An ES Cell–Based Functional Assay to Study BRCA2 Variants

TEDCO/NIH/NCI Technology Showcase

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Hereditary Breast Cancer: BRCA1 & BRCA2

- Lifetime risk of breast cancer in general population is ~13.2% but *BRCA1* and *BRCA2* carriers have 36-85%
- BRCA1 consists of 1863 amino acids and BRCA2 protein has 3418 amino acids
- Mutations are scattered throughout the gene: no hot spots

*BRCA1 and BRCA2 Mutation Spectrum**

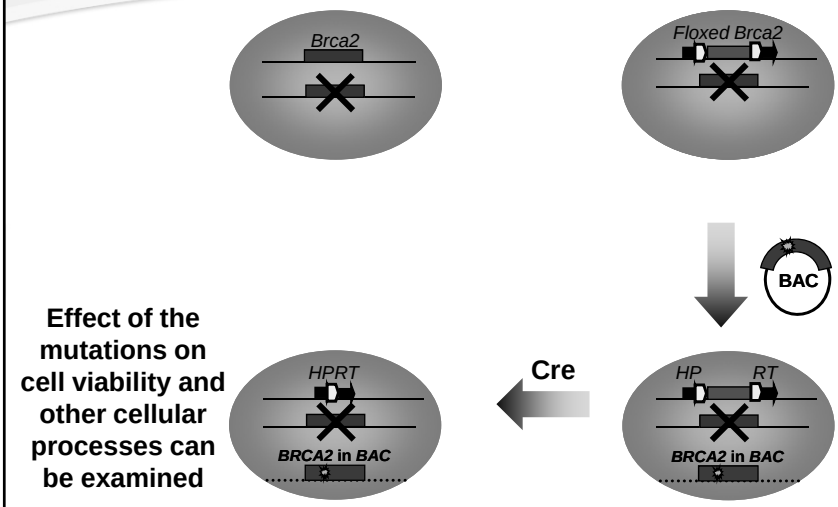
Mutation Type	Frequency	
	BRCA1	*BRCA2*
Frame-shift	5805	3436
Single aa change	3559	6168
Nonsense	1309	996
Intronic	1075	438
5´ UTR	5	88
In-frame deletion	64	46
In-frame insertion	3	16

*Source: Breast Cancer Information Core

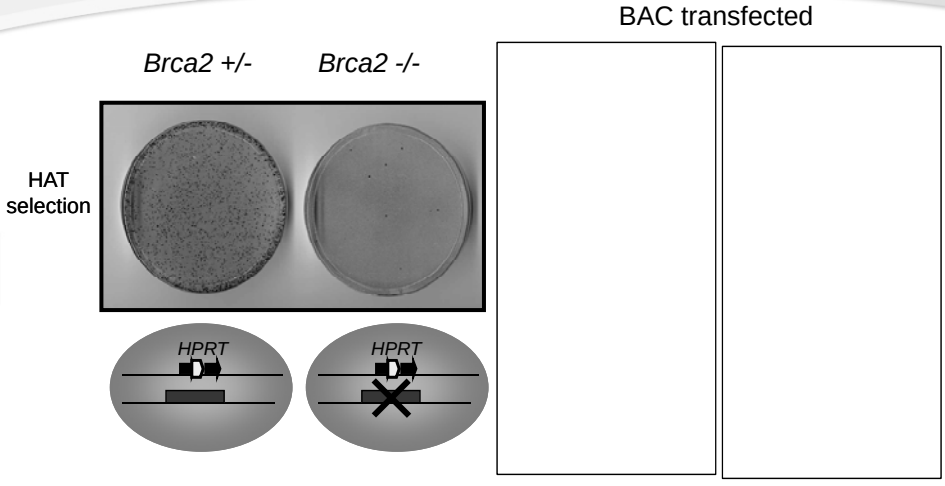
Cancer Predisposing Mutation or Polymorphism?

- Functional significance of mutations other than frame-shift alterations is unknown
- Lack of suitable functional assay
- Segregation of the disease with the mutation
- Prevalence of the mutation in general population

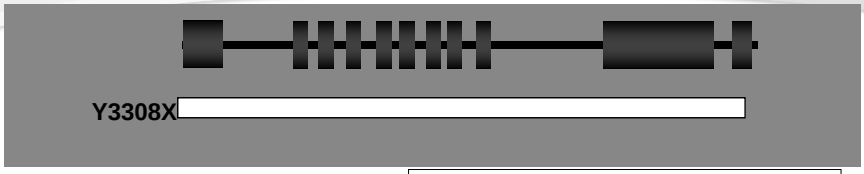
Analyzing functional significance of BRCA2 mutations in ES cells

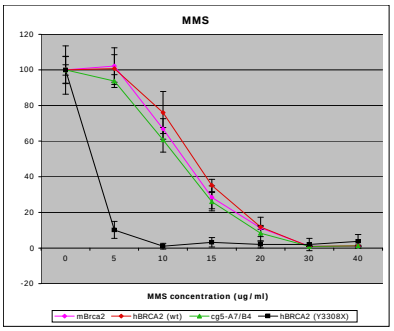


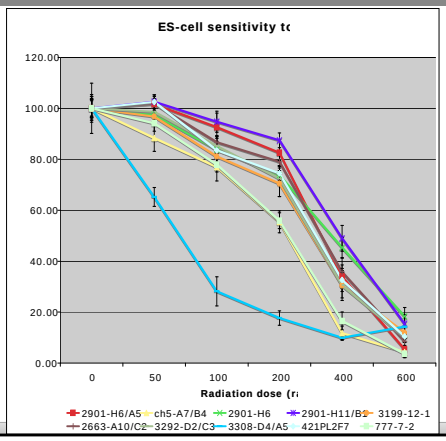
Human BRCA2 Can Rescue ES cell Lethality



Y3308X Mutant cells are Viable but Hypersensitive to Genotoxins

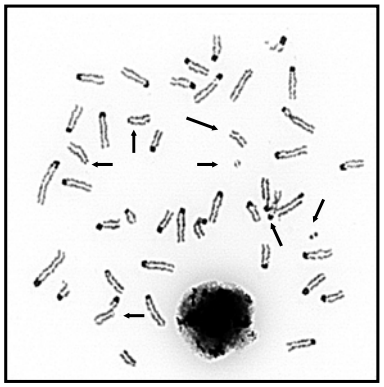






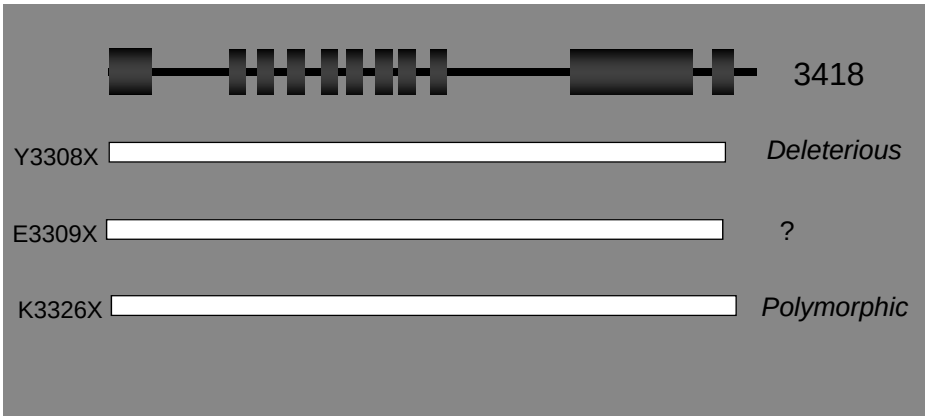
Y3308X cells Exhibit Genomic Instability

Brca2 +/-

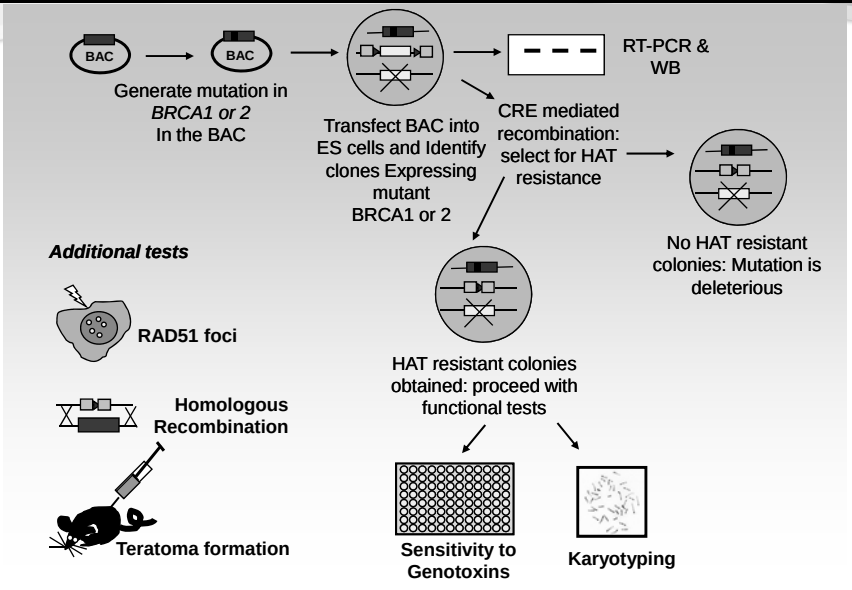


Y3308X

Is E3309X Deleterious or a Polymorphic Variation?



Scheme to Examine Human Missense Mutations in ES cells



Application in Genetic Counseling

- 10,000 individuals with personal or family history of cancer were screened for *BRCA1* and *BRCA2* mutations by Myriad Genetics*.
- 5503 indicated a personal history of breast or ovarian cancer
- 17% had deleterious mutations, 13% had one or more variants of unknown clinical significance
- The assay can be used to determine the functional significance of unknown variants

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