TEACHER GUIDE

PTC PCR

Polymerase Chain Reaction + Enzyme Digest

FRED HUTCH Science Education Partnership

Overview

In this lab, students will be using common biotech skills such as PCR, restriction enzyme digest, and gel electrophoresis, to genotype themselves. This lab can be used to help students understand many concepts:

- Relationship of genotype and phenotype
- Human genetics and inheritance
- Ethical concerns about genotyping
- Connection between single nucleotide polymorphisms and protein receptors
- Biology techniques (PCR, restriction enzymes, and gel electrophoresis)

TAS2R38

Taste 2 receptor member 38
Gene ID: 5726
FASTA: NC_000007.14
Location: 7q34
Length: 1,143 bp
Target sequence: 303 bp
refSNP: rs1726866

Lab Timeline

Part	Day	Time	Tasks	
Prelab prep		1 hour		Check all inventory for all reagents and supplies needed
Introduction	0	1-2 hours		Anchor phenomena with taste tests or taste survey Collect phenotypic class data using PTC and control tasting strips Have students predict genotype based on phenotypic evidence
DNA Isolation	1	1 hour	**Prelab:** **Lab:** **Post-lab:**	Prepare **0.9% saline solution** Set **heat block** to 99˚C Aliquot **Chelex** Set up stations Students isolate DNA Store DNA at 4˚C
Perform PCR	2	1 hour 30 min 2 hours	**Prelab:** **Lab:** **Post-lab:**	Mix **Taq MasterMix, water and primers** before class to make PCR Master Mix Aliquot reagents Set up **PCR machine** Students set up PCR reactions Run PCR and store product at 4˚C
Enzyme Digest	3	1 hour 30 min	**Prelab:** **Lab:**	Mix **Fnu4HI, Diluent A, and CutSmart buffer** before class. Aliquot reagents Set up **PCR machine** to hold at 37˚C Students set up restriction enzyme digest

		30-60 min	**Post-lab:**	Run PCR machine and store digest product at 4˚C
Gel Electrophoresis	4	30 min 20 min 20-25 min 15 min	**Prelab:** **Lab:** **Post-lab:**	Make 1X TAE running buffer Aliquot sample loading buffer Teacher or student prepares 2% gels Students load samples Run electrophoresis Take gel photos Store gels at 4˚C in airtight plastic bag (optional). Stain may diffuse and result in fuzzy bands.
Results and Discussion	5	60 min		Analyze gel photos Collect class results Compare class results with predictions

Inventory and Supplies

Freezer Box

- ❑ 5x Taq Mastermix
- ❑ TAS3R28 F Primer
- ❑ TAS3R28 R Primer
- ❑ PCR grade water
- ❑ LMW DNA Ladder
- ❑ Fnu4HI restriction enzyme
- ❑ Diluent A
- ❑ 10X Cutsmart Buffer

DNA Isolation

- ❑ 0.9% saline solution
- ❑ 10% Chelex solution
- ❑ Permanent markers
- ❑ Paper cups
- ❑ P1000 micropipette and tips
- ❑ 1.7 ml microtubes
- ❑ Centrifuge (shared)
- ❑ Heat Block 99°C (shared)
- ❑ Vortexer (optional)

PCR Supplies

- ❑ PCR Primer Master Mix (made by teacher)
- ❑ PCR grade water
- ❑ Permanent markers
- ❑ Gloves
- ❑ P10 micropipettes with tips
- ❑ PCR reaction tubes (0.2 ml)
- ❑ P20 micropipette and tips
- ❑ PCR reaction tubes
- ❑ Ice container
- ❑ 1.7 ml microtubes
- ❑ PCR machine (thermocycler)
- ❑ Microcentrifuge (shared)

Restriction Enzyme Digest

- ❑ PCR reaction tubes (0.2 ml)
- ❑ Gloves
- ❑ P20 micropipette and tips
- ❑ Restriction Enzyme Master Mix (made by teacher)
- ❑ PCR Machine (thermocycler)

Gel Electrophoresis

- ❏ Loading dye (Recommended: NEB Purple (6X), no SDS)
- ❏ Agarose powder
- ❏ LMW DNA ladder
- ❏ 1x TAE buffer
- ❏ Permanent marker
- ❏ GelGreen DNA stain
- ❏ P20 micropipette and tips
- ❏ Microcentrifuge (shared)
- ❏ Electrophoresis gel box
- ❏ Power supply or adapter
- ❏ UV/Blue LED transilluminator
- ❏ 0.5X TAE buffer (for MiniOne Gel Systems)
- ❏ Gloves

Safety Concerns and Technique

- Collecting samples from students requires informed consent that students will be genotyping themselves.
- Do not permit food or drink in the laboratory.
- To reduce risk of contamination of DNAases in the environment, make sure all surfaces are clean and students are wearing gloves.
- Students must be proficient with their micropipetting skills.

DNA Isolation

Students will be using a heat and Chelex protocol for isolating DNA from their own cheek cells. This is a quick and effective technique for obtaining DNA for PCR. The drawbacks of the Chelex method is the large variation in the amount of DNA that can be isolated, the inability to quantify the DNA via UV spectrophotometer, and potential for contaminants. These can be resolved using purification columns like the Qiagen DNeasy Kit which you may choose to use with your class.

To retrieve cheek cells, students will be doing a saline mouth wash. It is important to be aware of safety concerns. Students should only handle their sample to reduce the risk of spreading the Flu or other pathogens.

Use a centrifuge to create a cell pellet, pour off the supernatant, then add 250µl of 10% Chelex. Pipet up and down or vortex to gently resuspend. Incubate the tubes at 99˚C for 10 minutes. Spin the tubes again. The Chelex will pellet at the bottom of the tube and DNA will be in the supernatant. Make sure the students do not roughly handle their microtubes or contamination that could impact amplification will occur.

High-Speed Centrifuge Adaptation

This protocol can be done using a high-speed or a microcentrifuge. The student guide is set up for a microcentrifuge as that is what we provide in the gel kit. If you are using a high-speed centrifuge adjust your spin times. For the first spin to create the cell pellet spin samples at >10,000 rpm for 5 minutes. After the heat block step, spin for 3-5 mins at >10,000 rpm.

Lab prep: Prepare the 0.9% saline solution (refer to the PCR Recipe Guide). Each student will need 10-15 ml saline solution. Aliquot 10% Chelex, each student will need 250µl.

Amplification by PCR

Polymerase chain reaction is a technically challenging activity requiring very small and precise amounts of temperature-sensitive reagents. The 5x Taq Master Mix we are using already contains stable amounts of Taq polymerase, PCR buffer, dNTPs, and MgCl2.

Lab prep: Right before lab prepare the **PCR primer mastermix** using both primer sets and 5x Taq Master Mix (refer to PCR Recipe Guide). Once mixed together it should be used as soon as possible. You may choose to aliquot it beforehand. Each student will need 6 µl of PCR primer mastermix to which they will add 16.5 µl of PCR grade water and 2.5 µl of their extracted DNA.

See **PCR Recipe Guide** for instructions on making the primer mastermix.

Set the PCR machine to the following program. This should take less than 2 hours to run, depending on how quickly your PCR machine can ramp between temperatures.

Stages		Temperature	Time
Initial Denaturation		94˚C	180 s
35 cycles	Denaturation	94 ˚C	30 s
	Annealing	55˚C	30 s
	Extension	68˚C	60 s
Final Extension		68˚C	600 s

The PCR product can be kept at 4˚C overnight for storage.

TAS2R38 Primer Set

Forward Primer	5′ – AACTGGCAGATTAAAGATCTCAATTTAT – 3′
Reverse Primer	5′ – AACACAAACCATCACCCCTATTTT – 3′

Target Sequence (303 bp)

5′ AACTGGCAGATTAAAGATCTCAATTTATTTTATTCCTTTCTCTTCTGCTATCTGTGGTCTGTGCCTCCTT
TCCTATTGTTTCTGGTTTCTTCTGGGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGT
CTATACCAGAAACTCTCGTGACCCCAGCCTGGAGGCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTT
TTCTGCTTCTTTGTGATATCATCCTGTG [T/C] TGCCTTCATCTCTGTGCCCCTACTGATTCTGTGGCGCGAC
AAAATAGGGGTGATGGTTTGTGTT 3′

The yellow region marks the forward and reverse primers. The 303 base pair target sequence is between the primers. The Fnu4HI recognition sequence is underlined and the target SNP is designed by the 2 alleles [T/C].

Restriction Enzyme Digest

In this protocol, the restriction enzyme Fnu4HI will be used to identify the SNP associated with the ability to taste PTC. One allele of this SNP matches Fnu4HI's recognition site, GCTGC, resulting in a cut. The other allele GTTGC does not match Fnu4HI's recognition site resulting in no cut. This technique for genotyping using restriction enzymes is called restriction fragment length polymorphisms or RFLP.

SNP

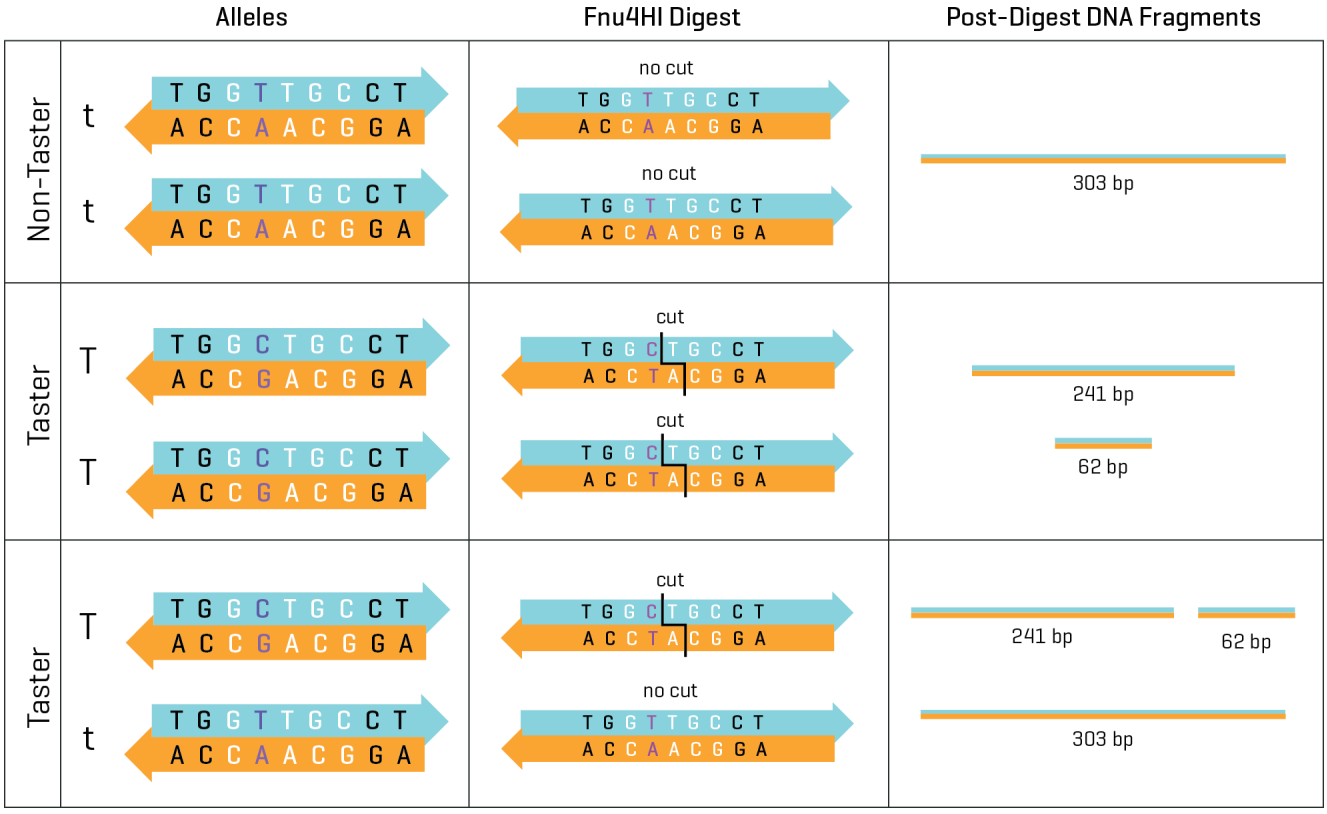
refSNP: rs1726866
Position: 141972905
NT Alleles: C > T
AA Alleles: Ala > Val
Missense

Lab-prep: Before the lab, prepare the Restriction Enzyme Master Mix that the students will use (refer to the PCR Recipe Guide). Restriction enzymes are extremely temperature-sensitive and should always be stored on ice while on the lab bench. **This is temperature sensitive, only make it at most a few hours in advance and keep it in the freezer until right before use.**

Once the students have combined the digest reagents and DNA, incubate the tubes at 37°C in the PCR machine (thermocycler). For complete digestion, we recommend incubating for at least 30 mins. If the incubation is less than 30 mins, a homozygous dominant (TT) may show a false positive for the 303 bp fragment. Samples can be incubated for several hours to overnight as the enzyme will denature and become inactive over time.

Fnu4HI
Recognition Site
5' GC|N GC 3'
3' CG N|CG 5'

In positive sample digestion the 303 bp fragment will be cut into a 62 bp fragment and a 241 bp fragment.



TAS2R38 Fnu4HI Digest Diagram. The arrows represent 5' -> 3'. The purple represents the SNP [C/T] and the white letters show the potential recognition site for Fnu4HI. At "GCNGC" (N representing any nucleotide), Fnu4HI will cut. The post-digest DNA fragments are shown for each genotype.

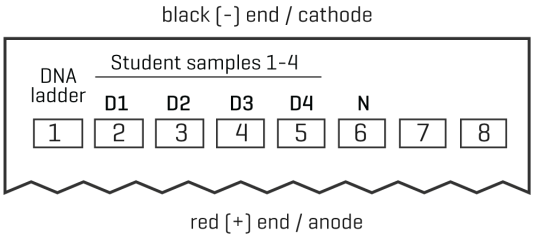
Gel Electrophoresis

In this lab students will run their Fnu4HI-cut sample and one "no-digest" DNA sample per lab group on a 2% agarose gel. The DNA band pattern they see will reveal their genotype for the TSA2R38 gene. Students will also load a DNA ladder containing DNA fragments of determined sizes on each gel. They can estimate DNA fragment size by comparing the bands from their digest with the ladder. If you are using Vernier probes and LoggerPro software, your software comes with gel analysis functions. A 2% gel is a high percent agarose gel, needed to separate small DNA fragments clearly during electrophoresis. At least one gel per class should also contain a positive and a negative control sample.

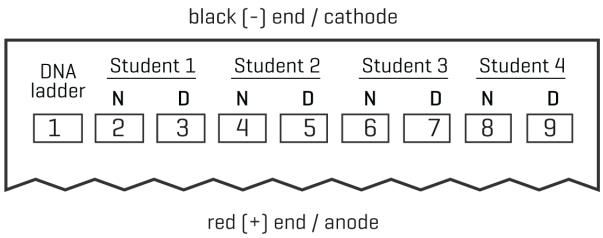
Note: It would be optimal for the teacher to set up a known Tt PTC PCR reaction. This would be DNA from a person that you know is a heterozygous taster. Run the product on a gel prior to doing these labs with students if possible. This would verify that all reaction components and equipment are working properly and would provide a gel for students to look at if for some reason none of their samples amplify or digest.

Due to the number of wells and samples, we suggest that each lab group of 4 students run their Fnu4HI digests and only 1 "no-digest" sample for the group (see Gel Diagram 1). If you have fewer students or gel boxes that can accommodate more wells you may choose to have all your students run both their digest and "no-digest" samples (see Gel Diagram 2).

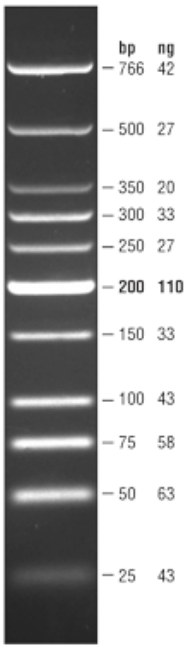
Gel Diagram 1



Gel Diagram 2



Lab prep: To save time some teachers may decide to prepare the agarose for the students to pour or make the gels before class. See the PCR Recipe Guide for directions on making a 2% gel with GelGreen. Aliquot the sample loading buffer and low-molecular weight (LMW) DNA ladder.



GelGreen: To visualize DNA fragments we supply GelGreen DNA stain that can be added directly to melted agarose. GelGreen is a fluorescent DNA stain and requires a UV or Blue LED transilluminator for visualization. Many all-in-one gel systems like the MiniOne allow students to turn on the Blue LED light and watch the DNA migrate as the gel is running.

Note: In Fall 2025, SEP began providing diluted GelGreen instead of SYBRsafe to use for making electrophoresis gels for its PCR labs. In our tests, we found that diluted GelGreen (provided at half the concentration of stock GelGreen) outperformed the undiluted SYBRsafe previously provided. We have adjusted the concentrations of our PCR DNA ladders provided so that teachers can use the diluted GelGreen provided as a direct swap for the SYBRsafe in our protocols, with better visualization than before.

Results:

Genotype	Phenotype	Bands (bp)
TT	Taster	241, 62
Tt	Taster	303, 241, 62
tt	Non-taster	303

Gel Procedure

Horizon Gel Box:
To visualize your results, run the samples in a 2% agarose gel made with 1x TAE. Add 2µl GelGreen DNA stain for every 10 ml of agarose used. Run with 1xTAE running buffer. Run your electrophoresis at 100V for 35-40 mins. Carefully watch the loading dye line, which will appear as a faint color, to determine how far DNA fragments have traveled. Stop the electrophoresis when the dye front has traveled about ⅔ of the length of the gel.

MiniOne Gel System:
To visualize your results, run the samples in a 2% agarose gel made with 1xTAE. Add 2µl of GelGreen DNA stain for every 10 ml of agarose used. Run in 0.5x TAE running buffer for 20 minutes or until the bands have clearly separated enough on the ladder to allow interpretation of results.

PCR Recipe Guide

At-a-glance Aliquoting Guide

Tube Label	Contents/Description	Amt per tube	# of tubes
PCR MM	5X Taq Mastermix, Forward and Reverse Primers (see below for recipe)	26 µl	8
PCR Grade Water	PCR Grade Water	66 µl	8
Ladder	100 bp ladder	10 µl	8
LD	Sample Loading Dye or Buffer	37 µl	8

0.9% Saline Solution

This solution will be used gargled by students. Make sure to use non-lab food-safe containers and ingredients.

1. Dissolve 9 g NaCl (non-iodized table salt) in 700 ml distilled water in a food-safe container.
2. Add water to bring total solution volume to 1000 ml.
3. Make 10 ml aliquots in disposable cups.

PCR Master Mix

PCR reagents are extremely temperature-sensitive. Mix all ingredients on ice. Class amounts = 32 rxns + 4 extra for pipetting error. For the separate reagents version of the lab please see this guide for the PCR Mastermix recipe. **This is temperature sensitive, only make it at most a few hours in advance and keep it in the freezer until right before use.**

Teacher Guide:

Reagent	Per Reaction	Per Class	Notes
5X Taq Master Mix	5 µl	180.0 µl	Contains Taq polymerase, PCR buffer, MgCl2, dNTPs. Very Temperature sensitive. Keep on ice!
TAS2R38 F	0.5 µl	18.0 µl	Forward primer. Thaw and mix.
TAS2R38 R	0.5 µl	18.0 µl	Reverse primer. Thaw and mix.
TOTAL	6.0 µl	216.0 µl	PCR Master Mix

Student Guide:

PCR Master Mix	6.0 µl
PCR Grade Water	16.5 µl
Student DNA	2.5 µl
TOTAL	25.0 µl

Fnu4HI Restriction Enzyme Master Mix

PCR reagents are extremely temperature-sensitive. Mix all ingredients on ice. **This is temperature sensitive, only make it at most a few hours in advance and keep it in the freezer until right before use.**

Teacher Guide:

Reagent	Per Reaction	Per Class	Notes
Fnu4HI	0.125 µl	4.5 µl	Restriction Enzyme. Temperature sensitive. Keep on ice!
Diluent A	1.125 µl	40.5 µl	Buffer for diluting Fnu4HI. Thaw and mix thoroughly.
10X CutSmart Buffer	1.25 µl	45.0 µl	Stabilizes Fnu4HI during digest.
TOTAL	2.5 µl	90.0 µl	Fnu4HI Enzyme Master Mix

Student Guide:

Fnu4HI Enzyme Master Mix	2.5 µl
Student PCR Product	12.5 µl
TOTAL	15.0 µl

Low Molecular Weight (LMW) Ladder

SEP supplies a Low Molecular Weight ladder from NEB. Label 8 tubes "Ladder" for the DNA ladder. Aliquot 10 µl of LMW ladder into the labeled tubes. Store tubes in the freezer.

Sample Loading Dye/Buffer

Label 8 tubes with "LD" or "LB" for Loading Dye or Loading Buffer. Each group will receive a tube. Aliquot 37 µl of Sample Loading Dye/Buffer into the labeled tubes (enough for 7 samples per gel). Store tubes at room temperature.

2.0% Agarose gel with GelGreen

Teachers may choose to prepare agarose gels ahead of time or prepare agarose for students to pour their own gels to save time. Gels should be stored in the running buffer in a Tupperware in the fridge.

If you are making the gels or agarose ahead of time, note that GelGreen is light-sensitive. Make sure to cover the gels in some running buffer (to make sure it doesn't dry out) and cover them in foil. Note: We provide materials for 8 student gels, 1 teacher gel, and 2 extra gels.

1. Calculate the number of gels needed.
    a. **Horizon system:** Each Horizon gel needs 25 ml. 8 gels plus an extra gel - about 225 ml of agarose gel.
    b. **MiniOne system:** Each MiniOne gel needs 15 ml. 8 gels plus an extra gel - about 135 ml of agarose gel.
2. Calculate the amount of agarose powder needed for your classes.
    a. **Horizon system:** 4.5 g agarose powder added to 225 ml 1X TAE for 2% agarose gels.
    b. **MiniOne system**: 2.7 g agarose powder added to 135 ml 1X TAE for 2% agarose gels.
3. Mix the agarose powder with the appropriate amount of 1X TAE in a large glass container. Loosen the cap.
4. Microwave, pause in 30-second intervals to keep the agarose from boiling over. Make sure that all the agarose is melted and no agarose particles are visible.
5. Add GelGreen directly to the melted agarose gel. Swirl until the dye is evenly distributed.
    a. 20 µl GelGreen per 100 ml agarose. This is a higher concentration of GelGreen than in most of our other labs. The goal is to maximize the chance of seeing the smallest, 62 bp band in this lab.

Components of a PCR Reaction using NEB 5X Master Mix*

*1X amounts listed below are final concentrations after primers, DNA template and water have been added to the Master Mix

Item	Function (info from NEB and Fischer websites)
Buffer: Tris-HCl	Tris buffers are temperature sensitive, maintaining a pH of 8.3-8.5 during cycling • 1X NEB MM uses 10 mM Tris-HCl
Buffer: KCl	• K+ helps decrease repulsive forces between primers (which are negatively charged) and template DNA (also negatively charged) • A higher salt concentration allows shorter DNA fragments to denature preferentially to longer fragments • 1X NEB MM uses 50 mM KCl
Buffer: MgCl2	• Mg++ is a cofactor of Taq required for enzymatic activity – it sits within the Taq active site and enables incorporation of dNTPs during extension • Mg++ also crucial for annealing of primers to template by stabilizing negative charges on their phosphate backbones • Optimal concentration 1.5-2.0 mM (1X NEB MM is 1.5 mM)
Primer	Short single-stranded DNA segments designed to anneal to complimentary sequences on target DNA 1. Generally 18-30 nucleotides in length 2. The melting temperature (Tm) is the temperature at which 50% of the primer and its target DNA are annealed 3. Tm depends on primer length and concentration of G/C content of primer 4. The Tm should not vary by more than +/- 5o C for the primer set 5. Secondary structures (primer-dimer and hairpins) can form if primers contain complementary sequences 6. Typically use 0.1 – 0.5 µM of each primer
Deoxynucleotide Triphosphates (dNTPs)	Each dNTP is made up of a phosphate group, a deoxyribose sugar and one of 4 nitrogenous bases • 1X NEB MM uses 200 µM of each dNTP
Taq Polymerase	A thermostable DNA polymerase from the bacterium Thermus aquaticus • Taq synthesizes a complementary DNA strand to template DNA by adding dNTPs to the primer-template complex in a 5'-3' direction • NEB 1X MM uses 1.25 units per 50 µl reaction
Target DNA	Only 1 ng – 1 µg of genomic template DNA is needed! Approx 104 copies of target DNA required to detect product in 25-30 cycles
PCR grade water	Must be sterile, DNase and RNase free
Other ingredients	These are also included in our NEB Master Mix
Glycerol (5%)	Stabilizes Taq polymerase
Tween-20 (0.05%)	Reduces secondary structures (primer dimers and hairpins)

IGEPAL CA-360 (0.08%)	A non-denaturing detergent • Prevents primers from non-specific annealing during the initial heating period before cycling begins