



WELCOME TO THE MICHAEL SMITH LABORATORIES

YOUR HOSTS: insert names here

THE EXPERIMENT

**DO YOU HAVE AN ALU INSERTION
IN THE GENE TPA-25 (TISSUE PLASMINOGEN
ACTIVATOR) ON CHROMOSOME 8 OF YOUR
GENOME?**

BASICALLY A “DNA” EXPERIMENT.

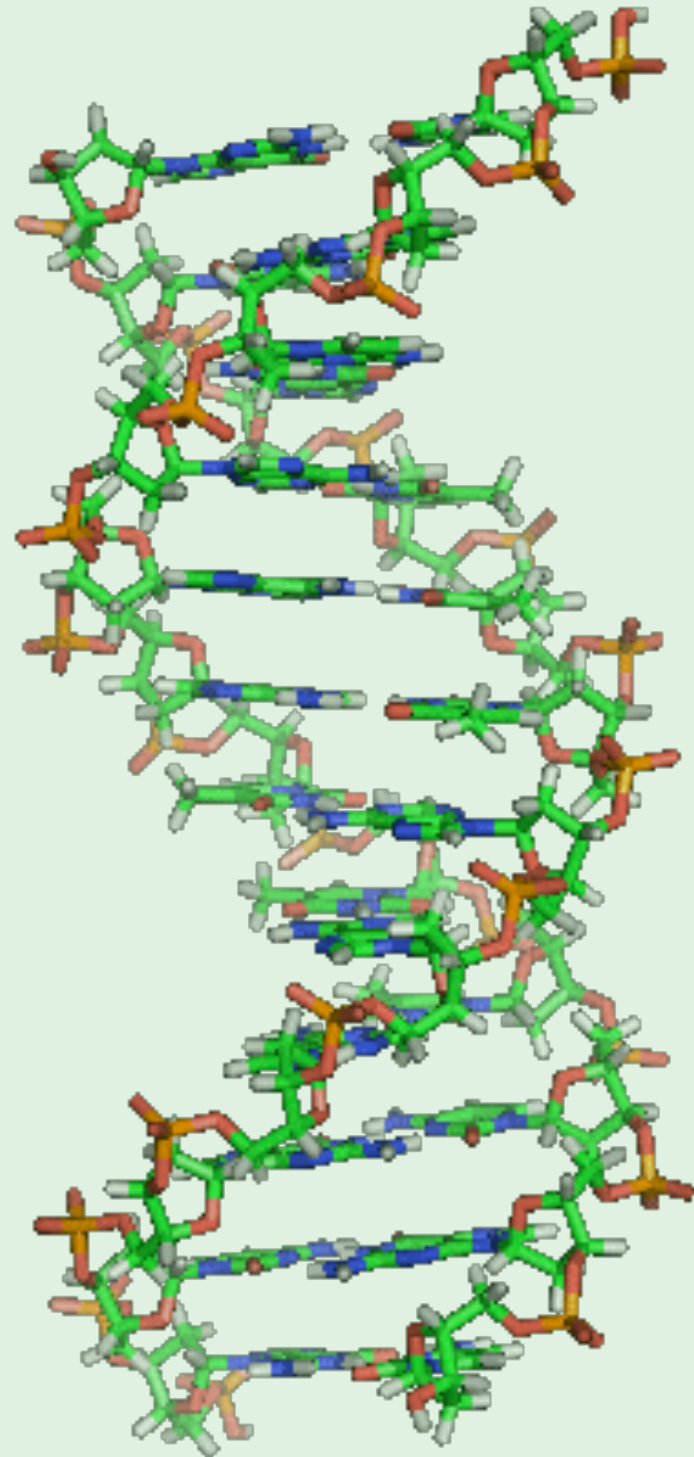
1. What is DNA?

**An instruction manual
or “blueprint.”**

**These instructions are
stored in a long twisted
molecule.**

Called a “double helix.”

**Composed of building
blocks called “nucleotides.”
(A, T, C, G)**

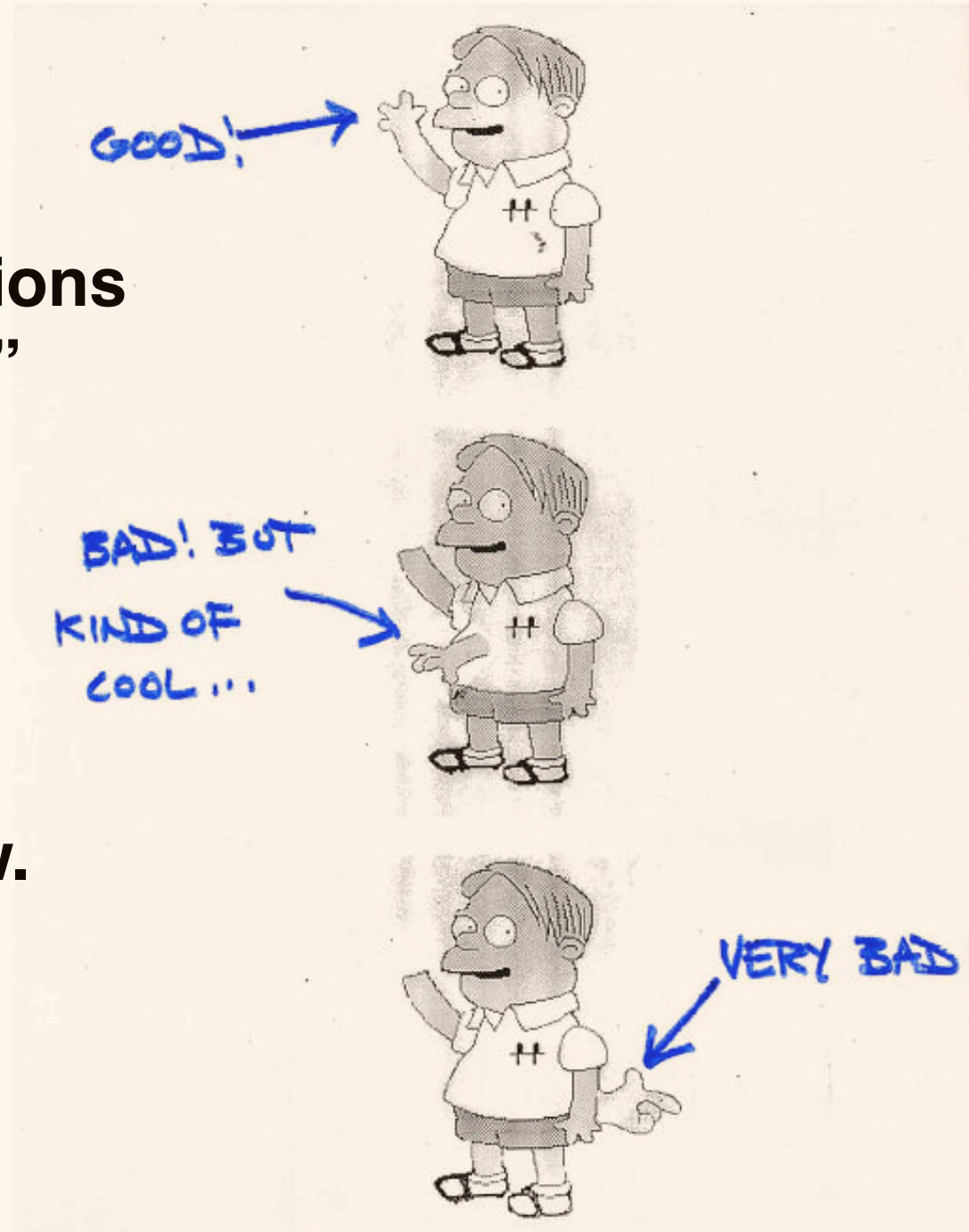


2. What does DNA do?

What do these instructions do? Essentially “build” you.

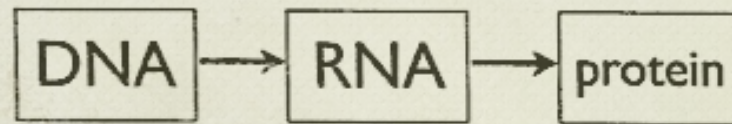
Physically.

As well as from a behaviour point of view.

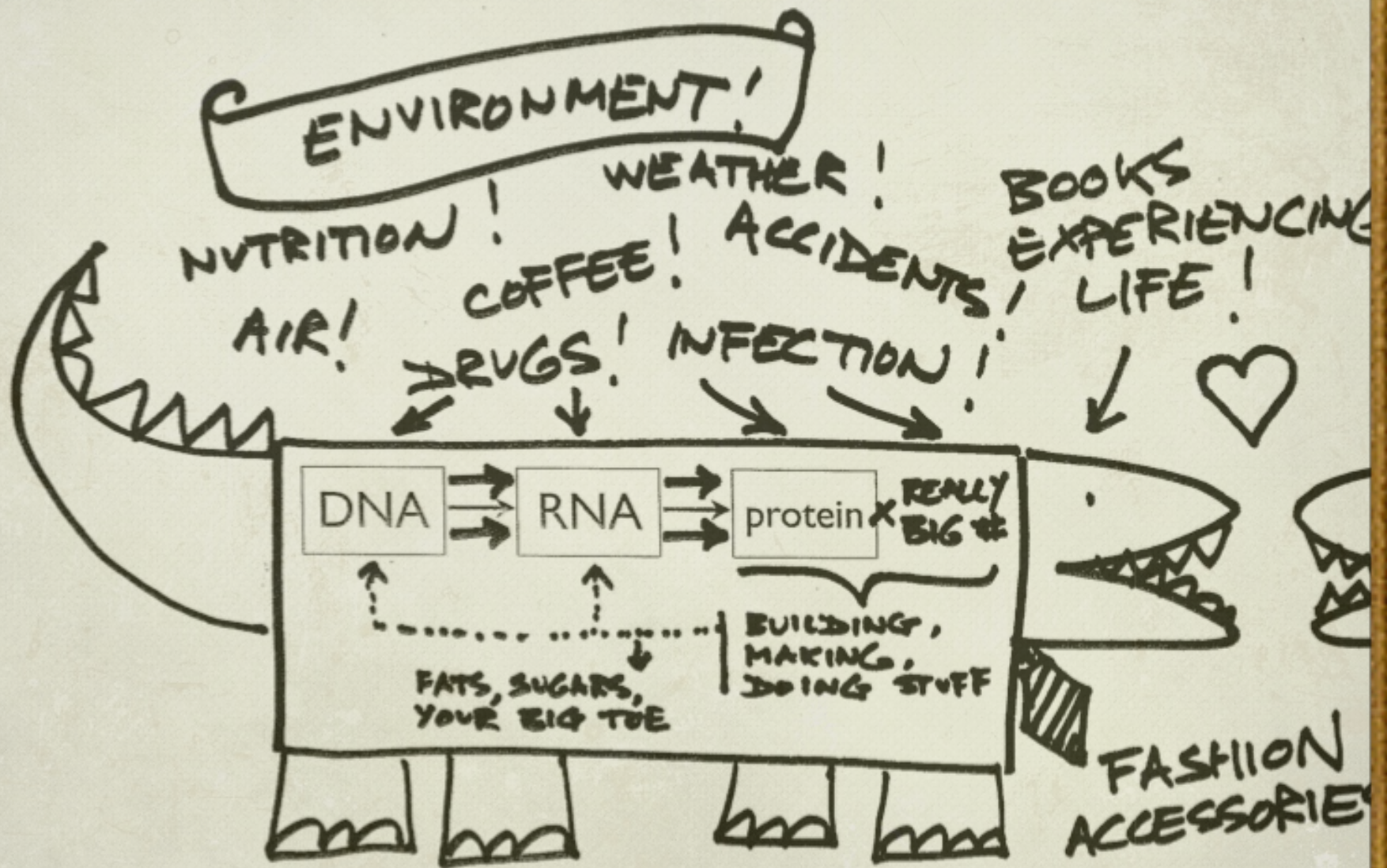


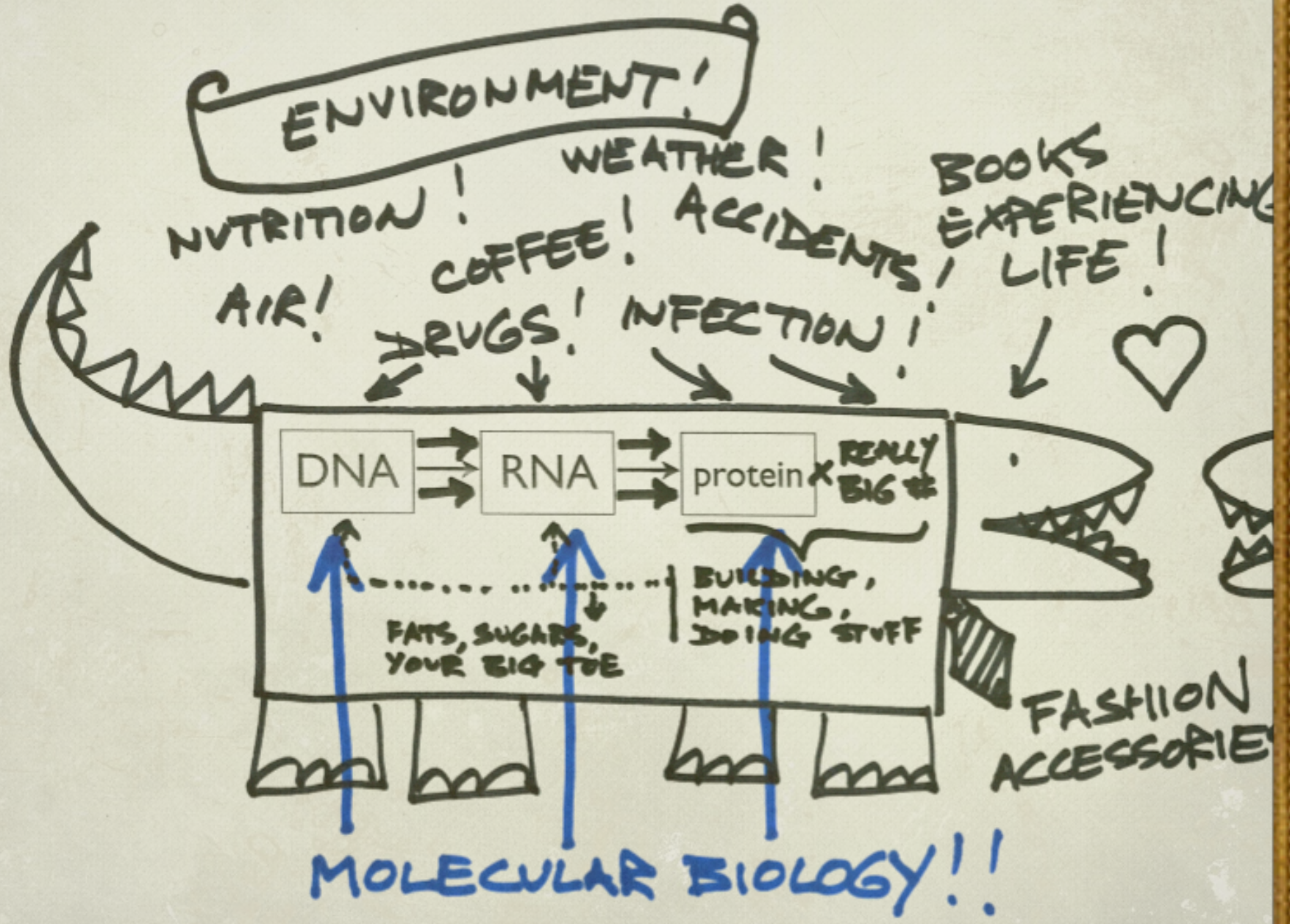
3. How does it do this?

The Central Dogma



Nature versus Nurture?





THE EXPERIMENT

**DO YOU HAVE AN ALU INSERTION
IN THE GENE TPA-25 (TISSUE PLASMINOGEN
ACTIVATOR) ON CHROMOSOME 8 OF YOUR
GENOME?**

BASICALLY A “DNA” EXPERIMENT.

**BASICALLY WE'RE DOING MOLECULAR
BIOLOGY.**

WHAT IS THIS ALU INSERTION?

300bp SEQUENCE IN YOUR GENOME

YOU ACTUALLY HAVE LOTS!

LOOKING FOR ONE IN PARTICULAR

CHROMOSOME 8, TPA-25 LOCI

GENOTYPE? + / + | - / - | + / -

WHAT DOES IT DO?

1. GET CELLS

2. EXTRACT DNA

3. PCR

4. RUN GEL

5. LOOK AT DATA

Cheek rinse using saline. Pellet cells by centrifugation.

Lysis via boiling
Purification via chelex beads + centrifugation

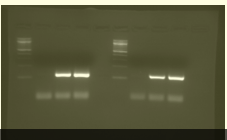
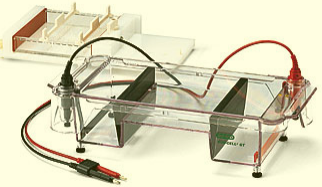
Set up PCR reactions. Allow to run over lunch

Load PCR reactions on “gel”
Apply current to gel.

What’s your genotype?

morning

afternoon



PICK UP ONE EACH



saline
pod



10ml
tube



paper
cup

WAIT FOR INSTRUCTOR BEFORE MOVING AHEAD

1.



- Remove tab.
- Squeeze saline into mouth.

2.



- Swish around cheek area for about 30sec

3.



- Spit “spit” into paper cup.

4.



- Pour spit into plastic tube.
- Throw cup away.
- Hang on to “your” tube.



LAB RULES

1. FOLLOW INSTRUCTIONS - **SAFETY FIRST**
 - a) EQUIPMENT
 - b) REAGENTS
 - c) PROTOCOL
2. NO EATING OR DRINKING IN LAB
3. NO GOOFING ON THE CHAIRS
4. IF YOU'RE NOT SURE, ASK SOMEONE

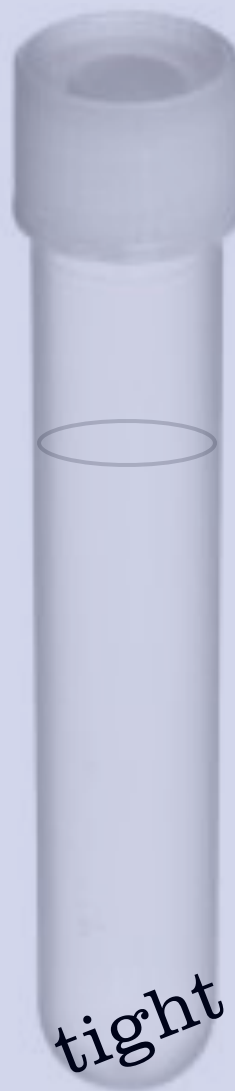
LABEL TUBE WITH PERMANENT MARKER LOAD YOUR “SPIT” TUBE INTO CENTRIFUGE

**CENTRIFUGATION
SEPARATES ON THE
BASIS OF DENSITY**

**YOU WANT A CELL
PELLET**

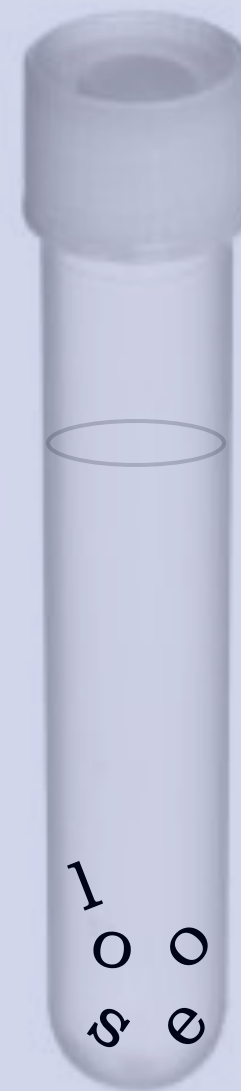
**1500 RPM
10 MINUTES**

**2 POSSIBILITIES
TIGHT VS LOOSE
PELLET**



tight pellet
- simply pour
off supernatant
into fresh paper
cup.

loose pellet
- TAs will help
transfer to a
microcentrifuge
tube and respin.



WITH YOUR PELLETT IN EITHER THE LARGE OR MICROCENTRIFUGE TUBE

use plastic pipette
to transfer pellet to
chelex beads

try to get most of the
pellet with minimal
fluid carry over



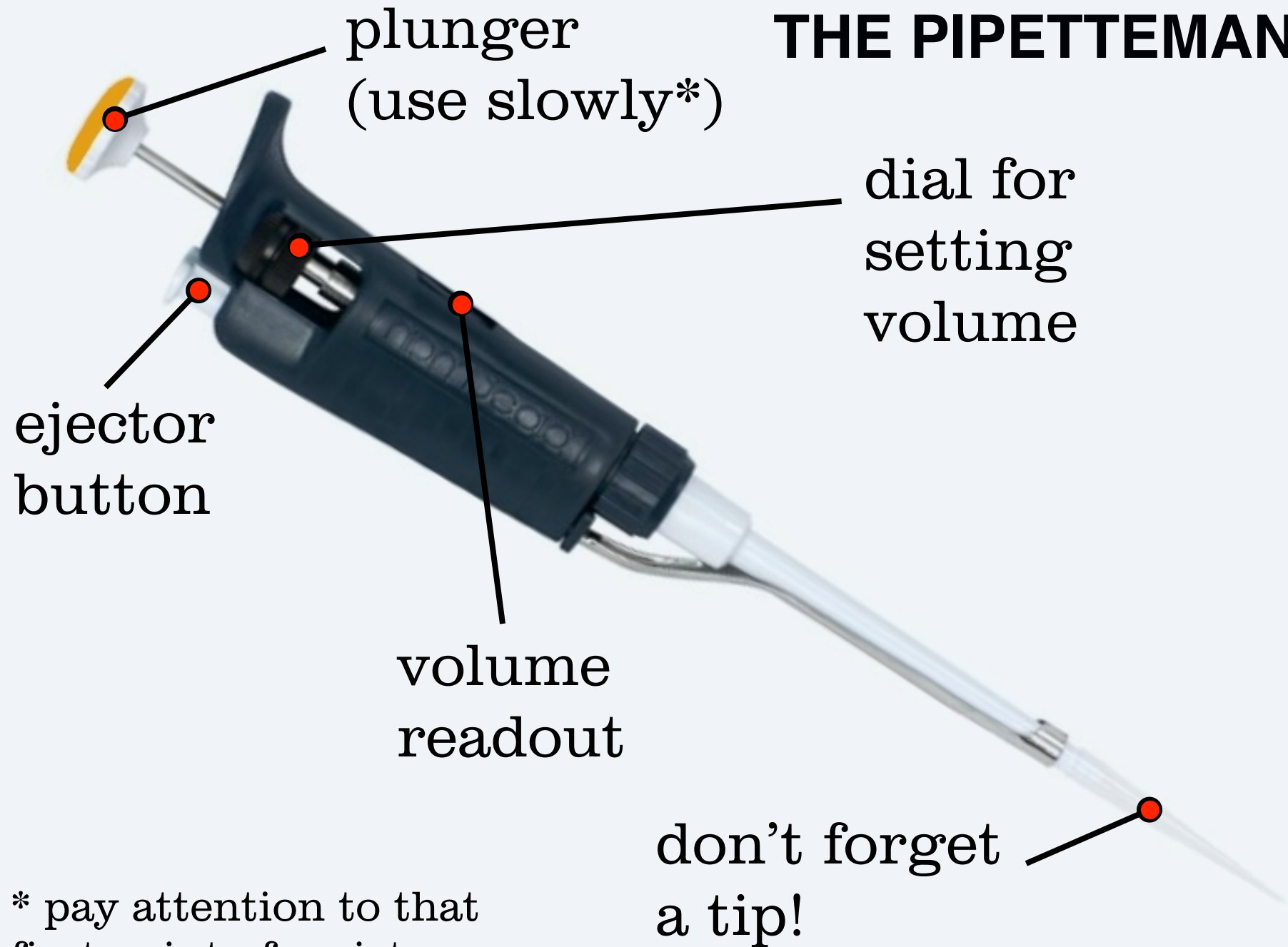
remove sticker
and relabel directly
on the top of the tube

**VORTEX 10 SECONDS
ADD LID LOCK**

**PLACE ON
FLOATING RACK
(CENTER CART)**

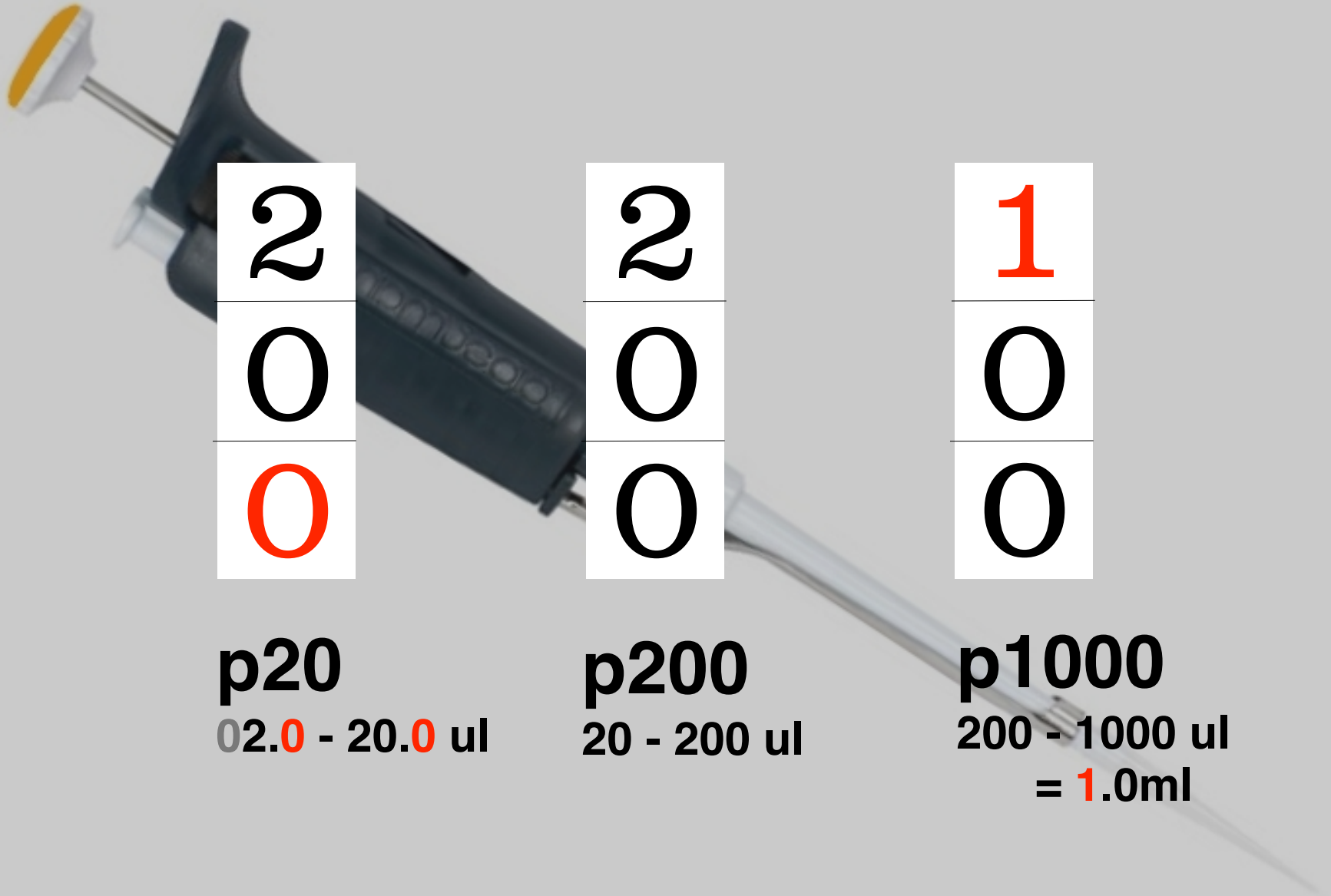
BOIL 10 MINUTES

THE PIPETTEMAN



* pay attention to that first point of resistance.

THE PIPETTEMAN



2
0
0

p20

02.0 - 20.0 ul

2
0
0

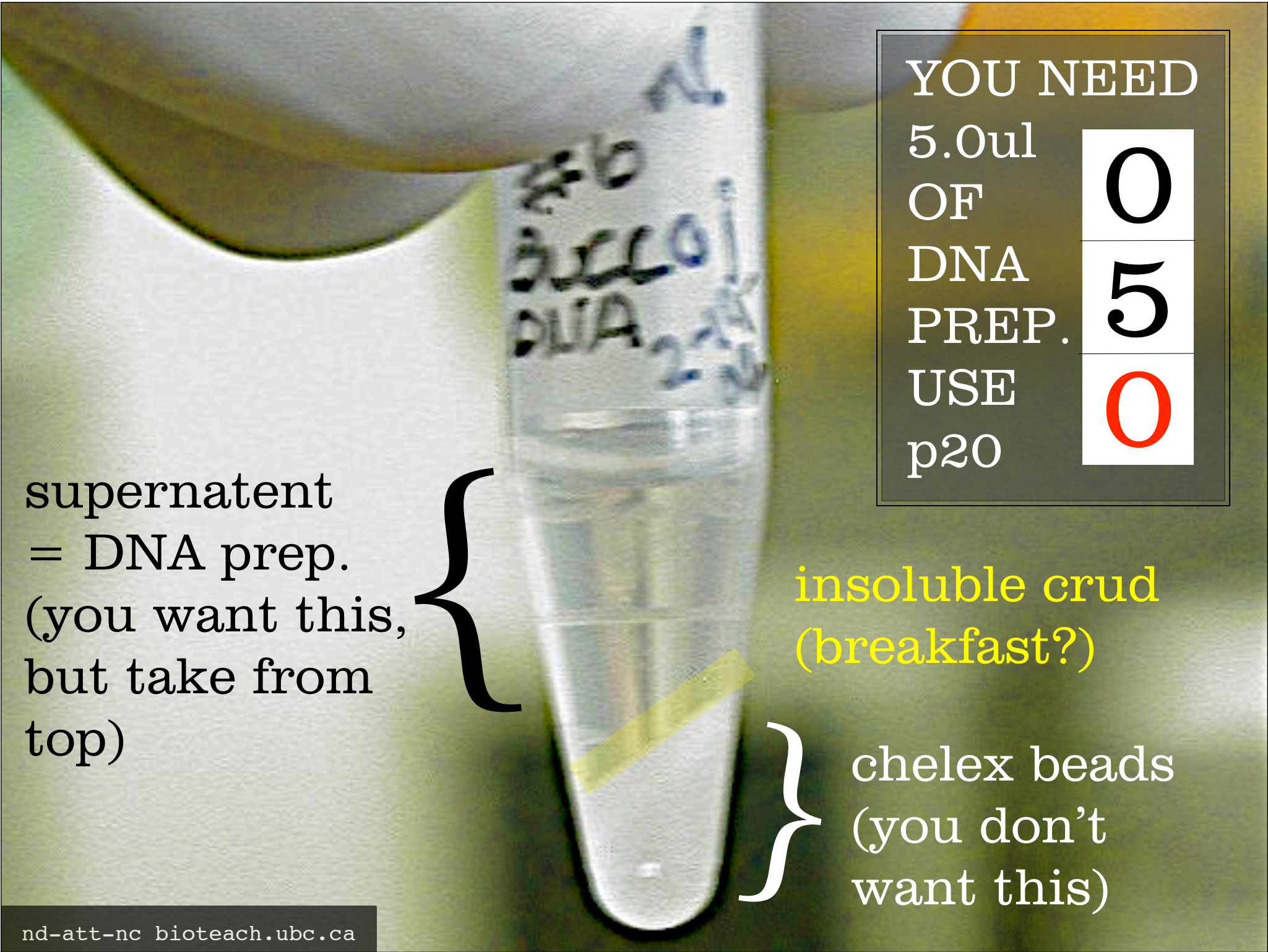
p200

20 - 200 ul

1
0
0

p1000

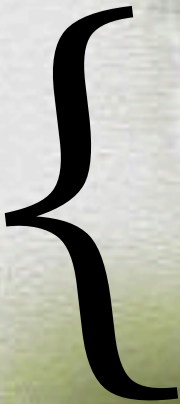
200 - 1000 ul
= 1.0ml



YOU NEED
 5.0ul
 OF
 DNA
 PREP.
 USE
 p20

0
 5
 0

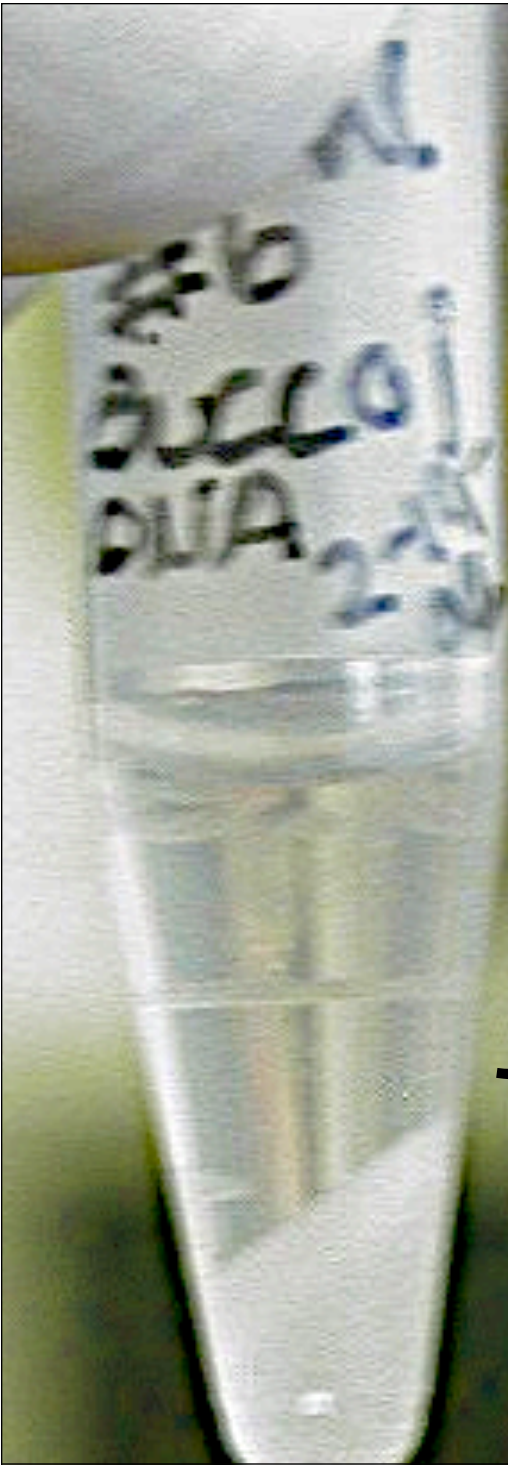
supernatant
 = DNA prep.
 (you want this,
 but take from
 top)



insoluble crud
 (breakfast?)

chelex beads
 (you don't
 want this)





YOU NEED

5.0ul

OF

DNA

PREP.

USE

p20

0

5

0

} supernatant
= DNA prep.
(you want this,
but take from
top)

Load 5ul
into small
PCR rxn
tube (on
yellow
rack)



Label tube, and
return to the yellow
rack - TAs will load
the PCR machine



4. Why molecular biology?

Things like DNA are a common thread connecting all life forms.



5. Using model systems.

Human 8 3
Homo sapiens



   Animalia, Chordata, Mammalia


3 POINTS




Play: *Homo sapien* has a **MOVE** of 2. Although an omnivore, this card can only be played next to **SPECIES CARD** that represent food that humans would actually eat.

Image by *Alexandria Neonakis*
alexneonakis.com/

**Cold, Cool,
Warm, Hot**

Escherichia coli 2 1
Escherichia coli



   Bacteria, Proteobacteria, Gammaproteob

2 POINTS

- *Escherichia coli* has a **SPREAD** of 1.
- *Escherichia coli* can be placed with any **TERRAIN**.

Image by *environmental-hazard*
environmental-hazard.deviantart.com/

**Cold, Cool,
Warm**

Which is easier to work with?

THIS IS HOW BIG YOUR GENOME IS...

~3,000,000,000!

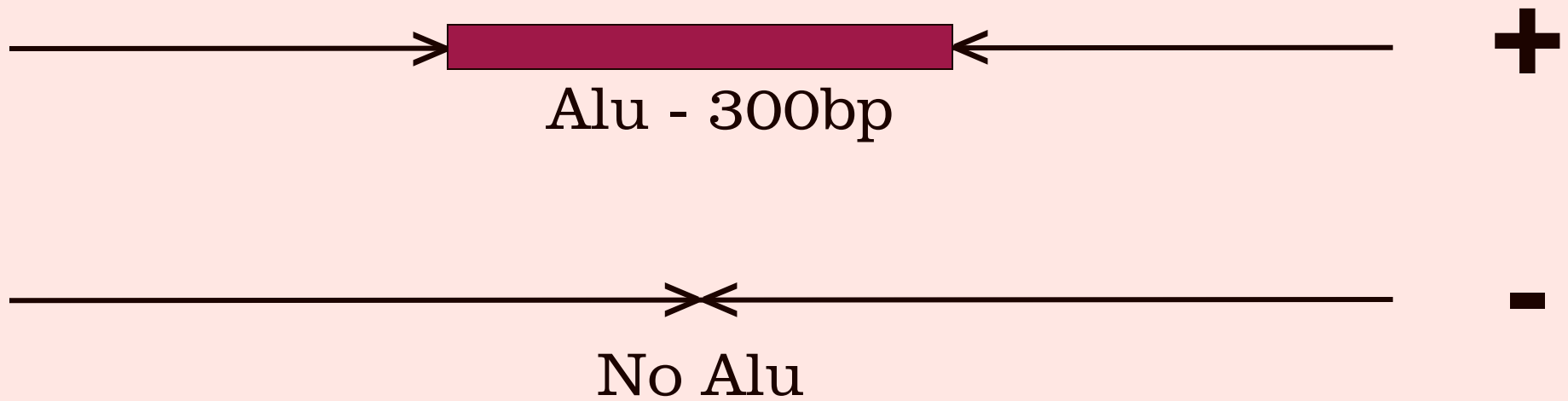
THIS INCLUDES ABOUT ~20,000 GENES

**ONLY A VERY SMALL PROPORTION OF THE
GENOME APPEARS TO BE “USEFUL.”**

ccccgttac caeggcaget agctctctgg agacctccag aggcacctct ggacccccctt ctaccatggt aactgtctct ctggagactt
ccaaaggcac ctctggacct cctgtttacca tggcaactga ctctctggag acctccactg ggaccactgg accccctgtt accatgacaa
ctggctctct ggagccctcc agcggggcca gtggacccca ggtctctagc gtaaaactat ctacaatgat gtctccaacg acctccacca
acgcaagcac tgtgcccttc cggaaaccag atgagaactc acgaggcatg ctgccagtgg ctgtgcttgt ggccctgctg gcggtcatag
tctctgtggc tctgctcctg ctgtggcgcc ggcggcagaa gcggcggact ggggccctcg tgctgagcag aggcggcaag cgtaacgggg
tgggtggacgc ctgggctggg ccagcccagg tccctgagga gggggccgtg acagtgaccg tgggaggggtc cggggggcgac aagggtctg
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ggcccacgct caccactttc tttggcagac ctggctctct ggagccctcc agcggggcca gtggacccca ggtctctagc gtaaaactat
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taacaagtga ccctaaggcc gacagcactg gggaccagac ctcagcccta cctccctcaa cttccatcaa tgagggatcc cctctttgga
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ctgtgcttgt ggccctgctg gcggtcatag tcctcgtggc tctgctcctg ctgtggcgcc ggcggcagaa gcggcggact ggggccctcg
tgctgagcag aggcggcaag cgtaacgggg tgggtggacgc ctgggctggg ccagcccagg tccctgagga gggggccgtg acagtgaccg
tgggaggggtc cggggggcgac aagggtctg ggttccccga tggggagggg tctagccgtc ggcccacgct caccactttc tttggcagac
ctggctctct ggagccctcc agcggggcca gtggacccca ggtctctagc gtaaaactat ctacaatgat gtctccaacg acctccacca
acgcaagcac tgtgcccttc cggaaaccag atgagaactc acgaggcatg ctgccagtgg ctgtgcttgt ggccctgctg gcggtcatag

DO THIS FOR 15minutes

CHROMOSOME 8, TPA-25 LOCI



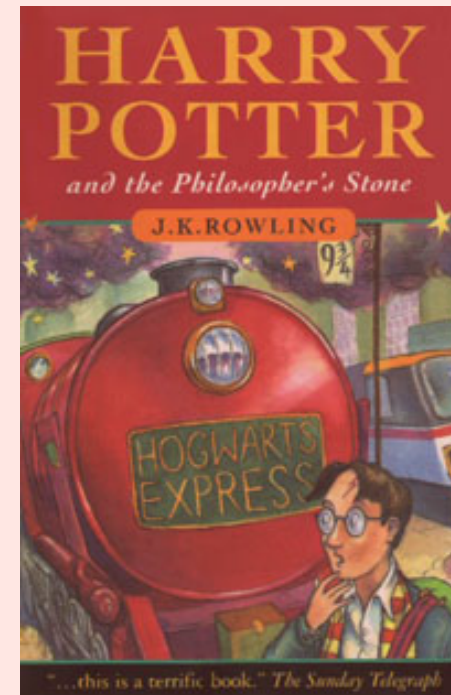
*I wandered lonely as a cloud
That floats on high o'er vales and hills,
When all at once I saw a crowd,
A host, of golden daffodils;
Beside the lake, beneath the trees,
Fluttering and dancing in the breeze.*

*Continuous as the stars that shine
And twinkle on the milky way,
They stretched in never-ending line
Along the margin of a bay:
Ten thousand saw I at a glance,
Tossing their heads in sprightly dance.*

*The waves beside them danced; but they
Out-did the sparkling waves in glee:
A poet could not but be gay,
In such a jocund company:
I gazed---and gazed---but little thought
What wealth the show to me had brought:*

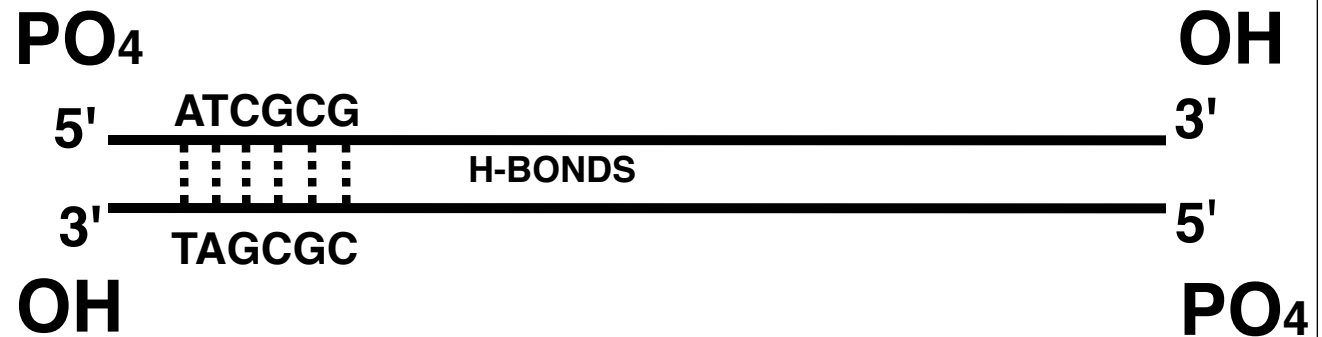
*For oft, when on my couch I lie
In vacant or in pensive mood,
They flash upon that inward eye
Which is the bliss of solitude;
And then my heart with pleasure fills,
And dances with the daffodils.*

William Wordsworth



I'm an athletic mature, independent, 33 yrs. old, with a youthful appearance of 23. I'm a single white male and a working professional in the research industry. I'm 5'10" tall, 175 lbs, with light brown hair and hazel eyes. I'm a non-drinker and non-smoker. I've never been married and have no dependants. Currently seeking a female companion who enjoys the outdoors and understands DNA replication. If interested in finding out more, call Box 1044.

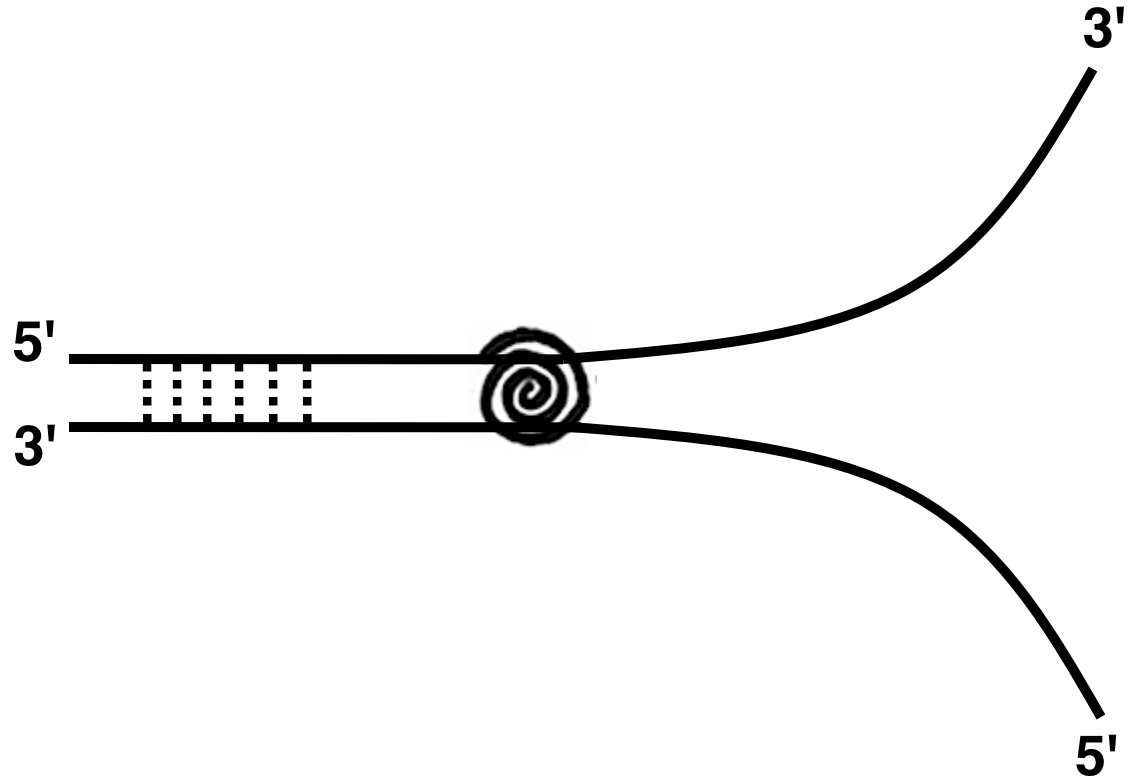
DNA BASICS





HELICASE

DNA REPLICATION

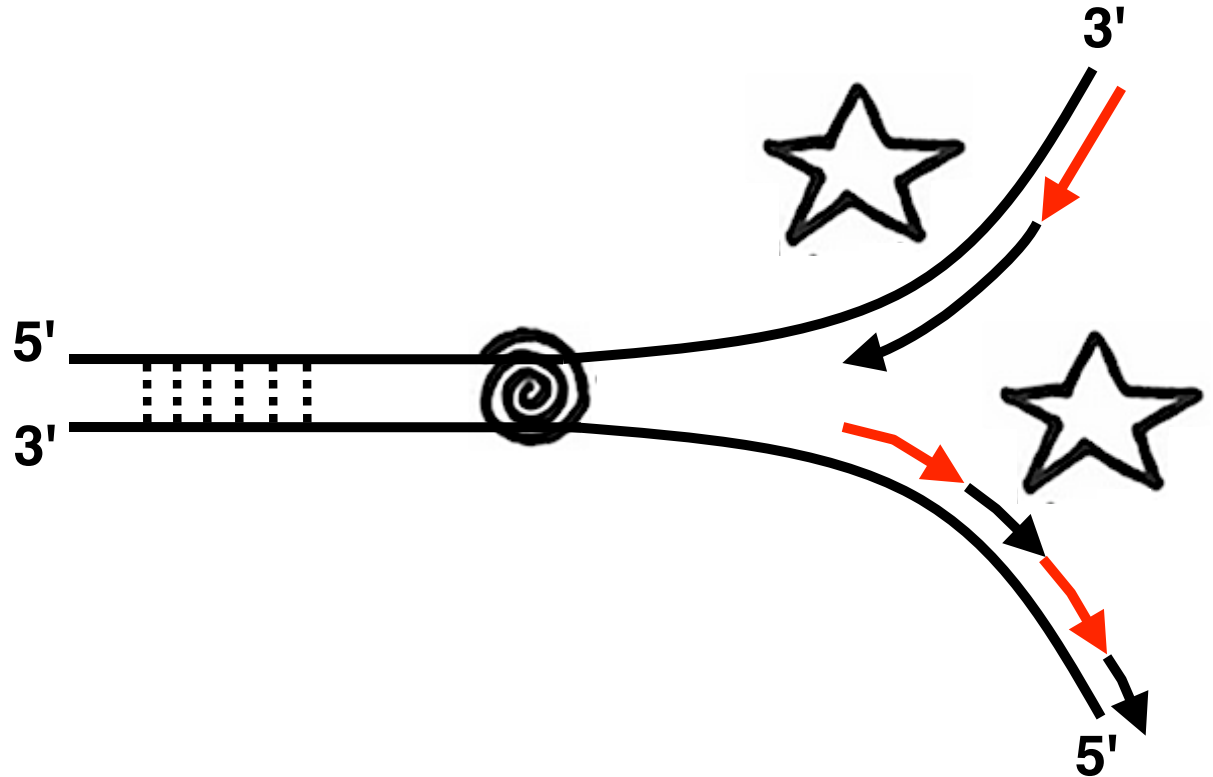


 HELICASE

 DNA POL III

 PRIMASE

DNA REPLICATION



DNA POL RULES:
+ dNTPs to 3' end
Primer + Template

 HELICASE

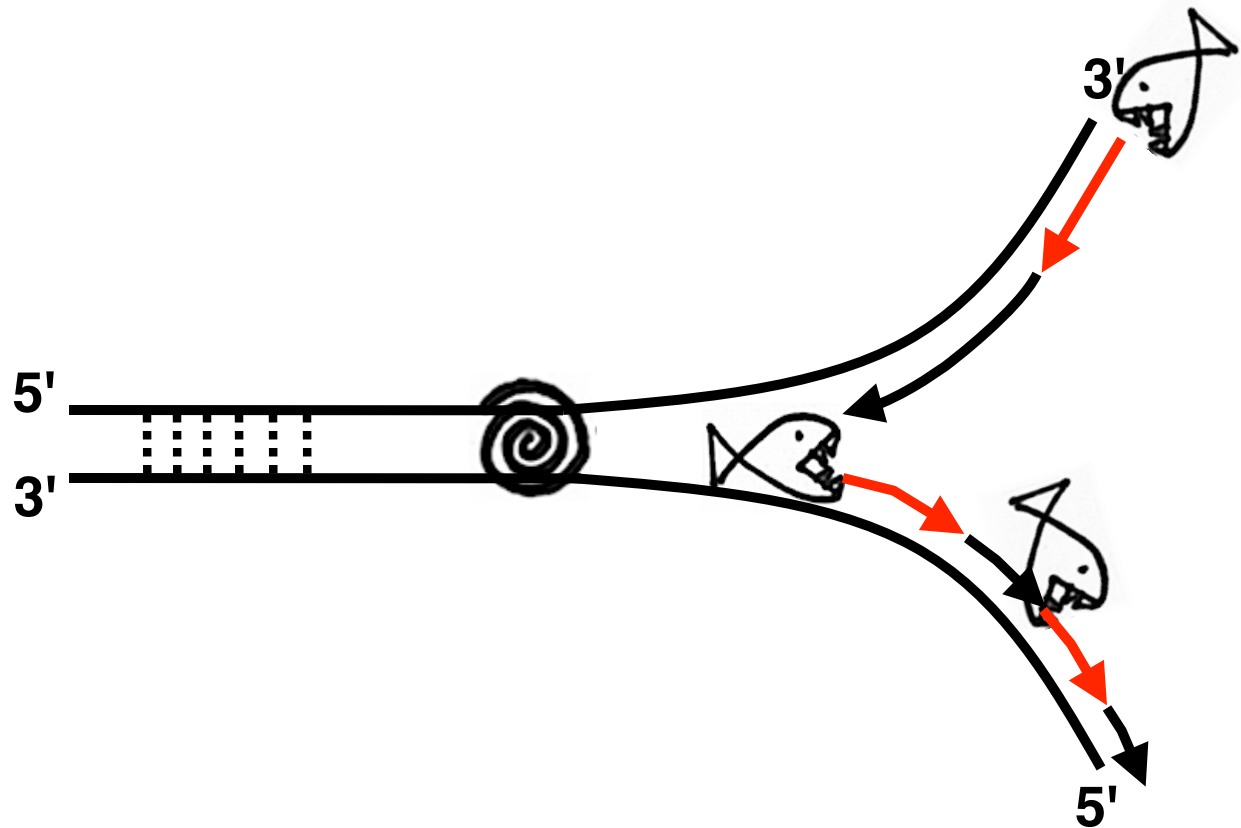
 DNA POL III

 PRIMASE

 DNA POL I

DNA POL RULES:
+ dNTPs to 3' end
Primer + Template

DNA REPLICATION



 HELICASE

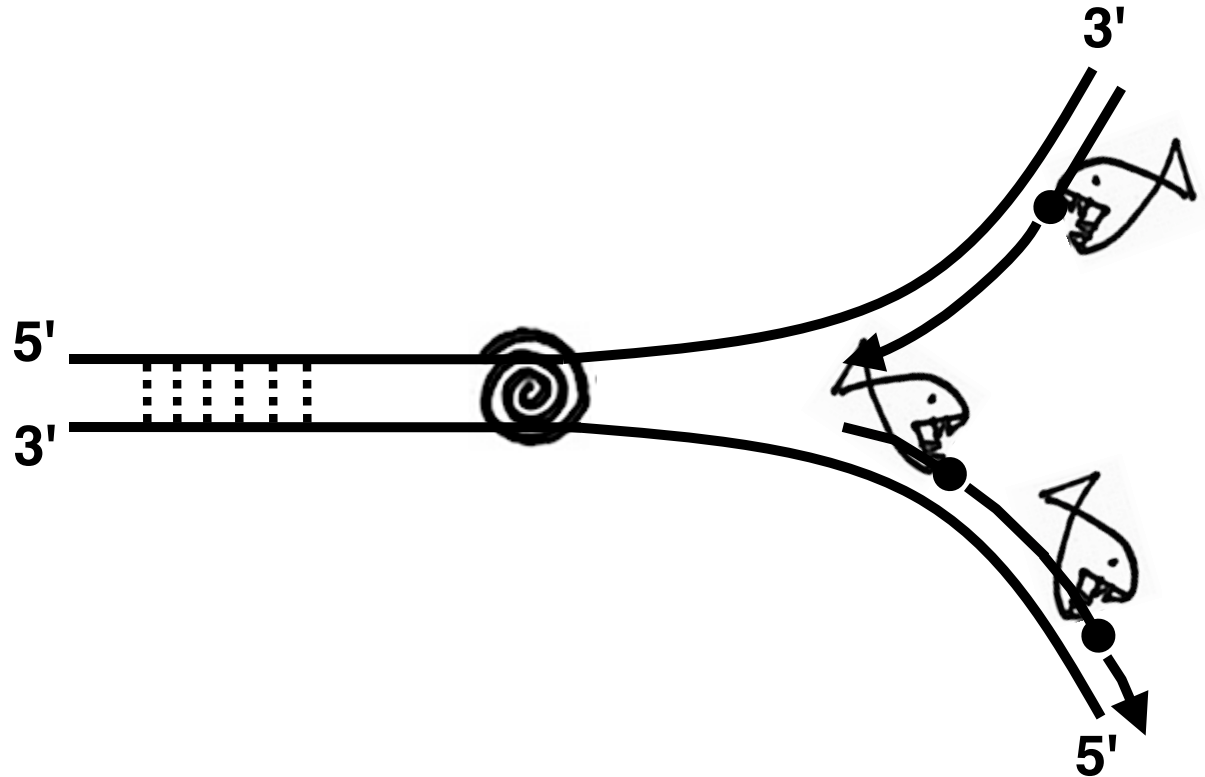
 DNA POL III

 PRIMASE

 DNA POL I

DNA POL RULES:
+ dNTPs to 3' end
Primer + Template

DNA REPLICATION



 HELICASE

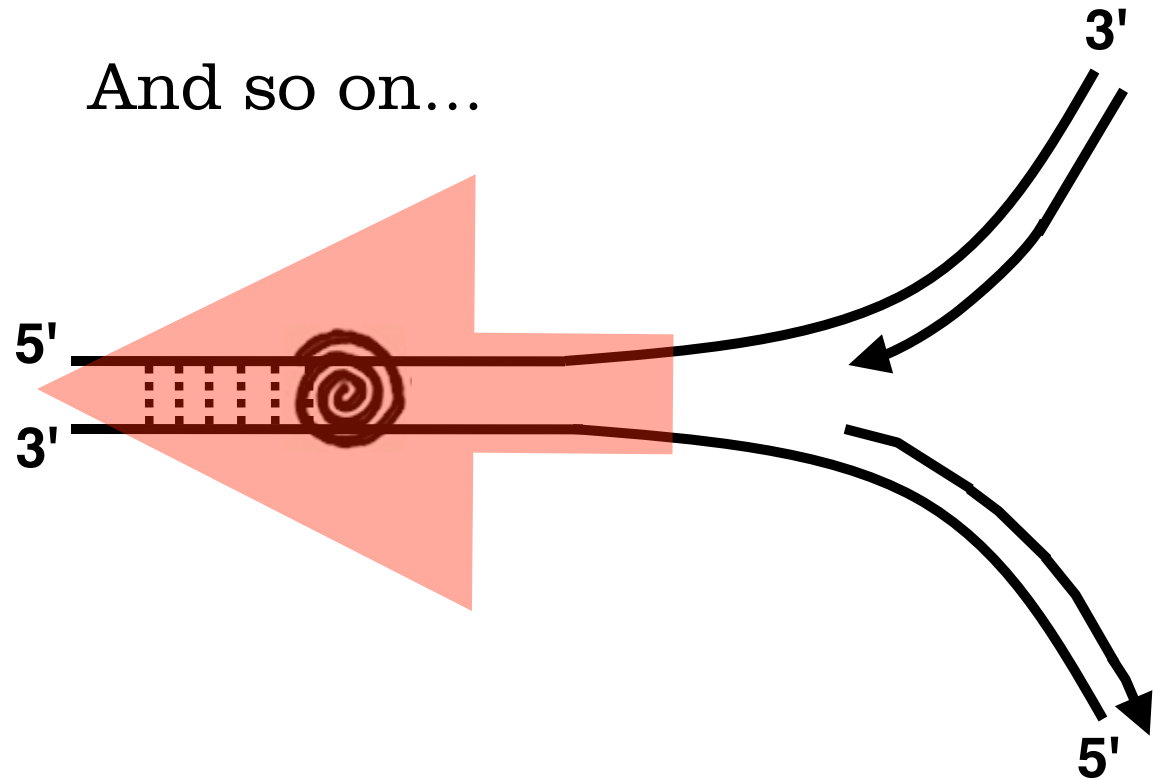
 DNA POL III

 PRIMASE

 DNA POL I

 LIGASE

DNA REPLICATION



Look! Two copies!

 HELICASE

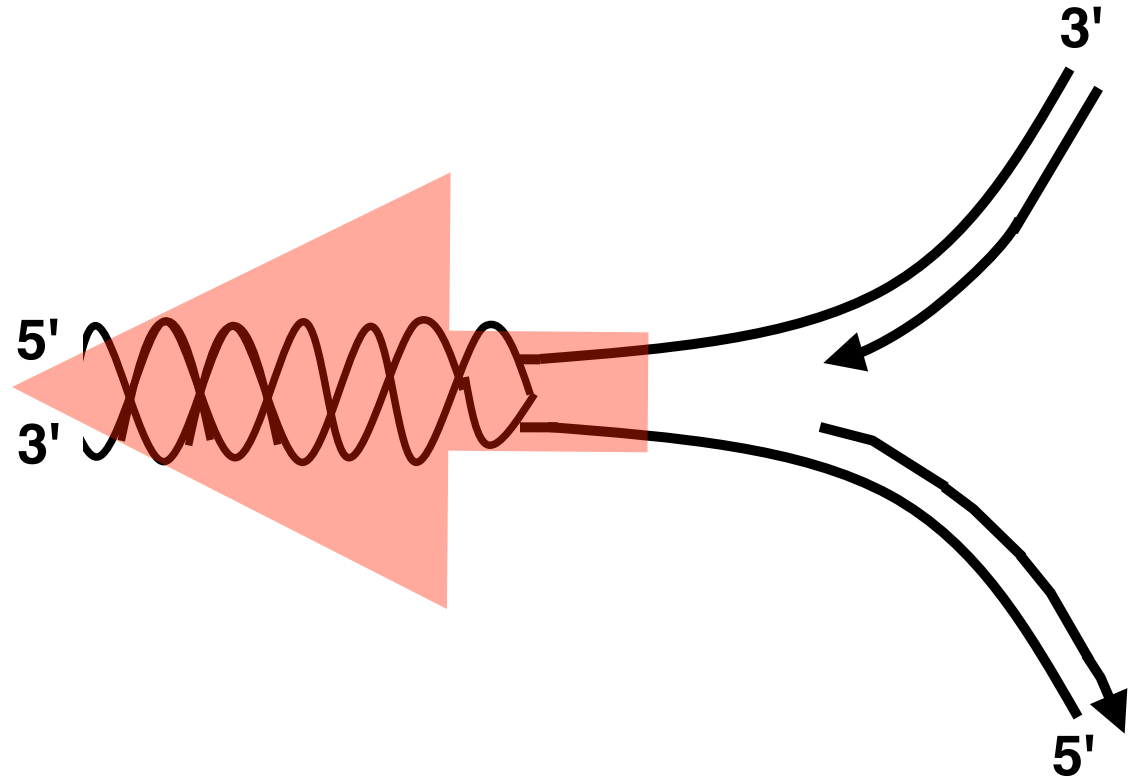
 DNA POL III

 PRIMASE

 DNA POL I

 LIGASE

DNA REPLICATION



 HELICASE

 DNA POL III

 PRIMASE

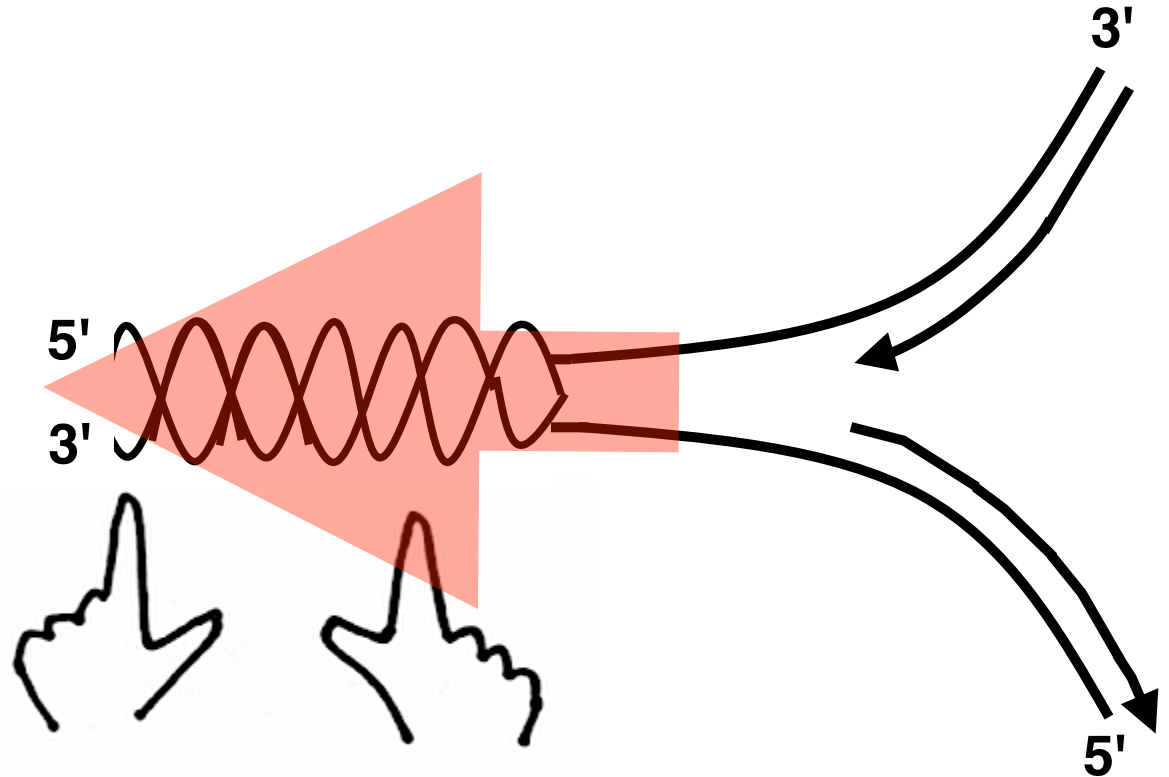
 DNA POL I

 LIGASE



TOPOISOMERASE

DNA REPLICATION





HELICASE

UNWINDS HELIX



DNA POL III

ADD NT's TO 3' (COPIES DNA)



PRIMASE

MAKES RNA PRIMER



DNA POL I

**REMOVES RNA PRIMER
REPLACES WITH DNA**



LIGASE

SEALS GAPS IN DNA



TOPOISOMERASE

RELIEVES STRUCTURAL STRESS

1. GET CELLS

2. EXTRACT DNA

3. PCR

4. RUN GEL

5. LOOK AT DATA

Cheek rinse using saline. Pellet cells by centrifugation.

Lysis via boiling
Purification via chelex beads + centrifugation

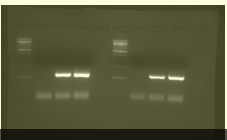
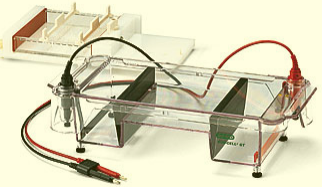
Set up PCR reactions. Allow to run over lunch

Load PCR reactions on “gel”
Apply current to gel.

What’s your genotype?

morning

afternoon



 HELICASE

 DNA POL III

 PRIMASE

 DNA POL I

 LIGASE



TOPOISOMERASE

Polymerase
Chain
Reaction = Replication
in a
test tube.

Can we
simplify
it?

Polymerase Chain Reaction

P.C.R.



Kary Mullis



~~HELICASE~~



DNA POL III



PRIMASE



DNA POL I

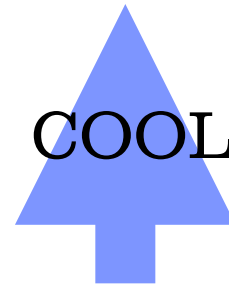
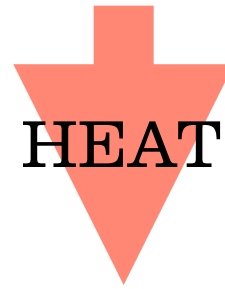
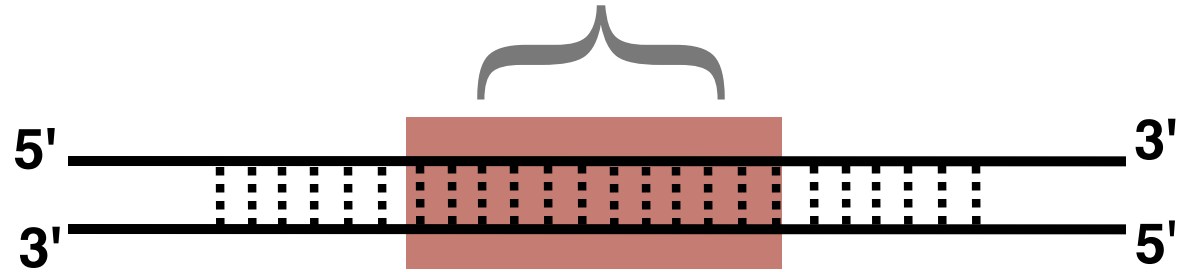


LIGASE



TOPOISOMERASE

DNA that I want to amplify





~~HELICASE~~



DNA POL III



~~PRIMASE~~



DNA POL I



LIGASE

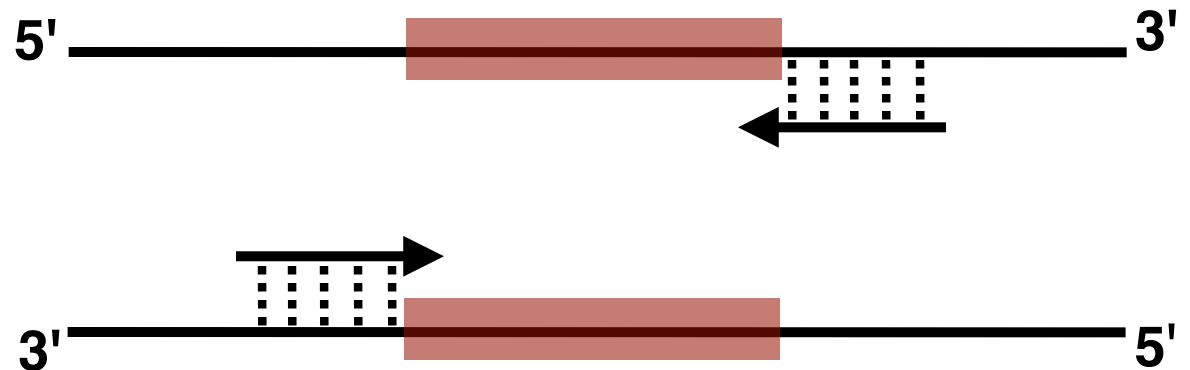


TOPOISOMERASE

OLIGONUCLEOTIDE SYNTHESIS

USE CHEMISTRY TO SYNTHESIZE
SMALL PIECES OF DNA

eg. MAKE AND DESIGN
YOUR OWN PRIMER!





~~HELICASE~~



~~PRIMASE~~



DNA POL III



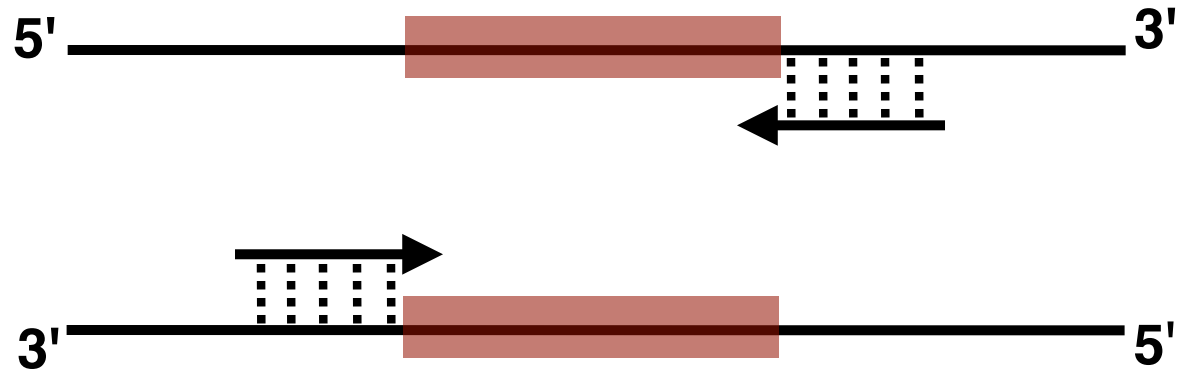
DNA POL I



LIGASE



TOPOISOMERASE



What about
the other 4?



~~HELICASE~~



~~PRIMASE~~



DNA POL III



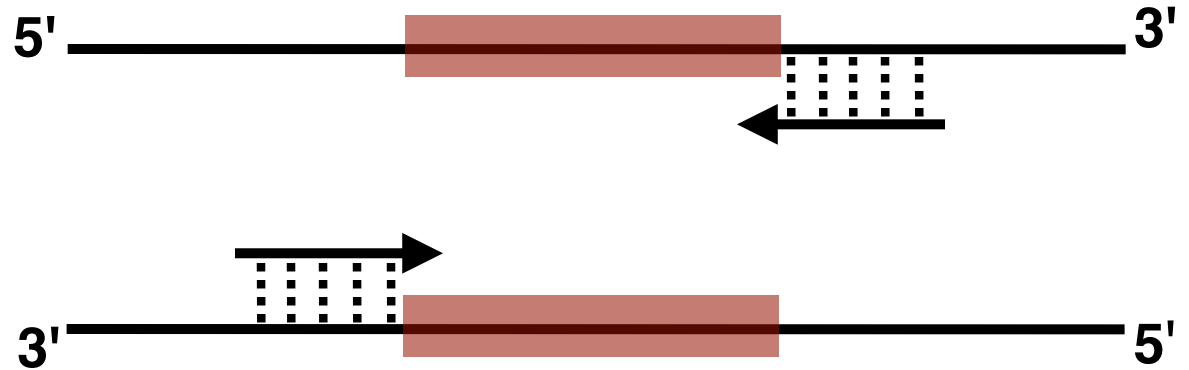
DNA POL I



LIGASE



TOPOISOMERASE



What about
the other 4?

~~HELICASE~~

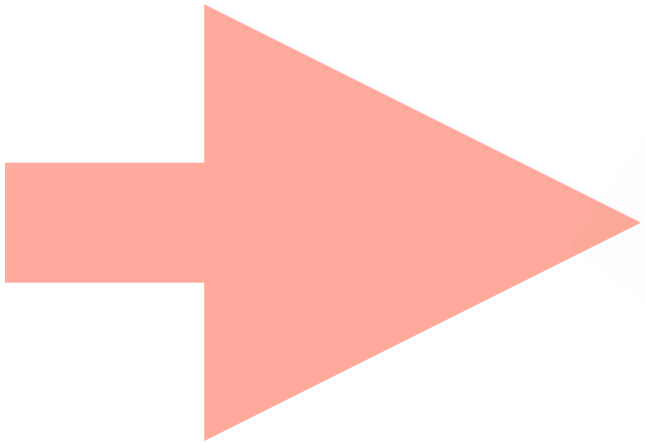
~~PRIMASE~~

DNA POL III

~~DNA POL I~~

~~LIGASE~~

~~TOPOISOMERASE~~



TAQ POL

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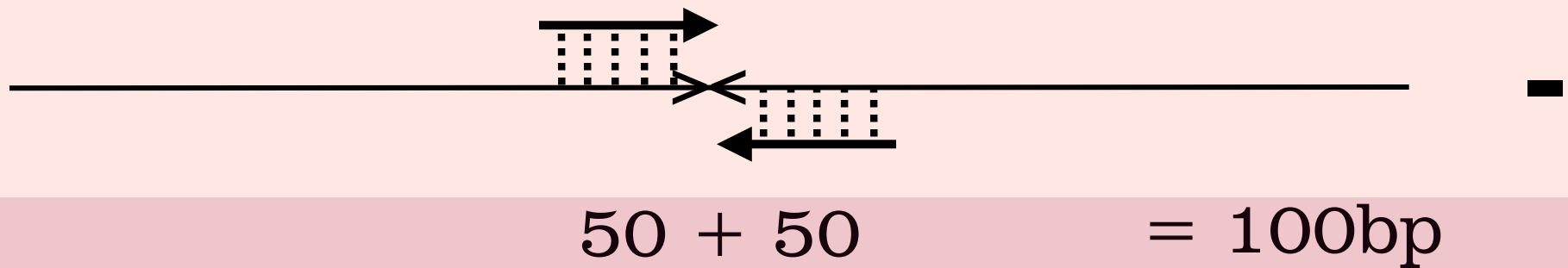
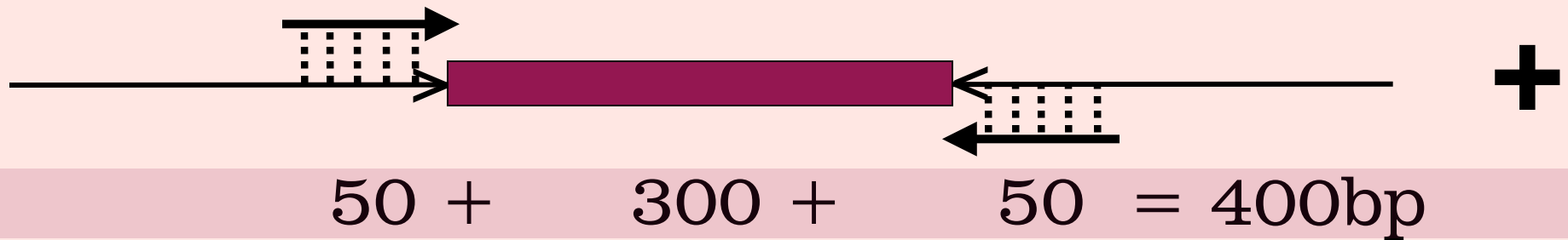
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Lysis via boiling
Purification via chelex beads + centrifugation

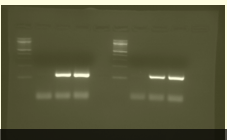
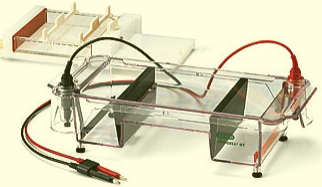
Set up PCR reactions. Allow to run over lunch

Load PCR reactions on “gel”
Apply current to gel.

What’s your genotype?

morning

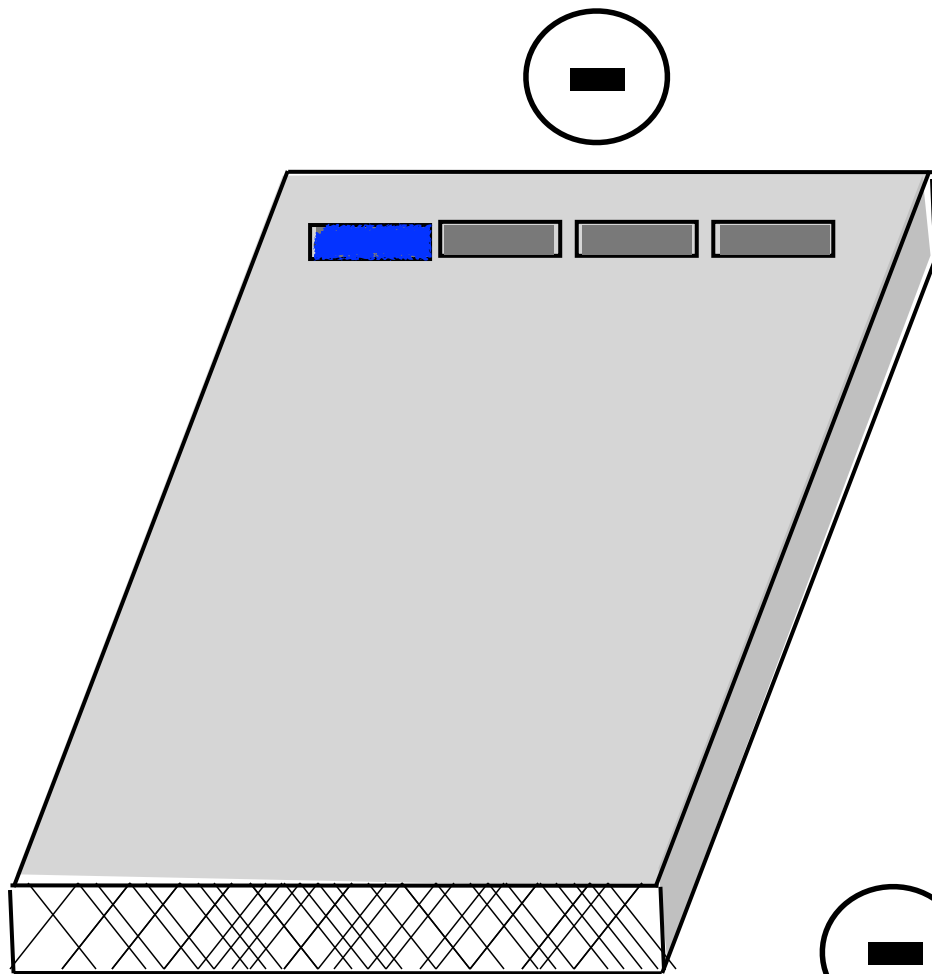
afternoon



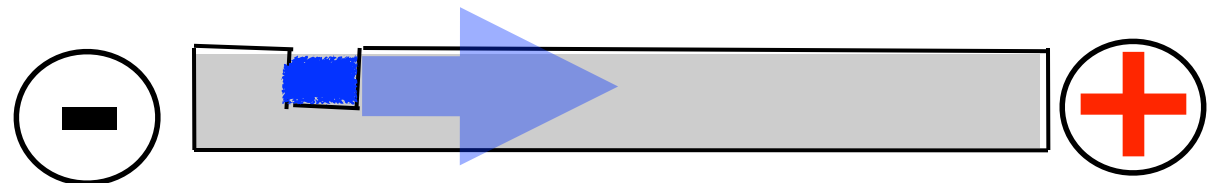
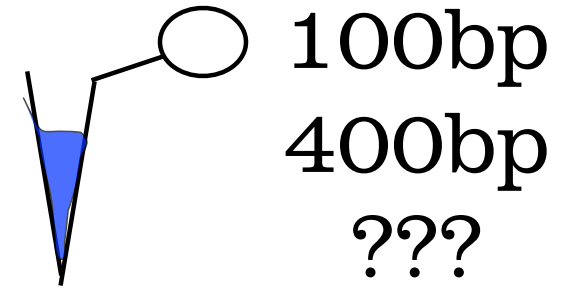




GEL ELECTROPHORESIS

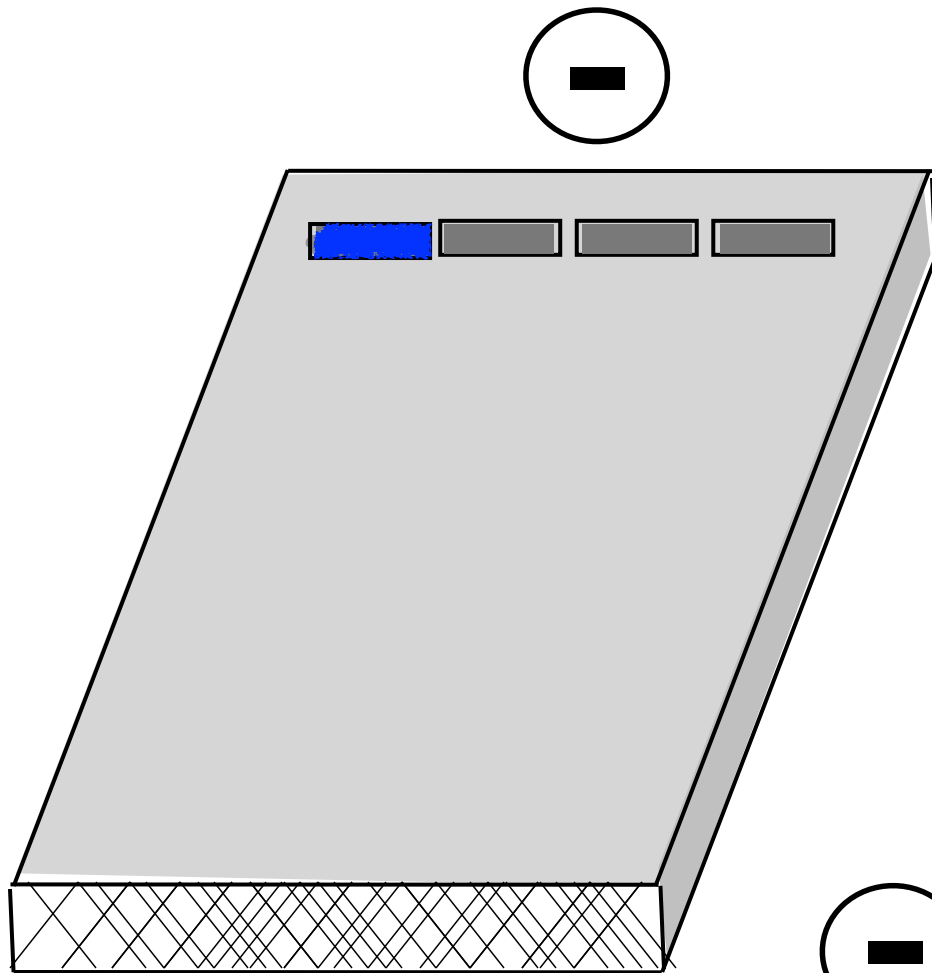


FRONT VIEW

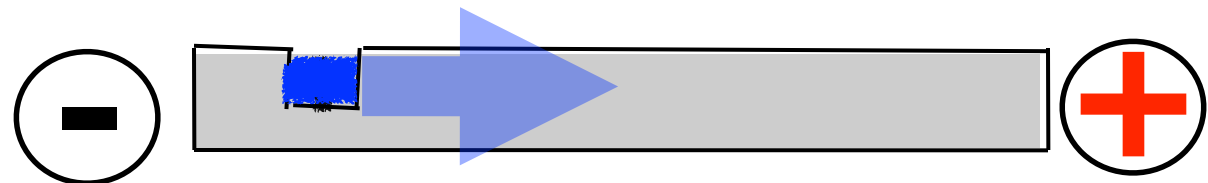
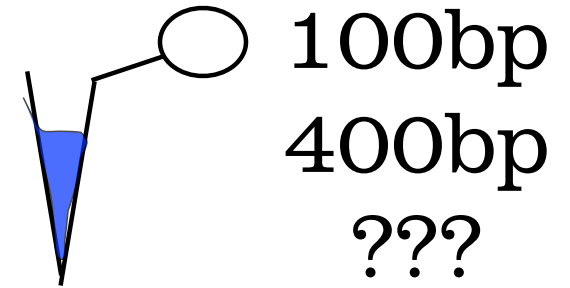


SIDE VIEW

GEL ELECTROPHORESIS

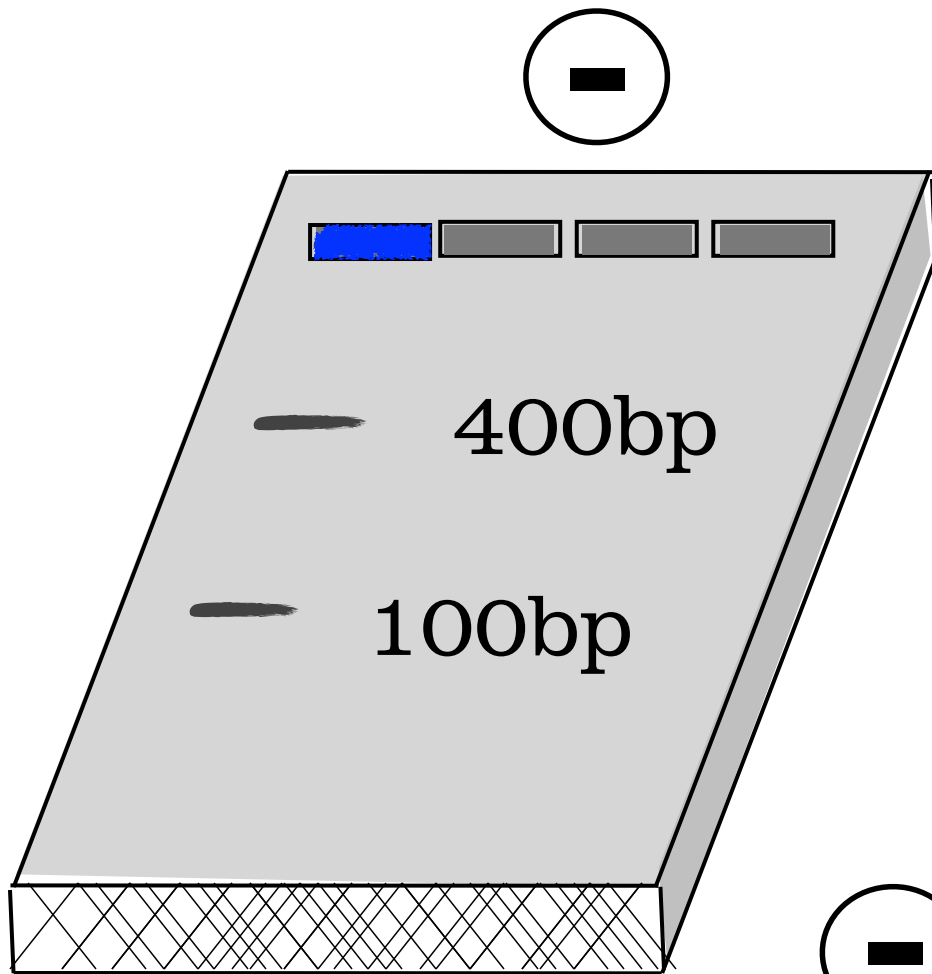



FRONT VIEW

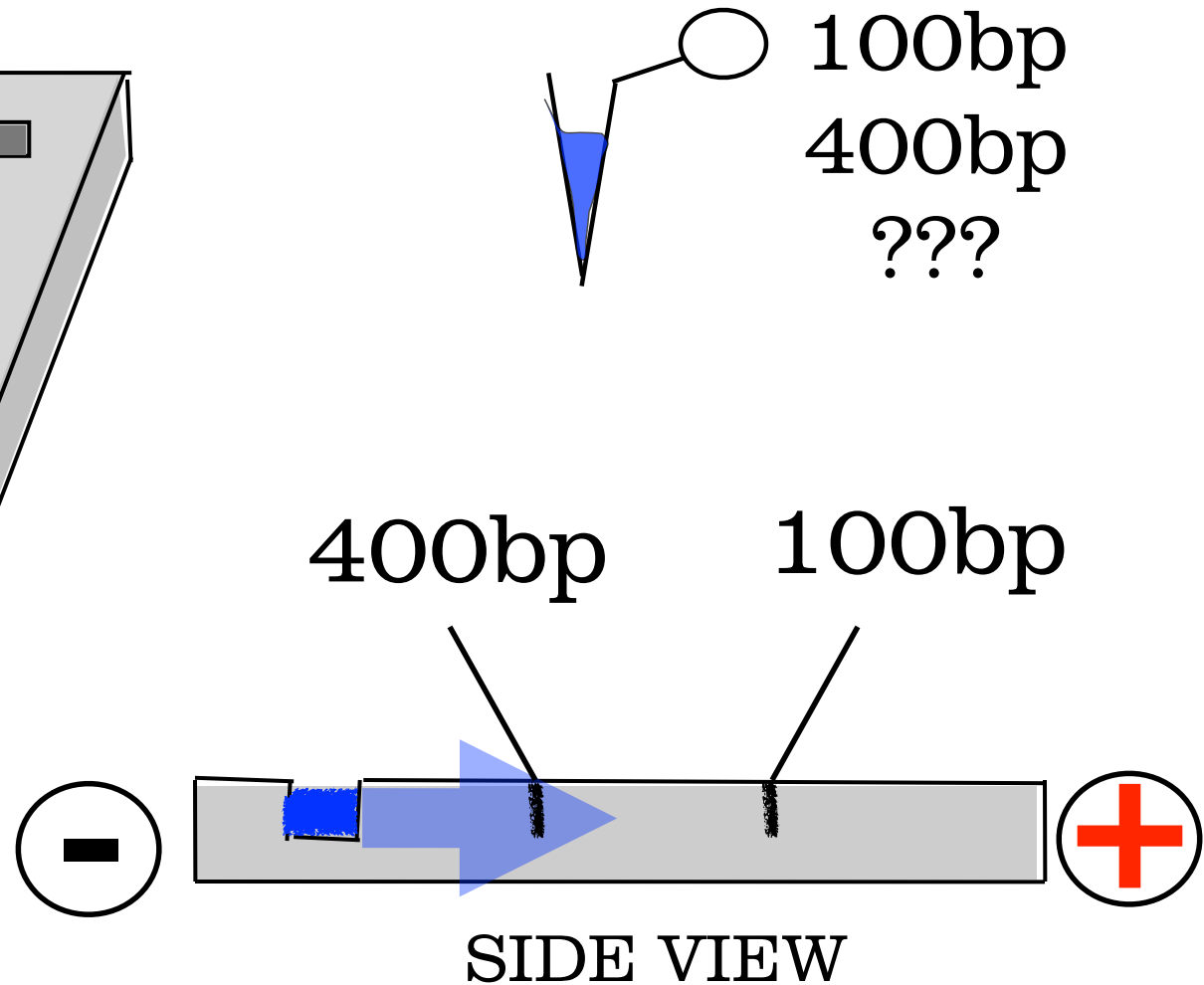


SIDE VIEW

GEL ELECTROPHORESIS



FRONT VIEW



YOU NEED
10.0ul
OF DNA
LOADING
BUFFER.
USE
p20

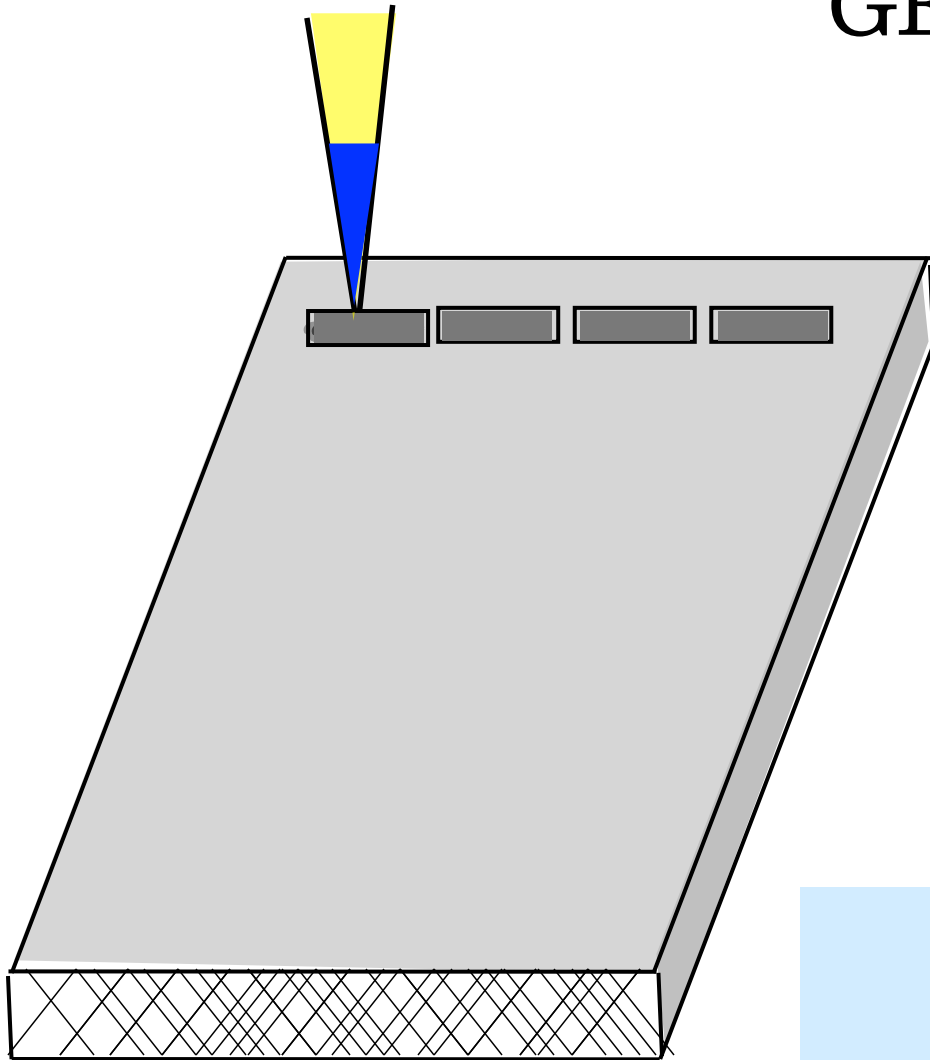
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0

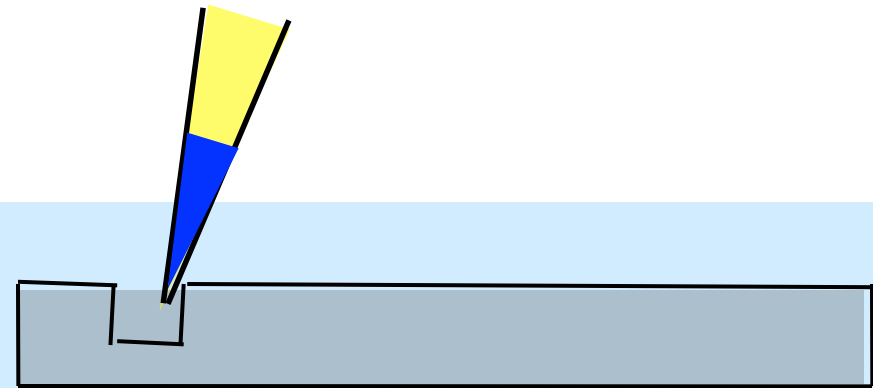
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GEL ELECTROPHORESIS (LOADING)

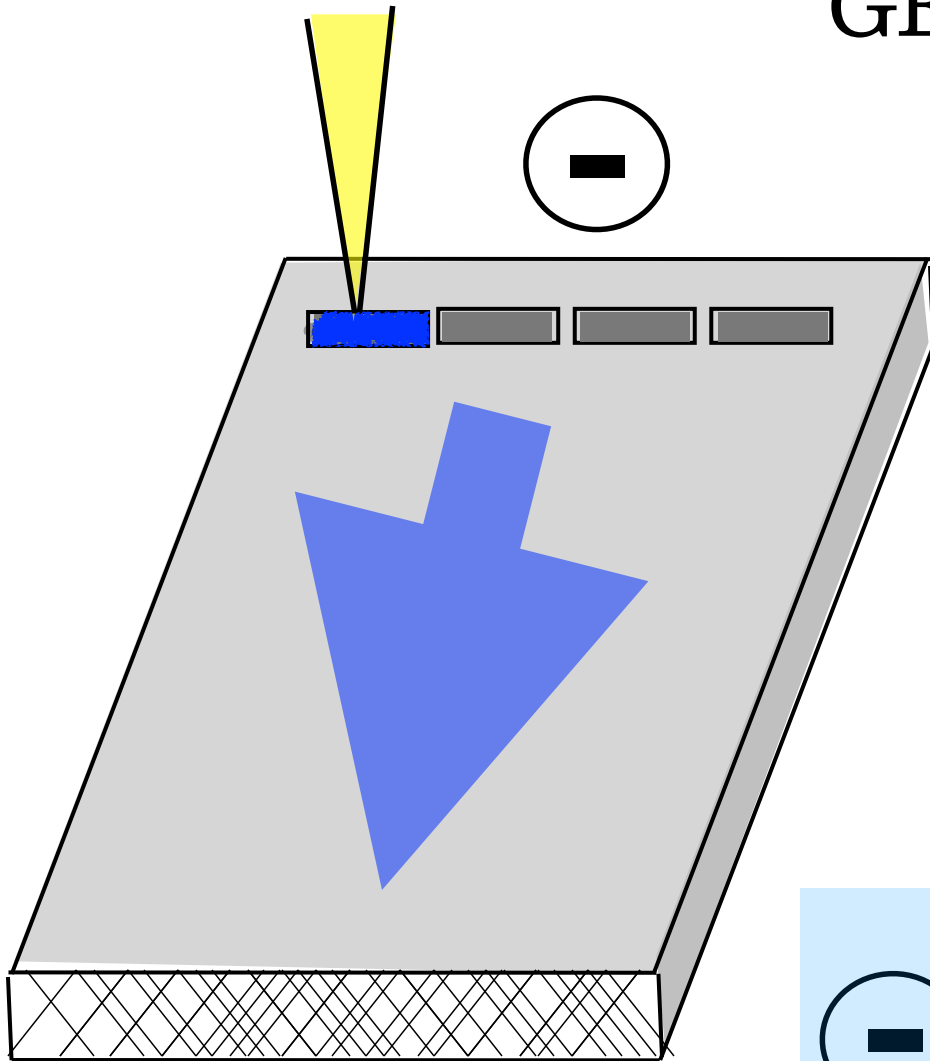


FRONT VIEW

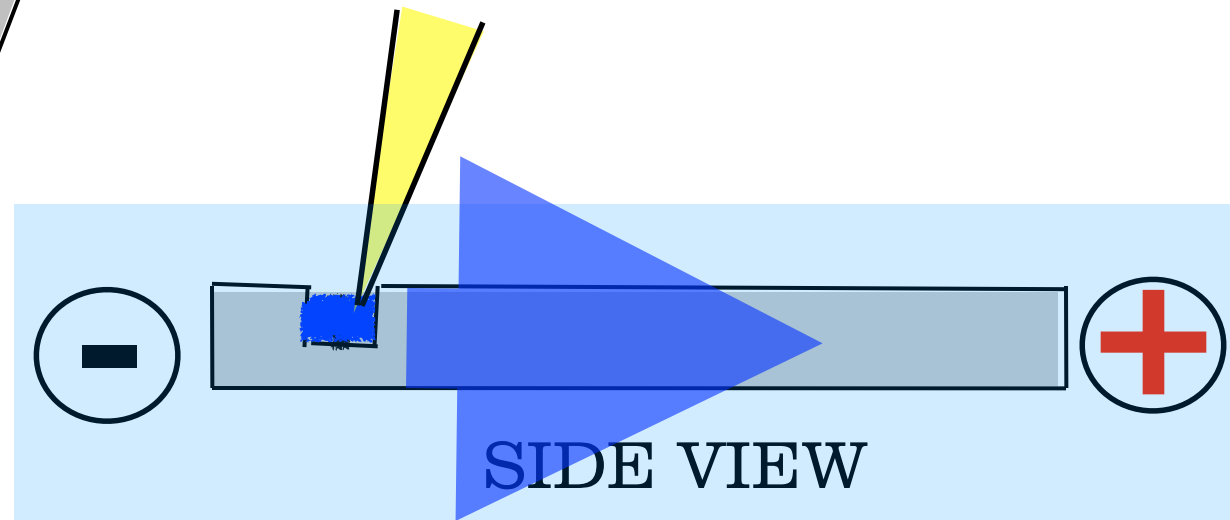


SIDE VIEW

GEL ELECTROPHORESIS (LOADING)

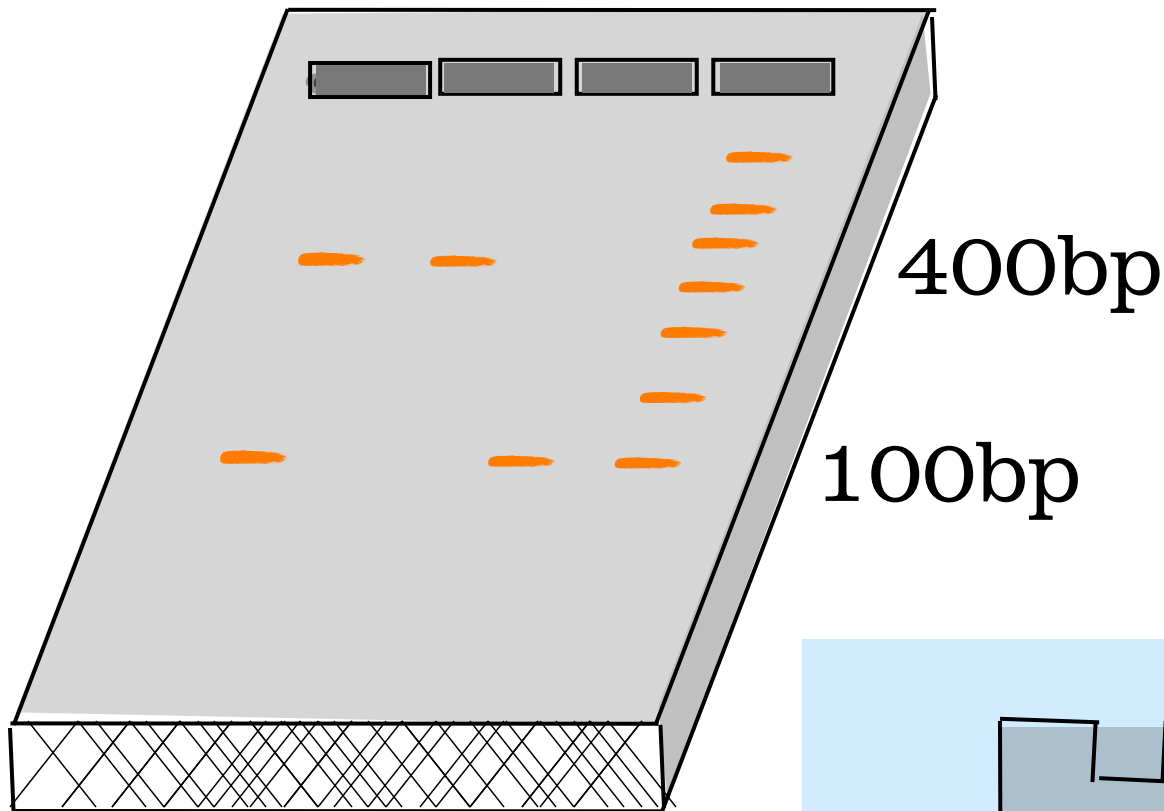


FRONT VIEW

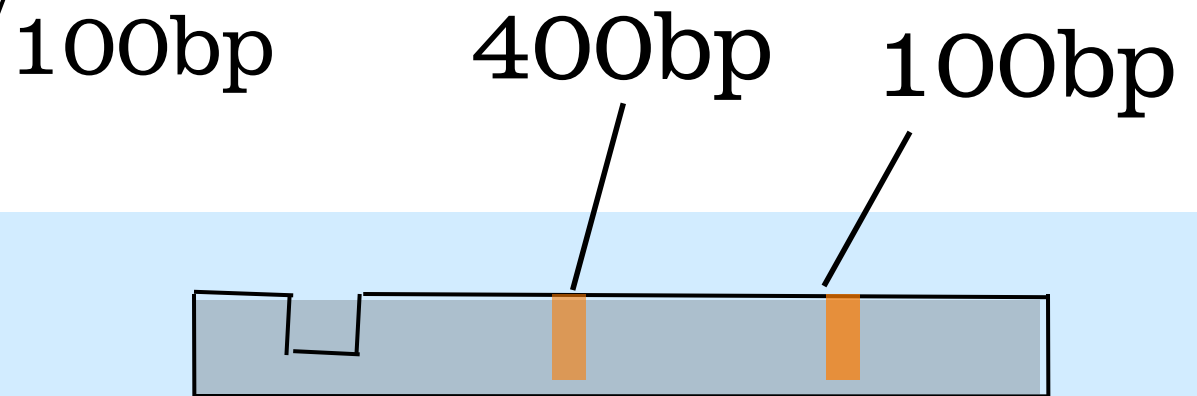


SIDE VIEW

GEL ELECTROPHORESIS (VISUALIZATION)



FRONT VIEW



SIDE VIEW

