

# Navodilo za delo

## 1. Poišči zapis za človeški protein BRCA1

- Zapiši UniProt ID za ta protein: P38398

- Zapiši dolžino proteina: 1863 ak

- Kaj je naloga tega proteina? Je E3 ubikvitinska ligaza, ki specifično sodeluje pri tvorbi Lys-6 označene poliubikvitinske verige in igra ključno vlogo pri popraviljanju DNA.

### P38398 · BRCA1\_HUMAN

Protein <sup>i</sup>	Breast cancer type 1 susceptibility protein	Amino acids	1863 ( <a href="#">go to sequence</a> )
Gene <sup>i</sup>	BRCA1	Protein existence <sup>i</sup>	Evidence at protein level
Status <sup>i</sup>	 UniProtKB reviewed (Swiss-Prot)	Annotation score <sup>i</sup>	 5/5
Organism <sup>i</sup>	Homo sapiens (Human)		

[Entry](#) [Variant viewer](#) [12,214](#) [Feature viewer](#) [Genomic coordinates](#) [Publications](#) [External links](#) [History](#)

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### Function<sup>i</sup>

E3 ubiquitin-protein ligase that specifically mediates the formation of 'Lys-6'-linked polyubiquitin chains and plays a central role in DNA repair by facilitating cellular responses to DNA damage (PubMed:[10500182](#), PubMed:[12887909](#), PubMed:[12890688](#), PubMed:[14976165](#), PubMed:[16818604](#), PubMed:[17525340](#), PubMed:[19261748](#)). It is unclear whether it also mediates the formation of other types of polyubiquitin chains (PubMed:[12890688](#)).

- Katere vrste mutacij najdemo pri raku dojke? Točkovne mutacije: zamenjave in delecije.

### Breast cancer (BC)

 22 Publications

Note	Disease susceptibility is associated with variants affecting the gene represented in this entry. Mutations in BRCA1 are thought to be responsible for 45% of inherited breast cancer. Moreover, BRCA1 carriers have a 4-fold increased risk of colon cancer; whereas male carriers face a 3-fold increased risk of prostate cancer. Cells lacking BRCA1 show defects in DNA repair by homologous recombination
Description	A common malignancy originating from breast epithelial tissue. Breast neoplasms can be distinguished by their histologic pattern. Invasive ductal carcinoma is by far the most common type. Breast cancer is etiologically and genetically heterogeneous. Important genetic factors have been indicated by familial occurrence and bilateral involvement. Mutations at more than one locus can be involved in different families or even in the same case.
See also	MIM: <a href="#">114480</a> 

### Natural variants in BC

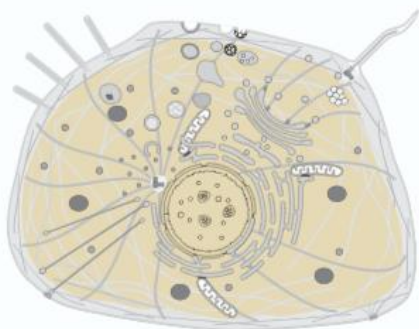
VARIANT ID	POSITION(S)	CHANGE	DESCRIPTION
VAR_007761	271	V>M	in BC; dbSNP: <a href="#">rs80357244</a>  1 Publication
VAR_008760	346	P>S	in BC; benign; dbSNP: <a href="#">rs80357015</a>  1 Publication
VAR_007763	369	missing	in BC; dbSNP: <a href="#">rs80358325</a> 
VAR_007765	461	F>L	in BC; benign; dbSNP: <a href="#">rs56046357</a>  1 Publication
VAR_007766	465	Y>D	in BC; dbSNP: <a href="#">rs397508869</a>  1 Publication
VAR_007768	552	G>V	in BC; dbSNP: <a href="#">rs397508893</a>  1 Publication

## 2. Za analiziranje mutacij si želimo izolirati protein BRCA1.

- Iz katerega dela celice bi morali izolirati protein BRCA1 za njegovo analizo? Iz jedra ali citoplazme.

UniProt Annotation

GO Annotation



**Nucleus** 6 Publications

**Chromosome** 3 Publications

**Cytoplasm** 1 Publication

Note: Localizes at sites of DNA damage at double-strand breaks (DSBs); recruitment to DNA damage sites is mediated by ABRAXAS1 and the BRCA1-A complex (PubMed:26778126). Translocated to the cytoplasm during UV-induced apoptosis (PubMed:20160719).

2 Publications

- Zapiši PDB ID za BRCA1 strukturo, ki je bila določena z NMR analizo: 1JM7
- Koliko meren je ta protein? Dimeren

**1JM7** | **pdb\_00001jm7**

Solution structure of the BRCA1/BARD1 RING-domain heterodimer

PDB DOI: <https://doi.org/10.2210/pdb1JM7/pdb>

Classification: **ANTITUMOR**

Organism(s): **Homo sapiens**

Expression System: **Escherichia coli BL21(DE3)**

Mutation(s): No

Deposited: 2001-07-17 Released: 2001-10-03

Deposition Author(s): **Brzovic, P.S., Rajagopal, P., Hoyt, D.W., King, M.-C., Klevit, R.E.**

Experimental Data Snapshot

Method: **SOLUTION NMR**

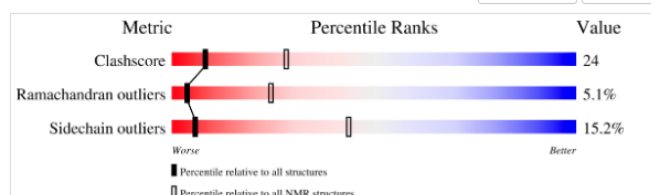
Conformers Calculated: **25**

Conformers Submitted: **14**

Selection Criteria: structures with the least restraint violations, structures with the lowest energy

wwPDB Validation

3D Report Full Report



- Protein smo izolirali tako, da smo celice spirali s PBS, nato dodali litični pufer RIPA z dodatkom proteaznih inhibitorjev. Po inkubaciji na ledu in centrifugiranju smo shranili supernatant. Za učinkovito izolacijo smo izvedli afinitetno kromatografijo. Čistost izoliranega proteina želimo potrditi z SDS-page, pri kateri velikosti pričakujemo liso na gelu? 26.25kDa



Explore in 3D: [Structure](#) | [Sequence Annotations](#) | [Validation Report](#) | [Ligand Interaction \(ZN\)](#)

#### Macromolecule Content

- Total Structure Weight: 26.25 kDa ⓘ
- Atom Count: 1,573 ⓘ
- Modelled Residue Count: 200 ⓘ
- Deposited Residue Count: 229 ⓘ
- Unique protein chains: 2

3. Za aktivnost BRCA1 proteina sta ključni dve domeni, in sicer BRCT ter RING.

- kje na proteinu se nahaja BRCT domena? Na C koncu proteina.

- Kje na proteinu se nahaja RING domena? Kateri ion je ključen za aktivnost te domene? Na N koncu proteina.  $Zn^{2+}$ .

► Families

cathgene3d G3DSA:3.30.40.10

▼ Domains

**Zinc/RING finger domain, C3HC4 (zinc finger)**

Integrated: IPR013083

Model: 1jm7A00

1 - 112

BRCT\_...

Representative families

Representative domains

IPR013083  
CATHGENE3D: G3DSA:3.30.40.10  
Unintegrated  
SSF: SSF57850  
Unintegrated  
CDD: cd16498

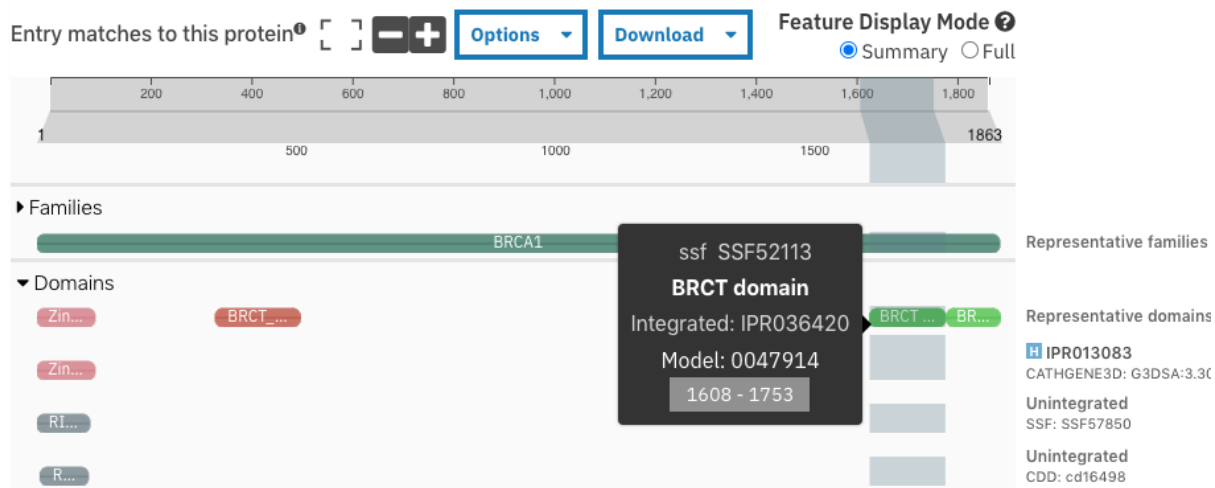
IPR001841  
SMART: SM00184  
PROFILE: PS50089

IPR018957  
PFAM: PF00097

IPR025994  
PFAM: PF12820

IPR036420  
CATHGENE3D: G3DSA:3.40.50.10  
SSF: SSF52113

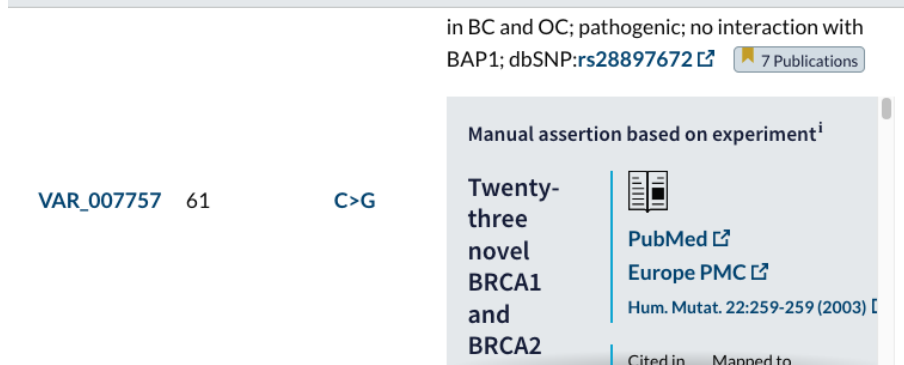
IPR001357  
PROFILE: PS50172



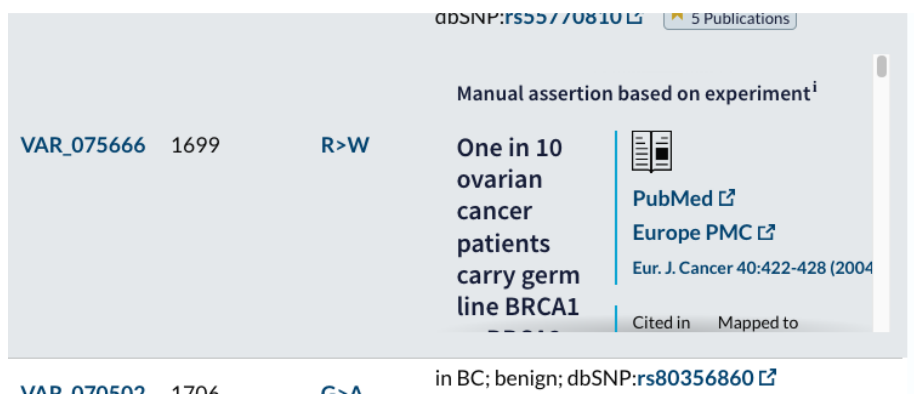
#### 4. Analiziranje mutacij.

- Za vsako izmed omenjenih domen izberi po eno patogeno mutacijo. Opiši, kaj se pri tej mutaciji zgodi in dodaj DOI enega izmed preiskovalnih (research) člankov na PubMedu, ki omenja to mutacijo.

Na RING domeni sva izbrali mutacijo na 61 mestu, kjer se cistein zamenja z glicinom. Prvi članek na PubMedu ima doi: 10.1002/humu.9174.



Na BRCT domeni sva izbrali mutacijo na 1699 mestu, kjer se arginin zamenja s triptofanom. Prvi članek na PubMedu ima doi: 10.1016/j.ejca.2003.09.016



- Želimo preveriti, kako dobro je ohranjena BRCT1 domena med sesalci. Zapiši imena organizmov, ki vsebujejo homolog te domene. (za database uporabi swissprot)

Pongo pygmaeus, Pan troglodytes, Gorilla gorilla gorilla, Macaca mulatta, Canis lupus familiaris, Bos taurus in Mus musculus.

Job Title: sp|P38398|BRCA1\_HUMAN Breast cancer type 1...

RID: 1VJBN7ZN016 Search expires on 05-10 17:23 pm Download All

Program: BLASTP Citation

Database: swissprot See details

Query ID: lcl|Query\_11802797

Description: unnamed protein product

Molecule type: amino acid

Query Length: 95

Other reports: Distance tree of results Multiple alignment MSA viewer

Filter Results

Organism: only top 20 will appear exclude

Homo sapiens (taxid:9606)

+ Add organism

Percent Identity: to E value: to Query Coverage: to

Filter Reset

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all 10 sequences selected GenPept Graphics Distance tree of results Multiple alignment MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Pongo pygmaeus	202	202	100%	1e-60	100.00%	1863	Q6J6J0.1
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Pan troglodytes	202	202	100%	1e-60	100.00%	1863	Q9GKK9.2
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Gorilla gorilla gorilla	199	199	100%	1e-59	98.95%	1863	Q6J6I8.1
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Macaca mulatta	194	194	100%	7e-58	94.74%	1863	Q6J6I9.1
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Canis lupus familiaris	184	184	100%	1e-54	88.42%	1878	Q95153.1
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Bos taurus	179	179	100%	1e-52	87.37%	1849	Q984U1.1
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Mus musculus	152	152	100%	3e-43	71.58%	1812	P48754.3
RecName: Full-Breast cancer type 1 susceptibility protein homolog; AltName: Full-RING-type E3 ubiquitin transfe...	Rattus norvegicus	149	149	98%	3e-42	70.97%	1817	Q54952.1
RecName: Full-Protein BREAST CANCER SUSCEPTIBILITY 1 homolog; Short=AIBRCA1; Arabidopsis thaliana	Arabidopsis thaliana	62.8	62.8	95%	9e-12	30.00%	941	Q8RXD4.1
RecName: Full-BRCA1-associated RING domain protein 1; Short=AIBARD1; Arabidopsis thaliana	Arabidopsis thaliana	60.5	60.5	99%	6e-11	29.79%	714	F4I443.1

- Ali je pri vseh teh organizmih ohranjena mutacija na mestu 1699? Ja, ta mutacija se pojavi pri vseh.

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

Query subrange

From To

Or, upload file Prebrskaj ... Datoteka ni izbrana

Job Title UNNAMED PROTEIN PRODUCT

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database Standard databases (nr etc.): Experimental databases

UniProtKB/Swiss-Prot(swissprot)

Organism Optional

Enter organism name or id--completions will be suggested exclude Add organism

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude Optional

Models (XM/XP) Non-redundant RefSeq proteins (WP) Uncultured/environmental sample sequences

Program Selection

Algorithm

blastp (protein-protein BLAST)

PSI-BLAST (Position-Specific Iterated BLAST)

PHI-BLAST (Pattern Hit Initiated BLAST)

DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

V blastp sva vnesli zaporedje domene BRCT in na mestu 1699 zamenjali R za W

## 5. Poravnava

- Želimo si ogledati ohranjenost celotnega proteina pri miših. Naredi poravnavo celotnega zapisa za človeški in mišji protein BRCA1 ter zapiši procent podobnosti med njima.

69.7%

```
#####
# Program: needle
# Rundate: Fri  9 May 2025 09:04:26
# Commandline: needle
#   -auto
#   -stdout
#   -asequence emboss_needle-I20250509-090355-0935-18818704-p1m.asequence
#   -bsequence emboss_needle-I20250509-090355-0935-18818704-p1m.bsequence
#   -datafile EBLOSUM62
#   -gapopen 10.0
#   -gapextend 0.5
#   -endopen 10.0
#   -endextend 0.5
#   -aformat3 pair
#   -sprotein1
#   -sprotein2
# Align_format: pair
# Report_file: stdout
#####

#=====
#
# Aligned_sequences: 2
# 1: BRCA1_HUMAN
# 2: BRCA1_MOUSE
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1876
# Identity:   1045/1876 (55.7%)
# Similarity: 1307/1876 (69.7%)
# Gaps:       77/1876 ( 4.1%)
# Score: 4888.0
#
#
#=====
```

V programu EMBOSS Needle sva vnesli celotno zaporedje človeškega in mišjega zaporedja za BRCA1, ki sva ju našli na Uni-Protu.