# UV-Exposure Induces Alternative Splicing of *BRC-1* RNA in Nematodes

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## ABSTRACT

Breast Cancer 1 protein 1 is a tumor suppressor protein with an important role is repairing DNA damage and is conserved in many species. The protein is encoded by *BRC1* in nematodes and *BRCA1* in humans. Mutations in *BRCA1* are linked to an increased risk in breast cancer. DNA damage, which can be induced by a variety of methods, including exposure to UV light, initiates cellular responses to repair damage. Many cellular responses occur by altering expression of genes. One point of gene regulation is alternative splicing in which different segments of the pre-mRNA are joined and may encode different proteins. To determine whether UV-exposure induced alternative splicing leading to alternative versions of the BRC-1 protein, nematodes were exposed to UV light or protected. RNA was examined using reverse transcription-polymerase chain reaction. Unexposed nematodes produced three fragments: 1) a 250 bp fragment, which corresponds to the reference sequence and likely encodes a functional protein, 2) a 150 bp, which was unpredicted, and 3) a 1600 bp fragment, which corresponds to unspliced RNA or genomic DNA. The exposed nematodes produced only the 250 bp, the reference sequence likely encoding the functional protein. These preliminary results clearly indicate that UV-exposure induced alternative splicing and that the splicing switched from producing some likely non-function or differently functional protein in non-exposed nematodes to producing only functional protein after UV exposure. Future studies should include determining the nature the unpredicted isoform, as well as quantifying levels of each isoform in the non-exposed nematodes.

## Hypothesis

The aim of this experiment is to investigate alternative versions of the *BRC-1* protein produced by alternative splicing in response to UV damage.

## INTRODUCTION

Breast Cancer gene1, *BRCA1,* is a tumor suppressor gene that was first discovered by Mary- Claire King in 1990 and cloned in 1994 (Alteri, 2019). About 80% of families prone to both ovarian and breast cancer and 50% of inherited breast cancer have mutations in *BRCA1*. *BRCA1* has also been implicated in sporadic breast cancers (Adamo, Montemauri, et al. 2008).In humans, *BRCA1* Is located on chromosome 17, has 24 exons and 22 transcripts which encode 1,863 amino acids (El Khachibi, 2015). These transcripts are produced by alternative promotors and alternative mRNA splicing. *BRCA1* phosphoprotein takes part in numerous important biological processes such as ubiquitination, transcription, DNA damage signaling, and checkpoint activation some *BRCA1* contain (Chen, Huang, et al. 2014). Ubiquitination is known to be known to be involved in the response to UV damage. E3 ubiquitin-protein ligase is the mediator for the formation of polyubiquitin chains whose function is to repair damaged DNA. It triggers cellular response damage sites when induced by Ultra-violet (UV) radiation. Since testing on human wouldn't be ethical for this experiment. UV induced expression will be studied on *BRC-1* in *C. elegans* because it is an ortholog of gene *BRCA1* (Boulton, 2003).

## METHODS AND MATERIALS

**Genomic Resources Bioinformatics Tools:**

- Reactome (https://reactome.org ) and UniProt (https://purl.uniprot.org/) databases were used to obtain genomic and protein information on *BRC-1*.
- Use Primer3plus software to design PCR primers that will detect splicing.
- Construct the full length of REFSEQ and Isoform with their calculated expected PCR sizes and then translate them into protein using Silico to determine the effects of the alternate protein on *BRC-1* domains.

**Wet Lab:**

- Two growth agar plates were inoculated with 250 uL of OP50 *E. coli* and inoculate nematodes after several days, afterward, one plate was exposed to UV radiation for 5 min. and then rested for 20 min. and the other plate was cover.
- Extract RNA and use reverse transcription to produce cDNA for both control and experimental conditions.
- Set up PCR with 6 different annealing temperatures and run gradient PCR products on an agarose gel electrophoresis to determine the best annealing temp.
- Finally, run another gradient PCR on the control and experimental cDNA on agarose gel electrophoresis.

## RESULTS

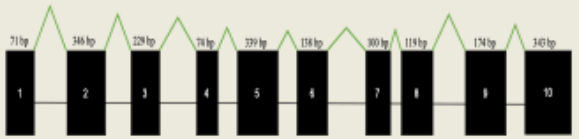


**Figure 1. *BRC-1* Gene Structure.**
Exons are shown as boxes while introns are shown as horizontal lines. Angled lines show constitutive splicing pattern and the number on the exons indicate expected RT-PCR in base paring. Gene structure was determined using information from ensembled Metazoa (Kersey at al, 2018).

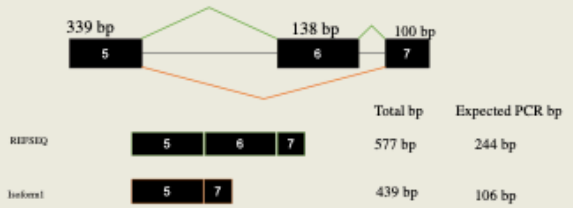


**Figure 2. Comparison of *BRC-1* Transcripts in Region of Interest.**
The total base pairs (bp) in the region and the expected total base pairs expected RT- PCR. Primers for RT-PCR to detect both transcript version were designed using Primer3 (Untergasser et al, 2007).

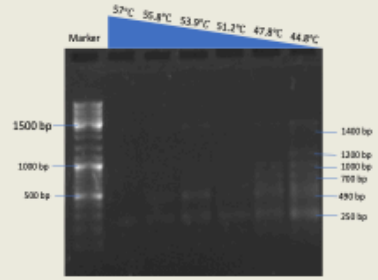


**Figure 3. Comparison of *BRC-1* Primers at Different Annealing Temperatures.**
Reverse transcription –polymerase chain reaction (RT-PCR) was performed on control RNA at different annealing temperature (noted above the well). Products were separated using agarose gel electrophoresis. OGeneRuler was used to measure RT-PCR products sizes in base pairs. The Marker is on the left and the products sizes are on the right (Thermo Fisher Scientific Inc. 2018).

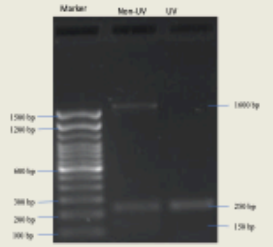


**Figure 4. Comparison of UV Exposure and Non- UV Exposure Nematodes cDNA.**
Gel electrophoresis was run for for 35 mins at 100v. The marker is on the left and the cDNA fragments on the right in base pairs (bp). TackIt 100 bp DNA Ladder was used to measure fragment sizes. (Thermo Fisher Scientific Inc. 2018).

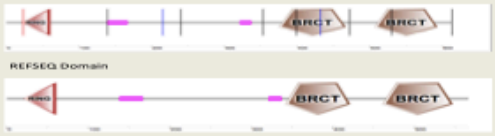


**Figure 6. Domain Structures of *BRC-1***
Breast cancer carboxy-terminal domain (BRCT) function in the cellular response to DNA damage. Ring finger (RING) is E3 ubiquitin protein ligases that transfer ubiquitin and mediates interaction between E2 Enzyme. The pink line indicate areas of low complexity (Artimo P, 2012).

**Figure 5. Multiple Alignment of REFSEQ and Isoform1.**
The letters represent encoded amino acids of *BRC-1* and Asterisk is none coded regions. The REFSEQ is above and Isoform1 is under (Wu, 2002).

## DISCUSSION

Isolation of RNA and RT to cDNA was successful. Annealing temp test showed 53.9°C as the best temp. Based on the result gathered from electrophoresis, the alternative splicing of the BRC-1 mRNA in UV exposed and not exposed nematodes is different. There is alternative splicing in the control sample because it contains three different cDNA fragments. One of the fragments of 1600 bp is likely un-spliced; one fragment of 250 bp is the reference sequence; the fragment of 150 bp is an unknown spliced event. The un-spliced RNA likely encoded alternate protein which likely doesn't function. On the other hand, the experimental sample contains only one fragment of 250 bp which is likely isoform sequence so, alternative splicing does occur in response to UV treatment. Domains present in both reference and isoform1 are RING and two BRCT.

## Future Direction

Isoform1 is likely functional, but it is unknown what alternate protein might be encoded in the new Isoform so its protein cannot be predicted without additional knowledge. For future research, I am interested in whether *BRC-1* can be expressed when induced with different UV wavelengths. Also, what would happen when exon 3 or 8 is skipped instead of exon 6.

## REFERENCES

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