Principles of Modern Biology SCI1210-02 Fall 2012
Dr. Jean Huang
Dr. Sadie Aznavoorian-Cheshire

PTC Phenotype and Genotype:
Pt. 2: PCR of the PTC gene from genomic DNA

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PCR of the PTC gene from genomic DNA

Overview of PTC haplotype differences

In this portion of the PTC module, each student will amplify the PTC gene (1106 bp PCR product) residing in the genomic DNA that was just isolated. The purpose of this is to obtain sufficient quantities of the PTC gene to allow for DNA sequencing analysis, and ultimately for determination of the PTC allele(s) that you carry.

Previous genetic analyses have revealed 3 polymorphic sites in the PTC gene: bp #145, bp #785, and bp #886 (see posted references). At these sites, changes in a single nucleotide result in changes in the corresponding amino acid (aa) in the protein product, as shown below:

Polymorphisms within the PTC gene

Base pair	Amino Acid	Allele	Amino Acid encoded
145	49	C	Proline (P)
		G	Alanine (A)
785	262	C	Alanine (A)
		T	Valine (V)
886	296	G	Valine (V)
		A	Isoleucine (I)

For example, 785C will result in an Alanine being inserted into the PTC gene product whereas a T at position 785 will code for a Valine. These different amino acids have been found to contribute to the taster or conversely non-taster phenotype, especially aa49 and aa262 (the amino acids at positions 49 and 262, respectively, in the protein chain)- see table 2 from the PTC gene cloning paper by Kim et al. 2003. By mechanisms still under investigation, the amino acids of the 'non-taster' version of the gene render the bitter taste receptor inactive (unresponsive to PTC and related ligands).

Alleles of the PTC gene are named according to the 3 amino acids at these polymorphic sites, i.e. "PAV" (colored red) is the allele most closely associated with PTC tasting ability, whereas "AVI" is the non-taster allele.

Diplotype analysis of the various combinations of alleles at the three loci is discussed in the PTC gene cloning paper.

Protocol: Amplification of Human Genomic DNA at using PCR

Lab equipment
Pipettemen and tips
Thermocycler
Thin walled PCR tubes

Reagents
- DNA purified from spin column
- Taq DNA Polymerase: (5 U/μl)
- 10X Taq buffer with $MgCl_2$
- dNTP mix (deoxynucleotides dATP, dTTP, dCTP, dGTP, at 10mM each)
- Primer PTC-3 (forward) (50 μM stock solution) (sequences of both primers listed below)
- Primer PTC-4 (reverse) (50 μM stock solution)
- dH_20 (sterile)

The PCR Reaction
A positive PCR control for this reaction will consist of amplification of "control" human genomic DNA (genomic DNA previously isolated and tested, and known to be intact). A negative control will consist of a tube that contains all of the components except for genomic DNA (i.e., dH_2O). Each team will share one 'positive' control sample; however, the number of negative control samples will depend on the available space in the PCR machine (i.e., the entire class may have to 'share' a single negative control so that there is room in the PCR machine for both sections in the same afternoon).

Reaction set-up

First, know that the components of each reaction tube need to be as follows:

• **5 µl genomic DNA** (~50 ng) (or 5 µl of dH_2O, for negative control)
• 5 µl 10X Taq buffer with $MgCl_2$
• 1µl dNTP mix
• 1 µl Primer PTC-3 (forward) (@50 µM)
• 1 µl Primer PTC-4 (reverse) (@50 µM)
• 36.8 µl dH_20
• 0.2 µl Taq Polymerase (@ 5 U/µl)
50 µl total per PCR tube

} **Master Mix (45 µl total per tube)**

1. Each team make up Master Mix, for the entire team (+1).
Each team should make up one "Master Mix" of the common reagents (in blue) in a regular eppendorf tube, labeled "Master Mix": if your team needs, for example, 5 samples total (one positive, one negative, one for each of three students), then make up enough Master Mix for 6 samples. Making up enough MM for one additional sample will cover what is lost to the sides of tubes and pipette tips. Likewise, if your team only requires 4 samples, make up MM for 5 reaction tubes. Simply multiply the volumes of each "blue" component x 5 (or 6, i.e. whatever is necessary) and add it to the eppendorf tube, *on ice at all times*.

Keep your Master Mix on ice!

After making up your Master Mix, set up and label your individual PCR reaction tubes (the small, thin-walled tubes) with your team initials (or some identifying logo) and the designation for "neg" (negative ctl), "pos" (positive ctl), and the individual student's initials for his/her own sample. Alternatively, simply number the tubes and record what the numbers stand for. Whatever labeling system you use, try to be clear! You will want to know which 5 or 6 tubes belong to your team, once they are all placed into the PCR machine. No need to add dates to the tubes- they will be processed and stored together, and the entire rack will be labeled with the date.

Do not forget that the final reaction tubes are set up in the small thin-walled PCR tubes, NOT regular eppendorfs):

2. Final Set-up of PCR Reaction tubes:
• keep the labeled PCR tubes on ice:
• 45 µl PCR Master Mix is added to each PCR tube
• 5 µl genomic DNA (~ 50 ng) or dH_2O for neg. control, is added last*
50 µl total

Mix by "flicking" tubes; then briefly microfuge down, using the centrifuge tube adaptors. You want to ensure that all the components are brought down to the bottom of the tubes before being placed in the PCR machine.

*B**efore** adding the genomic DNA to your samples, please coordinate with the other teams, so that most of the teams are ready to go at one time. Ideally, you want to add the DNA and then rather quickly (after mixing and pulse-spinning) get your samples into the PCR machine. Thirty samples at a time can be run together in the machine (2 independent blocks of 30). Given that we have 2 sections of student samples to run, we will try to accommodate all of section 02 in one half of the machine, and all of section 03 in the other half.

The PTC gene PCR primer sequences[1]:

PTC-3 (forward): 5'-AGCCAACTAGAGAAGAGAAG- 3'

PTC-4 (reverse): 5'-GCATATTTATGAAGACTCACAG- 3'

Stock concentrations = 50 μM, for each (1 μM final, in reaction tubes)

PCR program:
PCR program: called **"PTCMOD2"**:

Step	Temperature (°C)	Minutes
1	96	4
2	94	0.5
3	55	0.5
4	72	1.0
5	Repeat steps 2-4, 32X	
6	72	8 min
7	(cooling) 4	¥

After the end of the PCR reaction, tubes will be taken out of the machine and put in the -20^{o} freezer until the next lab period (11/14/12), when you will run an agarose gel on an aliquot of your PCR reactions to confirm successful amplification of the PTC gene (remainder will be purified and sent out for sequencing).

[1] Please remember to include the primer sequences, as written, in your lab reports on this topic; it is conventional and expected to give the reader your primer sequences, in order to allow for other scientists to replicate your reactions.