Welcome!

Taylor University Biology Department

February 28, 2025

Dr. Jan Reber

Dr. Rebecca Miles

Goals for today

- Learn the fundamentals of Polymerase Chain Reaction
- Learn the fundamentals of Gel Electrophoresis
- Discover your Bitter Taste Receptor Phenotype

Goals for today

- Learn how to operate and focus a light microscope
  - Identify and name the components
- Learn how to make a wet mount
  - human epithelial cells (cheek)
  - Identify the 3 major components of a eukaryotic cell
    - Membrane
    - Cytoplasm
    - Nucleus

PTC The Genetics of Bitter Taste

In 1931, a chemist named Arthur Fox was pouring some powdered PTC into a bottle. When some of the powder accidentally blew into the air, a colleague standing nearby complained that the dust tasted bitter. Fox tasted nothing at all. Curious how they could be tasting the chemical differently, they tasted it again. The results were the same. Fox had his friends and family try the chemical then describe how it tasted. Some people tasted nothing. Some found it intensely bitter, and still others thought it tasted only slightly bitter.

Dark chocolate and coffee are common bitter tasting foods.

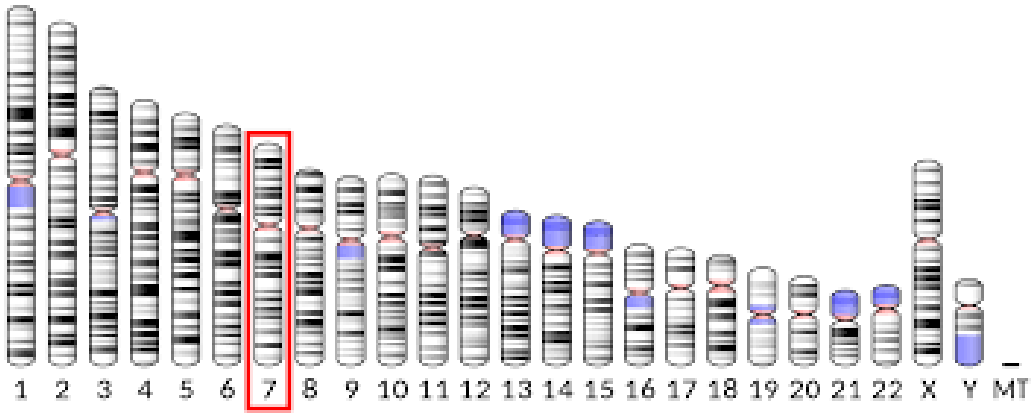
PTC paper is used to test whether a person is a "taster", "non-taster", or somewhere in between.

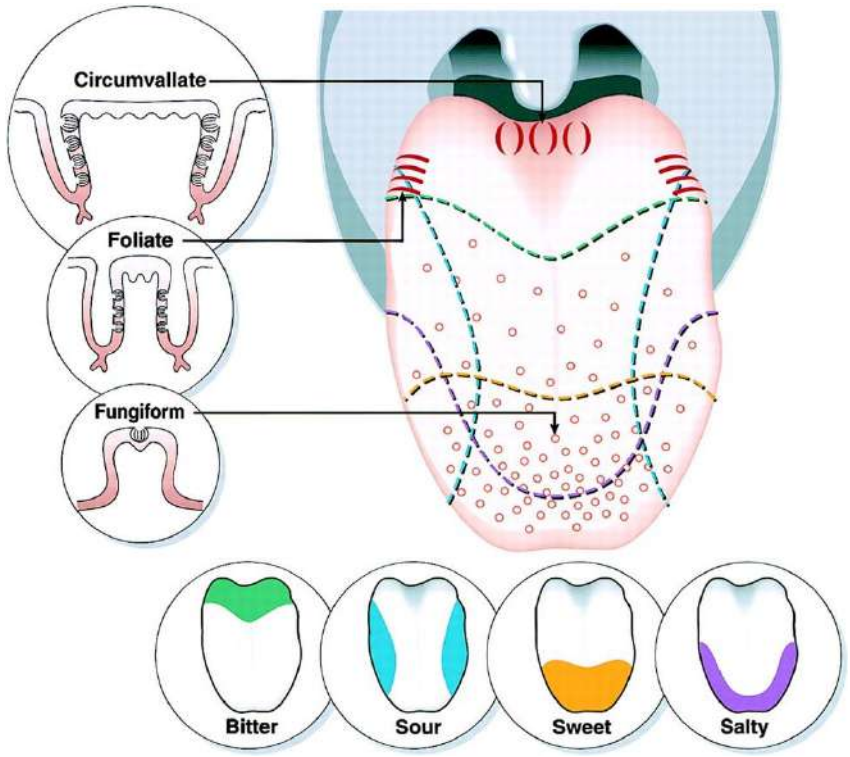
The ratio of tasters to non-tasters varies between populations, but every group has some tasters and some non-tasters. On average, 75% of people can taste PTC, while 25% cannot.

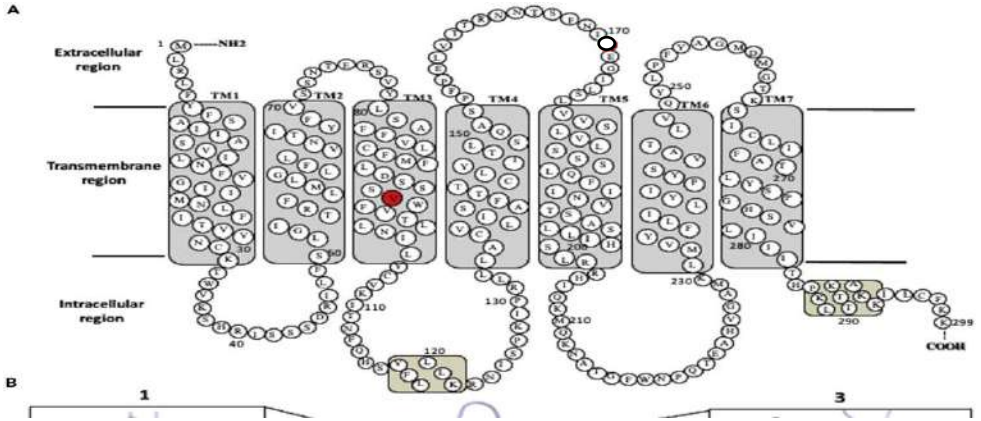
The gene for super tasting

- The TAS2R38 gene is associated with bitter taste and supertasting:
- Bitter taste
- The TAS2R38 gene produces a protein that functions as a receptor to perceive bitter compounds. This gene is associated with the perception of bitterness in vegetables like broccoli, cabbage, and Brussels sprouts.
- Super tasting
- Scientists believe that many supertasters have the TAS2R38 gene. This gene increases a person's perception of bitterness in foods and drinks. Supertasters are often extremely sensitive to a chemical called propylthiouracil (PTC).
- Alleles
- There are two common alleles of the TAS2R38 gene: the "taster" allele (T) and the "non-taster" allele (t). The "taster" allele confers the ability to detect the bitter chemical PTC, while the "non-taster" allele correlates with the inability to detect PTC.
- About 25% of the population qualifies as supertasters, and women are more likely to be supertasters than men.

TAS2R38 on chromosome 7

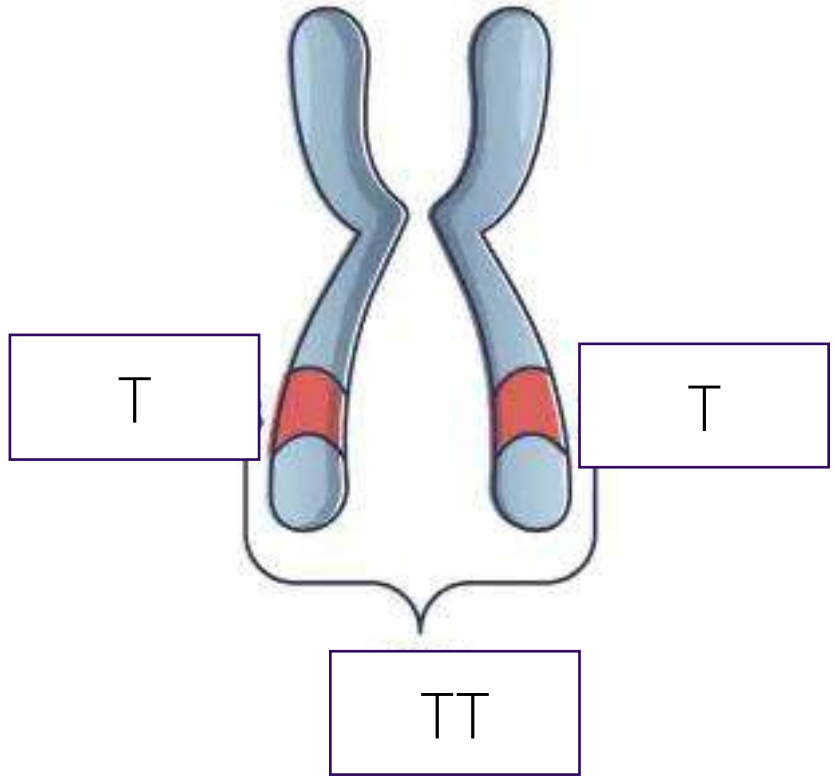






Two forms of the TAS2R38 gene: The T allele and the C allele

- TAS2R38 gene variations
- You will explore two forms of the TAS2R38 gene: The T allele and the C allele. C allele is associated with the presence of the receptor, which means you could taste PTC and bitterness. T allele is associated with the absence of the receptor, which means you could not taste PTC and bitterness.



Two forms of the TAS2R38 gene: The T allele and the t allele

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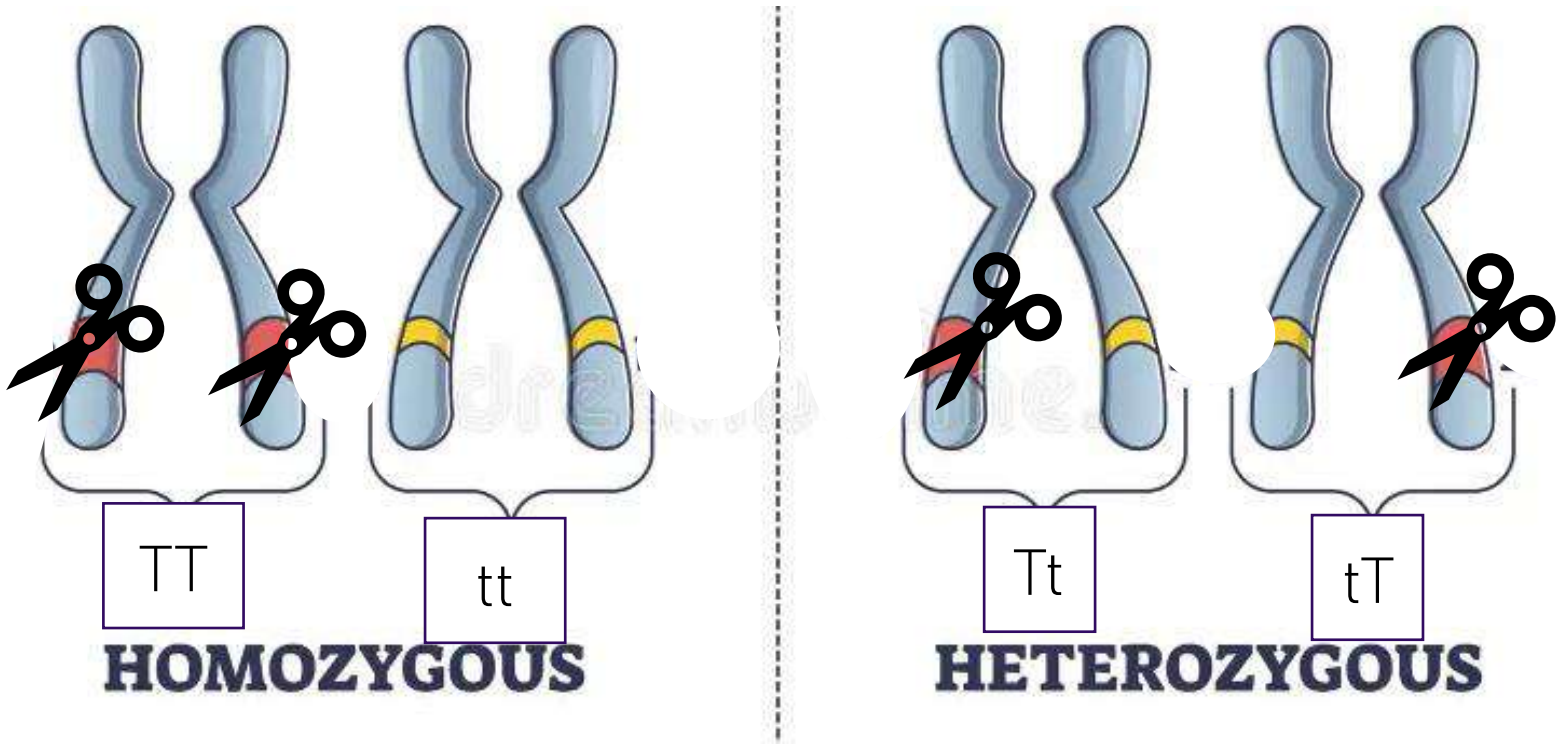


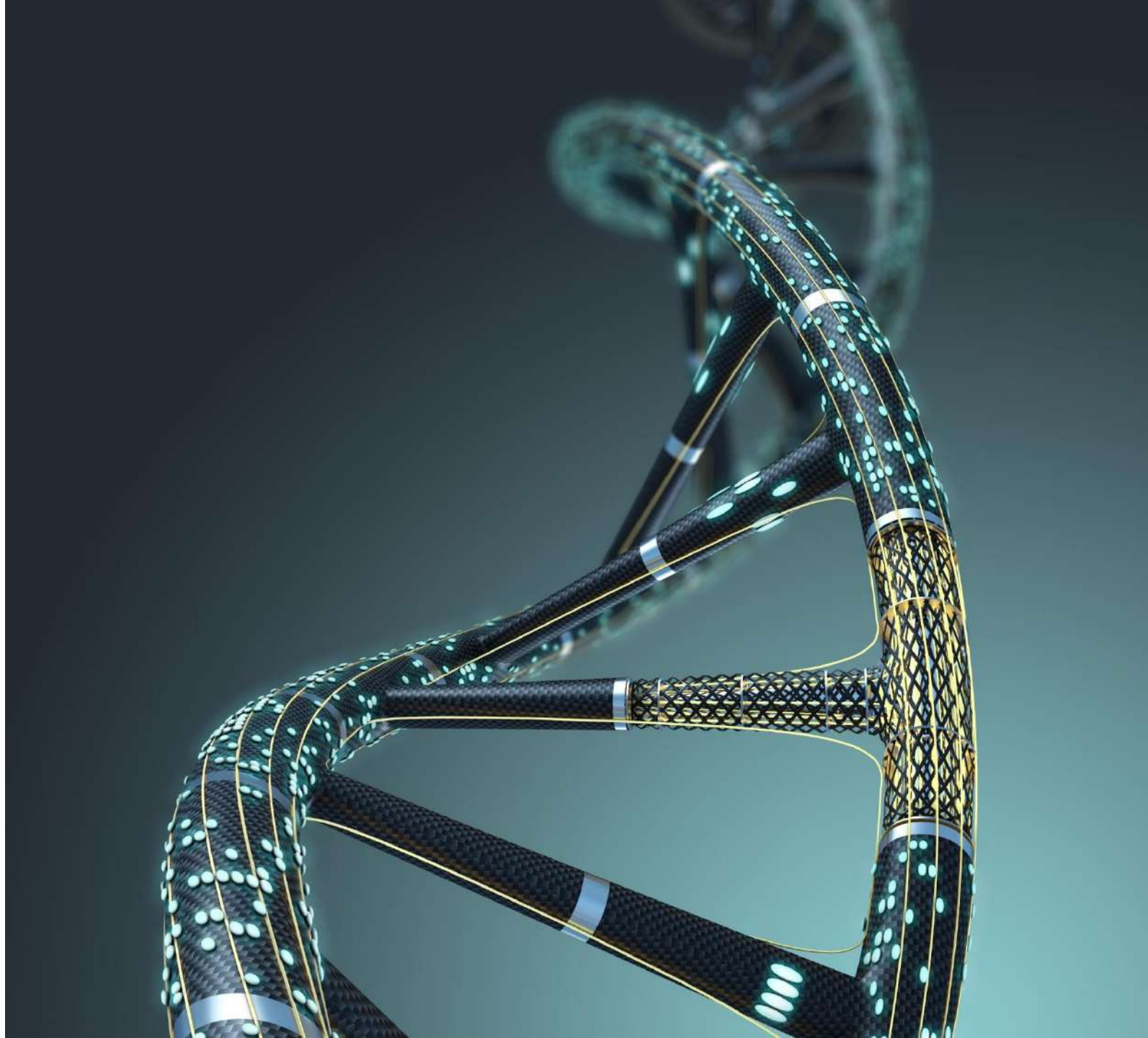
Taster TT /non-taster tt

Partial taster

Polymerase Chain Reaction

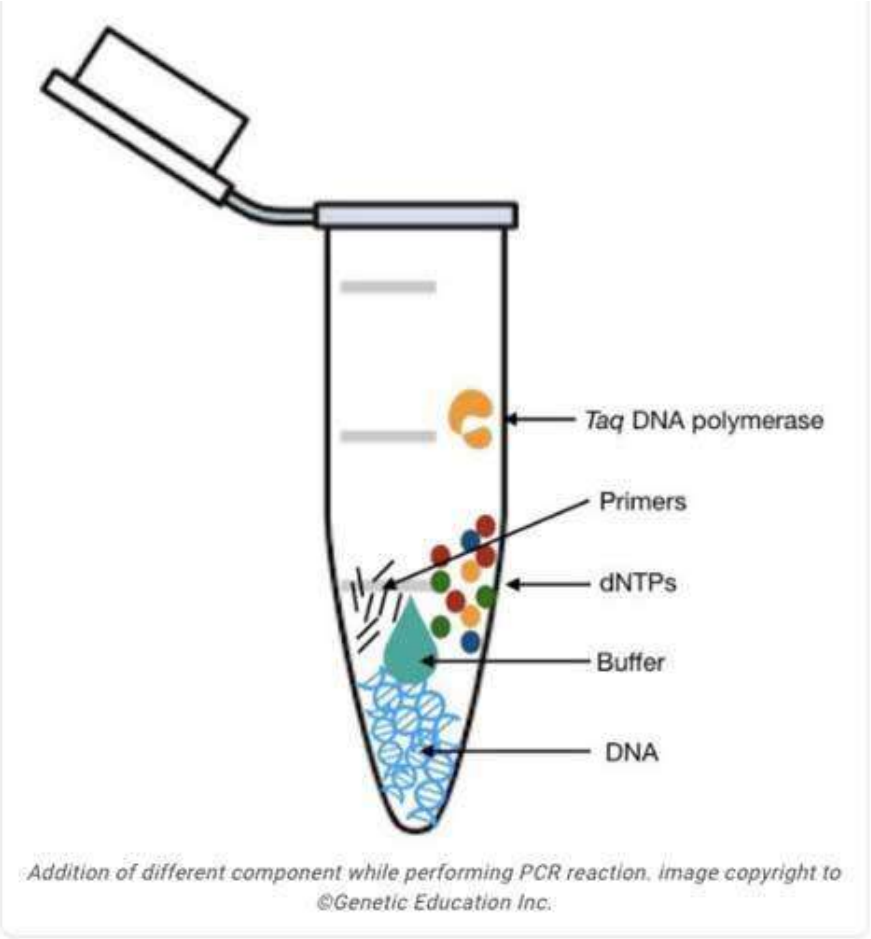
DNA Sample Number Code

1	Emily Steffen
2	Jazlyn Gillium
3	Gracie Reeves
4	Harley Barton
5	Caroline Rippesger
6	Bristol Chapman
7	Aulbach
9	Braden Vemer
8	Mr Butler
10	Kolbie Beavans
11	Shyanne Cook
12	Shalynn g
13	Lilly Gartin
14	Kane J
15	Aly



How do we figure out what your genes are?

- 1. We have to amplify your DNA to be able to test it and see it.
- 2 How do we amplify DNA?



Addition of different component while performing PCR reaction. image copyright to ©Genetic Education Inc.

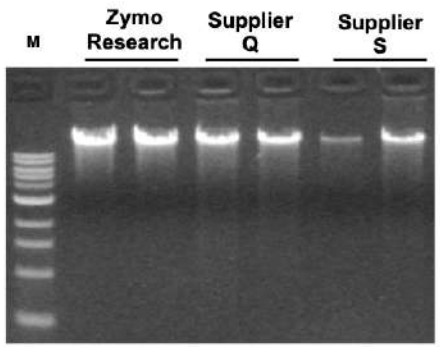
Product Description

The ***Quick*-DNA™ Microprep Kit** is a simple procedure for the rapid isolation of total DNA (e.g., genomic, mitochondrial, viral) from a variety of biological sample sources. This product has been optimized for maximal recovery of ultra-pure DNA without RNA contamination and is compatible with whole blood (fresh or stored), serum, plasma, buffy coat, buccal cells, cells from culture, and many biological liquid samples.

For processing, simply add the specially formulated **Genomic Lysis Buffer** to a sample, vortex, and transfer the mixture to the supplied **Zymo-Spin™ Column**. There is no need for organic denaturants or Proteinase K digestion because of the unique lysis buffer system. The product features **Zymo-Spin™ Technology** to yield high-quality, purified DNA in just minutes (see below). PCR inhibitors are effectively removed during the purification process. DNA purified using the ***Quick*-DNA™ Microprep Kit** is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bisulfite conversion/methylation analysis, and other downstream applications.

Ultra-pure DNA is ideal for…

- ✓ PCR
- ✓ Endonuclease Digestion
- ✓ Genotyping
- ✓ Bisulfite Conversion & Methylation Analysis



High yield/quality DNA is successfully isolated from porcine whole blood using the *Quick*-DNA™ Miniprep Kit (D3024). Equivalent amounts (100 µl) of blood were processed without Proteinase K using the *Quick*-DNA™ Miniprep Kit in half the time as compared to the kits from suppliers Q and S. Equal volumes of eluted DNA were then analyzed (in duplicate) in a 0.8% (w/v) TAE/agarose/ethidium bromide gel. The size marker "M" is a 1 kb ladder (Zymo Research).

Buccal Cells and Swabs

Buccal cells can be isolated using a rinse- or swab-based isolation method.

A. **Rinse Method**: Vigorously rinse 10-20 ml of saline solution or mouthwash orally for 30 seconds. The more vigorous the rinsing action, the more cells that will be recovered. Spit the saline into a 50 ml tube and pellet the cells at 1,500 rpm for 5 minutes. Discard the supernatant without disturbing the cell pellet. Add 500 µl of **Genomic Lysis Buffer** to the pellet then vortex 4-6 seconds, then let stand at room temperature for 5-10 minutes.

B. **Swab Isolation Method**: Thoroughly rinse mouth out before isolating cells. Brush the inside of the cheek with a *buccal swab* for 15 seconds (approximately 20 brushes), making sure to cover the entire area of the inner cheek. Rinse the brush into a microcentrifuge tube using 500 µl of **Genomic Lysis Buffer**, vortex 4-6 seconds, and then let stand at room temperature for 5-10 minutes.

1. Transfer the mixture[1] to a **Zymo-Spin™ IC Column** in a **Collection Tube**. Centrifuge at ≥ 10,000 *x g* for one minute. Discard the Collection Tube with the flow through.

2. Transfer the **Zymo-Spin™ IC Column** to a new Collection Tube. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at ≥ 10,000 *x g* for one minute.

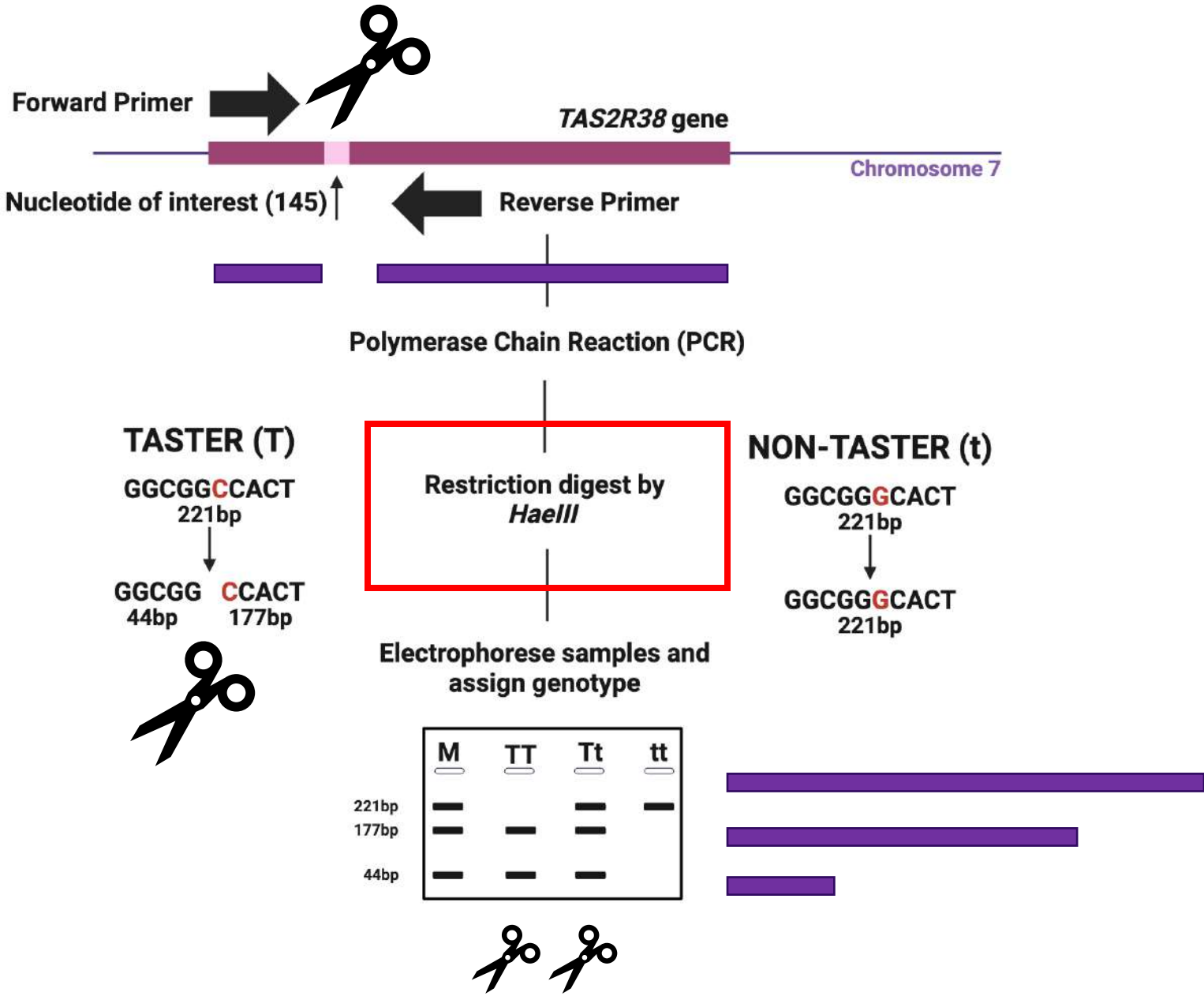
3. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at ≥ 10,000 *x g* for one minute.

4. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 10 µl **DNA Elution Buffer** or water[2,3] to the spin column. Incubate 2-5 minutes at room temperature and then centrifuge at ≥ 10,000 *x g* for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20 ˚C for future use.

[1] The column capacity is 800 µl.
[2] Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is >6.0. Also, the total yield may be improved by eluting the DNA with Elution Buffer or water pre-equilibrated to 60-70°C.
[3] The **DNA Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

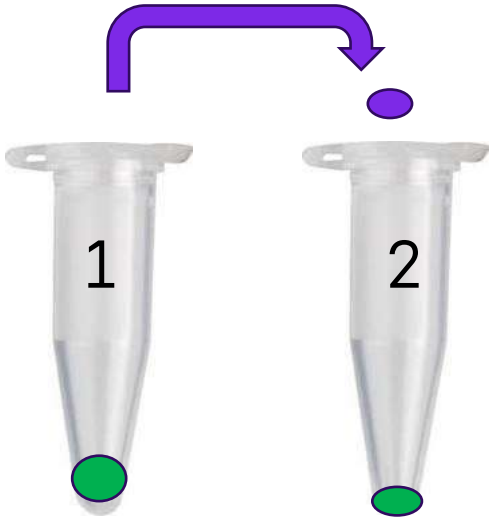

95
72
55
°C
Cycle 1
www.dnalc.org

In this step, you will use PCR to amplify part of the TAS2R38 gene. The sequence of gene fragment we are copying includes a C/T mutation, that contributes to taste perception. This C/T mutation is also known as a *single nucleotide polymorphism* (SNP) with the code rs1726866.



Take your prepared samples to the Thermocycler

Step 1: Add 10 uL from Tube 1 into a new empty Tube 2

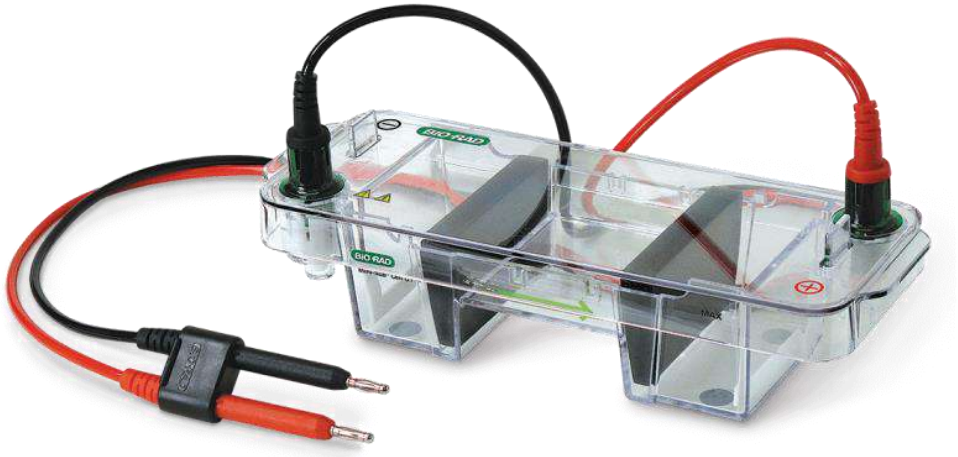


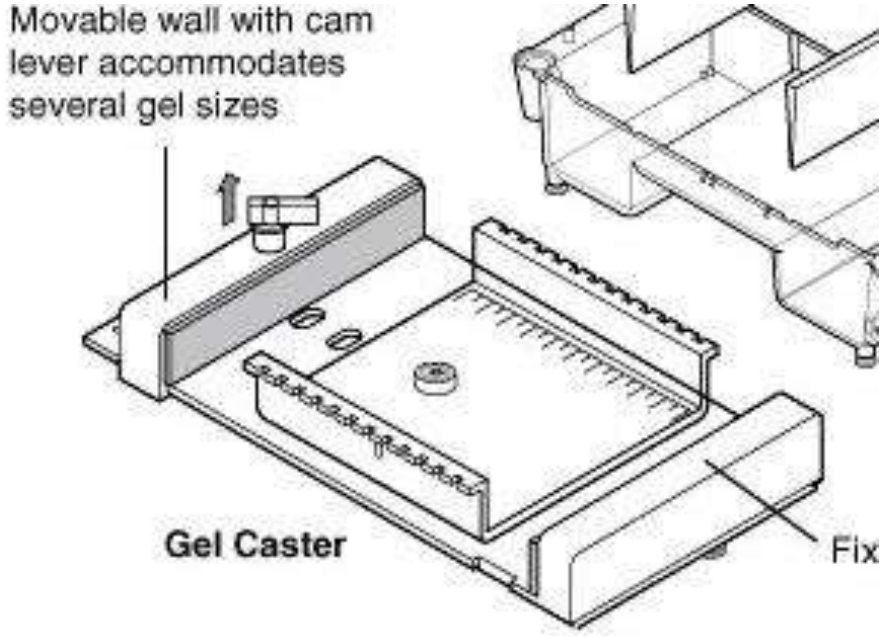
Step 1: Add 1 uL buffer
Step 2: Add 1 uL enzyme
Total will be 12 uL
Step 3: take to black PCR machine to digest for 30 Min at 37 C

Gel Electrophoresis


addgene
AGAROSE
GEL
ELECTROPHORESIS

Add 5 uL purple dye so we can see the sample in the water. It will be heavier than water and will sink into well.

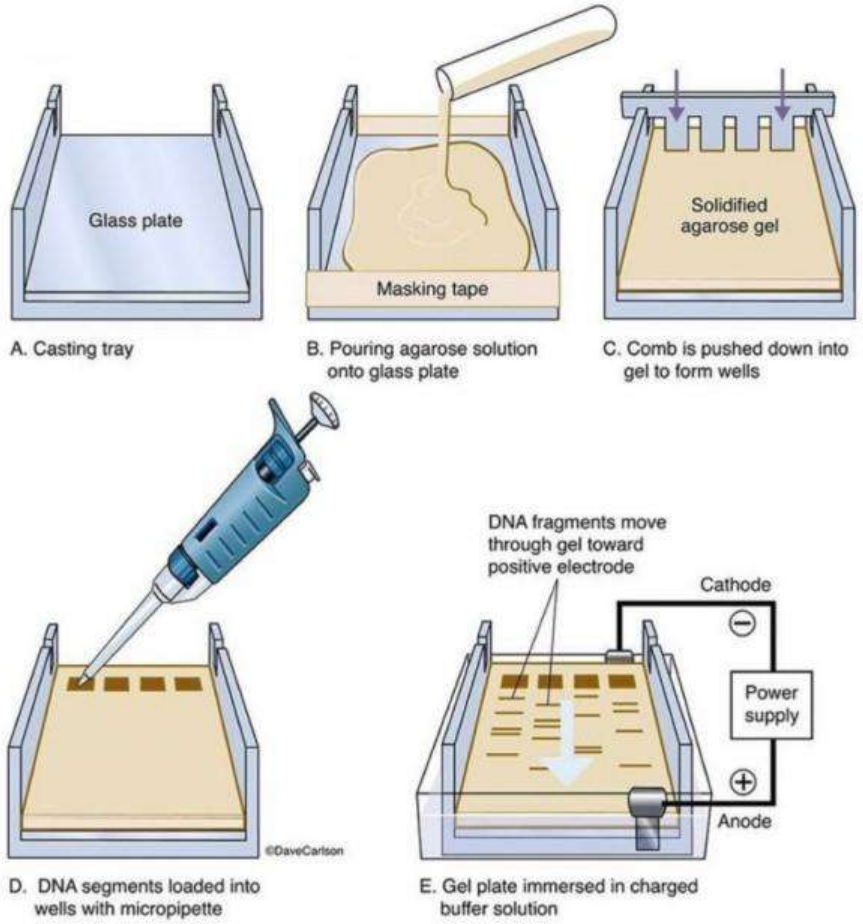




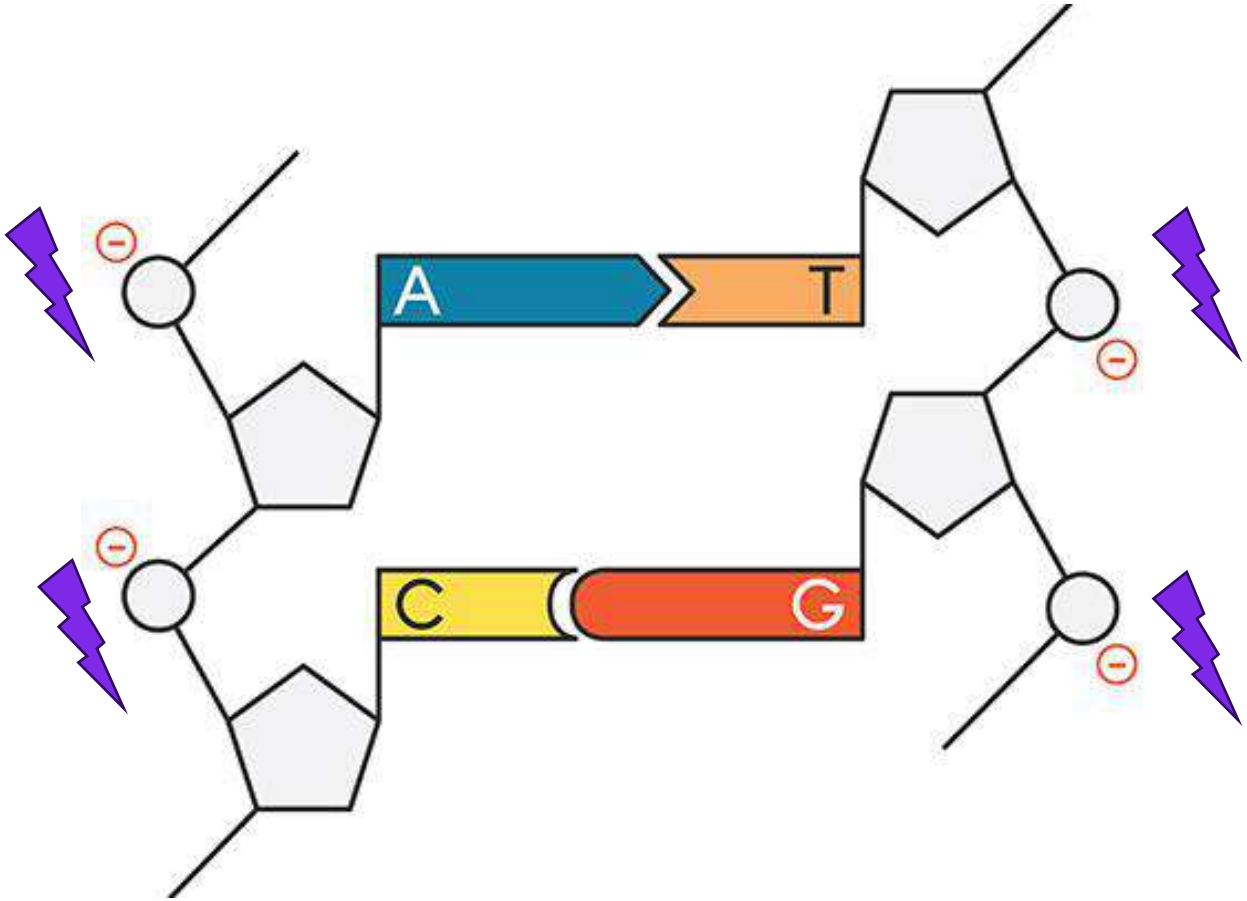
Protocol – Gel electrophoresis

Load 30 uL of reaction into one well of the gel. It will be heavier than water and will sink into well.

Agarose Gel Electrophoresis

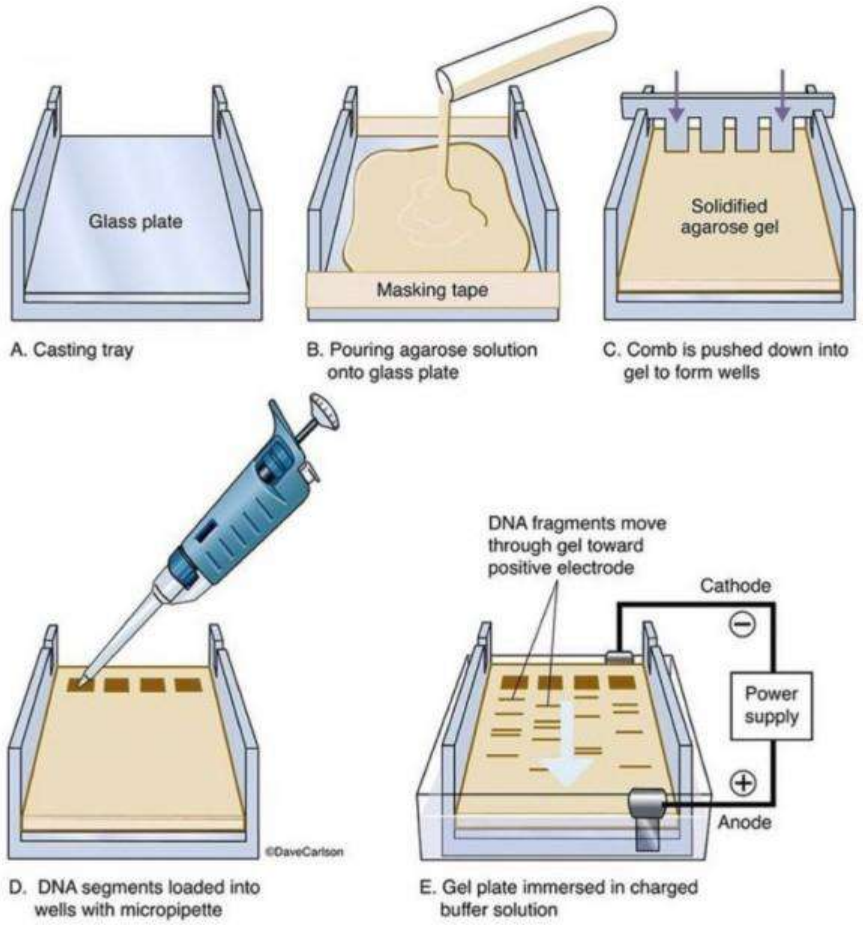


DNA is negatively charged on its backbone



Agarose Gel Electrophoresis

P



Take your tube and add 5 uL of purple dye

Load 30 uL of reaction into one well of the gel.
It will be heavier than water and will sink into well.

Run 30-45 min at 100 V

Image DNA in the gel and see if BAMHI site is present
In the DNA sample.

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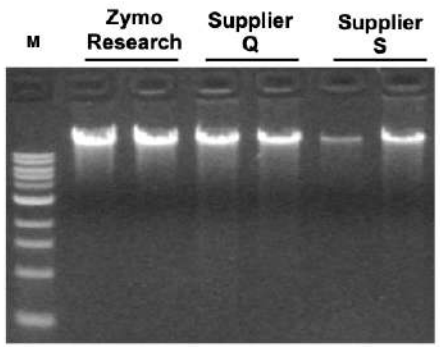

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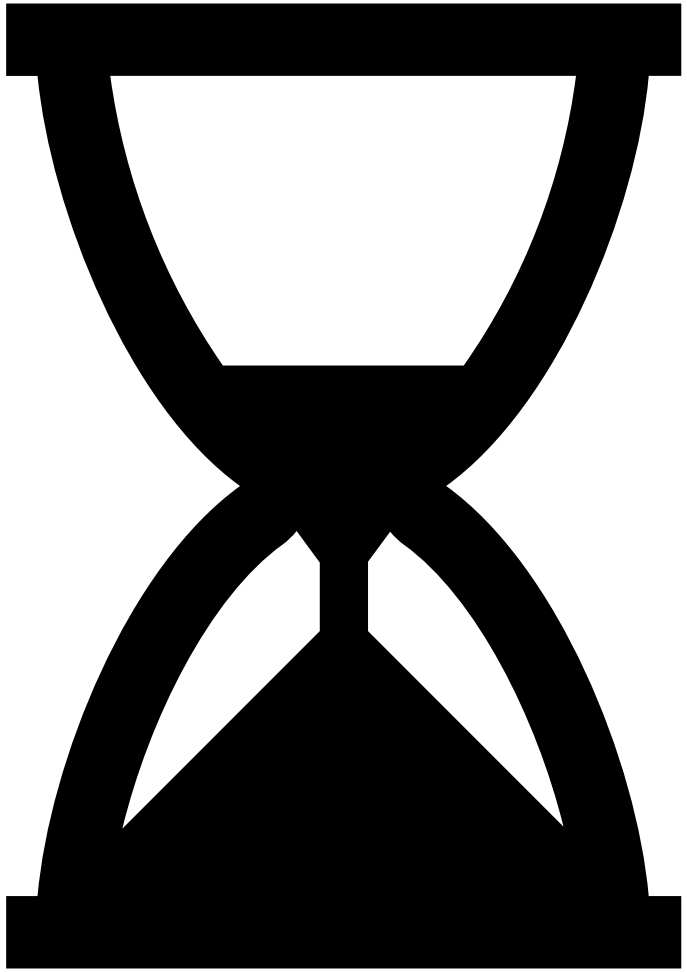
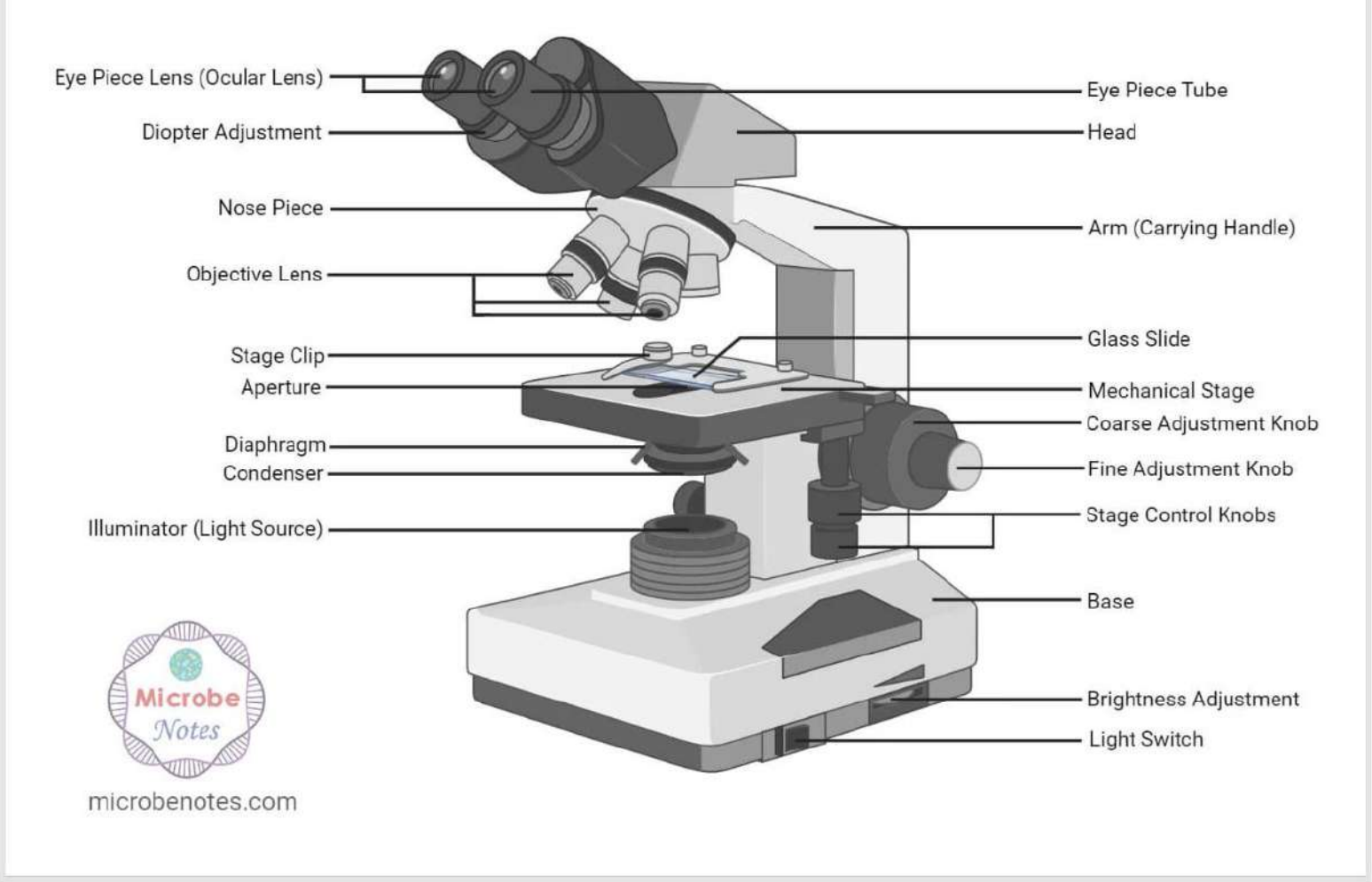


Observe your cells

Microscope Parts



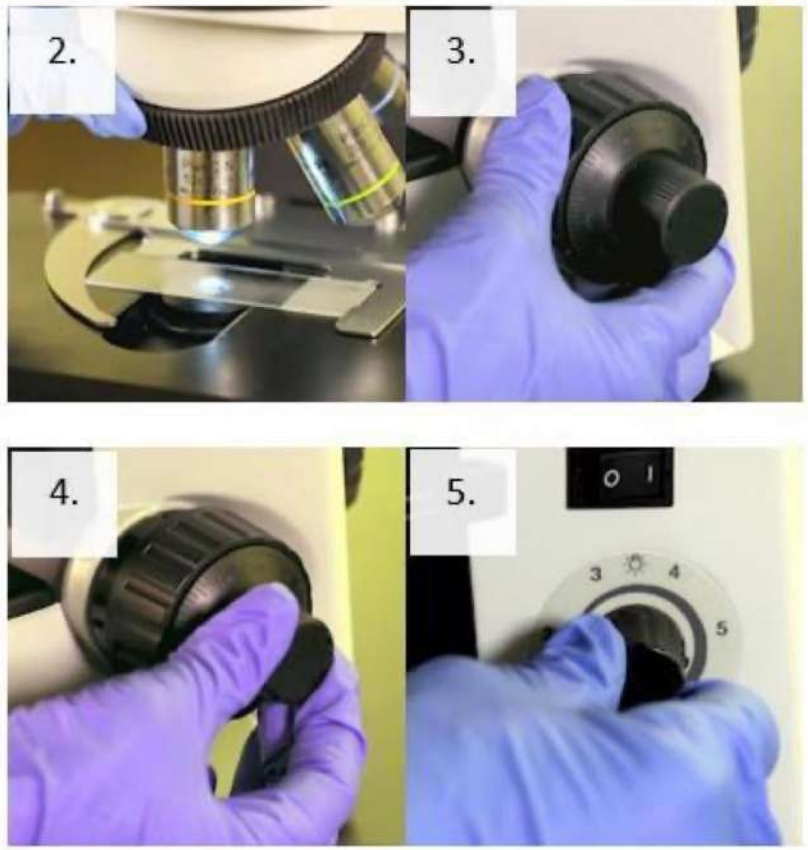
Focusing the Microscope

Introduction

It is necessary to focus the microscope when viewing specimens. Focusing the microscope is performed by using the fine and the coarse adjustment knobs near the base of the microscope.

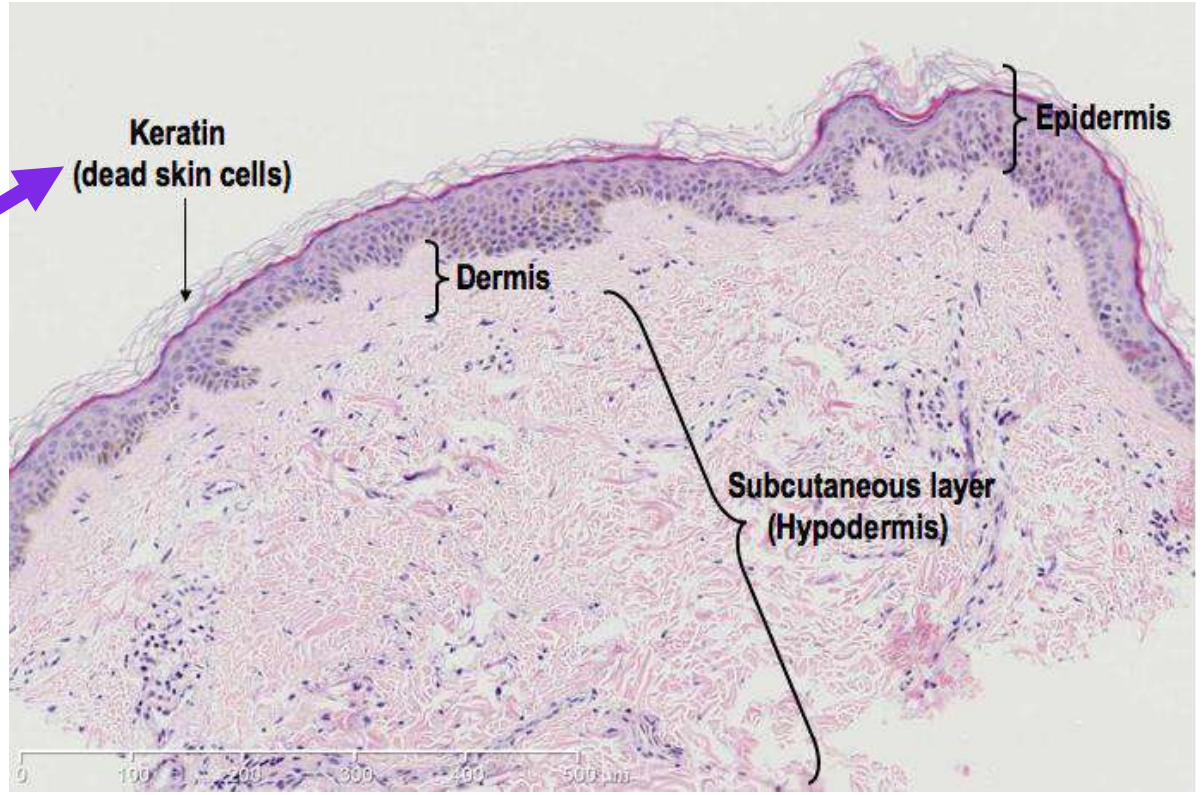
Instructions

1. Put a slide on the microscope stage, with the coverslip side up.
2. Bring the 10X objective over the slide.
3. Using the coarse adjustment knob, raise or lower the stage until you see an object come into view.
4. Use the fine adjustment knob to obtain a clearer view of the object.
5. Manipulate the light source to obtain the correct amount of light.



Whole skin

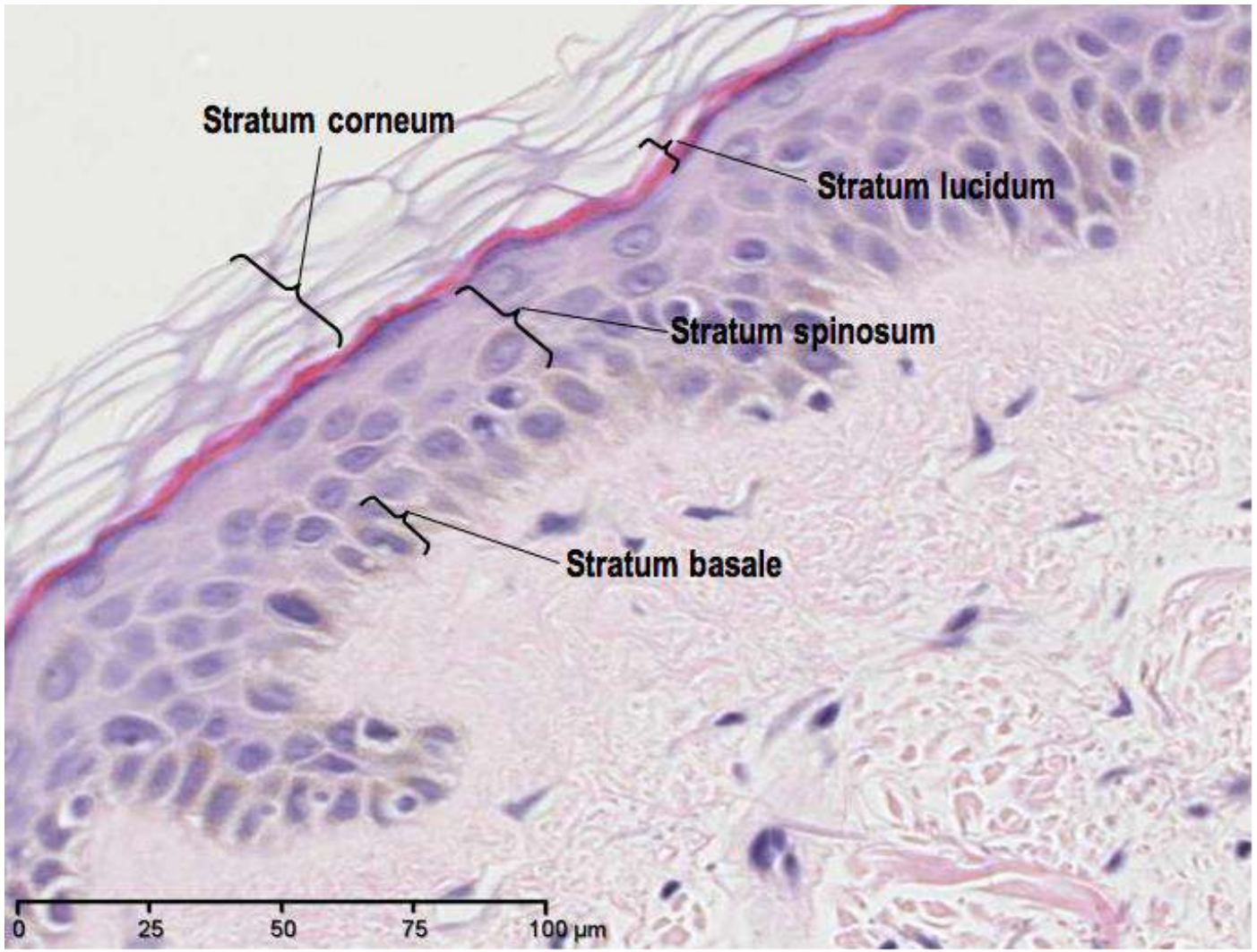
Skin has 3 layers:
Epidermis
Dermis
Hypodermis



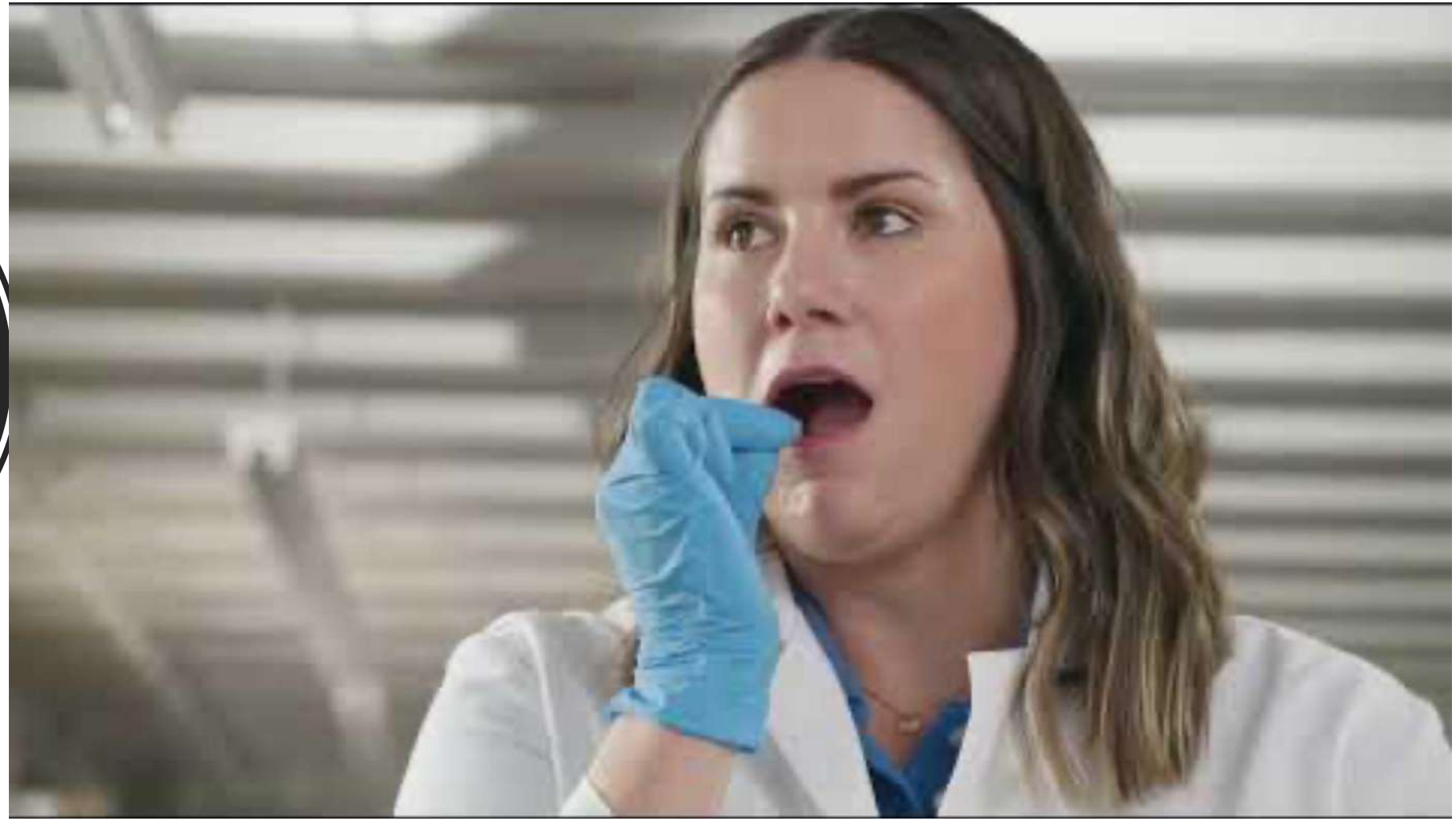
The cells of the epidermis (keratinocytes) are constantly dividing and then "dying" as they get pushed to the surface.
This creates a water barrier and a layer resistant to abrasion. This is what you will scrape off with your toothpick.

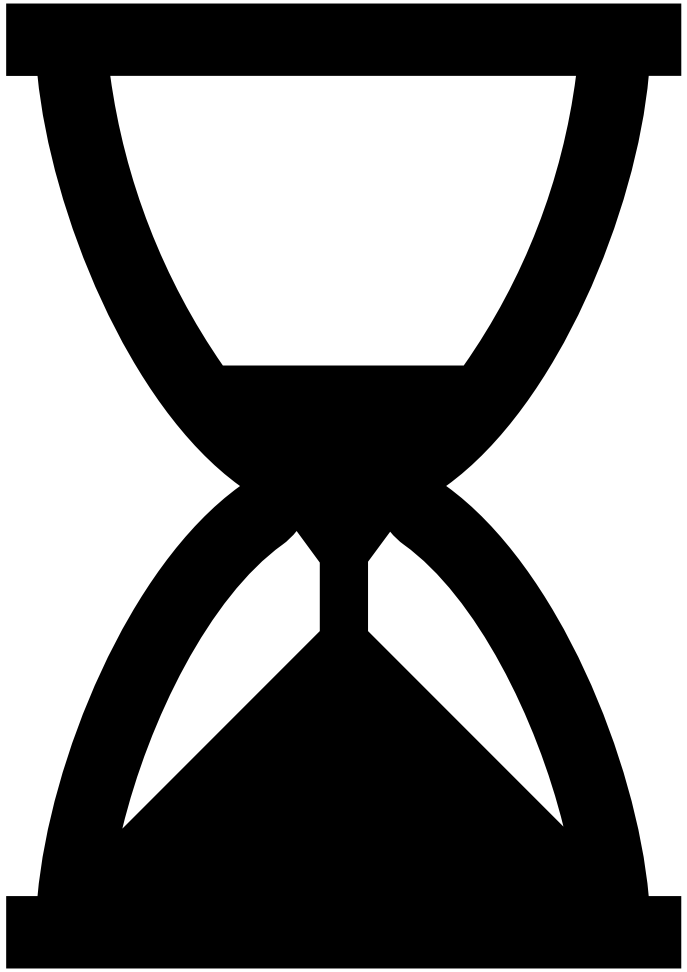
Fun fact: According to the American Chemical Society, the average adult loses about 500 million skin cells each day, or 0.001 to 0.003 ounces (0.03 to 0.09 grams) of skin flakes an hour.

Whole skin


Stratum corneum
Stratum lucidum
Stratum spinosum
Stratum basale
0
25
50
75
100 µm

How to stain cheek cells





Are you a Supertaster?
What's your phenotype?

PTC The Genetics of Bitter Taste

In 1931, a chemist named Arthur Fox was pouring some powdered PTC into a bottle. When some of the powder accidentally blew into the air, a colleague standing nearby complained that the dust tasted bitter. Fox tasted nothing at all. Curious how they could be tasting the chemical differently, they tasted it again. The results were the same. Fox had his friends and family try the chemical then describe how it tasted. Some people tasted nothing. Some found it intensely bitter, and still others thought it tasted only slightly bitter.

Dark chocolate and coffee are common bitter tasting foods.

PTC paper is used to test whether a person is a "taster", "non-taster", or somewhere in between.

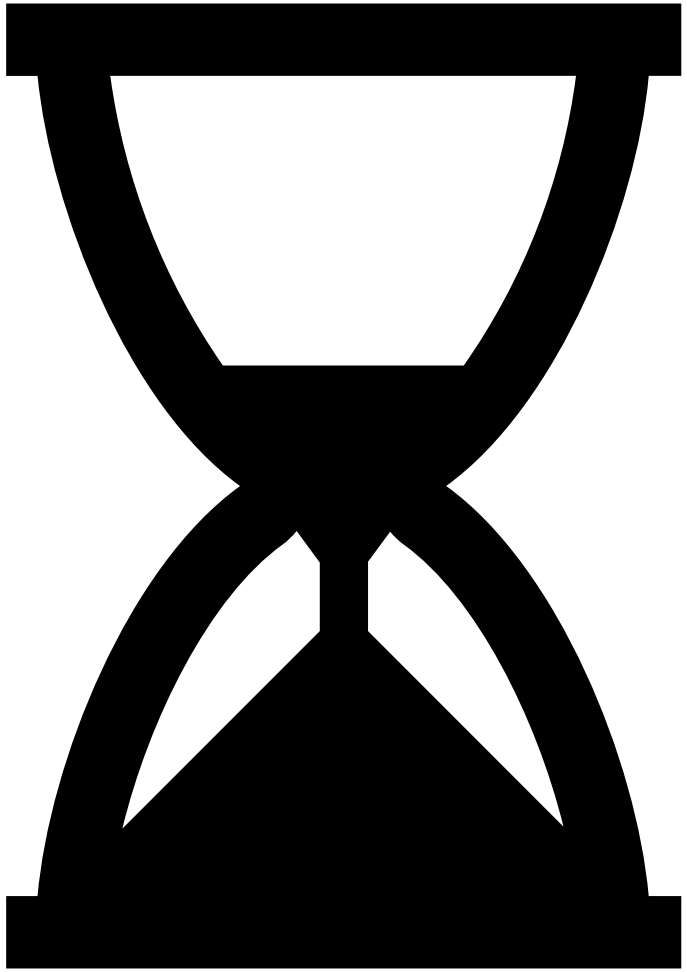
The ratio of tasters to non-tasters varies between populations, but every group has some tasters and some non-tasters. On average, 75% of people can taste PTC, while 25% cannot.

Are you a supertaster?

- To record a PTC test strip, you can note the taste or sensation you experience after placing the strip on your tongue:

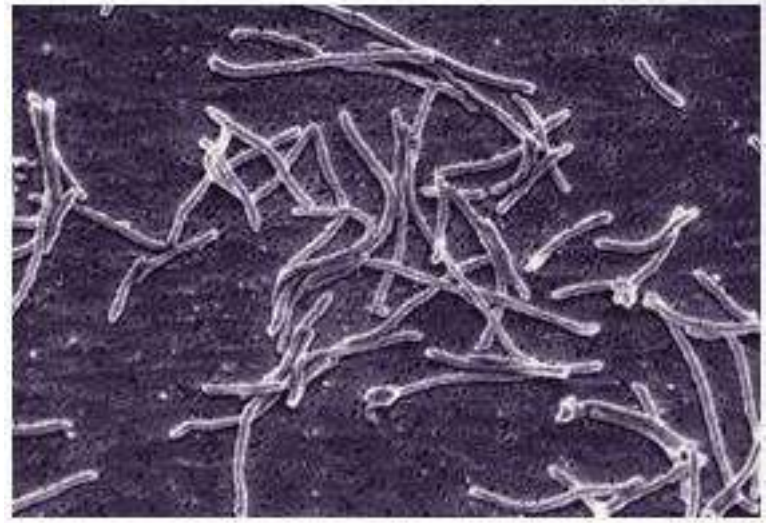
1. **Clear your mouth**: Take a sip of water to remove any other tastes.
2. **Place the strip**: Put the strip on your tongue and leave it there for a few minutes.
3. **Record your response**: Note the taste or sensation you experience. You can record the bitterness on a scale of 1 to 9.
4. **Dispose of the strip**: Discard the used PTC paper in the waste container provided.

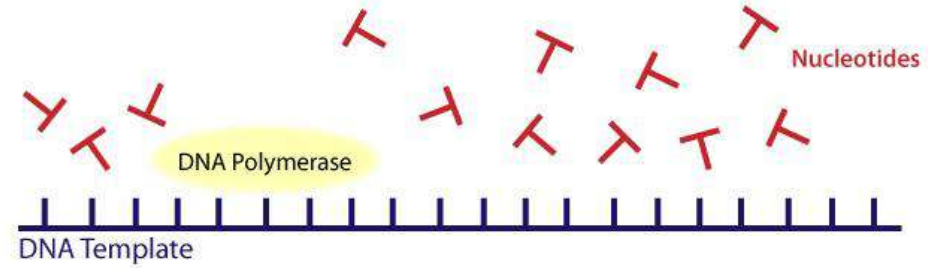
- You can also compare your PTC taste to a control paper. If you don't taste anything after 10-15 seconds, you are not a taster.
- The ability to taste PTC is a dominant trait, so about 2/3 to 3/4 of people should be able to taste it. People who have two copies of the tasting gene will have a very strong reaction to the PTC, while those with one copy of each gene will taste it, but not as strongly. People who are homozygous for the non-tasting allele will not be able to taste PTC



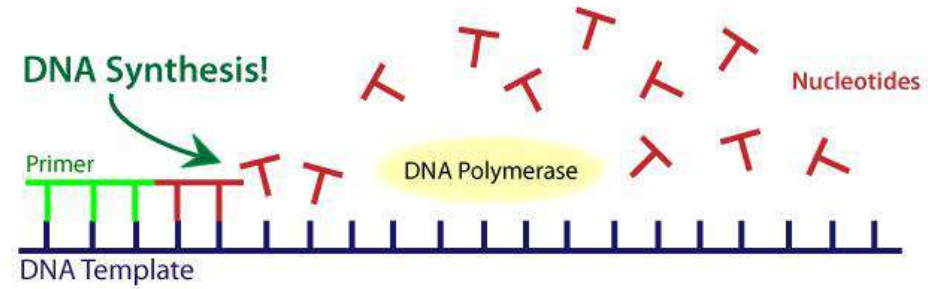
Are you a Supertaster?
Observe your genotype

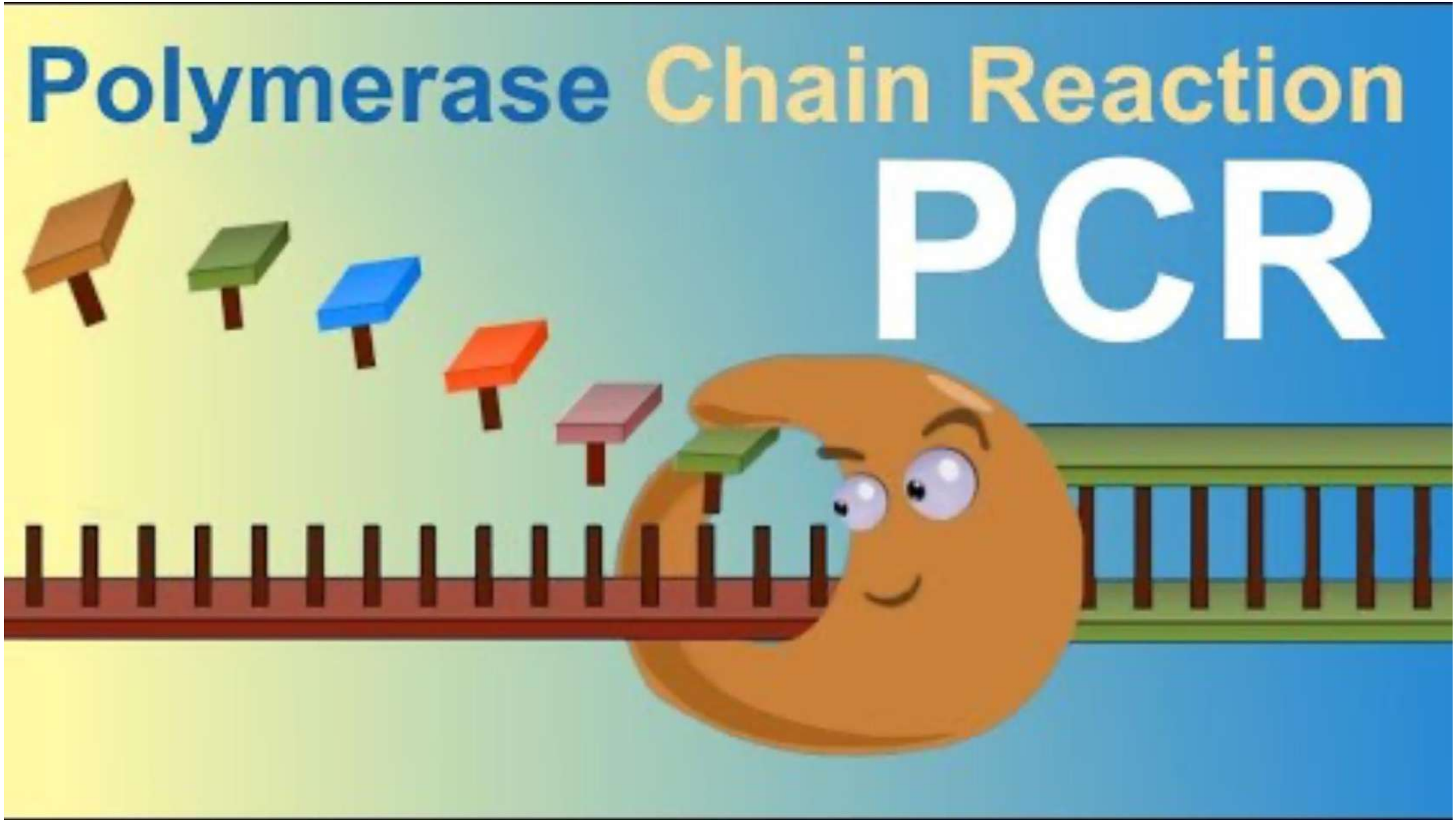
Taq DNA polymerase



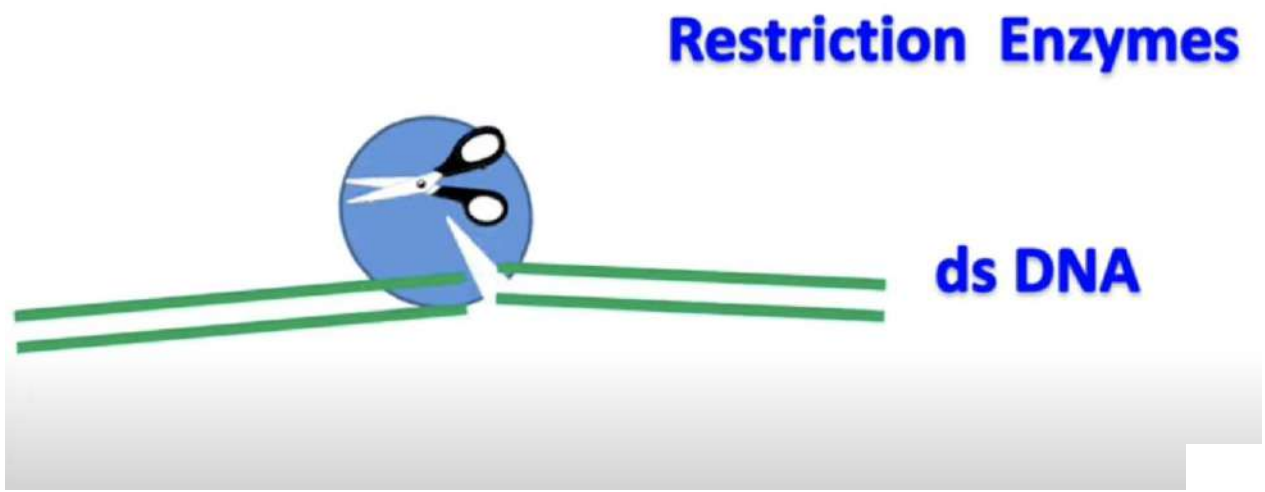


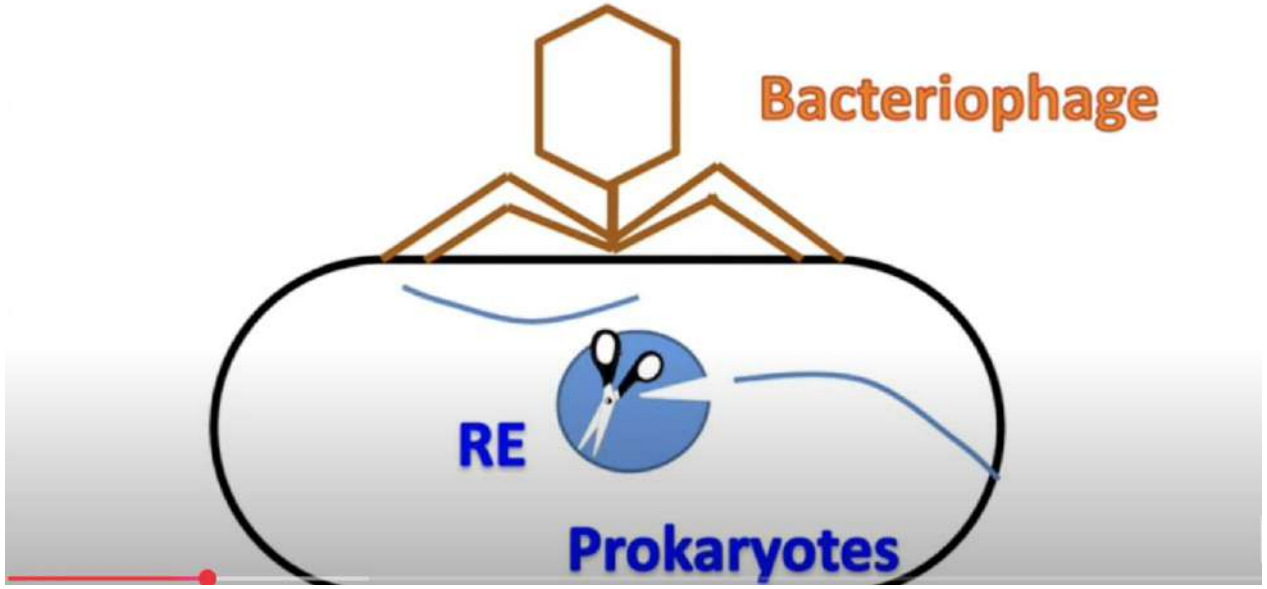
No DNA Synthesis



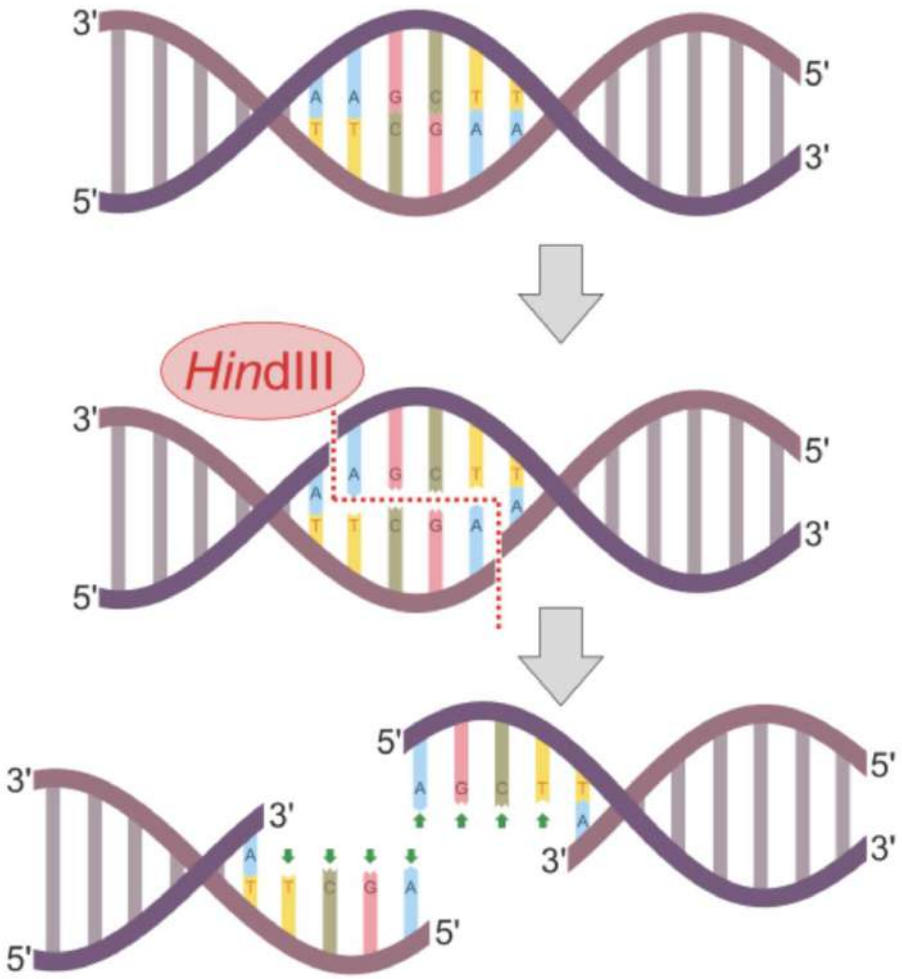

Polymerase Chain Reaction
PCR

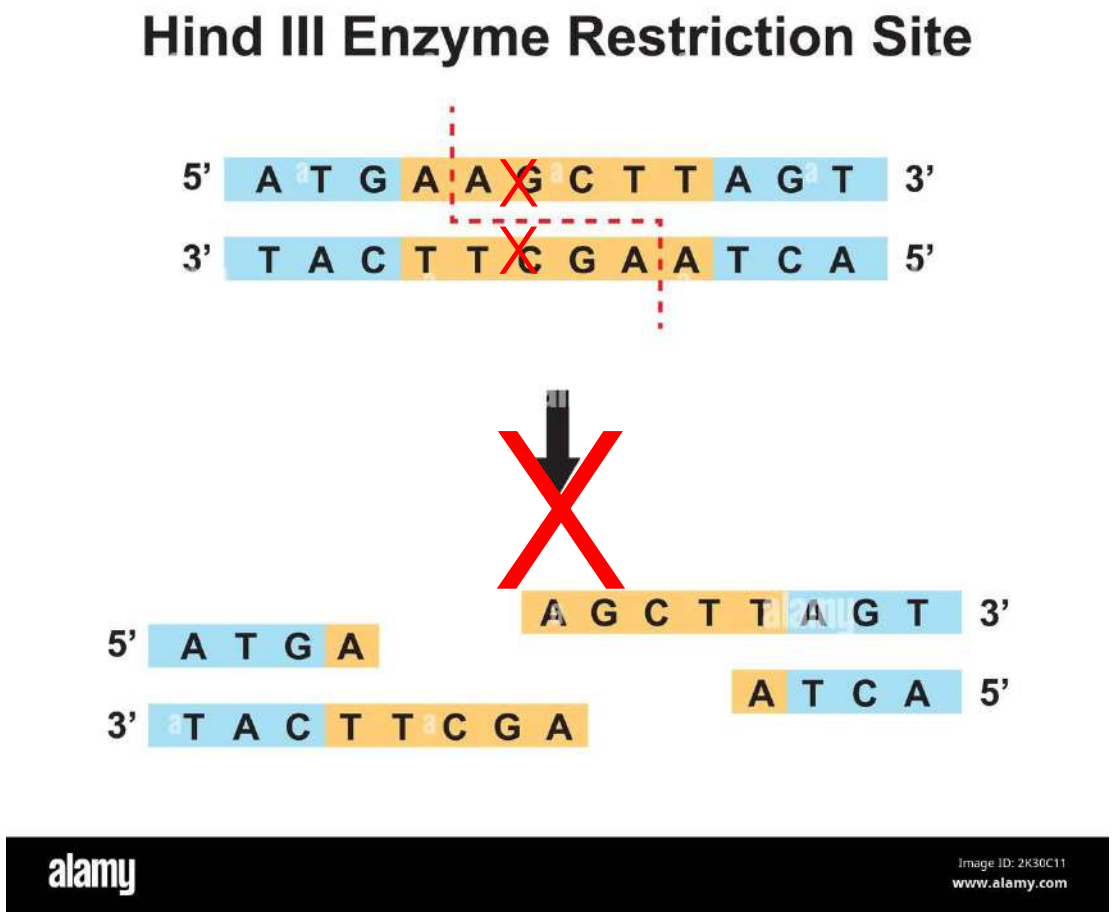
How do restriction enzymes work?





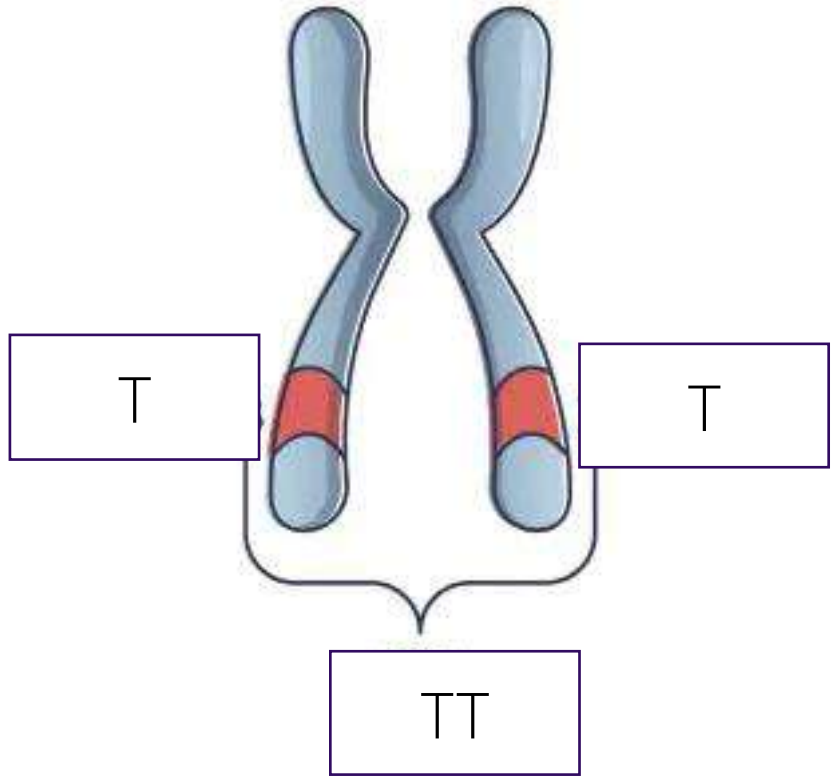
Restriction Enzymes only cut certain sequences





Two forms of the TAS2R38 gene: The T allele and the C allele

- TAS2R38 gene variations
- You will explore two forms of the TAS2R38 gene: The T allele and the C allele. C allele is associated with the presence of the receptor, which means you could taste PTC and bitterness. T allele is associated with the absence of the receptor, which means you could not taste PTC and bitterness.



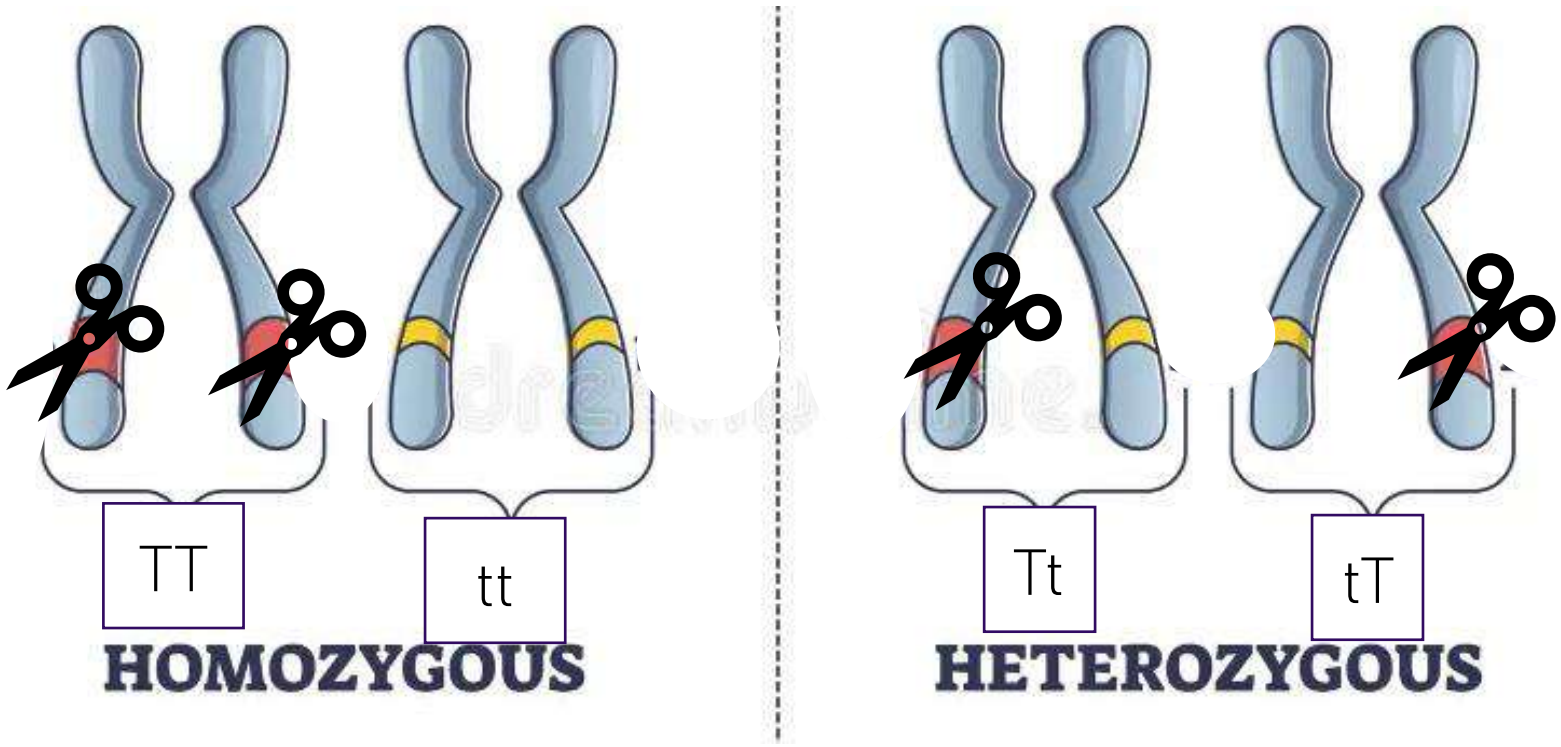
Two forms of the TAS2R38 gene: The T allele and the t allele

- TAS2R38 gene variations

Two forms of the TAS2R38 gene: The T allele and the t allele

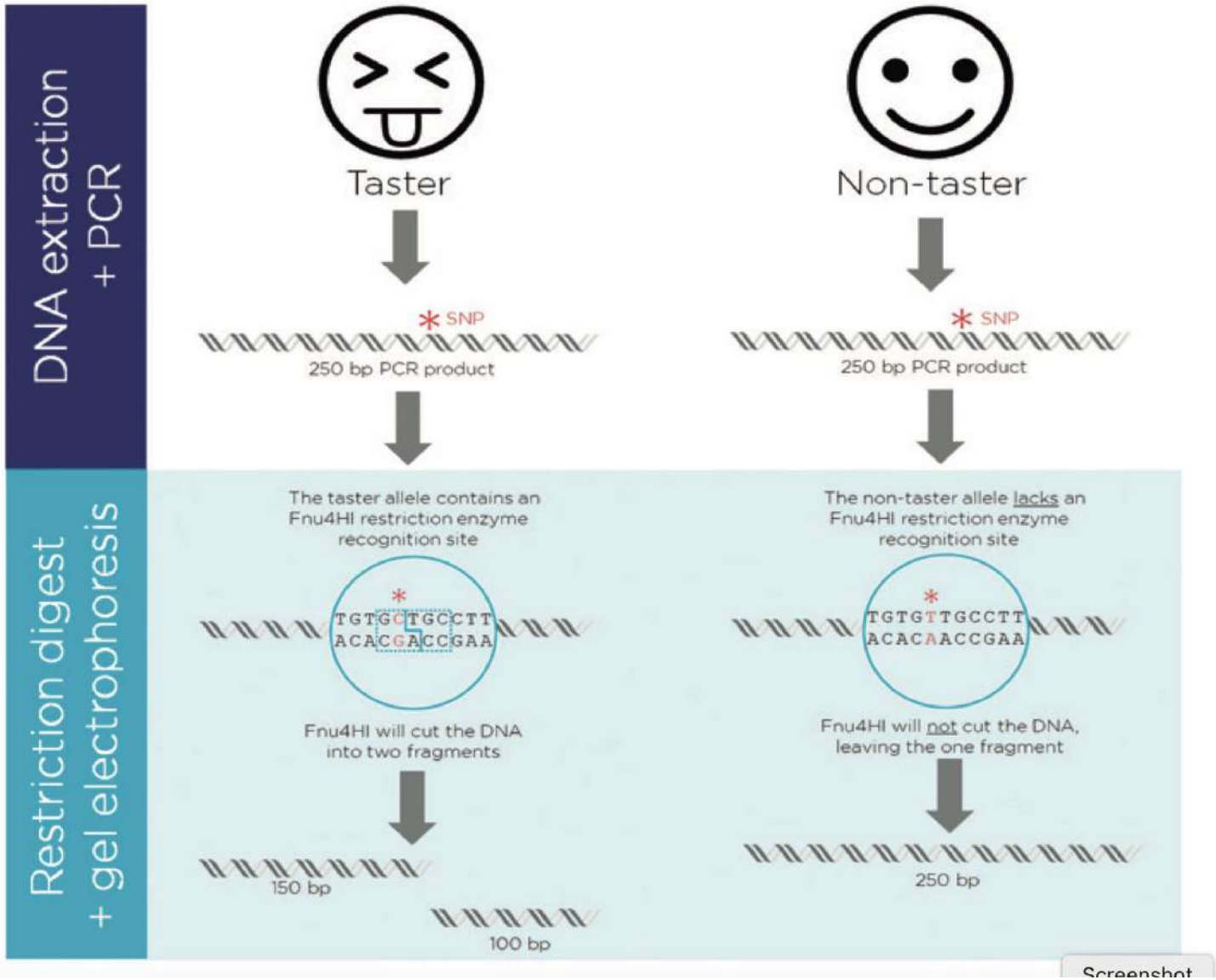
- TAS2R38 gene variations

Two forms of the TAS2R38 gene: The T allele and the t allele

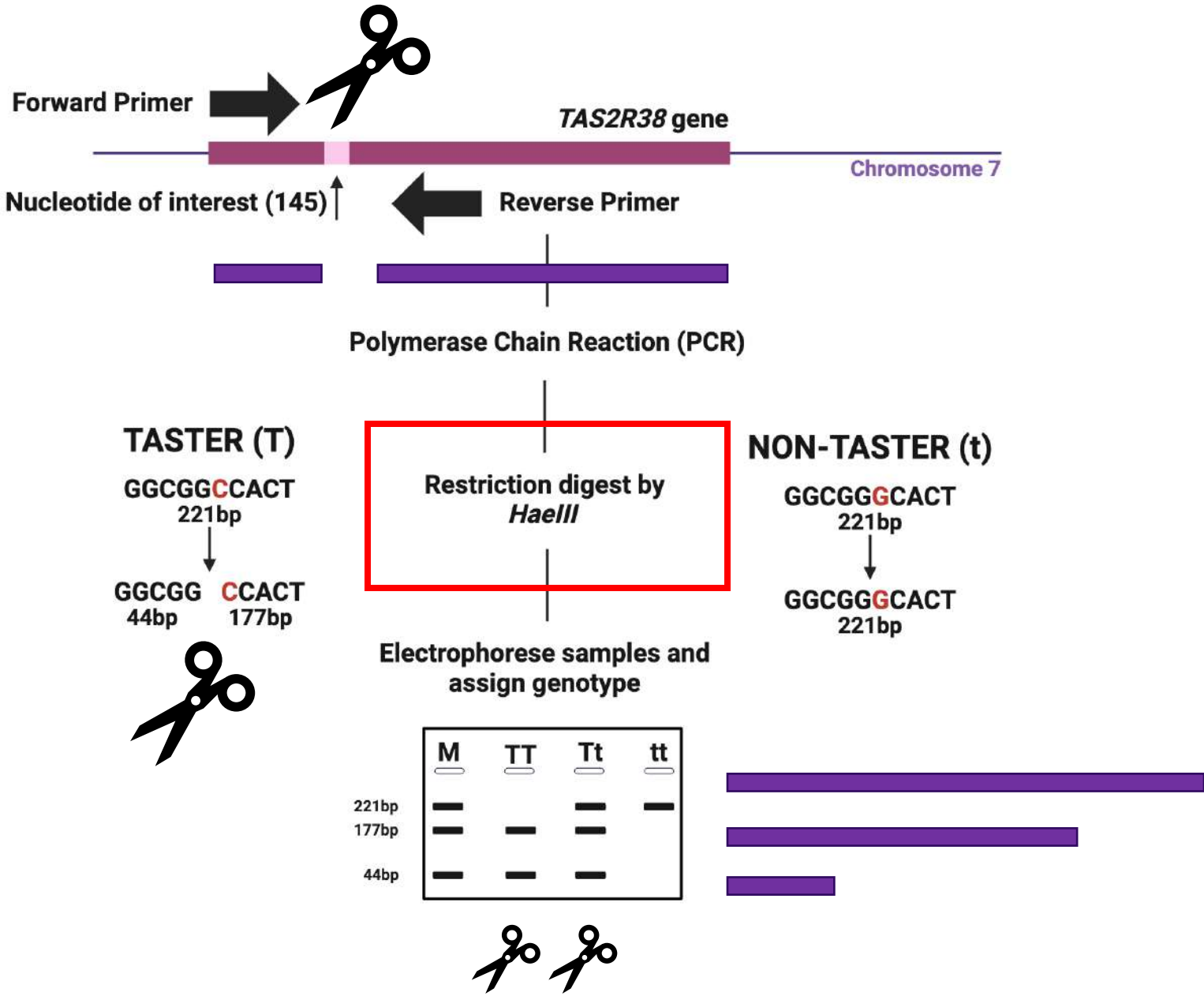


Taster TT /non-taster tt

Partial taster


DNA extraction
+ PCR
Taster
Non-taster
SNP
250 bp PCR product
SNP
250 bp PCR product
Restriction digest
+ gel electrophoresis
The taster allele contains an Fnu4HI restriction enzyme recognition site
TGTGCTGCCTT
ACACGACCGAA
Fnu4HI will cut the DNA into two fragments
150 bp
100 bp
The non-taster allele lacks an Fnu4HI restriction enzyme recognition site
TGTGTTGCCTT
ACACAACCGAA
Fnu4HI will not cut the DNA, leaving the one fragment
250 bp

In this step, you will use PCR to amplify part of the TAS2R38 gene. The sequence of gene fragment we are copying includes a C/T mutation, that contributes to taste perception. This C/T mutation is also known as a *single nucleotide polymorphism* (SNP) with the code rs1726866.



Tasters and Non-tasters

Tasters:
Anna
Wook
Erin
Preston
Reid

Non-tasters:
Cayden
Dakohta