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

Cheek rinse using saline. Pellet cells by centrifugation.

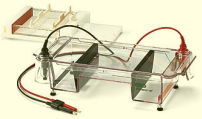
2. EXTRACT DNA

Lysis via boiling
Purification via chelex beads + centrifugation

3. PCR

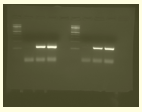
morning
afternoon

Set up PCR reactions. Allow to run over lunch



4. RUN GEL

Load PCR reactions on "gel" Apply current to gel.



5. LOOK AT DATA

What's your genotype?

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.
- Label and hang on to "your" tube.

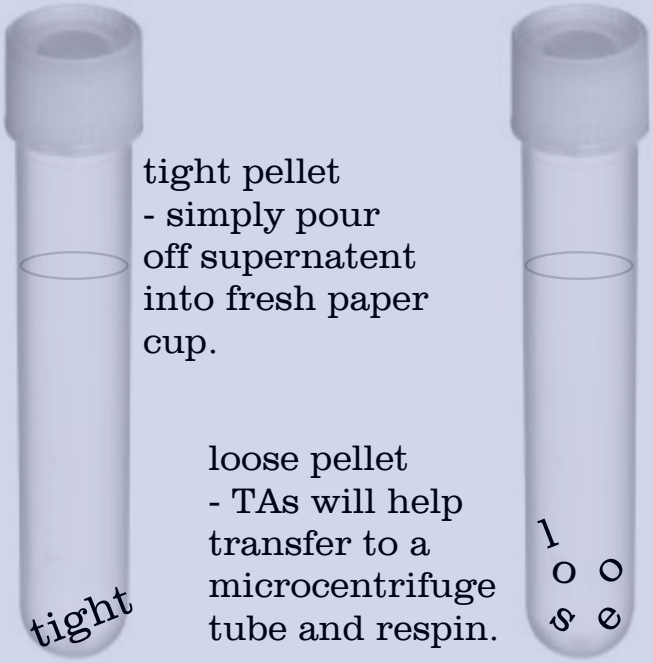
LABEL TUBE WITH PERMANENT MARKER
LOAD YOUR "SPIT" TUBE INTO CENTRIFUGE

CENTRIFUGATION SEPARATES ON THE BASIS OF DENSITY

YOU WANT A CELL PELLET

1500 RPM
10 MINUTES

2 POSSIBILITIES
TIGHT VS LOOSE PELLET



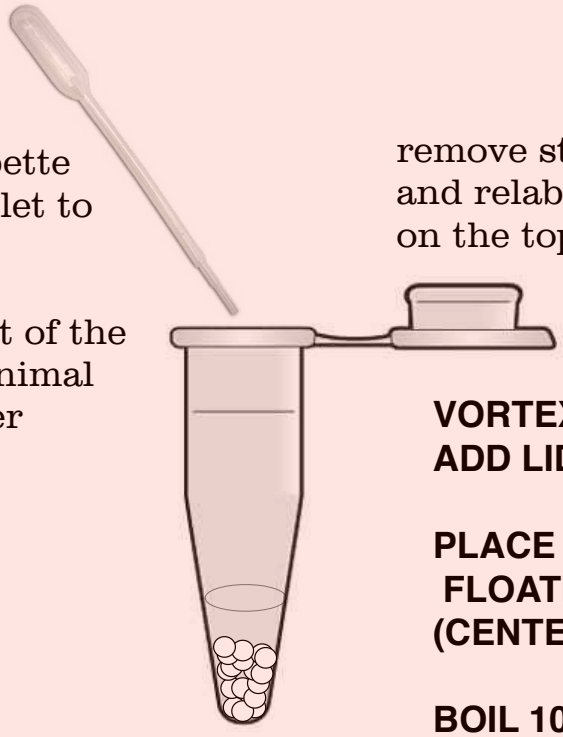
tight pellet
- simply pour off supernatent into fresh paper cup.

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

WITH YOUR PELLET
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



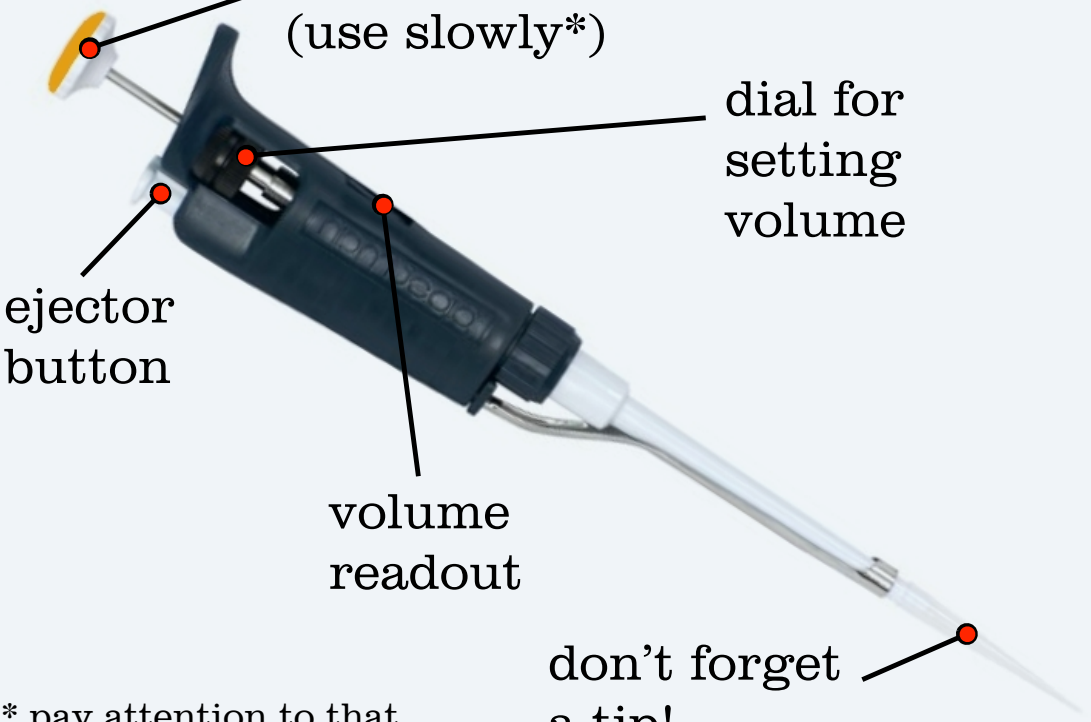
remove sticker and relabel directly on the top of the tube

VORTEX 10 SECONDS
ADD LID LOCK

PLACE ON FLOATING RACK (CENTER CART)

BOIL 10 MINUTES

THE PIPETTEMAN



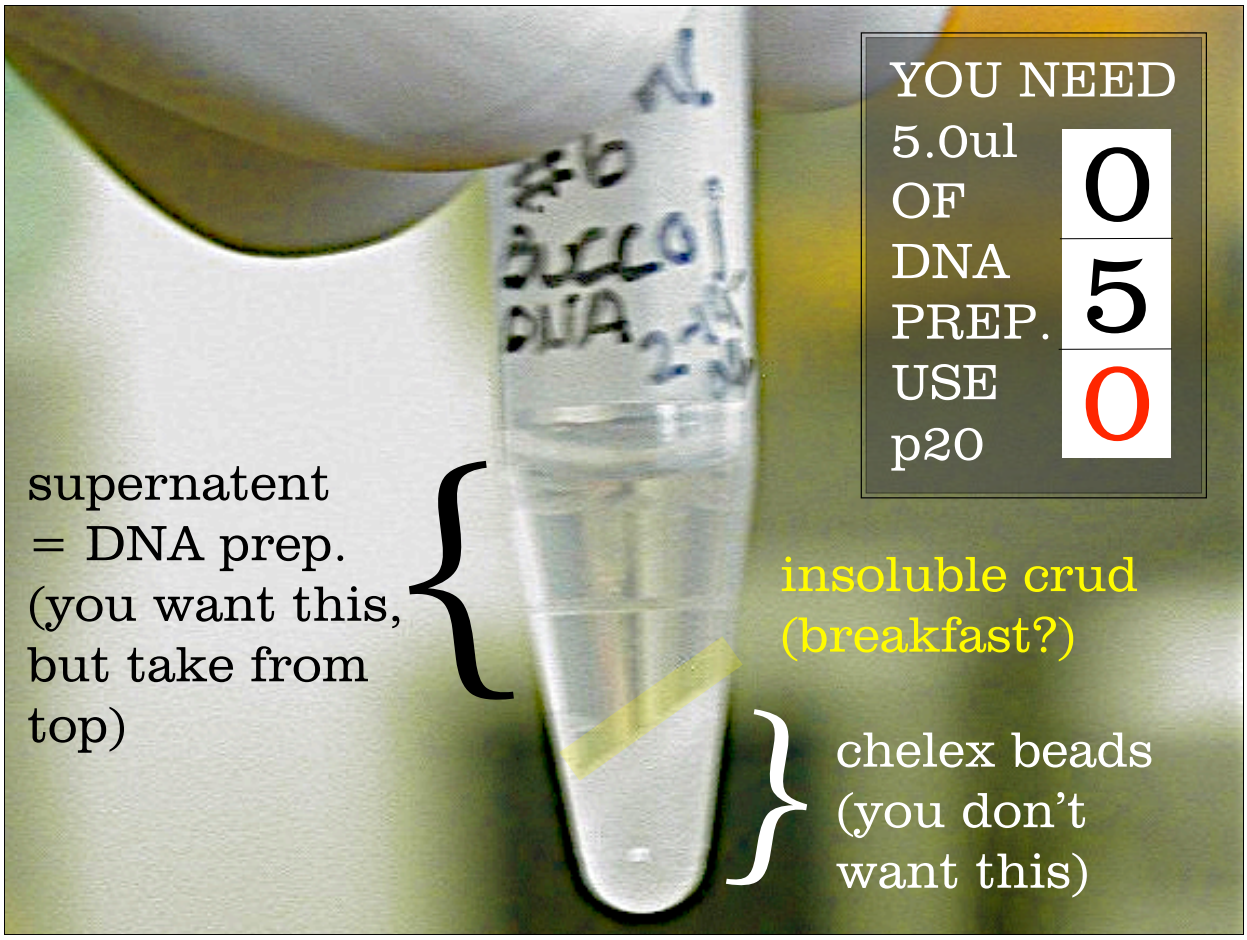
* pay attention to that
first point of resistance.

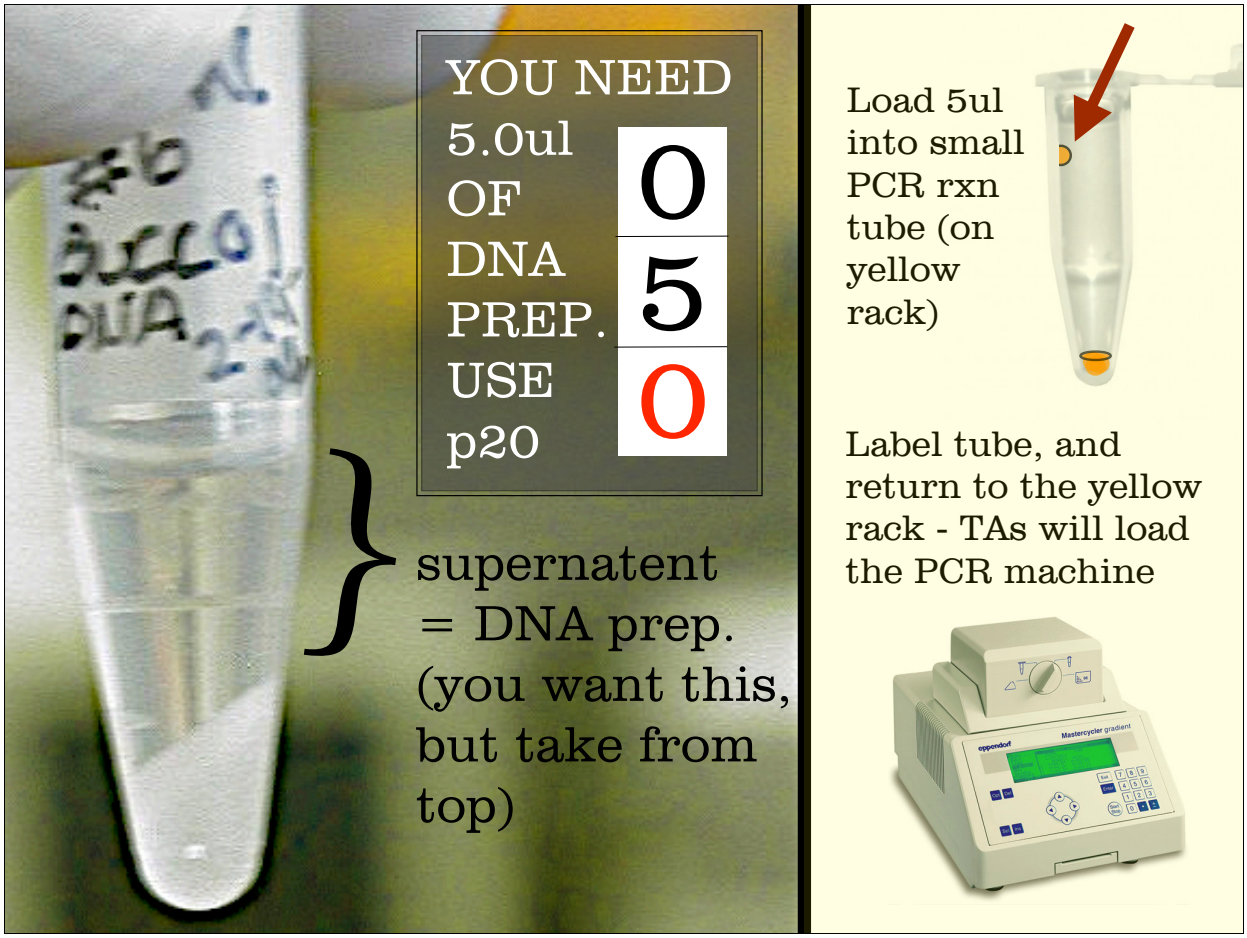
THE PIPETTEMAN

p20
02.0 - 20.0 ul

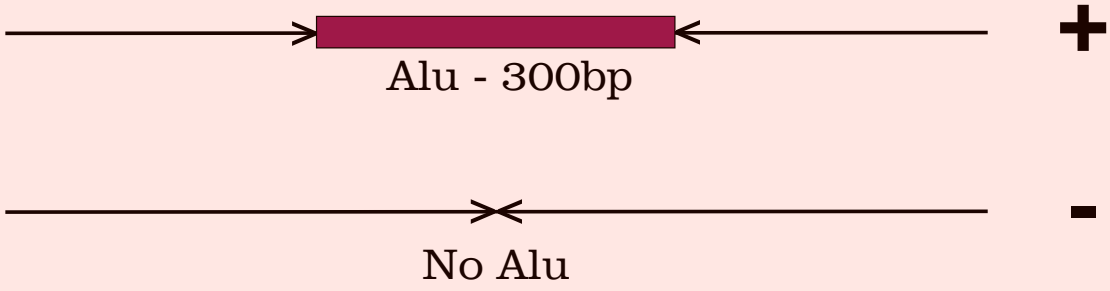
p200
20 - 200 ul

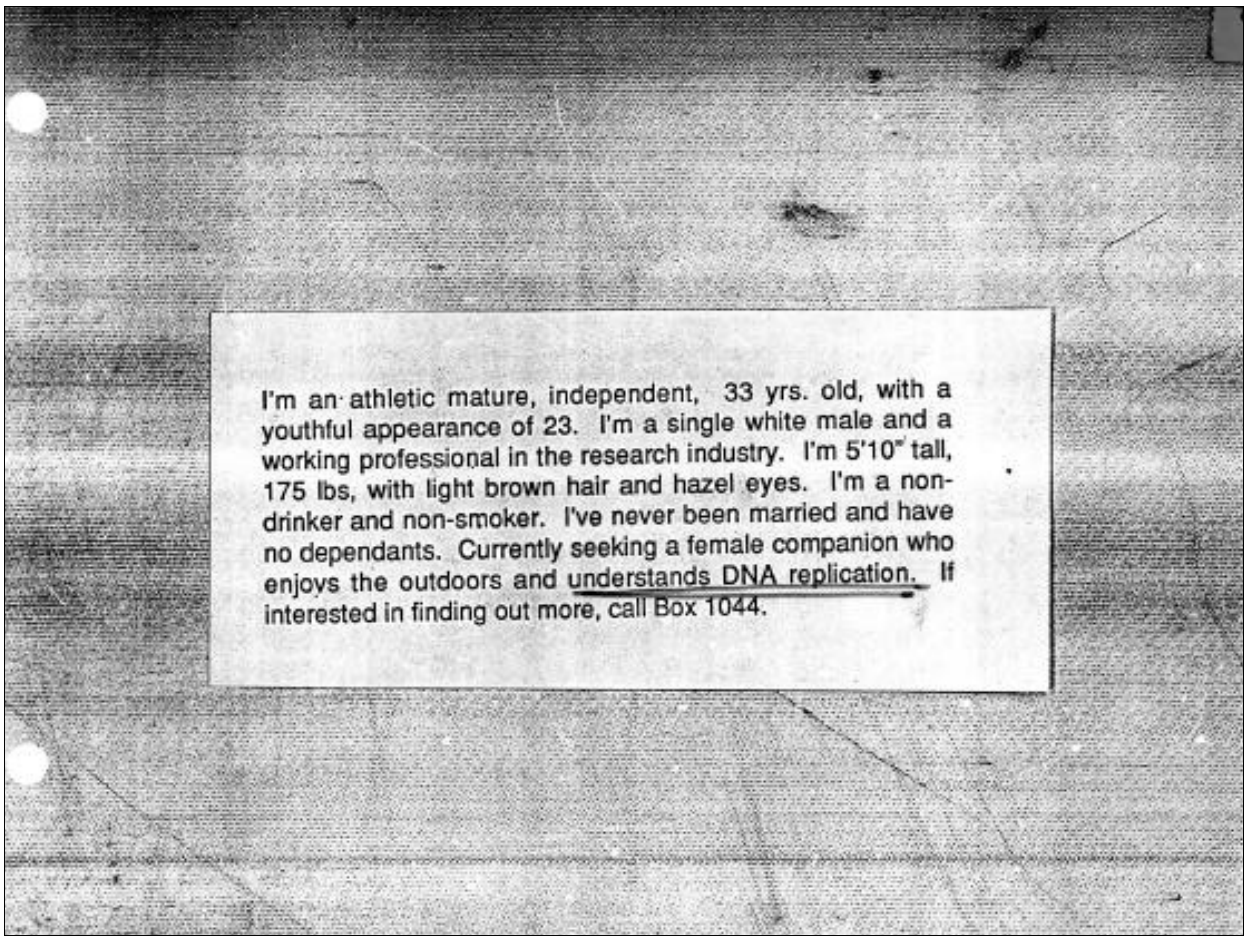
p1000
200 - 1000 ul
= 1.0ml


YOU NEED
5.0ul
OF
DNA
PREP.
USE
p20
0
5
0
supernatent
= DNA prep.
(you want this,
but take from
top)
insoluble crud
(breakfast?)
chelex beads
(you don't
want this)

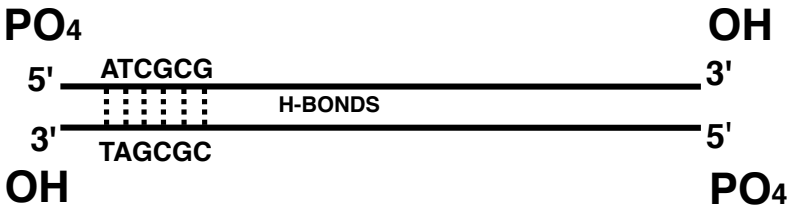

YOU NEED
5.0ul
OF
DNA
PREP.
USE
p20
0
5
0
supernatent
= DNA prep.
(you want this,
but take from
top)
Load 5ul
into small
PCR rxn
tube (on
yellow
rack)
Label tube, and
return to the yellow
rack - TAs will load
the PCR machine

CHROMOSOME 8, TPA-25 LOCI



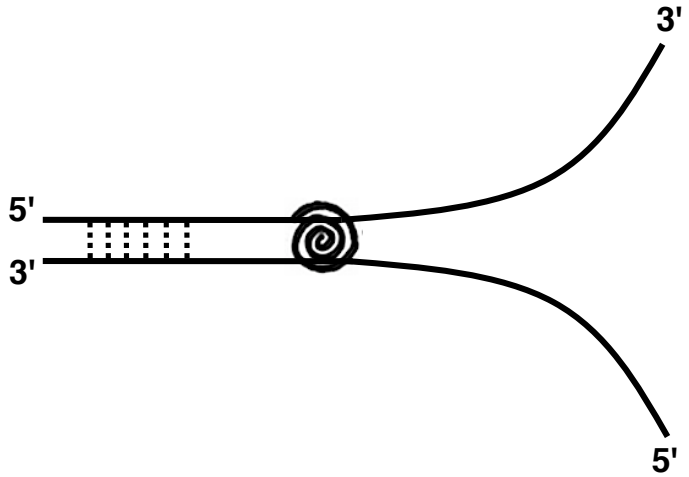

I'm an athletic mature, independent, 33 yrs. old, with a youthful appearance of 23. I'm a single white male and a working professional in the research industry. I'm 5'10" tall, 175 lbs, with light brown hair and hazel eyes. I'm a non-drinker and non-smoker. I've never been married and have no dependants. 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



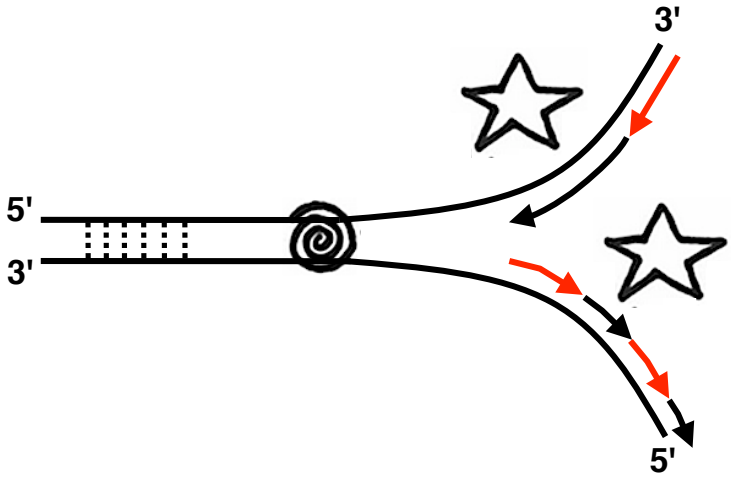
HELICASE

DNA POL III

PRIMASE

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

DNA REPLICATION



HELICASE

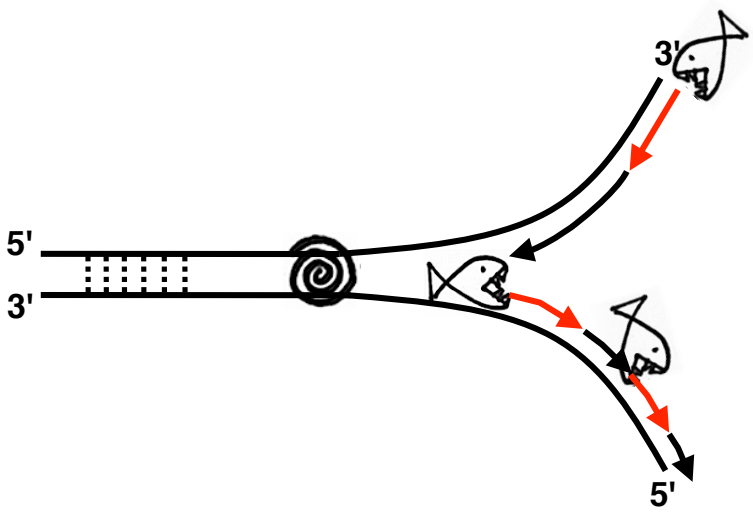
DNA POL III

PRIMASE

DNA POL I

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

DNA REPLICATION



HELICASE

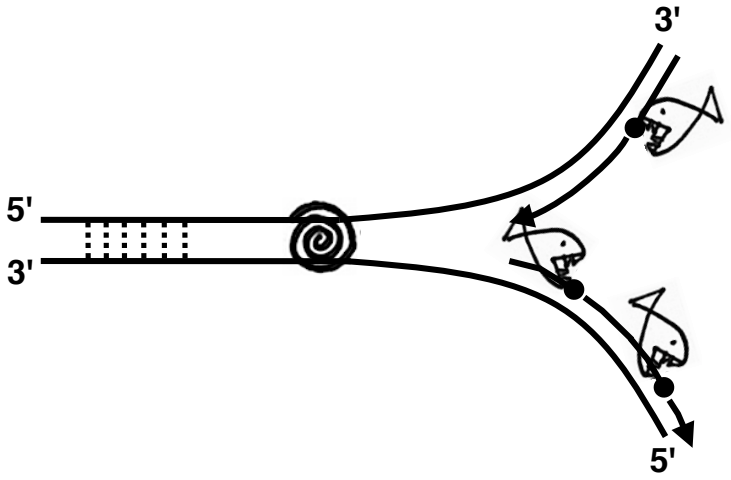
DNA POL III

PRIMASE

DNA POL I

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

DNA REPLICATION



HELICASE

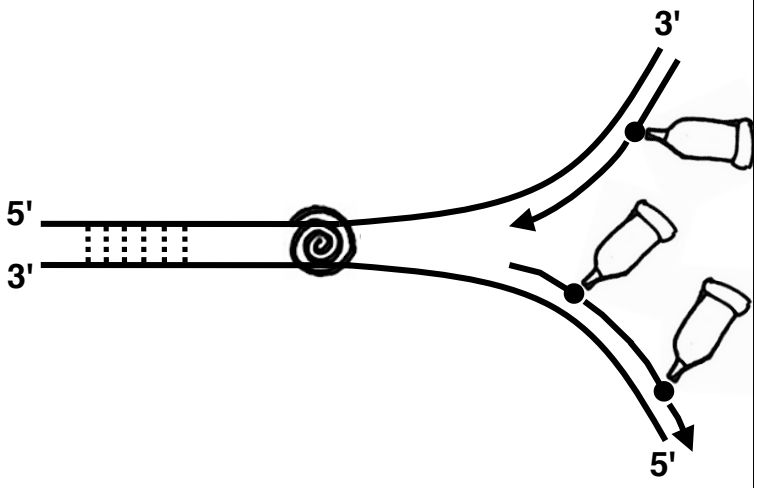
DNA POL III

PRIMASE

DNA POL I

LIGASE

DNA REPLICATION



HELICASE

DNA POL III

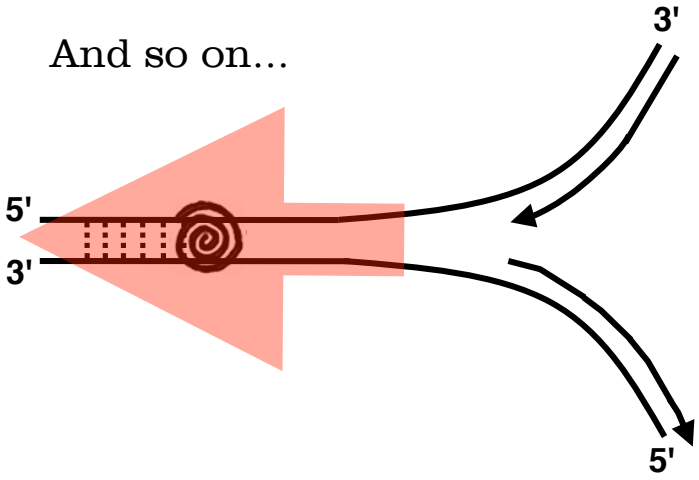
PRIMASE

DNA POL I

LIGASE

DNA REPLICATION

And so on…



Look! Two copies!

HELICASE

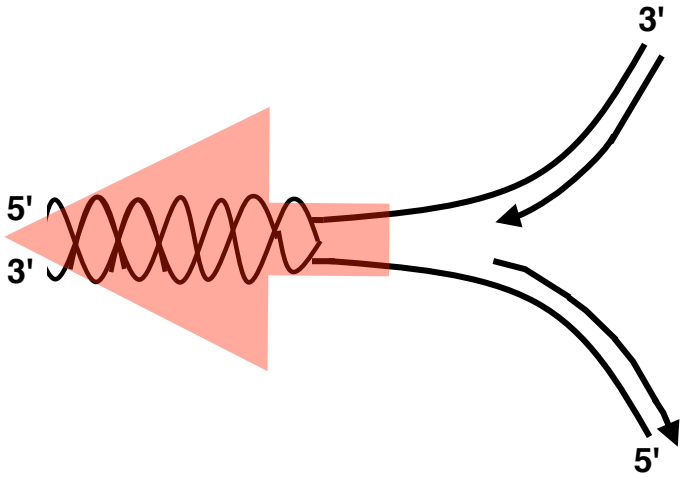
DNA POL III

PRIMASE

DNA POL I

LIGASE

DNA REPLICATION



HELICASE

DNA POL III

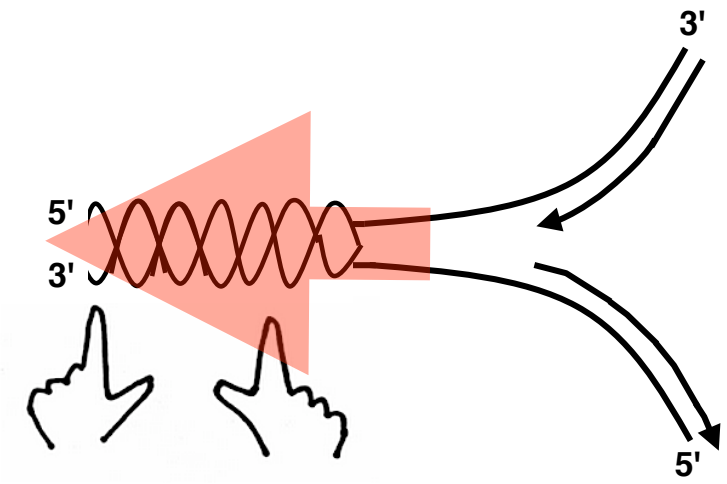
PRIMASE

DNA POL I

LIGASE

TOPOISOMERASE

DNA REPLICATION



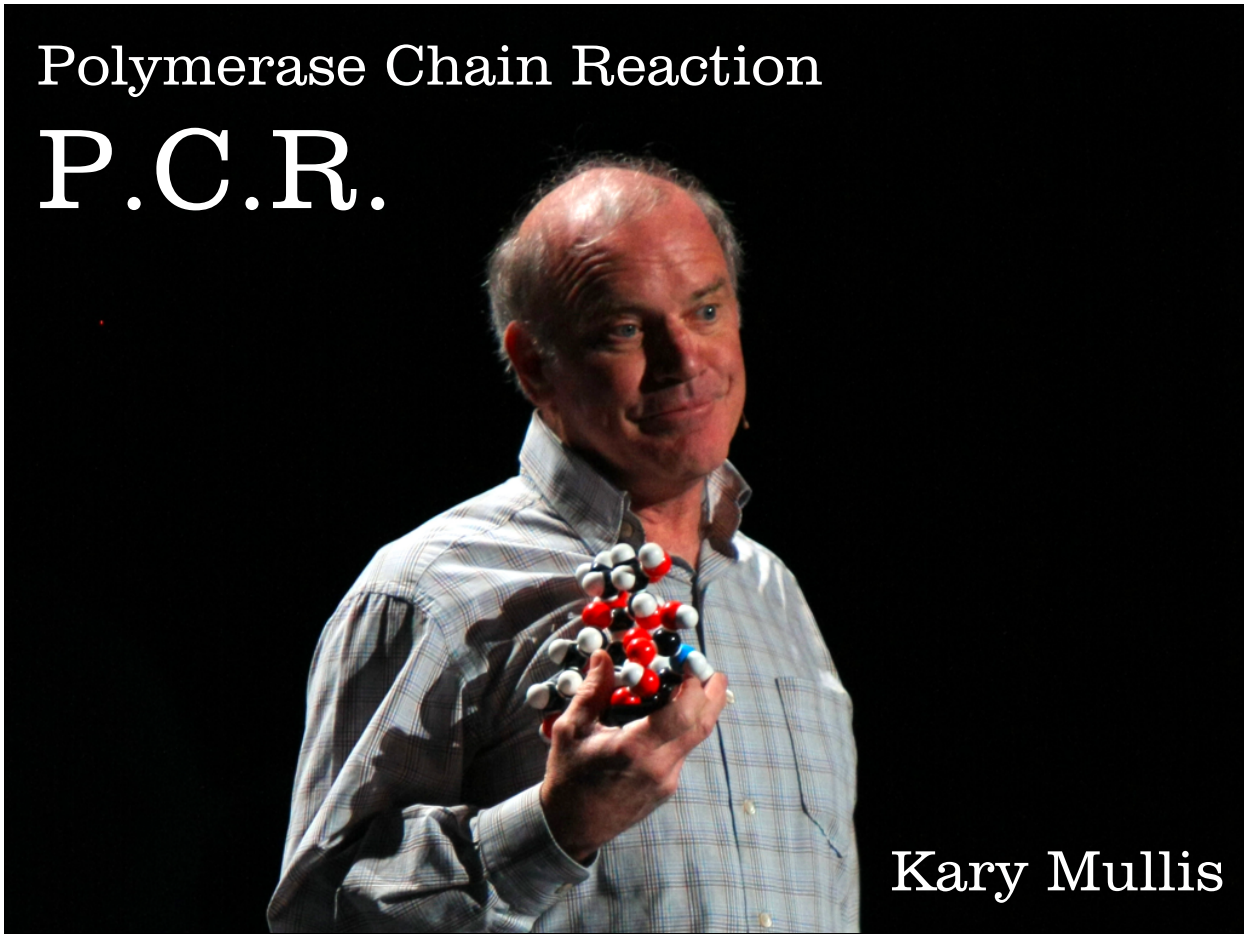
HELICASE	**UNWINDS HELIX**
DNA POL III	**ADD NT's TO 3' (COPIES DNA)**
PRIMASE	**MAKES RNA PRIMER**
DNA POL I	**REMOVES RNA PRIMER REPLACES WITH DNA**
LIGASE	**SEALS GAPS IN DNA**
TOPOISOMERASE	**RELIEVES STRUCTURAL STRESS**

Polymerase Chain Reaction = Replication in a test tube.

Can we simplify it?

Polymerase Chain Reaction

P.C.R.



~~HELICASE~~

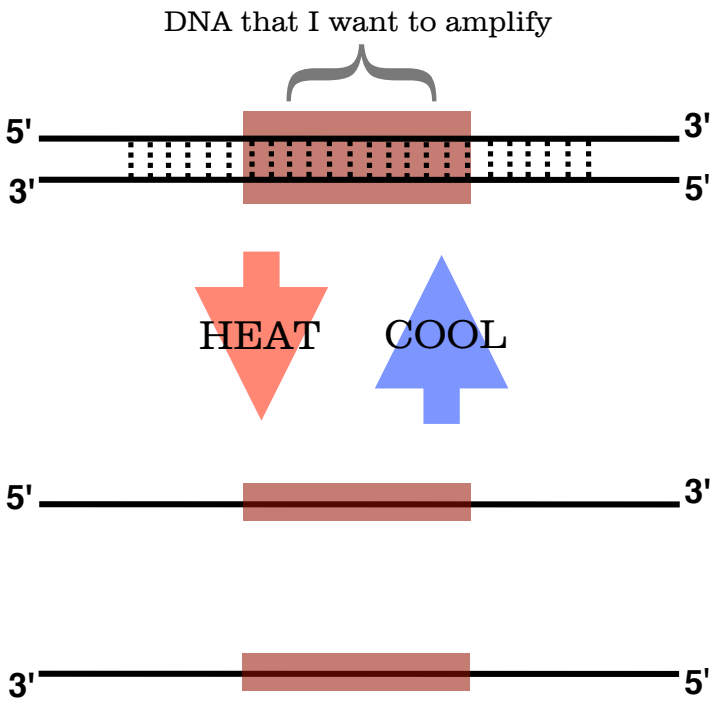
DNA POL III

PRIMASE

DNA POL I

LIGASE

TOPOISOMERASE



~~HELICASE~~

DNA POL III

~~PRIMASE~~

DNA POL I

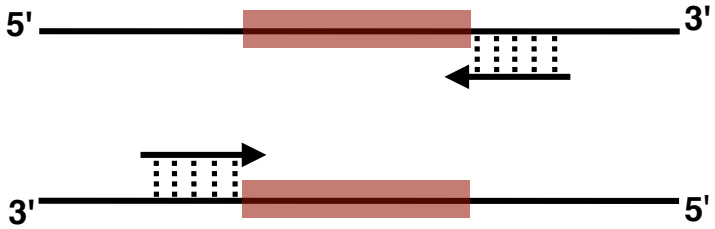
LIGASE

TOPOISOMERASE

OLIGONUCLEOTIDE SYNTHESIS

USE CHEMISTRY TO SYNTHESIZE SMALL PIECES OF DNA

eg. MAKE AND DESIGN YOUR OWN PRIMER!



~~HELICASE~~

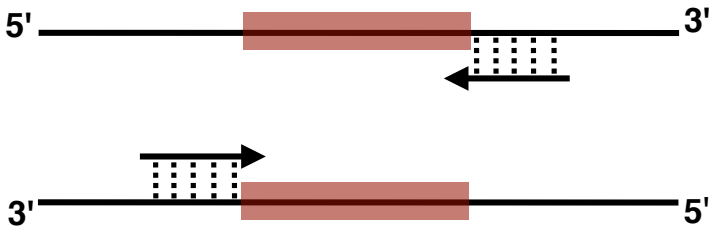
~~PRIMASE~~

DNA POL III

~~DNA POL I~~

~~LIGASE~~

TOPOISOMERASE



What about the other 4?

~~HELICASE~~

~~PRIMASE~~

DNA POL III

~~DNA POL I~~

~~LIGASE~~

~~TOPOISOMERASE~~

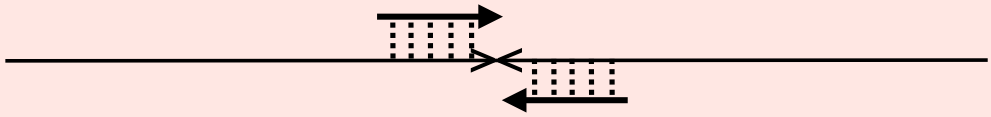
TAQ POL

GO WATCH 2DPCR.SWF

CHROMOSOME 8, TPA-25 LOCI

\+

50 + 300 + 50 = 400bp



\-

50 + 50 = 100bp

1. GET CELLS

Cheek rinse using saline. Pellet cells by centrifugation.

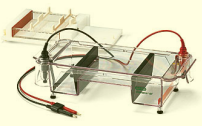
2. EXTRACT DNA

Lysis via boiling
Purification via chelex beads + centrifugation

3. PCR

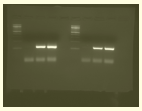
morning
afternoon

Set up PCR reactions. Allow to run over lunch



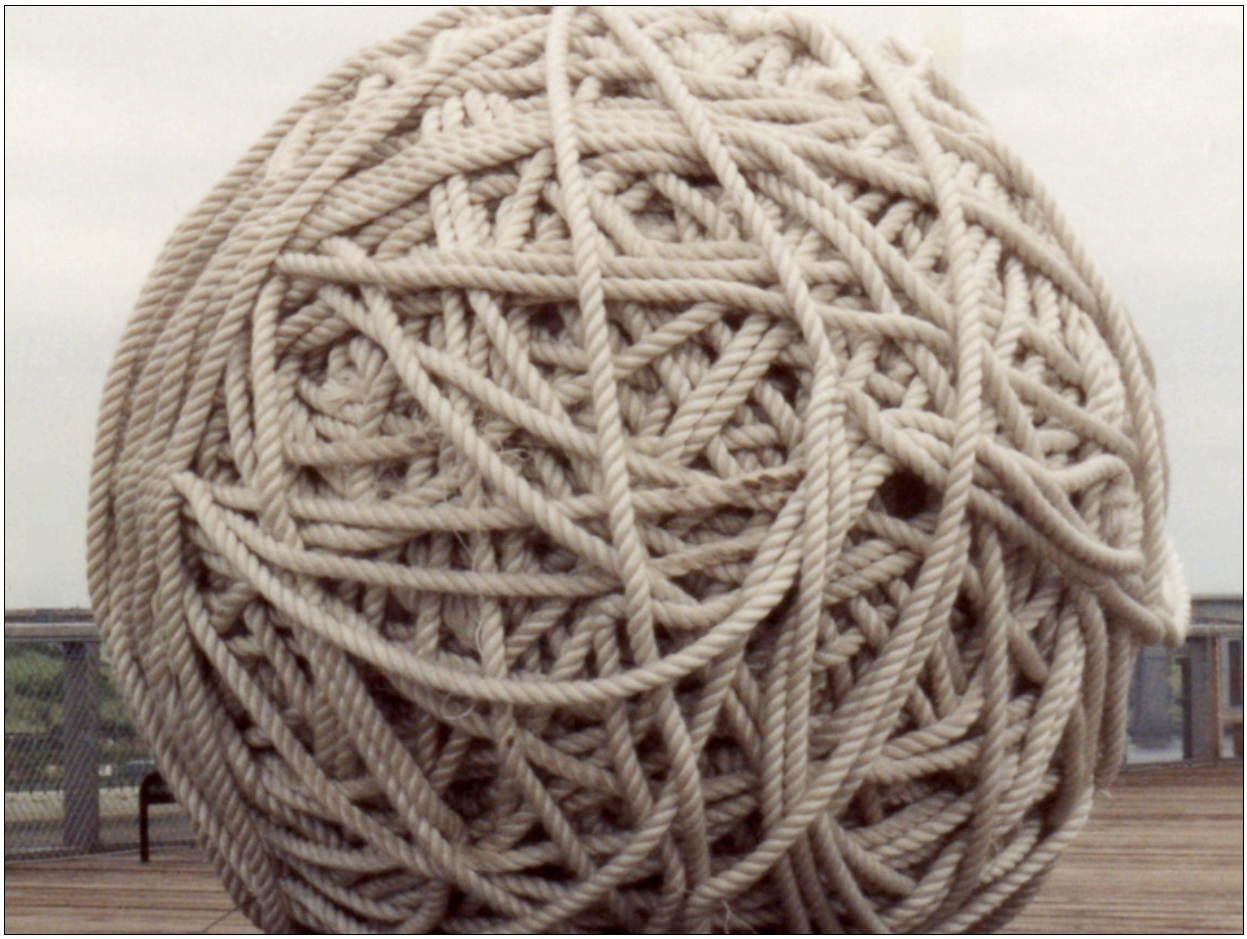
4. RUN GEL

Load PCR reactions on "gel" Apply current to gel.

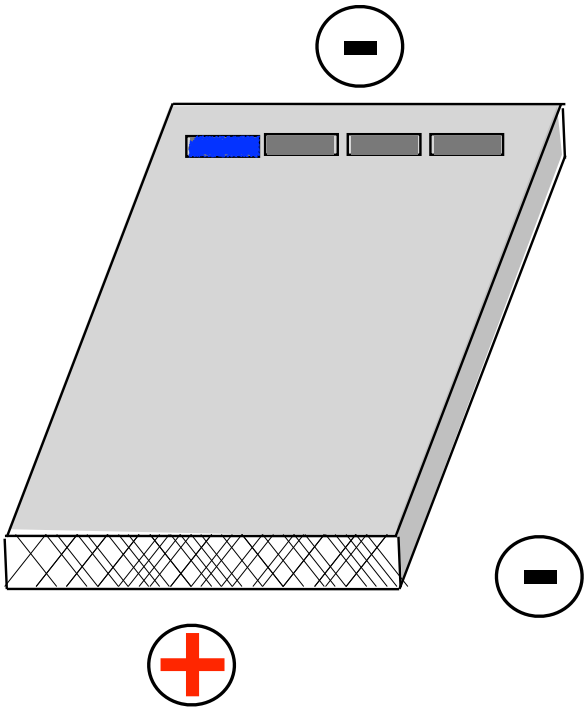


5. LOOK AT DATA

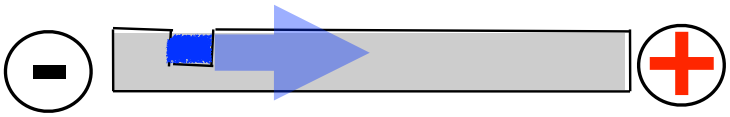
What's your genotype?



GEL ELECTROPHORESIS



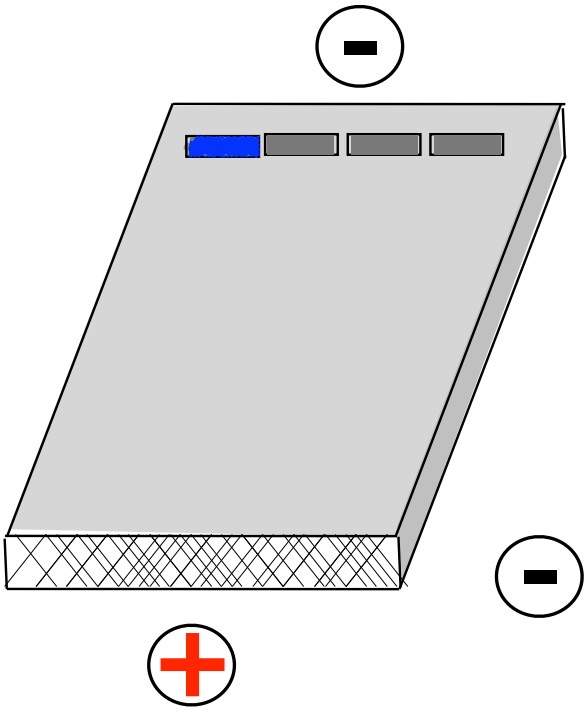
100bp
400bp
???



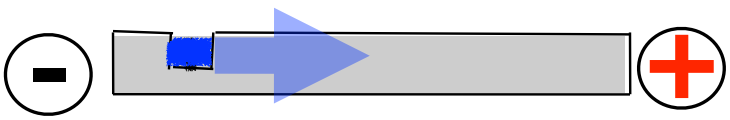
SIDE VIEW

FRONT VIEW

GEL ELECTROPHORESIS



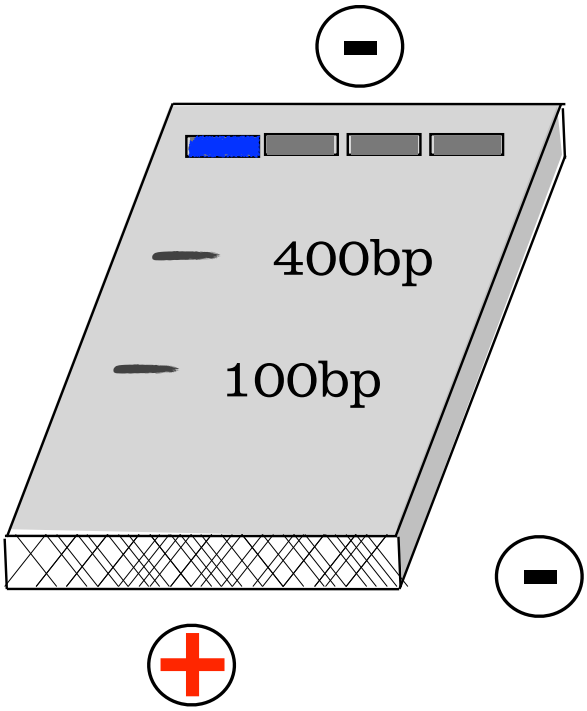
100bp
400bp
???



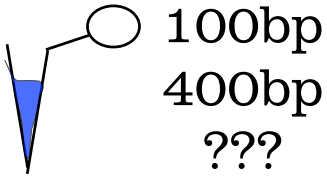
SIDE VIEW

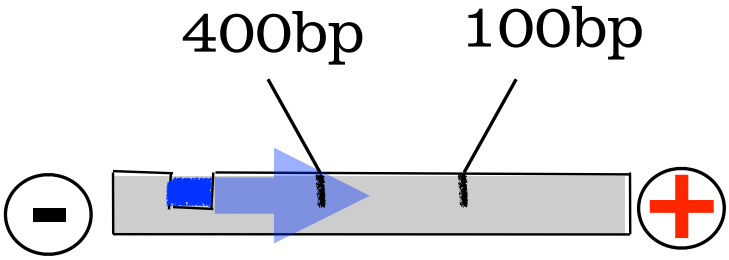
FRONT VIEW

GEL ELECTROPHORESIS

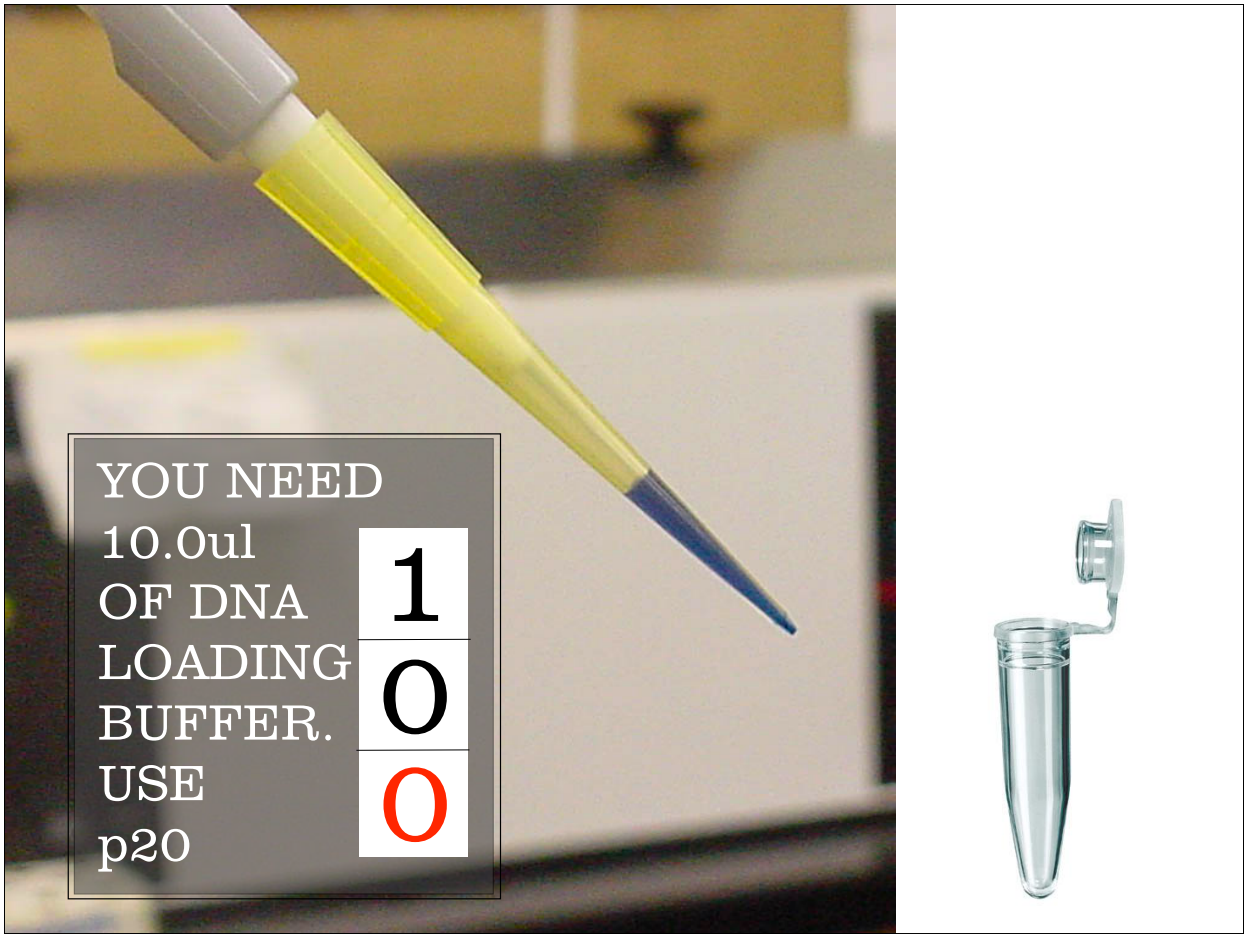


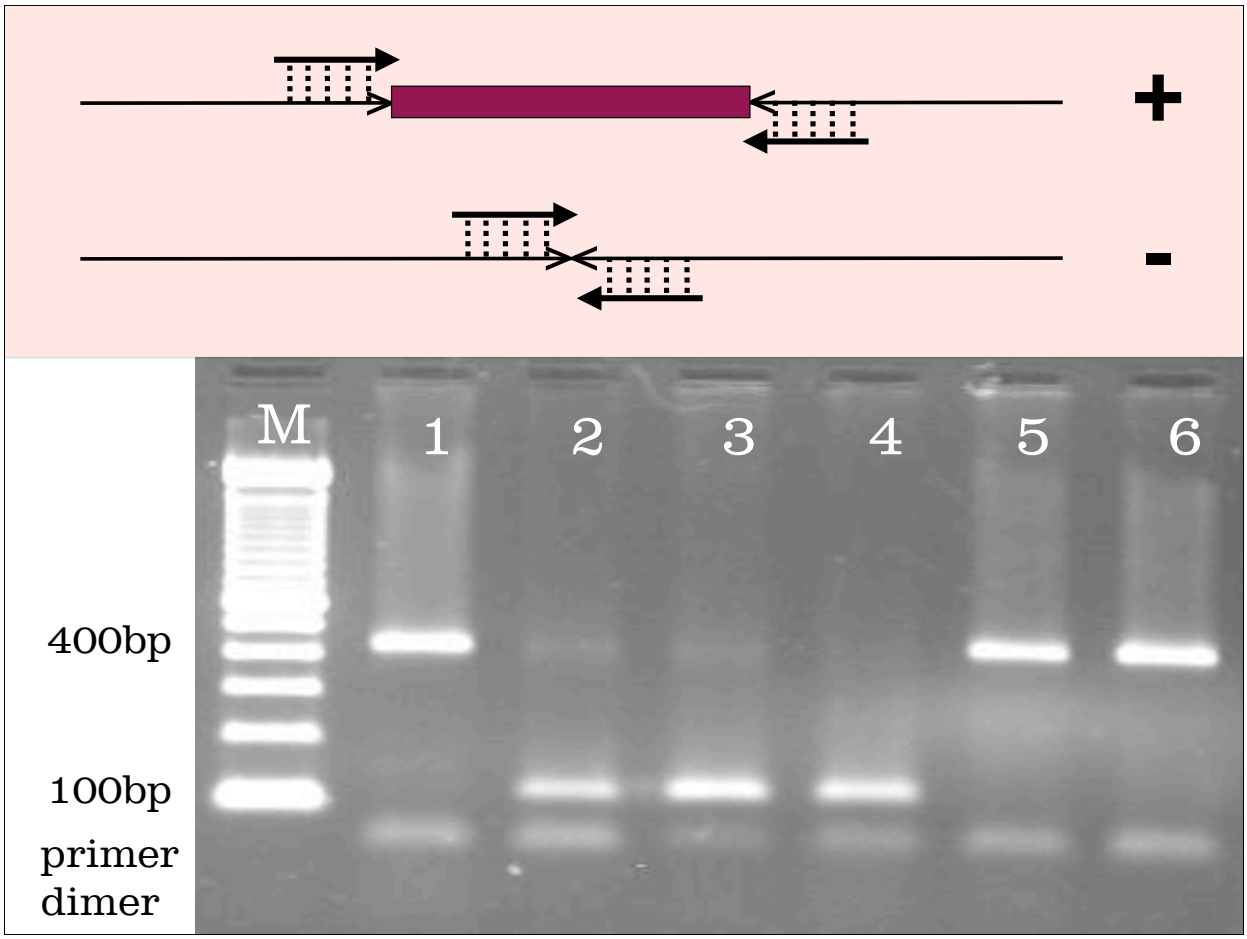
FRONT VIEW





SIDE VIEW





THE PCR LAB was intended to show you how molecular biology is, at its heart, fairly straightforward to do (see this below for information about the lab - lecture, etc), whilst being capable of generating some very weighty data. For this lab commentary, I'd like you to comment on the two queries presented below. You can write informally if you'd prefer, but we are looking for a relatively well thought out response (somewhere in the 300 to 500 word range for each). Ideas are strongly encouraged. Please send your answer to ASIC200@gmail.com (please use PCR as the subject heading). We'll do our best to reply to confirm receiving your email, but you may actually just receive your mark a week or so after you submit. Note that this assignment is due by midnight on the thursday after your lab (note that one need not fully get the mechanics of PCR to do this assignment).

1. It was important to stress that the experiment we did in the genetics lab aimed at looking for a genetic element with no real consequence. However, the methodology (PCR) can very easily be adapted to look at something of significant consequence - i.e. a diagnosis of a genetic disorder like Huntingtons (a fatal and nasty neurodegenerative disease). What type of ethical situations come to mind when an individual is put in the position of getting one of these genetic tests done.

2. If the opportunity presented itself, would you get one of these tests done? Why or why not?