# Detection of Mutations in the PAH, NF,-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 process generally 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 deoxyribonucleotideattare complementary to the beginning and end portions of the of thestergetneto be amplified through PCR. By developing primers specific to portions of certain genes, the presence of several disease-causing mutations can be examined in stempf patients' DNA. In this report, primewere designed to identify specific mutations in the PAH, NF1, and BRCgetnes These primers allowed for distinctions to be made between DNA containing the mutations and DNA that was mutatione.

Introduction

this gene leave a person more susceptible to developing breast and/or ovarian cancerson@ections have been made between mutations within this gene and the development of these cancers within familie&eading scientists to believe that it contributes to "inherited" cases of cancer (Ransburgh et al., 2010). Just asit is important to tryto determine if someones DNA contains diseaseausingmutations in their PAH and NF1 genes, the samean be said for the BRCA1 gene. The mutation of interest within this gene causes the deletion of a 40 base pair segmeting 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 -1mb microcentrifuge

placed in a boiling water bath for 15 minutes for 15 minutes, the tubes were remover of the boiling water cooledand used for the PCR procedure.

#### PCR Procedure

For each trial, 0.2mL Readyto-Go PCR tubes from GE Healthcarevere prepared with 22L of  $dH_2O$ , 2 L of primer mix, and 1 L of DNA.

The tubes for the PAH and BRCA1 trials were the thermocycled according to the following settings: 050 for 10 minutes; 35 cycles of:  $96^{\circ}$  for 45 seconds,  $55^{\circ}$  for 45 seconds, and  $72^{\circ}$  for 45 seconds;  $72^{\circ}$  for 10 minutes; hold at 4°C.

The tubes used for the NIF trails were thermocycled according to the following settings:  $\mathfrak{GF}$  for 10 minutes; 35 cycles of:  $\mathfrak{GC}$  for 45 seconds,  $\mathfrak{FC}$  for 45 seconds,  $\mathfrak{FC}$  for 1.5 minutes; 72° for 10 minutes; hold at 4°C.

T1/P

Digestion (for PAH trials only):ordi(7(I7 8997(:20 Td (°)Tj -0.004 Tc 0.004 Tw5uTc 0Tw5uTc 0Tw5u/P <m60Ct9.271.157 Td [(T

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 **th** this article was referenced in a much more recent article, published **O** inical Chemistry in 2010. In this article, it was stated that the **Ans**ertion was located 44 base pairs upstream of the<sup>nd</sup> **A** contained the predicted location of the mutation was then found using Genbank, which then provided a sequence that **Id d b** 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 shead 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 controlsr fthe experiment, isnce it was hown that neitherindividual suffered from Neurofibromatosis Type 1.

1 3 5 7 9

Figure 2. Photograph taken of the electrophoresed gel from the final successful NFF 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 seeni(a)atase (th)a(an)a8(att)(th)72))B(to 54(()560(fn)6(pTdd[iin)a)7f)18298(8)jTi)a(tr)2ta0F5(c)-3(e)-3(t)7hTm [(D 6 >>BD(t)7(() T2rilsF

(Nt) II 7 CNt Ntny Ntb T db (and the transmission of transmission of the transmission of the transmission of transmiss

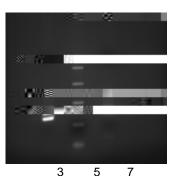


Figure 3. Photograph taken of the electrophoresed gel from the final successful BRCA1 trial. Lane **G**ontained mutant BRCA1 DNA, one band that was 205 base pairs long, and

one band that was 245 base pairs long were seen; Lane 5: PCR marker; Lane 7: Contained normal DNA, only one bad Parvin, J.D. 2010. Identification of breatstmor that was 245 base pairs in length was seen.

Gene	Primer Sequences
РАН	Forward: 5' GTGATTTCCCGAAAGTGAGAGC 3'
	Reverse: 5' ACTTTCTGCAGGGCCATTGA 3'
NF-1	Forward: 5' ATCACTTTTCCTTTTGCCCTGT 3'
	Reverse: 5' CAGCATCAGCATGTAGCGTG 3'
BRCA1	Forward: 5'AGAAACTGCCATGCTCAGAGAATC3'
	Reverse: 5'ATGAGGATCACTGGCCAGTAAGTC3'

Table 2. Primers that successfully identified mutations in the PAH, NF1 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.

