**Track 1 – Exon number**

Numbers for all translated *BRCA1* exons.

Exon numbering is based on refseq data – NM_007294.3 and U14680.1. For *BRCA1*, this entry includes 119 bases of 5'-untranslated sequence with protein translation starting at nucleotide 120. This is different from the current Locus Reference Genomic (LRG) annotation of the exons.

**Track 2 – BIC entries**

Breast Cancer Information Core (BIC): BIC maintains a database of documented BRCA1 and BRCA2 variants. The track in the Circos figure represents the number of entries for each variant in BIC (as of December 2013). The data is represented as a histogram to facilitate the identification of variants with the most entries in BIC. Frequency values can be found in the reference table.

**Further Reading:**

1) Couch FJ, Weber BL Mutations and polymorphisms in the familial early-onset breast cancer (BRCA1) gene. Breast Cancer Information Core *Hum Mutat*. 1996; 8(1):8-18

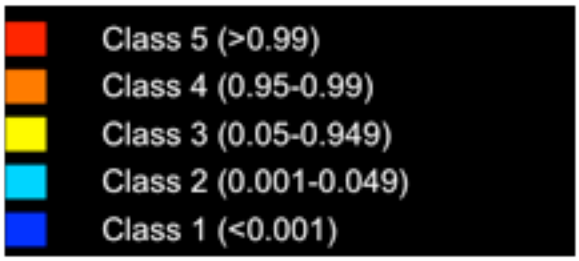
2) Friend S, Borresen AL, Brody L, *et al*. Breast cancer information on the web. *Nat Genet* 1995; 11(3):238-9.

3) Szabo C, Masiello A, Ryan JF, *et al*. The breast cancer information core: database design, structure, and scope. *Human Mutation* 2000; 16(2):123-31.

**Track 3 – IARC Class (Plon et al. 2008)**

In 2008, the unclassified genetic variant working group of the International Agency for Research on Cancer (IARC) proposed a five-class system based on degree of likelihood of pathogenicity derived from multifactorial models. Each class is associated with specific recommendations for clinical management of at-risk relatives that will depend on the syndrome. The five classes are Class 1 (not pathogenic or of no clinical significance), Class 2 (likely not pathogenic or of little clinical significance), Class 3 (uncertain), Class 4 (likely pathogenic), and Class 5 (definitely pathogenic). The probability of pathogenicity for each class is mentioned in parentheses.

Key:




**Reference:** Plon SE et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat. 2008; 29(11):1282-91

**Further Reading:**

1) Vallée MP et al. 2012. Classification of missense substitutions in the BRCA genes: a database dedicated to Ex-UVs. *Hum Mutat*. 2012; 33(1):22-8

2) Lindor NM et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS) *Hum Mutat*. 2012; 33(1):8-21.

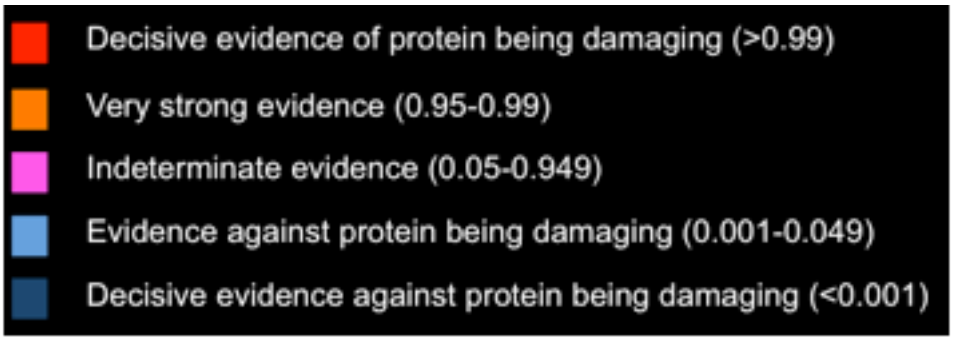
3) Goldgar DE, Easton DF, Deffenbaugh AM, et al. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. *Am J Hum Genet* 2004; 75(4):535-44.

4) Easton DF, Deffenbaugh AM, Pruss D, *et al*. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* 2007; 81(5):873-83.

5) Chenevix-Trench G, Healey S, Lakhani S, *et al*. Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. *Cancer Res*. 2006; 66(4):2019-27.
6) Spurdle AB, Lakhani SR, Healey S, *et al*. Clinical classification of BRCA1 and BRCA2 DNA sequence variants: the value of cytokeratin profiles and evolutionary analysis--a report from the kConFab Investigators. J Clin Oncol. 2000; 26(10):1657-63.

**Track 4 – Computational characterization of transcriptional assay data (Iversen et al. 2011)**
A computational tool, called VarCall that predicts the disease relevance for a variant of uncertain clinical significance (VUS) given data derived from a functional assay. The classification is performed based on the posterior probability that variant is protein damaging (pathogenic). The prior probability of a variant being protein damaging was assumed to be 0.5. The probability values (based on the thresholds for the IARC classes; see track 3) are interpreted as follows:

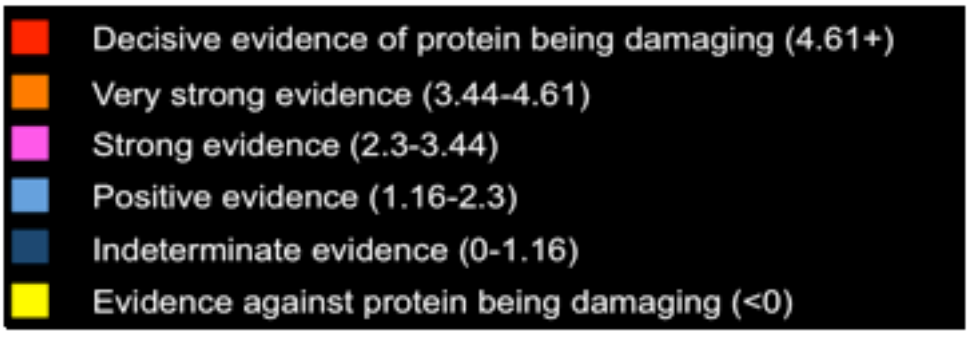
Key:




**Reference:** Iversen ES et al. 2011. A computational method to classify variants of uncertain significance using functional assay data with application to BRCA1 *Cancer Epidemiol Biomarkers Prev*. 2011; 20(6):1078-88.

**Track 5 – Bayes factor (Iversen et al. 2011)**
The Bayes factor is equivalent to the posterior odds that variant is protein damaging based on transcriptional assays. This track allows one to interpret the degree to which the data in Track 4 support the hypothesis (i.e. that a variant is protein damaging).
Below are the ranges for the Bayes factor:

Key:




**Reference:** Iversen ES et al. 2011. A computational method to classify variants of uncertain significance using functional assay data with application to BRCA1 *Cancer Epidemiol Biomarkers Prev*. 2011; 20(6):1078-88.

**Track 6 – NHLBI Exome Sequencing Project (ESP) *BRCA1* dataset**

This data has been obtained from the NHLBI Exome Sequencing Project (ESP) and indicates the allele frequencies of gene variants in the European American and African American populations. We indicate in the figure whether the variant is present in the Exome variant server (EVS) or not. Allele frequency values can be found in the reference table.

Key:


**Reference:** Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA http://evs.gs.washington.edu/EVS/ (ESP6500SI-V2)

**Track 7 – Reference amino acid**

The reference amino acid (refseq NP009225.1) corresponding to the codon number mentioned in Track 8.

**Track 8 – Codon**

The codon number of the variant.

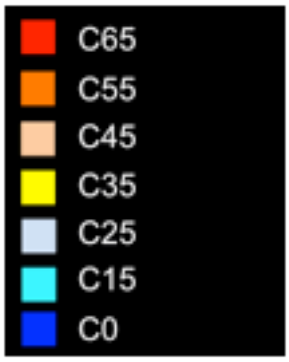
**Track 9 – Variant amino acid**

The variant amino acid corresponding to the codon number mentioned in Track 8.

**Track 10 – Align-GVGD**

Align-GVGD is a statistical model (implemented as a web-based program) that takes into consideration the biophysical characteristics of amino acids and protein multiple sequence alignments to predict where missense substitutions in genes of interest fall in a spectrum from deleterious to neutral. For BRCA1, the Align-GVGD scores are classified as below:
C65- highest probability of being pathogenic, C35 - C55 - lesser probability of being pathogenic, C15 - C25 - low probability of being pathogenic, C0 - least probability of being pathogenic.

Key:

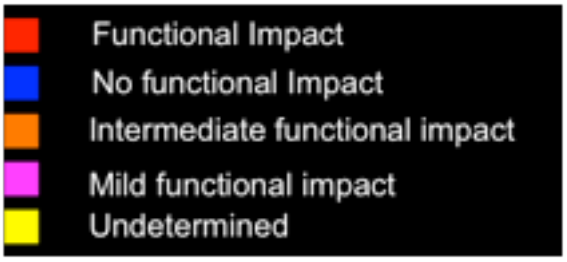



**Reference:** http://brca.iarc.fr/PRIORS/BRCA1/index.php

**Further Reading:**

1) Tavtigian SV et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. *J Med Genet*. 2006; 43(4):295-305.
2) Grantham R. Amino acid difference formula to help explain protein evolution. *Science* 1974; 185(4154):862-4.

**Track 11 – BARD Binding (Morris et al. 2006)**
The N-terminus BRCA1 associates with BARD1 to form a heterodimer, which exhibits ubiquitin ligase activity that is disrupted by known cancer-associated BRCA1 missense mutations. This assay evaluates how these missense mutations affect the ability of BRCA1 to interact with BARD1.
Key:

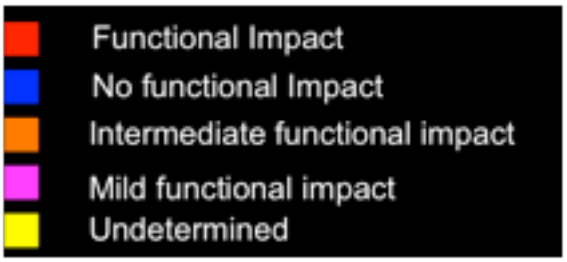



**Reference:** Morris JR et al. Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility.*Hum Mol Genet*. 2006; 15(4):599-606.

**Further reading:**
Wu LC, Wang ZW, Tsan JT, *et al*. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nature Genetics* 1996; 14(4):430-40.

**Track 12 – E2 Binding (Morris et al. 2006)**
By extensive missense substitution, the interaction of BRCA1 with the E2 ubiquitin-conjugating enzyme, UBcH5a was analyzed. BRCA1:E2 interaction is sensitive to substitutions in all structural elements of the BRCA1 N-terminus, whereas the BARD1 interaction is sensitive to a subset of BRCA1 substitutions. Variants that inhibit the BRCA1:E2 interaction show loss of ubiquitin ligase activity and correlate with disease susceptibility and theoretical predictions of pathogenicity.

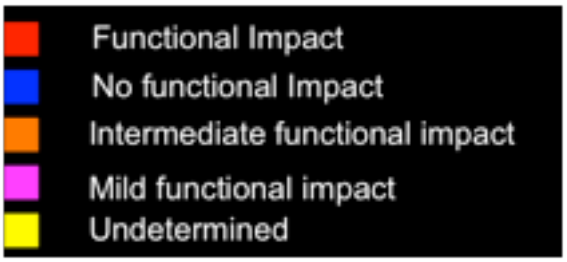
Key:




**Reference:** Morris JR et al. Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility.*Hum Mol Genet*. 2006; 15(4):599-606.

**Track 13 – Ubiquitin Ligase Activity (Morris et al. 2006)**
The BRCA1 ubiquitin ligase complex (with BARD1 and UBcH5a) is capable of forming covalently linked chains of ubiquitin on a substrate. Endogenous BRCA1-dependent ubiquitin conjugates occur at sites of double-stranded DNA breaks and DNA repair in cells suggesting that BRCA1 ubiquitin ligase activity is linked to the DNA damage repair role of BRCA1. Variants that inhibit the BRCA1:E2 interaction show loss of ubiquitin ligase activity and correlate with disease susceptibility and theoretical predictions of pathogenicity.

Key:

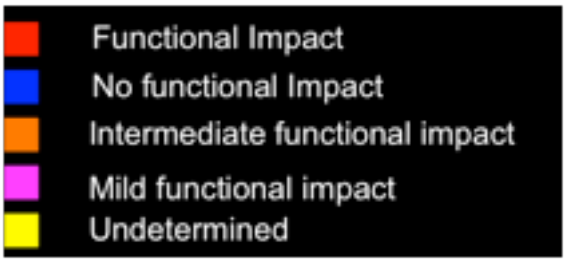


**Reference:** Morris JR et al. Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility.*Hum Mol Genet*. 2006; 15(4):599-606.

**Track 14 – Ubiquitin Ligase Activity (Ruffner et al. 2001)**

BRCA1 exhibits ubiquitin (Ub) protein ligase (E3) activity, and cancer-predisposing mutations within the BRCA1 RING domain abolish its Ub ligase activity. To determine the E3 Ub-ligase activity of the RING finger of BRCA1, GST fusion proteins encompassing residues 2–78 of wt human BRCA1 were generated. Purified, bacterially expressed GST-RING fusion protein was assayed for its ability to stimulate the synthesis of stable Ub conjugates. The paper suggests that the analysis of the Ub ligase activity of RING-domain mutations identified in patients can be used an assay to predict predisposition to cancer.

Key:

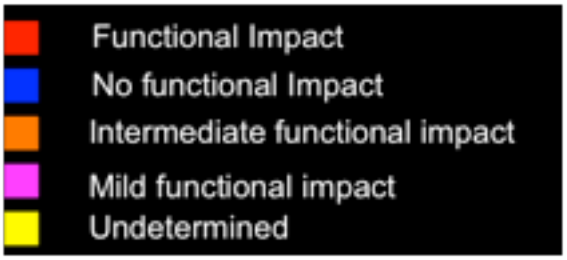



**Reference:** Ruffner H et al. 2006. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc Natl Acad Sci U S A*. 2001; 98(9):5134-9.

**Track 15 – Bimolecular fluorescence complementation (Sarkar & Magliery 2008)**

Split protein reassembly, also called Bimolecular Fluorescence Complementation (BiFC) is an *in vivo* probe of protein interactions where a GFP chromophore forms spontaneously on protein folding in cells. Split-GFP reassembly is used here to examine the determinants of association for a heterodimeric four-helix bundle between the N-terminal RING domains of BARD1 and BRCA1.

Key:

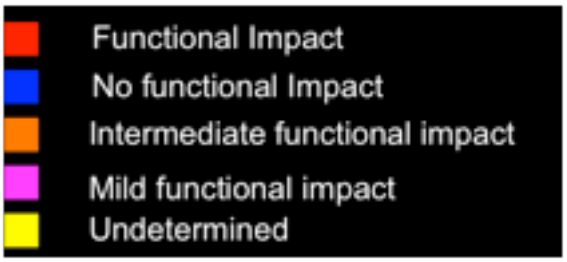



**Reference:** Sarkar M, Magliery TJ et al. Re-engineering a split-GFP reassembly screen to examine RING-domain interactions between BARD1 and BRCA1 mutants observed in cancer patients. *Mol Biosyst*. 2008; 4(6):599-605..

**Track 16 - Protease sensitivity (Lee et al. 2010)**

The protease sensitivity assay derives from an observation that BRCT domains containing a pathogenic mutation were more susceptible to protease (elastase, trypsin, or chymotrypsin) digestion than the wild-type counterpart. This assay can identify variants that cause defects in proper protein folding. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

Key:

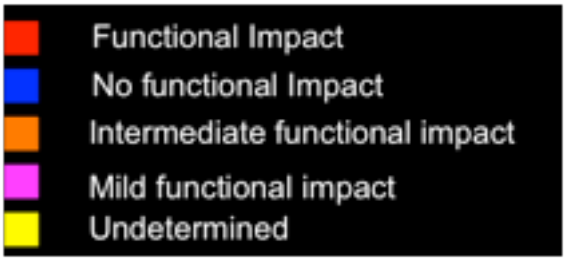



**Reference:** Lee MS et al. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. *Cancer Res*. 2010 Jun 15;70(12):4880-90.

**Further reading:**
1) Williams RS, Chasman DI, Hau DD, *et al*. Detection of protein folding defects caused by BRCA1-BRCT truncation and missense mutations. *Journal of Biological Chemistry* 2003;**278**(52):53007-16.
2) Williams RS, Green R, Glover JN. Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. *Nat StructBiol* 2001;**8**(10):838-42.

**Track 17 - Phosphopeptide binding activity (Lee et al. 2010)**
The BRCT domains in BRCA1 interact with phosphorylated protein targets containing the sequence pSer-x-x-Phe. In this assay, the BRCT domain is transcribed and translated in vitro, pulled down with a biotinylated pSer-x-x-Phe containing peptide coupled to streptavidin agarose beads. VUS are then tested for their binding activity. "Binding activity" corresponds to the pSer-x-x-Phe binding activity of the variant compared to the wild-type. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

Key:



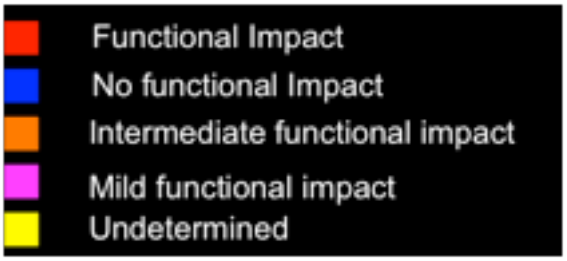

**Reference:** Lee MS et al. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. Cancer Res. 2010 Jun 15;70(12):4880-90.

**Further reading:**
1) Williams RS, Lee MS, Hau DD, *et al*. Structural basis of phosphopeptide recognition by the BRCT domain of BRCA1. *Nat StructMolBiol* 2004;11(6):519-25.
2) Manke IA, Lowery DM, Nguyen A, *et al*. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science* 2003; 302(5645):636-9.
3) Clapperton JA, Manke IA, Lowery DM, *et al*. Structure and mechanism of BRCA1 BRCT domain recognition of phosphorylated BACH1 with implications for cancer. *Nat StructMolBiol* 2004; 11(6):512-8.
4) Yu X, Chini CC, He M, *et al*. The BRCT domain is a phospho-protein binding domain. *Science* 2003; 302(5645):639-42.

**Track 18 - Phosphopeptide binding specificity (Lee et al. 2010)**

The BRCT domains in BRCA1 interact with phosphorylated protein targets containing the sequence pSer-x-x-Phe. In this assay, the BRCT domain including the VUS is transcribed and translated in vitro, pulled down with a biotinylated pSer-x-x-Phe containing peptide coupled to streptavidin agarose beads. VUS are then tested for their binding specificity. "Binding specificity" compares the binding of the variant to the phosphorylated peptide versus binding to an unphosphorylated peptide. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

Key:



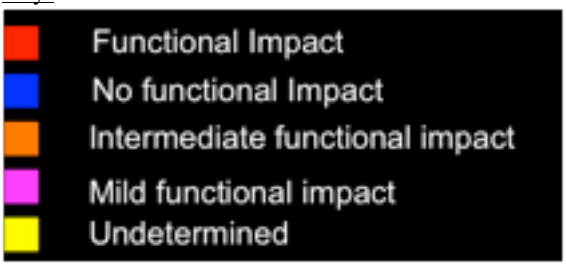

**Reference:** Lee MS et al. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. Cancer Res. 2010; 70(12):4880-90.

**Track 19 - Transcription assay (Lee et al. 2010)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*.

This track reports results using the GAL4 DBD fusion to the aa1646-1859 region.

Key:




**Reference:** Lee MS et al. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. Cancer Res. 2010; 70(12):4880-90.

**Further reading:**

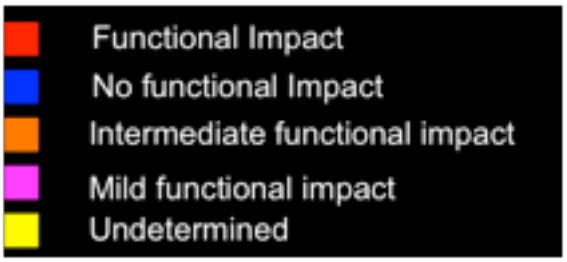
1) Monteiro AN, August A, Hanafusa H. Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci USA* 1996; 93(24):13595-9.
2) Chapman MS, Verma IM. Transcriptional activation by BRCA1. Nature. 1996; 382(6593):678-9.
3) Monteiro AN, August A, Hanafusa H. Common BRCA1 variants and transcriptional activation. *Am J Hum Genet* 1997; 61(3):761-2.
4) Vallon-Christersson J, Cayanan C, Haraldsson K, *et al*. Functional analysis of BRCA1 C-terminal missense mutations identified in breast and ovarian cancer families. *Human Molecular Genetics* 2001;10(4):353-60.

5) Phelan CM, Dapic V, Tice B, *et al*. Classification of BRCA1 missense variants of unknown clinical significance. *J Med Genet* 2005; 42(2):138-46.
6) Carvalho MA, Marsillac SM, Karchin R, *et al*. Determination of Cancer Risk Associated with Germ Line BRCA1 Missense Variants by Functional Analysis. *Cancer Research* 2007; 67(4):1494-501.

**Track 20 - Transcription assay (Carvalho et al. 2009)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa1396-1863 region.

Key:



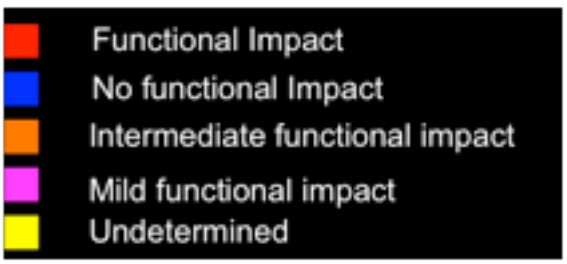

**Reference:** Carvalho MA et al. Analysis of a set of missense, frameshift, and in-frame deletion variants of BRCA1. *Mutat Res*. 2009; 660(1-2):1-11.

**Track 21 - Transcription assay (Tischkowitz et al. 2008)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa1396-1863 region.

Functional assays in yeast and mammalian cells were performed to show that BRCA1 BRCT domains carrying the amino-acid change p.Met1775Lys displayed markedly reduced transcriptional activity, indicating that this variant represents a deleterious variant. Importantly, the p.Met1775Lys mutation disrupted the phosphopeptide-binding pocket of the BRCA1 BRCT domains, thereby inhibiting the BRCA1 interaction with the proteins BRIP1 and CtIP, which are involved in DNA damage-induced checkpoint control.

Key:



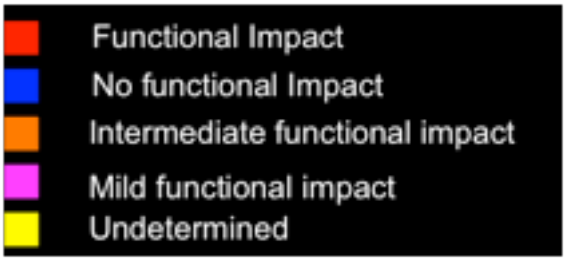
**Reference:** Tischkowitz M et al. Pathogenicity of the BRCA1 missense variant M1775K is determined by the disruption of the BRCT phosphopeptide-binding pocket: a multi-modal approach..*Eur J Hum Genet*. 2008; 16(7):820-32.

**Track 22 - Transcription assay (Carvalho et al. 2007)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa1396-1863 region.

Key:




**Reference:** Carvalho MA et al. Determination of cancer risk associated with germ line BRCA1 missense variants by functional analysis. *Cancer Res*. 2007 Feb 15;67(4):1494-501.

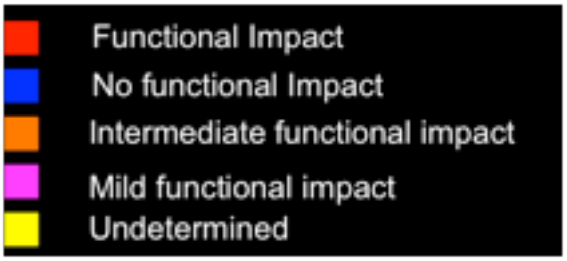
**Track 23 - Transcription assay (Kaufman et al. 2006)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*.

This track reports results using the GAL4 DBD fusion to the aa 1396-1863 region.

This assay assessed the pathogenicity of the BRCA1 p.Pro1812Ala mutation in vitro using reporter gene assays in yeast and mammalian cells. It was found that the BRCA1 p.Pro1812Ala variant activity assays yielded a slightly reduced reporter gene activity as compared to wild-type.

Key:



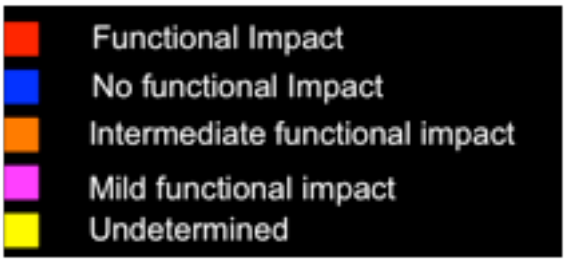
**Reference:** Kaufman B et al. The P1812A and P25T BRCA1 and the 5164del4 BRCA2 mutations: occurrence in high-risk non-Ashkenazi Jews *Genet Test*. 2006; 10(3):200-7.

**Track 24 - Transcription assay (Phelan et al. 2005)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa 1396-1863 region.

Key:



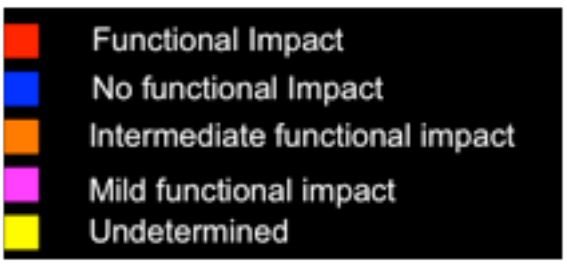

**Reference:** Phelan CM et al. Classification of BRCA1 missense variants of unknown clinical significance. *J Med Genet*. 2005;42(2):138-46.

**Track 25 - Transcription assay (Mirkovic et al. 2004)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa 1560-1863 region.

Key:

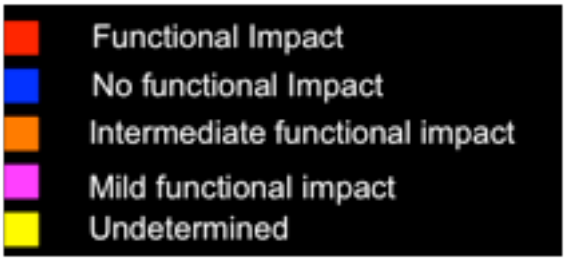



**Reference:** Mirkovic N et al. Structure-Based Assessment of Missense Mutations in Human BRCA1 Implications for Breast and Ovarian Cancer Predisposition *Cancer Res*. 2004; 64(11):3790-7.

**Track 26 - Transcription assay (Worley et al. 2002)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa 1560-1863 region.

It was shown that the protein was able to activate transcription of a reporter gene to levels observed for wild type BRCA1 at the permissive temperature (30°C) but exhibited significantly less transcription activity at the restrictive temperature (37°C) in both yeast and mammalian cells.
Key:

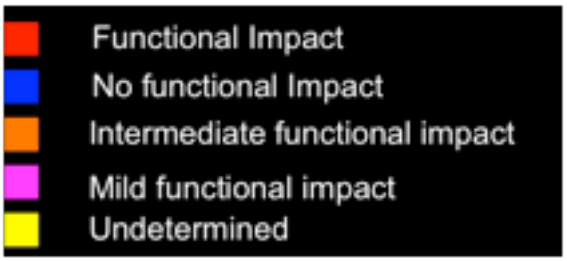



**Reference:** Worley T et al. A naturally occurring allele of BRCA1 coding for a temperature-sensitive mutant protein. *Cancer Biol Ther*. 2002; 1(5):497-501.

**Track 27 - Transcription assay (Vallon-Christersson et al. 2001)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa 1560-1863 region.

Key:



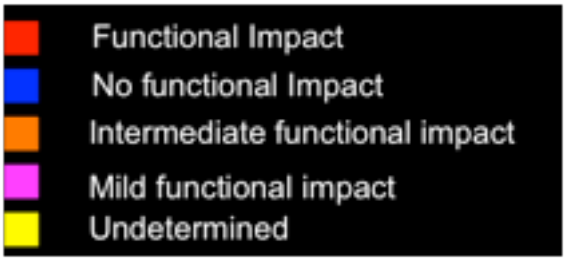

**Reference:** Vallon-Christersson et al. Functional analysis of BRCA1 C-terminal missense mutations identified in breast and ovarian cancer families. *Hum Mol Genet*. 2001; 10(4):353-60.

**Track 28 - Transcription assay (Monteiro et al. 1996)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

This track reports results using the GAL4 DBD fusion to the aa 1560-1863 region.

Key:

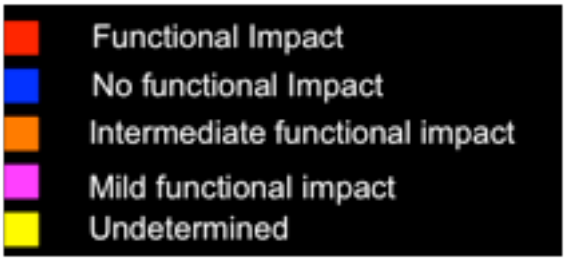



**Reference:** Monteiro AN et al. Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci U S A*. 1996; 93(24):13595-9.

**Track 29 - Transcription assay (Lovelock et al. 2006)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*.

This assay was used to elucidate the effect of two BRCA1 variants, p.Gly1706Ala and p.Ala1708Glu on BRCA1 function. Mammalian cells co-transfected with BRCA1 expression constructs to analyze the effect on reporter activity between the cells transfected with the wt BRCA1 or p.Gly1706Ala variant BRCA1.
Key:

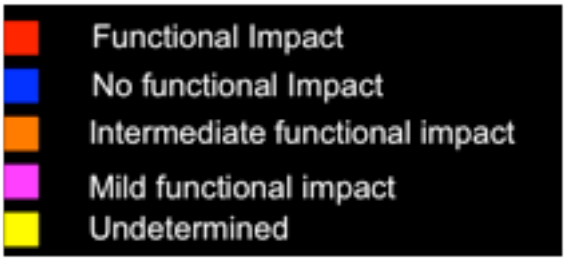



**Reference:** Lovelock PK et al. Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci U S A*. 1996; 93(24):13595-9.

**Track 30 - Transcription assay (Ostrow et al. 2004)**

The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay

provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.
p.Val1804Asp and p.Met1628Thr BRCA1 mutants were tested for transcriptional activation by cloning into pcDNA3.1. Wild-type, mutant, and empty vector constructs were tested in human kidney 293 cells using a p53-responsive luciferase reporter.
Key:

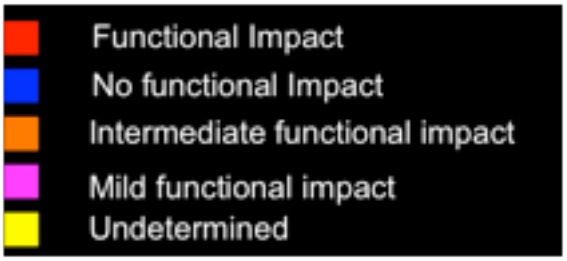



**Reference:** Ostrow KL et al. The effects of BRCA1 missense variants V1804D and M1628T on transcriptional activity. *Cancer Genet Cytogenet*. 2004 Sep;153(2):177-80.

**Track 31 - Transcription assay (Quiles et al. 2013)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription assay*.

This track reports results using the GAL4 DBD fusion to the aa 1396-1863 region. Transcriptional activity was determined for seven BRCA1 variants – p.Gln1409Leu, p.Ser1473Pro, Glu1586Gly, p.Arg1589His, p.Tyr1703Ser, p.Trp1718Leu and Gly1770Val.

Key:



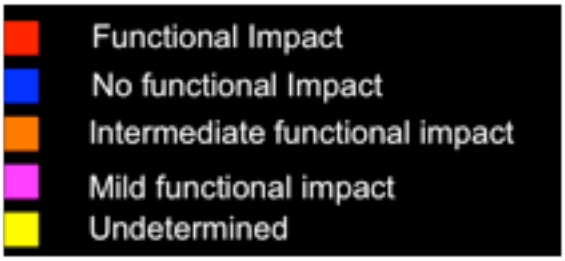

**Reference:** Quiles F et al. Functional and structural analysis of C-terminal BRCA1 missense variants**.** *PLoS One*. 2013; 8(4):e61302

**Track 32 - Transcription assay (Kawaku et al. 2013)**
The carboxy-terminus of BRCA1 encompasses one coiled-coil motif, a disordered region and two BRCT (BRCA1 Carboxy Terminal) domains in tandem (aa 1646-1736 and aa 1760–1855). This region can activate transcription of a reporter gene when fused to a heterologous DNA binding domain. Importantly, known pathogenic variants disrupt this activity while non-pathogenic variants do not. Thus, the assay provides a functional assessment that can inform on the likelihood of pathogenicity of variants located in the carboxy-terminal region to of BRCA1. This set-up is the basis for all tracks designated as *Transcription*

*assay*. For manuscripts reporting the assay using yeast and mammalian cells to assess the same variant, only the mammalian results are shown.

Segregation, conservation, transcription and structure analyses were performed in Japanese families of patients detected with breast cancer. Two VUS were analyzed – p.Ala1752Gly and p.Tyr1853Cys.

Key:

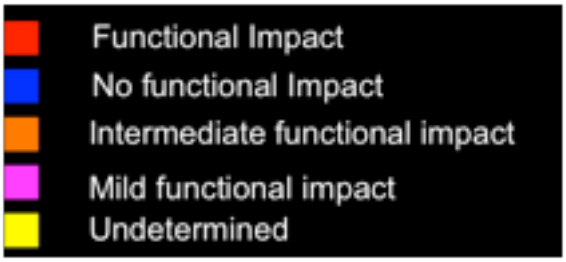



**Reference:** Kawaku et al Functional analysis of BRCA1 missense variants of uncertain significance in Japanese breast cancer families. *J Hum Genet*. 2013;58(9):618-21

**Track 33 - Small Colony Phenotype (Coyne et al. 2004)**

The small colony phenotype (SCP) assay is derived from the observation that BRCA1 expression inhibits yeast growth leading to the formation of small size colonies. The phenotype can be assessed qualitatively by looking at the size of the colonies or quantitatively by determining the number of cells in each colony. There is growth suppression with expression of wt or variants with no clinical significance but not with truncations or missense mutations known to be pathogenic. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

Key:




**Reference:** Coyne RS et al Functional characterization of BRCA1 sequence variants using a yeast small colony phenotype assay *Cancer Biol Ther*. 2004; 3(5):453-7.

**Further reading:**

1) Monteiro AN, Humphrey JS. Yeast-based assays for detection and characterization of mutations in BRCA1. *Breast Disease* 1998;10:61-70.
2) Humphrey JS, Salim A, Erdos MR, *et al*. Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing. *Proc Natl Acad Sci U S A* 1997; 94(11):5820-5.

**Track 34 - Small Colony Phenotype (Humphrey et al. 1997)**

The small colony phenotype (SCP) assay is derived from the observation that BRCA1 expression inhibits yeast growth leading to the formation of small size colonies. The phenotype can be assessed qualitatively by looking at the size of the colonies or quantitatively by determining the number of cells in each colony. There is growth suppression with expression of wt or variants with no clinical significance but not with truncations or missense mutations known to be pathogenic. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

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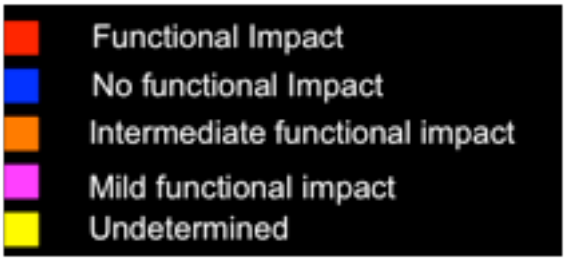


**Reference:** Humphrey JS et al Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing *Proc Natl Acad Sci U S A*. 1997; 94(11):5820-5.

**Track 35 - Small Colony Phenotype (Caligo et al. 2009)**

The small colony phenotype (SCP) assay is derived from the observation that BRCA1 expression inhibits yeast growth leading to the formation of small size colonies. The phenotype can be assessed qualitatively by looking at the size of the colonies or quantitatively by determining the number of cells in each colony. There is growth suppression with expression of wt or variants with no clinical significance but not with truncations or missense mutations known to be pathogenic. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

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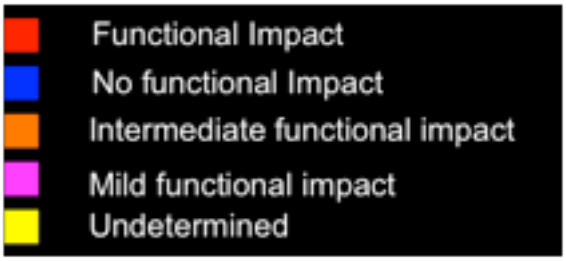


**Reference:** Caligo MA *et al*. A yeast recombination assay to characterize human BRCA1 missense variants of unknown pathological significance. *Hum Mutat*. 2009; 30(1):123-33.

**Track 36 - Small Colony Phenotype (Millot et al. 2011)**

The small colony phenotype (SCP) assay is derived from the observation that BRCA1 expression inhibits yeast growth leading to the formation of small size colonies. The phenotype can be assessed qualitatively by looking at the size of the colonies or quantitatively by determining the number of cells in each colony. There is growth suppression with expression of wt or variants with no clinical significance but not with truncations or missense mutations known to be pathogenic. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

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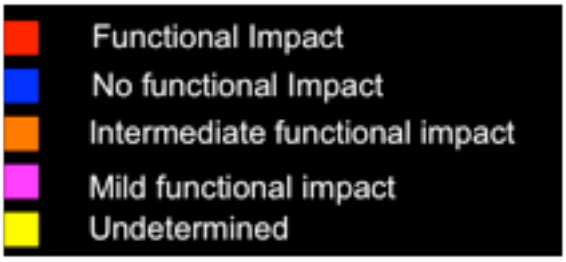


**Reference:** Millot GA *et al*. Assessment of human Nter and Cter BRCA1 mutations using growth and localization assays in yeast. *Hum Mutat*. 2011; 32(12):1470-80.

**Track 37 - Yeast localization phenotype (Millot et al. 2011)**

In the yeast localization phenotype (YLP) assay, the BRCA1 wt protein fused to the mCherry red fluorescent protein accumulates in a single inclusion body in the yeast nucleus providing qualitative information concerning the cellular localization of BRCA1 This event can be assessed quantitatively by determining the proportion of cells showing the nuclear aggregate in fluorescent microscopy. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

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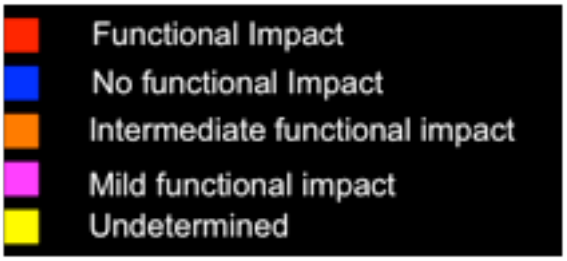


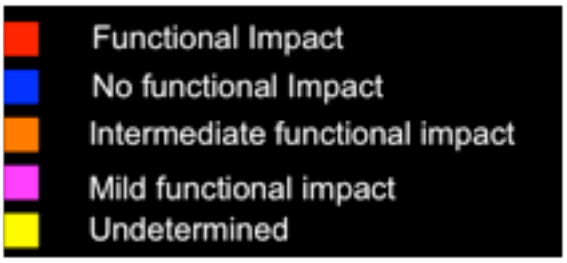

**Reference:** Millot GA *et al*. Assessment of human Nter and Cter BRCA1 mutations using growth and localization assays in yeast. *Hum Mutat*. 2011; 32(12):1470-80

**Track 38 - ES Viability (Chang et al 2009)**

Brca1 function is required for Mouse embryonic stem (ES) cell viability. The assays reported in the BRCA1 Circos follow a similar overall approach in which murine *Brca1* is inactivated to interrogating whether an ectopic human BRCA1 carrying a variant is able to rescue viability (Tracks 38 and 39). If ES containing the variant does not generate viable clones it indicates a significant functional impact. Viable clones can also be screened further to determine homology-directed repair (Track 44), cisplatin sensitivity (Track 52), and PARP inhibitor sensitivity (Track 53).

This track reports viability.

Key:



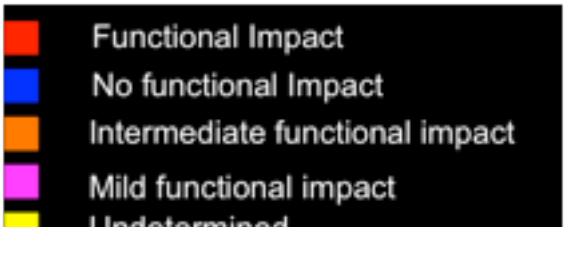

**Reference:** Chang S *et al*. Expression of human BRCA1 variants in mouse ES cells allows functional analysis of BRCA1 mutations *J Clin Invest*. 2009; 119(10):3160–3171.

**Track 39 - ES Viability (Bouwman et al. 2013)**

Brca1 function is required for Mouse embryonic stem (ES) cell viability. The assays reported in the BRCA1 Circos follow a similar overall approach in which murine *Brca1* is inactivated to interrogating whether an ectopic human BRCA1 carrying a variant is able to rescue viability (Tracks 38 and 39). If ES containing the variant does not generate viable clones it indicates a significant functional impact. Viable clones can also be screened further to determine homology-directed repair (Track 44), cisplatin sensitivity (Track 52), and PARP inhibitor sensitivity (Track 53).

This track reports viability.

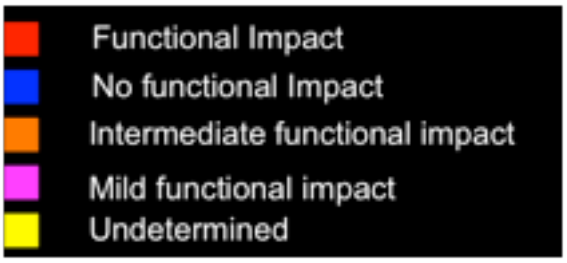
Key:



**Reference:** Bouwman P et al. A High-Throughput Functional Complementation Assay for Classification of *BRCA1* Missense Variants *Cancer Discov*; 3(10); 1142–55

**Track 40 - Restoration of radiation resistance (Scully et al. 1999)**
Retrovirus infected cells coexpressing green fluorescent protein (GFP) and BRCA1 (at levels comparable to human BC cell line MCF-7) and GFP-negative (noninfected control HCC1937) cells were mixed and treated with IR. After 3-24 days, cells were assayed for growth advantage compared to parental cells by fluorescence-activated cell sorting (FACS) analysis. The enrichment of GFP positive cells indicates a restoration of radiation resistance, discriminating the biological behavior of wt BRCA1 alleles from functionally impaired ones. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

Key:

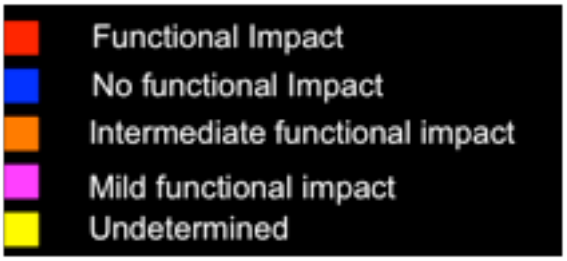



**Reference:** Scully R et al. Genetic analysis of BRCA1 function in a defined tumor cell line. *Mol Cell*. 1999; 4(6):1093-9.

**Further reading:**
Tomlinson GE, Chen TT, Stastny VA, *et al*. Characterization of a breast cancer cell line derived from a germ-line BRCA1 mutation carrier. *Cancer Res* 1998; 58(15):3237-42.

**Track 41 - Increase/restoration in radiation resistance (Ruffner et al. 2001)**
HCC1937 cells which have truncated BRCA1 alleles and are hypersensitive to IR were stably reconstituted with wt or mutant BRCA1 harboring individual N-terminal mutations (BRCA1 RING finger). HCC1937 cells were infected with the various recombinant viruses and assayed for increased IR resistance.

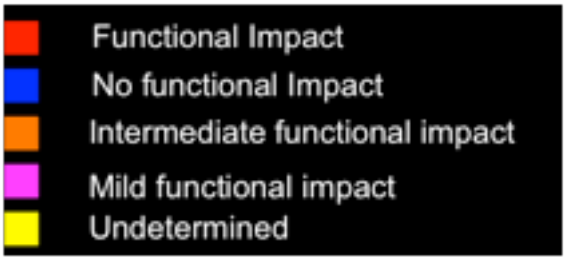
Key:




**Reference:** Ruffner H et al. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc Natl Acad Sci U S A*. 2001; 98(9):5134-9.

**Track 42 - Homology-directed repair (Ransburgh et al. 2010)**
HeLa-derived cell lines with a genomically integrated recombination substrate in which the endogenous BRCA1 is targeted by RNA interference are used as host to assess the function of an ectopically expressed (RNAi-resistant) BRCA1 variant. These cells can then be assessed for their ability to perform homology-directed repair (Track 42 and 43) and single strand annealing (Track 45).

Key:

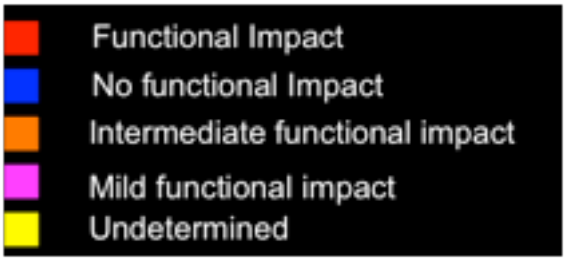



**Reference:** Ransburgh DJ *et al*. Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. *Cancer Res*. 2010; 70(3):988-95.

**Track 43 - Homology-directed repair (Towler et al. 2013)**

HeLa-derived cell lines with a genomically integrated recombination substrate in which the endogenous BRCA1 is targeted by RNA interference are used as host to assess the function of an ectopically expressed (RNAi-resistant) BRCA1 variant. These cells can then be assessed for their ability to perform homology-directed repair (Track 42 and 43) and single strand annealing (Track 45).

Key:



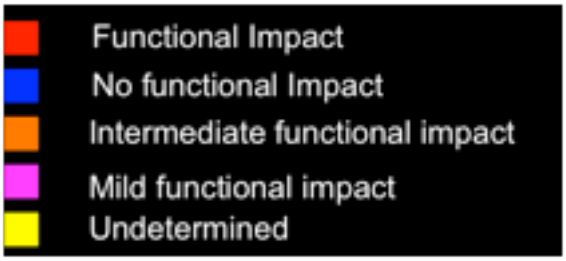

**Reference:** Towler WI et al. Analysis of BRCA1 Variants in Double-Strand Break Repair by Homologous Recombination and Single-Strand Annealing *Hum Mutat*. 2013; 34(3):439-45.

**Track 44 - Homology-directed repair (Bouwman et al. 2013)**

Brca1 function is required for Mouse embryonic stem (ES) cell viability. The assays reported in the BRCA1 Circos follow a similar overall approach in which murine *Brca1* is inactivated to interrogating whether an ectopic human BRCA1 carrying a variant is able to rescue viability (Tracks 38 and 39). If ES containing the variant does not generate viable clones it indicates a significant functional impact. Viable clones can also be screened further to determine homology-directed repair (Track 44), cisplatin sensitivity (Track 52), and PARP inhibitor sensitivity (Track 53).

In this track ES variant clones are assessed for the ability to support homology-directed repair.

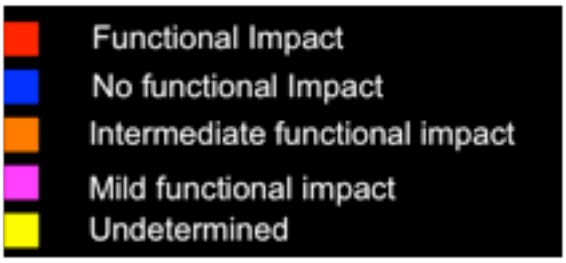
Key:




**Reference:** Bouwman P et al. A High-Throughput Functional Complementation Assay for Classification of *BRCA1* Missense Variants *Cancer Discov*; 3(10); 1142–55

**Track 45 – Single Strand Annealing (SSA) (Towler et al. 2013)**
HeLa-derived cell lines with a genomically integrated recombination substrate in which the endogenous BRCA1 is targeted by RNA interference are used as host to assess the function of an ectopically expressed (RNAi-resistant) BRCA1 variant. These cells can then be assessed for their ability to perform homology-directed repair (Track 42 and 43) and single strand annealing (Track 45).

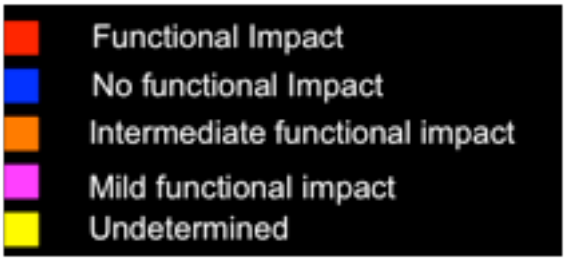
Key:




**Reference:** Towler WI et al. Analysis of BRCA1 Variants in Double-Strand Break Repair by Homologous Recombination and Single-Strand Annealing *Hum Mutat*. 2013; 34(3):439-45

**Track 46 - Centrosome number (Kais et al 2012)**
This functional assay relies on the observation that depletion or down regulation of BRCA1 leads to centrosome amplification. The percentage of cells with centrosome amplification was determined for 14 variants located in the RING domain. Variants were cotransfected with GFP-centrin 2 in the Hs578T cell line, in which the endogenous BRCA1 was silenced using RNA interference against the BRCA1 3' UTR. Expression
of all variants located at residues that coordinate zinc binding resulted in centrosome amplification. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

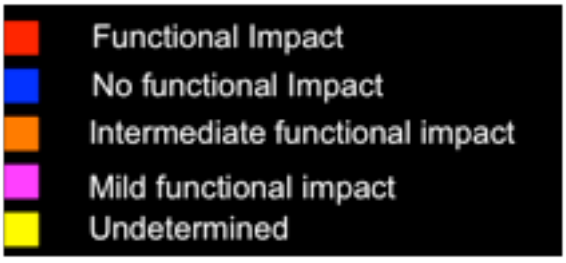
Key:




**Reference:** Kais Z et al. Functional differences among BRCA1 missense mutations in the control of centrosome duplication.Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. *Oncogene*. 2012; 31(6):799-804.

**Track 47 - Yeast Intrachromosomal recombination (Caligo et al. 2009)**
The yeast recombination assay evaluates the effect of the expression of BRCA1
missense variants located throughout the entire coding region on yeast homologous recombination (HR) using an intrachromosomal reporter assay in a diploid yeast strain. The assay measures recombination events between two *his3* direct repeats located in the same chromosome. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

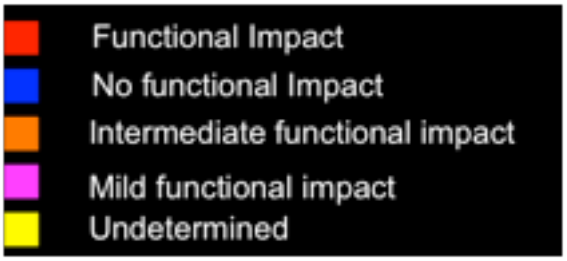
Key:



**Reference:** Caligo MA et al A yeast recombination assay to characterize human BRCA1 missense variants of unknown pathological significance. Hum Mutat. 2009; 30(1):123-33.

**Track 48 - Yeast Interchromosomal recombination (Caligo et al. 2009)**
The yeast recombination assay evaluates the effect of the expression of BRCA1 missense variants located throughout the entire coding region on yeast homologous recombination (HR) using an interchromosomal reporter assay in a diploid yeast strain. The assay measures recombination events between two *ade2* alleles located in two homologous chromosomes. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

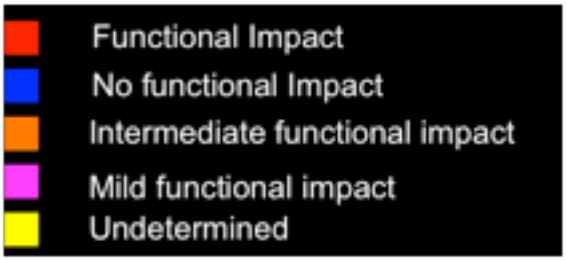
Key:




**Reference:** Caligo MA et al A yeast recombination assay to characterize human BRCA1 missense variants of unknown pathological significance. *Hum Mutat*. 2009; 30(1):123-33.

**Track 49 - Subcellular localization/formation of foci after IR (Au & Henderson 2005)**
BRCA1 is a predominantly nuclear localized protein that has also been detected in the cytoplasm. To examine whether a variant changes the subcellular location of BRCA1, the localization of GFP-tagged variant BRCA1 in transiently transfected cells is examined by confocal laser scanning microscopy. The cells are then scored as nuclear, cytosolic, or nuclear/cytosolic in mock or IR-treated cells. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

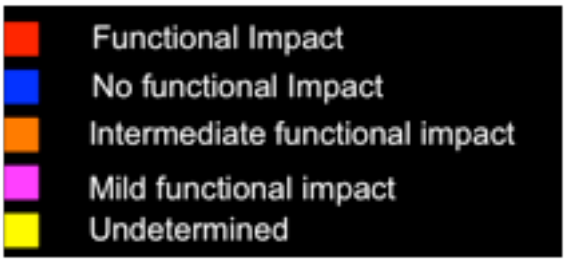
Key:




**Reference:** Au WW, Henderson BR. The BRCA1 RING and BRCT domains cooperate in targeting BRCA1 to ionizing radiation-induced nuclear foci. *J Biol Chem*. 2005; 280(8):6993-7001.

**Track 50 - Subcellular localization/cytoplasmic mislocalization (Rodriguez et al 2004)**
BRCA1 is a predominantly nuclear localized protein that has also been detected in the cytoplasm. To examine whether a variant changes the subcellular location of BRCA1, the localization of GFP-tagged variant BRCA1 in transiently transfected cells is examined by confocal laser scanning microscopy. The cells are then scored as nuclear, cytosolic, or nuclear/cytosolic in mock or IR-treated cells. (Excerpted from Millot GA, Carvalho MA, Caputo SM, *et al*. Hum Mutat. 2012; 33(11):1526-37).

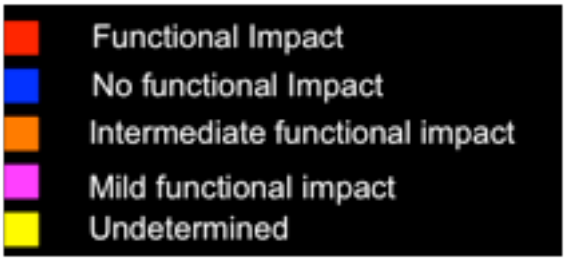
Key:



**Reference:** Rodriguez JA et al..Cytoplasmic mislocalization of BRCA1 caused by cancer-associated mutations in the BRCT domain. *Exp Cell Res*. 2004; 293(1):14-21

**Track 51 - G2/M Checkpoint proficiency (Ruffner et al. 2001)**
To test whether expression of BRCA1 in HCC1937 cells affects cell-cycle progression, that authors compared the cell-cycle profiles of non transduced or BRCA1-reconstituted HCC1937 cells at various time points after IR with 4 Gy. Cells expressing wt BRCA1 progressing through $G_2$ + M were compared with non-transduced cells.

Key:

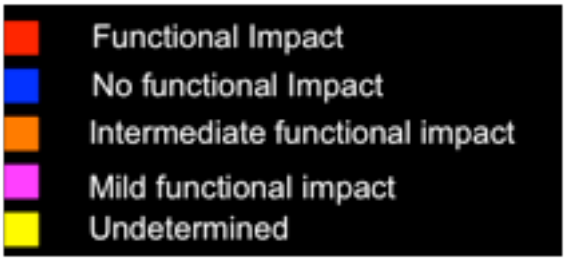



**Reference:** Ruffner H et al. 2006. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc Natl Acad Sci U S A*. 2001 Apr 24;98(9):5134-9.

**Track 52 – Cisplatin Response (Bouwman et al. 2013)**
Brca1 function is required for Mouse embryonic stem (ES) cell viability. The assays reported in the BRCA1 Circos follow a similar overall approach in which murine *Brca1* is inactivated to interrogating whether an ectopic human BRCA1 carrying a variant is able to rescue viability (Tracks 38 and 39). If ES containing the variant does not generate viable clones it indicates a significant functional impact. Viable clones can also be screened further to determine homology-directed repair (Track 44), cisplatin sensitivity (Track 52), and PARP inhibitor sensitivity (Track 53).

This track reports cisplatin sensitivity.

Key:

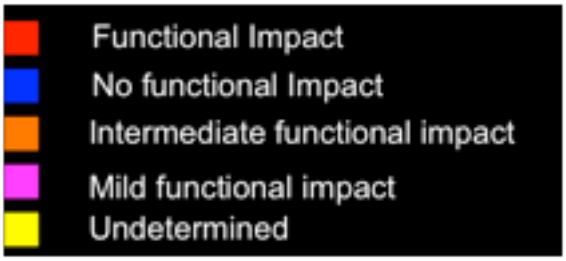



**Reference:** Bouwman P et al. A High-Throughput Functional Complementation Assay for Classification of *BRCA1* Missense Variants *Cancer Discov*; 3(10); 1142–55

**Track 53 – PARP Inhibitor sensitivity (Bouwman et al. 2013)**
Brca1 function is required for Mouse embryonic stem (ES) cell viability. The assays reported in the BRCA1 Circos follow a similar overall approach in which murine *Brca1* is inactivated to interrogating whether an ectopic human BRCA1 carrying a variant is able to rescue viability (Tracks 38 and 39). If ES containing the variant does not generate viable clones it indicates a significant functional impact. Viable clones can also be screened further to determine homology-directed repair (Track 44), cisplatin sensitivity (Track 52), and PARP inhibitor sensitivity (Track 53).

This track reports Olaparib (AZD2281) sensitivity.

Key:




**Reference:** Bouwman P et al. A High-Throughput Functional Complementation Assay for Classification of *BRCA1* Missense Variants *Cancer Discov*; 3(10); 1142–55