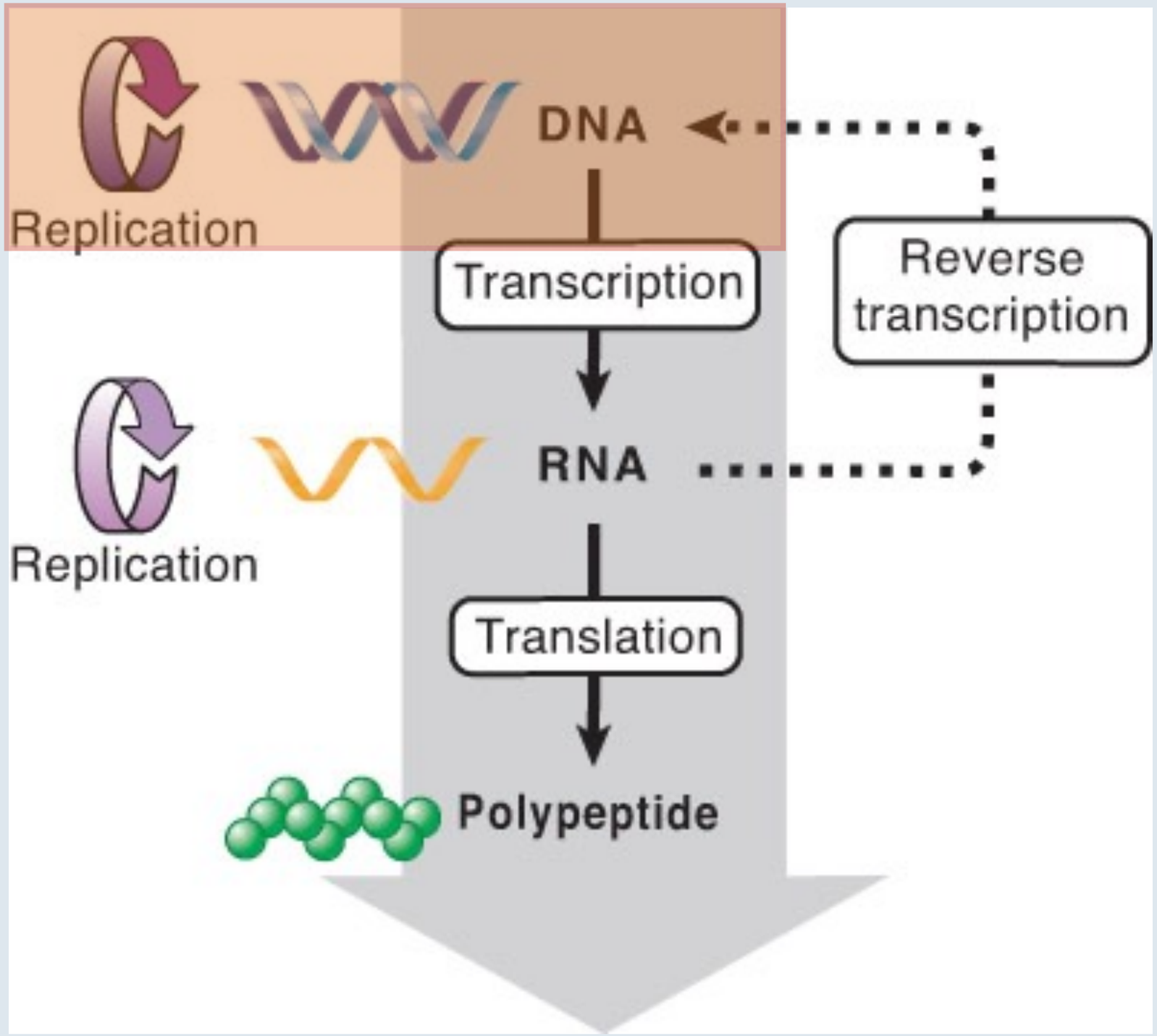
Concentration = 305.95 µg/ml of nucleic acid

Calculate

Formula:

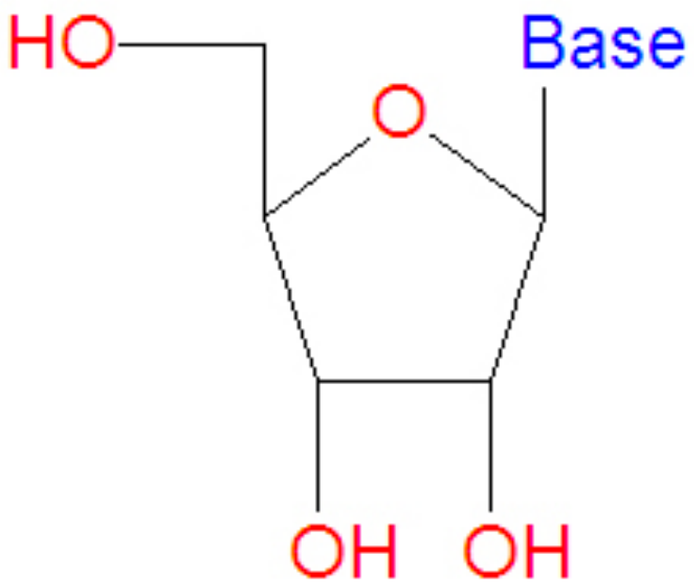
OD_{260} x conversion factor = µg/ml of nucleic acid

1 OD_{260} Unit = 50µg/ml for dsDNA
1 OD_{260} Unit = 40µg/ml ssRNA
1 OD_{260} Unit = 35µg/ml ssDNA
1 OD_{260} Unit = 20µg/ml for single-stranded oligo

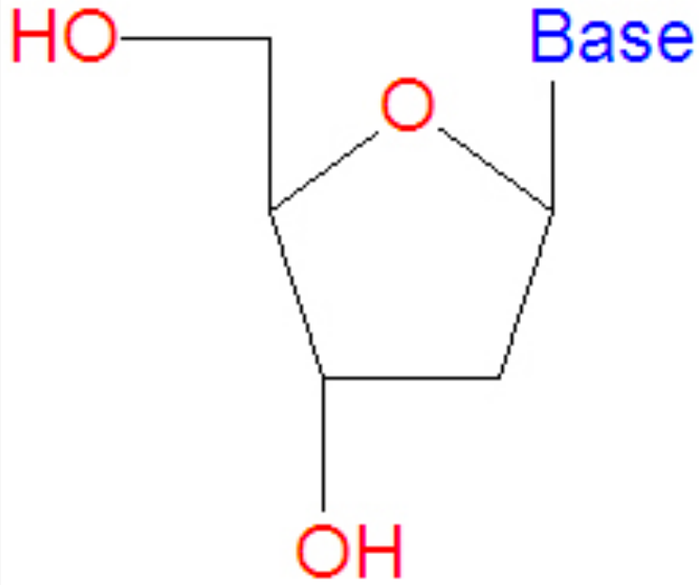


Information in nucleic acid can be perpetuated or transferred, but the transfer of information into a polypeptide is irreversible.

RNA Preparation

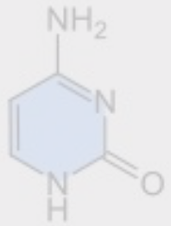


Ribonucleic acid (RNA)
Base= A, C, G or T

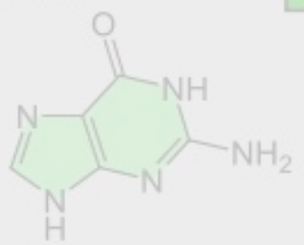


Deoxyribonucleic acid (DNA)
Base = A, C, G or U

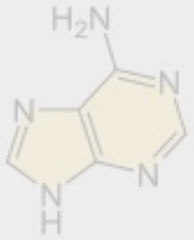
Cytosine C



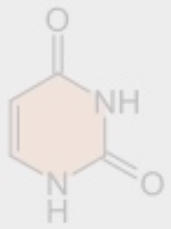
Guanine G



Adenine A



Uracil U



Nucleobases of RNA

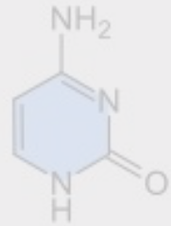
RNA

Ribonucleic acid

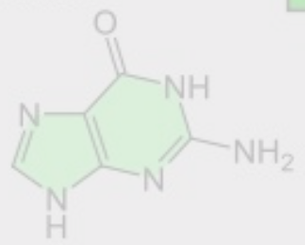
DNA

Deoxyribonucleic acid

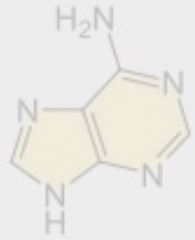
Cytosine C



Guanine G



Adenine A



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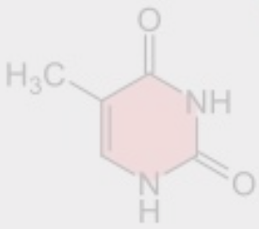
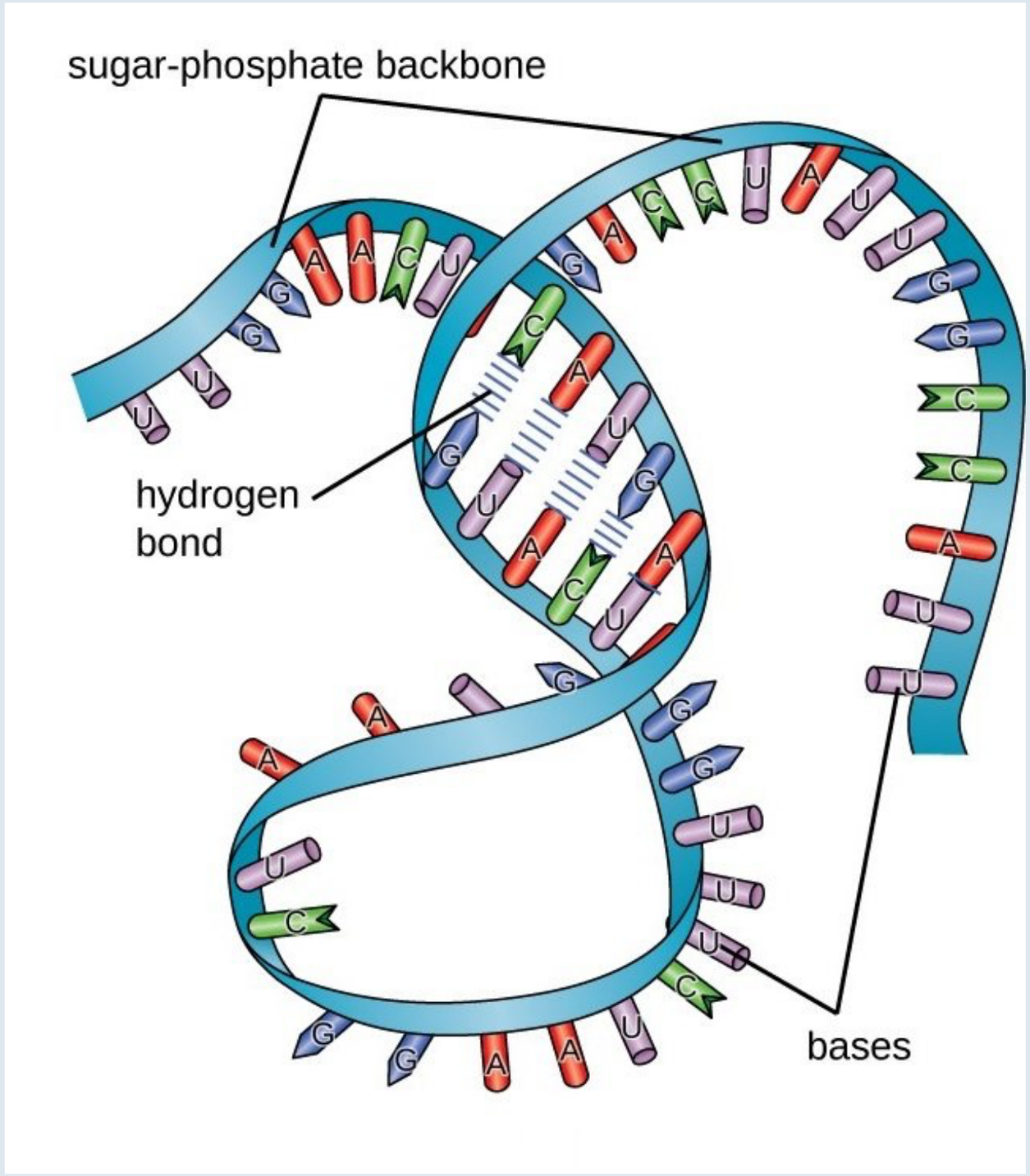
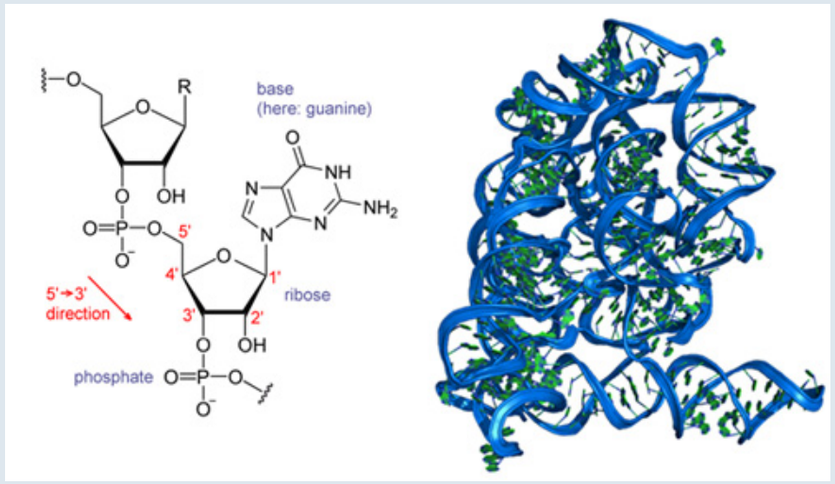
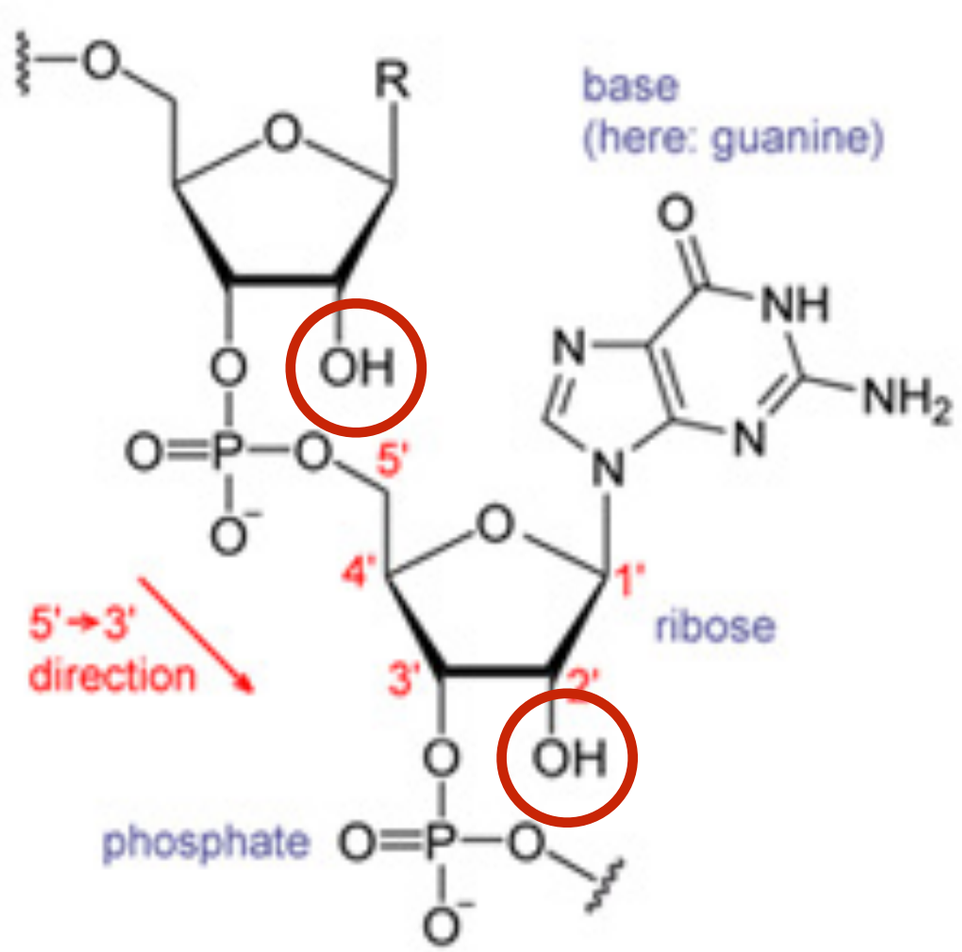
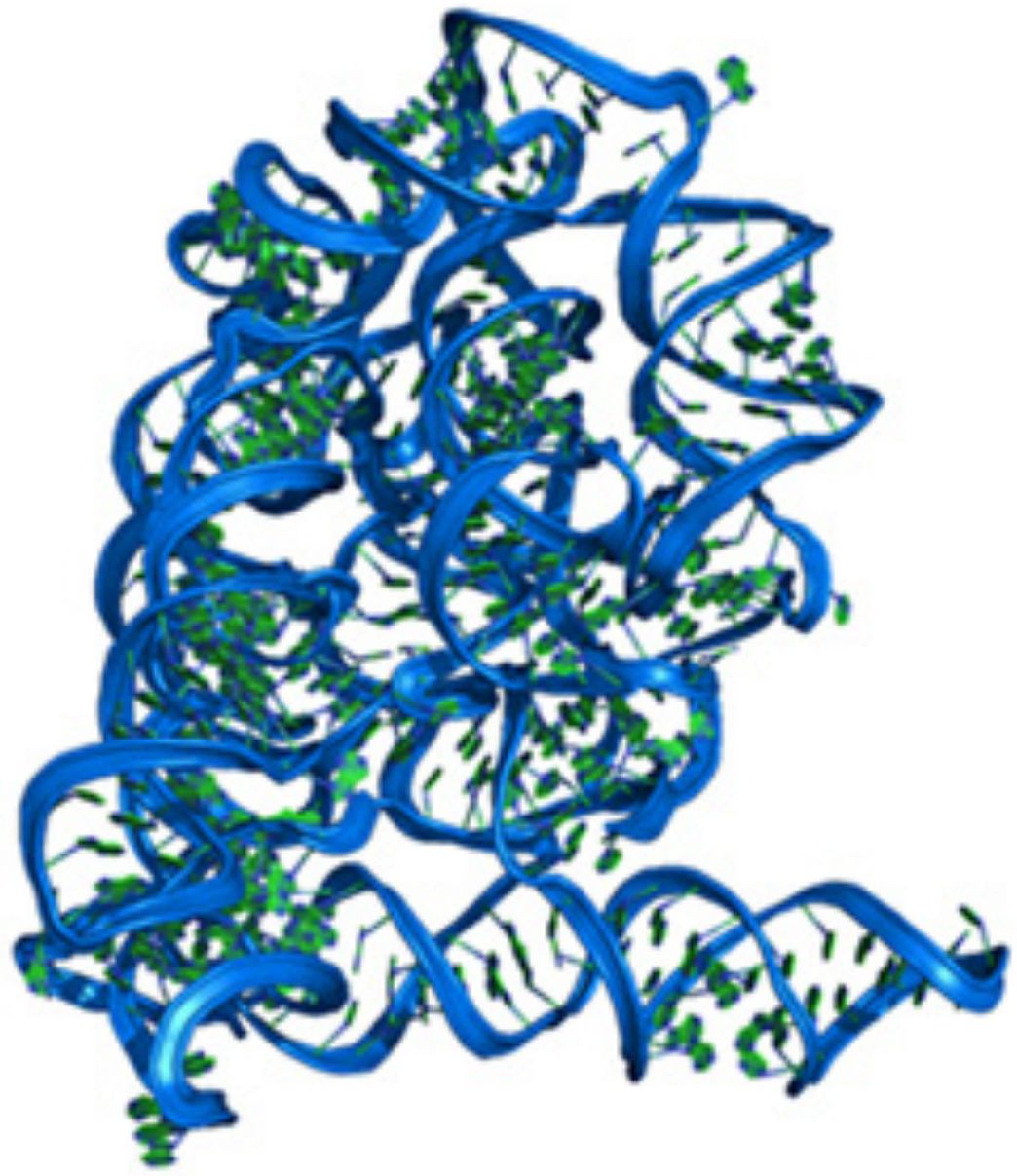


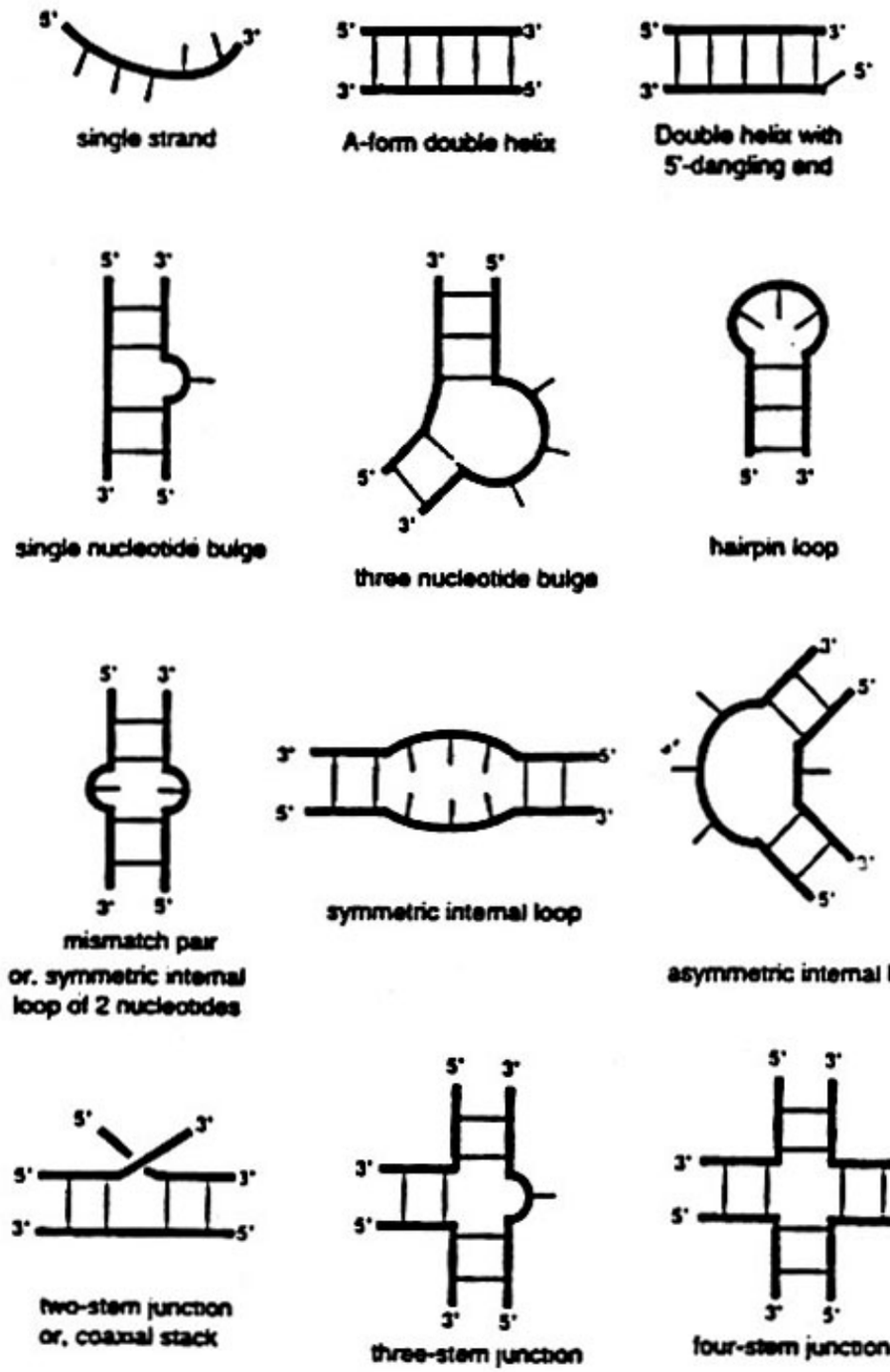
Nucleobases of DNA


sugar-phosphate backbone
hydrogen bond
bases

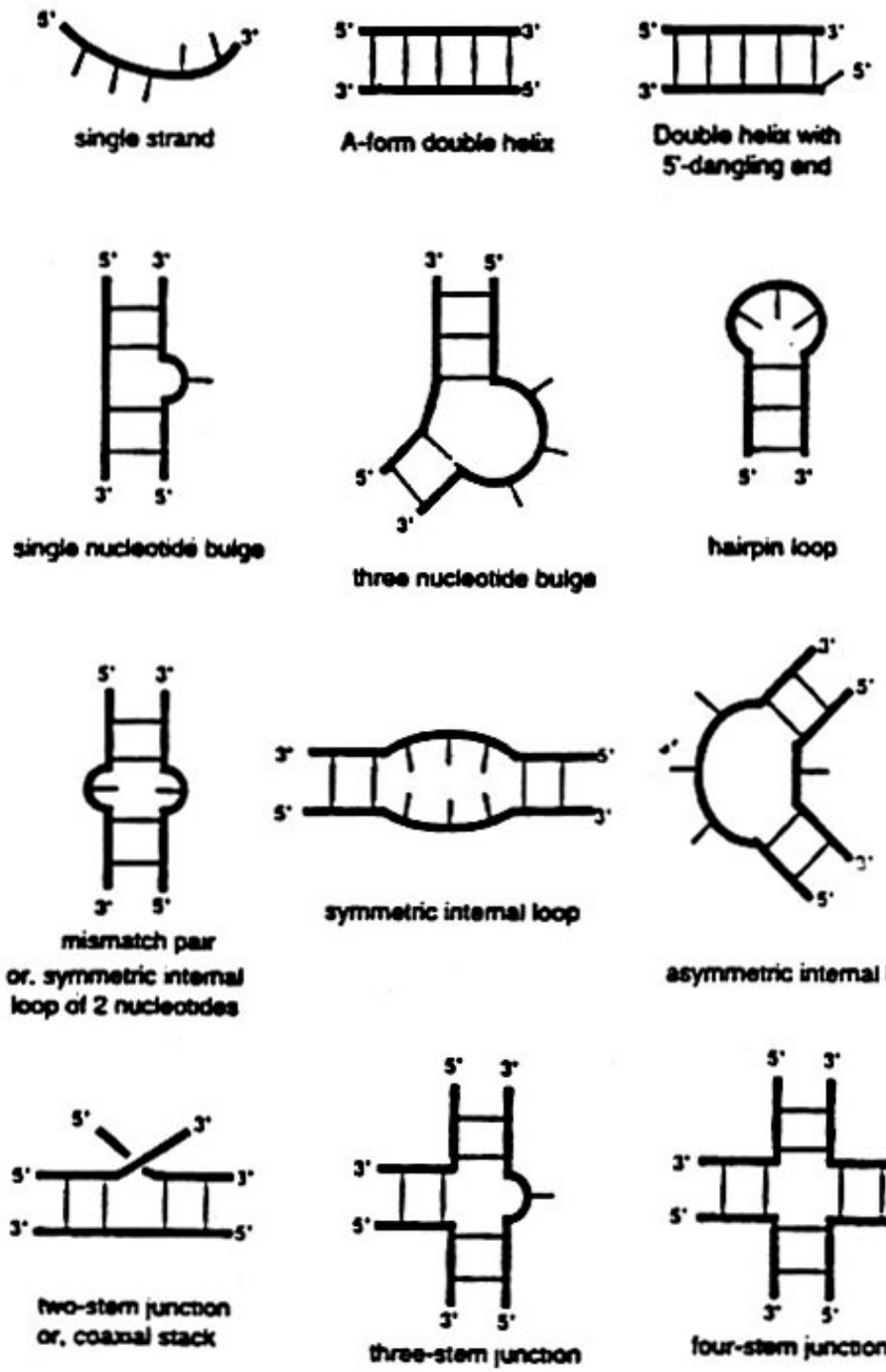

base
(here: guanine)
ribose
5'→3' direction
phosphate


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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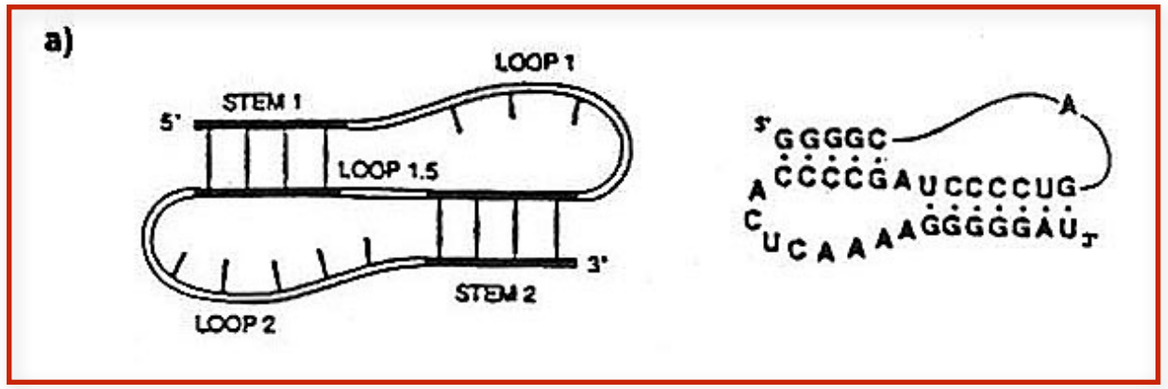
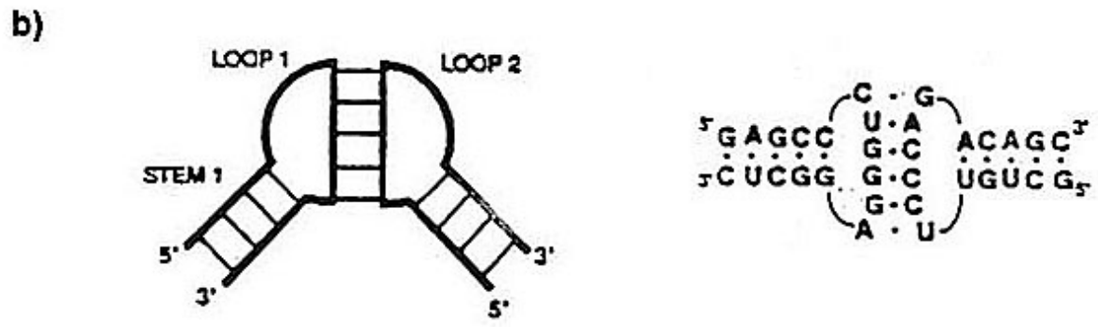
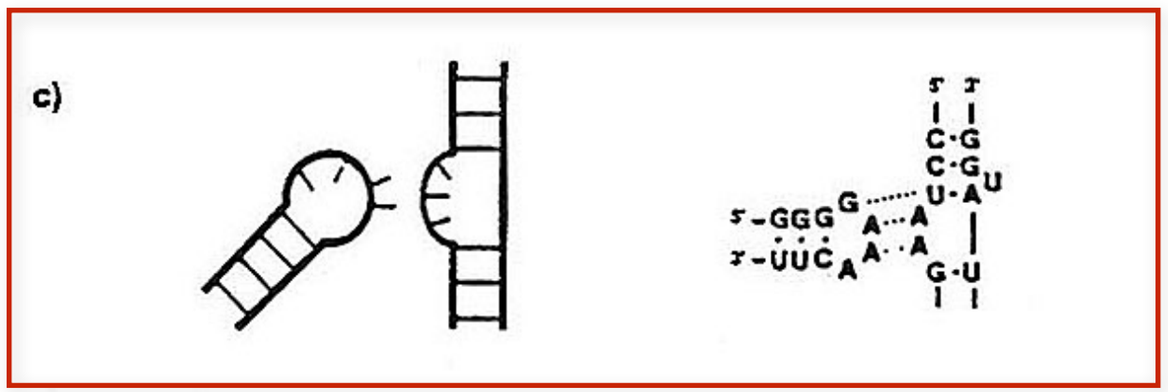


Pseudoknot

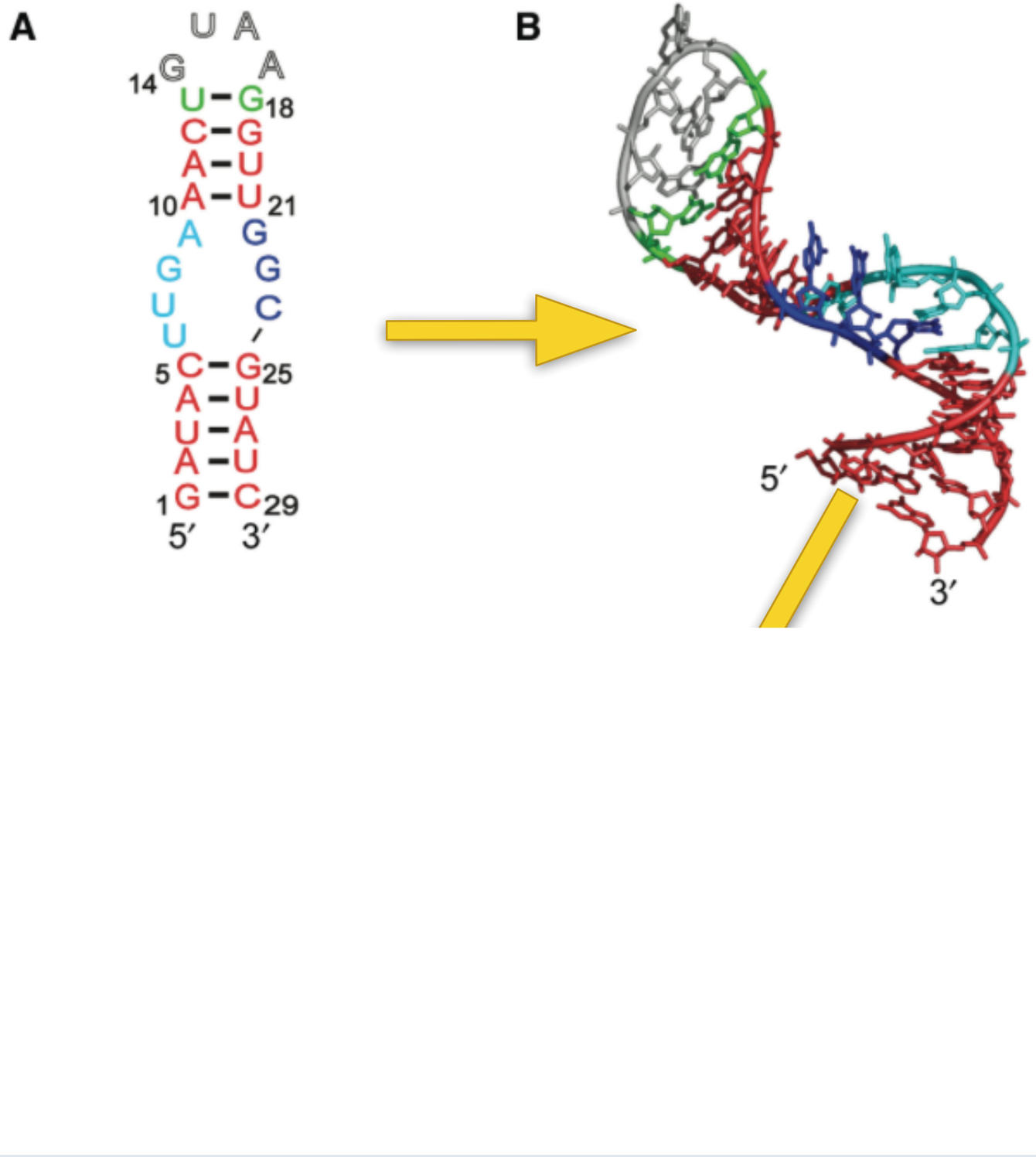


Kissing hairpins

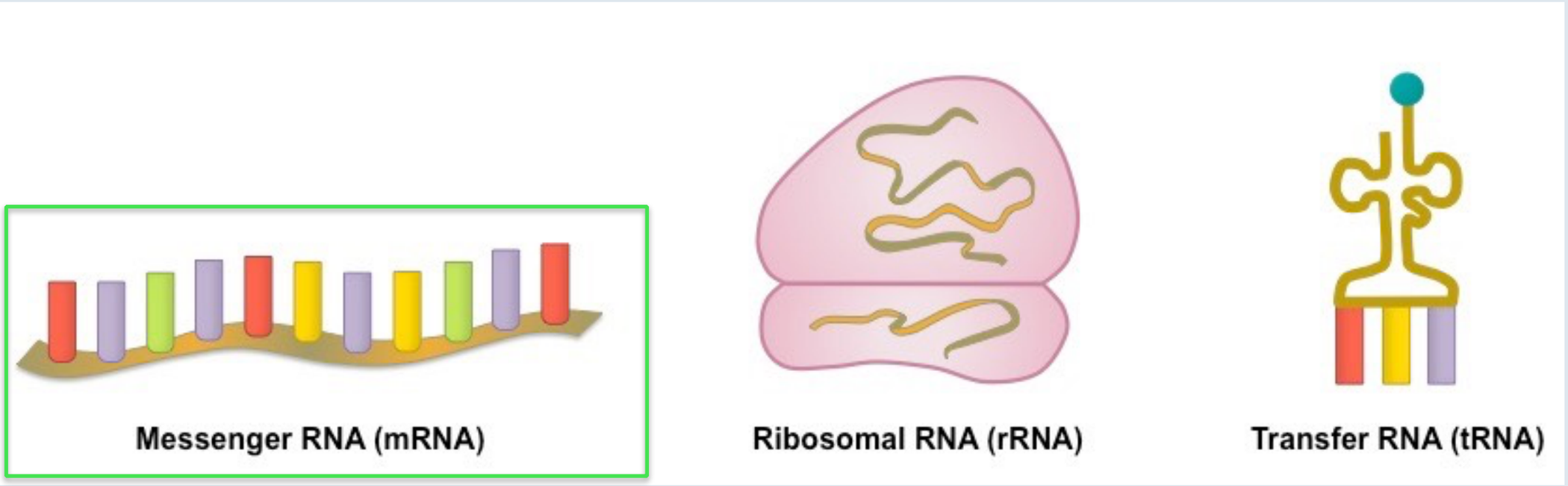


Hairpin loop - bulge contact

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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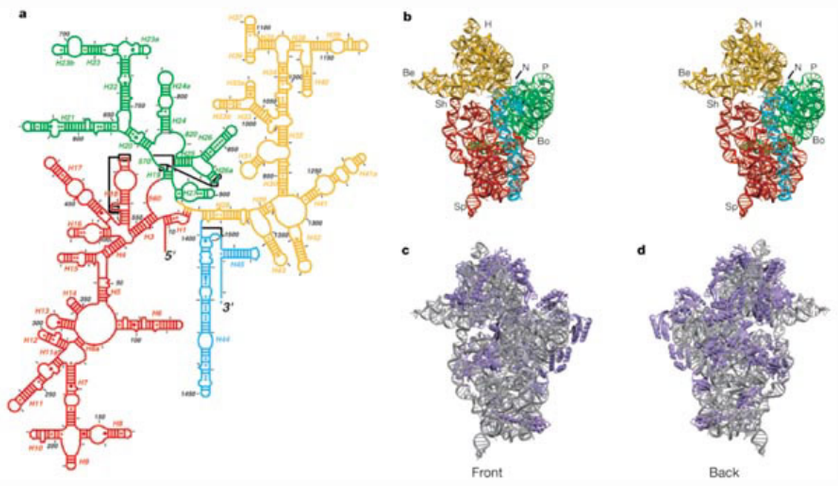

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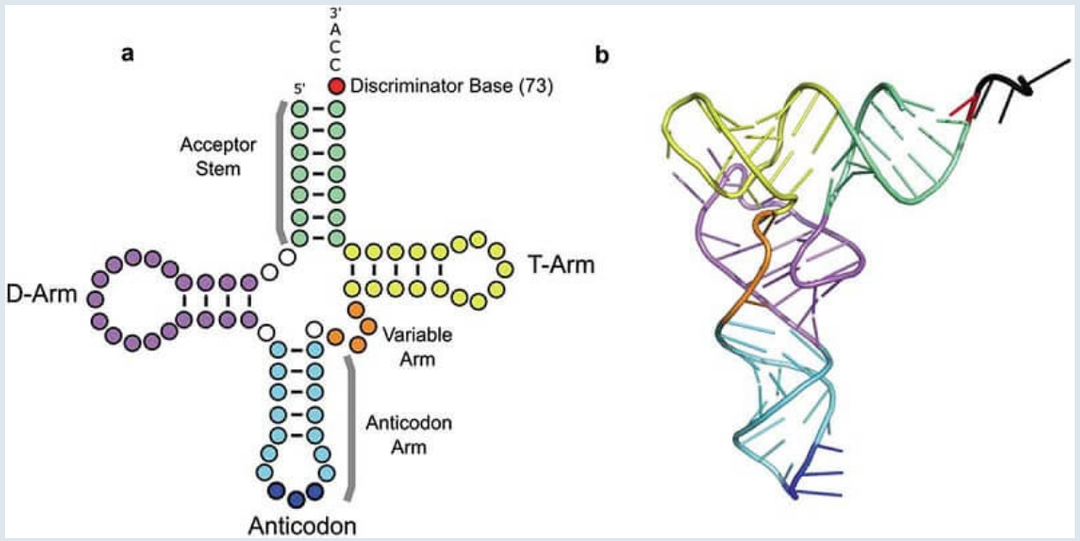
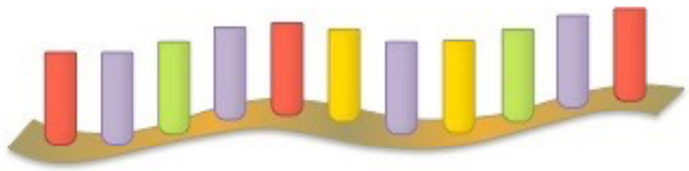
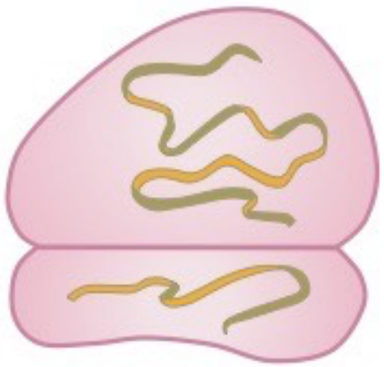




Messenger RNA (mRNA)



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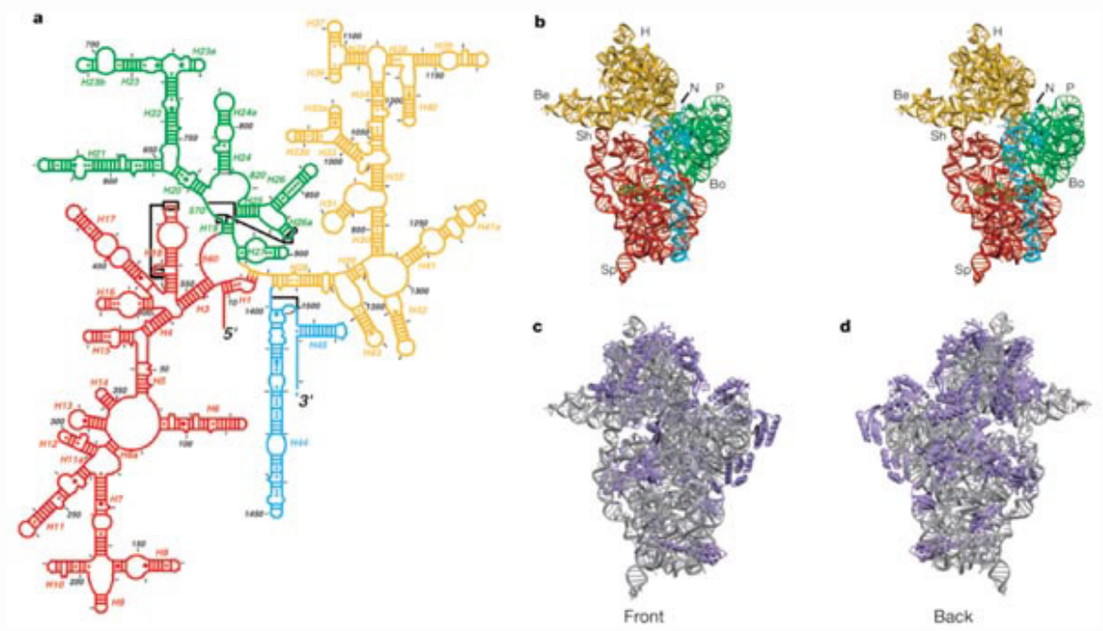

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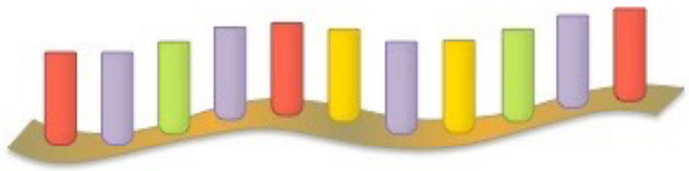

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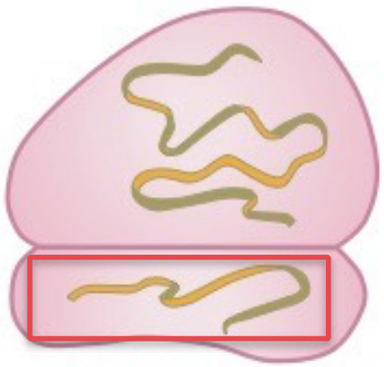

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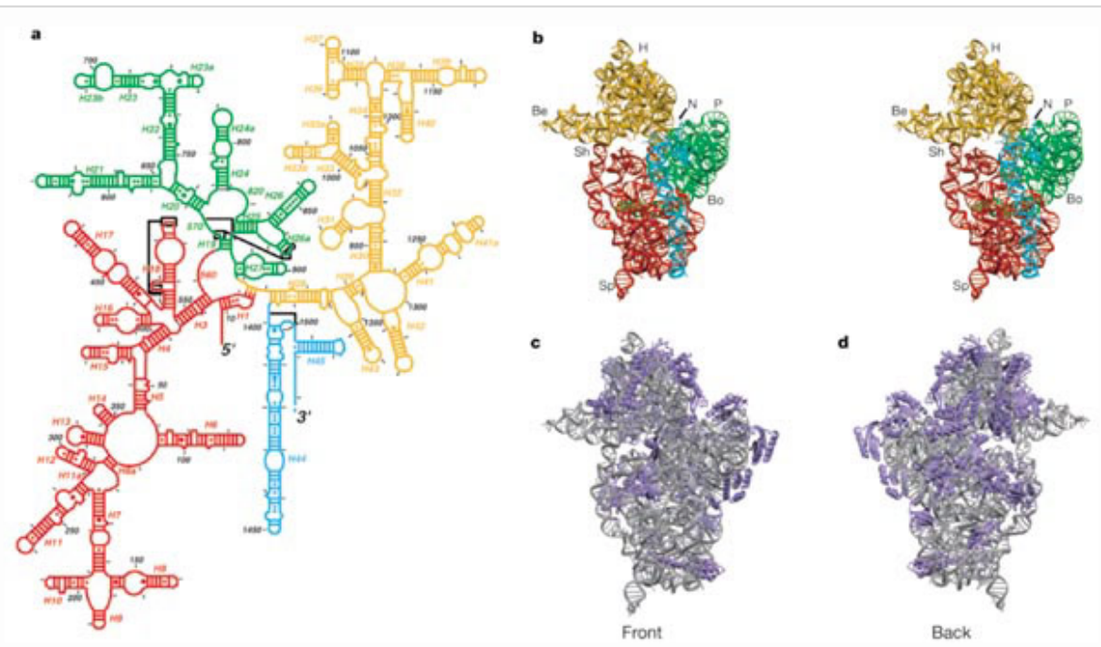

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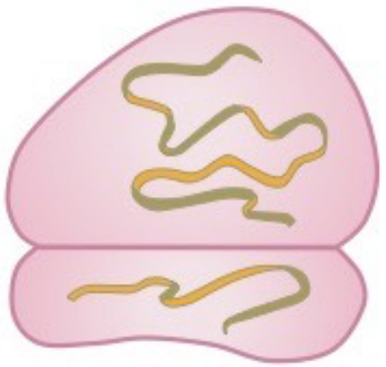

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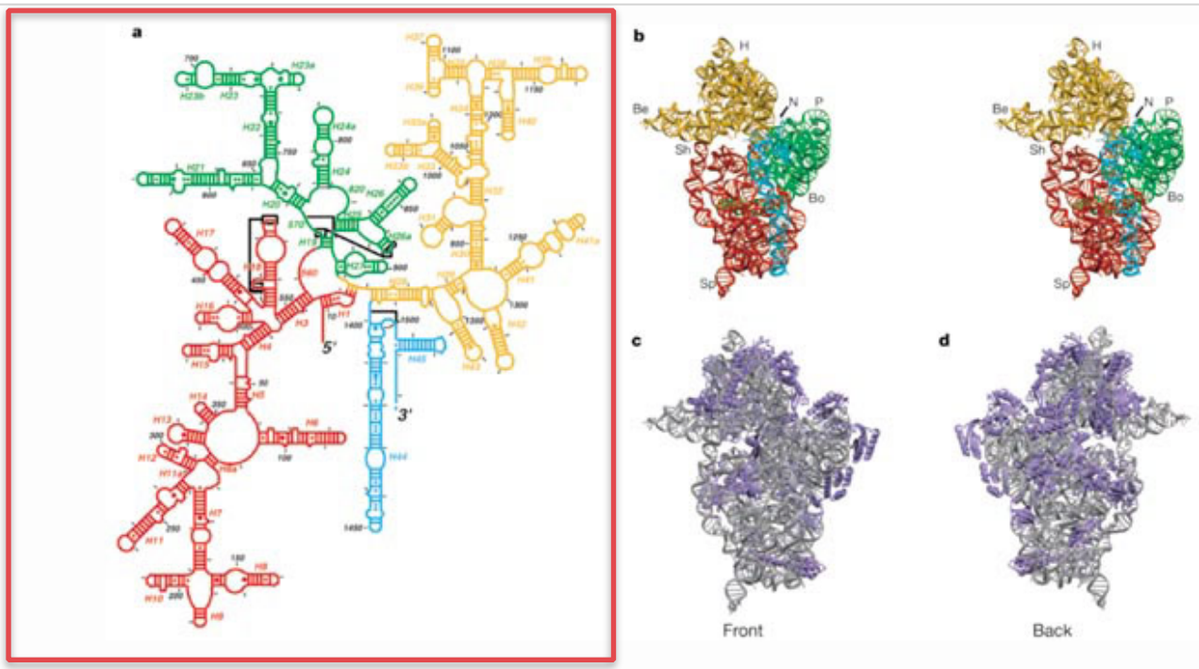

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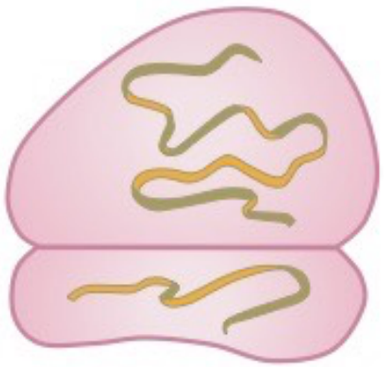

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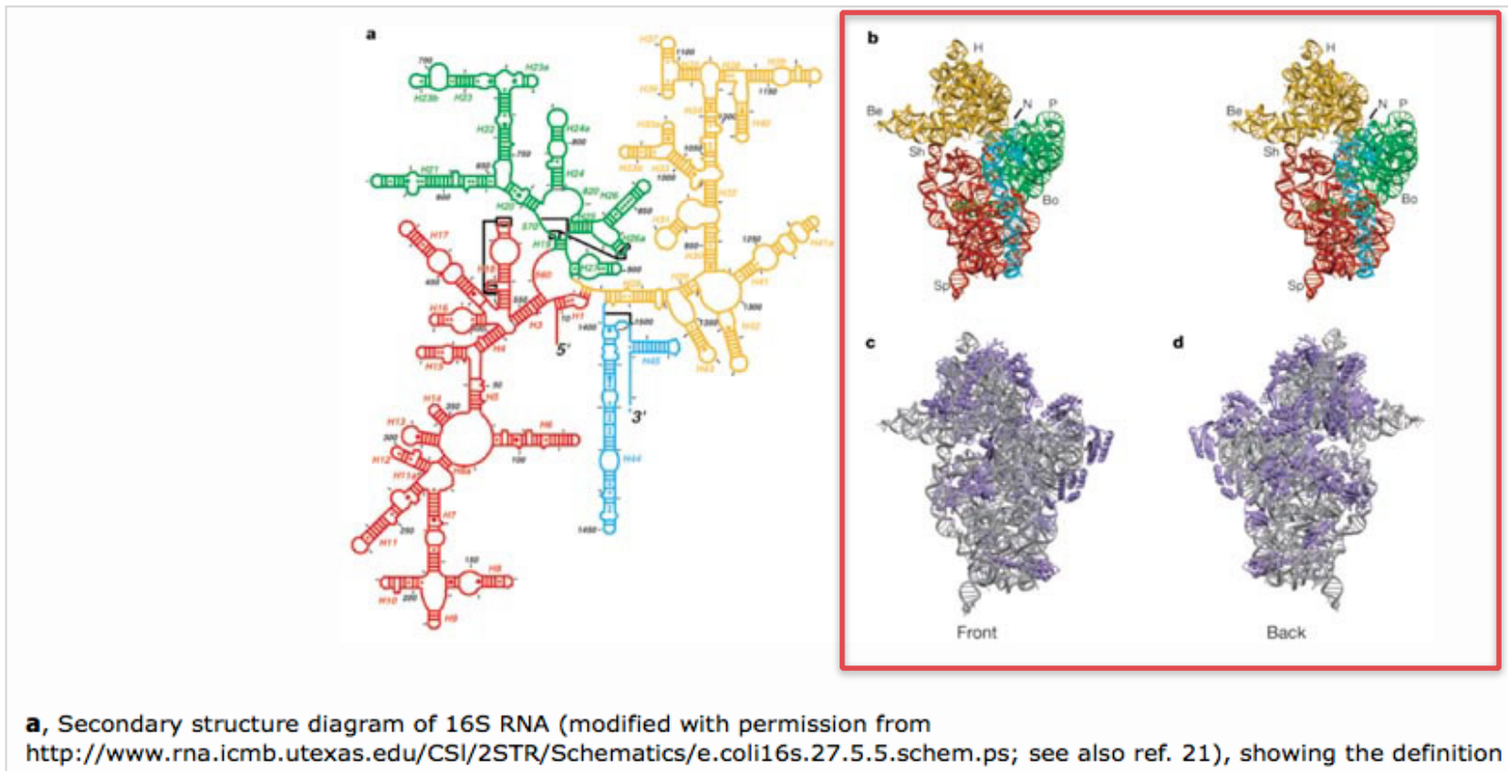

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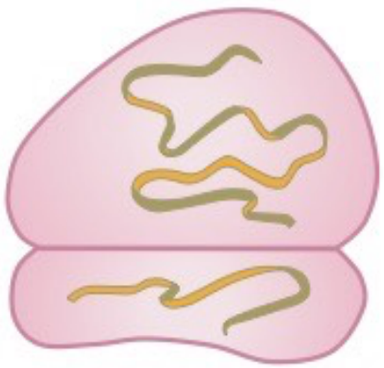

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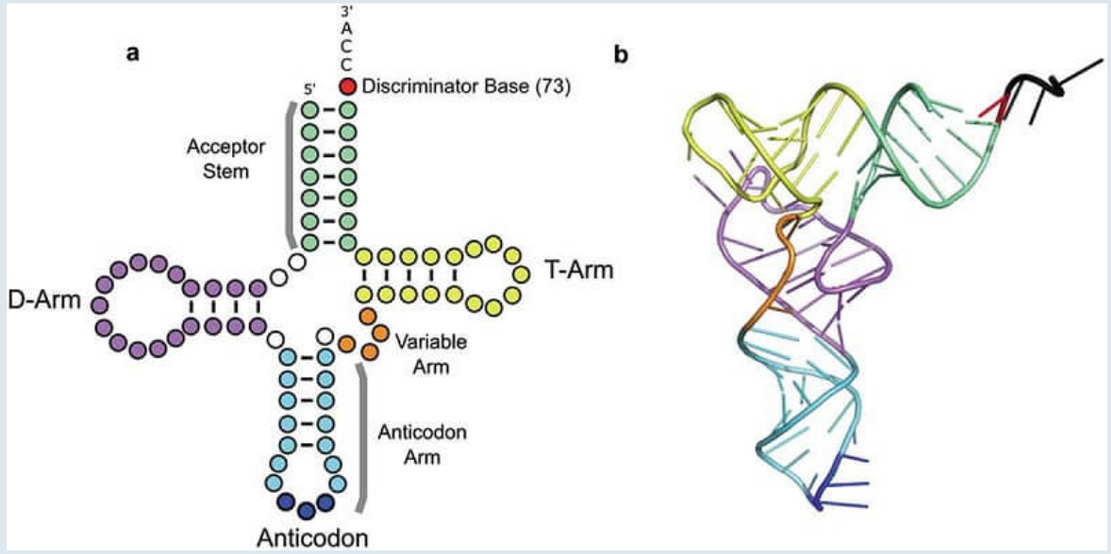

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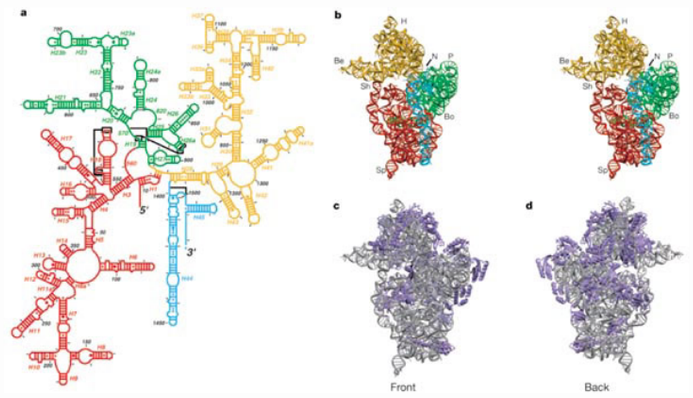

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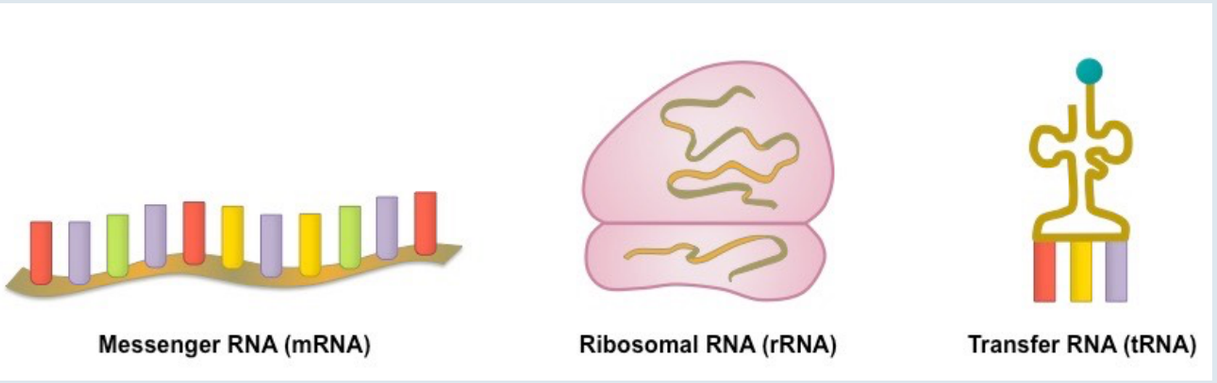
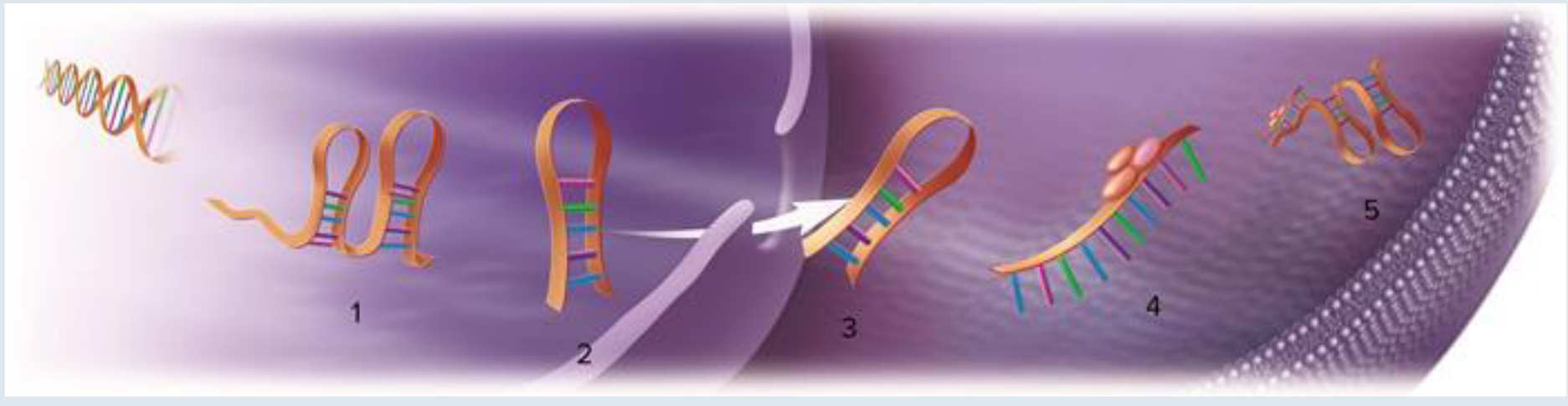
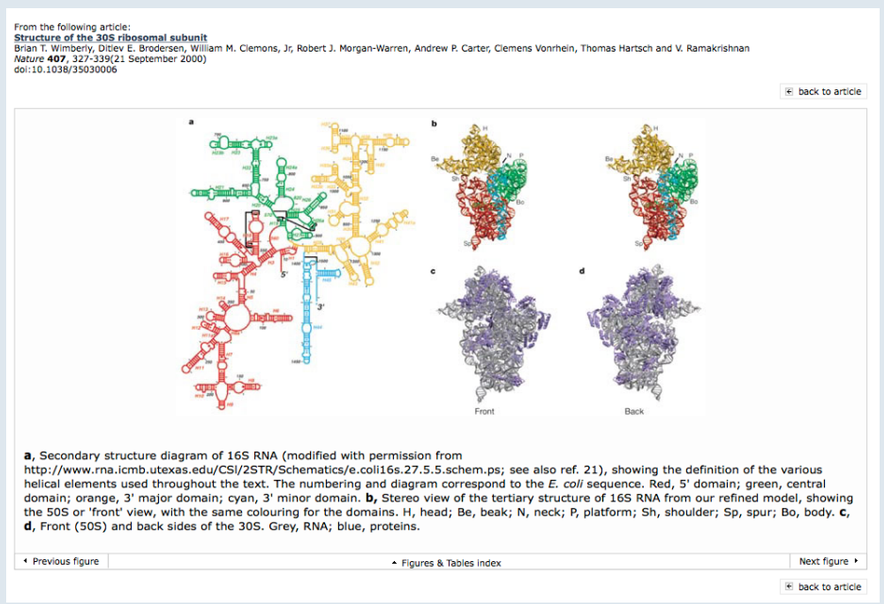




miRNA/siRNA

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



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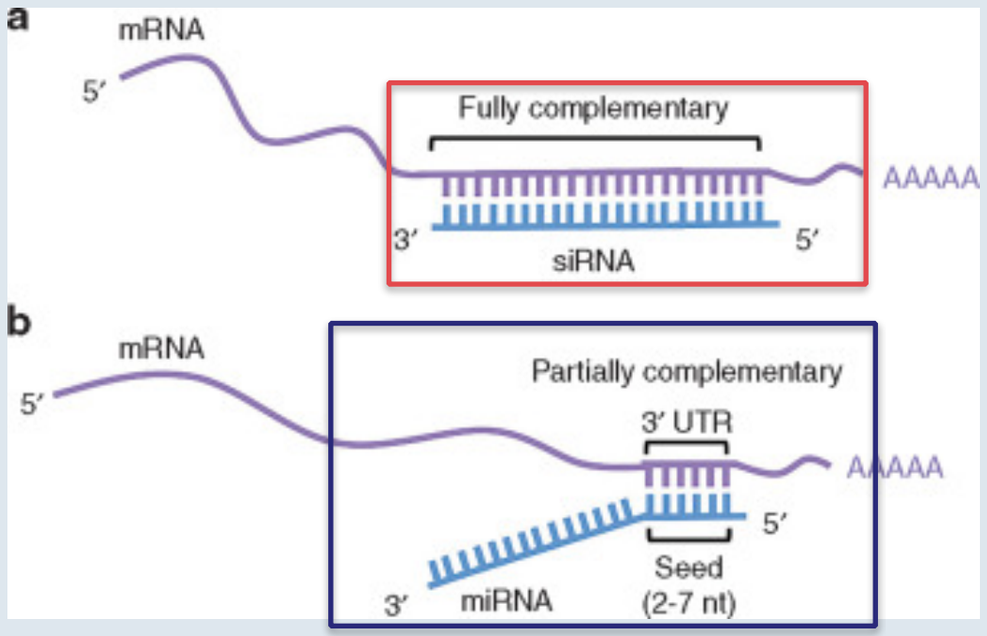


GSU Biology Core Facility

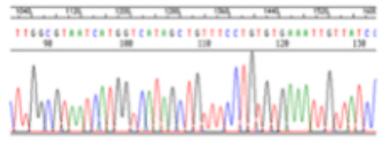
Supporting Life Sciences at GSU

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

Georgia State University

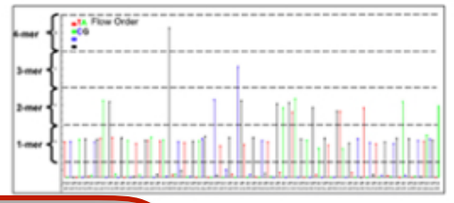
DNA Sequence Analysis:

Profiling DNA



Sanger Sequencing –
>800 base pairs/run

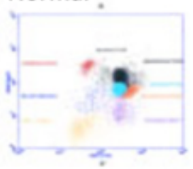
High Throughput Genomic Sequencing –
100,000 base pairs/run



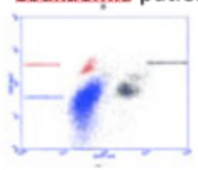
Flow Cytometry

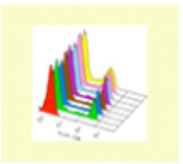
Profiling Cells

Normal



Leukaemia patient

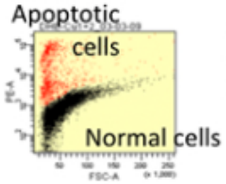




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

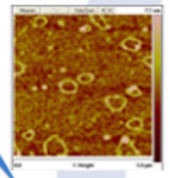


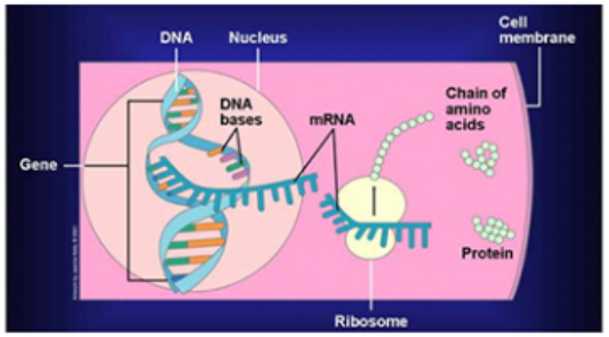
Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis





DNA

Replication

RNA

Expression

Protein

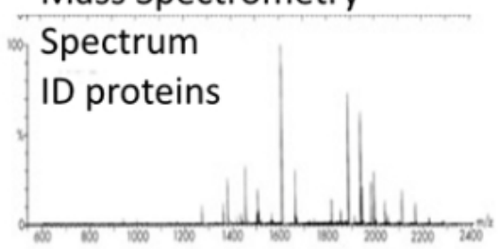
Expression

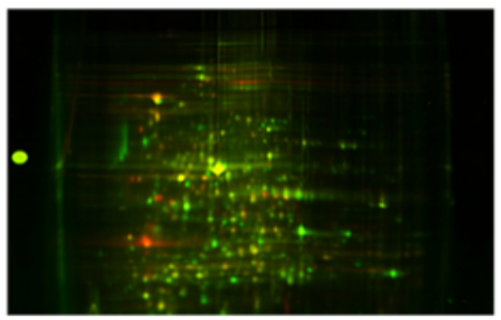
Cellular Functions

Mass Spectrometry

Spectrum

ID proteins





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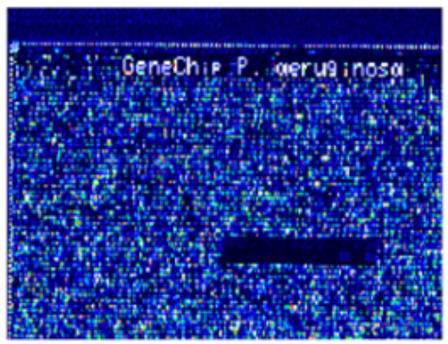


Colour of pin-point dots demonstrates the presence / absence of gene sequences

GSU Biology Core Facility

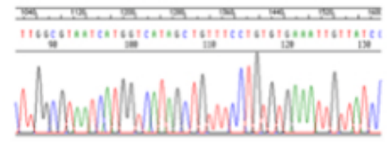
Supporting Life Sciences at GSU

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

Georgia State University

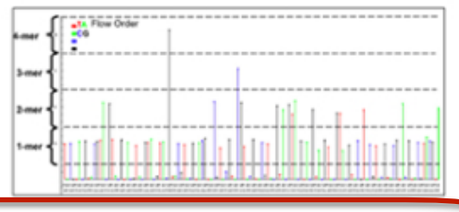
DNA Sequence Analysis:

Profiling DNA



Sanger Sequencing –
>800 base pairs/run

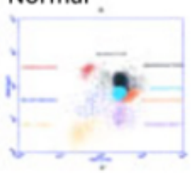
High Throughput Genomic Sequencing –
100,000 base pairs/run



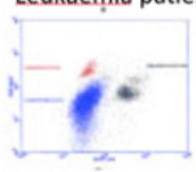
Flow Cytometry

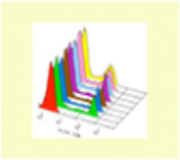
Profiling Cells

Normal



Leukaemia patient



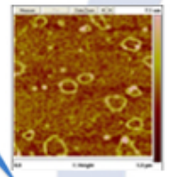


See effects of different drugs on Cell cycle

Atomic Force Microscopy

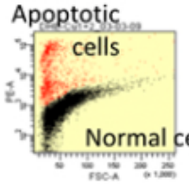
Imaging at the Ångström level

Protein structure analysis

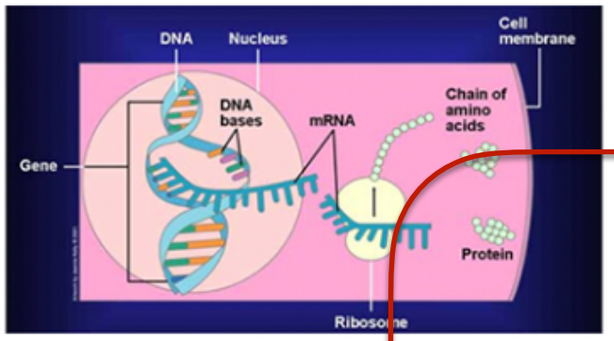


Apoptotic cells

Normal cells



Apoptosis -programmed cell death



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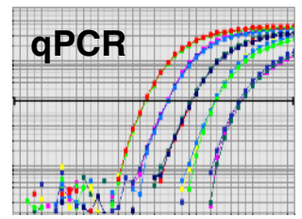
RNA Expression



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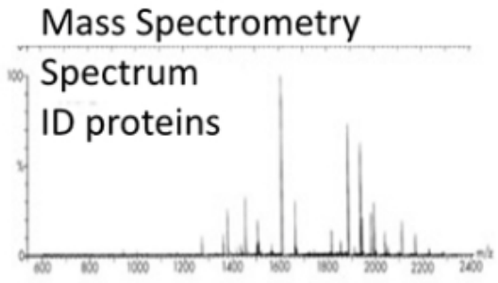
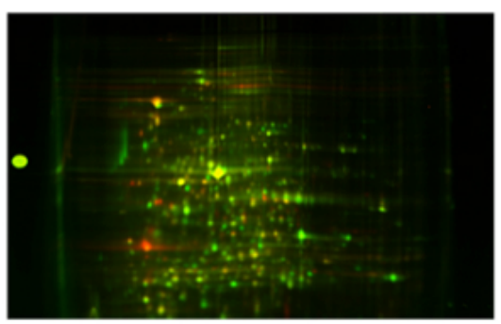
Spectrum

ID proteins





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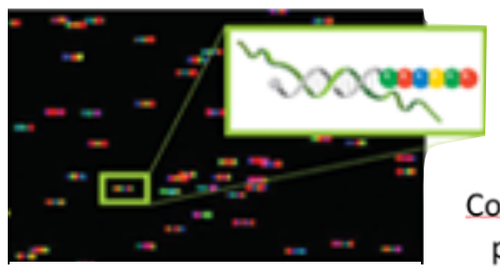
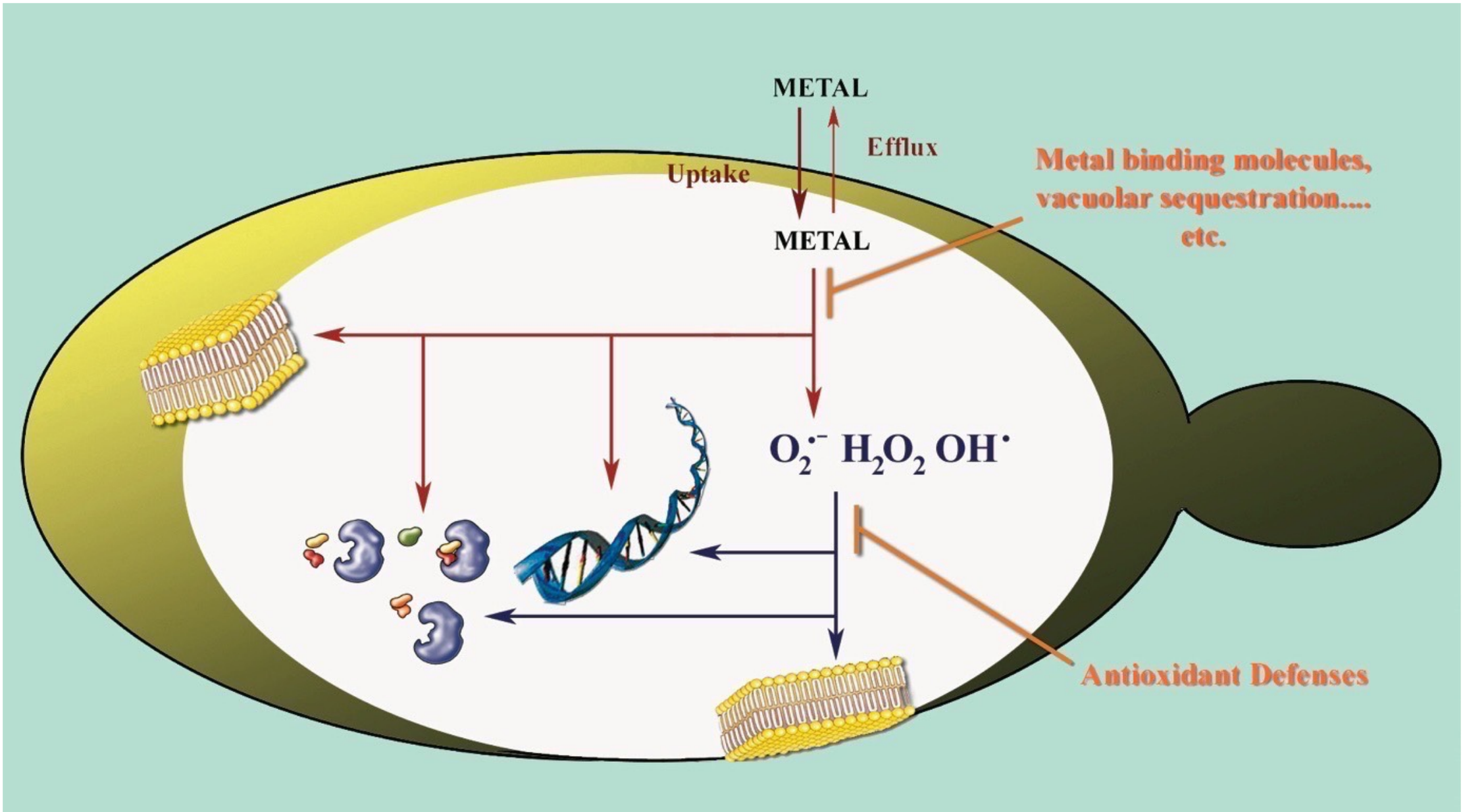


Nanostring

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)

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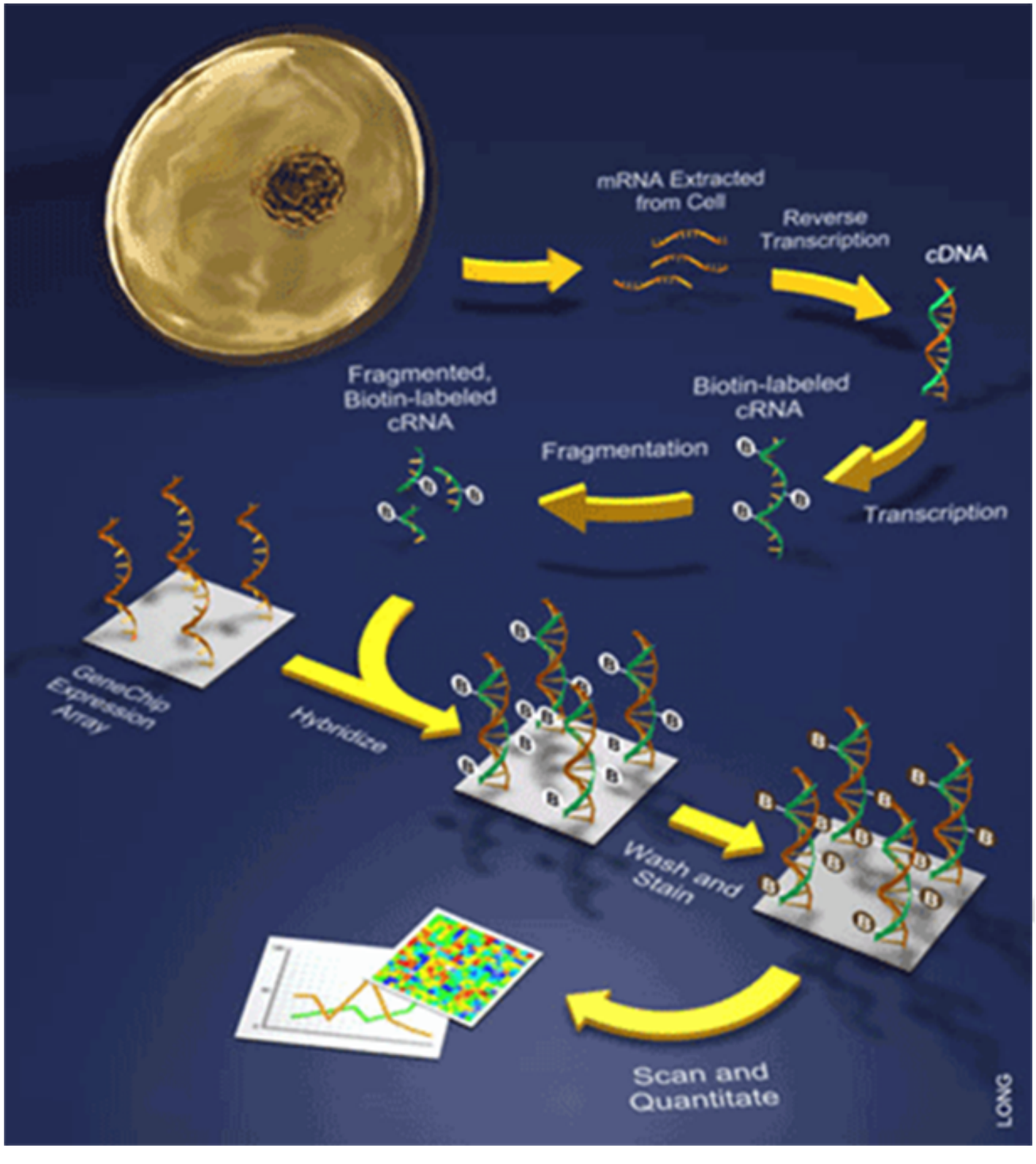
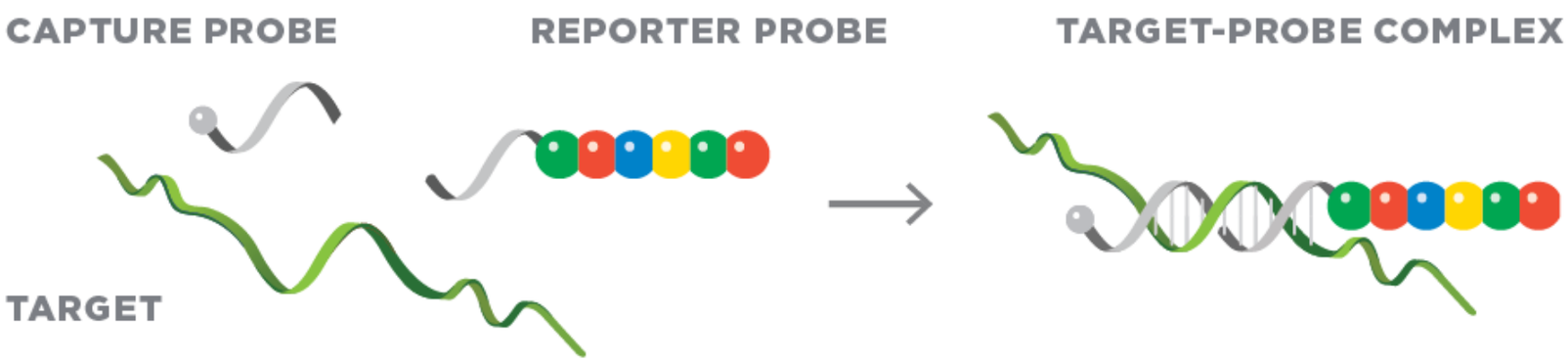
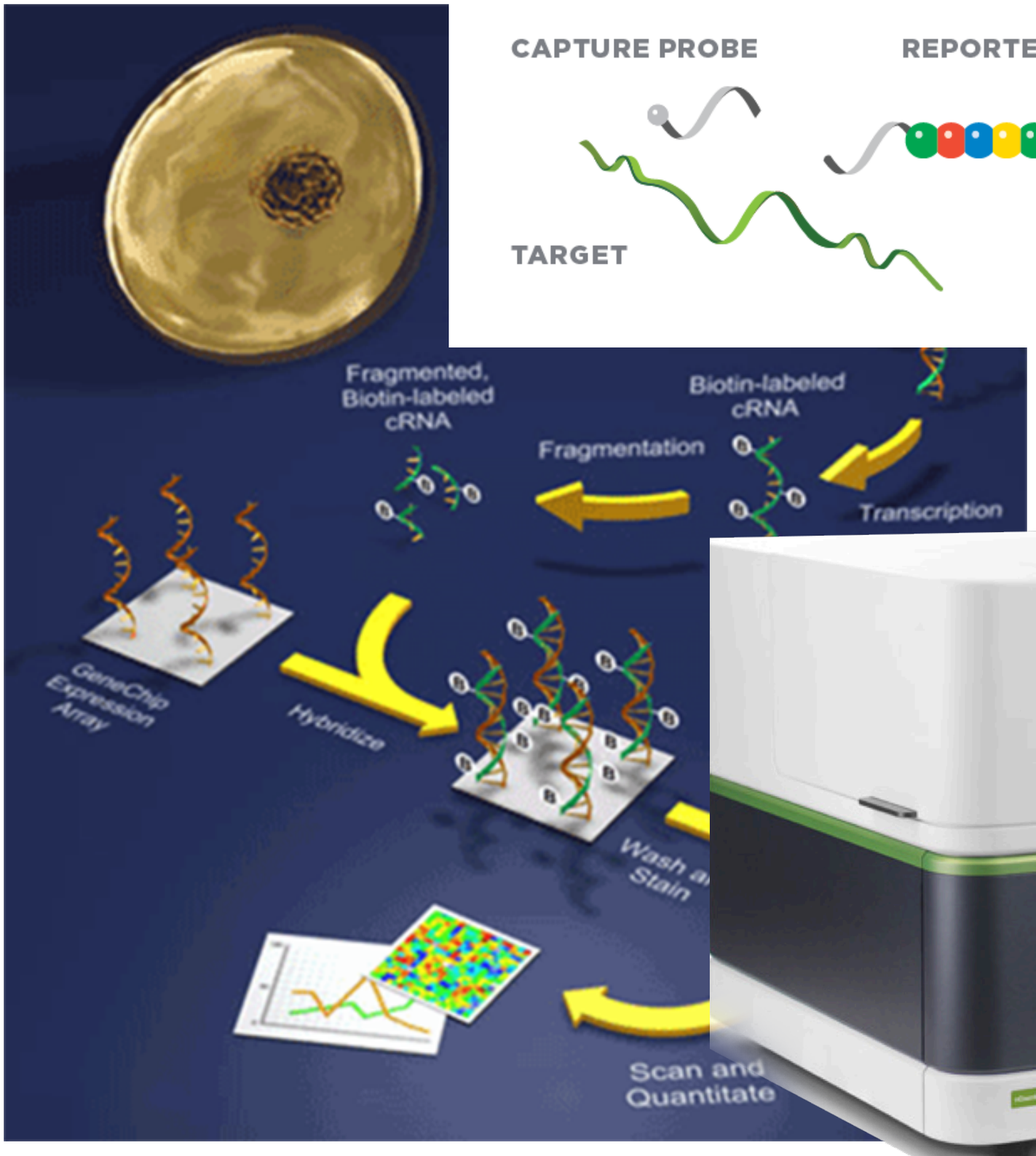
Microarray technology at GSU



Nanostring technology at GSU

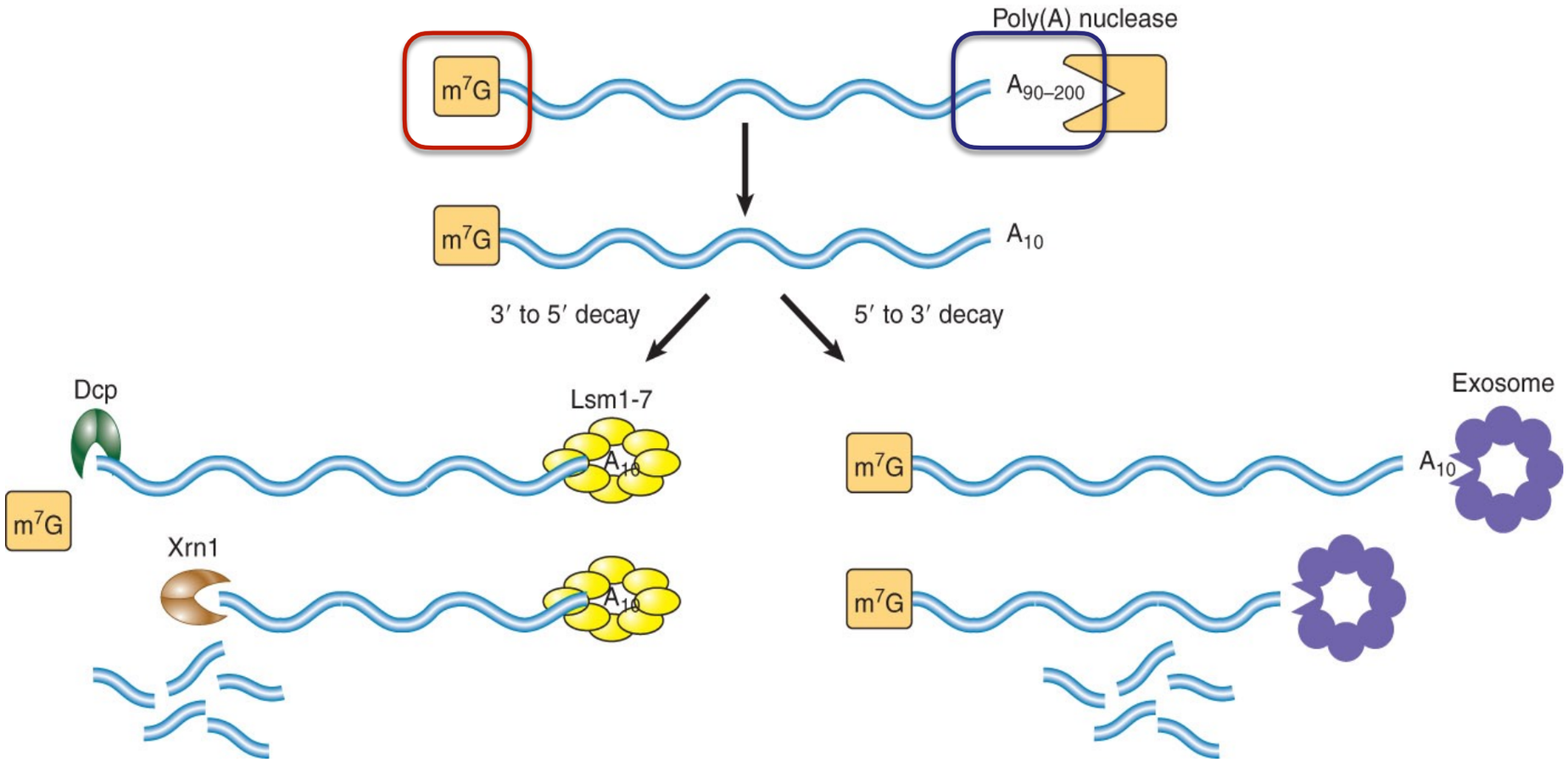





RNeasy® Mini Kit (50)
Cat. No. 74104
Store at room temperature (15-25°C)
QIAGEN
Tissue disruption in RNAwiz™
Add chloroform (RT, 5 min)
Centrifuge (10 min)
Isopropanol precipitate (RT, 5 min)
Centrifuge (10 min)
Drain, resuspend pellet
Total RNA
Northern Analysis, Nuclease Protection, RT-PCR, Translation

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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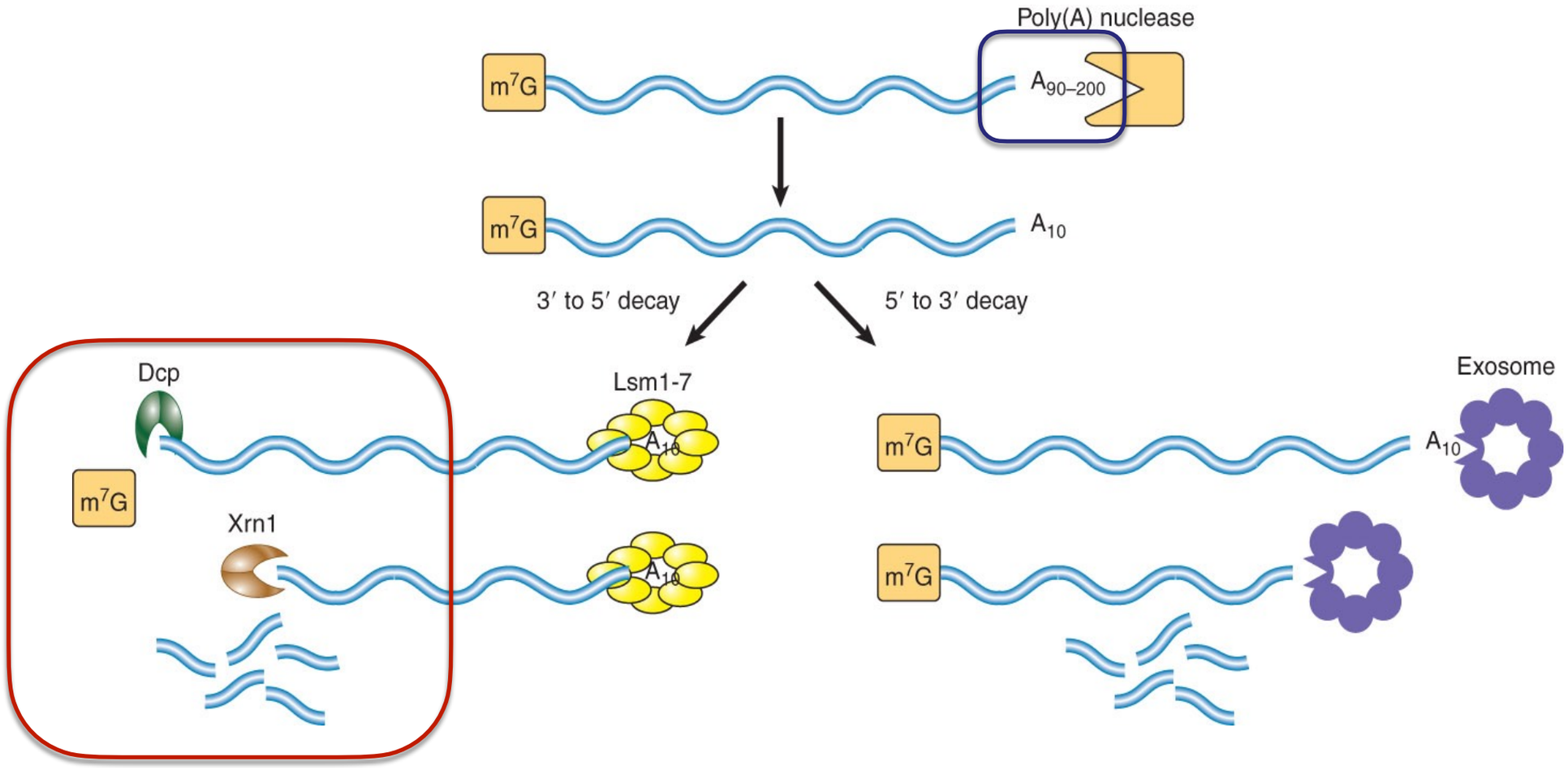

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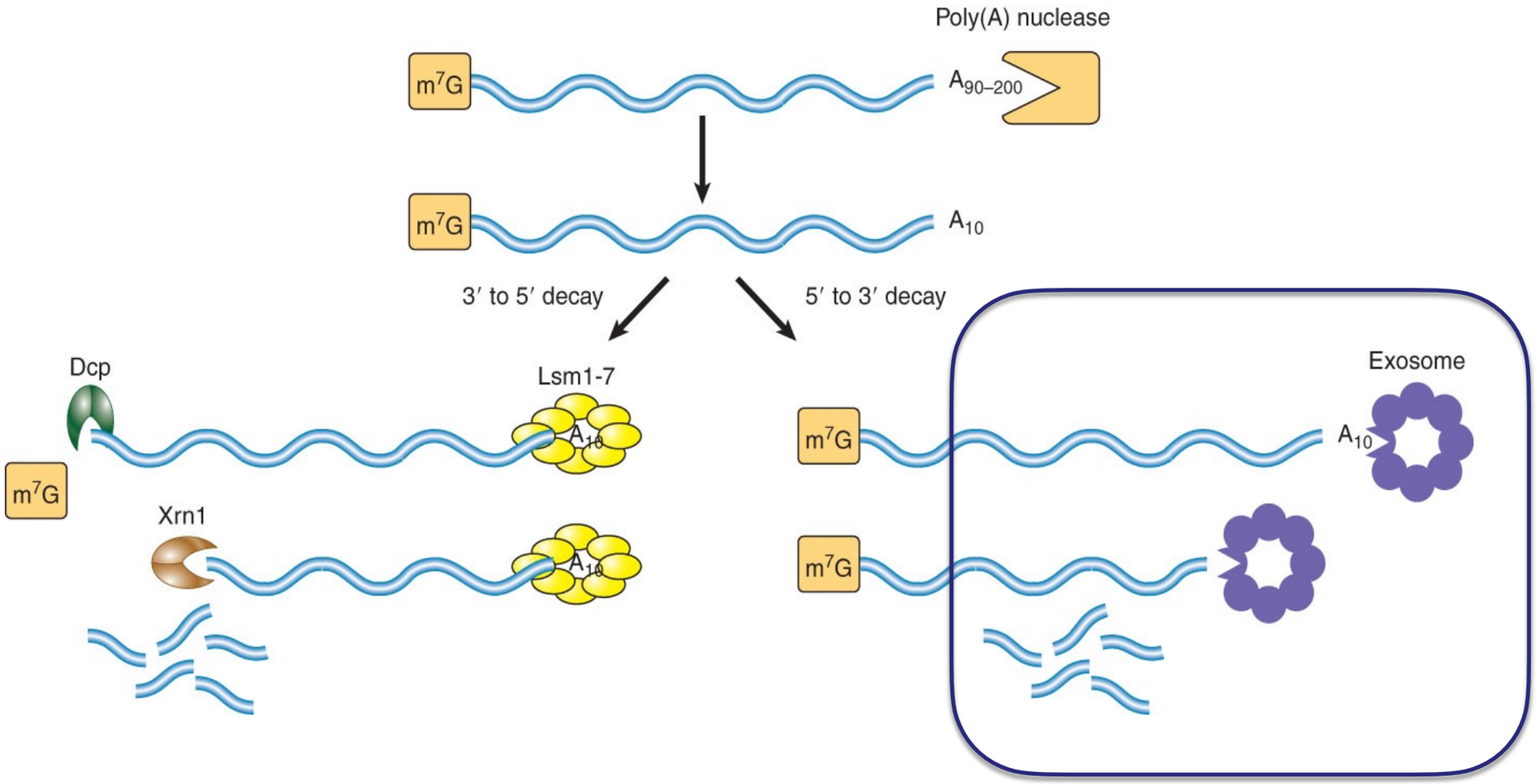
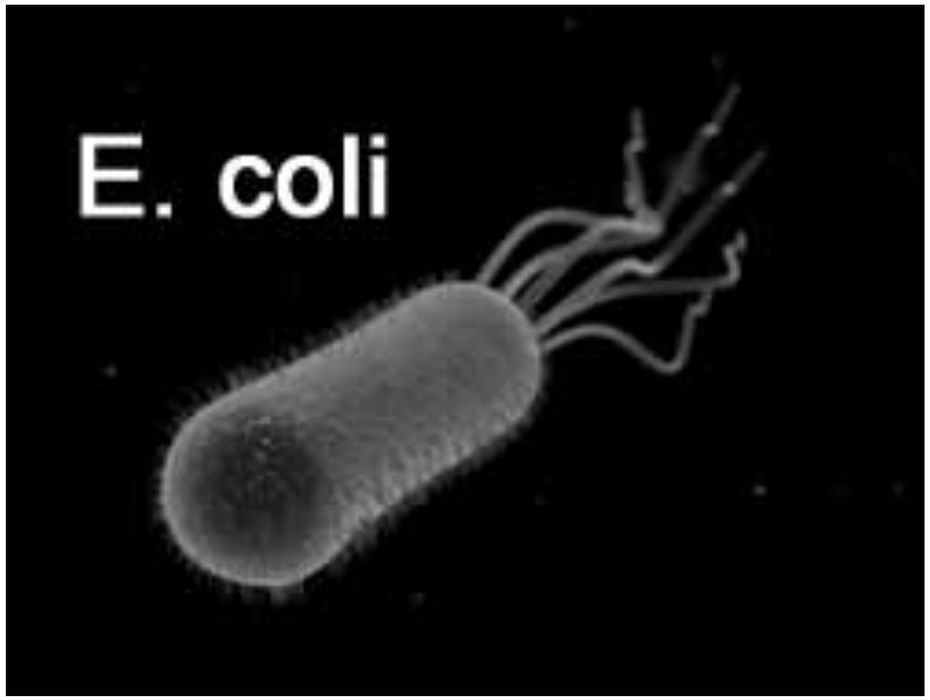


The major deadenylation-dependent decay pathways in eukaryotes



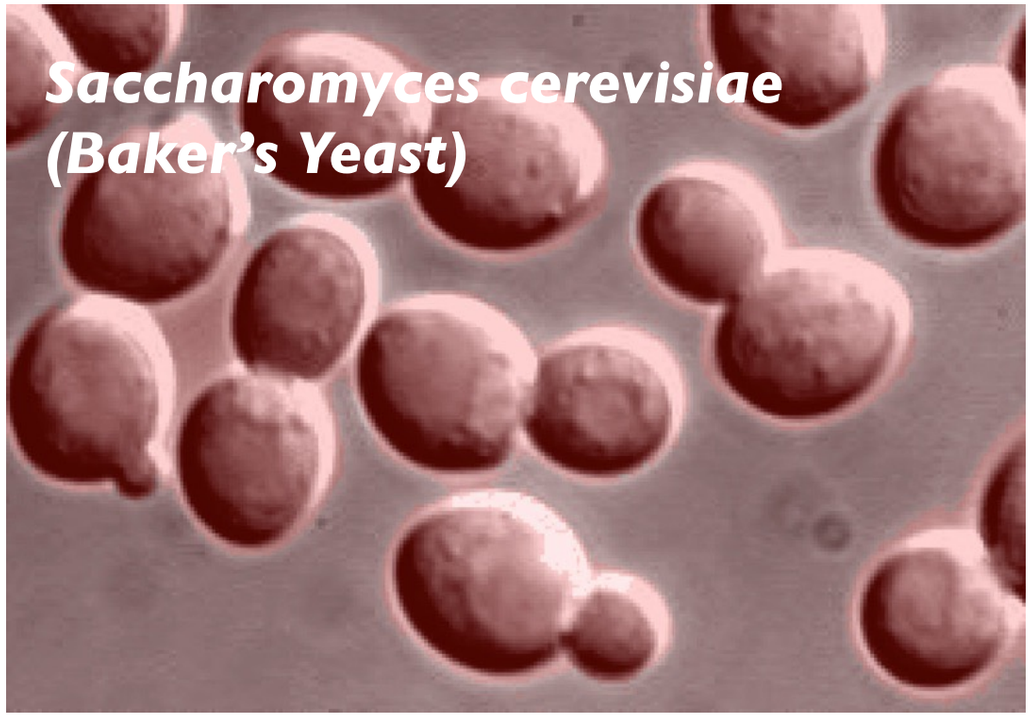
Bacteria thrive on many different types of food. But most yeasts can live only on sugars and starches. From these, they produce carbon dioxide gas and alcohol. Thus, they have been useful to man for centuries in the production of certain foods and beverages. They are responsible for the rising of bread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

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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.**

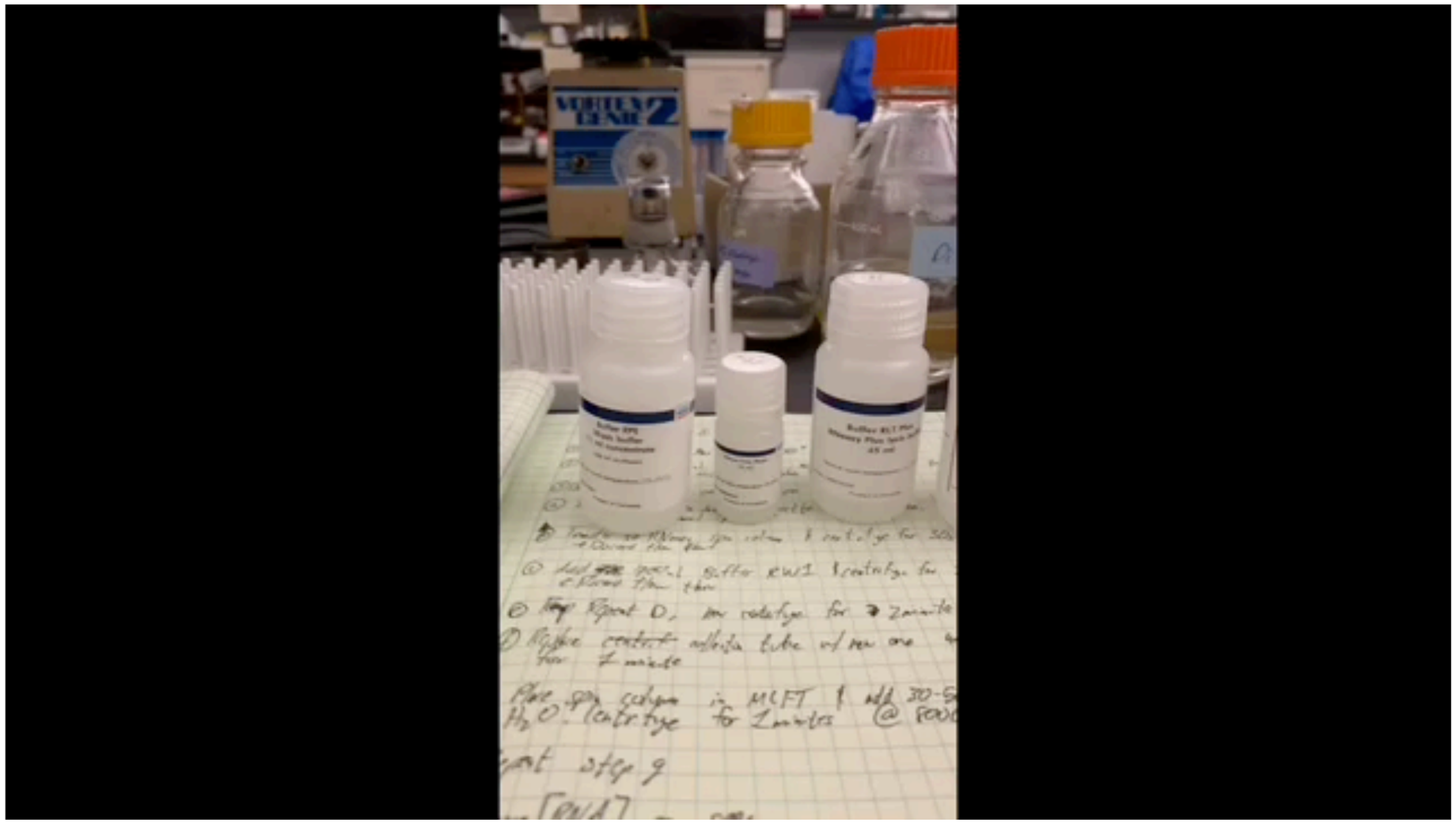
e. Repeat (d), -this time **centrifuging for 2 min at 8,000 x g**.

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

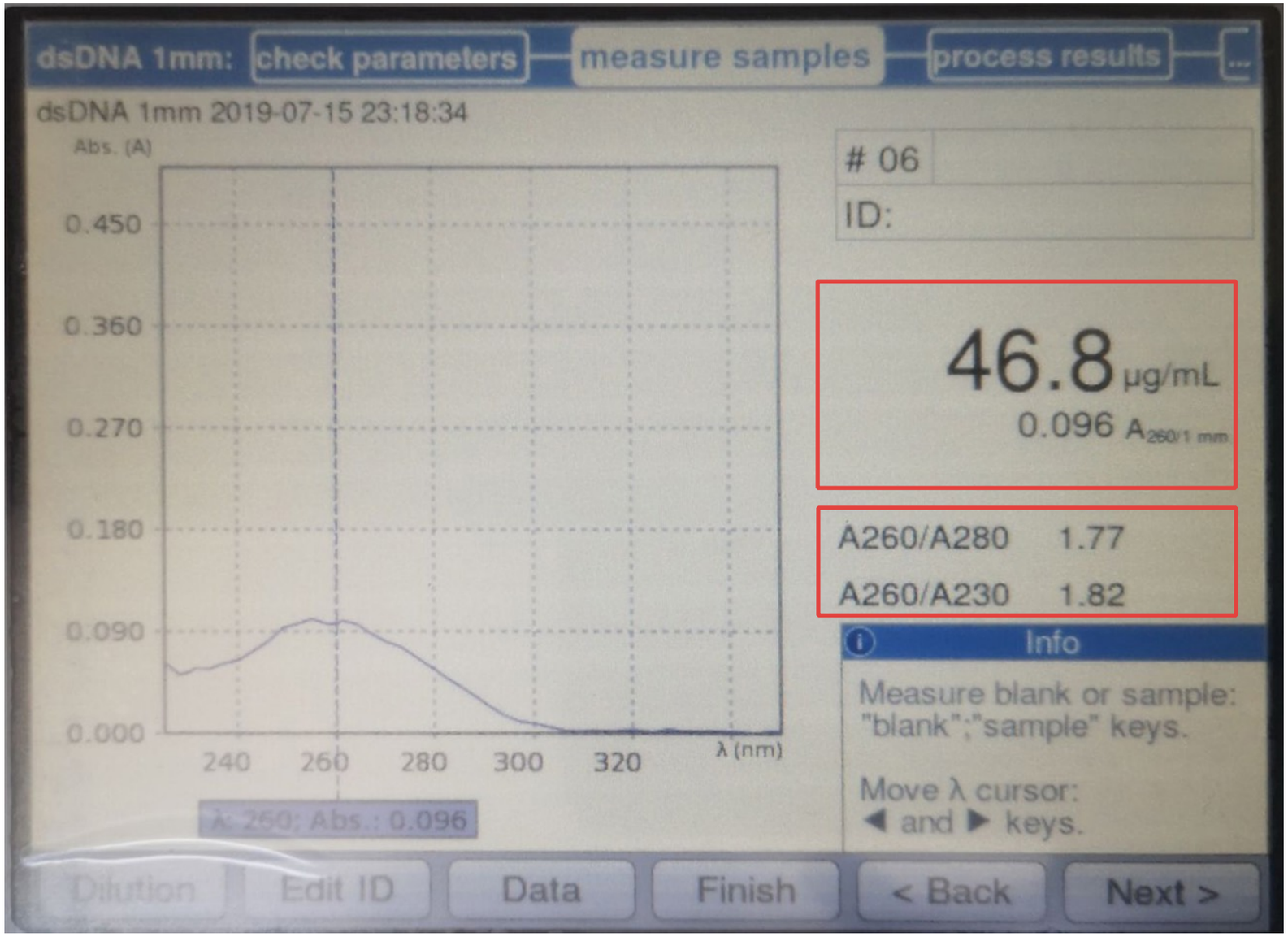
g. Place spin column in microcentrifuge tube and **add 30-50 µl of RNase free H_2O**. **Centrifuge for 1 min at 8,000 x g** to elute the RNA.

h. Repeat (g)

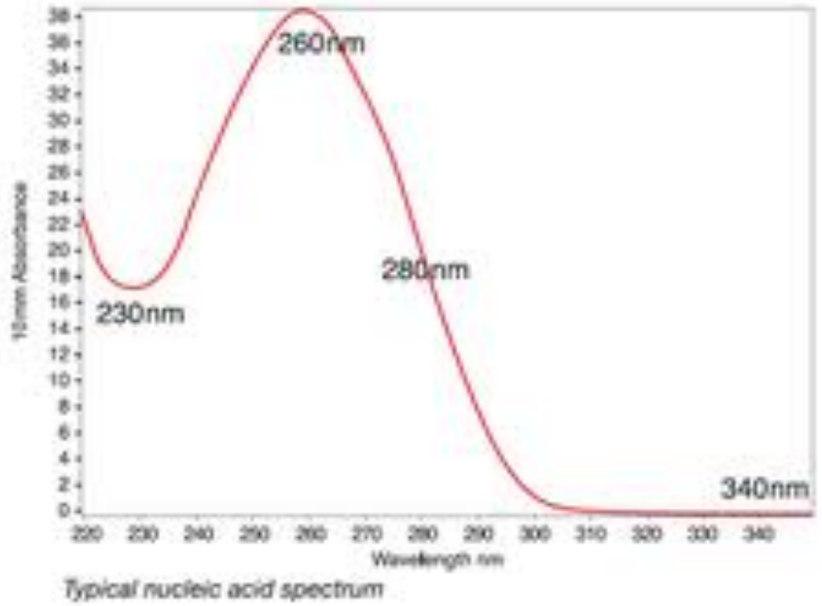
i. Measure RNA concentration using the Spectrophotometer.



RNA preparation… **Shawn**

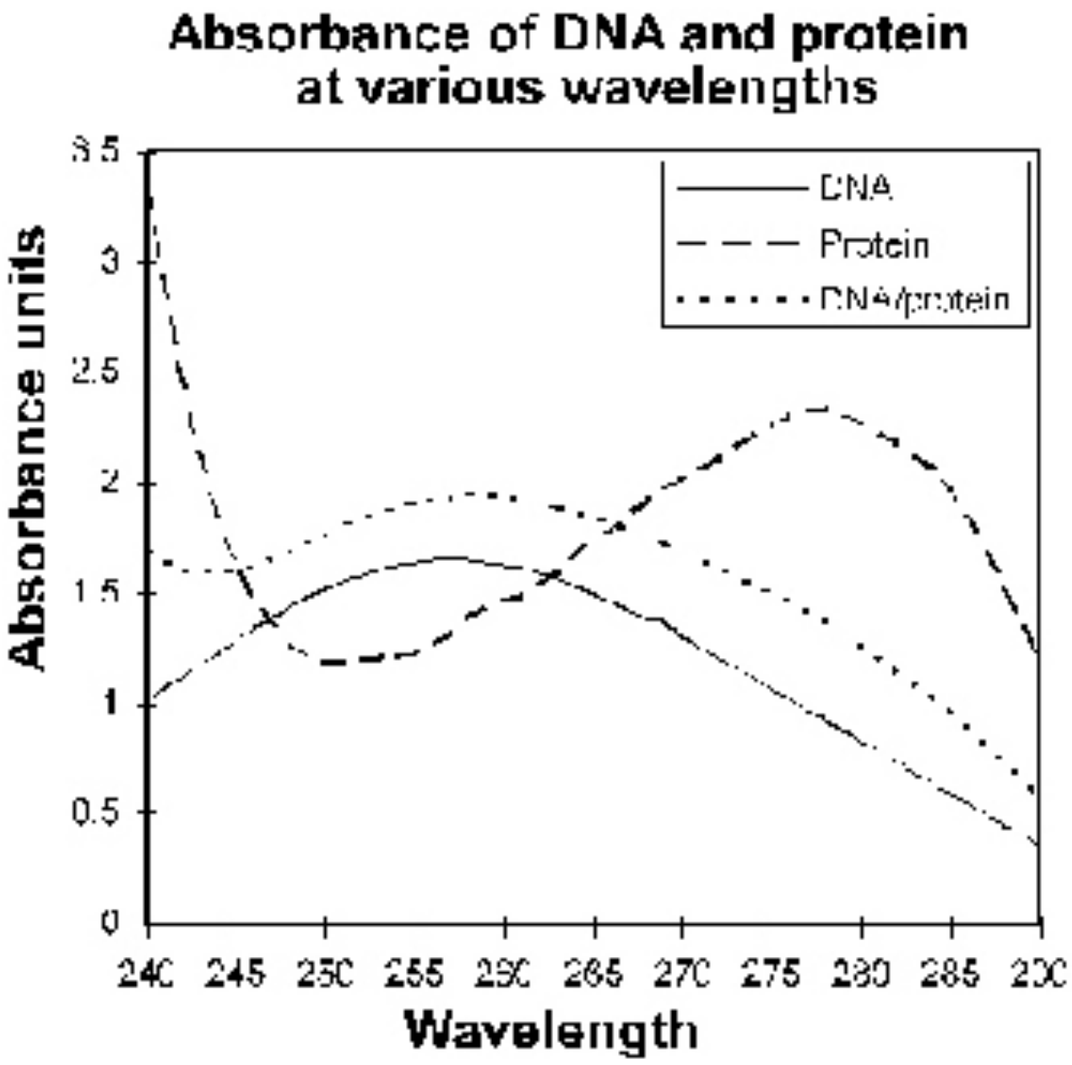


Biophotometer D 30
Eppendendorf



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