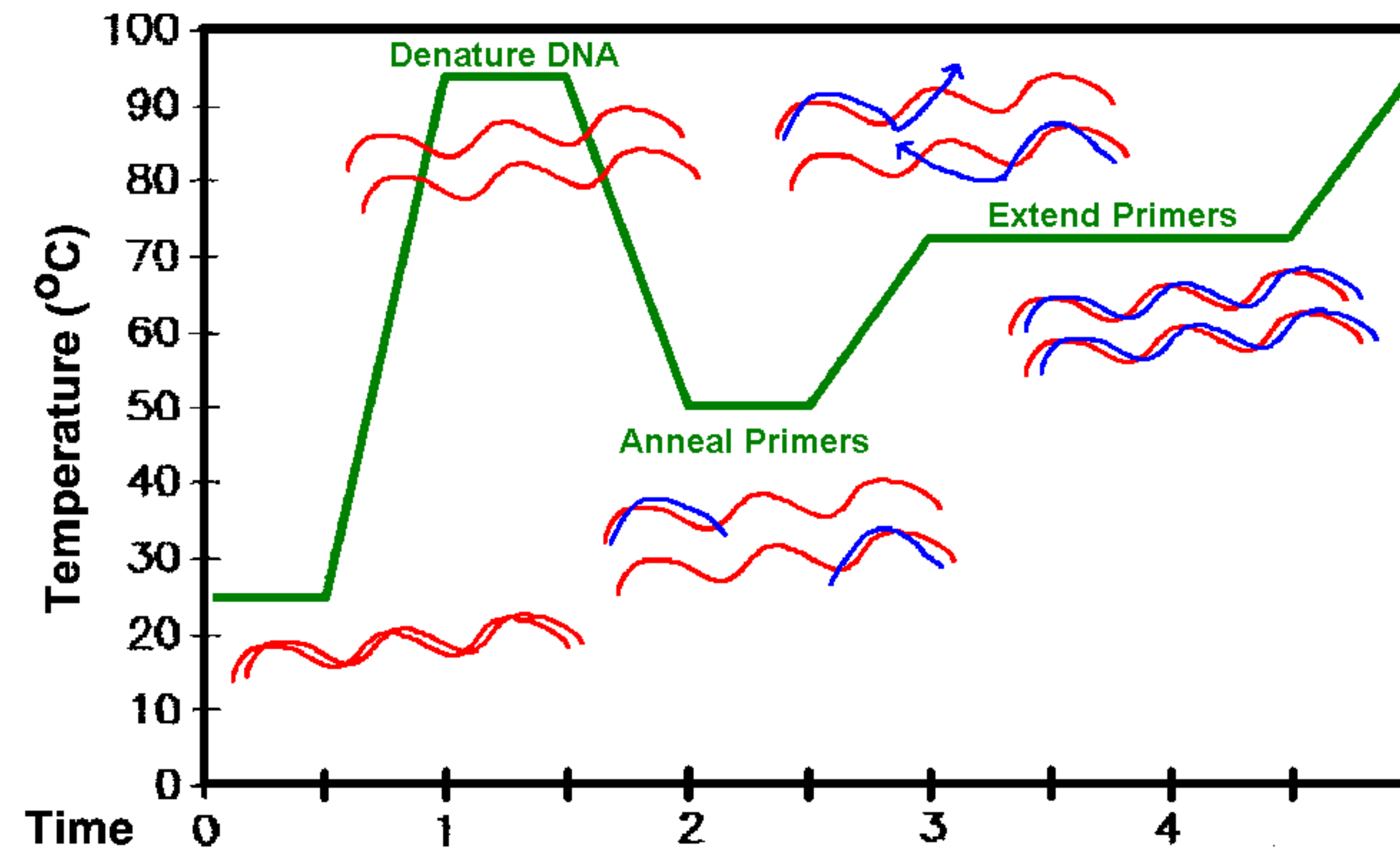
1. Pick up Name Folder
• Pick up your name folder and set it up in front of you at your seat.
2. Clicker Attendance
• Launch your Top Hat and get ready for a Pop Quiz! on the reading.
3. Laptops closed (unless TopHat)
• (open if need 4 TopHat, don't distract others)

“Pop Quiz”: **Reward** for those who prepared.

(3 questions, 60 seconds each)



If primer #1 is calculated to anneal at 50°C and primer #2 at 60°C what's a good temp to try for your annealing temperature in the first PCR experiment you do?

a. 45°C

b. 50°C

c. 55°C

d. 60°C

e. 65°C

Survey: Got bands?

- With PCR primers vs. lambda genomic DNA

A. Not yet
B. Some fuzzy ones that might be correct
C. Yep done with that. Our group rocks!
D. Should we be doing PCR in lab, didn’t know?

Survey: Got distracted folks?

- (Does it appear you have at least one under-achiever not pulling their weight in your research group)

A. Nope my group is doing OK.
B. Yep, I think “firing” will be an important tool.
C. Yep, I am considering *quitting* my group.

1. **Pick up** Name Folder

- Pick up your name folder and set it up in front of you at your seat.

2. **Clicker** Attendance

- Launch your Top Hat and get ready for a Pop Quiz! on the reading.

3. Laptops closed (unless TopHat)

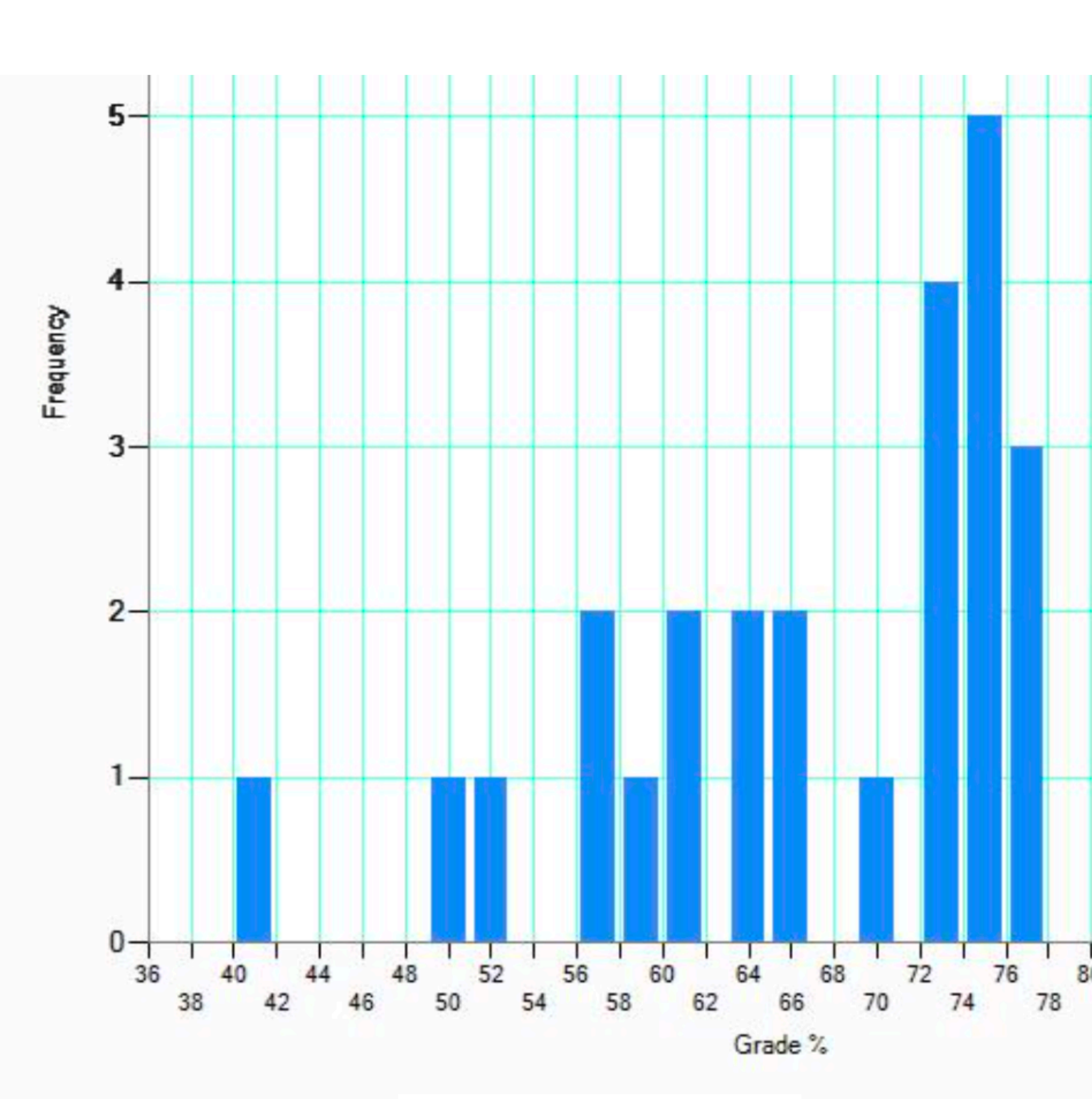
- (open if need 4 TopHat, don't distract others)

Please now set-up Name Folder

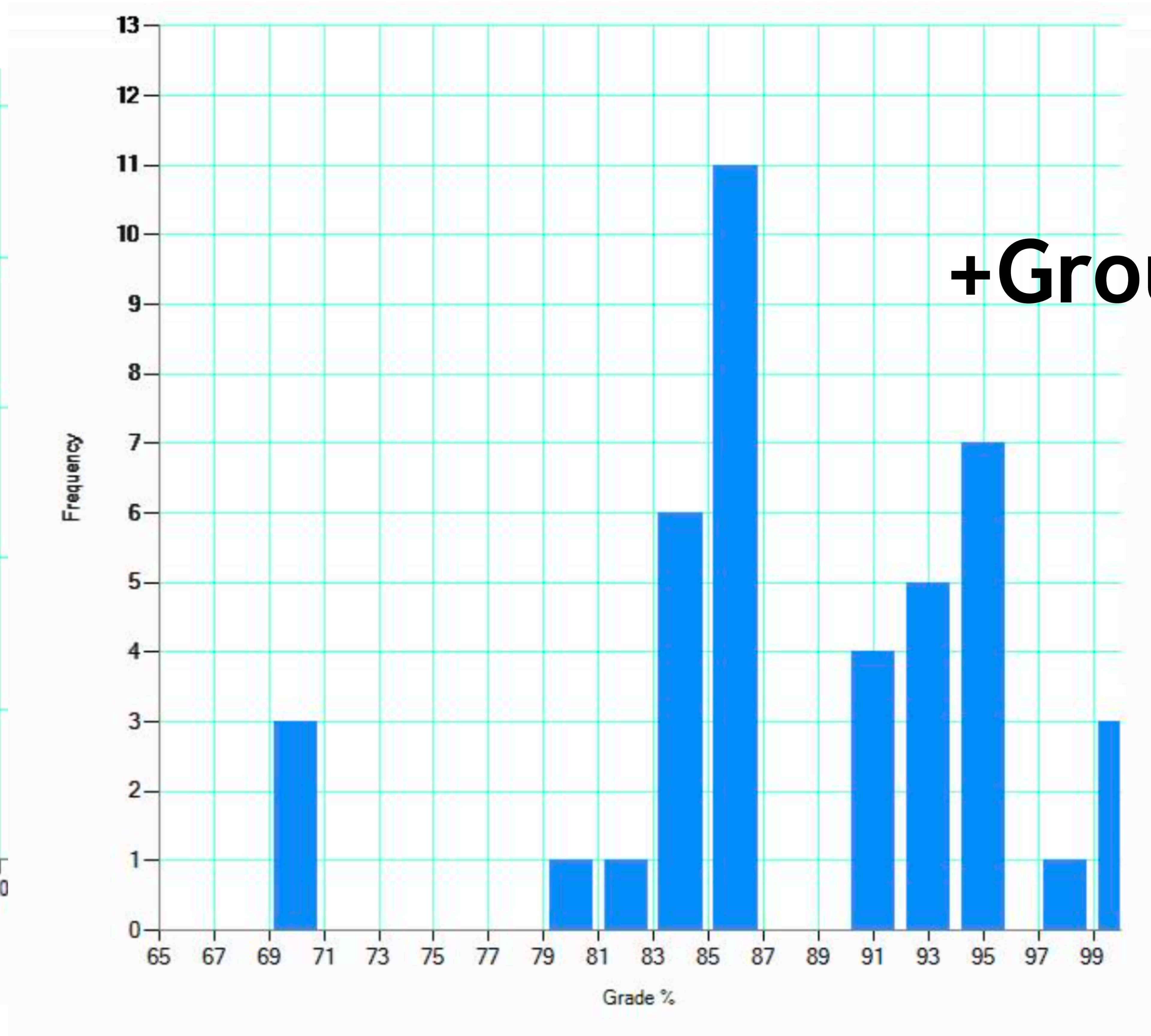
(so it's easier for both instructors and peers to call you by name)

Announcements

1. ***Lab Course: Reminder*** *ONLY buy two primers*
2. *Prof Interviews start today, during lab*
3. ***Exam I****: Pretty strong scores, Way to go.*



Did it alone



Did it with group

+Group Bonus!

EXAM1 final score	
108.64	#
106.36	#
106.36	#
106.36	#
106.36	#
105.45	#
104.09	#
101.82	#
101.82	#
100.91	#
98.18	#
97.27	#
97.27	#
95.91	#
95.91	#
95.00	#
92.73	#
92.27	#
91.36	#
90.00	#
89.09	#
86.82	#
86.82	#
85.91	#

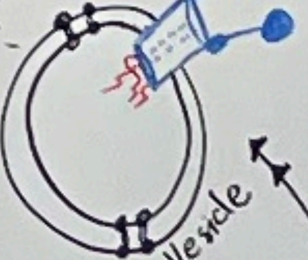
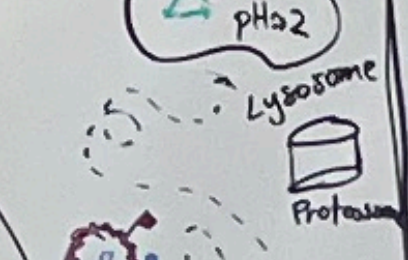
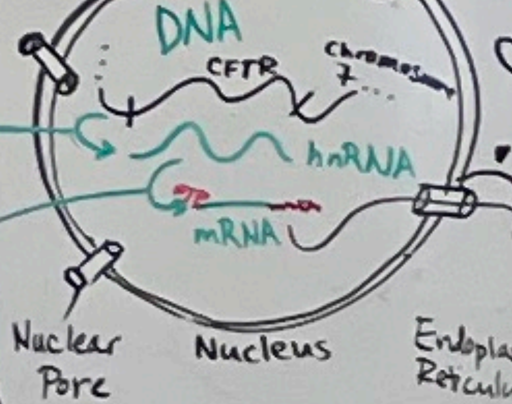
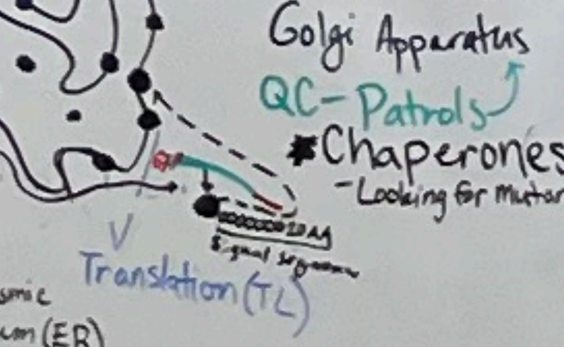
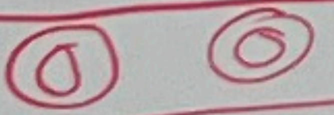
Pyramid Exam

Now: Take the exam as a group
(can increase your grade by up to **20%**)

Rewards:

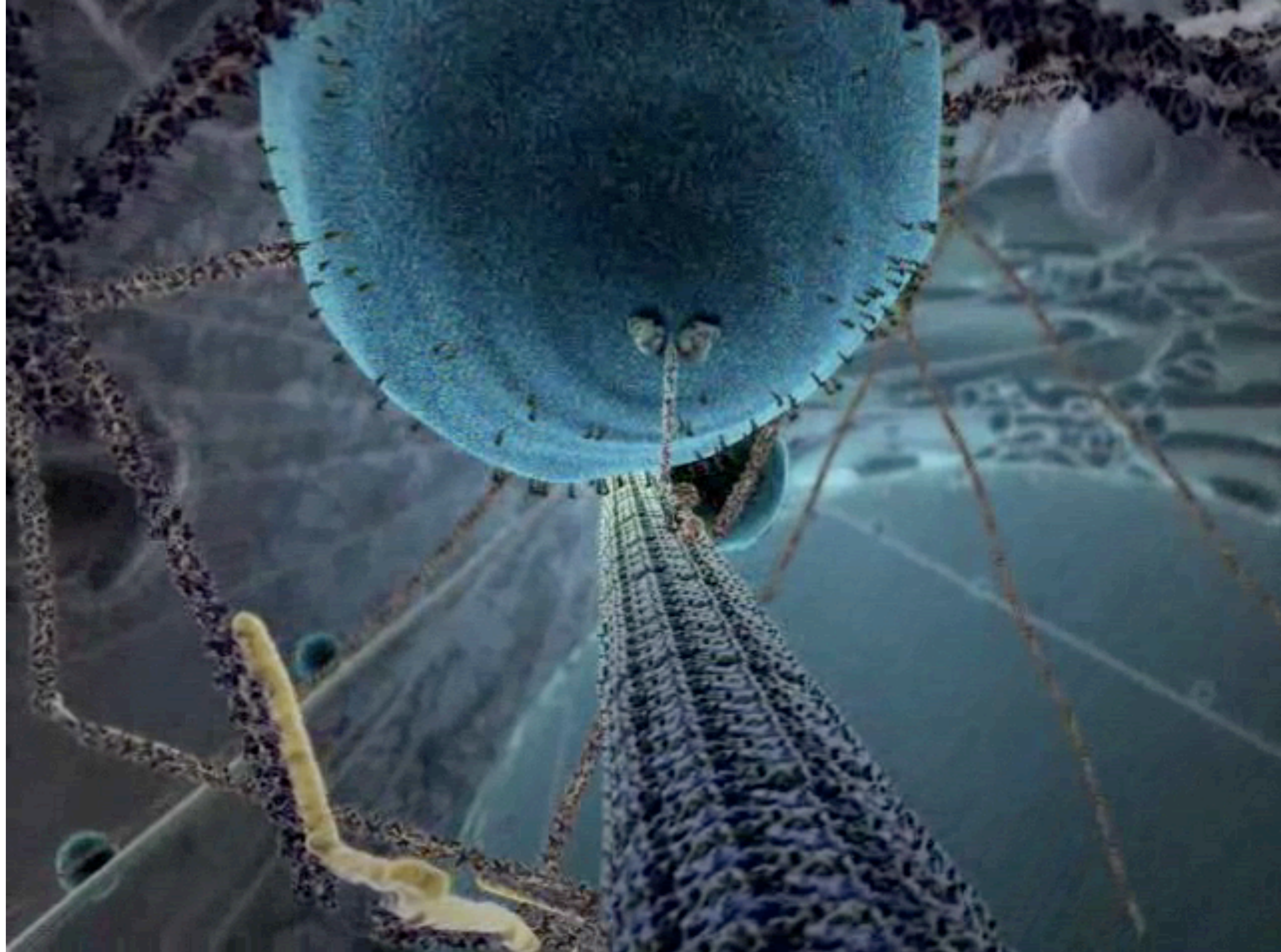
- If **all** members **increase** their exam score **+5%**
- If all members have group score **> 80%** **+5%**
- If all members have group score **> 90%** **+5%**
- If all members have group score **> Jillian*** **+5%**

*"Jillian" is a nickname for smartest student in a group

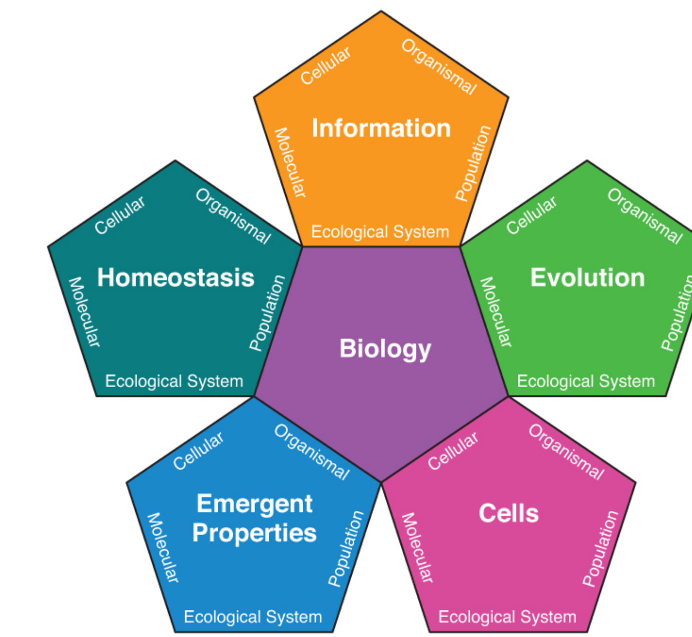






In air way
CFTR
Na⁺ Channel
Apical
Opens or Closes CFTR
Image Not to Scale ;)
pH 0.2
Lysosome
Proteasome
Vesicle
Lateral
GTP Cap
Poly A Tail
GTP mRNA AAAA
Transcription (TS)
DNA
CFTR
hnRNA
mRNA
Splicing
Nuclear Pore
Nucleus
Endoplasmic Reticulum (ER)
Translation (TL)
Golgi Apparatus
Mutant Path
PTM (Post translational modifications)
QC - Patrols
Chaperones
- Looking for mutants/misfolding
Lateral
Basolateral
Basal
PTM + QC (by chaperones)
- Folding
- Glycosylation
- Phosphorylation
- disulphide bridges
mRNA
20aa
free ribosome
TL
free
ER
R
"bound"

1.
2.
3.
4.
5.



Integrating Concepts in Biology



Evolution Applied

5.1 How do genetic diseases arise?

by A. Malcolm Campbell, Laurie J. Heyer, & Christopher Paradise

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(Preparing for) **Tuesday's lecture:**

Budgeting homework time (60 min): Chapter 5 section 5.1 is 2090 words in length with several data tables and figures that require thinking and notetaking for the Trifecta. Reading at 200 words per minute would mean the section might take 10 minutes to read. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 60 minutes. It could be shorter if you have been doing homework regularly, ie. training like an athlete, and getting stronger, better, faster at this now that it is week 7.

1. _______ **For Tuesday's lecture**, read **Chapter 5: Evolution Applied**, section 5.1: "How do genetic diseases arise?" and as you read it be sure to take handwritten notes.

2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (aloud) Figure 5.1, and Tables 5.1, 5.2 and 5.3 in class** (Purpose, Methods, Findings).

4. _______ **Advanced**: Take a peek at several of the published research papers in the Bibliography at the bottom of the page. This may help you understand how to make taq polymerase function more efficiently in your own PCR research project.

LB-145 ICB 5.1 2-23-25 Polymerases + mutations

Chapter 5 - How do genetic diseases arise?

→ imperfect information transfer produces variation
→ DNA replication is susceptible to errors → variation genotypes → passed on

L.O.s - construct a model that explains how mutations can accumulate in DNA
- explain the role of primers in DNA polymerases

Q. How do children have genetic mutations if parents did not?
↳ DNA replication makes mistakes

David Baltimore + Donna Smoler (1971) MIT Massachusetts USA
E. coli In vitro
Purpose: Determine how DNA polymerase starts replication.

Methods: (A) three tubes added: polymerase, dNTPs, DNA template
+ small amount ^{32}P-dGTP → label all new DNA
Figure 5.1 - each tube different amounts of primer 84, 210, 420 pmoles
As time passed aliquots were removed.

(B) similar but diffed types of primers (same concs) w/ poly A - RNA, poly dA - DNA, poly dA no 3'OH group

Found: 1. more primers more products
2. need DNA + 3'OH to 'prime' (E. coli:) - in vitro — in vivo RNA is fine

~~Linn~~ Stuart Linn, Michael Kairis + Robin Holiday (1976)
National Instit. Medical Research, London, England

Purpose: Does DNA polymerase 'age' and slow speed? etc
Could this contribute to cell age/death due to errors?

Method: Grew human skin cells in petri dishes over time
Isolated DNA polymerases from young vs old cells.

Table 5.1 speed of replication - young way faster

Linn et al (cont.) (their Table 2).

Table 5.2 young vs old, + Mg^{2+} vs Mn^{2+}, speed + error rates
polymerase needs $Mg^{2+}Cl$ (or Mn)
but no statistical tests so?

young good with either Mg or Mn (errors) but faster w/ Mn
old slower with Mg but more accurate (yet 3+ times faster)

Gita Seal + Clyde Shearman + Lawrence Loeb
Seal et al (1979) Philly + Seattle (1978) Cancer Research Centers
DNA polymerase isolated from human placenta
Radioactive $\alpha^{32}P$-dTTP

Table 5.3 Purpose: Test different ions for impact human DNA polymerase activity
Methods: use Ni^{2+} Cd^{2+} Ca^{2+} on accuracy of young poly
No details in textbook Disposable batteries

Finding: error rates best w/ Mg^{2+}. 1 in 41,000 bases
higher Nickel 2mM vs 1mM = worst 1 in 1,850 bases
Cd^{2+} 0.1 mM 0.2 mM higher worse Ca^{2+} 0.6, 1.0, 2.5

Wrap up each child inherits (acquires?) 100 mutations not present in parents

Biology Learning Objectives

- Construct a model that explains how mutations can accumulate in DNA.
- Explain the role of primers in DNA polymerization.

you are here		Big Ideas of biology				
		Information	Evolution	Cells	Homeostasis	Emergent Properties
levels of the biological hierarchy	molecules	1	4	7	10	13
	cells	2	5	8	11	14
	organisms I	3	6	9	12	15
	organisms II	16	19	22	28	25
	populations	17	20	23	29	26
	ecological systems	18	21	24	30	27

Trifecta

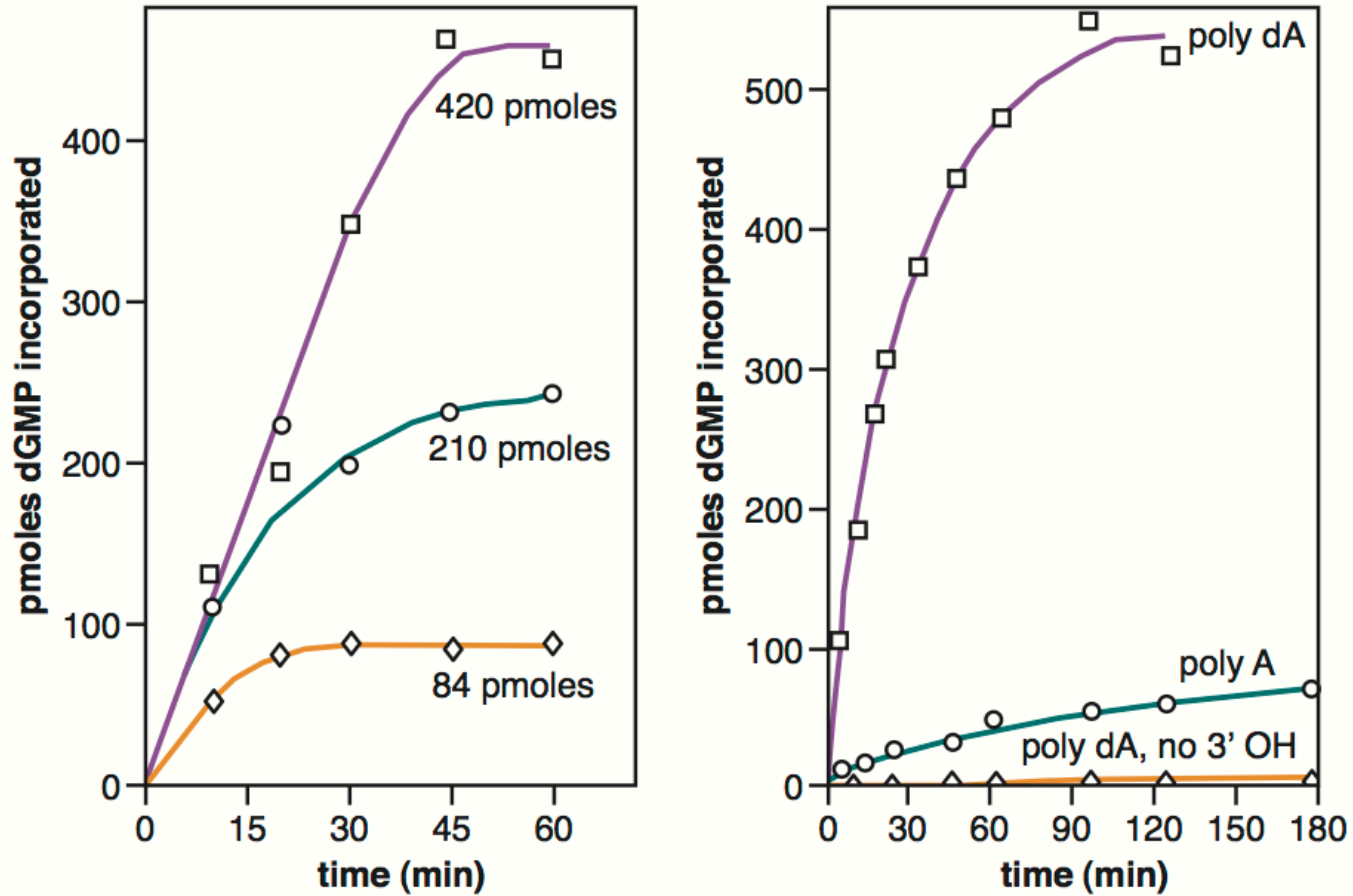


Fig. 5.1

modified from Baltimore and Smoler, 1971

Trifecta

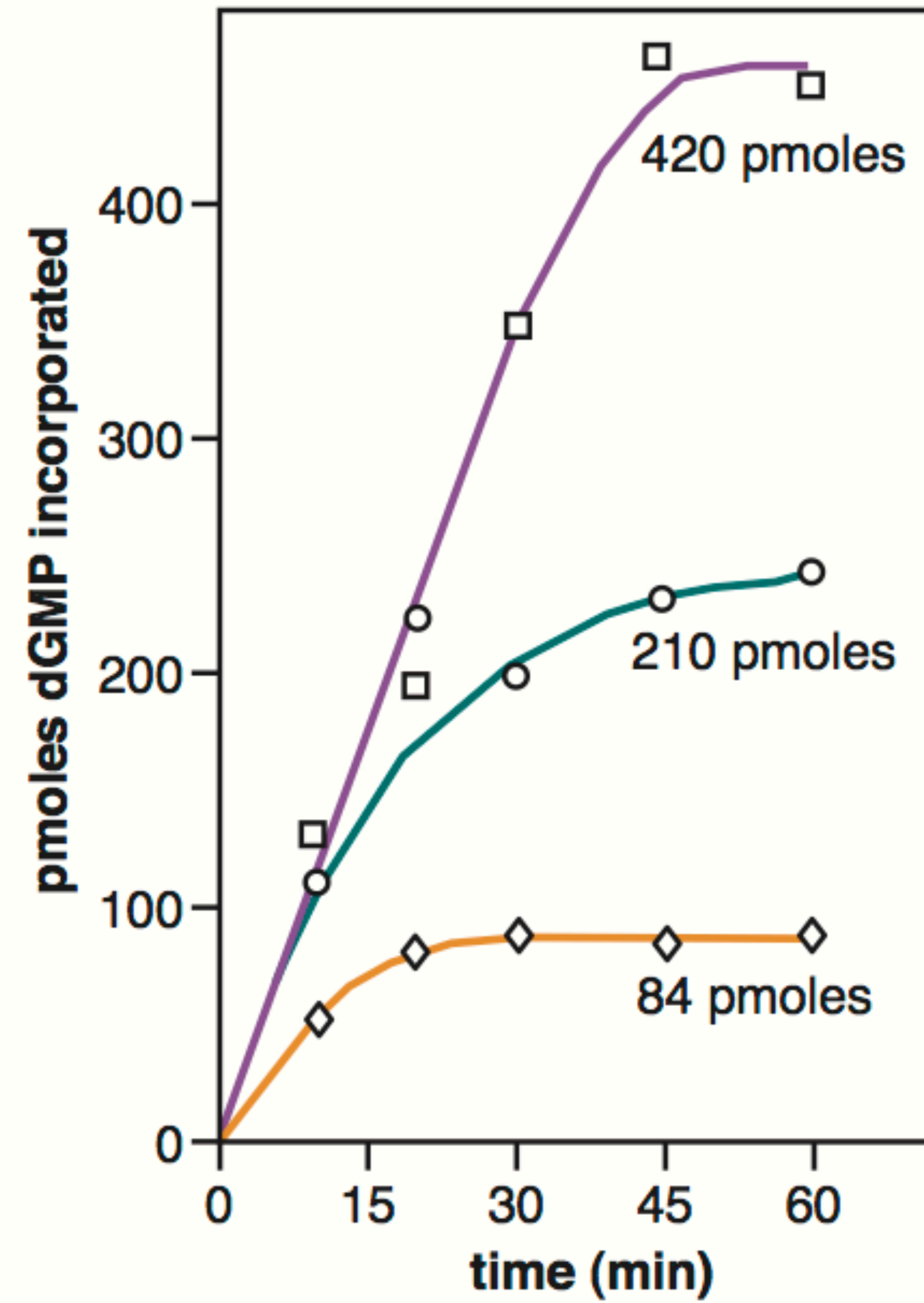


Fig. 5.1

modified from Baltiore and Smoler, 1971

Primer? 1:00 Show Correct Answer

What is the role of a primer in DNA Replication?

A	Primers are annealed to assemble dsDNA
B	Primers are ligated together to form dsDNA
C	Primers provide the monomer dNTPs for DNA polymerase
D	Primers provide a starting place for DNA polymerase
E	None of the above

Trifecta

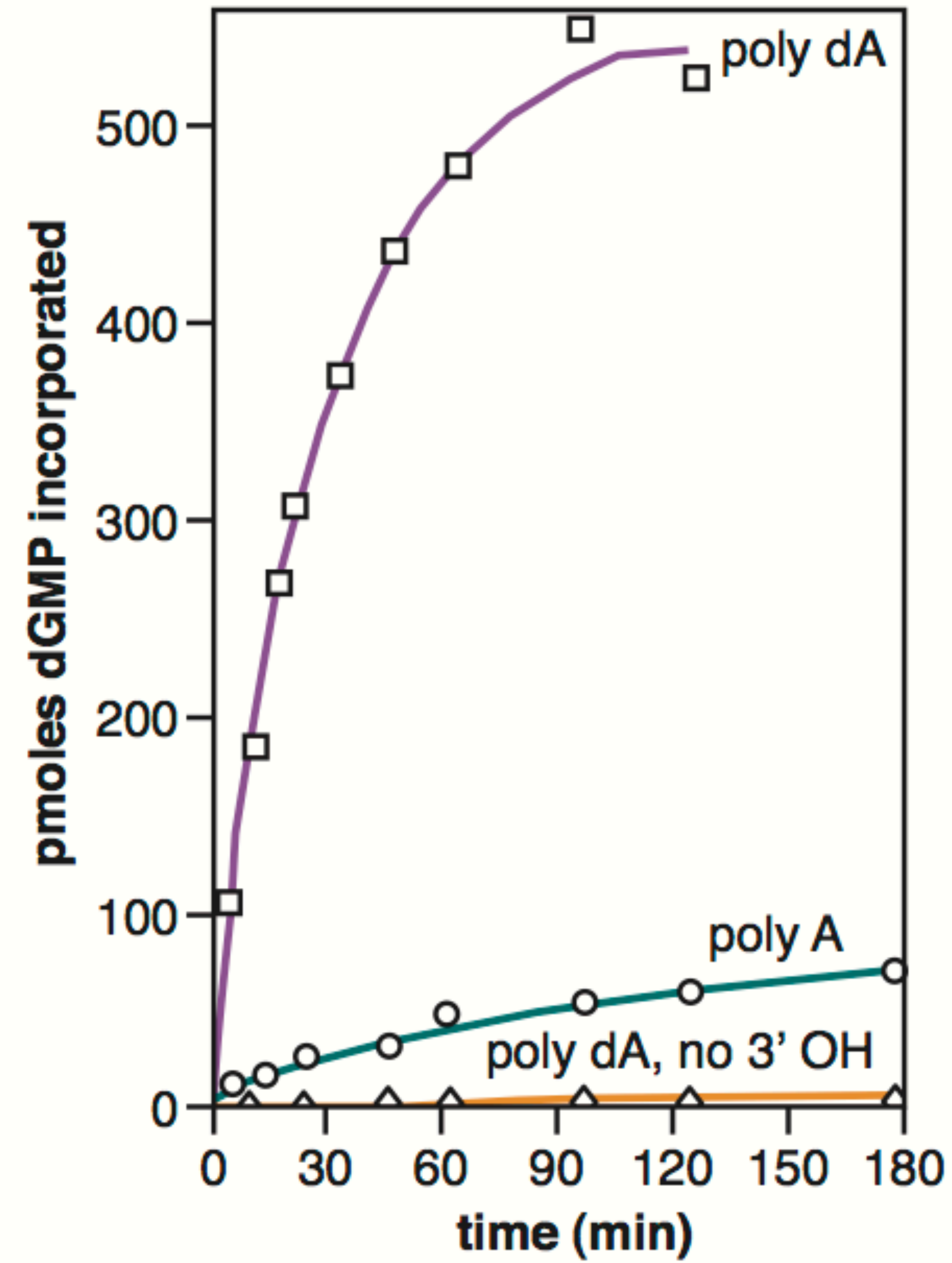


Fig. 5.1

modified from Baltiore and Smoler, 1971

What is required of all primers used by DNA polymerase?

All results ▾

A	2'-OH
B	3'-H
C	5' phosphate
D	4' phosphate
E	None of the above

DNA Polymerase Activity

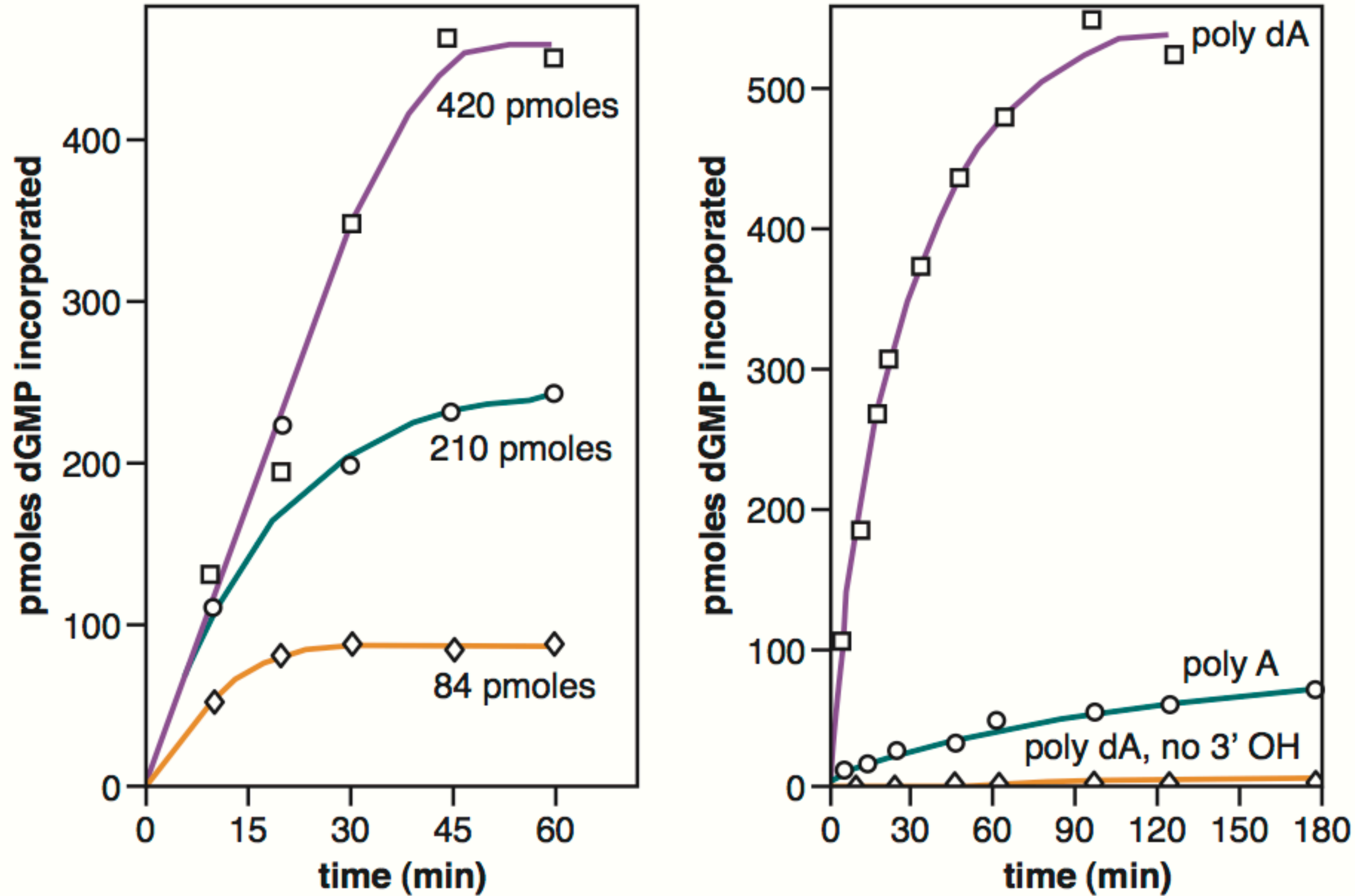
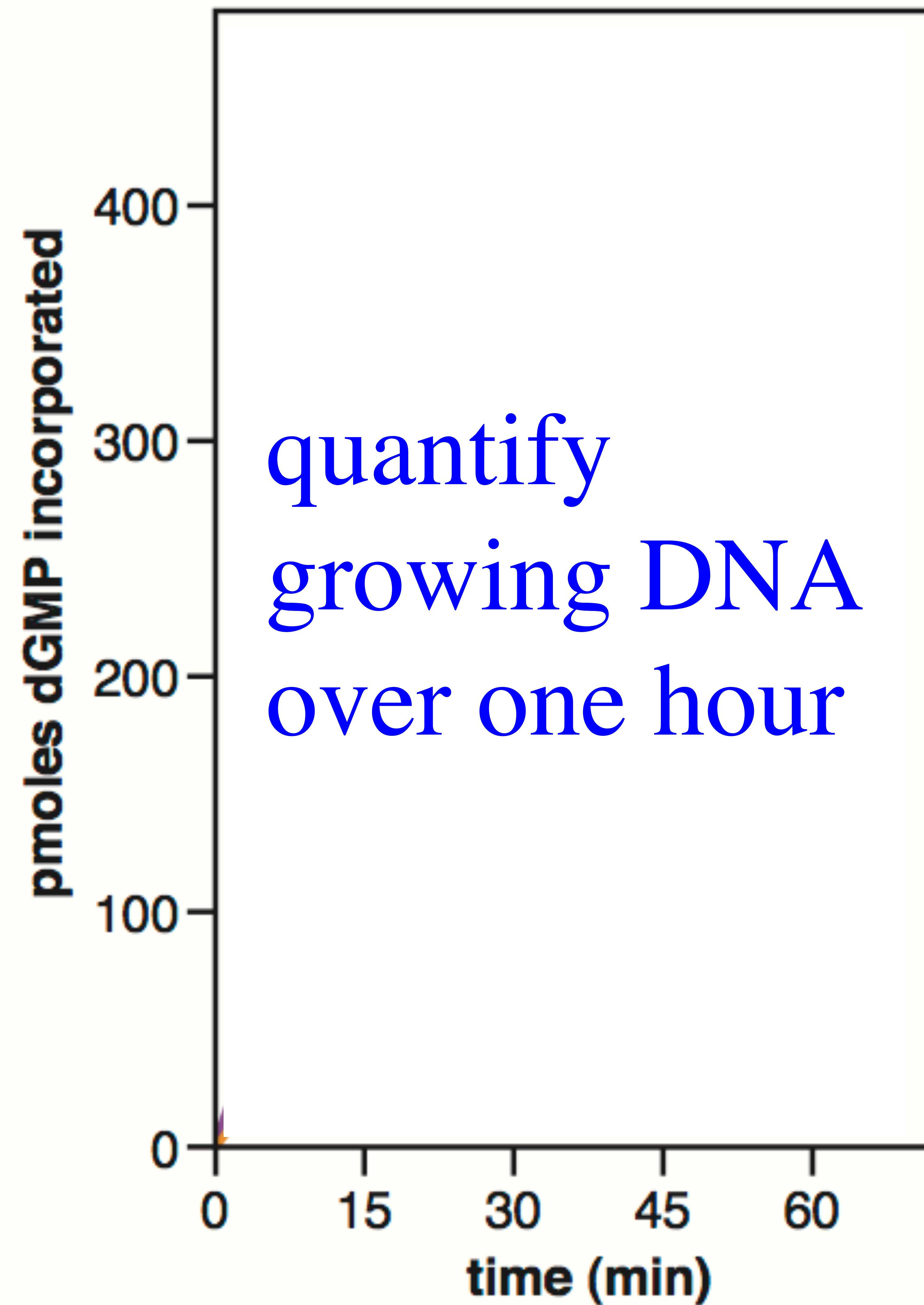


Fig. 5.1

modified from Baltiore and Smoler, 1971

What is the role of primers?



Mix in a tube:

- DNA polymerase
- DNA template
- dNTP monomers
- primer (oligonucleotide)
- buffer

Fig. 5.1

modified from Baltiore and Smoler, 1971

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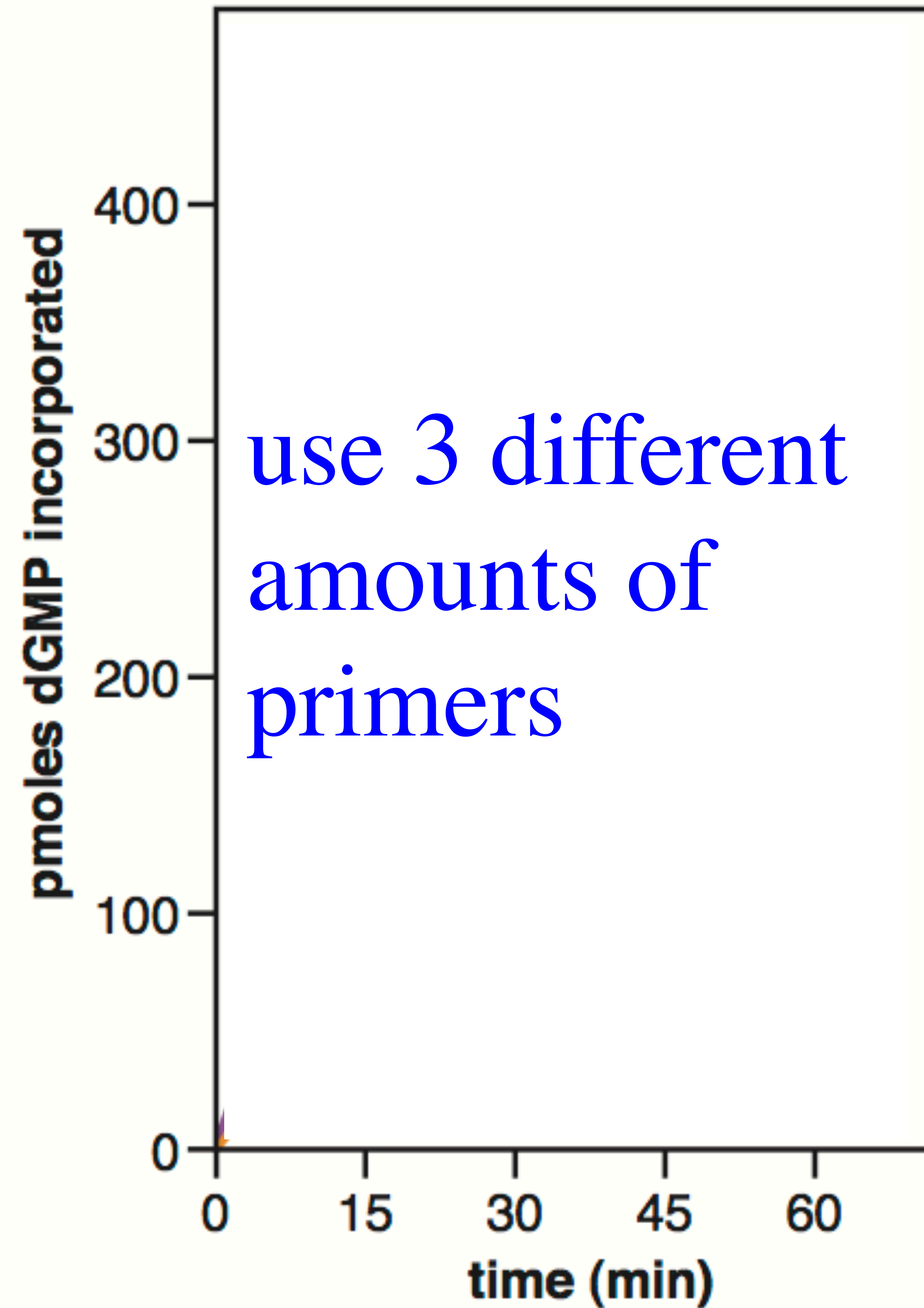
What is the role of primers?



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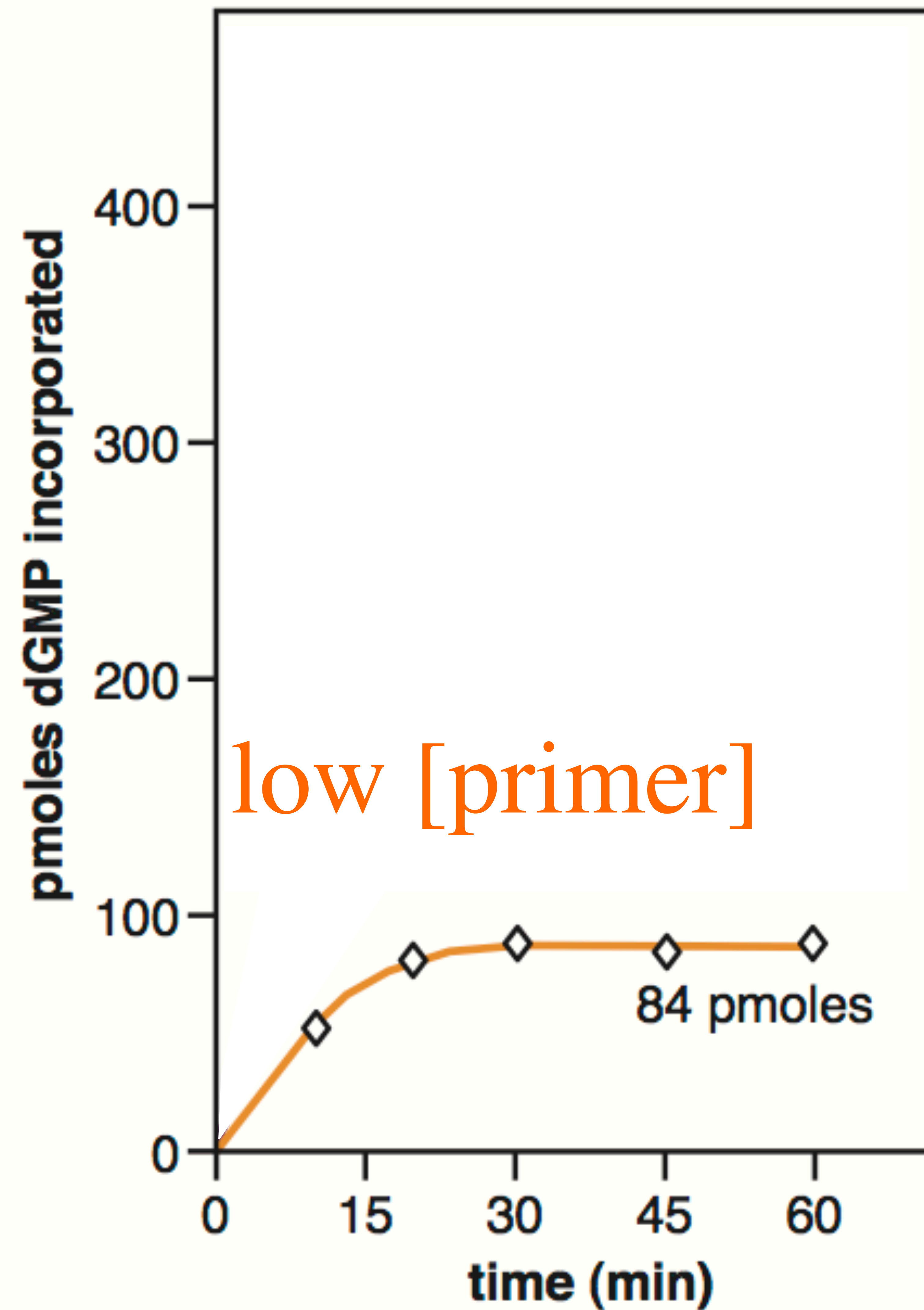
Change Primer Concentration



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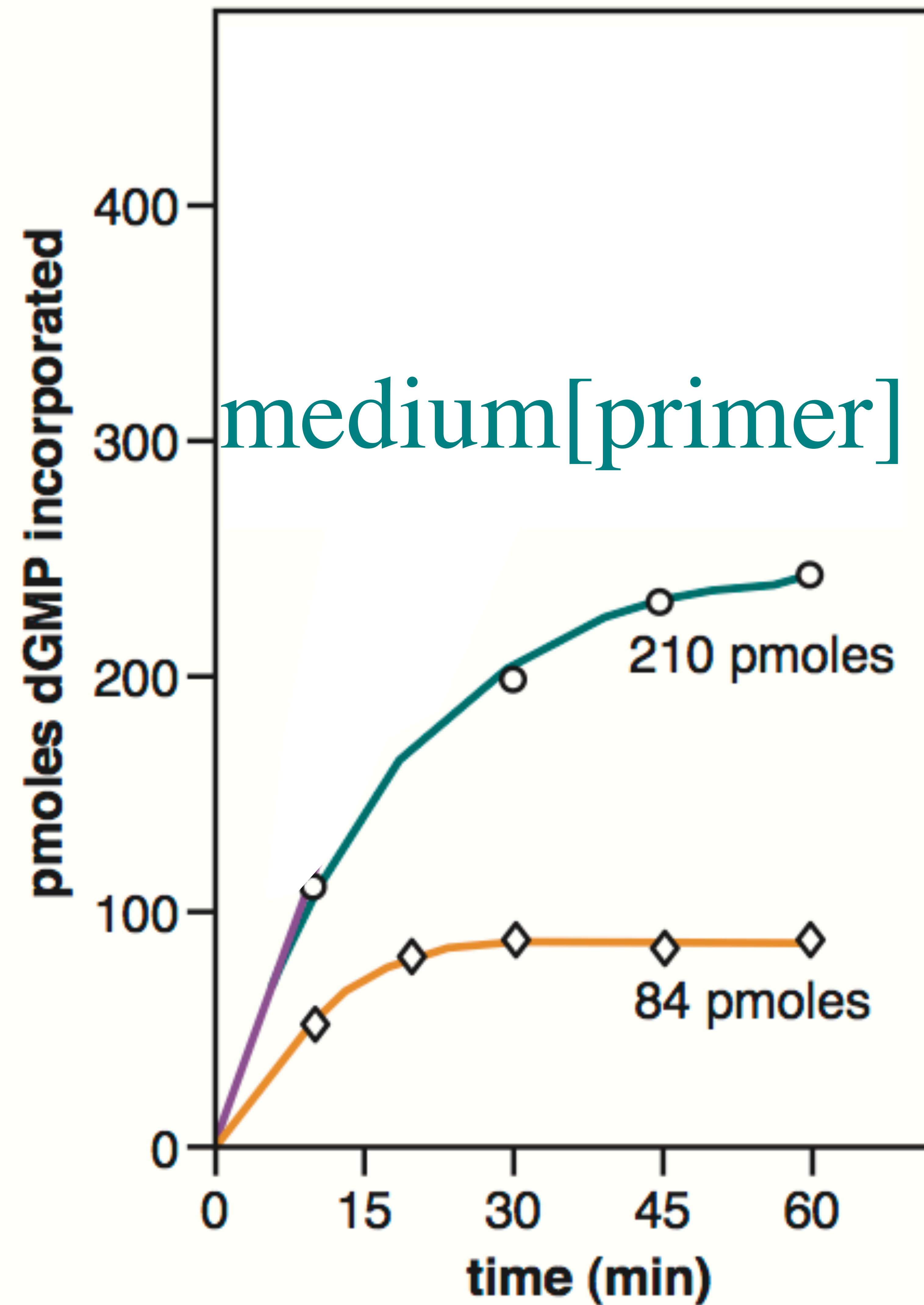

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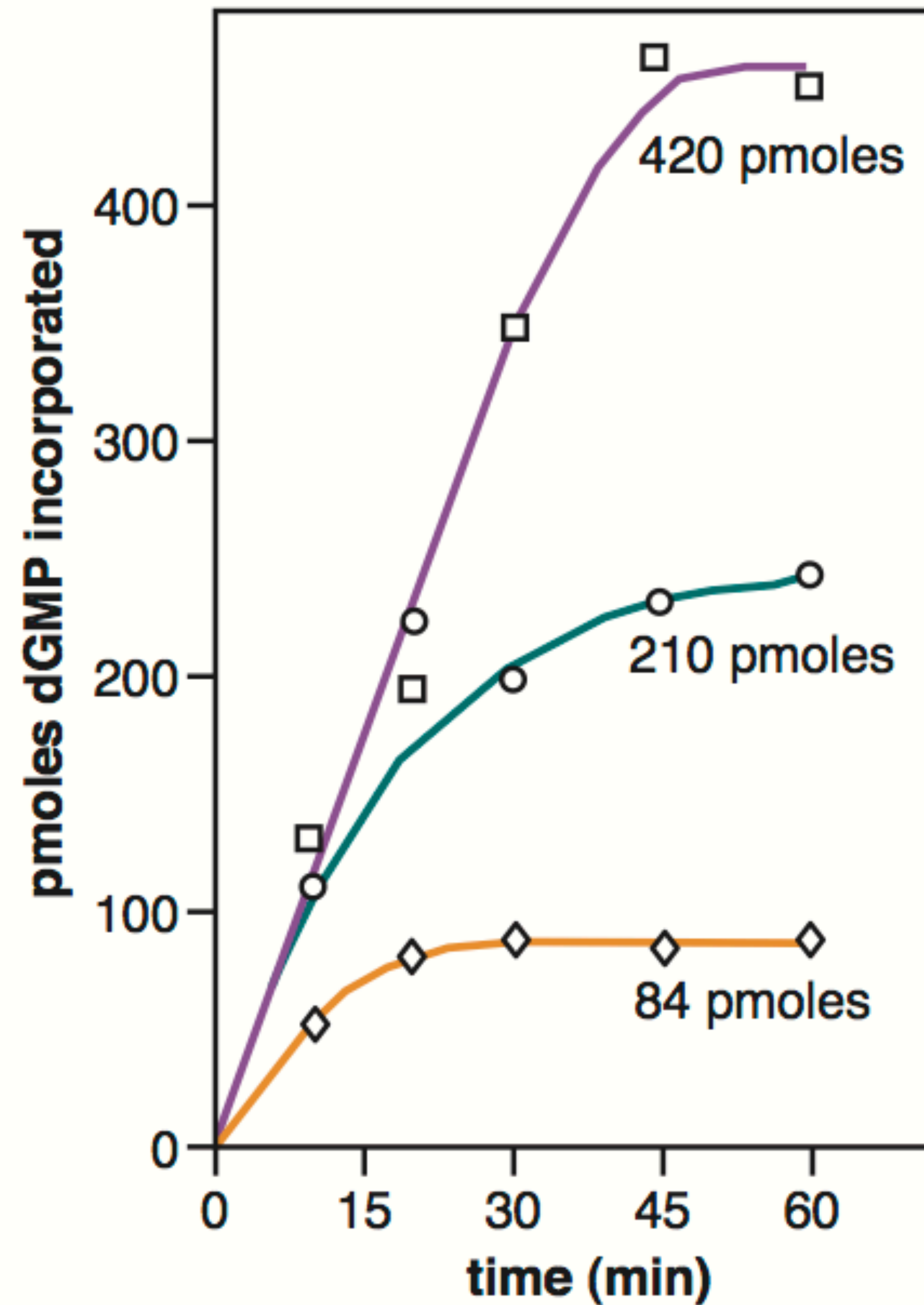

high [primer]

Mix in a tube:

- DNA polymerase
- DNA template
- dNTP monomers
- primer (oligonucleotide)
- buffer

Fig. 5.1

modified from Baltiore and Smoler, 1971

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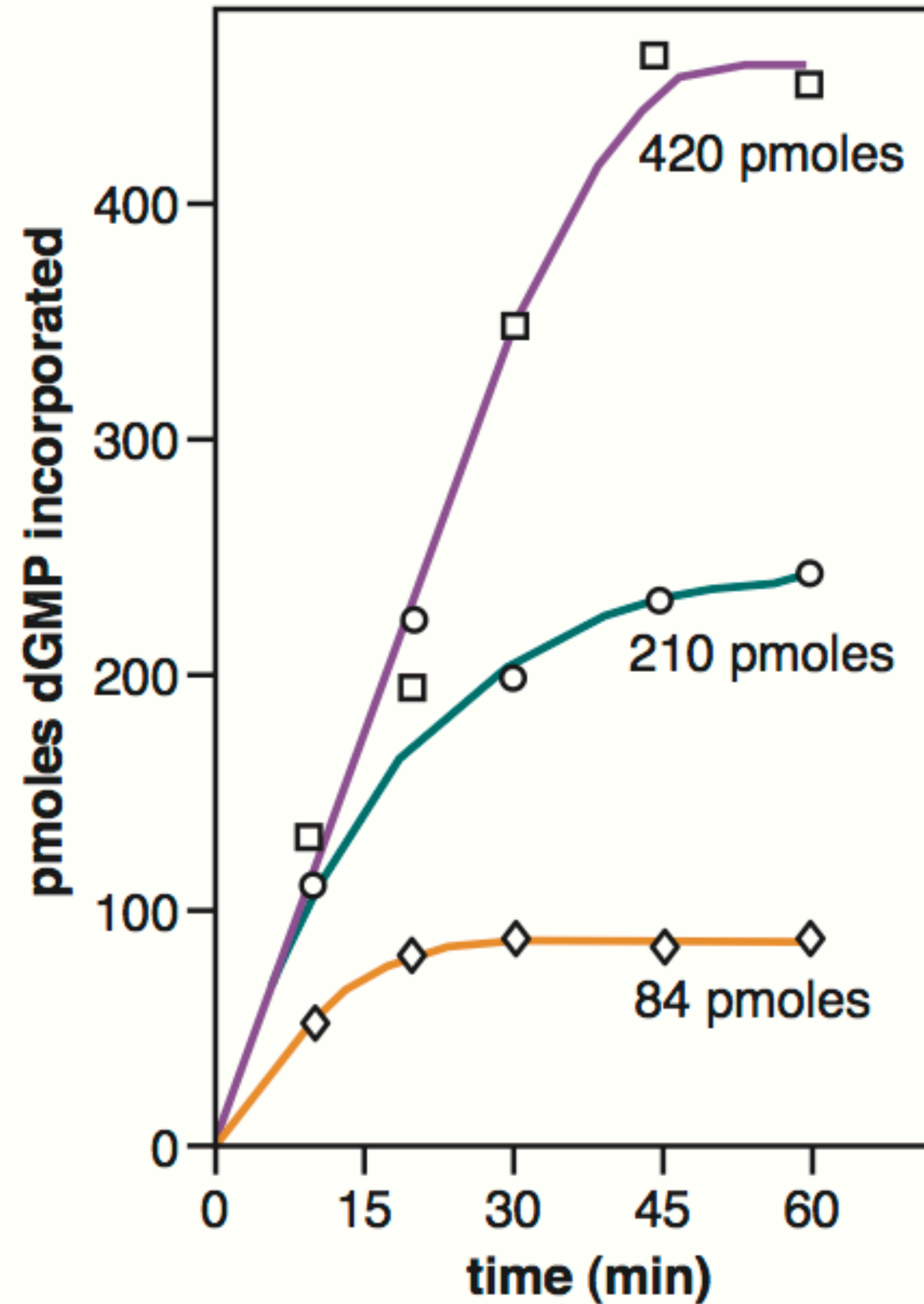
DNA Polymerase Function



DNA synthesis requires a primer: the more primer the more DNA produced

Fig. 5.1

modified from Baltiore and Smoler, 1971

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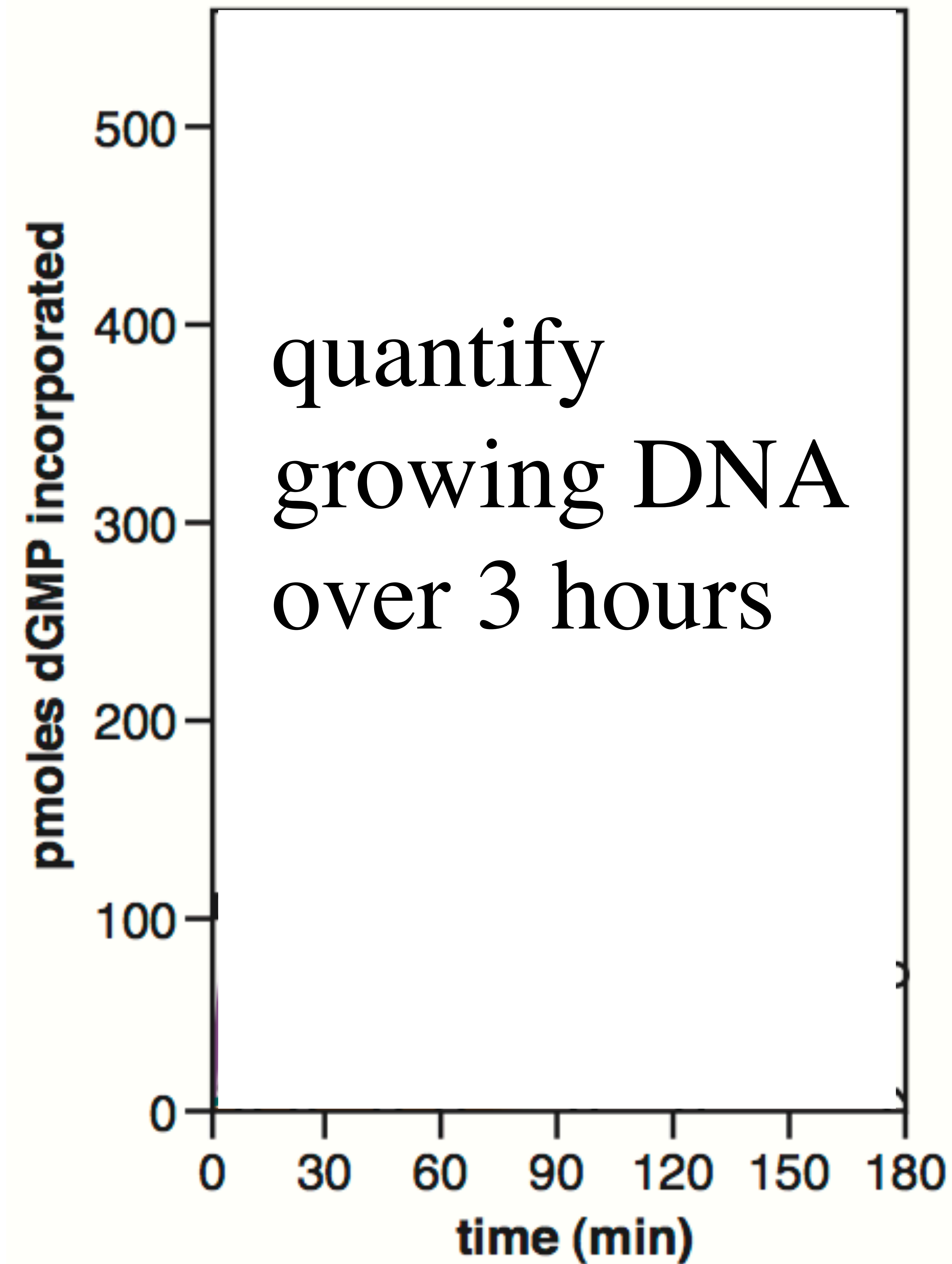
What Makes a Good Primer?

Mix in a tube:

- DNA polymerase
- DNA template
- dNTP monomers
- primer (oligonucleotide)
- buffer



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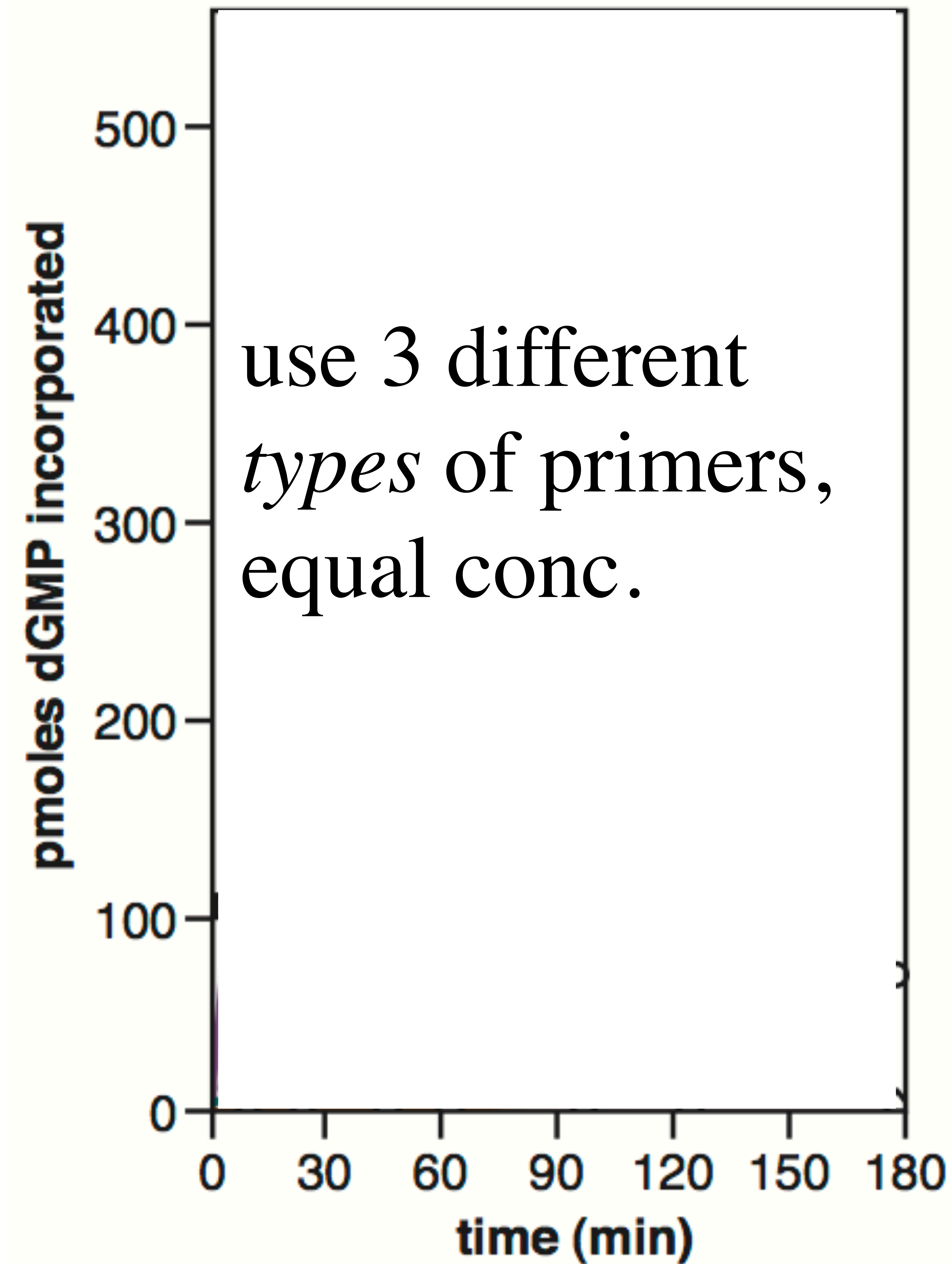

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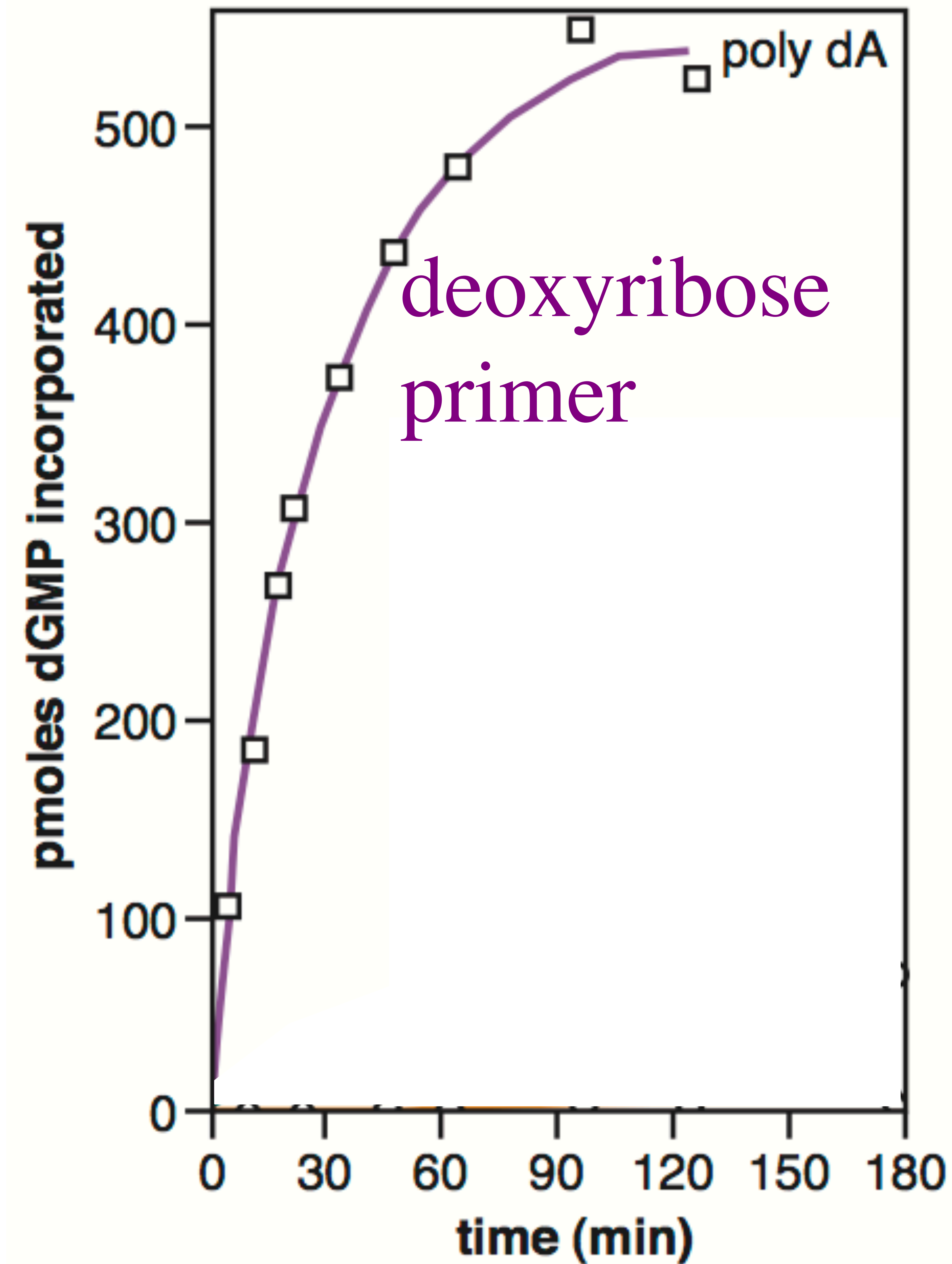

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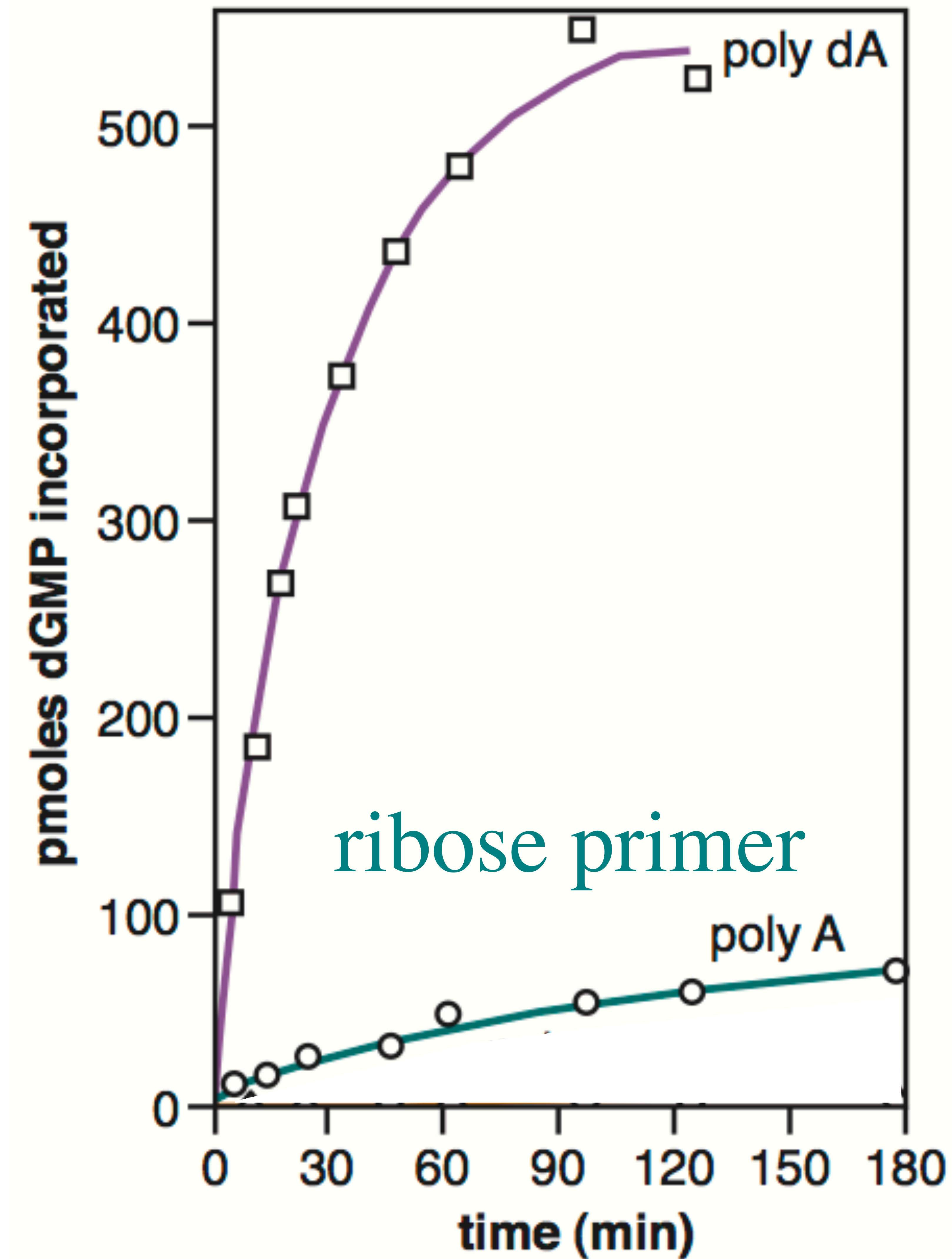

Fig. 5.1

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What Makes a Good Primer?

Mix in a tube:

- DNA polymerase
- DNA template
- dNTP monomers
- primer (oligonucleotide)
- buffer

deoxyribose primer, 3' OH removed

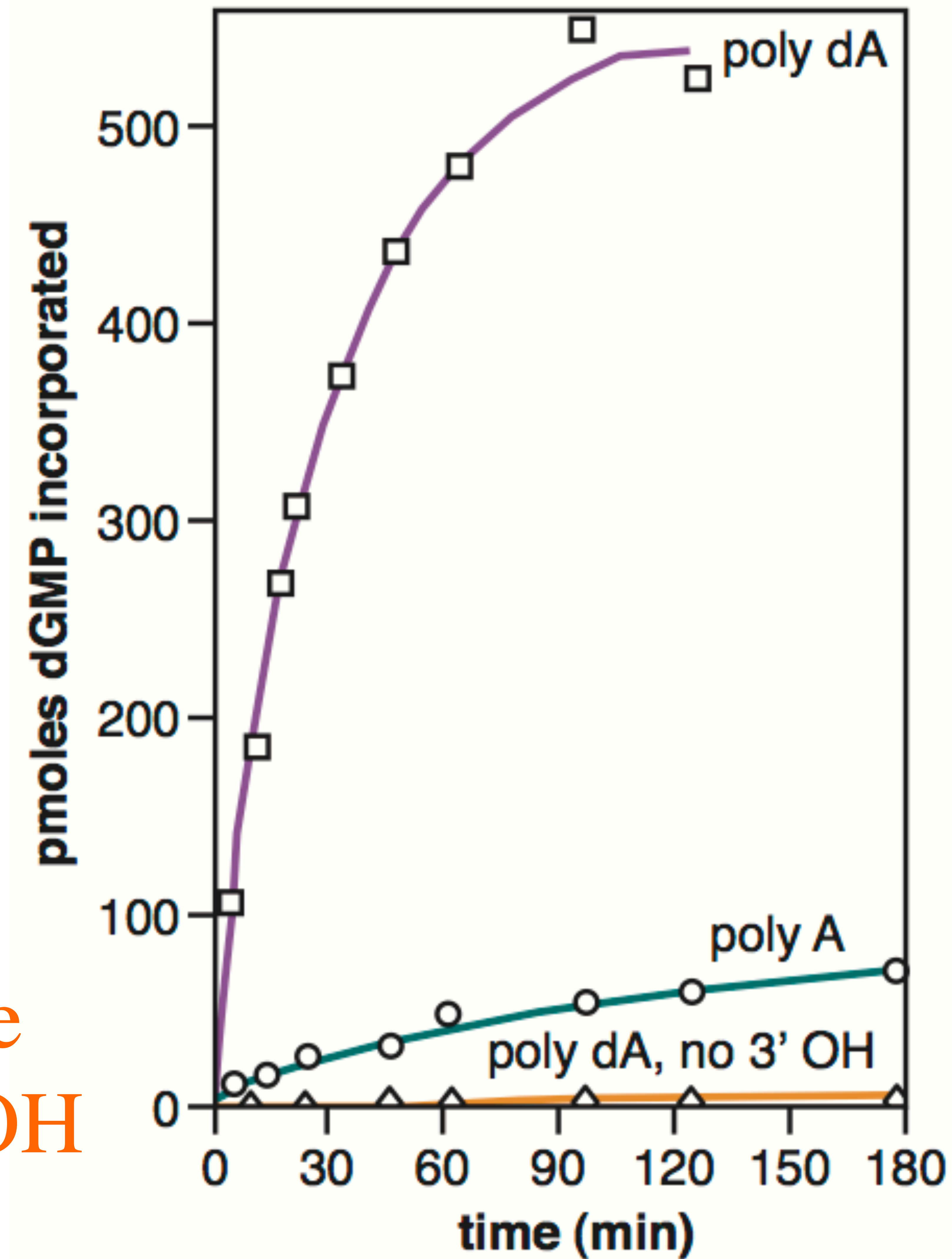

Fig. 5.1

modified from Baltiore and Smoler, 1971

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Which Primer Is Best?

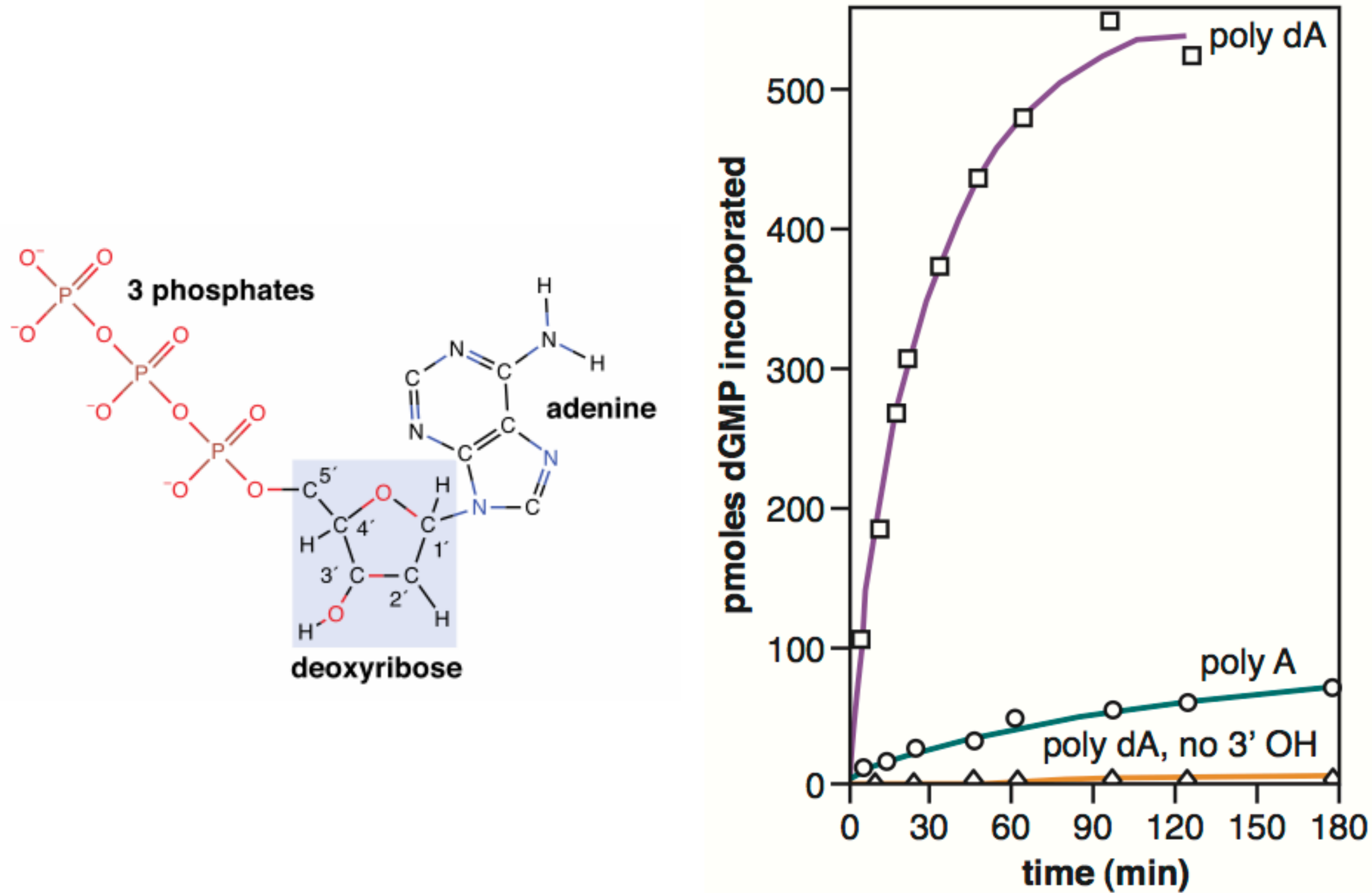

Fig. 5.1

modified from Baltiore and Smoler, 1971

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Which Primer Is Best?

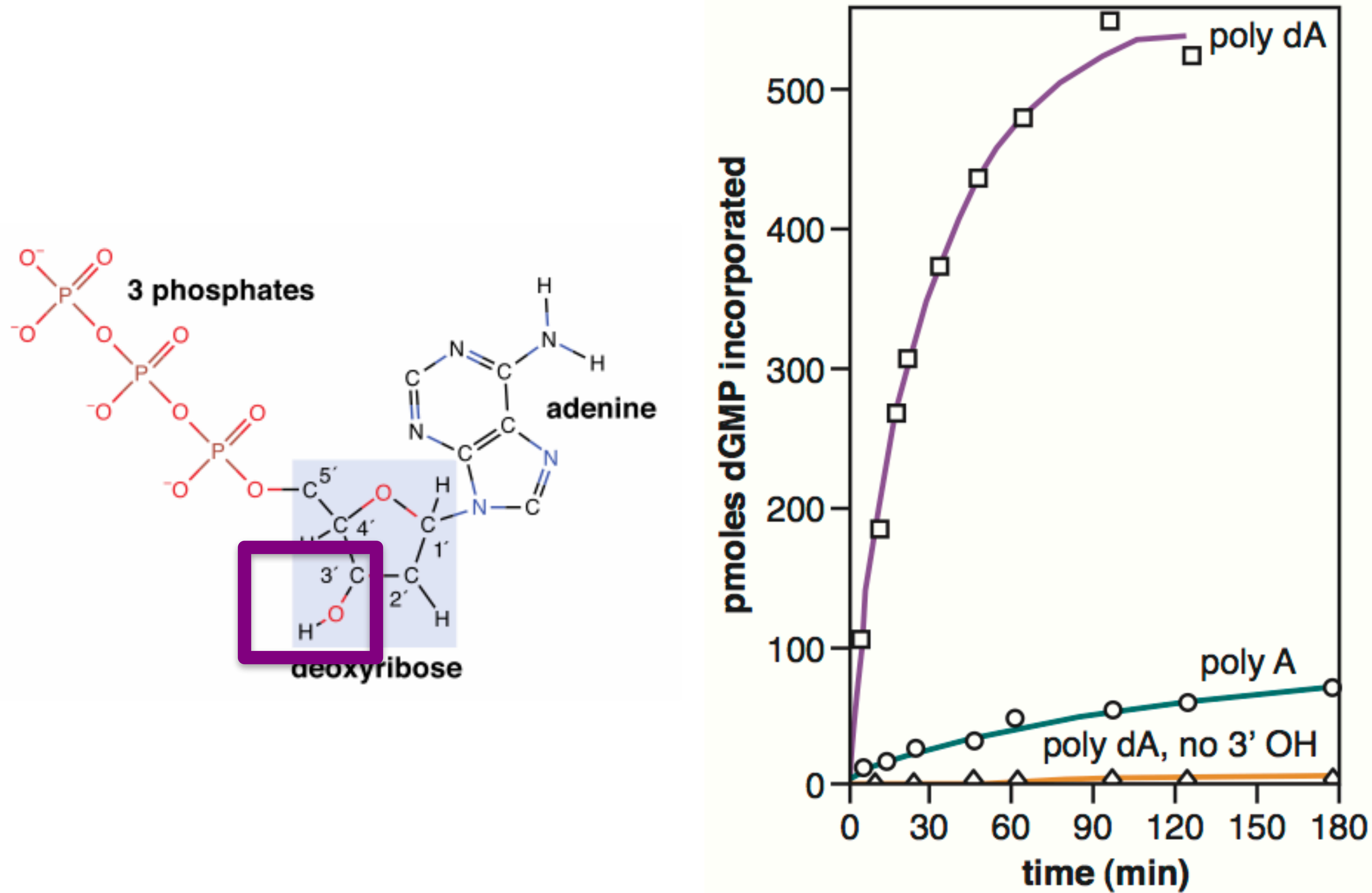

Fig. 5.1

modified from Baltiore and Smoler, 1971

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Trifecta

Table 5.1 DNA polymerase activity comparison.

cell extracts	activity
young	982 units
old	58 units

from Linn *et al.*, 1976

Table 5.1

DNA Polymerase Activity

Does age affect DNA polymerase?

Table 5.1 DNA polymerase activity comparison.

cell extracts	activity
young	982 units
old	58 units

Table 5.1

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DNA Polymerase Activity

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DNA Polymerase Activity

cause *vs* correlation

Table 5.1 DNA polymerase activity comparison.

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old	58 units

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Proc. Natl. Acad. Sci. USA
Vol. 73, No. 8, pp. 2818–2822, August 1976
Cell Biology

Decreased fidelity of DNA polyme ing human fibroblasts

(DNA nucleotidyltransferase/error-prone replication/cell cultu

STUART LINN*, MICHAEL KAIRIS, AND ROBIN HOLLI

Genetics Division, National Institute for Medical Research, Mill Hill, London NV

Communicated by Bruce N. Ames, June 7, 1976

ABSTRACT **DNA polymerase (deoxynucleosidetriphosphate:DNA nucleotidyltransferase, EC 2.7.7.7 or DNA nucleotidyltransferase) activity, isolated from late and early passage cells of the diploid human fibroblast line, MRC-5, was compared. The level of activity dropped with increasing passage. In addition, when the fidelity of polymerization was monitored with four synthetic templates under a variety of conditions, it was observed that the enzyme from late passage cells was more error-prone. The possible relation of these observations to "senescence" of the fibroblasts is discussed.**

Diploid human fibroblasts have a defined lifespan in culture that is somewhat related to the potential life expectancy of the

MATERIALS AND METHODS

Growth of Cells. MRC-5 human male fetal lung fibroblasts (5) were grown in Eagle's basal medium containing 10% fetal calf serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 50 μg/ml of aureomycin. Cells were monitored to assure the absence of mycoplasmic contamination (11). For collection, cells that had not reached confluence, as judged by microscopic examination, were rinsed with 5 mM Tris·HCl (pH 7.5), 0.15 M NaCl, scraped off of the glass with a piece of soft plastic, then harvested and washed by centrifugation in the same buffer. The pellet could be stored at −70° or used immediately with no effect upon the subsequently fractionated activity.

Assays for Enzyme Activity. Reaction mixtures (0.1 ml) contained 50 mM Tris·HCl (pH 8.5), 7.5 mM $MgCl_2$, 0.1 M KCl, 0.5 mM dithiothreitol, 0.5 mg/ml of bovine serum albumin (Sigma, Fraction V), 0.13 μmol of "activated" salmon sperm DNA (12), and 5 nmol each of dATP, dGTP, dCTP, and dTTP. One of the triphosphates was labeled with ^3H at 50–100 cpm/pmol. After 30 min at 37°, the reaction mixtures were chilled, then mixed with 0.2 ml of 0.1 M $Na_4P_2O_7$ and 0.7 ml of 10% trichloroacetic acid. After at least 5 min, 3 ml of 1 M HCl, 0.1 M $Na_4P_2O_7$ were added. The contents were filtered through a Whatman GF/C glass filter that had been soaked in 0.1 M $Na_4P_2O_7$, then washed with the HCl-$Na_4P_2O_7$. The filter was then successively washed 12 times with 3 ml of the HCl-$Na_4P_2O_7$ and then with ethanol and dried under a heat lamp. Radioactivity was measured in 2 ml of toluene scintillator fluid. One unit of enzyme incorporates 1 nmol of total nucleotide in 30 min at 37°. The assay was linear with fraction I up to about 1 unit, whereas it was linear to about 0.2 unit of fraction II due to the presence of inhibitor(s). Early fractions give a nonlinear response, and less than 0.1 unit is generally used.

Other Materials. Synthetic polymers and unlabeled nucleotides were from P-L Biochemicals. Radioactive nucleotides were from Amersham.

ml of 1 M
ere filtered
soaked in
. The filter
f the HCl-
heat lamp.
llator fluid.
cleotide in
up to about
tion II due
nonlinear

Trifecta

Table 5.2 Comparison of old and young DNA polymerase capacity

DNA polymerase	ion	bases polymerized	error rate
young	Mg^{2+}	17,300	1 in 1821 bases
old	Mg^{2+}	5,400	1 in 474 bases
young	Mn^{2+}	26,800	1 in 1848 bases
old	Mn^{2+}	18,800	1 in 556 bases

from Linn *et al.*, 1976

Table 5.2

DNA Polymerase Fidelity

Does age affect accuracy of DNA polymerase?

Table 5.2 Comparison of old and young DNA polymerase capacity

DNA polymerase	ion	bases polymerized	error rate
young	Mg^{2+}	17,300	1 in 1821 bases
old	Mg^{2+}	5,400	1 in 474 bases
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Table 5.2

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DNA Polymerase Fidelity

Table 5.2 Comparison of old and young DNA polymerase capacity

DNA polymerase	ion	bases polymerized	error rate
young	Mg^{2+}	17,300	1 in 1821 bases
old	Mg^{2+}	5,400	1 in 474 bases

young DNA polymerase is faster and more accurate

Table 5.2

from Linn *et al.*, 1976

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DNA Polymerase Fidelity

Table 5.2 Comparison of old and young DNA polymerase capacity

DNA polymerase	ion	bases polymerized	error rate
young	Mn^{2+}	26,800	1 in 1848 bases
old	Mn^{2+}	18,800	1 in 556 bases

more productive with Mn^{2+} than Mg^{2+}, similar error rates

Table 5.2

from Linn *et al.*, 1976

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DNA Polymerase Makes Errors

Table 5.2 Comparison of old and young DNA polymerase capacity

DNA polymerase	ion	bases polymerized	error rate
young	Mg^{2+}	17,300	1 in 1821 bases
old	Mg^{2+}	5,400	1 in 474 bases
young	Mn^{2+}	26,800	1 in 1848 bases
old	Mn^{2+}	18,800	1 in 556 bases

from Linn *et al.*, 1976

Table 5.2

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Trifecta

Table 5.3 Comparison of ions on human DNA polymerase capacity.

ions (concentration in mM)	error rate
Mg^{2+} (1.0)	1 in 41,000
Ni^{2+} (1.0)	1 in 5,030
Ni^{2+} (2.0)	1 in 1,850
Cd^{2+} (0.1)	1 in 7,810
Cd^{2+} (0.2)	1 in 5,070
Ca^{2+} (0.6)	1 in 7,520
Ca^{2+} (1.0)	1 in 5,500
Ca^{2+} (2.5)	1 in 3,760

from Gita Seal *et al.*, 1979

Table 5.3

Changes in DNA pol. Error Rate

Table 5.3 Comparison of ions on human DNA polymerase capacity.

ions (concentration in mM)	error rate
Mg^{2+} (1.0)	1 in 41,000
Ni^{2+} (1.0)	1 in 5,030
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from Seal *et al.*, 1979

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from Seal *et al.*, 1979

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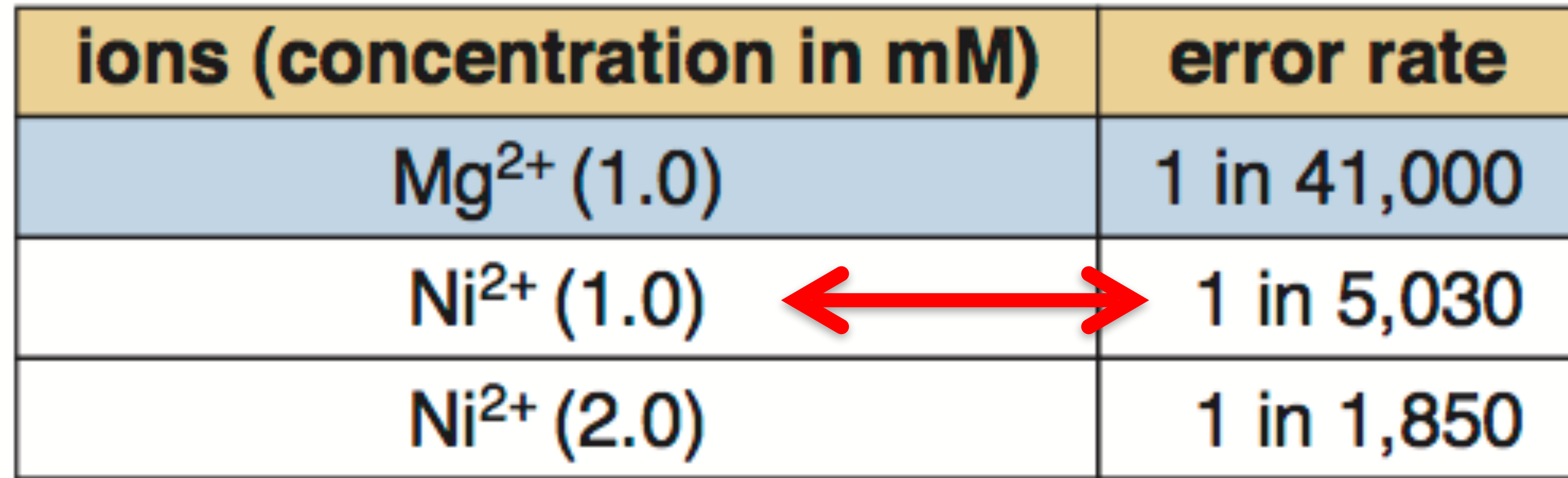
Changes in DNA pol. Error Rate



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Changes in DNA pol. Error Rate

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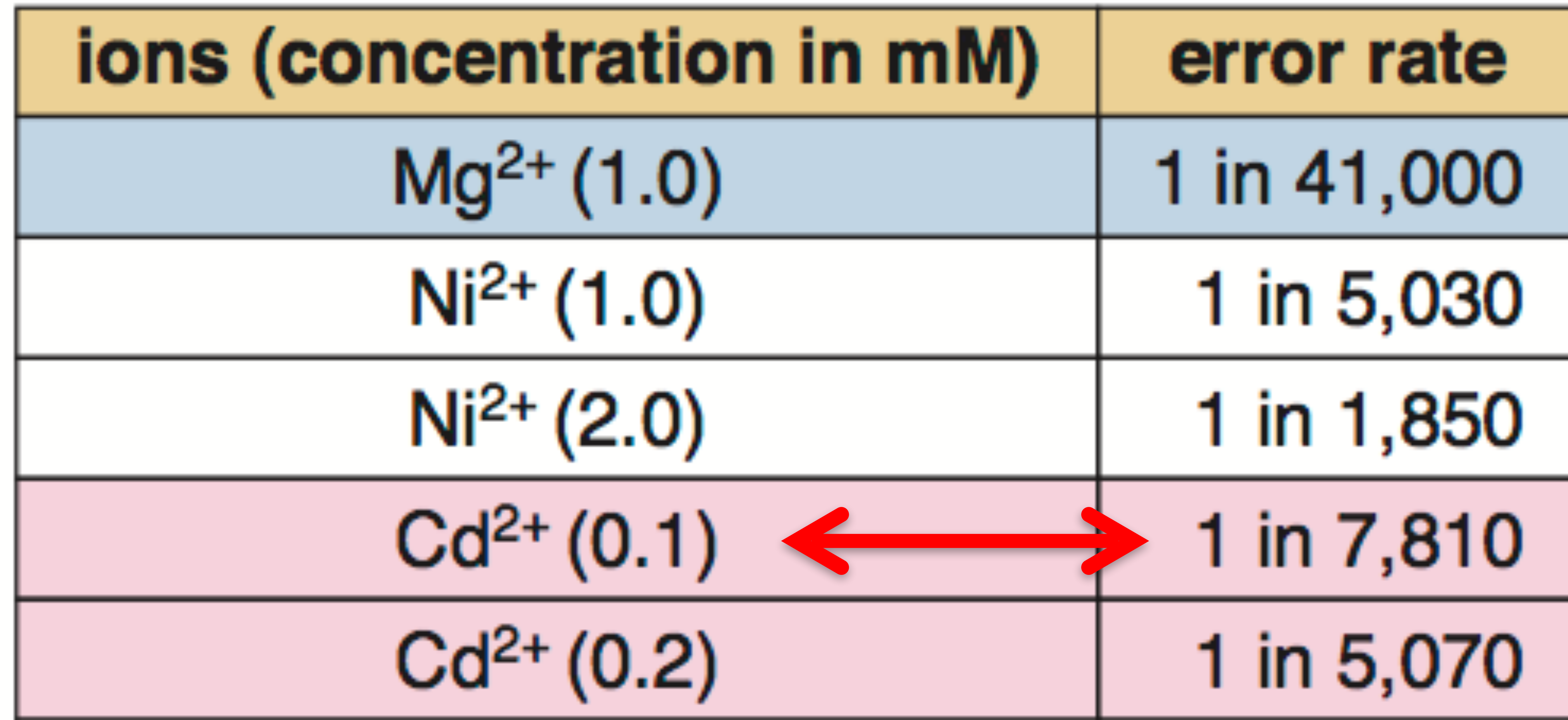

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How does this connect to our course?

Table 5.3 Comparison of ions on human DNA polymerase capacity.

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THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Methods

Step 1: Cell Homogenization and Extraction—Normal human placentas were obtained immediately after birth and were placed in ice-cold 0.15 M KCl. After removal of covering membranes and visible connective tissue, the placentas were drained of blood, weighed, and homogenized in a Waring Blendor (4-liter capacity cooled with circulating ice water) for 10 min in Buffer A (50 mM Tris-HCl, pH 7.8, 4 mM β-mercaptoethanol, 0.5% Triton X-100, and 0.2 M KCl). The ratio of buffer to placenta was 4:1 (v/w). The homogenate was centrifuged for 60 min at 17,000 rpm using a No. 19 rotor. The pellet was resuspended, extracted using 4 volumes of Buffer A, and centrifuged a second time. The supernatants from the two extractions were combined and glycerol was added to a final concentration of 10% (v/v).

Assays were incubated at 37°C for 30 min. Incorporation of the radioactive deoxynucleotides into an acid-insoluble precipitate was determined after precipitating the polynucleotides with 1.0 N perchloric acid and solubilizing with 0.2 N NaOH three times as previously described (14). All reactions were carried out in triplicate and the incorporation of the [^{3}H]dGTP in the absence of enzyme or incubation, typically less than 0.002 pmol, was subtracted from each sample.

Exam sequence

- **Exam I: multiple-select [lower level knowledge]**
- **Exam II: long form essay [higher Bloom scale]**
- **Verbal Final: verbal format [highest Bloom]**

[Traditional Final: multiple-choice format]

Verbal Final Exam

page 34

1. **Draw and Explain Light Reactions**: *"Take your time and draw an illustration of the photosystems and carriers etc important for light reactions. This illustration is so you have something to point at when you explain light reactions to me."*

When you are done explaining light reactions I will ask you questions. First I'll ask about stuff you said that didn't quite make sense to me, then questions like these: What is an absorption spectrum vs action spectrum? Why does a pigment prefer certain colors of light? How are electrons and orbitals involved? Where are we in the cell/leaf? If we had 100 protons in the stroma and 200 in the lumen, how much ATP can we make?

2. **Draw and Explain Calvin Cycle**: "*Take your time and draw an illustration of the Calvin Cycle. This illustration is so you have something to point at when you explain light-independent reactions of photosynthesis to me."*

When you are done explaining the reactions I will again ask questions. First I'll ask about stuff you said that didn't quite make sense to me or forgot to include [like enzyme names], then questions like these: What does PGA taste like? What are the names of the phases and why? What if we only fixed 1 CO_2 molecule, how would that change things?

Transition: OK, let's pretend the glucose you just made in the Calvin Cycle turns into a donut. Preferably a warm Krispy Kreme original glazed donut. Let's eat it.

3. **Draw** {if you'd like} **and Explain Digestion & Absorption**: "*Eat the donut and explain how digestion works in one organ* (I'll pick the organ, you explain function, cells, enzymes, hormones etc) *and then explain absorption at the epithelial villus cell*."

When you are done explaining digestion or absorption I will again ask questions. First I'll ask about stuff you said that didn't quite make sense or you forgot to include [like enzyme & hormone names] then questions like these: Draw a parietal cell and explain how it makes HCl and the same for the villus cell and how it absorbs glucose. What would happen if the glucose transporter didn't co-transport Na^{2+}, ie it was just a simple channel? How does the glucose molecule get out of the villus cell and into the capillary?

4. **Draw & Explain the Biosynthesis of a protein** (I'll choose which) **and your disease**. *Draw a pancreatic beta cell [or epithelial cell] and explain how it makes/secretes the*

Verbal Final Exam

page 34

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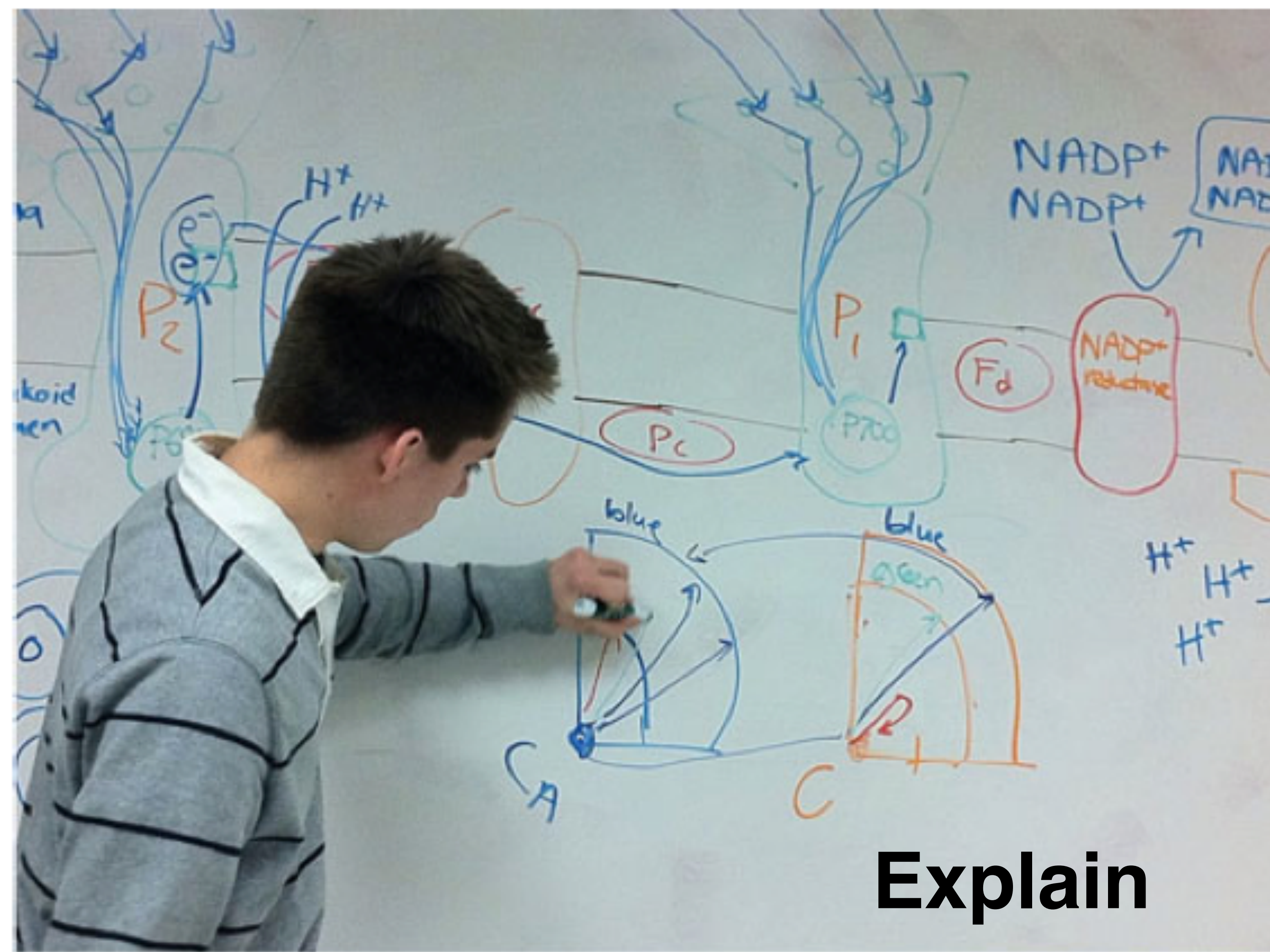
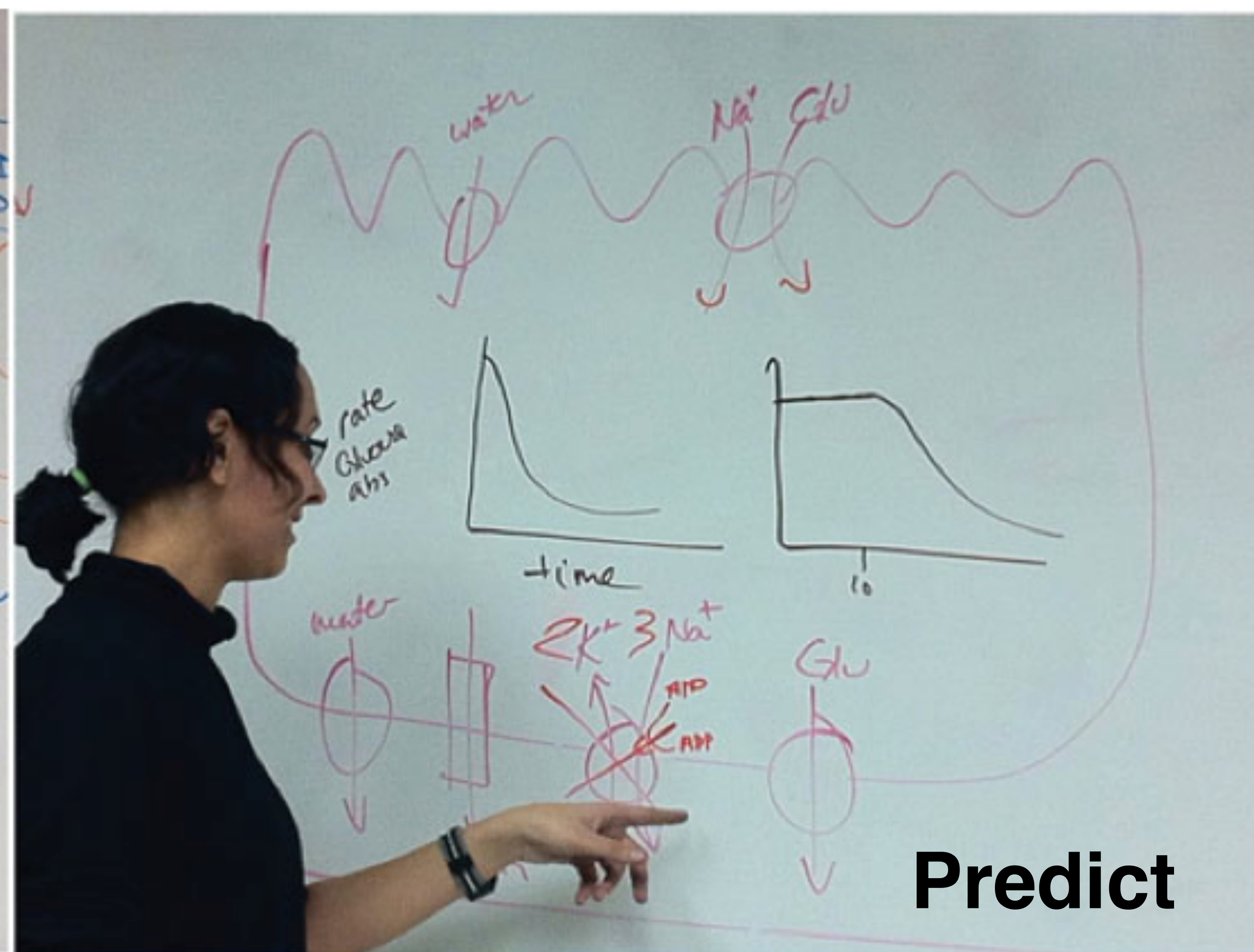
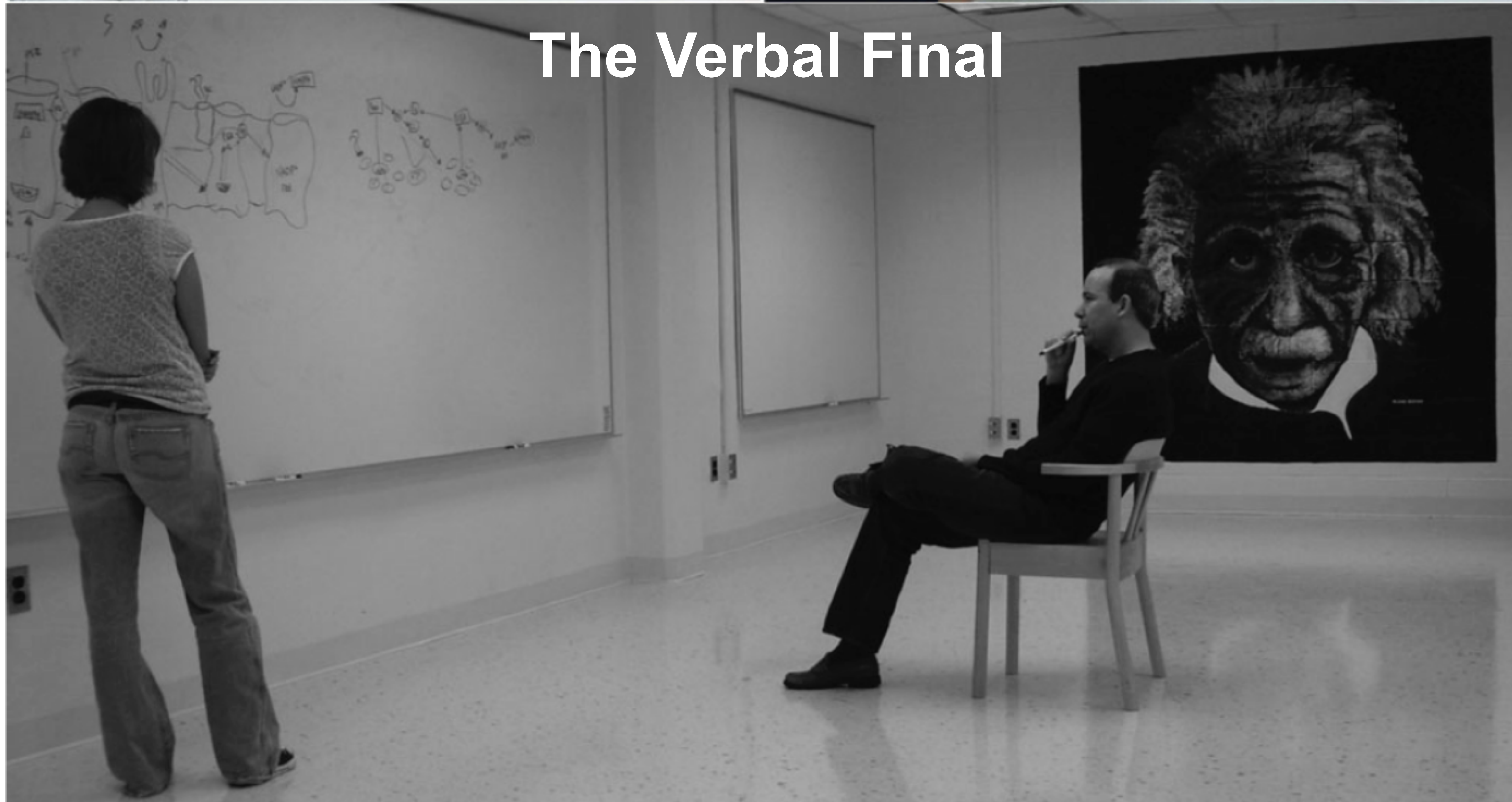
4. **Draw & Explain the Biosynthesis of a protein** (I'll choose which) **and your disease**. *Draw a pancreatic beta cell [or epithelial cell] and explain how it makes/secretes the protein (ie the path DNA -> RNA -> protein -> organelles what they each do and why).*

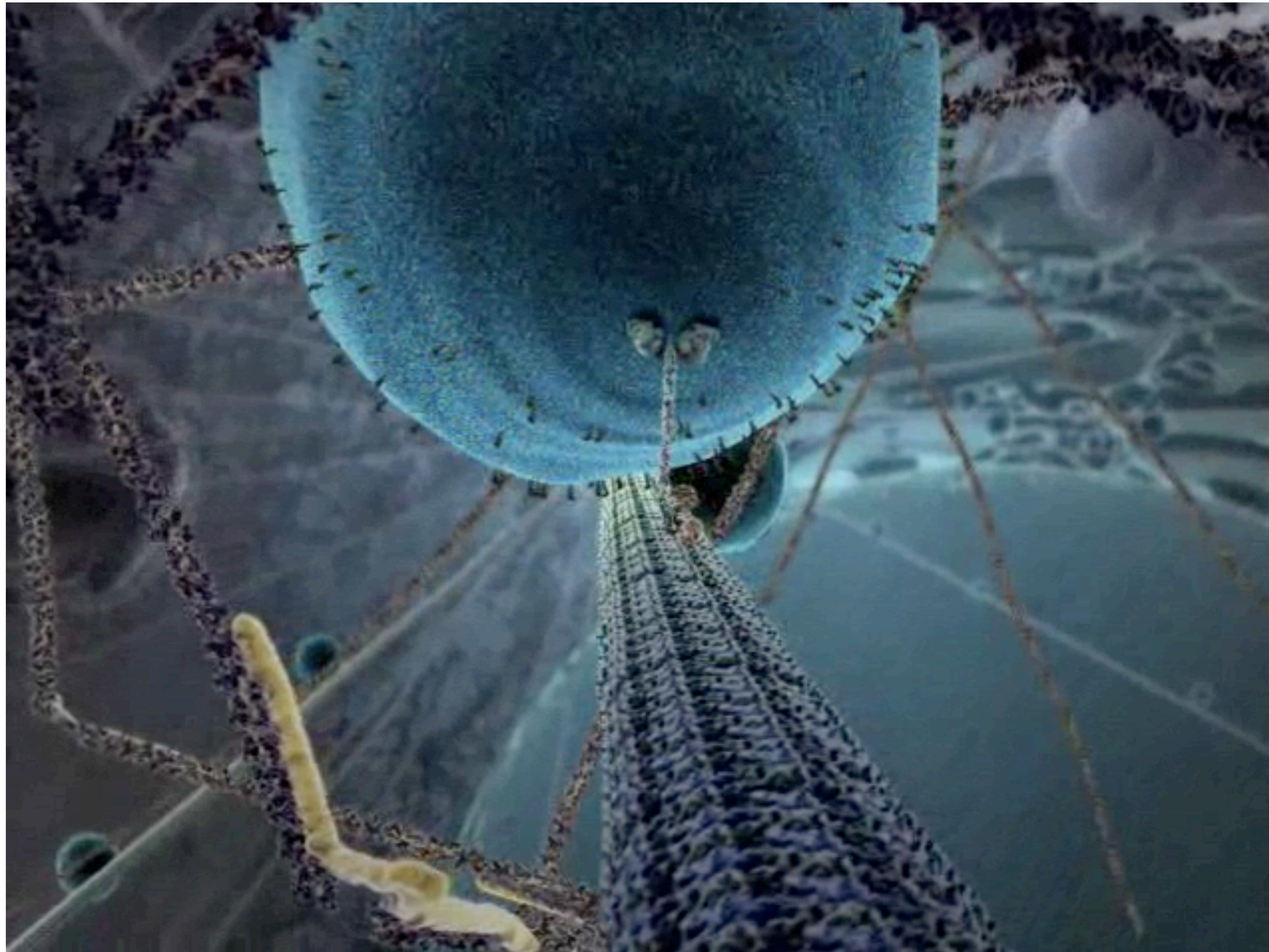
When you are done I'll ask questions, first about stuff you said that didn't quite make sense to me or forgot to include and then stuff like: What is splicing? How does an hnRNA differ from a mRNA? What is an intron? What is your disease and mutation? What do you predict would happen if we add/subtract some hydrophobic domains/parts to the gene you're studying?


Explain


Predict


The Verbal Final

Prepare Paper "Structure" "Function" *Pre-test*



1.
2.
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10.

(Aloud)

Which “class” is your group’s mutation? (explain why to Mom)

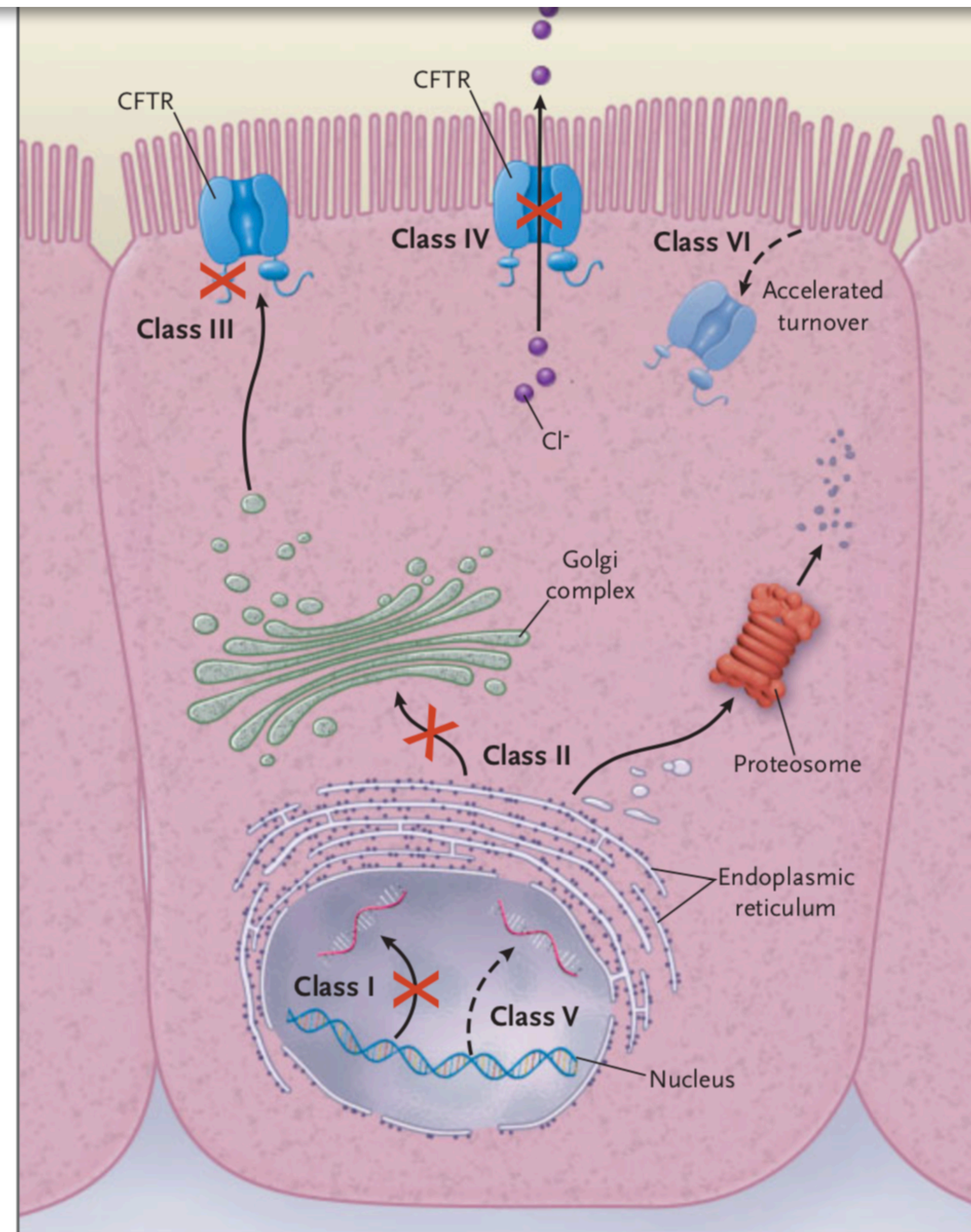

Figure 5. Categories of *CFTR* Mutations.

Classes of defects in the *CFTR* gene include the absence of synthesis (class I); defective protein maturation and premature degradation (class II); disordered regulation, such as diminished ATP binding and hydrolysis (class III); defec-