

# Detection of Mutations in the PAH, NF-1, and BRCA1 Genes

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## Abstract

Several mutations occurring within the human genome can be easily detected through the use of a process known as PCR, or polymerase chain reaction. PCR allows scientists to take small fragments of DNA and amplify, or copy, them so that an adequate amount of the sample is available for further examination. This process generally only takes a few hours, and can provide scientists with millions of copies of the sample in question.

The specificity of the region the scientists can attempt to amplify depends on the primers used. Primers are short sequences of deoxyribonucleotides that are complementary to the beginning and end portions of the of the target sequence to be amplified through PCR. By developing primers specific to portions of certain genes, the presence of several disease-causing mutations can be examined in samples of patients' DNA. In this report, primers were designed to identify specific mutations in the *PAH*, *NF1*, and *BRCA1* genes. These primers allowed for distinctions to be made between DNA containing the mutations and DNA that was mutation-free.

## Introduction

Phenylketonuria (PKU) can be caused by approximately 600 different variations of mutations, and is present in about 1 in every 10,000 people on earth. It is commonly identified as "the most common inborn error of amino acid metabolism in European-descended populations" (Gersting, *et al.*, 2008). The gene affected by these mutations is known as the *phenylalanine hydroxylase (PAH)* gene, which is responsible for breaking down phenylalanine. When phenylalanine cannot be properly broken down and removed from our bodies, it builds up to dangerous levels. The presence of too much phenylalanine in one's body can lead to varying degrees of mental impairment (Cunningham, 1996).

If the disease is caught early, doctors can prescribe for patients an extremely restrictive, low phenylalanine diet. Once on this strict regimen, patients can escape the possibility of mental impairment and live an otherwise normal, productive life. As a result, all infants are screened for the disease within days of their birth.

The specific PKU-causing mutation being examined in this report involves a change at the gene's 526<sup>th</sup> base pair, from a cytosine to a thymine. This change results in the loss of a restriction site for the enzyme *TaqI*. With the creation of appropriate primers, PCR amplification of DNA containing this mutation would yield a product that clearly resists *TaqI* digestion, while non-mutated DNA does not.

Neurofibromatosis, another genetic disorder, affects approximately 1 in every 2500 to 3000 people in the world (Williams *et al.*, 2009). Mutations within the *Neurofibromin 1 (NF1)* gene can lead to the development of a disease known simply as Neurofibromatosis Type 1, sometimes called von Recklinghausen disease (Shen *et al.*, 1996). If a mutation is present within the gene, which is located on chromosome 17, symptoms of varying degrees of severity can develop, from harmless café-au-lait skin spots to the growth of malignant tumors, nodules on the skin, and neurocognitive defects (Williams *et al.*, 2009). It has been speculated that the *NF1* gene acts as a tumor suppressor gene, and that it may play a part in cell growth and differentiation, which could explain why some of its

symptoms involve the development of unusual bodily growths (Shen *et al.*, 1996).

In order to ensure that patients receive the proper care and treatment for this disease, it is imperative that it is caught early. Currently, the disease is diagnosed based on the presence of the aforementioned symptoms, along with several other symptoms (Williams *et al.*, 2009). Being able to identify the specific mutation behind a patient's disease can prove to be very fruitful. The mutation involved with the disease that is being examined involves what is called a *de novo* Alu insertion, the presence of an extra 300 or so base pairs derived from an Alu transposable element inserted within the *NF1* gene.

The third and final genetic disorder to be examined concerns a mutation within the *breast cancer-associated* gene, or *BRCA1* (Ransburgh *et al.*, 2010). Mutations within this gene leave a person more susceptible to developing breast and/or ovarian cancers. Connections have been made between mutations within this gene and the development of these cancers within families, leading scientists to believe that it contributes to "inherited" cases of cancer (Ransburgh *et al.*, 2010). Just as it is important to try to determine if someone's DNA contains disease-causing mutations in their *PAH* and *NF1* genes, the same can be said for the *BRCA1* gene. The mutation of interest within this gene causes the deletion of a 40 base pair segment, beginning at the gene's 1294<sup>th</sup> base pair.

## Materials and Methods

### *DNA Extraction (for NF-1 Trials only)*

Flat toothpicks were rubbed vigorously against the inside of the cheek in order to dislodge cells. The toothpicks were then immersed in individual 1.5-mL microcentrifuge tubes filled containing 500  $\mu$ L of QuickExtract DNA Extraction Solution from Epicenter Corporation. The tubes were each vortexed for approximately 15 seconds and were then incubated in a 65°C water bath. After 15 minutes in the water bath, the tubes were removed, inverted several times, and then placed back in the bath for another 20 minutes. At the end of the 20 minutes, the tubes were removed from the 65°C bath and

placed in a boiling water bath for 15 minutes. After 15 minutes, the tubes were removed from the boiling water, cooled and used for the PCR procedure.

#### PCR Procedure:

For each trial, 0.2-mL Ready-to-Go PCR tubes from GE Healthcare were prepared with 22 $\mu$ L of dH<sub>2</sub>O, 2  $\mu$ L of primer mix, and 1  $\mu$ L of DNA.

The tubes for the PAH and BRCA1 trials were both thermocycled according to the following settings: 95°C for 10 minutes; 35 cycles of: 95°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds; 72°C for 10 minutes; hold at 4°C.

The tubes used for the NF-1 trials were thermocycled according to the following settings: 95°C for 10 minutes; 35 cycles of: 95°C for 45 seconds, 57°C for 45 seconds, 72°C for 1.5 minutes; 72°C for 10 minutes; hold at 4°C.

#### Digestion (for PAH trials only):

The 4 tubes that were thermocycled for the PAH trials were then prepared for digestion using the enzyme *TaqI*. Each digestion reaction was prepared in a 1.5mL microcentrifuge tube, as outlined in Table 1. All 4 tubes were then incubated in a 37° water bath overnight.

PAH Digestion	PAH Undigested	Normal Digestion	Normal Undigested
-1 $\mu$ L dH <sub>2</sub> O	-3 $\mu$ L dH <sub>2</sub> O	-1 $\mu$ L dH <sub>2</sub> O	-3 $\mu$ L dH <sub>2</sub> O
-2 $\mu$ L 10X Buffer	-2 $\mu$ L 10X Buffer	-2 $\mu$ L 10X Buffer	-2 $\mu$ L 10X Buffer
-16 $\mu$ L PAH PCR Product	-15 $\mu$ L PAH PCR Product	-16 $\mu$ L Normal PCR Product	-15 $\mu$ L Normal PCR Product
-1 $\mu$ L <i>TaqI</i> enzyme	-0 $\mu$ L <i>TaqI</i> enzyme	-1 $\mu$ L <i>TaqI</i> enzyme	-0 $\mu$ L <i>TaqI</i> enzyme

**Table 1.** Contents of the 4 1.5mL-tubes prepared for digestion during the PAH trials.

#### Electrophoresis and Analysis:

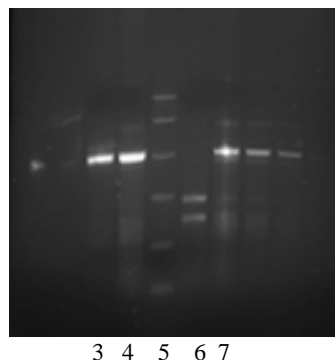
For each trial, a 2% agarose gel was prepared. The PCR/digestion products were then mixed with 4  $\mu$ L of Blue/Orange Loading Dye, and inserted into the gel. Each gel was electrophoresed at approximately 120V until the products moved  $\frac{3}{4}$  of the way through them. Upon the completion of electrophoresis, the gels were taken and analyzed using a ProteinSimple FluorChem™ E System, under its ethidium bromide setting.

## Results and Discussion

#### PAH Trials:

According to information found on the Coriell Cell Repository site, the point mutation being examined within the *PAH* gene was located at the gene's 526<sup>th</sup> base pair. This change, from a cytosine to a thymine, would result in the loss of a restriction site for the enzyme *TaqI*. The loss of this site would stop the DNA from being cleaved in two, resulting in a single band when electrophoresed. In order to better detect this mutation, primers were needed that would amplify the section in which the mutation was found, while

also amplifying portions of the gene on either side of the mutation's location. After accessing GenBank and pinpointing where the mutation should be located, the segment of the genetic code to be amplified was then input into PrimerBlast, and two primers (listed in Table 2) were generated. It was predicted by the PrimerBlast site that the PCR product amplified with those primers should have been approximately 497 base pairs long. If the aforementioned mutation was not present, then *TaqI* would have cut the segment between its 279<sup>th</sup> and 280<sup>th</sup> base pairs, yielding a 279-base pair long piece of DNA. The second segment would therefore have been 218 base pairs long.



**Figure 1.** Photograph taken of the electrophoresed gel from the final successful PAH trial. Lane 3: Contained digested PAH DNA, only 1 497-base pair band seen; Lane 4: Contained undigested PAH, only 1 497-base pair band seen; Lane 5: PCR marker; Lane 6: Contained digested normal DNA, one band of 279 base pairs, one band that was 218 base pairs in length; Lane 7: Contained undigested normal DNA, only 1 497-base pair band seen.

After amplifying the DNA with these primers and then allowing them to digest with *TaqI* overnight, the “normal” DNA, which contained no mutations, was seen to contain two very prominent bands of DNA, which appeared to fall in the 218 and 279 base pair regions, respectively. The DNA that contained the C>T mutation showed only one very prominent band of DNA, which was approximately 497 base pairs long, even though it too had been digested overnight in *TaqI* (Figure 1). Just as predicted, the loss of the restriction site led to a singular DNA band, which was easily identifiable when compared to the “normal” DNA.

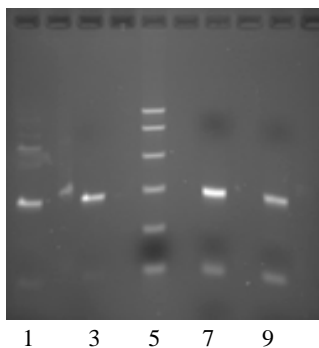
Two controls that were not digested in *TaqI* were also examined in order to make comparisons; both showed only one band of DNA that was 497 base pairs long, just like the mutated DNA. It could then be concluded that these primers were excellent for determining when a sample of DNA contained either a “normal” or mutated *PAH* gene.

#### NF-1 Trials:

The Coriell Cell Repository site stated that there was an Alu insertion located 44 base pairs upstream of exon 6. This information was correlated back to the article “A de novo Alu insertion results in neurofibromatosis type 1,” published in *Nature* in 1991. However, this information yielded primers that did not detect the roughly 300 base

pairs that were supposedly present in the mutated genome. After careful research, it was determined that the information cited by both Coriell and the article itself were outdated, since the article was published over a decade before the human genome was even fully sequenced. It was then discovered that this article was referenced in a much more recent article, published in *Clinical Chemistry* in 2010. In this article, it was stated that the *Alu* insertion was located 44 base pairs upstream of the 42<sup>nd</sup> exon, not the 6<sup>th</sup> (Chou *et al.*, 2010). With this newfound information, the predicted location of the mutation was then found using Genbank, which then provided a sequence that could be entered into PrimerBlast.

The primer sequences generated by PrimerBlast (listed in Table 2) were predicted to amplify a segment of DNA that was 275 base pairs long without the mutation. With the mutation, the DNA segment that would be amplified would be approximately 575 base pairs long. These results were clearly seen upon electrophoresis of the four amplified samples of DNA. The “normal” DNA only produced a prominent band in the 275 base pair range, while the DNA containing the insertion showed two bands; one in the 275 base pair range, and the other in the 575 base pair range. The two DNA samples extracted from Angelica and Jessica showed only one band each in the 275 base pair range (Figure 2). These acted as controls for the experiment, since it was known that neither individual suffered from Neurofibromatosis Type 1.



**Figure 2.** Photograph taken of the electrophoresed gel from the final successful NF-1 trial. Lane 1: Contained mutant NF-1 DNA, 2 bands seen, one was around 275 base pairs long, and the other was around 575 base pairs long; Lane 3: Contained normal DNA, one band around 275 base pairs long was seen; Lane 5: PCR marker; Lane 7: Angelica’s extracted DNA, only one 275-base pair long band seen; Lane 9: Jessica’s extracted DNA, one band that was 275 base pairs long was seen.

Based on the presence of the two bands in the lane containing the mutated DNA, it was concluded that the sample was obtained from someone who was a heterozygote. A heterozygote would have one copy of “normal” DNA and a copy of the mutated DNA, and would therefore show both bands of the expected lengths. According to this conclusion, the primers produced were

effective in amplifying and identifying DNA containing this specific, Neurofibromatosis Type 1-causing mutation.

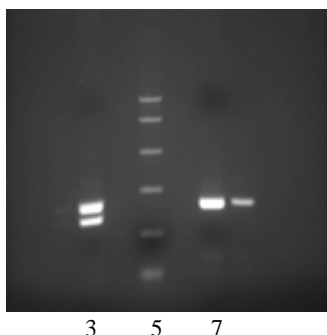
#### *BRCA1 Trials:*

The Coriell Cell Repository described the mutation being examined in the *BRCA1* gene as a 40 base pair deletion located at the gene’s 1294<sup>th</sup> base pair. However, when primers were produced based on this information, multiple bands and smears were visualized in the lanes for both the mutated and non-mutated DNA samples. It was concluded that these primer sequences were causing nonspecific amplification, which was causing segments of the DNA that were similar to the primer sequences to amplify. Since DNA often contains repetitions of similar sequences, multiple bands can sometimes occur if the wrong primers are used for amplification.

After sifting through various articles, it was discovered that a primer was used to amplify this specific mutation in an experiment in which several *BRCA1* mutations were identified and sequenced in order to determine the type of genomic change they caused (Castilla *et al.*, 1994). It was decided that this sequence would then be used to see if a change could be detected between DNA containing this deletion and DNA that had retained those 40 base pairs. It was predicted in the article that these primers, listed in Table 2, were capable of producing a PCR product that was 245 base pairs in length when all of the correct bases were present (Castilla *et al.*, 1994). That meant that if the DNA being examined contained the mutation, only 205 base pairs would be seen in the final amplified product.

Upon electrophoresis, it could clearly be seen that the lane containing the DNA that was not mutated showed only a single band in the predicted 245 base pair range. The mutated DNA, on the other hand, contained two bands: one in the 245 base pair range, and the other in the 205 base pair range (Figure 3). The definite distinction between the two types of DNA allowed for the conclusion that this set of primers was indeed effective for determining whether a sample of DNA being tested contained this specific, 40 base pair deletion mutation.

The identification of these three different mutations may one day serve to help with more than the early detection and treatment of possible life-threatening diseases. By beginning to identify not only the disease, but the specific mutation that causes a person’s suffering, doctors can begin to create medical regimens personalized to work with the patients’ individual bodies, instead of prescribing treatments that may not be as effective.



**Figure 3.** Photograph taken of the electrophoresed gel from the final successful BRCA1 trial. Lane 3: Contained mutant BRCA1 DNA, one band that was 205 base pairs long, and one band that was 245 base pairs long were seen; Lane 5: PCR marker; Lane 7: Contained normal DNA, only one band that was 245 base pairs in length was seen.

Gene	Primer Sequences
PAH	<b>Forward:</b> 5' GTGATTCCCGAAAGTGAGAGC 3'
	<b>Reverse:</b> 5' ACTTTCTGCAGGGCCATTGA 3'
NF-1	<b>Forward:</b> 5' ATCACTTTTCCTTTTGCCCTGT 3'
	<b>Reverse:</b> 5' CAGCATCAGCATGTAGCGTG 3'
BRCA1	<b>Forward:</b> 5'AGAAACTGCCATGCTCAGAGAATC3'
	<b>Reverse:</b> 5'ATGAGGATCACTGGCCAGTAAGTC3'

**Table 2.** Primers that successfully identified mutations in the PAH, NF-1 and BRCA1 genes.

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### Biography

Jessica Imperato is currently a junior, and will graduate in May 2015 with dual B.S. degrees in Forensic Science and Biology. Originally from Thiells, New York, she hopes to pursue PhD in cellular and molecular biology, and eventually enter the clinical research field.

