# Developing a worm model to investigate breast cancer

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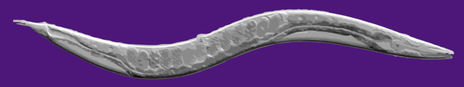


## INTRODUCTION

The gene *BRCA1* plays a crucial role in cancer prevention by coding for a tumor suppressor protein. Mutations in this gene that disrupt BRCA1 protein function can produce an 80% increase in the likelihood of developing breast or ovarian cancer. *Caenorhabditis elegans*, a microscopic worm that acts as a model organism, also contains a *BRCA1* gene that appears to have some shared functions as the human gene, such as ubiquitin ligase activity. [1]

One critical function of BRCA1 in humans is an enzymatic activity that attaches a signaling protein, ubiquitin, to histones. The exact mechanism driving histone ubiquitylation by BRCA1 in worms is currently unknown. By affirming conservation between worm and human BRCA1 enzymatic activity towards histones, we can open the possibility of testing human mutations *in vivo*.

### BRCA1 Ubiquitylation Pathway

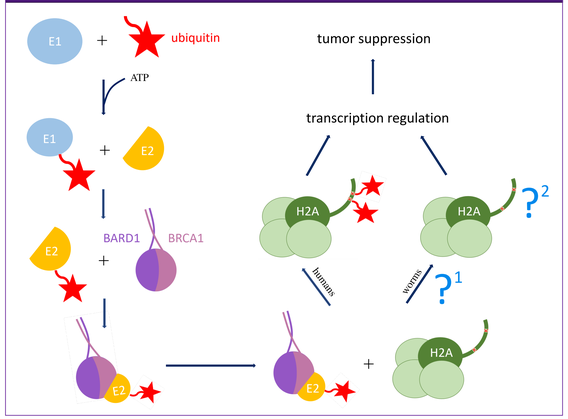
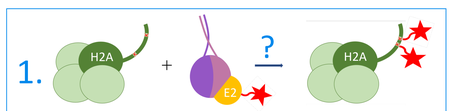



**Figure 1. (above)**: BRCA1 ubiquitylation pathway. [2] E1 charges ubiquitin (ub) then passes it on to E2. E2-ub binds to BRCA1, which then transfers ub to lysines (K) 127 and 129 on human histone H2A to prevent gene expression. [3] The exact location of the lysines is unknown in worms. **Figure 2. (below)**: Alignment showing the tail sequence of human and worm H2A. Lysines 127 and 129 (red) in humans are not a conserved ubiquitination site in worms. [4]

Human H2A: GVLPNIQAVLLPKKTESHHKAKGK
Worm H2A: GVLPNIQAVLLPKKTGGDKE

## OBJECTIVES

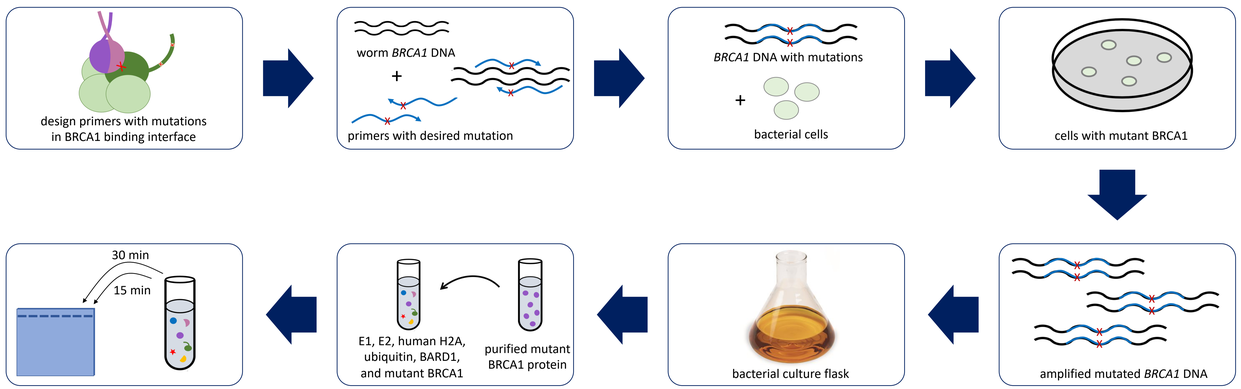



Determine if BRCA1 enzymatic function is conserved in worms

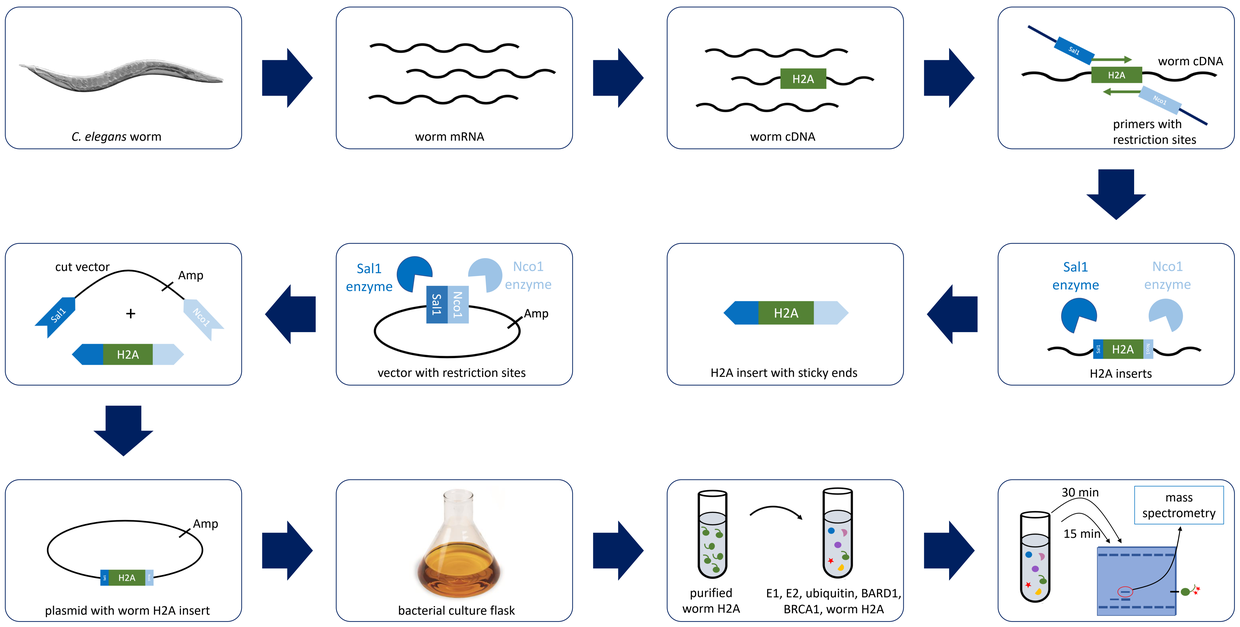

Determine the exact location of ubiquitylated lysines in worm H2A

**Importance**: this will allow us to see if we can use worms as a model system for breast cancer mutation testing

## METHODS: OBJECTIVE 1




## METHODS: OBJECTIVE 2




## RESULTS: OBJECTIVE 1

### BRCA1 enzymatic function is conserved in worms

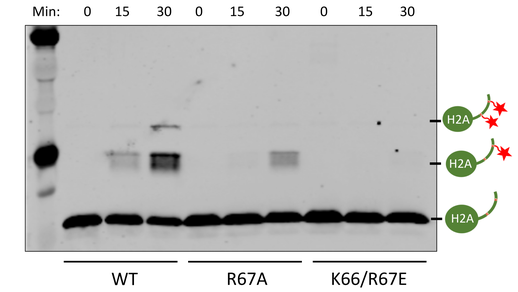



**Figure 3**: Western blot showing BRCA1 ubiquitylation activity toward human H2A: WT BRCA1 has normal ubiquitylation activity, R67A mutant shows disrupted function, K66/R67E mutants show complete loss of function.

## RESULTS: OBJECTIVE 2

### Worm histone genes are amplified

- We have successfully isolated the worm H2A and H2B inserts with sticky ends (**figure 4**).
- Future plans: continue remaining steps in Objective #2 to conduct enzymatic assay on worm histones.
- Determine exact positions of ubiquitylated lysines using mass spectrometry.

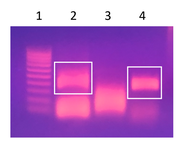



**Figure 4.** Gel electrophoresis shows relative lengths of gene inserts after restriction enzyme digestion. Lane 1: 100 base pair ladder, Lane 2: H2A, Lane 3: H2AV, Lane 4: H2B.

## REFERENCES & FUNDING

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