EASTER BUSH SCIENCE OUTREACH CENTRE

Get hands-on with real-life science

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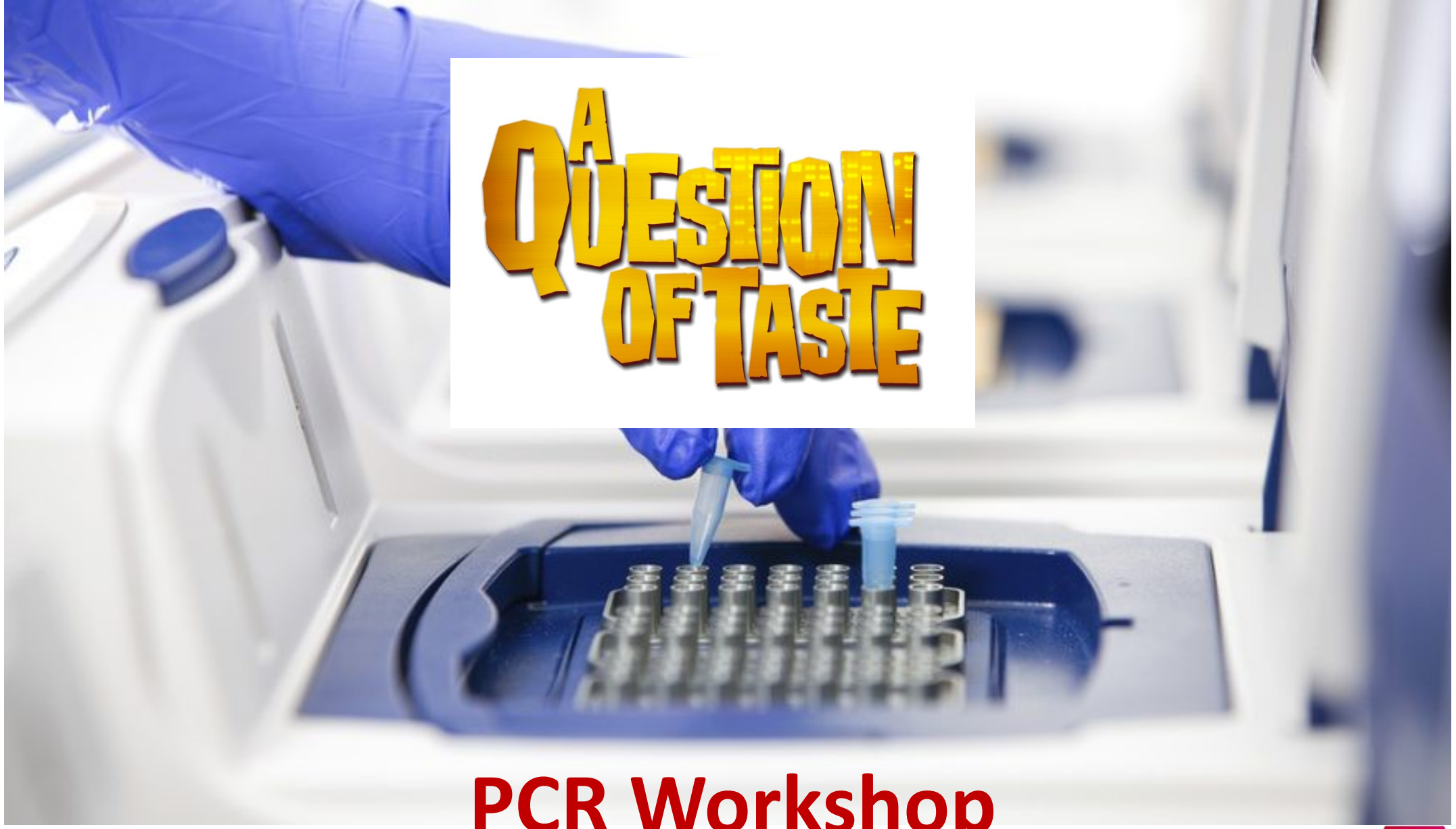

PCR Workshop

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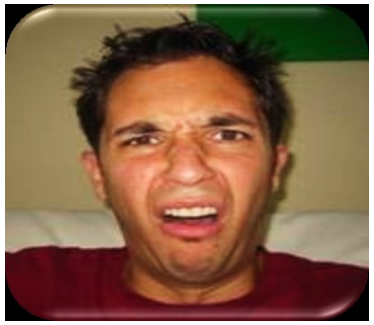
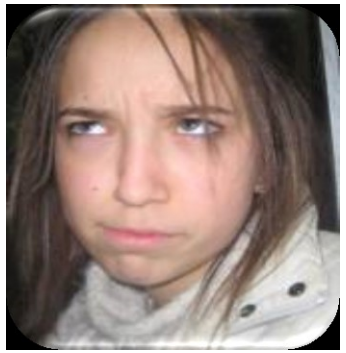
Let’s do a taste test!

What are your taste test results?



Strong taster

- Quick negative reaction
- Very bitter
- *Ratings 4-5*



Weak taster

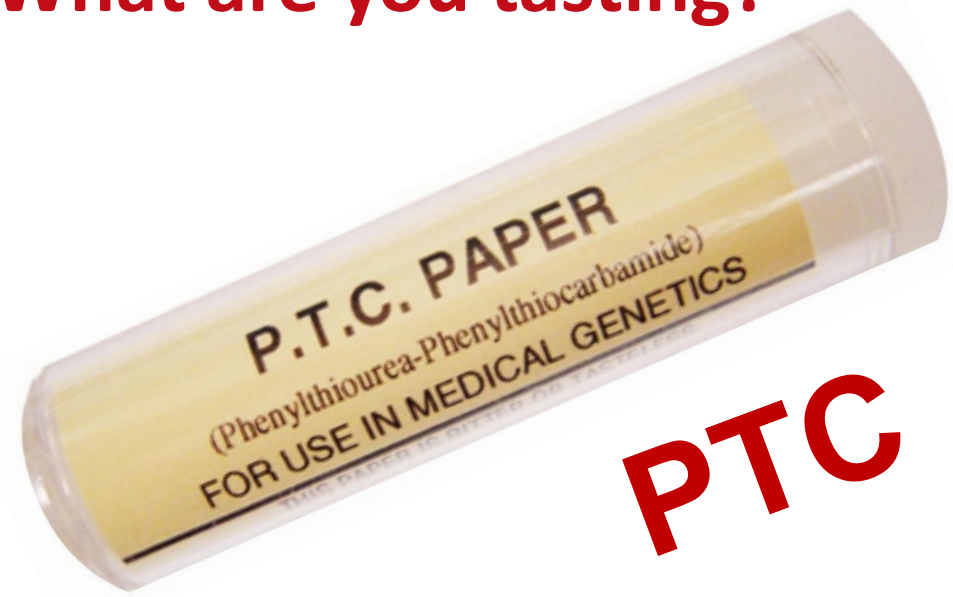
- Unsure at first, but then find the taste unpleasant
- *Ratings 1-3*

Non-taster

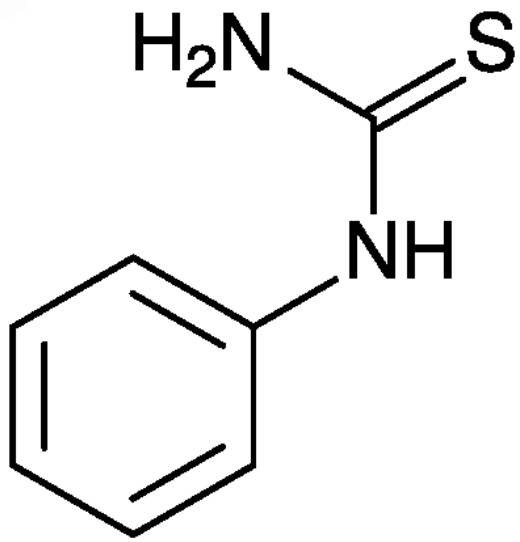
- Cannot taste anything bitter
- *Rating 0*

Record your own result on your sheet

What are you tasting?



PTC

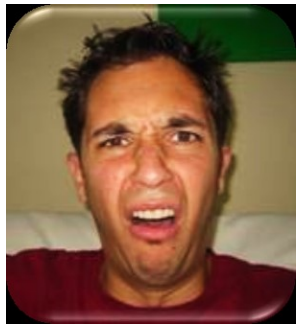


Phenylthiocarbamide

PTC is similar to the natural compounds found brussels sprouts

PTC only tastes bitter to around 70% of people. To the other 30% it is completely tasteless.

What was your phenotype?



Why can only some of you taste it?

The ability to taste PTC has a **genetic basis**.

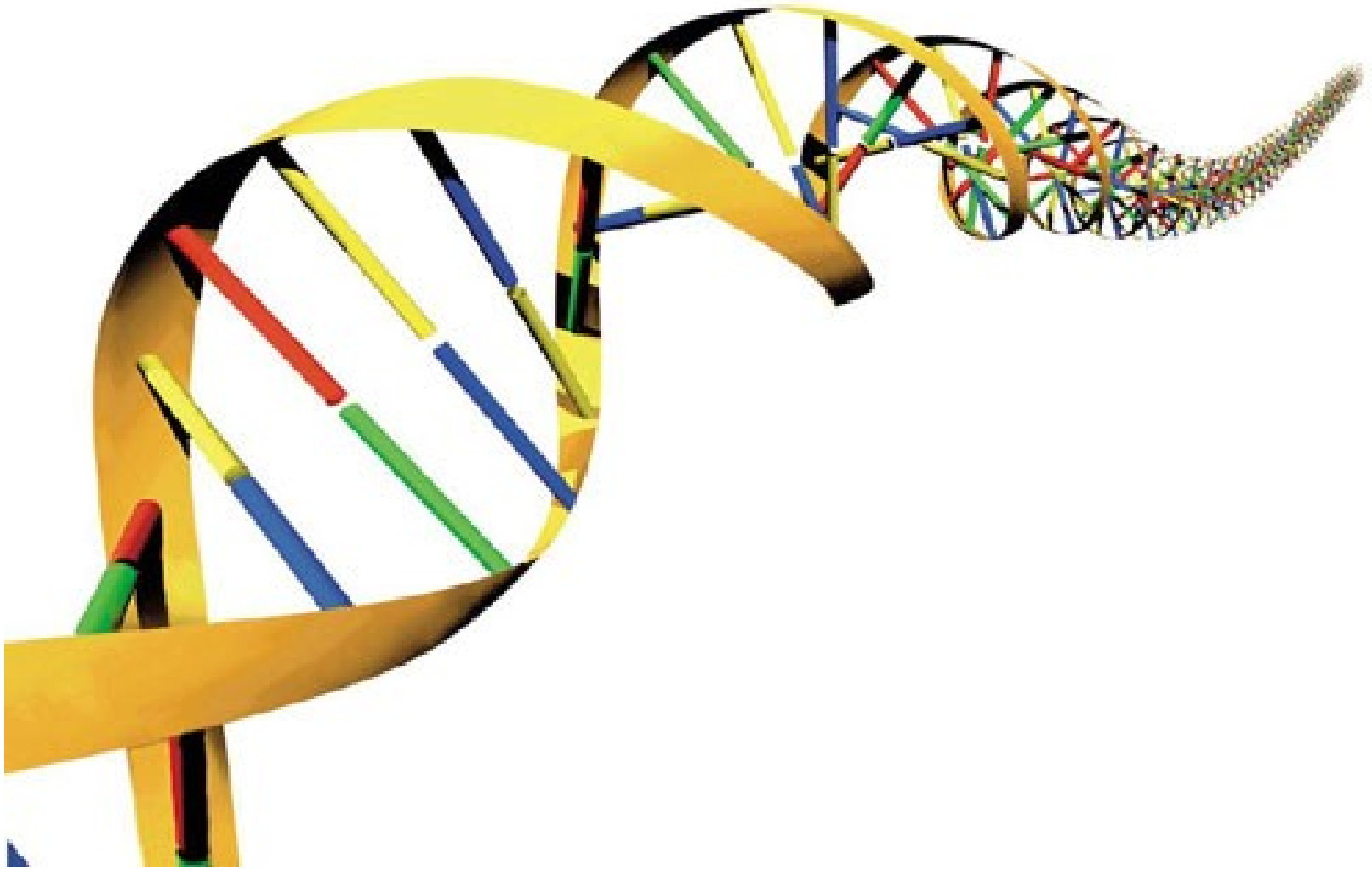
What is your genotype?

Your phenotype is determined by your genotype.

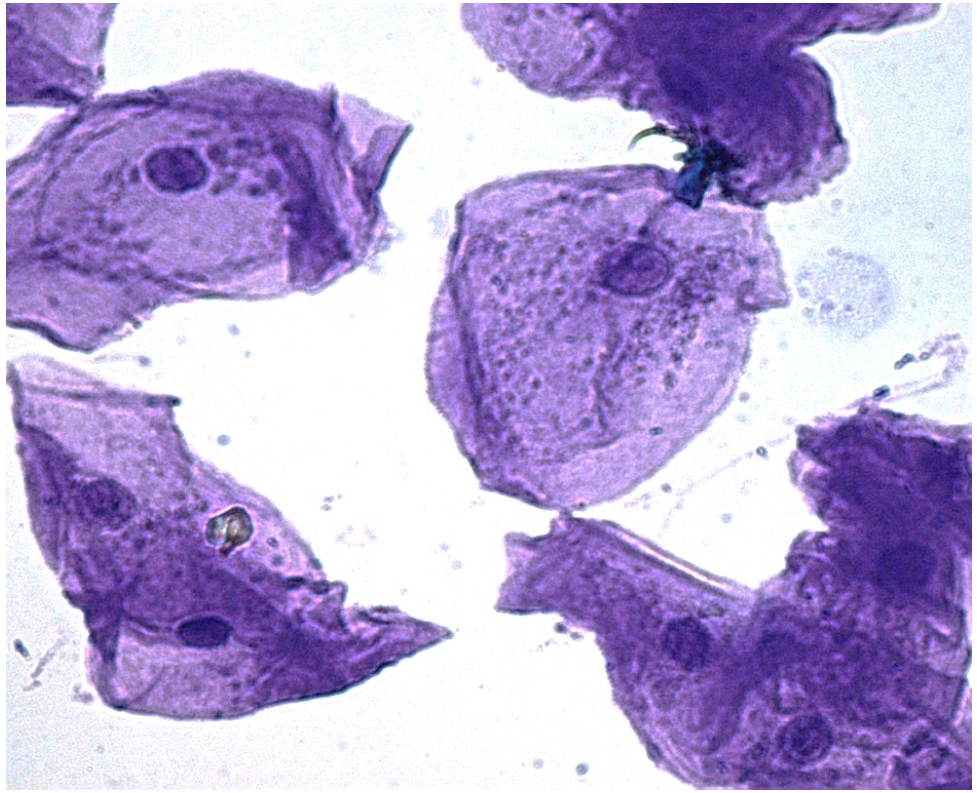
Today, you will see if your genotype matches your phenotype.

Genotype = An organism's unique DNA
Phenotype = Observable characteristic of an organism

What do we need to see your genotype?



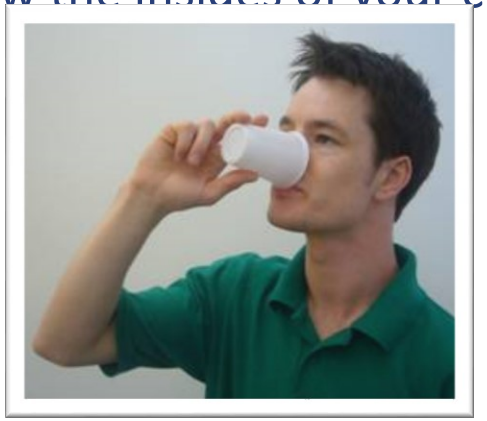
Collecting your cheek cells



1. Write your lab number on the cup.

2. Swallow any excess saliva, clean your mouth with your tongue.

3. Gently chew the insides of your cheeks for 1 minute.



4. Swill your mouth with the salt water in the cup for 30 seconds. **Don't swallow it.**

5. Gently dribble liquid back into cup.

Make sure you only handle your own sample

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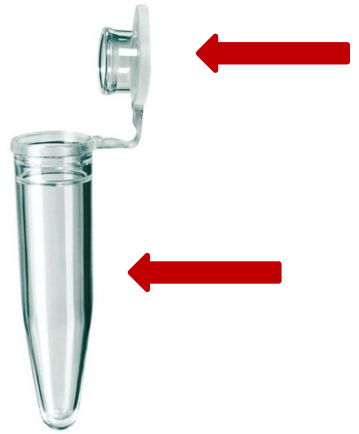
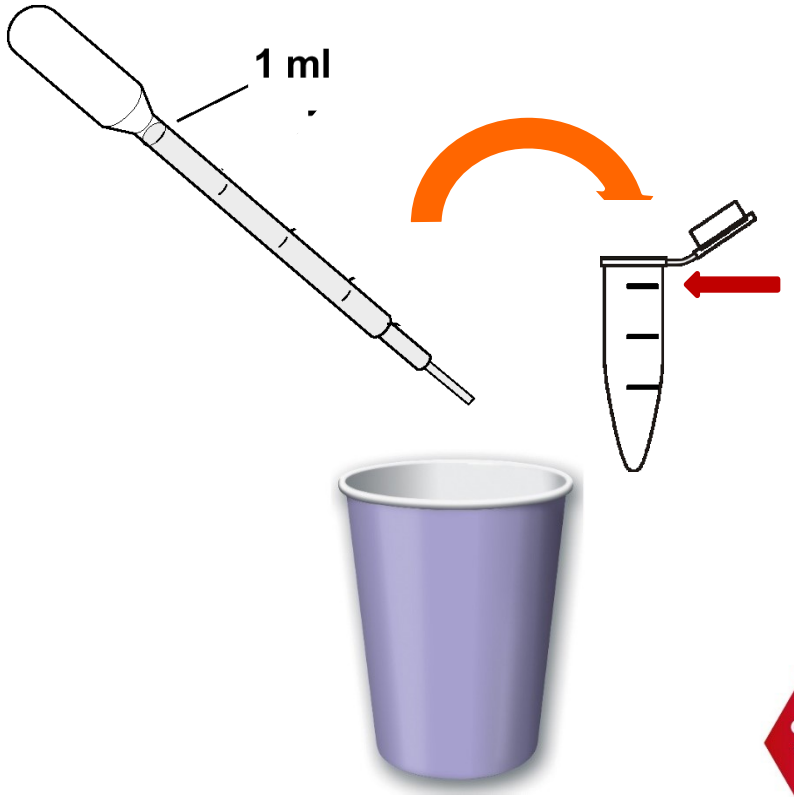
Concentrate your cheek cells



1) Use marker pen to write your lab number on the **top and side** of a tube

2) Pipette the cell sample into the tube. Make sure you **take sample from bottom of cup and fill it to the top line (1.5ml)**



3) Close lid and place tube in small white foam rack, **one person** take it to the centrifuge.

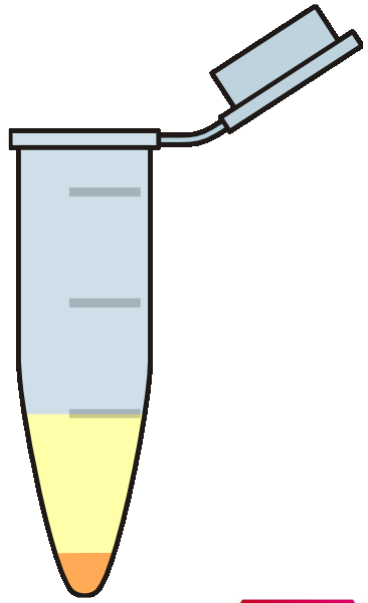
Samples will be centrifuged to concentrate cells at bottom of tube

Centrifuge spins tubes at 14,000 revolutions per minute (rpm) for 2 minutes

Heavier material (the cells) is thrown outwards and collects to form a **pellet**

Liquid
(saliva, salt water)

Pellet
(cheek cells)



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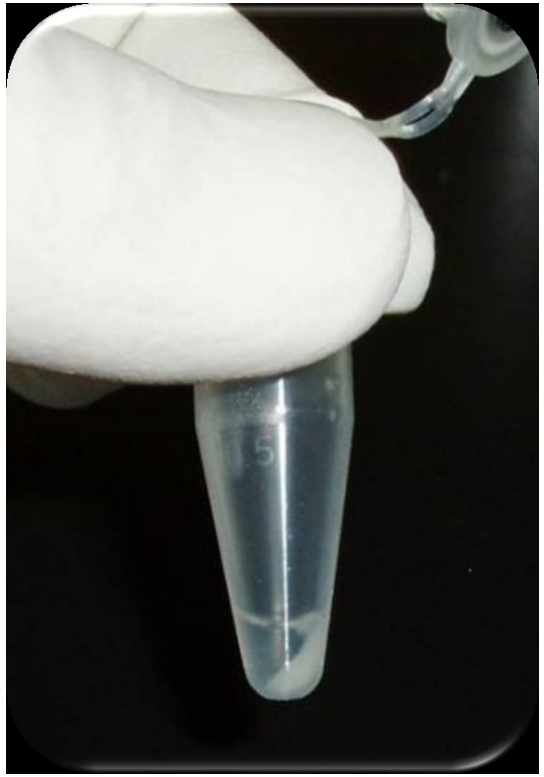
Discard the liquid



1) Pour most of the liquid **back into cup**.

2) Place the end of the tube on a **tissue** (to get out more liquid)

3) **Close** lid

Repeat if there are not enough cells.

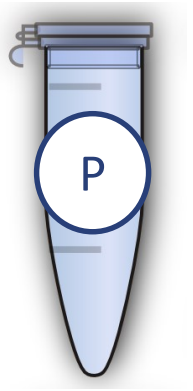
Create a Cell Suspension (soup)

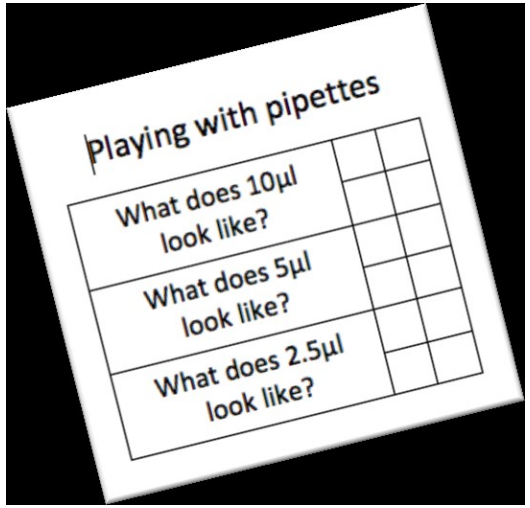
1) Break up the pellet of cells.

2) The cell suspension should be a **cloudy liquid with no lumps**.

3) When finished, place tube in your plastic rack.

Using Micropipettes- gather your equipment





P20 pipette

P200 pipette

Tips

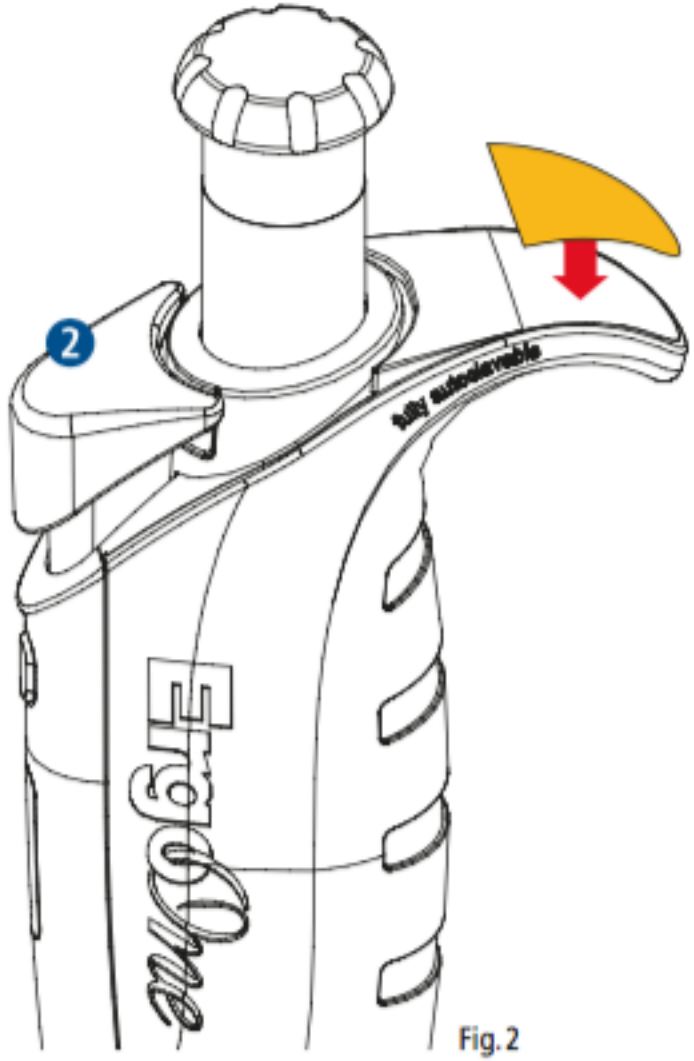
Practice dye

Practice card

How to hold a micropipette

Micropipette identification

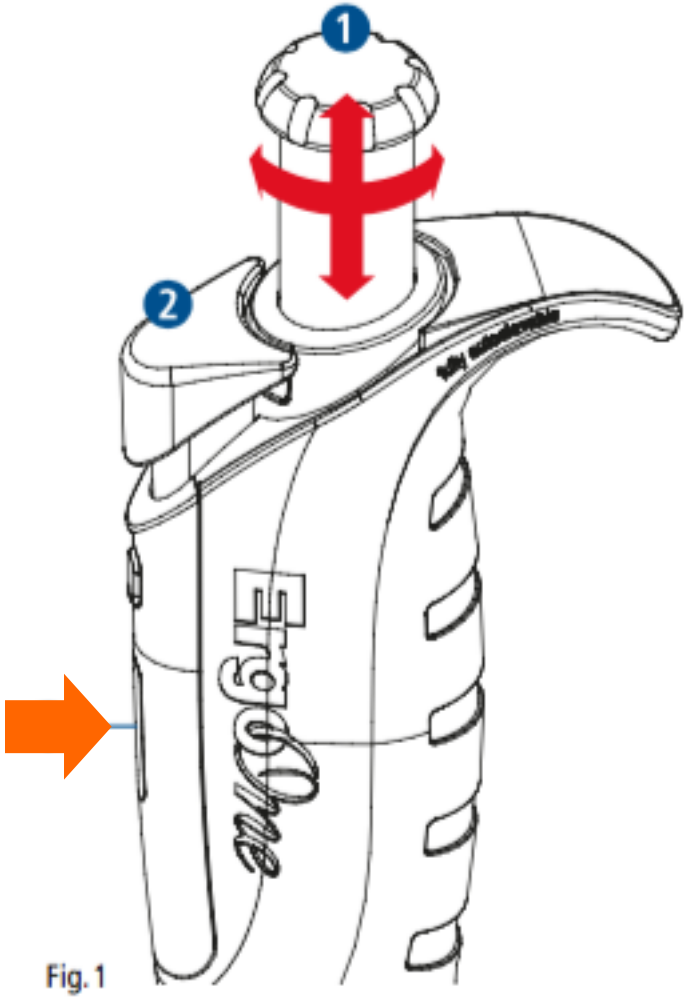
1 - 20 µl 20-200 µl



Setting the volume

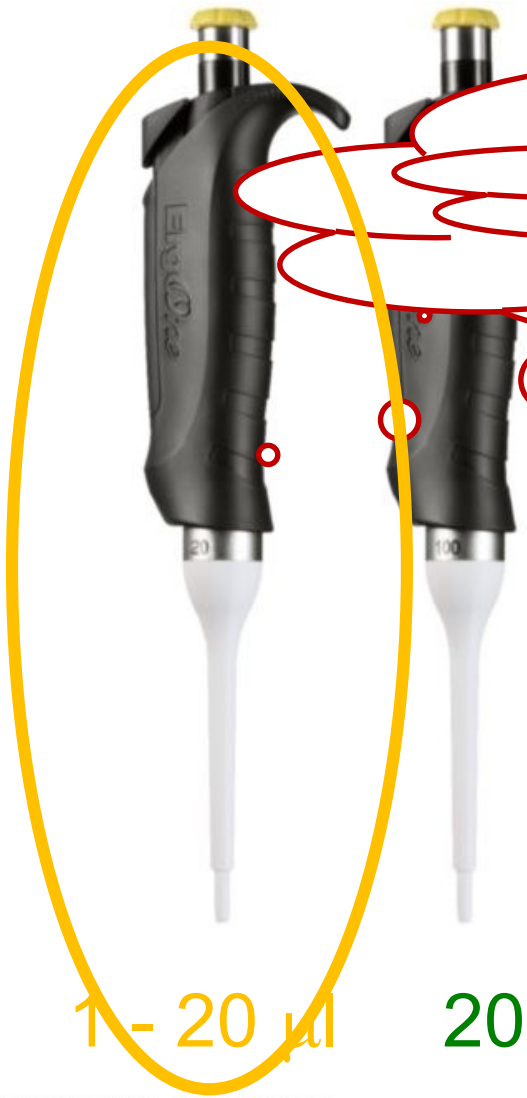
1 - 20 µl

20-200 µl



Let's practice setting the micropipettes!

1 ml =1000 µl



Can you set the pipette for 10 µl?

Pipette set for 10 µl

1- 20 µl

20-200 µl

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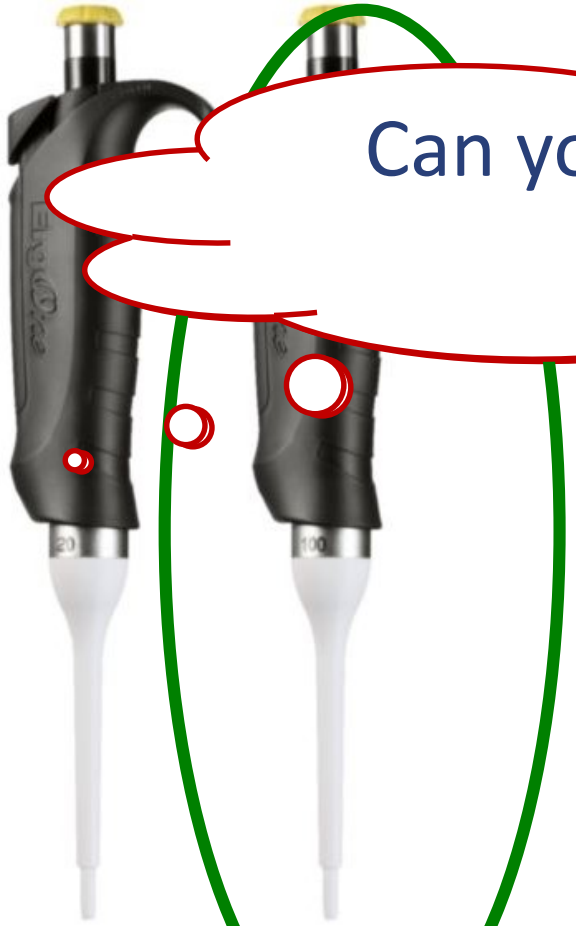


Can you set the pipette for 30µl?

1 - 20 µl

20-200 µl

Pipette set for 30 µl

Taking up liquid

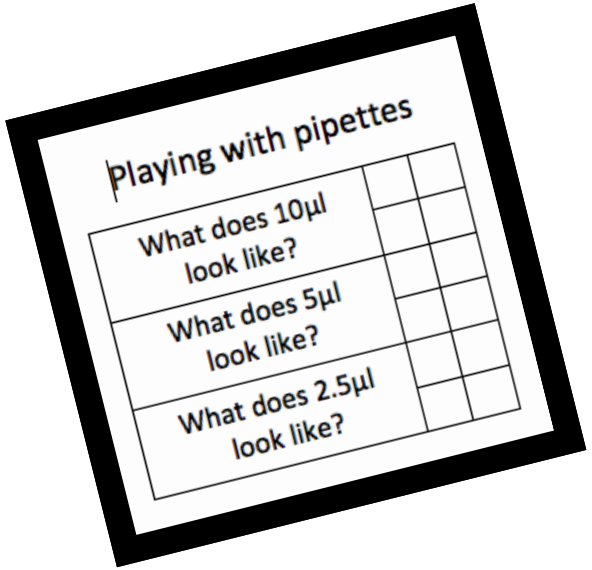
- Put tip on pipette
- Press plunger down to 1st stop
- Place tip in liquid
- Release plunger SLOWLY

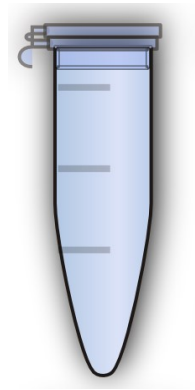
Dispensing liquid onto card

- Place tip where you want liquid to go
- Press plunger right down to 1st stop (when **putting liquid into a tube** we use the 2nd stop!)
- Move tip away from liquid
- Release plunger

Volume control
Plunger

Disposable tip





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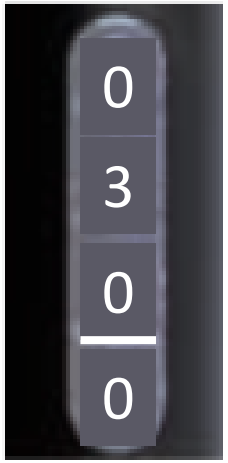
Extract DNA from cells

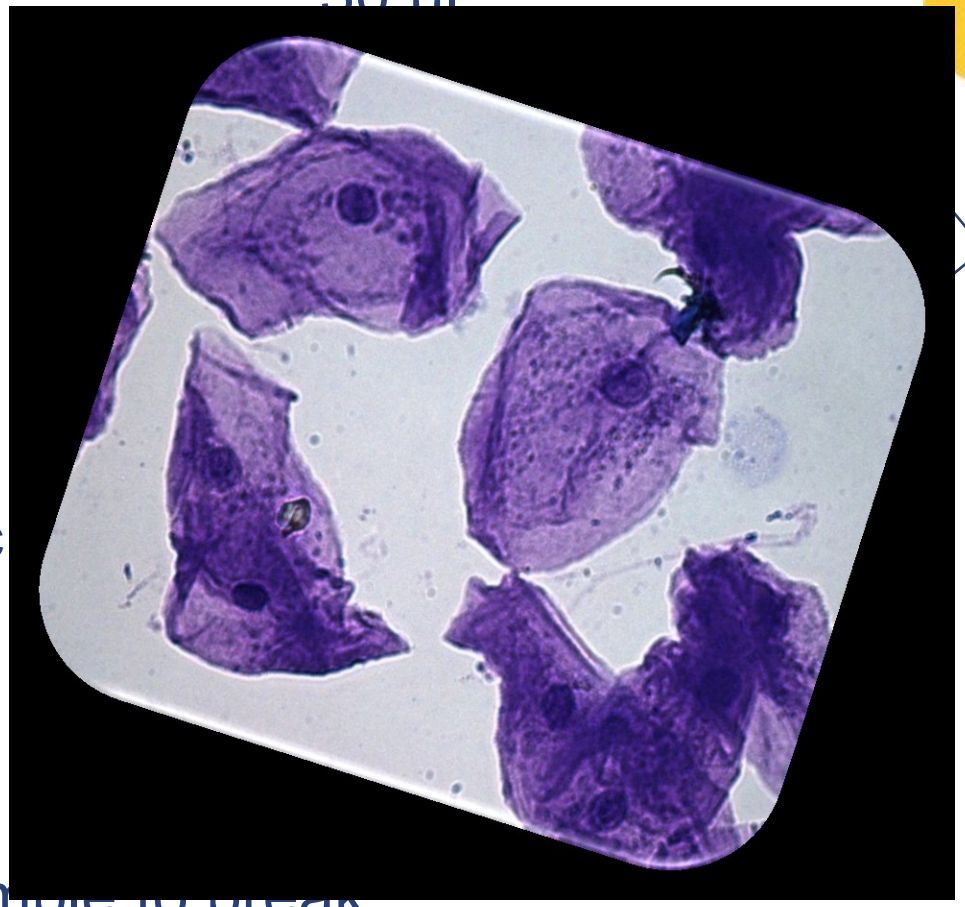
1. Write your number on the lid **and** side of the screw cap tube containing Chelex beads.

2. Check that micropipette is set to 30µl.



3. Place a tip on pipette and cut the end of it at an angle.

30 µl

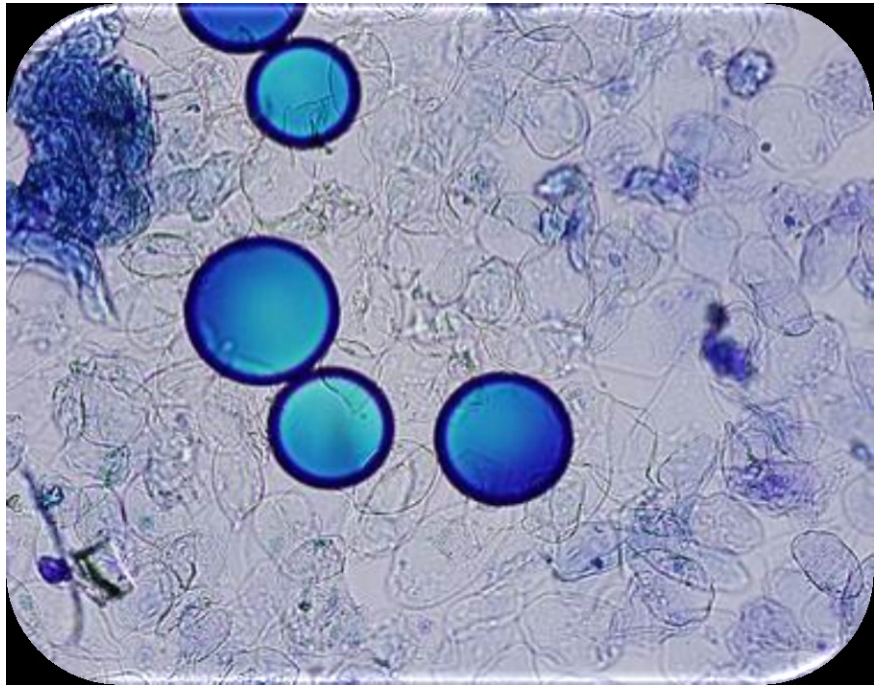


4. Transfer 30µl of cell suspension **from** your sample tube **to** your new screw cap tube.

5. Screw lid down **tightly** on screw-cap tube, **flic**
it and place in foam rack.

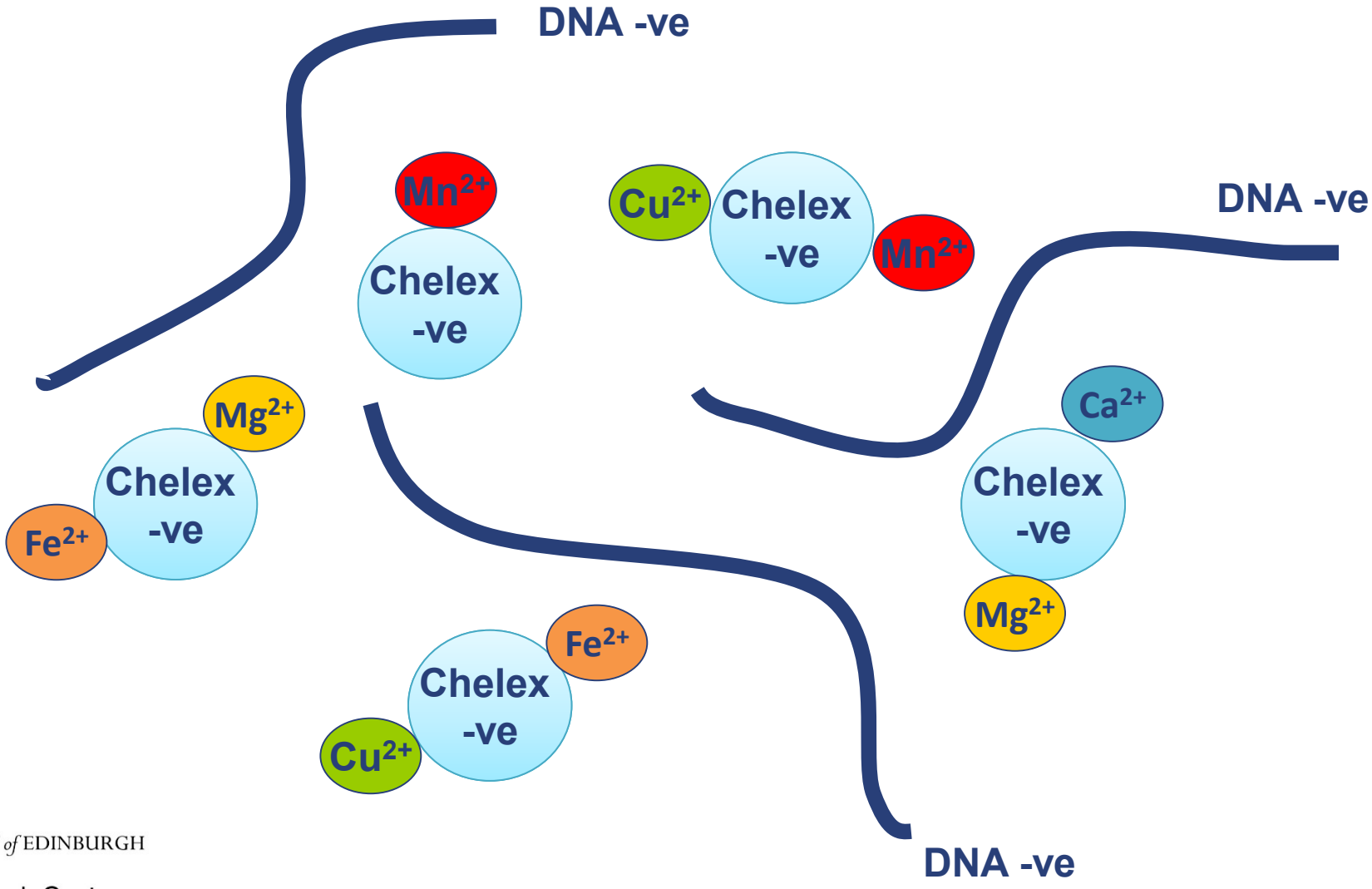
6. One person take it to the **vortex** and heat sample to break cell membranes and release DNA (100°C for 10 min)

What are the beads doing?

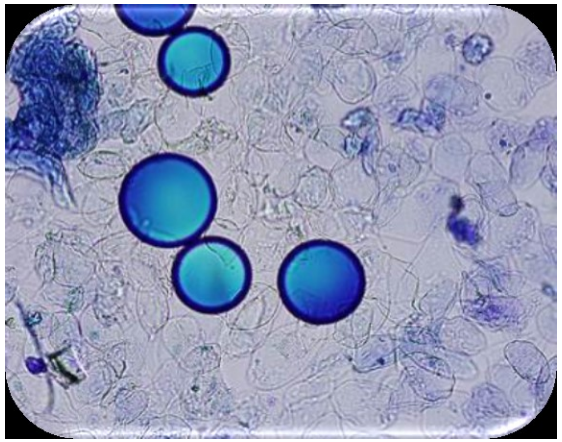


Chelex beads trap things which might help cut the DNA

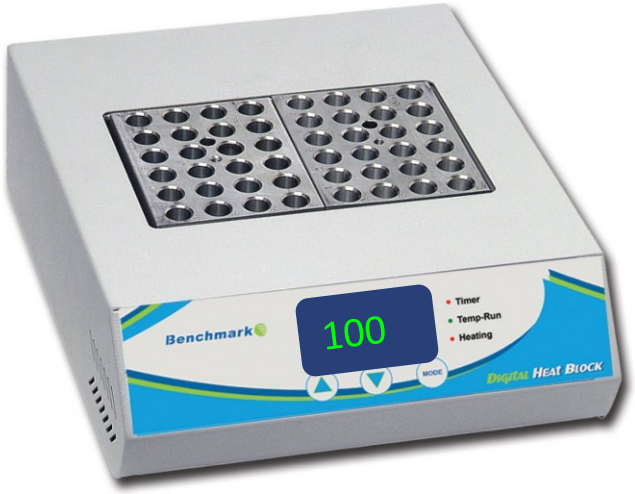
Chelex beads bind positive metal ions



So at the moment…



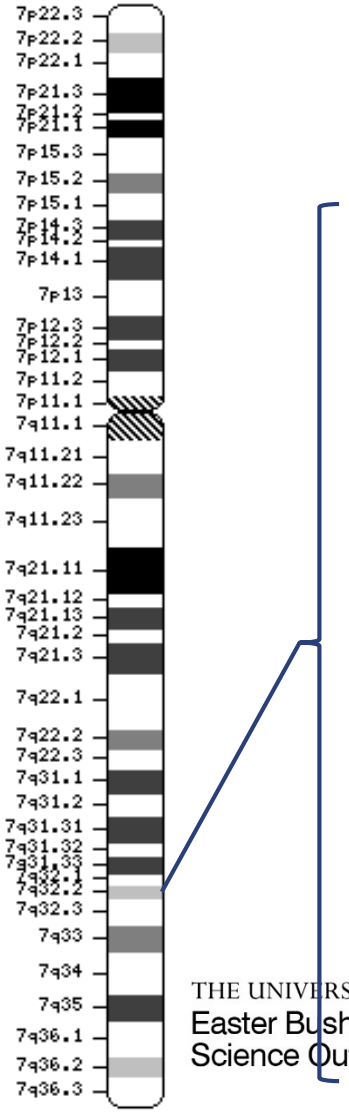
Chelex beads



heating

Centrifuge to remove used beads and cell debris

Today we are looking at the gene TAS2R38



CCTTTCTGCACTGGGTGGCAACCAGGTCTTTAGATTAGCCAACTAGAGAAGAGAAGTAGA
ATAGCCAATTAGAGAAGTGACATC**ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTAT**
GAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACC
AATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCAGCCACTGAGCAAC
AGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTC

This is the gene which encodes for a taste receptor protein that detects PTC

AACTCTCGTGACCCCAGCCTGGAGGCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTT
TTCTGCTTCTTTGTGATATCATCCTGTGCTGCCTTCATCTCTGTGCCCCTACTGATTCTG
TGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGCTTGTCCCTCTGGG
CATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTG
CTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACACTG
TGCTGAGAATGGACATGAAATGAGCTCTTCATTAATACGCCTGTGAGTCTTCATAAATAT
GCC

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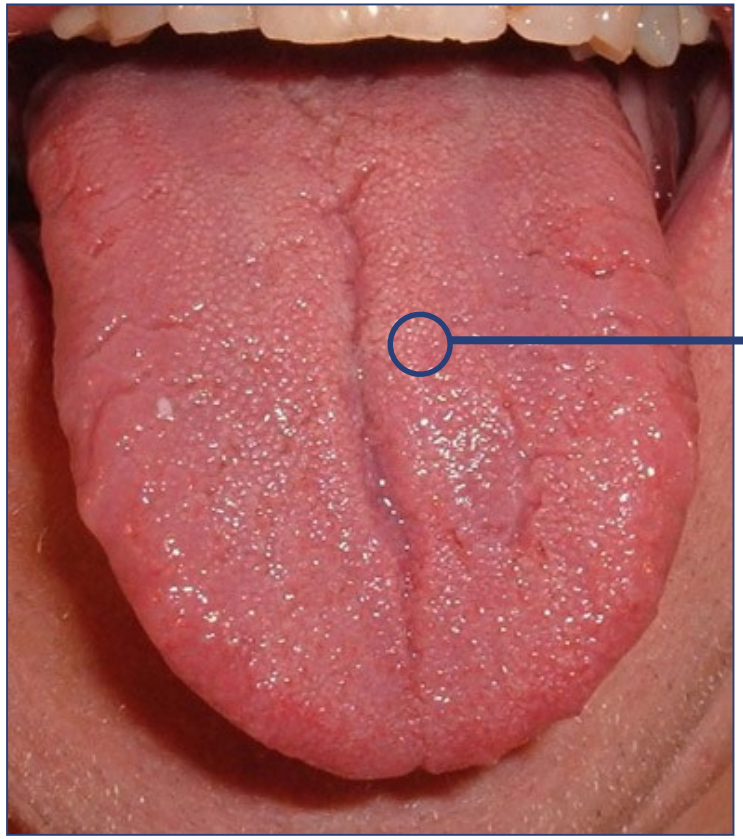
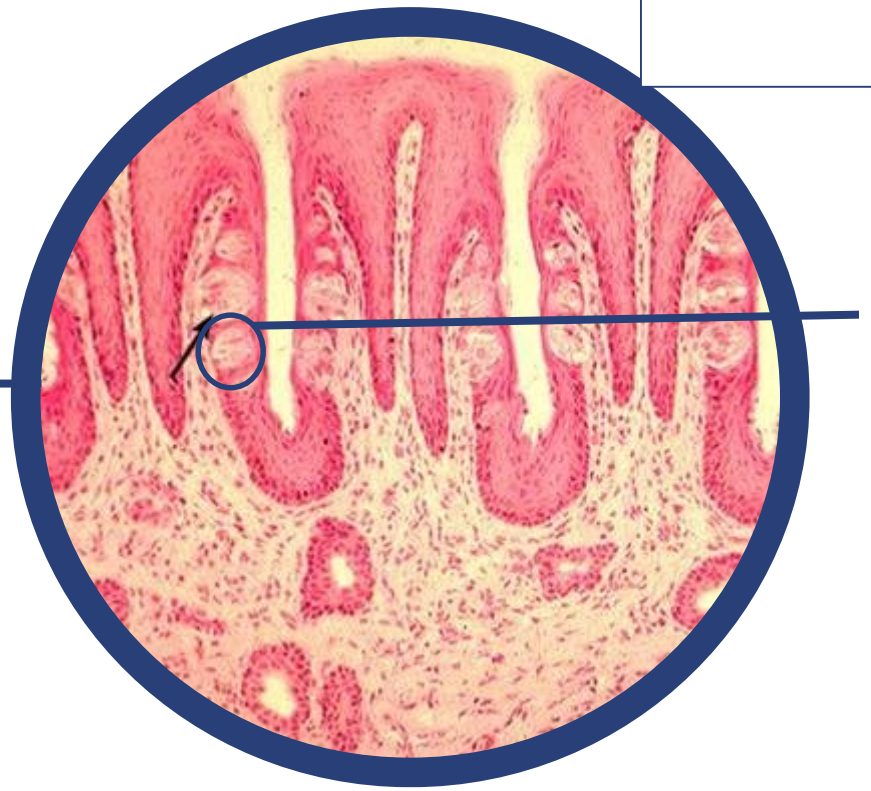
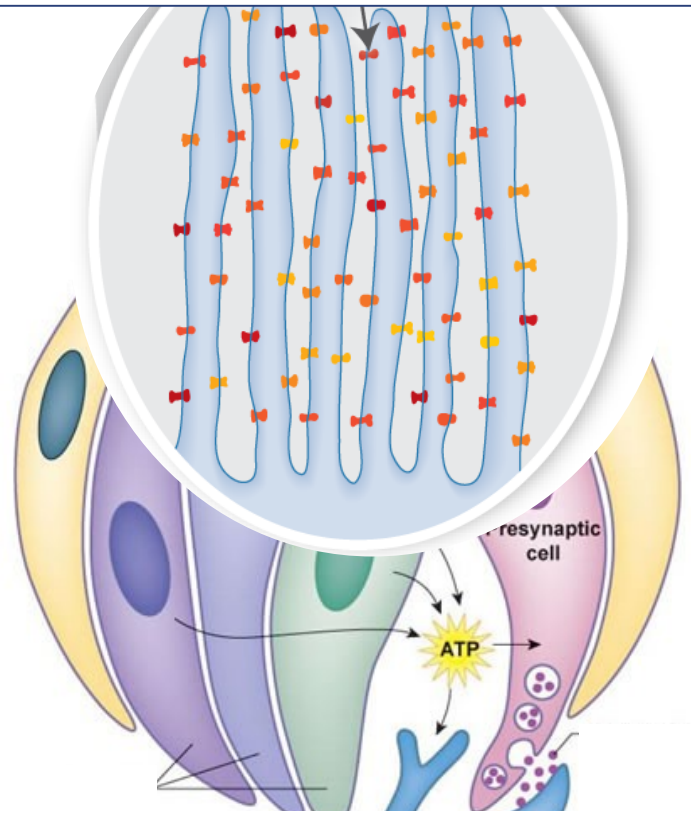
Does your tongue have taste receptors for PTC?

TAS2R38 taste receptor protein detects PTC







This is a single taste bud.

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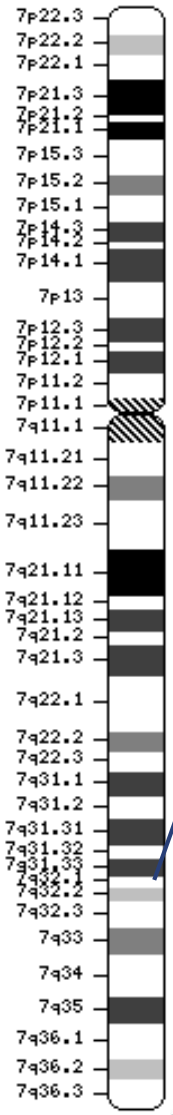
Today we're going to make billions of copies of this part of the gene



CCTTTCTGCACTGGGTGGCAACCAGGTCTTTAGATTAGCCAACTAGAGAAGAGAAGTAC
ATAGCCAATTAGAGAAGTGACATC**ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTA**
GAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACC
AATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCAGCCACTGAGCAAC

You will use PCR to make many copies of the part of the TAS2R38 gene we want to investigate.

CATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTG
CTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACACTG
TGCTGAGAATGGACATGAAATGAGCTCTTCATTAATACGCCTGTGAGTCTTCATAAATAT
GCC

How?

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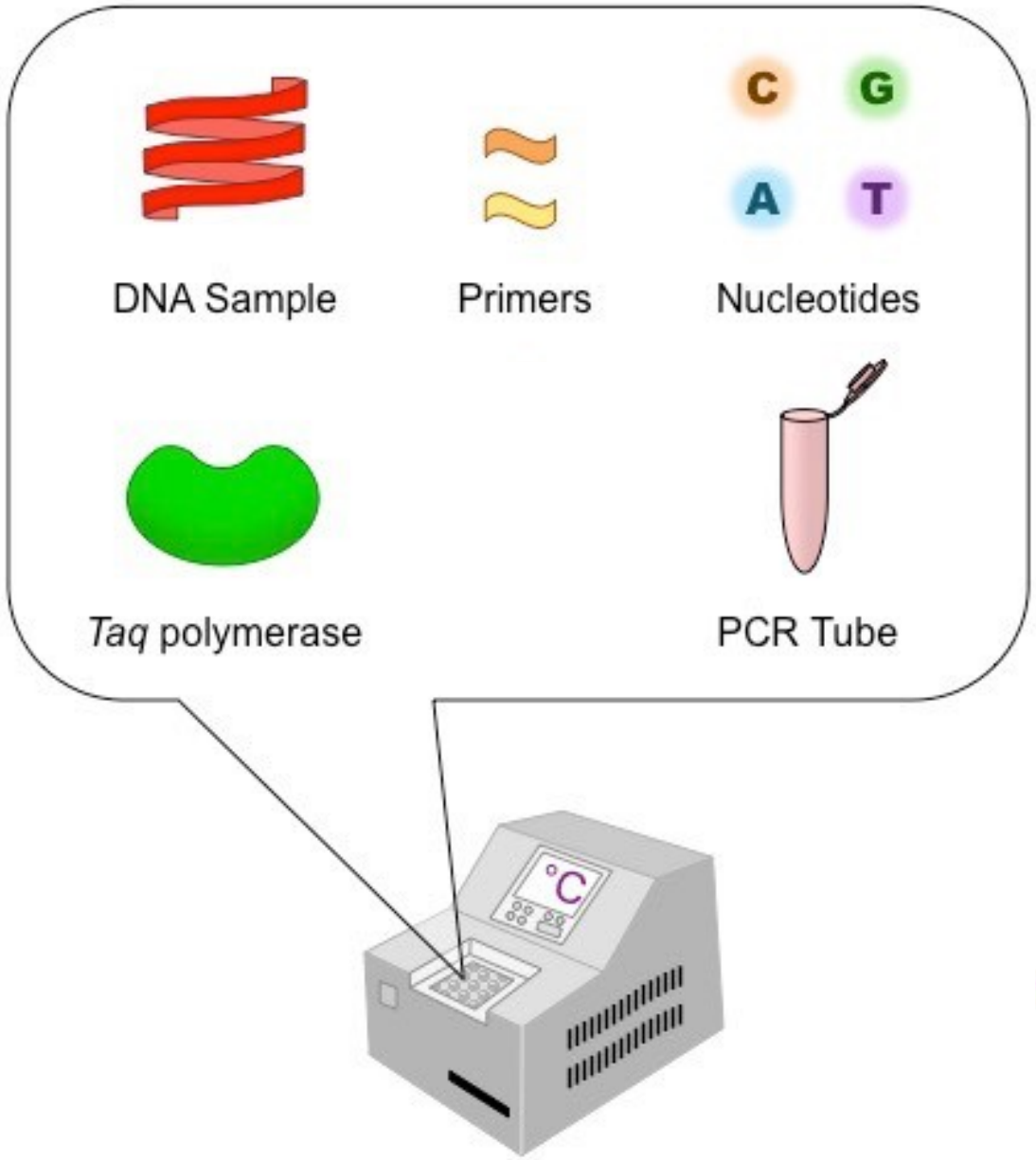
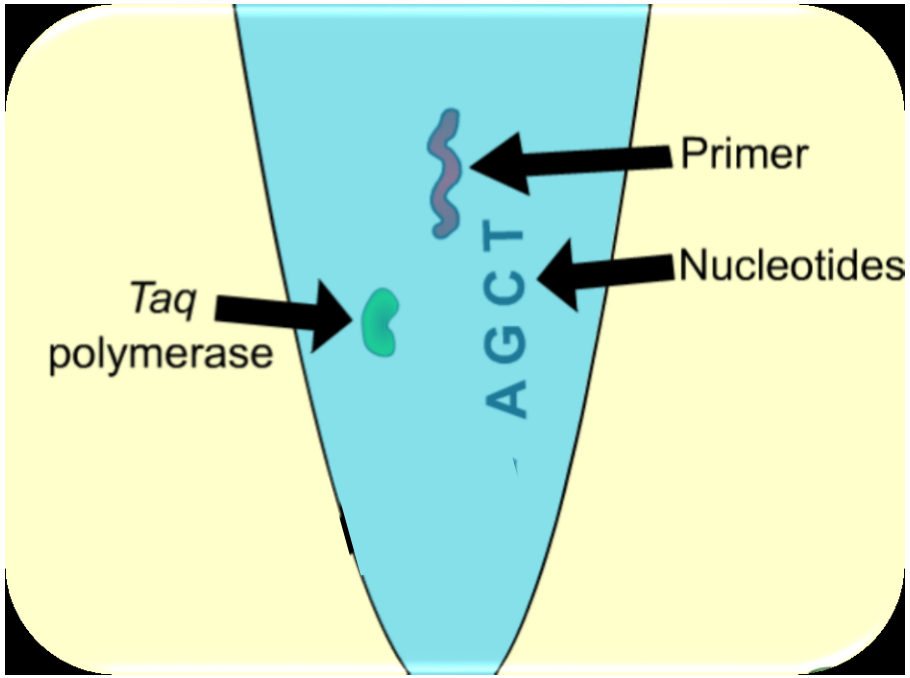
What are the *ingredients* for PCR?



Setting up PCR reaction

Your will each receive a PCR tube containing



You will add the final ingredient:

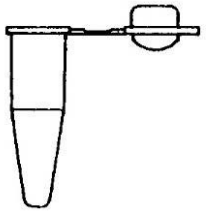
your DNA!

Short break

Remove your lab coat if leaving the lab.

Setting up PCR reaction

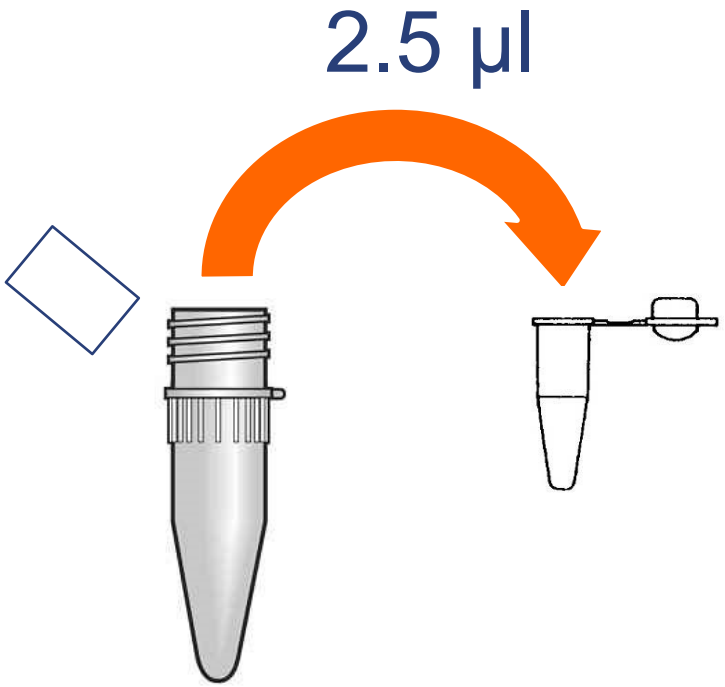
1. Your tiny PCR tube is on ice



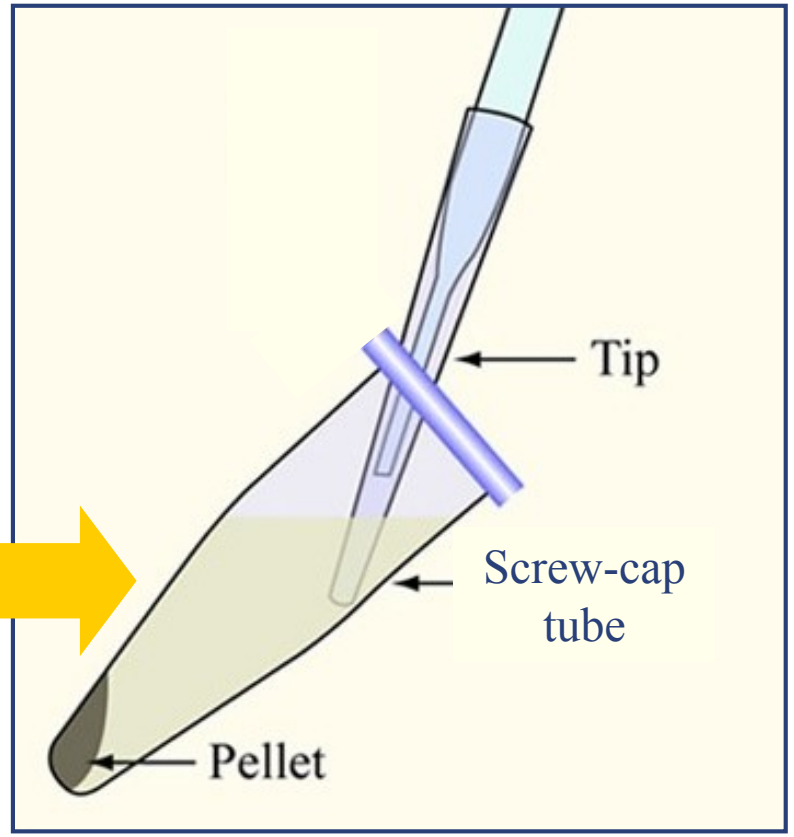
2. Write your number on lid **and** side, then place back on ice

3. Set pipette for 2.5 μl

Add 2.5µl of your DNA to the PCR mix – check you have DNA!



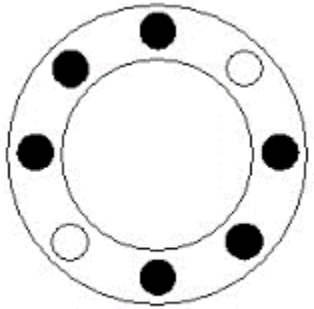
Take DNA from top of sample, avoiding the beads



Centrifuge your tubes

Open the centrifuge

Put in the tubes, make sure they are balanced!



6 Tubes

Close the centrifuge and turn on for 30 seconds then turn off

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Your DNA now goes in the PCR machine

The PCR machine is programmed to run through all the required temperatures, and repeats the cycle 35 times

A reminder about PCR

Recreate the PCR reaction

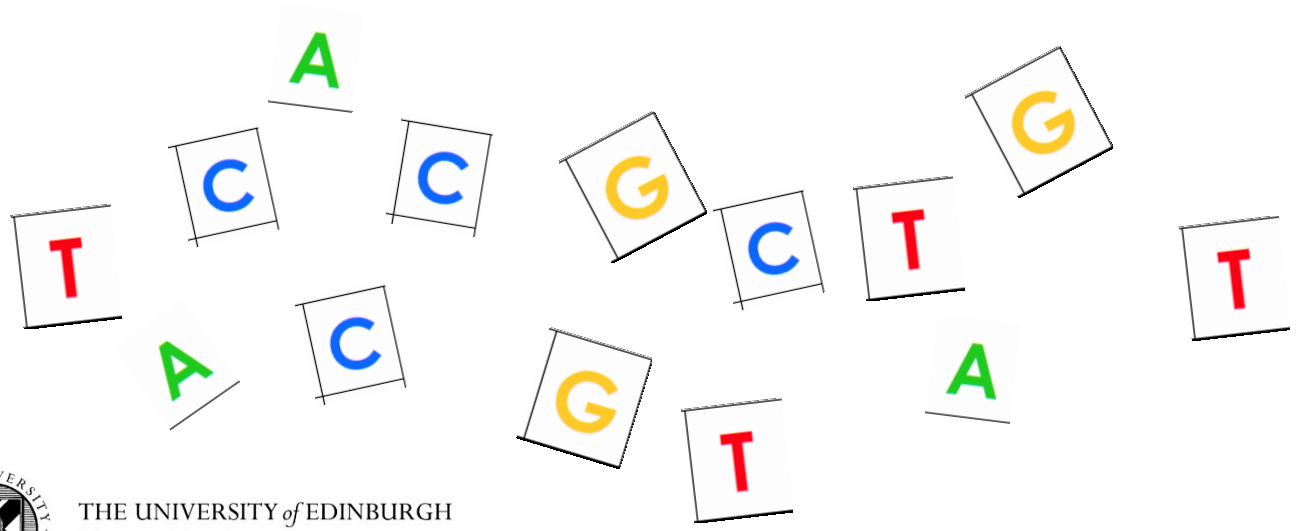
G	A	C	C	G	A	T	G	A	C	A	G	A	T	G	A	T	T	A	G	G	A	A	A
C	T	G	G	C	T	A	C	T	G	T	C	T	A	C	T	A	A	T	C	C	T	T	T

DNA

C	C	G	A	T

A	T	C	C	T

primers



nucleotides

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LUNCH

Please be back here for 1pm!

What have we done so far?

Discovered our phenotype via PTC taste test

Sampled our own DNA

Burst open the cells and removed impurities from sample

Run a PCR to amplify a specific region of the PTC tasting gene

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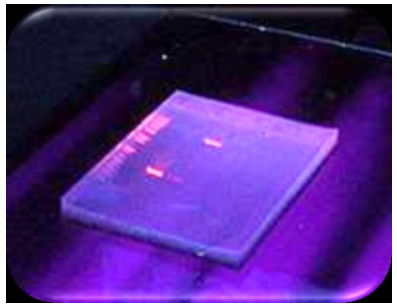
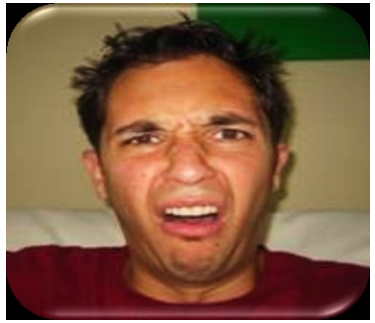
What will you do next?

Use molecular biology techniques to analyse your DNA



Compare the taste test results with your DNA analysis

How does the TAS2R38 gene make us a….

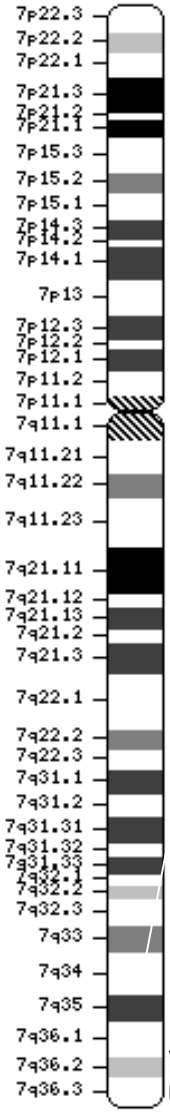


Strong taster

Weak taster

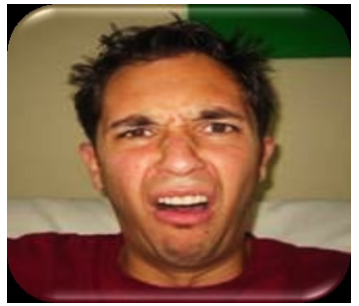
Non-taster

A difference in just one nucleotide of *TAS2R38* gene

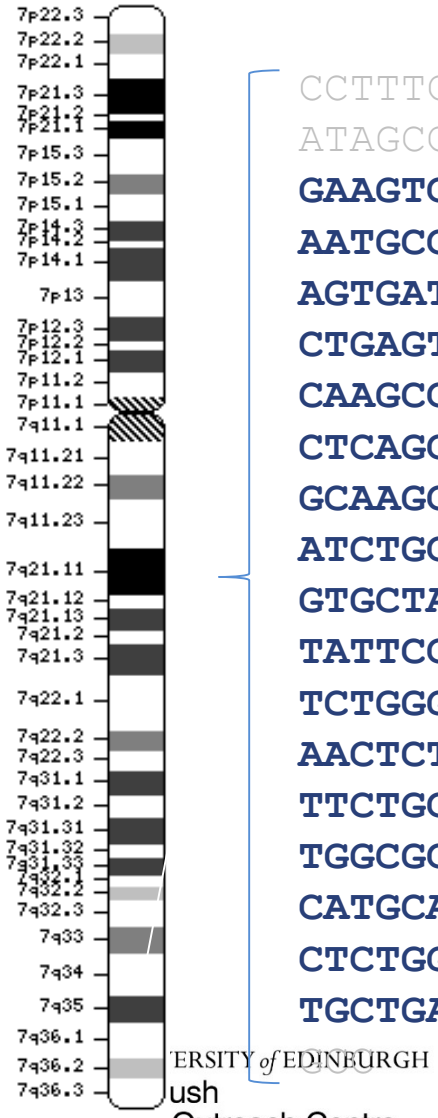


Point mutation at nucleotide 145

CCTTTCTGCACTGGGTGGCAACCAGGTCTTTAGATTAGCCAACTAGAGAAGAGAAGTAGA
ATAGCCAATTAGAGAAGTGACATC**ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTAT**
GAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACC
AATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGGCCACTGAGCAAC
AGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTC
CTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCACTGAACCACAGCTAC
CAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTTGCTGCCTGC
CTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTG
GCAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGC
ATCTGCACTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGACCTCACTTCACAGTCACAACT
GTGCTATTCATGAATAACAATACAAGGCTCAACTGGCAGATTAAAGATCTCAATTTATTT
TATTCCTTTCTCTTCTGCTATCTGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCT
TCTGGGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGTCTATACCAGA
AACTCTCGTGACCCCAGCCTGGAGGCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTT
TTCTGCTTCTTTGTGATATCATCCTGTGCTGCCTTCATCTCTGTGCCCCTACTGATTCTG
TGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGCTTGTCCCTCTGGG
CATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTG
CTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACACTG
TGCTGAGAATGGACATGAAATGAGCTCTTCATTAATACGCCTGTGAGTCTTCATAAATAT



A difference in just one nucleotide of *TAS2R38* gene



Point mutation at nucleotide 145

CCTTTCTGCACTGGGTGGCAACCAGGTCTTTAGATTAGCCAACTAGAGAAGAGAAGTAGA
ATAGCCAATTAGAGAAGTGACATC**ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTAT**
GAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACC
AATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGGGCACTGAGCAAC
AGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTC
CTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCACTGAACCACAGCTAC
CAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTTGCTGCCTGC
CTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTG
GCAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGC
ATCTGCACTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGACCTCACTTCACAGTCACAACT
GTGCTATTCATGAATAACAATACAAGGCTCAACTGGCAGATTAAAGATCTCAATTTATTT
TATTCCTTTCTCTTCTGCTATCTGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCT
TCTGGGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGTCTATACCAGA
AACTCTCGTGACCCCAGCCTGGAGGCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTT
TTCTGCTTCTTTGTGATATCATCCTGTGCTGCCTTCATCTCTGTGCCCCTACTGATTCTG
TGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGCTTGTCCCTCTGGG
CATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTG
CTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACACTG
TGCTGAGAATGGACATGAAATGAGCTCTTCATTAATACGCCTGTGAGTCTTCATAAATAT

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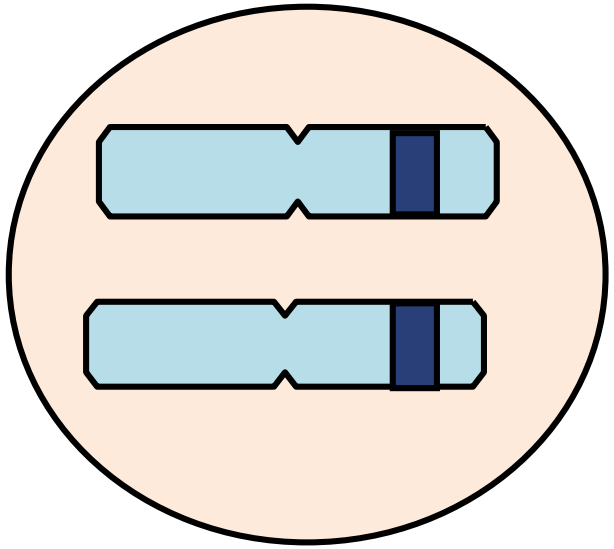
How many chromosomes do you have?

What do we call different forms of the same gene?

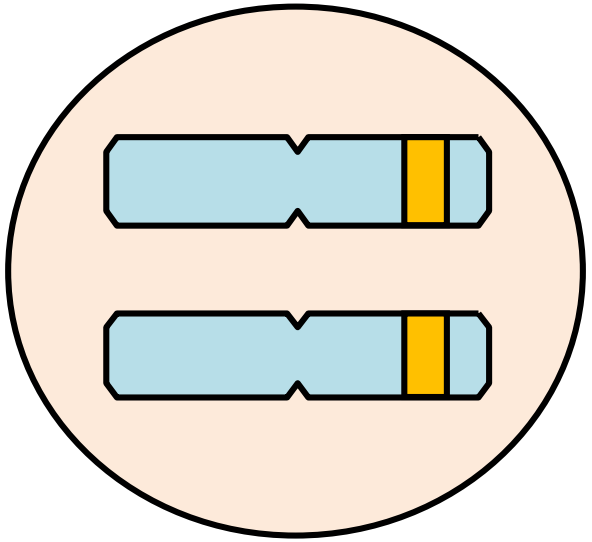
Humans have 23 pairs of chromosomes

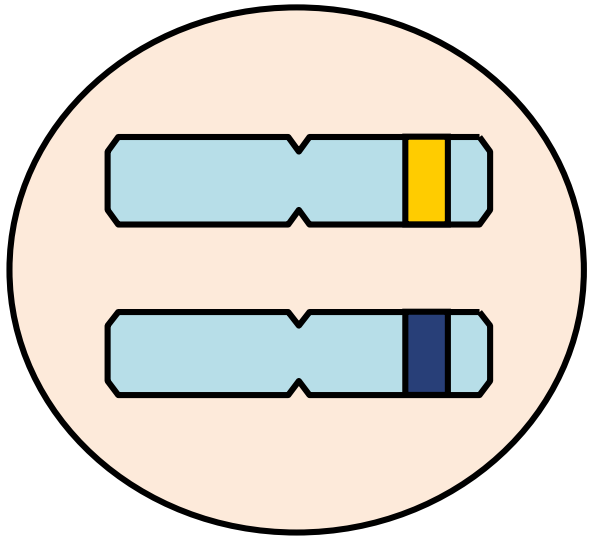
→ We have **2 copies of each gene**

Alleles are different forms of the same gene



Same alleles:





Different alleles:

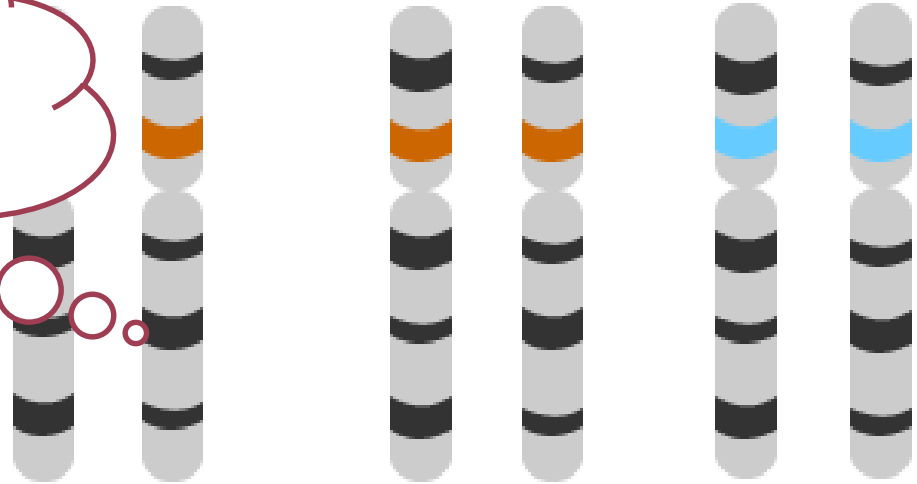
Alleles can be dominant or recessive

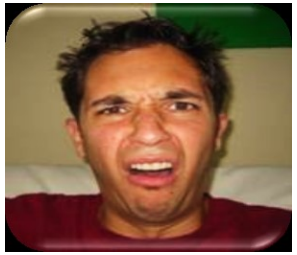
Allele for not tasting PTC (t)

Allele for tasting PTC (T)

PTC taster allele is dominant

Make a prediction… what do you think your genotype is?



GENOTYPE	Tt Heterozygous	TT Homozygous	tt Homozygous
PHENOTYPE			
	Weak taster	Strong taster	Non-taster

How can we tell if our DNA contains a C or G at position 145?

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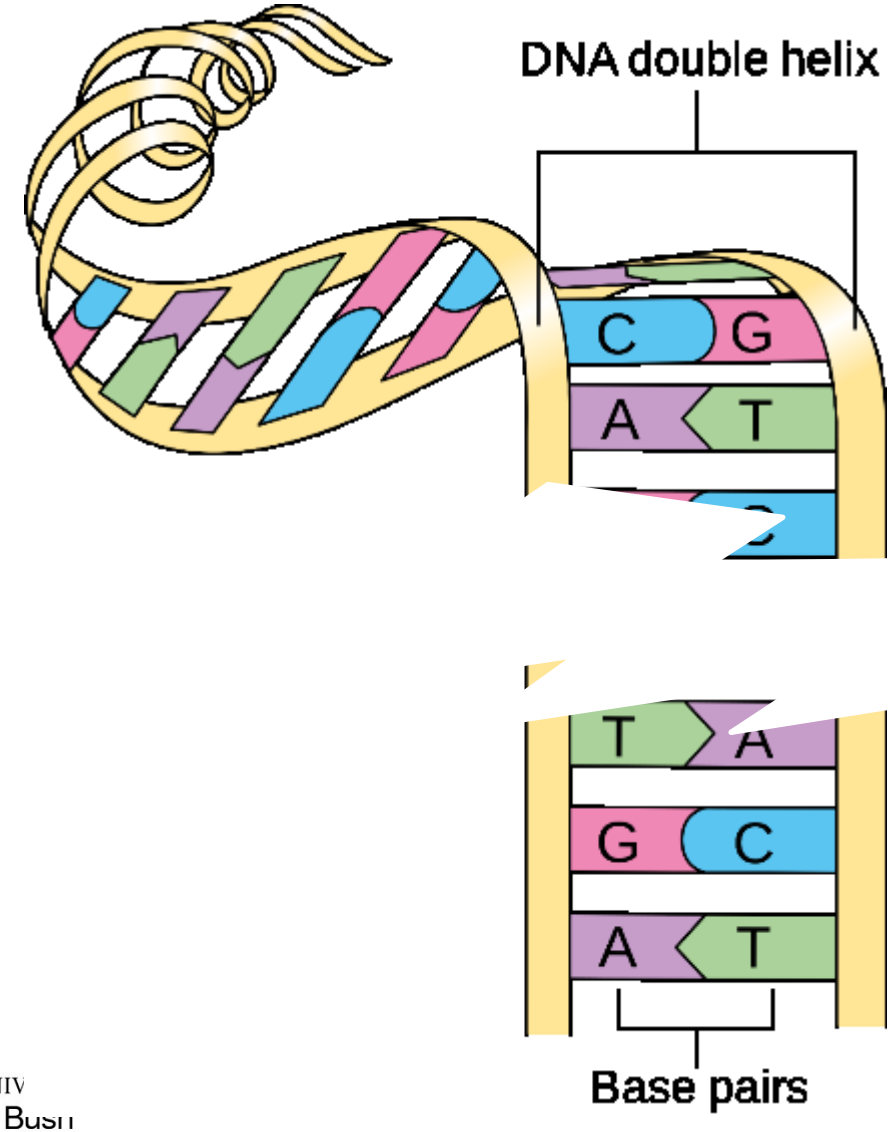
We can use restriction enzymes



Restriction enzymes cut the DNA at specific sequences

Different restriction enzymes cut different sequences

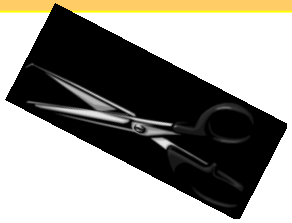
EcoRI

G A A T T C
C T T A A G

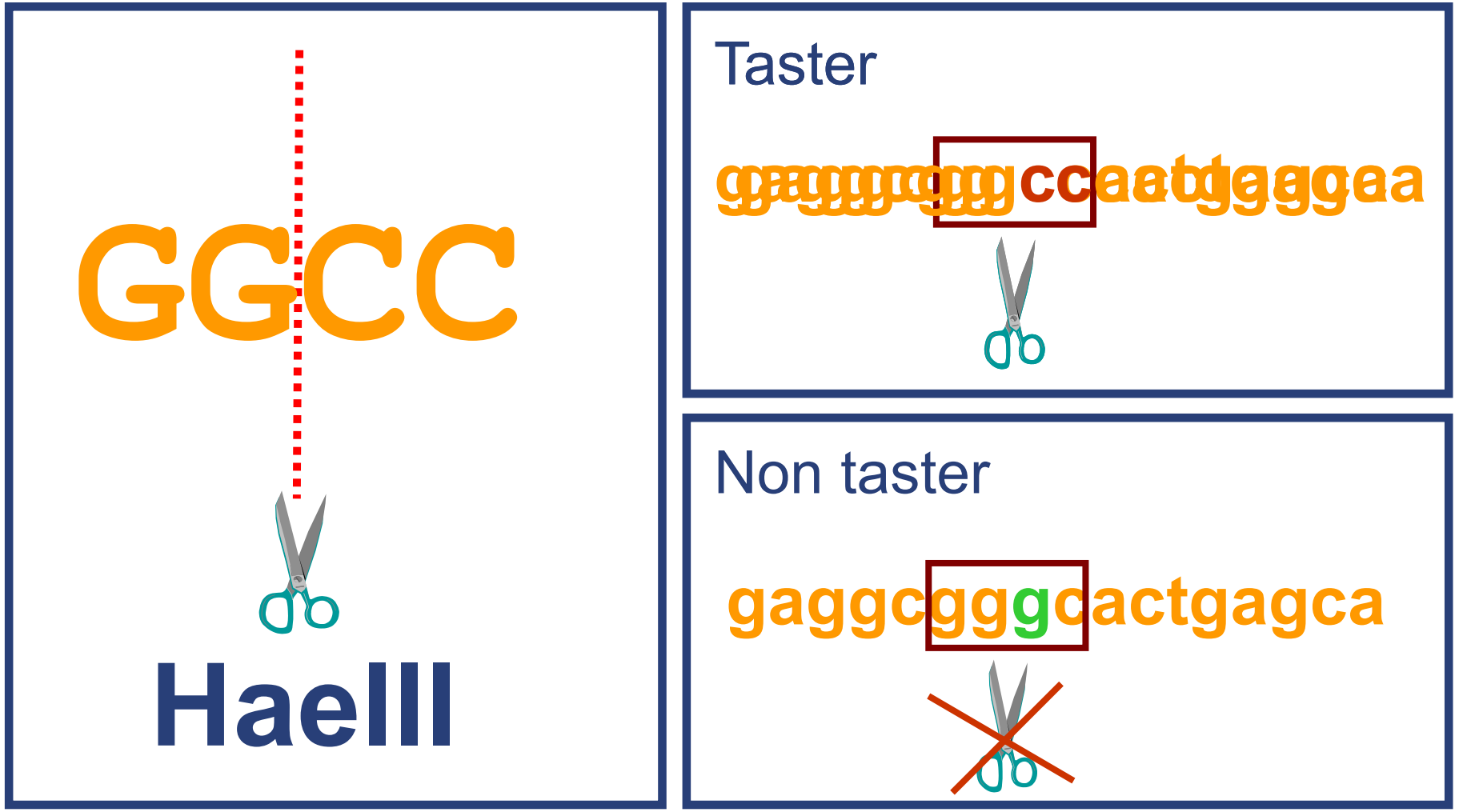
PstI

C T G C A G
G A C G T C

G A A T T C A C G T C T G C A G C C A A A T G G C G A A T T C C A
C T T A A G T G C A G A C G T C G G T T T A C C G C T T A A G G T



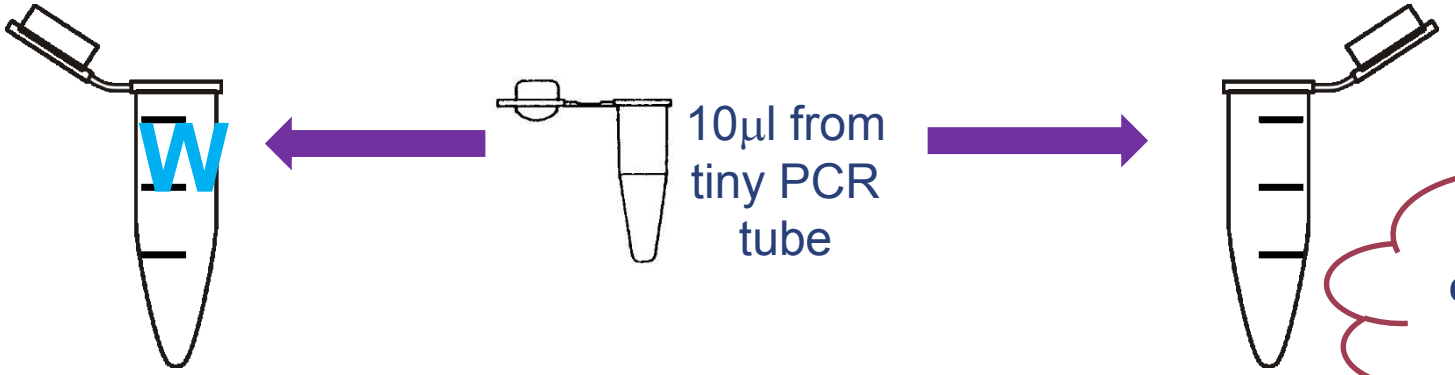
Which allele with HaeIII cut?



Safety first!

Setting up the enzyme reaction

1. Add 10µl of your PCR product to bottom of each tube



Why do we have no enzyme (water) in one tube?

2. To **W**, Add 5µl **water**

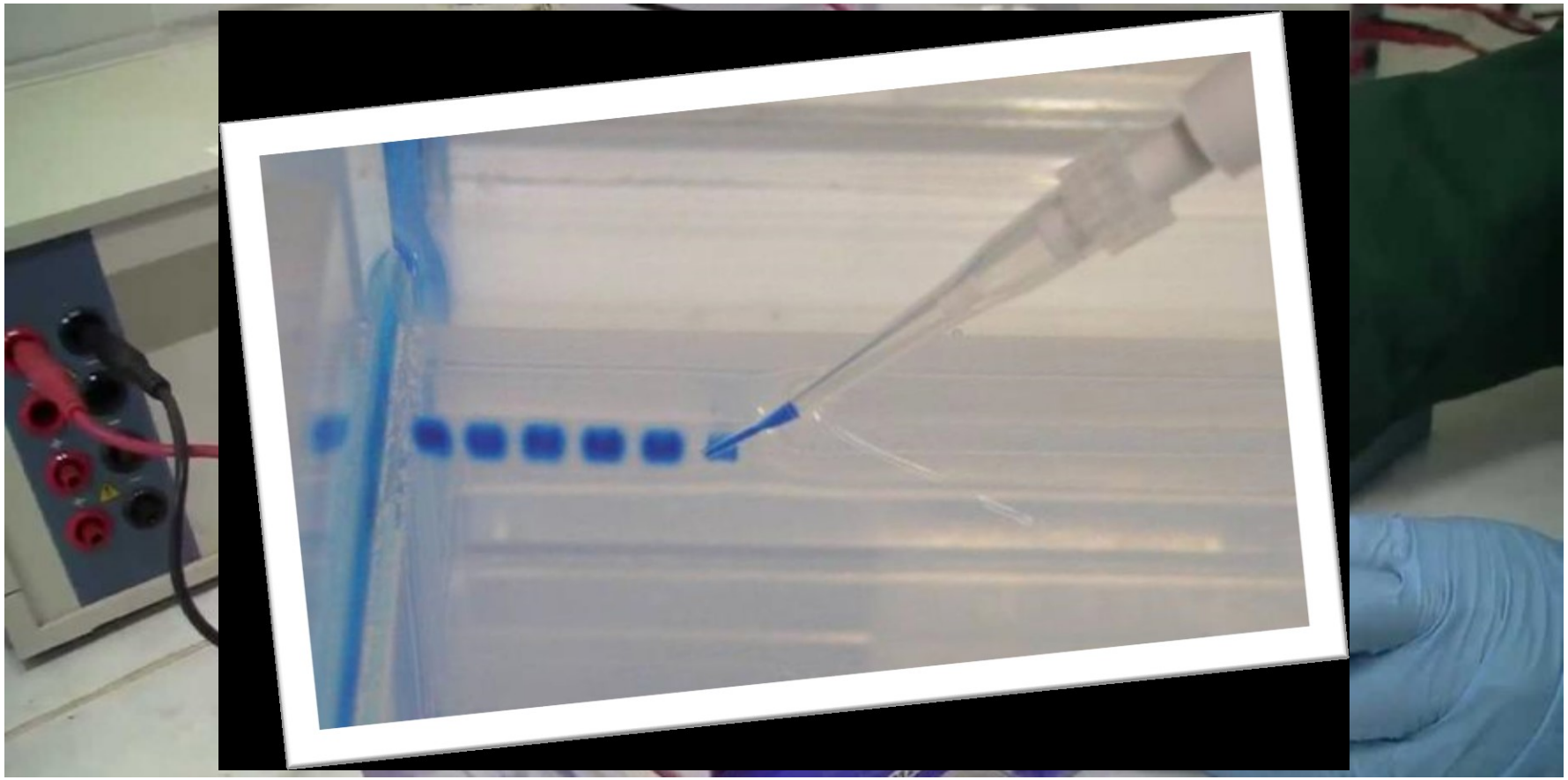
3. To **E**, Add 5µl **enzyme**

Change pipette tips between tubes!

4. Flick the tubes then centrifuge for about 30 sec and put it in a foam rack.

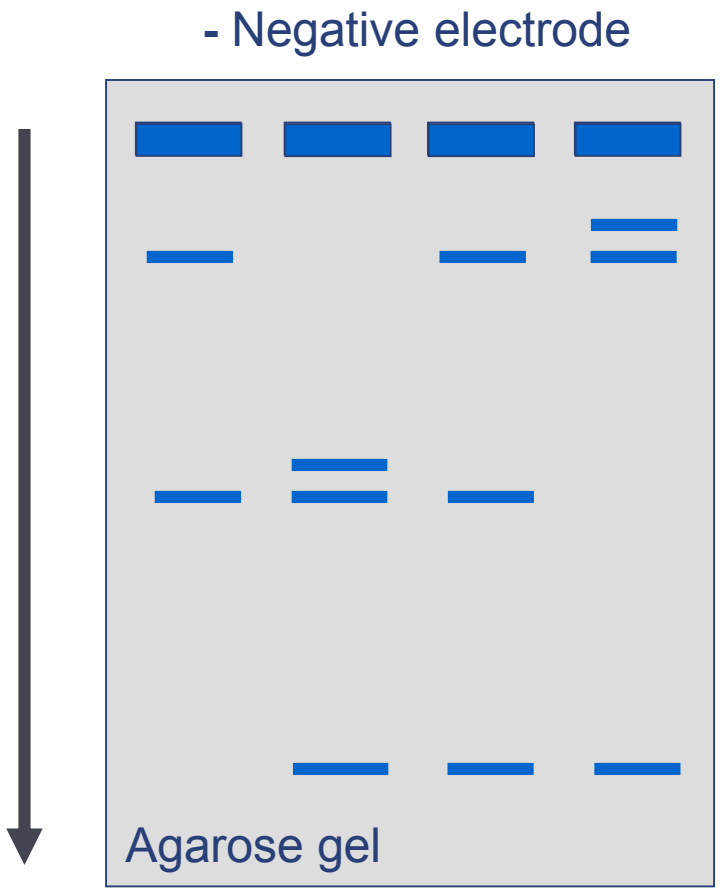
5. **One person** take the tubes to the water bath and then incubate at 37°C for 30 minutes

How can we tell if the enzyme has cut the DNA?



Gel Electrophoresis

Gel electrophoresis

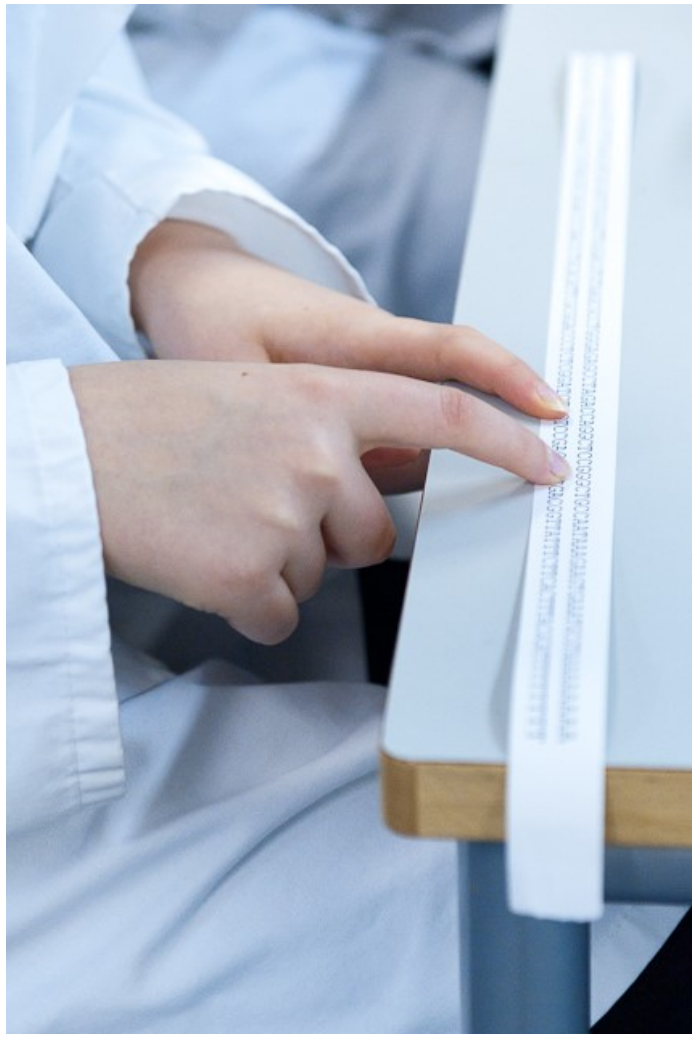


Larger DNA fragments move slower

Molecules of the same size travel the same distance and form a band

Smaller DNA fragments move faster

Group activity



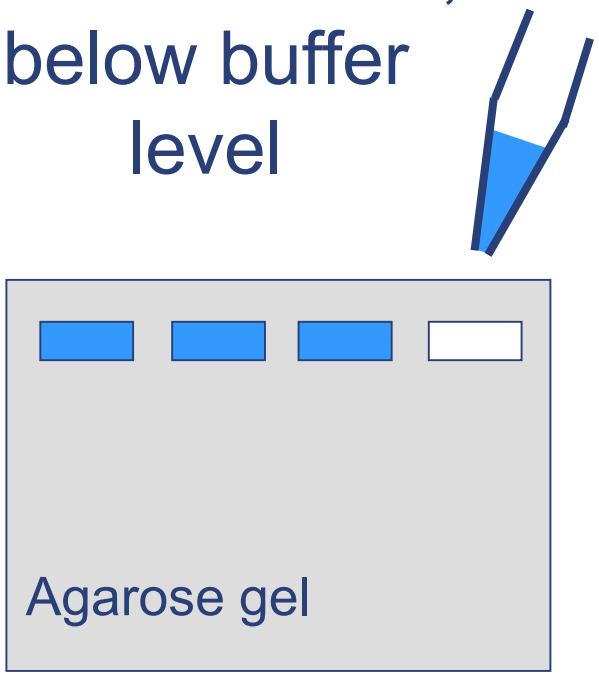
GGCC

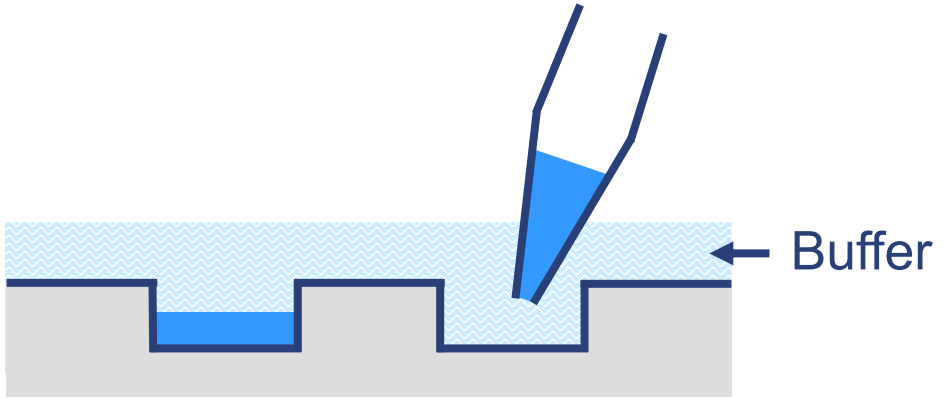
CCGG

HaeIII

Loading sample on to gel

Hold pipette tip just above well, below buffer level



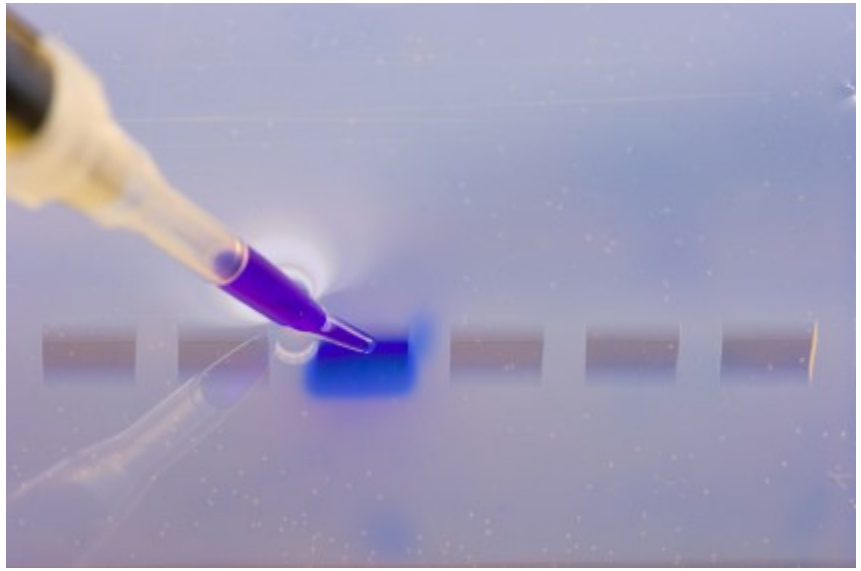


Be careful not to pierce bottom of well with pipette tip!

This time you push down to the 1st stop to fill the well with the DNA

Have a go!

Load 10µl practice dye (tube marked P) to each well.



Push down to the 1st stop to fill the well with the DNA

Prepare the DNA for analysis

1. Add 2.5µl orange loading dye (tube marked LD) to sample W and E

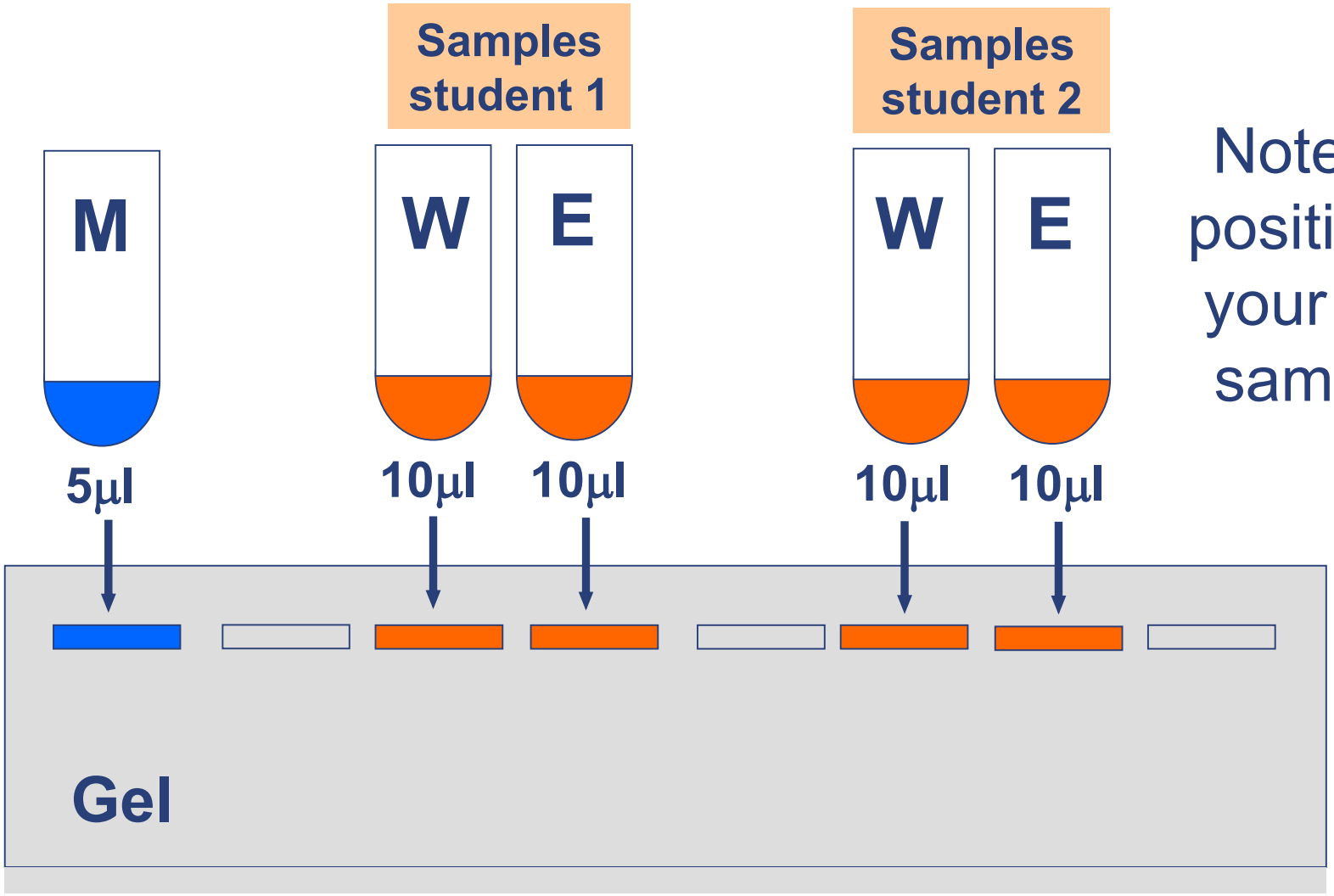
2. **Change the pipette tip** for each sample

3. Flick then centrifuge samples (make sure they are balanced)

Meet the Scientists

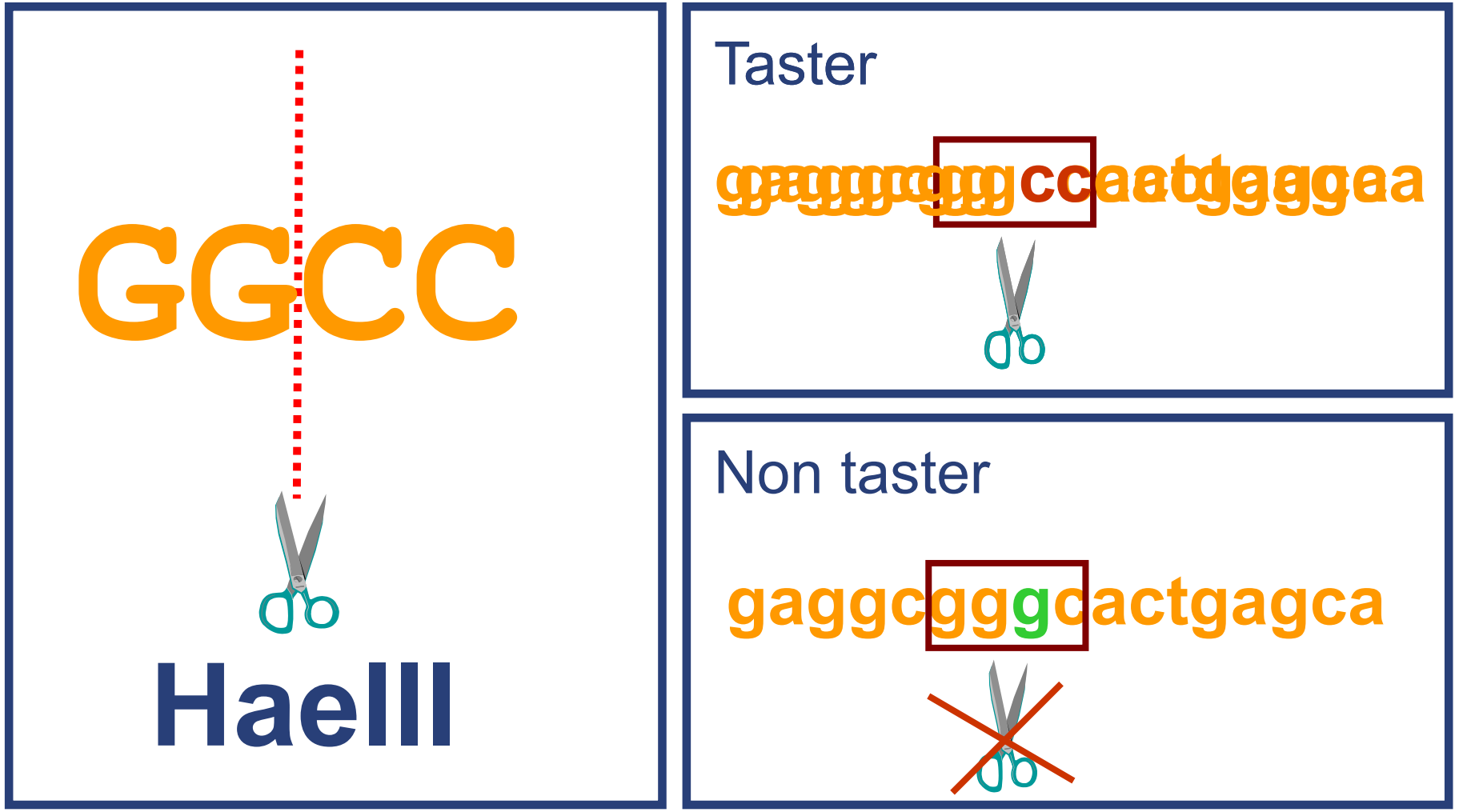
ROSLIN

Loading DNA on to gel



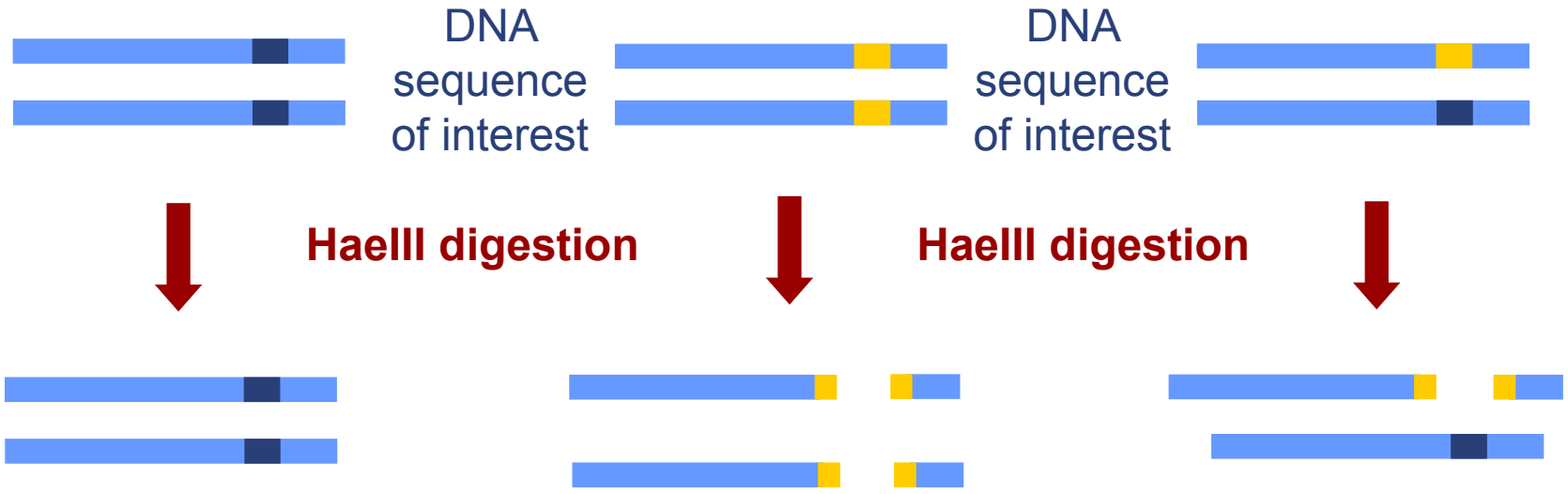
Note the position of your own samples

Which allele with HaeIII cut?



Possible digest results

Homozygous non-taster	Homozygous taster	Heterozygous taster
tt	**TT**	**Tt**



1 size of DNA fragment	2 sizes of DNA fragment	3 sizes of DNA fragment

Practice your analysis…

1) What is the phenotype of the tongue?

Taster

2) What is the genotype of the tongue?

TT or Tt

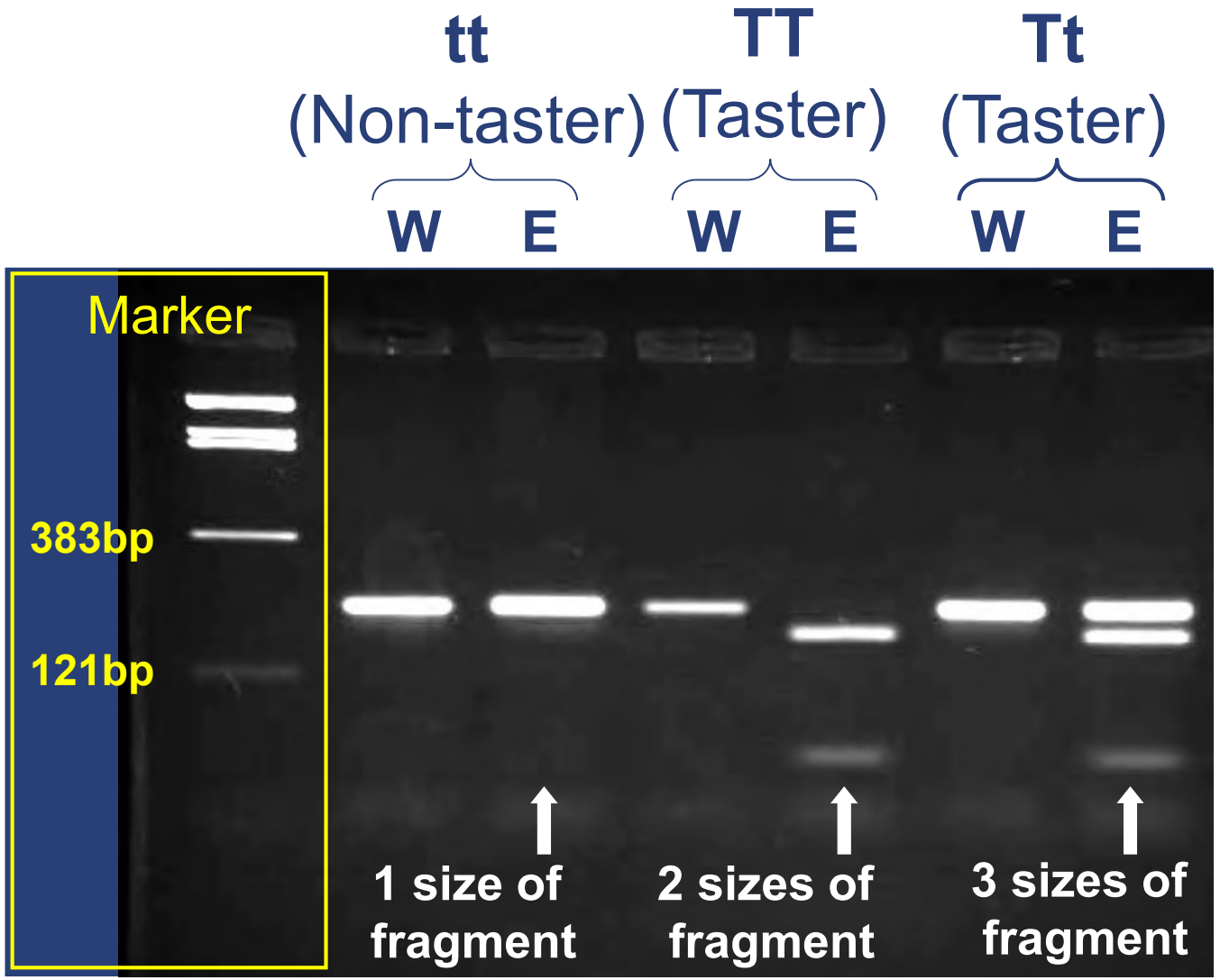
3) Will the enzyme cut?

Yes

4) How many fragments of DNA will there be?

If TT then two fragments
If Tt then three fragments

This is what it will really look like…



W – water only (control)

E - treated with HaeIII enzyme

Image from Carolina Biological Supply Ltd.

Safety check!

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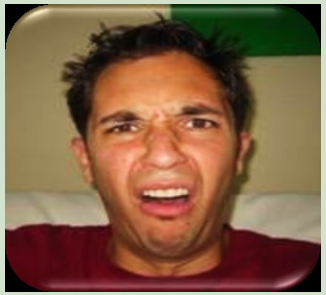
Viewing the DNA

A DNA stain was added to the agarose when the gels were made

Unexpected results?

Phenotype	Expected Genotype	Actual Genotype	Possible reasons
	TT	Tt	• High density of taste buds • Other genes involved
	Tt	TT	• Low density of taste buds • Dry mouth • Have a cold • Other genes involved
	tt	Tt	• Low density of taste buds • Dry mouth • Have a cold • Other genes involved

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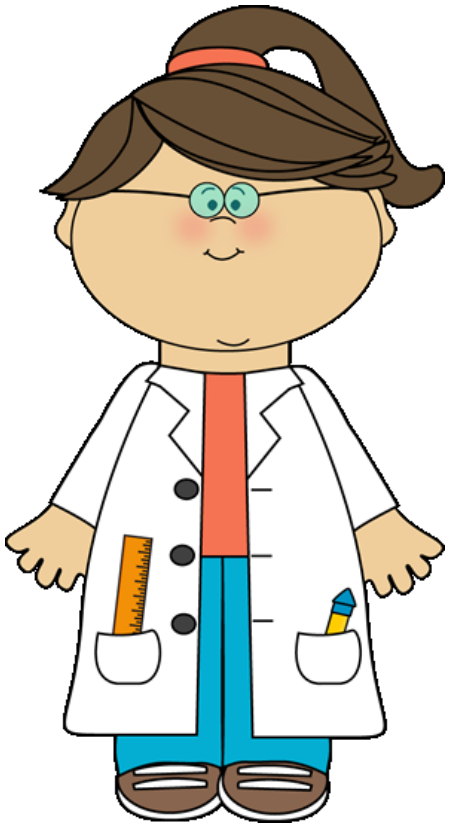

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Fun

Boring

Informative

Inspiring

Rewarding

Uninteresting

Interesting

Confusing

Enjoyable

Difficult

Thought-provoking

Frustrating

Dull

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