



GenoSensor Corporation

Genotype vs. Phenotype: Genetics of Taste

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User Manual

Genotype vs. Phenotype: Genetics of Taste Kit Manual

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Literature Citation

When describing a procedure for publication using these products, we would appreciate that you refer to them as the GenoSensor Genetics of Taste Kit.

Notes for Instructors

Kit Components and Storage Conditions:

| Component | | Storage |
|--------------|---|------------|
| Clear Box | Solution A | Room temp. |
| | Solution B | |
| | Cotton Swabs | |
| | PTC Test Strips | |
| Aluminum Bag | 2X PCR Master Mix | -20 °C |
| | 2X Res. Dig. Mater Mix (in plastic bag) | |
| | Positive Control WT | |
| | Positive Control MT | |
| | DNase- RNase-free H ₂ O (Negative Control) | |
| | DNA Ladder | |

Preparation for DNA isolation and PCR for up to 24 students

Set heat block or water bath to 95°C

Instruct the students in cheek cell collection step to make sure proper technique and timing is used to ensure sufficient collection for DNA isolation.

Thaw 2X PCR Master Mix on ice (not 2X Res Dig Master Mix). Before opening tube, spin 10 sec at 6,000 rpm or greater in a microcentrifuge. Vortex 10 sec, then spin again for 10 sec.

Aliquot the 2X PCR Master Mix as necessary *after* doing the above preparation.

Each package contains enough 2X PCR Master Mix for 30 reactions. Use 10 ul of 2X PCR Master Mix with 8 ul H₂O and 2 ul isolated DNA for a final PCR volume of 20 ul.

Two reactions of both Positive controls, WT (wild type), and MT (mutant), four total, are available for PCR reaction (10 ul from Positive Control tube + 10 ul 2X PCR Master Mix for one reaction).

Not thaw 2X Res. Dig. Master Mix (in plastic bag) until use.

Electrophoresis

Electrophoresis reagents are not provided in the kit. Please refer to the required materials list.

Best results are obtained by adding DNA dye (i.e. Gel Red or Sybr Safe) to molten agarose.

Avoid exposing the agarose gel to light. It is best to store and run the gel in a dark room, or cover the gel with a box during gel polymerization and the whole electrophoresis process.

There is enough DNA ladder to load 3 lanes with 10 ul.

After PCR and digestion, load as much as 20 ul of student PCR reaction into a lane.

Shipping, Storage and Safety

Shipping and Storage

Genetics of Taste kits are shipped on blue ice. Components should be stored at temperatures shown in the above table. At proper storage conditions, components are stable for 1 year from the date received. Expiration dates are also noted on product labels.

Safety Warnings and Precautions

This product is intended for research use only. It is not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water (Material Safety Data Sheet for products is available upon request).

Genetics of Taste Kit Overview

The Genetic Taste Test Kit introduces Polymerase Chain Reaction (PCR) techniques to students, or anyone wishing to learn PCR and its uses. It contains all reagents necessary for DNA isolation and PCR. PCR is an extremely important and valuable skill to have in contemporary biological and related sciences. In this particular kit, the experiment generates varying results from person to person, demonstrating the basis for the process of creating DNA profiles that are used to differentiate one person from another. After completing this experiment, one should be able to proficiently perform PCR and understand the concepts behind it.

Kit Components and Storage Conditions

(for a lab of 24 students)

| Component | | Amount | Storage |
|--------------|--|-------------|------------|
| Clear Box | Solution A | 6 mL | Room temp. |
| | Solution B | 0.6 mL | |
| | Cotton Swabs | 28 | |
| | PTC Test Strips | 28 | |
| Aluminum Bag | 2X PCR Master Mix | 300 μ L | -20°C |
| | 2X Res. Dig. Mix (in plastic bag) | 300 μ L | |
| | Positive Control WT | 20 μ L | |
| | Positive Control MT | 20 μ L | |
| | DNase- RNase-free H ₂ O (Negative Control) | 300 μ L | |
| | DNA ladder | 30 μ L | |

Additional Required Materials

- Thermal Cycler
- Heat Block or (heat plate, Beaker with de-ionized water; water bath, Tube floater; Thermometer)
- Microcentrifuge
- Microcentrifuge tubes
- Vortex
- Micropipettes (p10, p200, p1000)
- Pipette tips
- PCR tubes
- Tube Racks
- Ethanol or ethanol wipes
- Electrophoresis equipment
- Electrophoresis supplies: agarose, TBE, DNA loading buffer, running buffer, gel dye (eg. Sybr safe, Gel Red)
- Scissors and tweezers
- UV light box or “Gel Doc” equipment and program

Introduction: Theory and Background

Ever wonder why your best friend likes coffee but you can't stand the taste of it? The acquired taste you're supposed to develop for certain foods might just be a myth. Our ability to taste certain chemical compounds is actually related to our genetics! Foods that contain these chemical compounds will not taste the same for people with different genotypes. Generally, there are a number of genes involved in determining a person's ability to taste a chemical compound, but in some cases it can be narrowed down to just one gene. For example, the ability to taste Phenylthiocarbamide (PTC) is known to be caused by the gene TAS2R38. The correlation between PTC and this gene was discovered back in the 1930's.

Dr A. L. Fox had occasion to prepare a quantity of phenyl-thio-carbamide... As he was placing this compound in a bottle some of it was dispersed into the air as dust. Thereupon another occupant of the laboratory complained of the bitter taste of the dust. This surprised Fox, who being much closer to the scene of operations had of course inhaled more of the dust, but had perceived no taste. He was so positive that the stuff was tasteless that he went so far as to taste some of the crystals directly, finding them as tasteless as chalk. Nevertheless the other chemist was convinced the substance was bitter and was confirmed in this impression when he in turn tasted the crystals and found them to be intensely bitter. Naturally a lively argument arose. In an attempt to settle it, the two chemists called in various other laboratory workers, friends and other people with whom they could establish contact. Some people declared the substance was tasteless and some again found it bitter.

After further investigation, it was found that the correlation between this gene and people's ability to taste PTC was so strong that it could be used in paternity testing, and so it did for many years. Although PTC is not present in the food that we eat, there are other chemical compounds with very similar structures that are naturally occurring and are part of our diet.

Now, taking a look at the molecular side of things we see that the physical variations in this gene among the population are extremely tiny. How tiny? These variations are due to a single nucleotide difference in the DNA template strand that is thought to affect the protein product when its mRNA is translated. This type of variation is called a Single-Nucleotide Polymorphism (SNP), commonly referred to as "snips". Snips can occur at any point along the DNA strand and are what characterize the differences between alleles. There are 3 known snips in the gene TAS2R38. The most common one, located at the 785 nucleotide position of the DNA template strand, is associated with a loss of function in the protein product. This particular snip is a transition mutation from the pyrimidine Cytosine to the pyrimidine Thymine as seen here: GCTGC to GTTGC. Tasters have the 'C' while non-tasters have the 'T'.

By using Polymerase Chain Reaction and Restriction Digest enzymes we are able to amplify a small region of DNA, called an amplicon, then cut it into pieces based on the presence of a snip. The restriction enzyme ApeKI binds to the sequence GCNGC, where N is an 'A' or a 'T'. In this case, the ApeKI enzyme will be able to cut any amplicon that does not contain this particular snip. The result will be two pieces of DNA, each containing a portion of the original amplicon. Amplicons with the snip will not be recognized by the ApeKI enzyme; the result will be a full length amplicon. Therefore non-tasters who are homozygous for this gene and have two alleles both containing the snip will have only undigested (uncut) amplicons. Meanwhile tasters who

are homozygous for this gene and have two alleles without the snip will have only digested (cut) amplicons. Tasters that are heterozygous for this gene and have one allele with the snip and one without it will have some digested amplicons and some undigested amplicons.

Genotype vs. Phenotype: Genetics of Taste - Protocol

Preparation

1. Set heat block or water bath to 95 °C. For a heat block, it is recommended to add water or sand to ensure proper heat transfer. For a water bath, be sure tubes are tightly sealed and not fully submerged to avoid contamination.
2. Thaw 2X PCR Master Mix on ice. **Before opening tube, spin 10 sec at 6,000 rpm or greater in a microcentrifuge. Vortex 10 seconds, then spin again for 10 seconds.**
3. Not thaw 2X Restriction Digest Master Mix (in plastic bag) until use.

Taste Testing

1. Obtain a single PTC test strip.
2. Place the strip on your tongue.
3. Make note of what the strip tastes like to you.
4. Dispose of the strip and proceed with the experiment.

DNA Preparation

****Do not eat or brush teeth one hour prior to cheek cell collection. Wear gloves and handle solutions carefully****

1. Add 200µl of **Solution A** (red label) to a marked microcentrifuge tube.
2. Lightly chew or scrape your teeth against the inside of your cheek to loosen cells.
3. **Thoroughly** roll provided cotton swab inside cheek for no less than **10 seconds** to collect cheek cells.
4. Place swab into marked tube with Solution A.
5. Cut swab above tube to a length that will fit inside the tube. Make certain the cap will shut tightly.
6. Press the tube against the vortex machine to thoroughly mix the sample, for at least 10 seconds. *Solution A contains components which chemically disrupt cell membranes and begin to unravel proteins. Under these conditions, the cheek cells will begin to lyse or break open, spilling cell contents into the solution in the tube.*
7. Place sample in heat block to incubate at 95°C for 10 minutes. Immediately place tube in ice until ready for next step. *This process continues to destroy proteins, particularly, those that damage DNA.*
8. Load sample into microcentrifuge (mini centrifuge works as well), taking care that there is another sample directly across from your sample to keep the centrifuge in balance as it spins. Close internal and external centrifuge lids.
9. Spin briefly (~10 seconds) to pool condensation that has collected on the cap.
10. Remove swab with tweezers (tweezers should be rinsed with ethanol between samples to prevent contamination).
11. Add 20 µl **Solution B** (green label) to the sample tube. *Solution B neutralizes the harsh conditions needed for lysis, preparing the solution for DNA isolation and PCR to follow.*
12. Close the tube lid tightly and vortex to mix for at least 10 seconds.
13. Load sample into microcentrifuge with the tube hinge pointing out, balancing out your sample tube, and closing the lids.
14. Spin sample for 1 minute at 12,000 rpm.

15. Look for a small clear round pellet near the bottom of the tube under the hinge. This pellet contains cellular debris. The aqueous solution (supernatant) that has not precipitated into the pellet contains cellular DNA.

PCR Reaction

Wear gloves and handle solutions carefully

1. Prepare and label a small PCR tube with your name. Label both the top and side of the PCR tube to ensure clarity.
2. Ensure that the “2X PCR Master Mix” is on ice and has been spun at 6,000 RPM or greater in a microcentrifuge for 10 seconds, vortexed for 10 seconds, then spun again for 10 seconds before opening the Master Mix tube.
3. Add 10 μl of “2X PCR Master Mix”, 8 μl H₂O, and 2 μl of supernatant from step 14 above (avoid the pellet) to the labeled PCR tube for a total of 20 μl as indicated in the table below. *The supernatant contains your genomic DNA for PCR.* (Note: It is preferred that the PCR reaction mix preparation is done on ice).
4. Mix the 20 μl PCR reaction mixture by pipetting in and out with the pipette, and close the lid tightly.
5. Store the sample on ice until it is ready to be loaded into the thermal cycler.
6. (as directed by instructor) Label and prepare PCR tubes for controls. At least one of each Positive control WT (wild type) and MT (mutant) should be experimented, and one negative tested. Follow the table below

PCR reaction mixture tables

| Student Sample PCR Mixture | |
|-----------------------------------|------------------|
| 2x PCR Master Mix | 10 μl |
| H ₂ O (provided) | 8 μl |
| Genomic Template (Supernatant) | 2 μl |
| Volume total | 20 μl |

| Control Samples PCR Mixture | | | | | |
|------------------------------------|------------------|------------------------------|------------------|-------------------------------------|------------------|
| Positive Control (WT) | | Positive Control (MT) | | Negative Control | |
| 2X PCR Master Mix | 10 μl | 2X PCR Master Mix | 10 μl | 2X PCR Master Mix | 10 μl |
| Positive control DNA | 10 μl | Positive control DNA | 10 μl | Negative control (H ₂ O) | 10 μl |
| Volume total | 20 μl | Volume total | 20 μl | Volume total | 20 μl |

PCR Parameters

Program your thermal cycler as follows:

1. 94°C – 2 minutes
2. 94°C denaturing – 20 seconds}

3. 58°C annealing – 20 seconds} **repeat steps 2, 3, & 4 for 40 cycles**
4. 72°C extension – 20 seconds}
5. 72°C – 5 minutes
6. 4°C – finished / hold



Stopping point. Store PCR products at -20 °C. Or directly proceed to the following steps.

Restriction Digest

1. Cut open plastic bag containing 2X Restriction Digest Master Mix tube.
2. Thaw the provided 2X Restriction Digest Master Mix tube by warming it with your hand and place on ice till finished. (store -20°C)
3. Before opening tube, spin 10 sec at 6,000 rpm or greater in a microcentrifuge.
4. Add all (9 ml) ApeKI restriction enzyme from PCR tube to 2X Res Dig Master Mix tube.
5. Vortex 20 seconds, then spin again for 10 seconds.
6. Aliquot as necessary for lab groups.
7. After PCR reaction is finished take out 10 µl and transfer to marked new tube.
8. Add 10 of the 2X Restriction Digest Master Mix and mix by pipetting.

| Digestion Reaction Mixture | |
|----------------------------|-------|
| 2X Res. Dig. Master Mix | 10 µl |
| Finished PCR Reaction | 10 µl |
| Volume Total | 20 µl |

9. Incubate final mixture at **75°C** for 1 hour. Or it can be safely left overnight.
10. Load samples on the gel to proceed to the next “agarose gel electrophoresis” steps or store at -20 °C for later use.

Agarose Gel Electrophoresis

General Procedure, detailed directions as given by instructor

- Prepare a 2.5 – 3% agarose gel.
- Set up electrophoresis apparatus and pour the 2.5 – 3% molten agarose for gelation.
- For staining, use a DNA dye which is added directly to the molten agarose (adding more dyes, e.g. double amount, might have a better result as the digested products are small <100bp). For light sensitive dyes, keep the gel in the dark during gelation, either by performing in a dark room or placing a box over the gel.

- Use at least 10 μL of digested product to visualize results by electrophoresis on agarose gel. If gel well volume will accommodate more than 10 μL , a higher volume is preferred. Loading dye is added to the sample to ensure that the sample will sink to the bottom of the well and properly enter the agarose gel.
- Mix sample with loading dye according to instructor directions before carefully pipetting into a well in the gel. Be sure to record which well holds your sample.
- Run at $\sim 100\text{V}$ for ~ 30 minutes and stop before loading dye has run off gel. Depending on the DNA dye used, caution may need to be taken to reduce exposure of gel to light.
- Visualize under UV and record the results manually or by photography
- Compare individual experimental bands.

DNA ladder is a standard which is used like a ruler to show how DNA of a certain length will migrate through a gel during electrophoresis.

Example of Gel Setup and Loading (example of 12 well gel):

| | |
|-------------|--|
| Lane 1: | 10 μL DNA Ladder |
| Lane 2: | 10 μL Positive Control WT |
| Lane 3: | 10 μL Positive Control MT |
| Lanes 4 -11 | Up to 20 μL of each digested student sample |
| Lane 12: | 10 μL Negative Control |

Result

Three types of band patterns can be observed: homozygous non-digested band (a single band, 129bp) for non-tasters; homozygous digested band (two smaller bands, 52 and 77bp) for tasters; and heterozygous bands (three bands combining previous two genotypes) for either non-tasters or tasters.

Keep in mind, the accuracy of the test is only about 60-70%. Most phenotypes are usually composed of multiple genes with multiple genotypes.

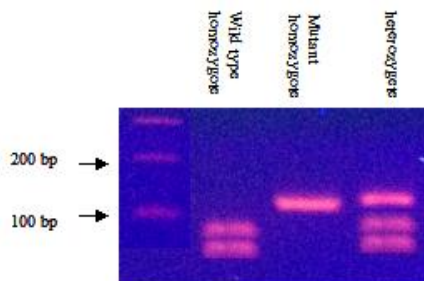


Figure: a gel image of different genotypes

Results and Discussion

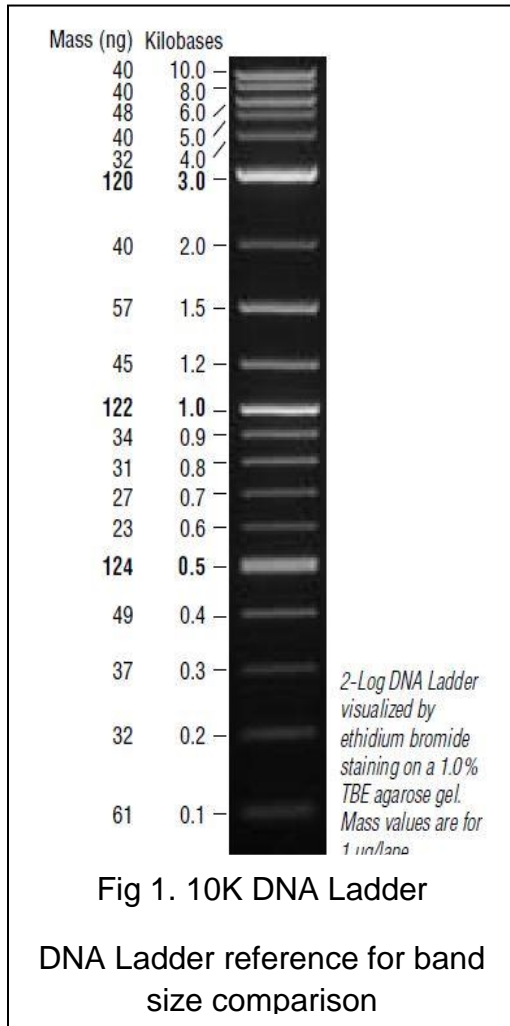
What did the PTC paper taste like to you?

Did your PCR result cut?

The ApeKI restriction enzyme is expected to cut the DNA of those able to taste PTC and to not cut those unable to taste PTC.

Do your results match up with this expectation? If no, what are the reasons the experiment might not have worked as expected?

What does this experiment tell you about the link between your genes and the phenotypes that result?



What else can this type of test be used for?

Genetics of Taste Kit: Technical Background

Introduction to PCR

In 1983, during his time working at Cetus Corporation, Kary Mullis developed a technique that has changed the field of genetics and biological science in general. This revolutionary process was termed “polymerase chain reaction,” or PCR. He earned the Nobel Prize in Chemistry in 1993 due to his innovation. His technique enabled researchers, besides a few expert microbiologists, to amplify DNA. Before that, amplification of DNA was extremely difficult and time consuming. Now, scientists in any field can incorporate molecular biology into their research with PCR.

Currently, PCR is used in a wide variety of areas, for example, gene mapping, DNA sequencing, gene expression, gene detection, forensics, criminal investigation, medical diagnostics, and genome sequencing. Very few of these applications were practically possible before PCR. The experiment does require an initial investment in specially made machinery, but with the proper equipment, nearly anyone can perform a successful PCR experiment without significant cost.

PCR and Biotechnology — Revolutionizes an Entire Research Community

PCR is capable of producing large amounts of targeted DNA from an extremely small amount of starting material, known as your template. DNA can be obtained from any cell such as blood cells, hair cells, cheek cells etc., and after proper treatment to isolate DNA, PCR can be applied to create millions of copies of virtually any desired DNA sequence. That is one of the most significant powers of PCR, specificity. Although you may put an entire genome worth of DNA into a PCR, it amplifies the exact piece of DNA desired and leaves the rest out.

The basic components of PCR:

- Reaction Buffer
- DNA nucleotides (dNTP's) of each adenine, guanine, thymine and cytosine
- DNA polymerase
- 2 DNA oligonucleotide primers
- Template DNA (starting material)

PCR Makes Use of Two Basic Processes in Molecular Genetics

1. Complementary DNA strand hybridization

For DNA to be amplified, one must have a known sequence which flanks the gene of interest upstream and downstream. These sequences are used to create what are known as

'oligonucleotide primers,' meaning a short ~20 base pair nucleotide sequence which is used as a starting point for DNA replication. The primers are said to be complementary to their target regions, so they will anneal (attach) to those regions specifically. Primers are required by DNA polymerase because it cannot add nucleotides without a preexisting chain.

Complementary Strand Hybridization occurs when two different oligonucleotide primers anneal to each of their respective complementary base pair sequences on the template. They are designed specifically to anneal at opposite ends of opposite strands of the specific sequence of DNA that is desired to be amplified.

2. DNA strand synthesis via DNA polymerase

In a PCR, a special type of DNA polymerase is used that is able to withstand the temperature fluctuations required for thermal cycling. Most DNA polymerases cannot tolerate the high temperatures and fluctuations from ~60°C-94°C. The breakthrough in PCR came with the isolation of DNA polymerase from a thermophilic bacterium known as *Thermus aquaticus*. This bacterial species lives in high temperature steam vents and therefore its DNA polymerase evolved to withstand extremely high temperatures.

During PCR, DNA is synthesized and multiplies by 2 each cycle, thus the growth of DNA copy # over the reaction is exponential. In theory, after 30 cycles there will be 2^{30} . This is over a billion copies of DNA. Yielding this amount of DNA allows the possibility of visualization through a variety of means. One of the most popular visualization methods is agarose gel electrophoresis.

Genes and DNA

The human genome contains 23 pairs of chromosomes that contain a total of thirty to fifty thousand genes, most of which generally code for proteins. However, those genes only comprise about 5% of the genome, leaving 95% as so-called non-coding DNA. This noncoding DNA is found not only between, but within genes, splitting them into segments. In eukaryotes, non-coding DNA found within genes is known as **introns**. The sequences that do code for proteins are called **exons**. In eukaryotes, genomic DNA is transcribed into RNA molecules containing both introns and exons for a particular gene. While the RNA is still in the nucleus (before being transported out of the nucleus), the introns (in = stay within the nucleus) must be removed from the RNA while the exons (ex = exit the nucleus) are spliced together to form the complete coding sequence which will soon be translated into the protein. This process is called RNA splicing. Some genes may contain a few introns, others may contain dozens. Interestingly, it is the non-coding 'junk' DNA that is useful to us when considering a DNA profile of an individual, instead of the DNA that actually codes for life.

As discussed, functional segments of genes (exons) code for proteins. Proteins are molecules that carry out most cellular functions. Exon sequences are therefore very similar among individuals, because even slight difference can change the function of the protein in a

potentially harmful way (many diseases are caused by mutated proteins). Introns, however, often vary in size and number among individuals. Intron sequences are thought to be the result of the differential accumulation of mutations throughout evolution that are silently passed to descendants through the hereditary code. It is this difference in intron sequences that allows us to determine human genetic diversity. The identification of these distinctive characteristics in DNA represents the molecular basis for human identification and population genetics. Throughout evolution, intron sequences have been the target of random insertions by short repetitive interspersed elements, also known as SINEs. SINEs have become randomly inserted within our introns over millions of years.

PCR Stages

The machinery required to perform PCR is known as a thermal cycler. The thermal cycler enables the steps of PCR to be automated. The reaction involves a repetitive series of cycles, each of which consists of template denaturation, primer annealing, and extension of the annealed primer by Taq DNA polymerase. Before beginning DNA amplification, genomic DNA is prepared from students' cells. The students' DNA is then added to a mixture of the necessary reagents: oligonucleotide primers, thermostable DNA polymerase (Taq), the four deoxynucleotides (A, T, G, C), and reaction buffer. These reagents are pre-mixed. The tubes are placed into the thermal cycler. These thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across extreme temperature differences. The rapid heating and cooling of this thermal block is called temperature cycling or thermal cycling.

The first step of the PCR temperature cycling procedure involves heating the sample to 94°C. At this high temperature, the template strands separate. This is called the denaturation step.

The thermal cycler then rapidly cools to 60°C to allow the primers to anneal to the separated template strands. This is called the annealing step. The two original template strands may reanneal to each other or compete with the primers for the primers complementary binding sites. However, the primers are added in excess such that the primers actually out-compete the original DNA strands for the primers' complementary binding sites.

Lastly, the thermal cycler heats the sample to 72°C for Taq DNA polymerase to extend the primers and make complete copies of each template DNA strand. This is called the extension step. Taq polymerase works most efficiently at this temperature. Two new copies of each complementary strand are created. There are now two sets of double-stranded DNA (dsDNA). These two sets of dsDNA can now be used for another cycle and subsequent strand synthesis. At this stage, a complete temperature cycle (thermal cycle) has been completed.

Each step takes about 30 seconds to 1 minute, and this process continues for roughly 30-40 cycles depending on how the user has programmed the thermal cycler. Each step is

repeated in that order each cycle until it is completed. At the end, the product is put on hold at a low temperature, generally 4°C, until the user is ready to proceed to the analysis of the product.

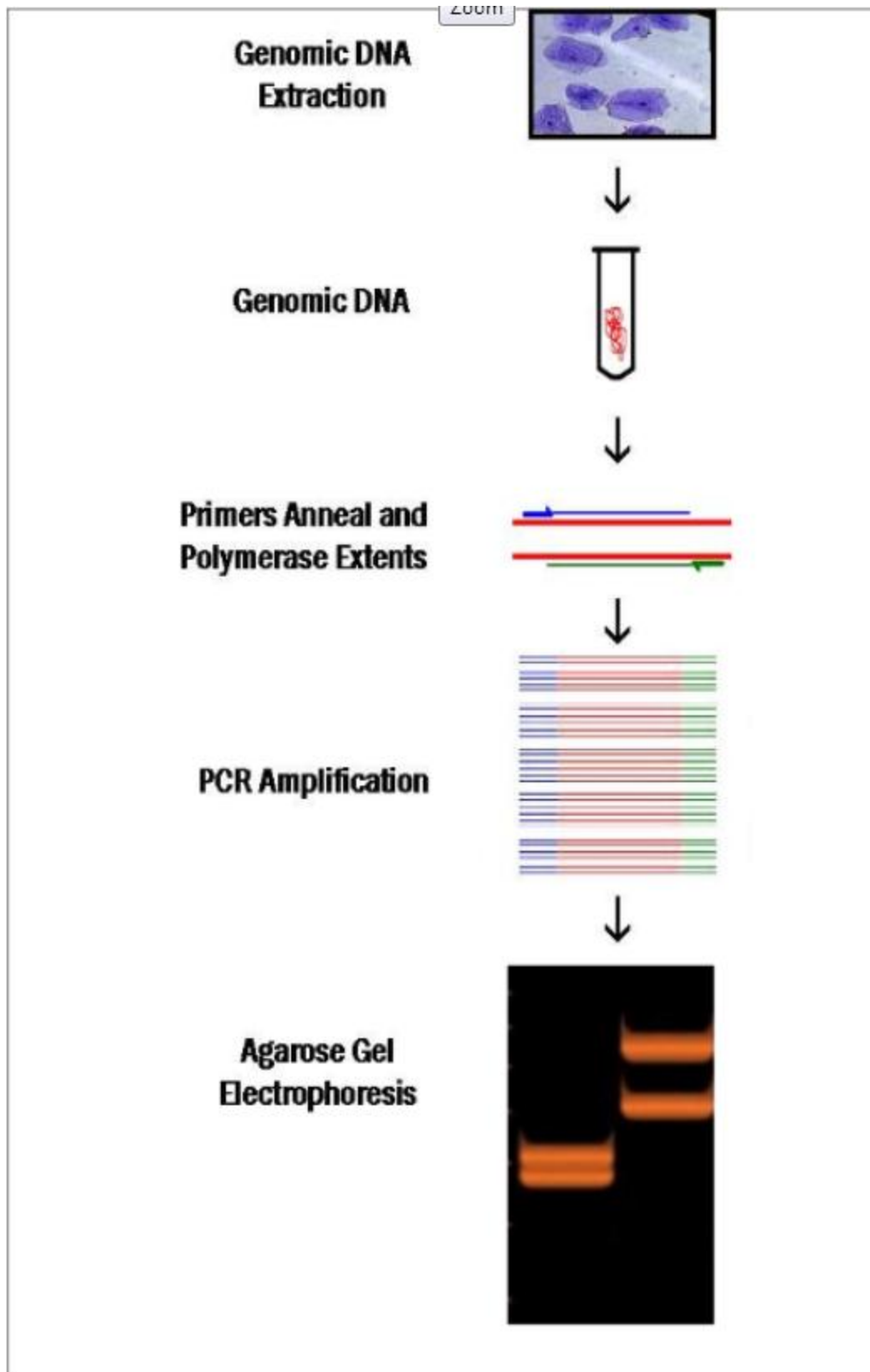


Figure 3. Experiment flowchart from start to finish

Troubleshooting

| Symptom | Possible causes | Solutions |
|------------------------------------|---|---|
| No amplification product | Questionable template quality | Analyze starting material |
| | Inhibitory Substance in reaction | Decrease sample volume |
| | Insufficient cycle # | Run additional cycle |
| | Incorrect thermal cycler program | Verify times and temperatures |
| | Errors in heat block incubation | Calibrate heating block, use sand or water to maximize contact with tube for proper heat transfer |
| | Contaminated tubes/solutions | Autoclave tubes and use filter tips |
| Weak bands/faint signal | Primer annealing temperature too high | Lower annealing temperature in 2 ^o increments |
| | Low concentration of DNA template | Increase swabbing time, thoroughly swab. |
| | DNA Dye degradation during preparation | Light sensitive dyes should be kept in the dark during gel preparation. Prepare in dark room or place a box over the electrophoresis apparatus during gelation and electrophoresis. |
| Non-specific amplification product | Expired, contaminated or degraded DNA dye | Verify that the DNA dye has not degraded in storage, been contaminated or expired. |
| | Premature Taq-polymerase replication | Mix solutions on ice, place rxn directly to 94 ^o thermal cycler |
| | Primer annealing temperature too low | Raise annealing temperature in 2 ^o increments |
| | Insufficient mixing of reaction solution | Mix solutions thoroughly before beginning the reaction |
| Exogenous DNA contamination | | -Wear gloves -Use dedicated area for sample preparation -Use non-aerosol tips |

Technical Service

For more information or technical assistance, please call, write, fax, or email.

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