

# Use of allele-specific PCR to investigate allele parentage in cases of potential germline mosaicism in *BRCA2*

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## Introduction

Breast cancer is one of the most common cancers affecting woman in the developed world. It has been estimated that up to 10% of cases are familial and roughly 30-40% of these familial cases are thought to have a genetic predisposition<sup>1</sup>. In the mid 1990's, two breast cancer susceptibility genes were identified, *BRCA1* and *BRCA2*<sup>2,3</sup>. The term Hereditary Breast/Ovarian Cancer syndrome (HBOC) was termed to encompass the wide range of cancers that have been linked to mutations within these genes. Pathogenic mutations within *BRCA1* and *BRCA2* are most commonly frameshift or nonsense mutations causing a premature termination codon and a truncated protein.

Identification of a pathogenic mutation within one of these genes allows patients to make an informed decision regarding screening programmes and prophylactic surgery as well as the possibility to offer presymptomatic testing to at risk family members. However due to the incomplete penetrance of *BRCA* mutations genetic counselling should always be offered both before and after genetic testing to discuss the possible implications of results.

## The *BRCA* genes

The Scottish regional genetic laboratories work together as a consortium for large high throughput screens such as the *BRCA* genes. The Glasgow laboratory sequence all coding exons of the *BRCA2* gene whilst the Aberdeen laboratory sequence the *BRCA1* gene. The Aberdeen laboratory also performs Multiplex Ligation-dependent Probe Amplification (MLPA) for both genes to detect large scale deletions and duplications.

## Clinical details and genetic screening

Patient WB was a 26 year old female referred to Clinical Genetics with Grade III, triple negative breast cancer. A slight family history of cancer on the maternal side was identified (possible pancreatic and skin cancers two generations previous) although no cases of breast or ovarian cancer were found. Sequencing of the two *BRCA* genes was requested due to the triple negative status of the breast cancer and the young age of onset, the results of which identified a nonsense mutation located within exon 11 of the *BRCA2* gene (c.3599\_3600delGT; p.Cys1200X).

Parental blood samples were received to determine the origin of the mutation and allow presymptomatic testing to be offered to at risk relatives. The results of this analysis indicated that both parents were negative for the mutation. A report was re-issued for WB stating that the mutation most probably arose *de novo*, although the possibility of germline mosaicism in either parent could not be discounted.

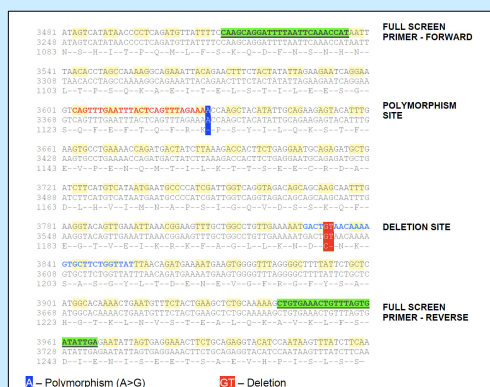
As the patient's mother had a second daughter (patient WB's half-sister) from a different marriage, investigations were warranted to see if she would be at risk of inheriting the mutation.

## Allele specific PCR design

The identification of an A/G polymorphism 200 bp upstream of the deletion site allowed primer sets to be designed which would be specific for each allele and allow a distinction to be made between the maternal and paternal allele (Figure 1).



Primer sets were designed as detailed in Figure 2 using Primer3 to optimise  $T_m$  and G/C content. Four different combinations of primer sets were used as described in Table 1 to offer conclusive results.



■ - Polymorphism (A>G)    ■ - Deletion  
SNP\_A - CAGTTTGAATTTACTCAGTTTGAACA (Paternal allele)  
SNP\_G - CAGTTTGAATTTACTCAGTTTGAACG (Maternal allele)  
(Forward orientation. Second to last base is C to increase stringency)

WITH DELETION - ATAACCAGAAGCACTTTTGTAGTC  
WITHOUT DELETION - ATAACCAGAAGCACTTTTGTAGTC  
(Reverse complement, allele specific region highlighted)

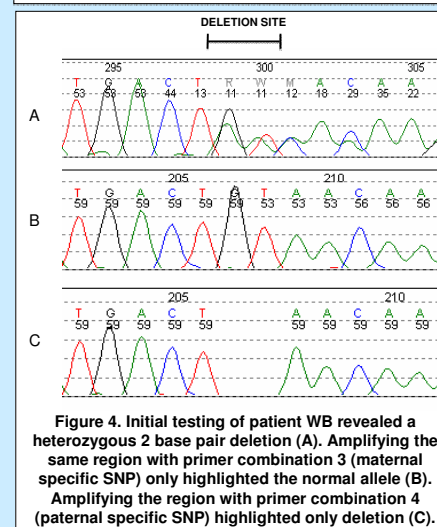
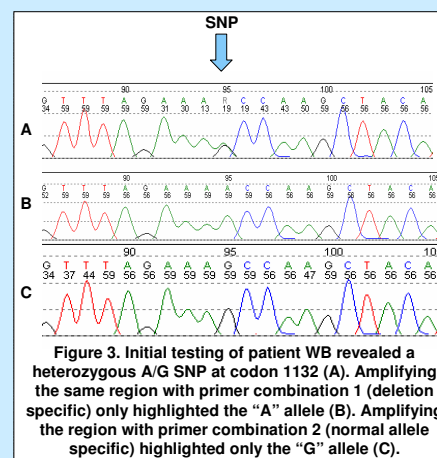
Figure 2. Primer design utilising the polymorphism at codon 1132 and the deletion at codon 1200 of the *BRCA2* gene. Full screen primers are those which are routinely used in the laboratory for *BRCA2* sequencing.

COMBINATION	FORWARD	REVERSE
1	Full Screen	With Deletion
2	Full Screen	Without Deletion
3	SNP_G (maternal)	Full Screen
4	SNP_A (paternal)	Full Screen

Table 1. The combination of primer sets used in the analysis.

## Results & Interpretation

Bi-directional sequencing of the DNA from patient WB using the primers sets highlighted the specificity of the primers. Figure 3 clearly illustrates that with primer combinations 1 & 2, each allele is separately amplified. The results of primer combinations 3 & 4 in figure 4 again re-instate the fact that each allele can be separately amplified.



The presence of only the "A" allele when sequencing with the deletion primer (Figure 3b) and the apparent homozygous deletion detected with the paternal specific SNP primer (Figure 4c) suggests that the deletion is present on the paternal allele. These results illustrate that the patient's half-sister was not at risk of having the deletion.

## Conclusion

We have demonstrated here the use allele specific PCR to identify the parental origin of a mutated allele. This work allows identification of at risk family members where germline mosaicism may be suspected.

The application of this approach is available to any genetic study and is not *BRCA2* specific, however testing is limited to those families where a discriminating polymorphism is present near the mutation site.

- References  
(1) Lux et al. Hereditary breast and ovarian cancer: review and future perspectives. *J. Mol. Med.* (2006) 84: 16-28  
(2) Miki et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* (1994) 266:66-71  
(3) Wooster et al. Identification of the breast cancer susceptibility gene *BRCA2*. *Nature* (1995) 378: 789-792