KRAS kot potencialna, a zahtevna tarča za PROTAC terapijo- REŠITVE

V raziskovalnem laboratoriju preučujejo potencialne tarče za zdravljenje rakavih obolenj z uporabo tehnologije PROTAC (proteolysis-targeting chimeras), ki omogoča usmerjeno razgradnjo proteinov. PROTAC je molekula, sestavljena iz treh osnovnih komponent: liganda za tarčni protein, povezovalca (linkerja) in liganda za E3 ubikvitin ligazo, ki posreduje prenos aktiviranega ubikvitina z E2 na specifičen substratni protein. Ligand za tarčni protein selektivno veže protein interesa (POI), ligand za E3 ligazo pa rekrutira E3 ubikvitin ligazo. Tvori se ternarni kompleks POI–PROTAC–E3 ligaza, v katerem E3 ligaza ubikvitinira POI, kar ga označi za razgradnjo v proteasomu.

Tvoja raziskovalna skupina je izolirala neznano aminokislinsko zaporedje proteina iz tumorskega vzorca, za katerega sumiš, da ima vlogo pri razvoju raka. Tvoja naloga je, da protein identificiraš, analiziraš njegove značilnosti ter oceniš njegovo primernost kot potencialno PROTAC tarčo.

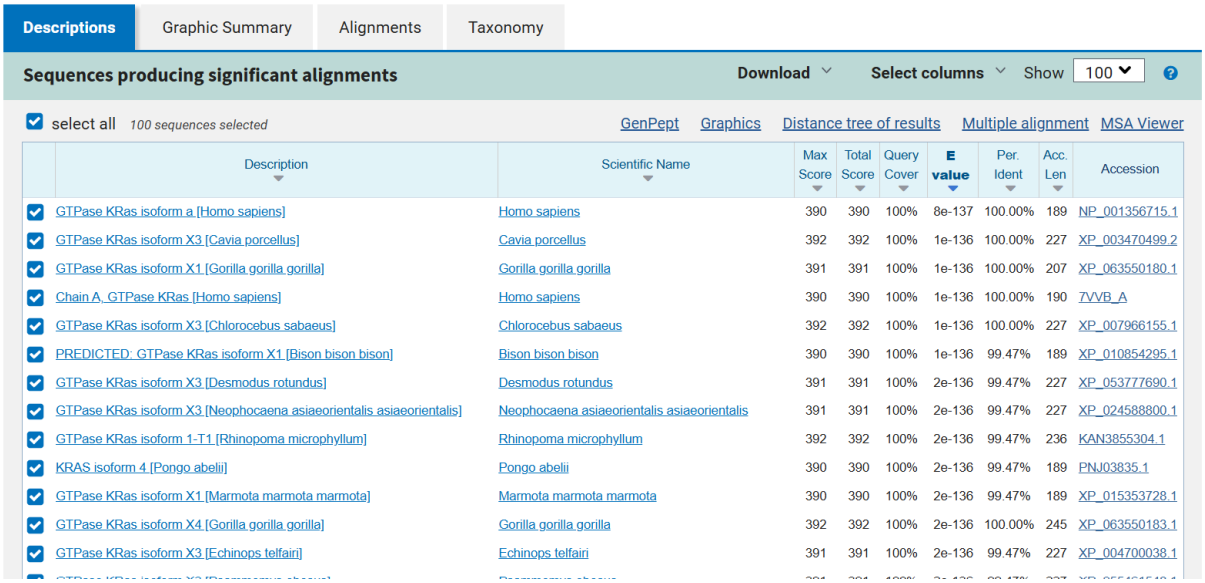
Identifikacija proteina in ocena aktualnosti

1. Proteinu pripadajoče zaporedje je pripeto spodaj.

    1. Identificiraj protein, ki ga kodira dano zaporedje, in

    Gre za KRAS protein.

    Informacijo o tem najdeš z iskanjem z zaporedjem po pblastu:



Descriptions | Graphic Summary | Alignments | Taxonomy

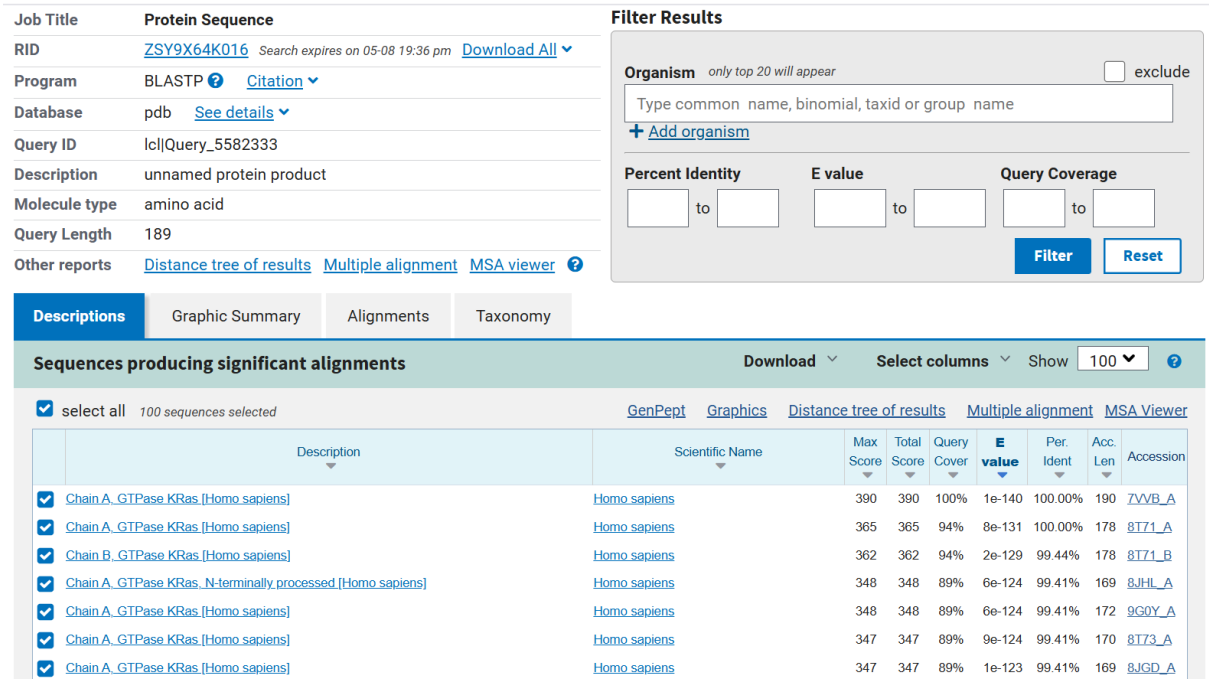
Sequences producing significant alignments Download Select columns Show 100

select all *100 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
GTPase KRas isoform a [Homo sapiens]	Homo sapiens	390	390	100%	8e-137	100.00%	189	NP_001356715.1
GTPase KRas isoform X3 [Cavia porcellus]	Cavia porcellus	392	392	100%	1e-136	100.00%	227	XP_003470499.2
GTPase KRas isoform X1 [Gorilla gorilla gorilla]	Gorilla gorilla gorilla	391	391	100%	1e-136	100.00%	207	XP_063550180.1
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	390	390	100%	1e-136	100.00%	190	7VVB_A
GTPase KRas isoform X3 [Chlorocebus sabaeus]	Chlorocebus sabaeus	392	392	100%	1e-136	100.00%	227	XP_007966155.1
PREDICTED: GTPase KRas isoform X1 [Bison bison bison]	Bison bison bison	390	390	100%	1e-136	99.47%	189	XP_010854295.1
GTPase KRas isoform X3 [Desmodus rotundus]	Desmodus rotundus	391	391	100%	2e-136	99.47%	227	XP_053777690.1
GTPase KRas isoform X3 [Neophocaena asiaeorientalis asiaeorientalis]	Neophocaena asiaeorientalis asiaeorientalis	391	391	100%	2e-136	99.47%	227	XP_024588800.1
GTPase KRas isoform 1-T1 [Rhinopoma microphyllum]	Rhinopoma microphyllum	392	392	100%	2e-136	99.47%	236	KAN3855304.1
KRAS isoform 4 [Pongo abelii]	Pongo abelii	390	390	100%	2e-136	99.47%	189	PNJ03835.1
GTPase KRas isoform X1 [Marmota marmota marmota]	Marmota marmota marmota	390	390	100%	2e-136	99.47%	189	XP_015353728.1
GTPase KRas isoform X4 [Gorilla gorilla gorilla]	Gorilla gorilla gorilla	392	392	100%	2e-136	100.00%	245	XP_063550183.1
GTPase KRas isoform X3 [Echinops telfairi]	Echinops telfairi	391	391	100%	2e-136	99.47%	227	XP_004700038.1

2. preveri, ali ima znano strukturo.

Njegova struktura je znana. To preverimo z iskanjem s pblastom po PDB.



Job Title	Protein Sequence
RID	ZSY9X64K016 *Search expires on 05-08 19:36 pm* Download All
Program	BLASTP Citation
Database	pdb See details
Query ID	lcl|Query_5582333
Description	unnamed protein product
Molecule type	amino acid
Query Length	189
Other reports	Distance tree of results Multiple alignment MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to | E value to | Query Coverage to

Filter Reset

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Sequences producing significant alignments — Download — Select columns — Show 100

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment MSA Viewer

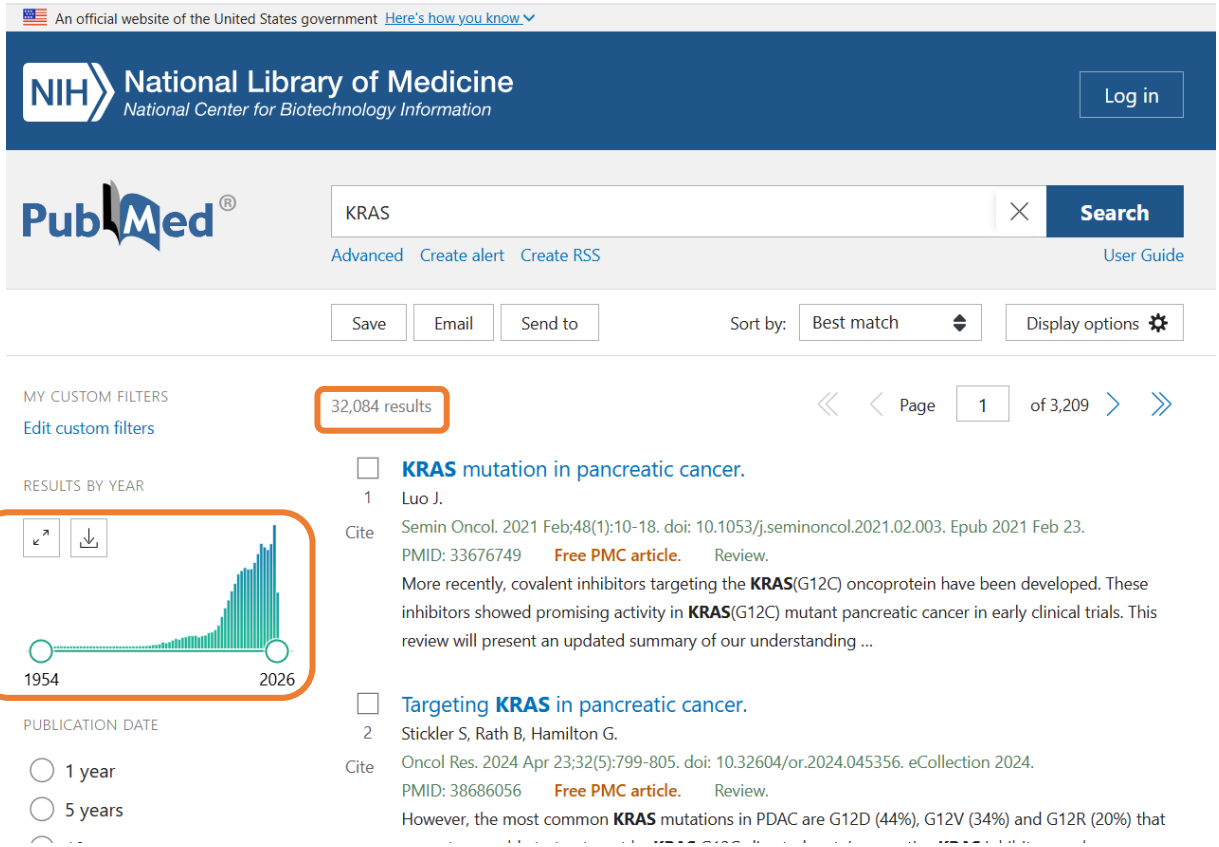
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	390	390	100%	1e-140	100.00%	190	7VVB_A
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	365	365	94%	8e-131	100.00%	178	8T71_A
Chain B, GTPase KRas [Homo sapiens]	Homo sapiens	362	362	94%	2e-129	99.44%	178	8T71_B
Chain A, GTPase KRas, N-terminally processed [Homo sapiens]	Homo sapiens	348	348	89%	6e-124	99.41%	169	8JHL_A
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	348	348	89%	6e-124	99.41%	172	9G0Y_A
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	347	347	89%	9e-124	99.41%	170	8T73_A
Chain A, GTPase KRas [Homo sapiens]	Homo sapiens	347	347	89%	1e-123	99.41%	169	8JGD_A

Protein KRAS ima pomembno vlogo pri uravnavanju celične proliferacije in signalnih poteh ter je pogosto mutiran pri različnih vrstah raka, zato predstavlja pomembno tarčo sodobnih protirakavih terapij.

Odgovore na vprašanja iz sledeče naloge od 2. dalje najdeš z iskanjem po PubMed-u.

2. Preveri koliko je vseh člankov, ki so bili objavljeni na temo proteina KRAS.

32084



An official website of the United States government Here's how you know

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KRAS — Search

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Edit custom filters

32,084 results

Page 1 of 3,209

RESULTS BY YEAR

1954 2026

PUBLICATION DATE
1 year
5 years

1 **KRAS** mutation in pancreatic cancer.
Luo J.
Cite
Semin Oncol. 2021 Feb;48(1):10-18. doi: 10.1053/j.seminoncol.2021.02.003. Epub 2021 Feb 23.
PMID: 33676749 Free PMC article. Review.
More recently, covalent inhibitors targeting the **KRAS**(G12C) oncoprotein have been developed. These inhibitors showed promising activity in **KRAS**(G12C) mutant pancreatic cancer in early clinical trials. This review will present an updated summary of our understanding ...

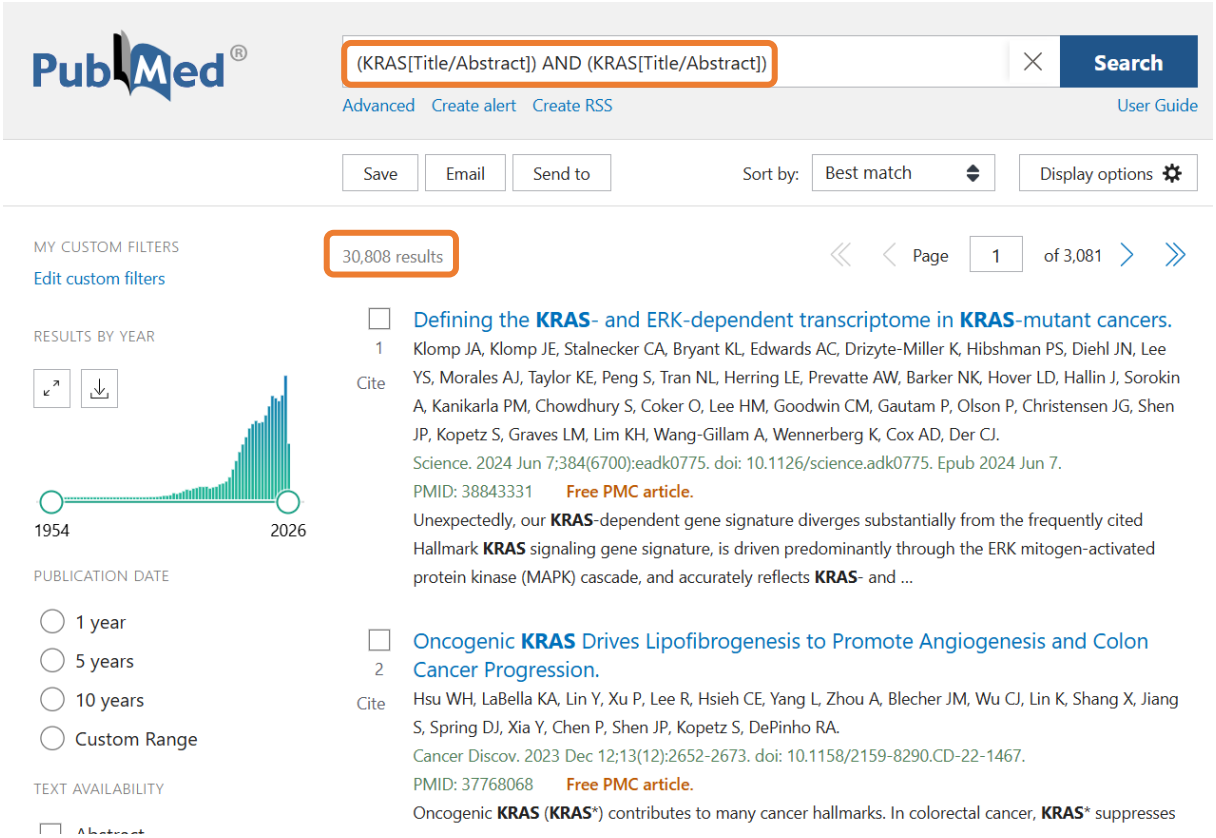
2 Targeting **KRAS** in pancreatic cancer.
Stickler S, Rath B, Hamilton G.
Cite
Oncol Res. 2024 Apr 23;32(5):799-805. doi: 10.32604/or.2024.045356. eCollection 2024.
PMID: 38686056 Free PMC article. Review.
However, the most common **KRAS** mutations in PDAC are G12D (44%), G12V (34%) and G12R (20%) that

3. Ali gre za aktualno raziskovalno področje?

Da, v zadnjih letih število objavljenih člankov na leto narašča.

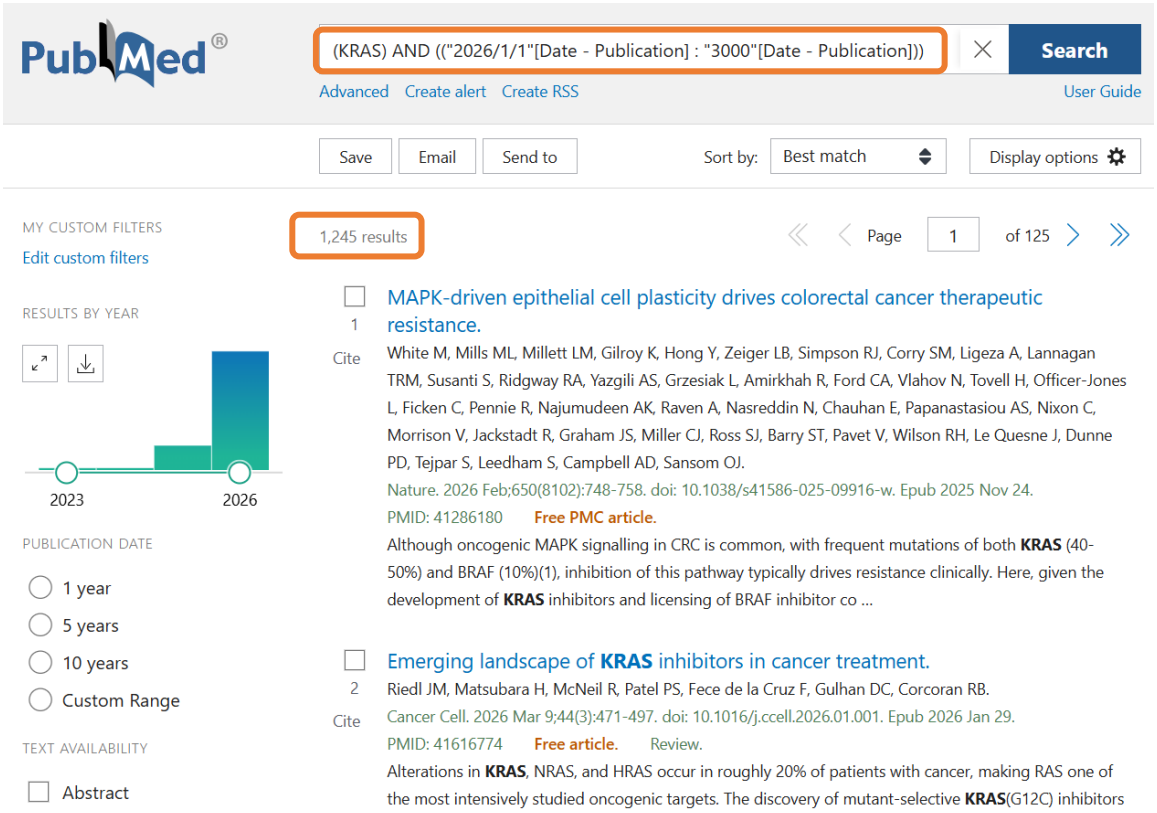
4. Koliko člankov je takih, kjer se ime proteina pojavi že v samem naslovu ali povzetku članka?

30808



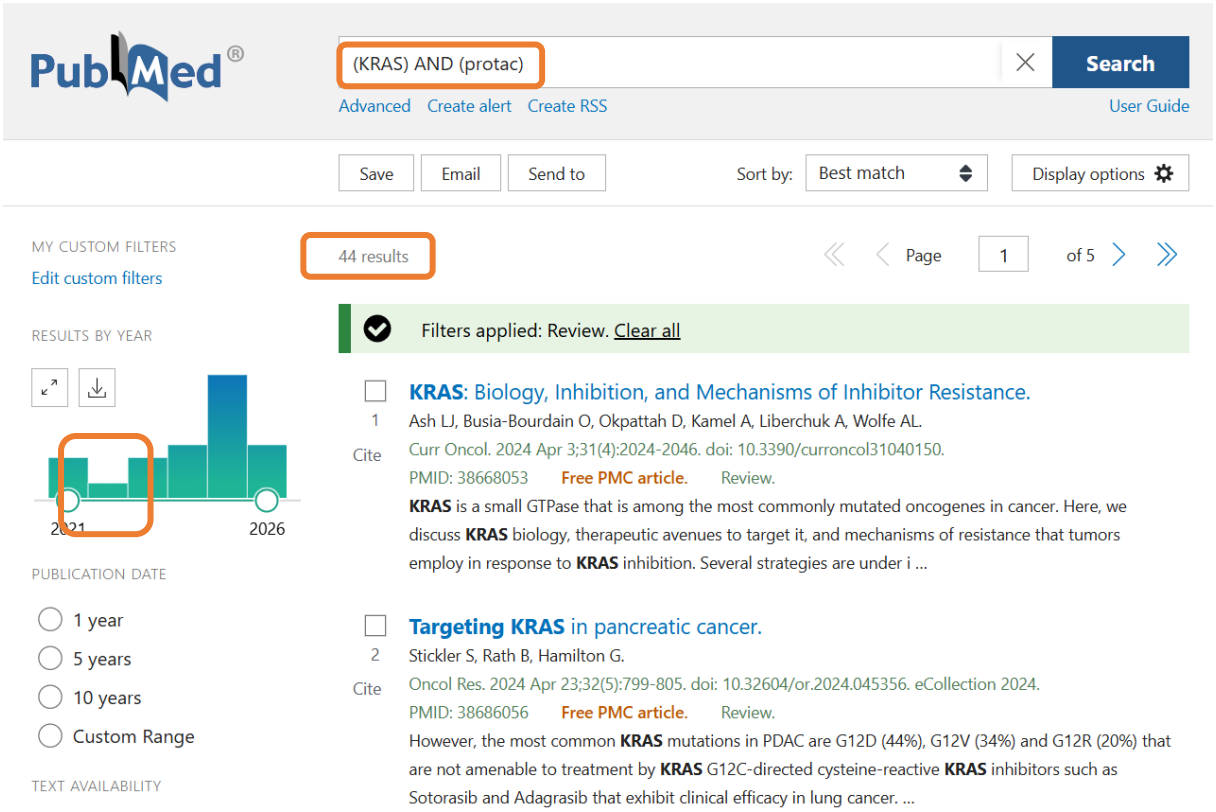
5. Koliko člankov je bilo z omenjenim proteinom objavljenih v letu 2026?

1245



6. Koliko člankov pa je takih, ki so pregledni in hkrati vsebujejo tako besedo *KRAS* kot tudi *PROTAC*?

44



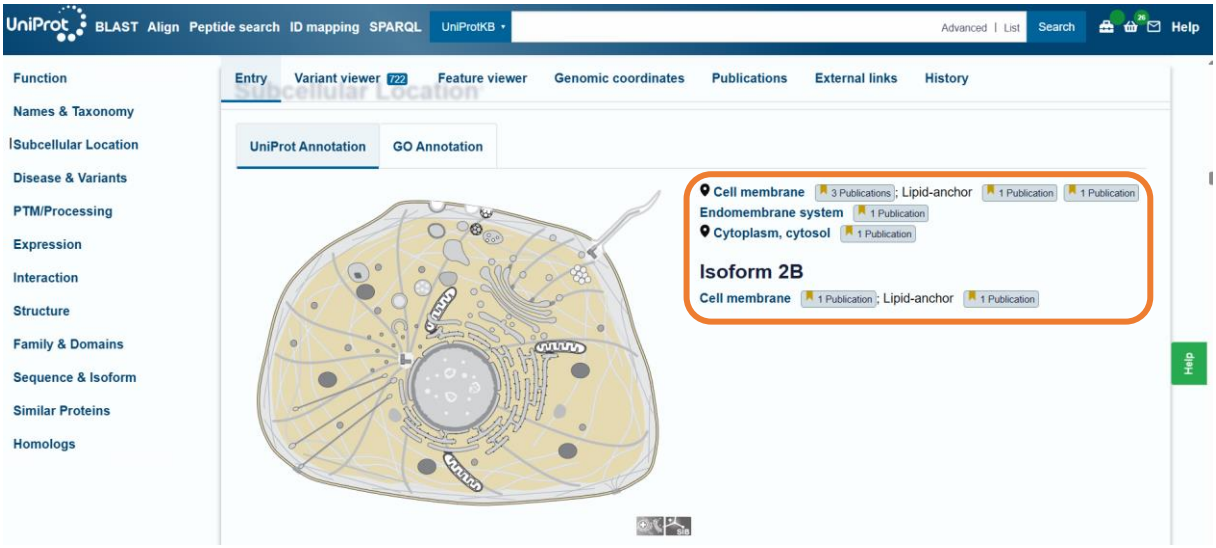
7. V katerem letu sta bila izdana le dva taka članka (pregledna, hkrati pa vsebujeta besedi *KRAS* in *PROTAC*)?

2022 (razvidno iz levega diagrama).

Značilnosti identificiranega proteina

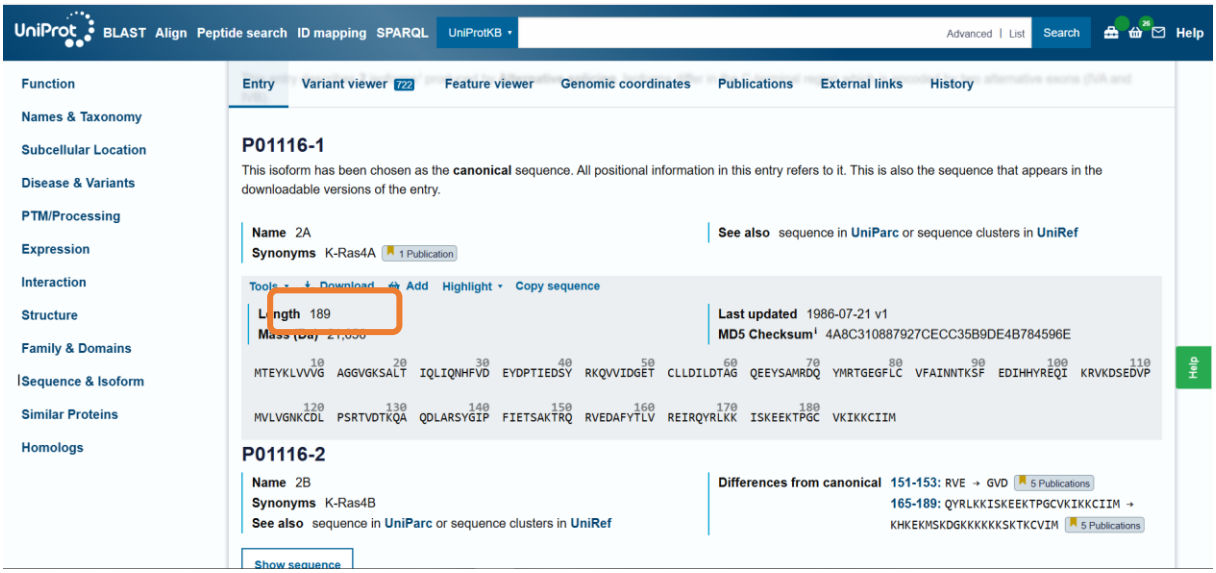
1. Kje v celici oziroma izven nje v človeku najdemo protein KRAS? Gre morda za transmembranski protein?

Protein se nahaja v celični membrani in citoplazmi. Ne gre za transmembranski protein.



2. Koliko aminokislinskih ostankov tvori ta protein?

189



3. Iz koliko domen je sestavljen ta protein?

Informacijo najdeš v Prosite. Sestavljen je iz ene, RAS domene.

P01116 **RASK_HUMAN** (189 aa)
GTPase KRas. *Homo sapiens (Human)*

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSA
MRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQD
LARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGCVKIKKCIIM

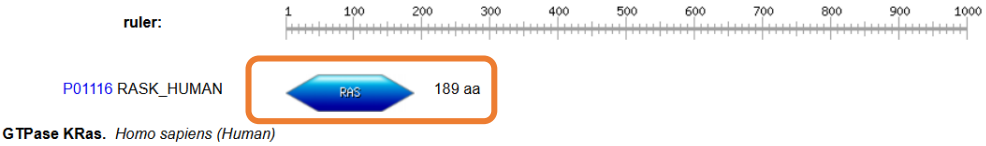
Legend:

disulfide bridge | active site | other 'ranges' | other sites

Please note that the graphical representations of domains displayed hereafter are for illustrative purposes only, and that their colors and shapes are not intended to indicate homology or shared function.
For more information about how these graphical representations are constructed, go to https://prosite.expasy.org/mydomains/.

hits by profiles: [1 hit (by 1 profile) on 1 sequence]

Upper case represents match positions, lower case insert positions, and the '-' symbol represents deletions relative to the matching profile.



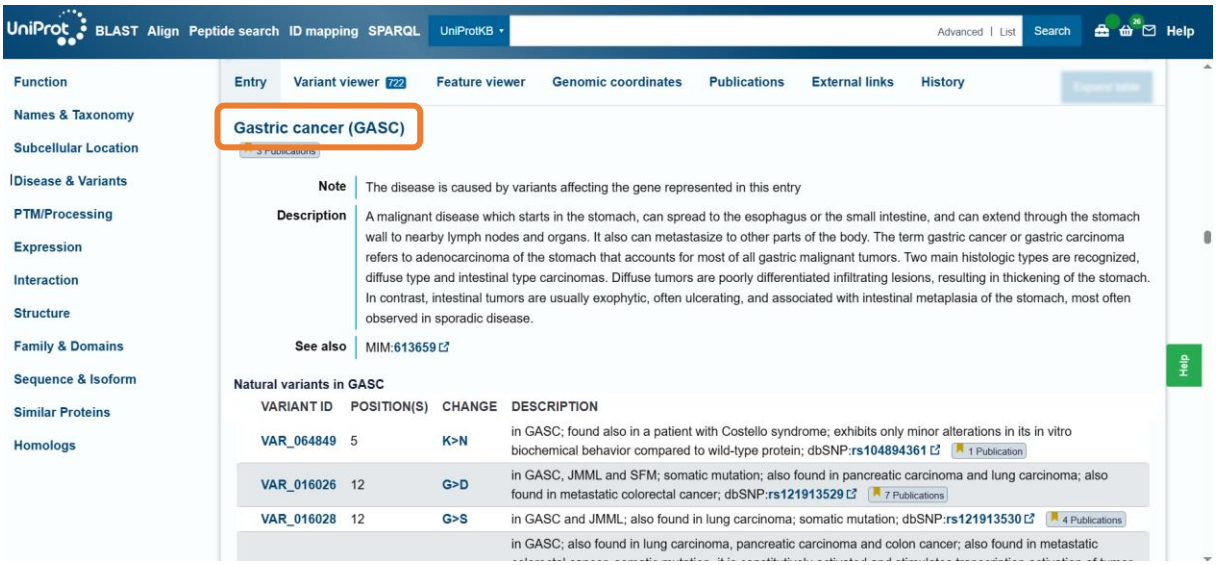
GTPase KRas. *Homo sapiens (Human)*

Hits of PS51421 on AlphaFold-P01116-F1-v4

PS51421 **RAS** *Small GTPase Ras domain profile* :
Hits of PS51421 on PDB 3D structures x-referenced by P01116: pdb_00002msc_B, pdb_00002msd_B, pdb_00002mse_B, pdb_00003gft_A, pdb_00003gft_B, pdb_00003gft_C, pdb_00003gft_D, pdb_00003gft_E, pdb_00003gft_F, pdb_00004dsn_A, pdb_00004dso_A, pdb_00004epr_A, pdb_00004ept_A, pdb_00004epv_A, pdb_00004epw_A, pdb_00004epx_A, pdb_00004epy_A, pdb_00004l8g_A, pdb_00004ldj_A, pdb_00004lpk_A, pdb_00004lpk_B, pdb_00004lrw_A, pdb_00004lrw_B, pdb_00004luc_A, pdb_00004luc_B, pdb_00004lv6_A, pdb_00004lv6_B, pdb_00004lyf_A, pdb_00004lyf_B, pdb_00004lyf_C, pdb_00004lyh_A, pdb_00004lyh_B, pdb_00004lyh_C, pdb_00004lyj_A, pdb_00004m1o_A, pdb_00004m1o_B, pdb_00004m1o_C, pdb_00004m1s_A, pdb_00004m1s_B, pdb_00004m1s_C, pdb_00004m1t_A, pdb_00004m1t_B, pdb_00004m1t_C, pdb_00004m1w_A, pdb_00004m1w_B, pdb_00004m1w_C, pdb_00004m1y_A, pdb_00004m1y_B, pdb_00004m1y_C, pdb_00004m21_A, pdb_00004m21_B, pdb_00004m21_C, pdb_00004m22_A, pdb_00004m22_B, pdb_00004m22_C, pdb_00004nmm_A, pdb_00004obe_A, pdb_00004obe_B, pdb_00004pzy_A, pdb_00004pzy_B, pdb_00004pzz_A, pdb_00004q01_A, pdb_00004q01_B, pdb_00004q02_A, pdb_00004q03_A, pdb_00004ql3_A,

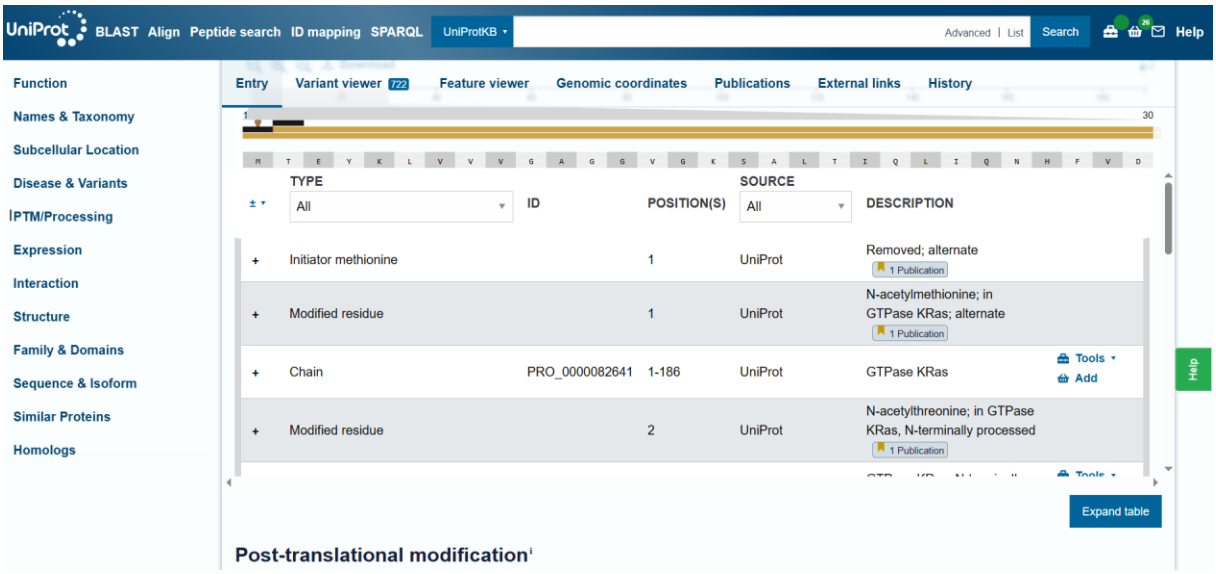
4. V potencialni razvoj katerega raka je vključen? Podatek poiščite v UniProt-u.

V razvoja raka želodca.

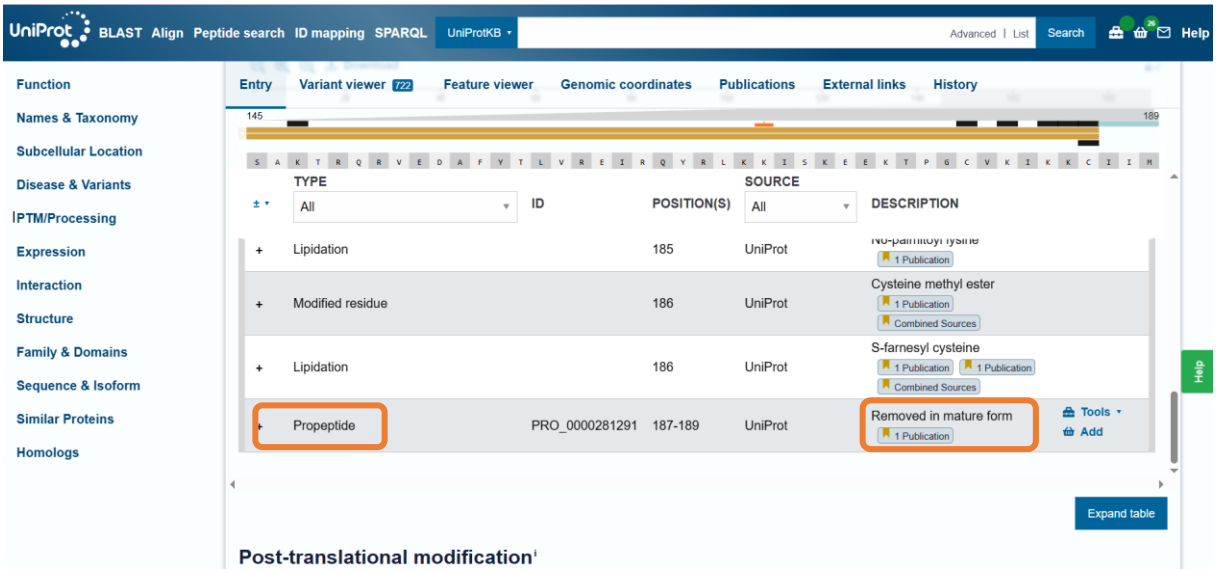


5. Ali protein vsebuje signalni peptid? Se (tudi) kateri drug del zaporedja v zreli obliki peptida odcepi?

Protein ne vsebuje signalnega peptida,

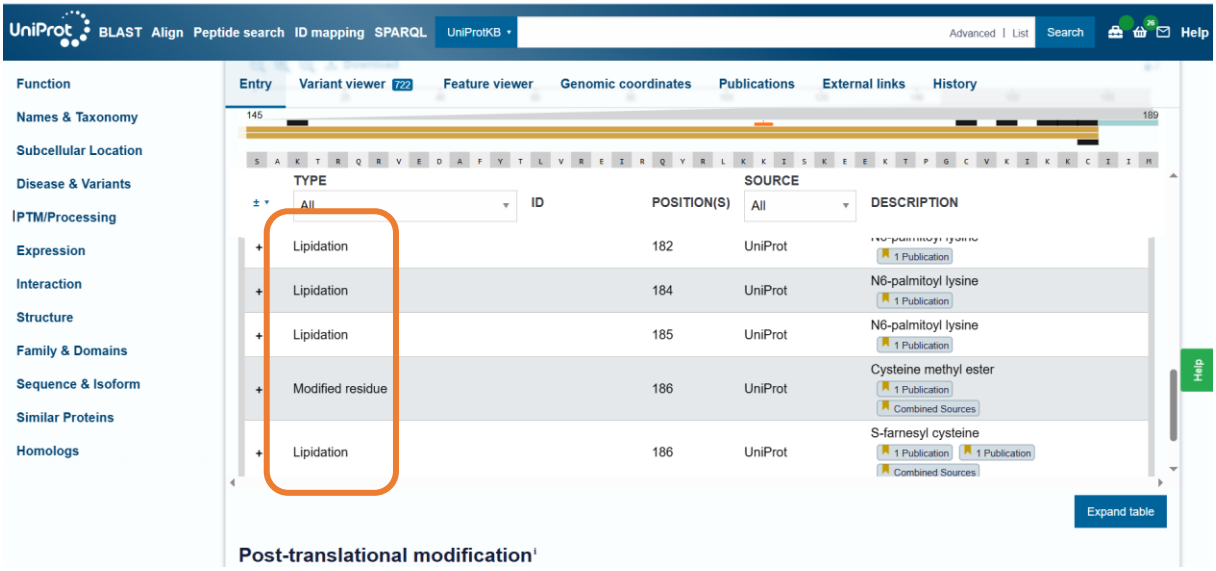


vsebuje pa propeptid, ki v zreli obliki proteina ni prisoten.



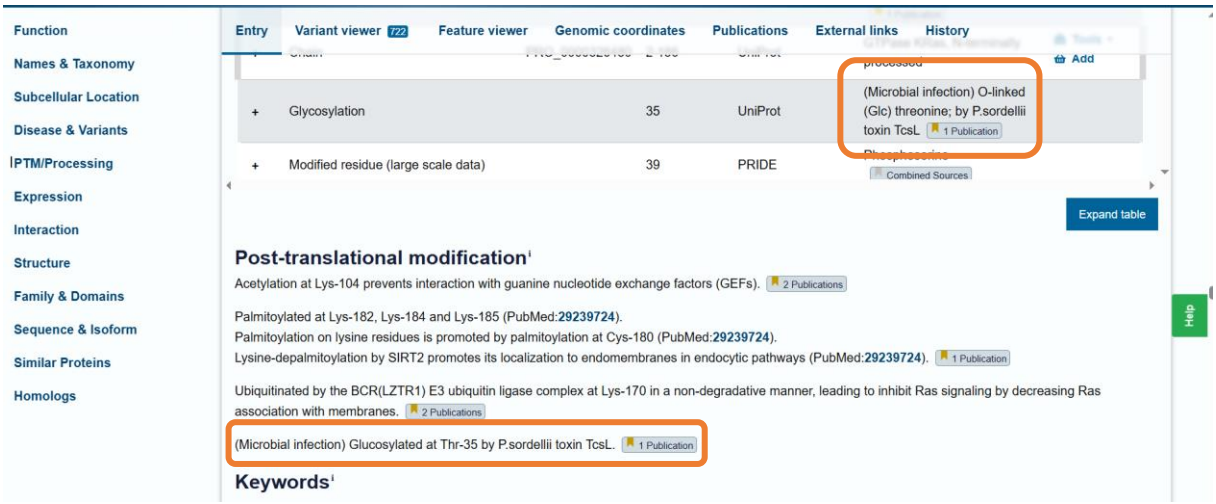
6. Katera posttranslacijska modifikacija je najbolj pogosta na C-terminalnem koncu proteina?

Lipidacija.



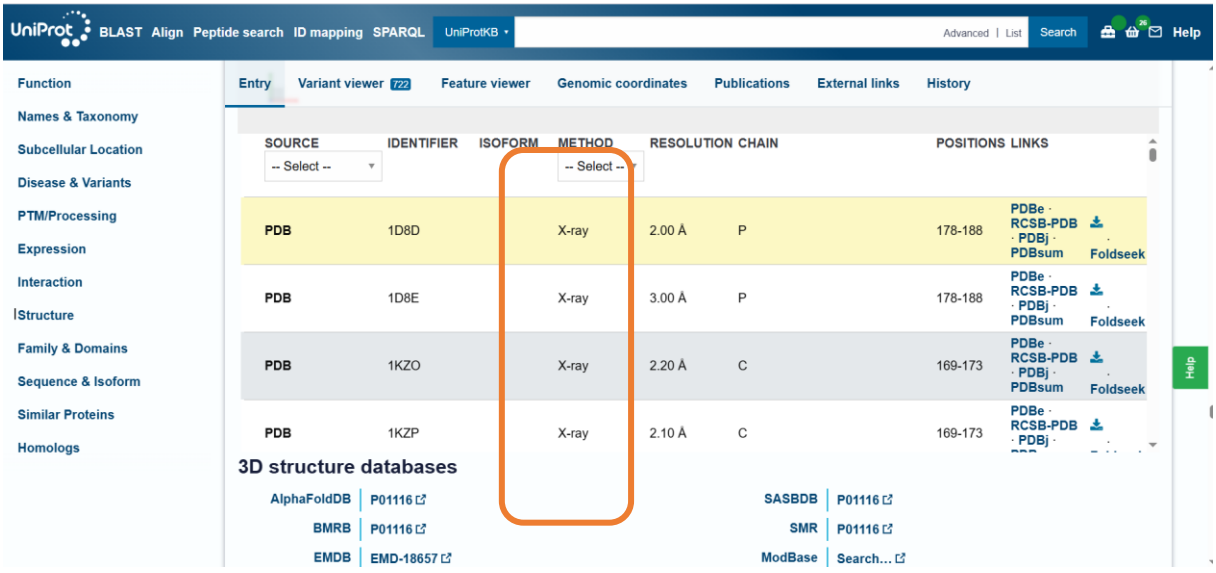
7. Kaj povzroči glikozilacijo Thr35?

Toksin TcsL (iz bakterije Paeniclostridium sordellii).



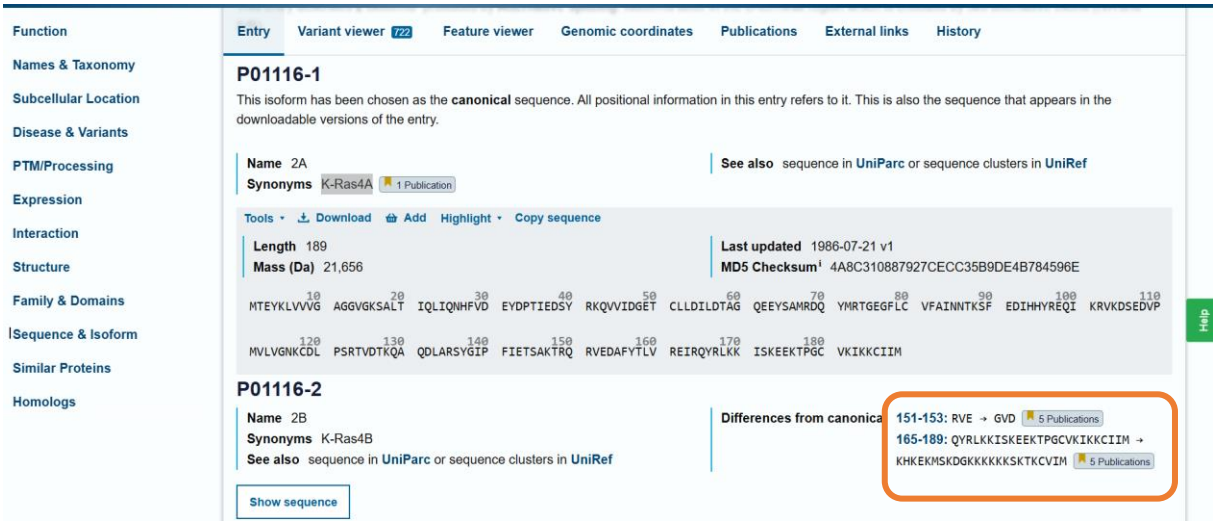
8. S katero metodo je bilo določeno največ struktur tega proteina?

Z XRD.



9. V katerih aminokislinskih ostankih (zapiši le interval od kje do kje) se kanonična struktura razlikuje od njene druge izoformne oblike?

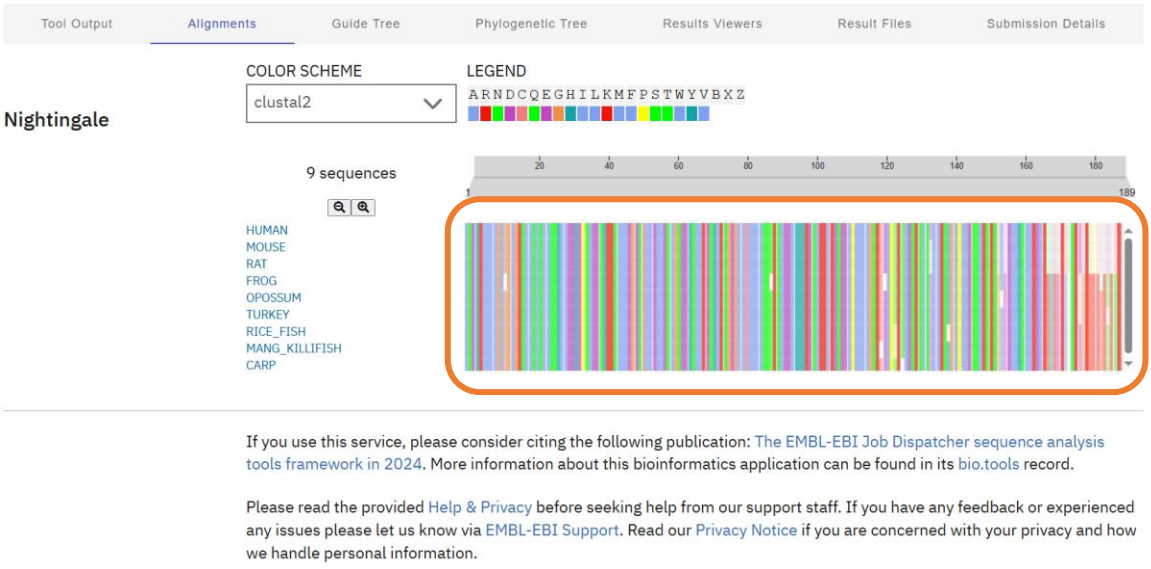
Od 151 do 153 in od 165 do 189.



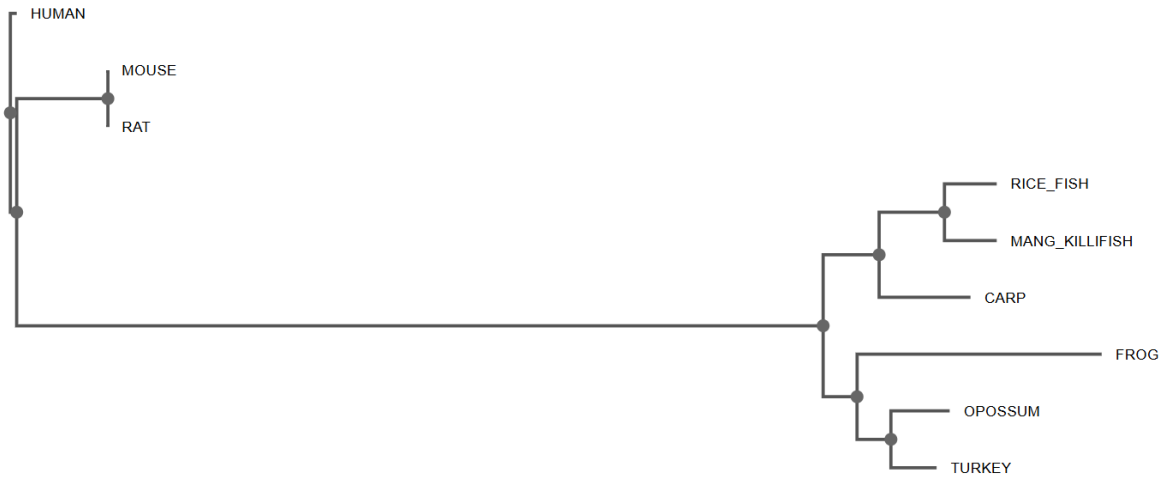
Primerjava KRAS z drugimi proteini z RAS domeno

1. Poišči več KRAS proteinov iz drugih organizmov in ugotovi, kateri konec proteina (N- ali C-končni del) je evolucijsko bolj ohranjen (omejite se na zbirko Swiss-Prot).

Bolj je ohranjen N-končni del proteina (poravnava narejena s ClustalOmega).



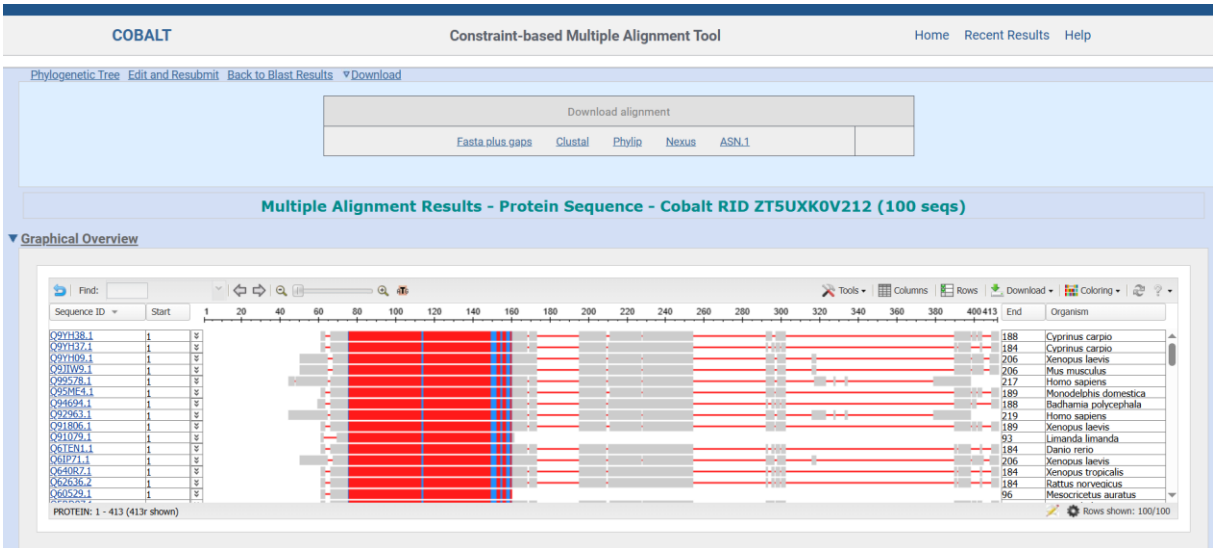
2. Na podlagi KRAS proteinov iz izbranih organizmov prikaži filogenetsko drevo, tako da je človeški KRAS protein na 'začetku/izvoru drevesa'.

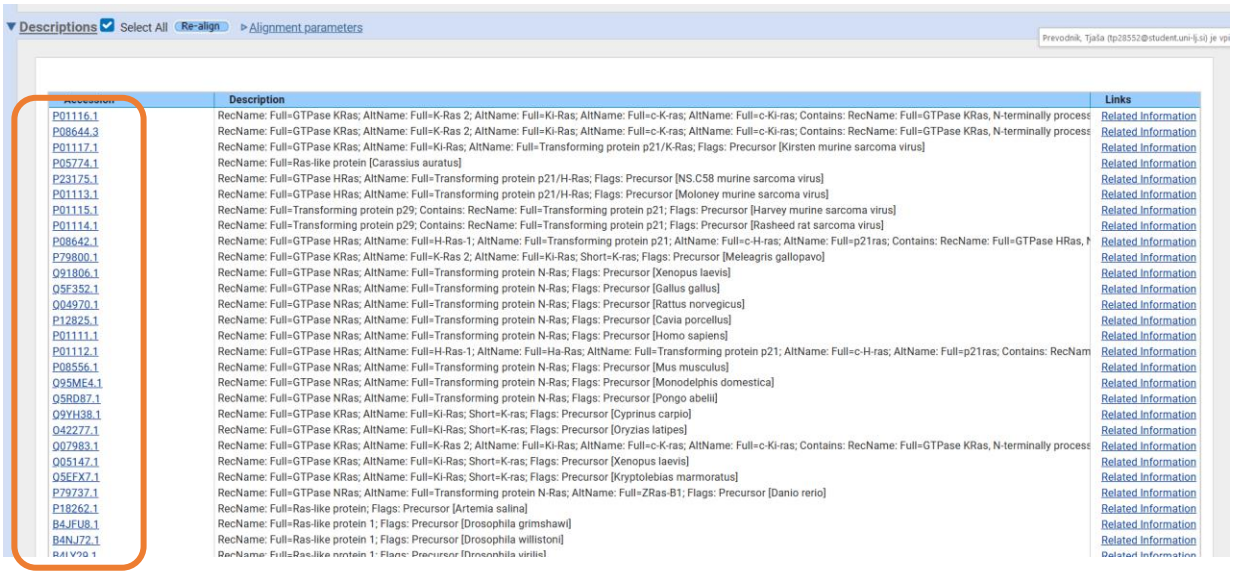


3. Protein KRAS vsebuje RAS (GTPazno) domeno, ki je ključna za vezavo GTP/GDP in s tem za uravnavanje njegove aktivnosti v signalnih poteh. Identificiraj proteine (vsaj 15), ki prav tako vsebujejo tako RAS domeno (lahko iz poljubnih organizmov) in s primerjavo teh proteinov med seboj oceni ohranjenost vezavnih mest znotraj RAS družine. Ohranjenost aminokislinskih ostankov prikaži tudi s Sequence Logo-m.

S pomočjo pblast identifikacija proteinov, ki vsebujejo tako RAS domeno kot jo ima protein KRAS. Sledi poravnava zadetkov z orodjem Cobalt in izbira (vsaj 15) proteinov, ki jih prek accession code odpreš v UniProtu. Tam jih shraniš v košarico in jih poravnaš. Na človeškem proteinu KRAS (P01116) označiš 'binding site' in ga primerjaš z ostalimi proteini.

Iz poravnave je razvidno, da so vezavna mesta med proteini z RAS domeno vsaj delno ohranjena, nekatera sicer bolj (116-119 aminokislinskega ostanka), druga manj (29-35).




Descriptions
Select All
Re-align
Alignment parameters
Accession
Description
Links
P01116.1 RecName: Full=GTPase KRas; AltName: Full=K-Ras 2; AltName: Full=Ki-Ras; AltName: Full=c-K-ras; AltName: Full=c-Ki-ras; Contains: RecName: Full=GTPase KRas, N-terminally process Related Information
P08644.3 RecName: Full=GTPase KRas; AltName: Full=K-Ras 2; AltName: Full=Ki-Ras; AltName: Full=c-K-ras; AltName: Full=c-Ki-ras; Contains: RecName: Full=GTPase KRas, N-terminally process Related Information
P01117.1 RecName: Full=GTPase KRas; AltName: Full=KI-Ras; AltName: Full=Transforming protein p21/K-Ras; Flags: Precursor [Kirsten murine sarcoma virus] Related Information
P05774.1 RecName: Full=Ras-like protein [Carassius auratus] Related Information
P23175.1 RecName: Full=GTPase HRas; AltName: Full=Transforming protein p21/H-Ras; Flags: Precursor [NS.C58 murine sarcoma virus] Related Information
P01113.1 RecName: Full=GTPase HRas; AltName: Full=Transforming protein p21/H-Ras; Flags: Precursor [Moloney murine sarcoma virus] Related Information
P01115.1 RecName: Full=Transforming protein p29; Contains: RecName: Full=Transforming protein p21; Flags: Precursor [Harvey murine sarcoma virus] Related Information
P01114.1 RecName: Full=Transforming protein p29; Contains: RecName: Full=Transforming protein p21; Flags: Precursor [Rasheed rat sarcoma virus] Related Information
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P79800.1 RecName: Full=GTPase KRas; AltName: Full=K-Ras 2; AltName: Full=Ki-Ras; Short=K-ras; Flags: Precursor [Meleagris gallopavo] Related Information
Q91806.1 RecName: Full=GTPase NRas; AltName: Full=Transforming protein N-Ras; Flags: Precursor [Xenopus laevis] Related Information
Q5F352.1 RecName: Full=GTPase NRas; AltName: Full=Transforming protein N-Ras; Flags: Precursor [Gallus gallus] Related Information
Q04970.1 RecName: Full=GTPase NRas; AltName: Full=Transforming protein N-Ras; Flags: Precursor [Rattus norvegicus] Related Information
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P01111.1 RecName: Full=GTPase NRas; AltName: Full=Transforming protein N-Ras; Flags: Precursor [Homo sapiens] Related Information
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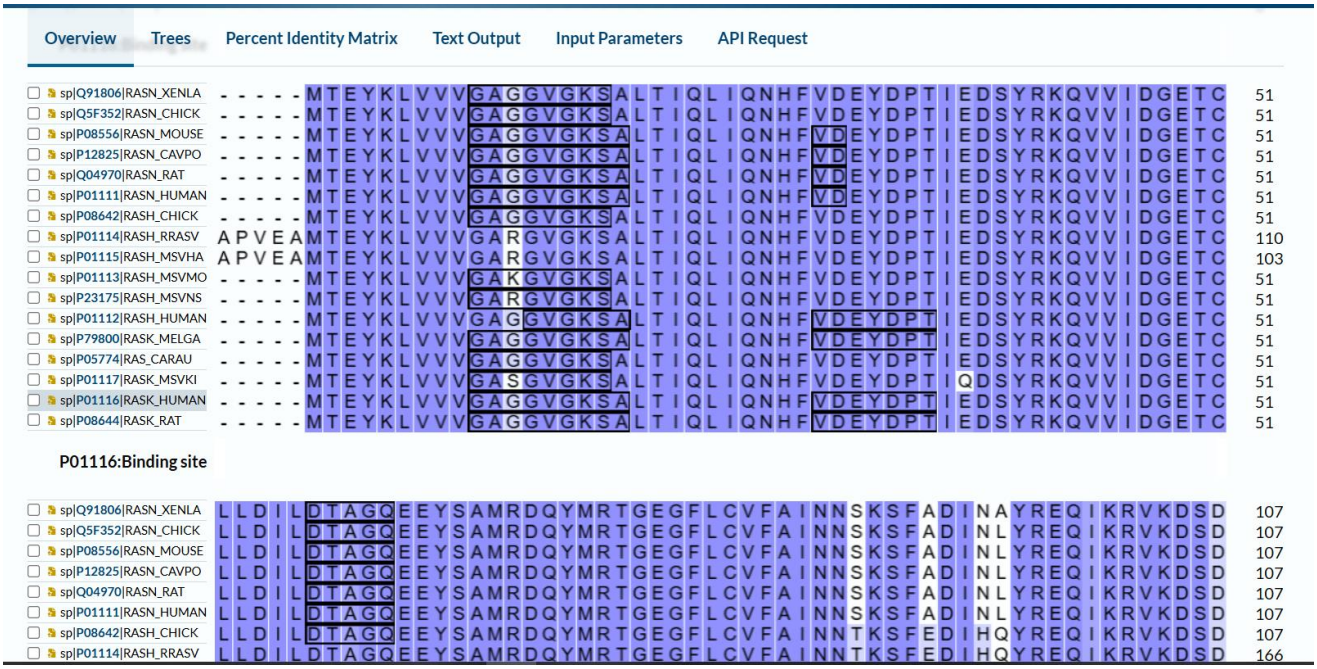
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P01116:Binding site

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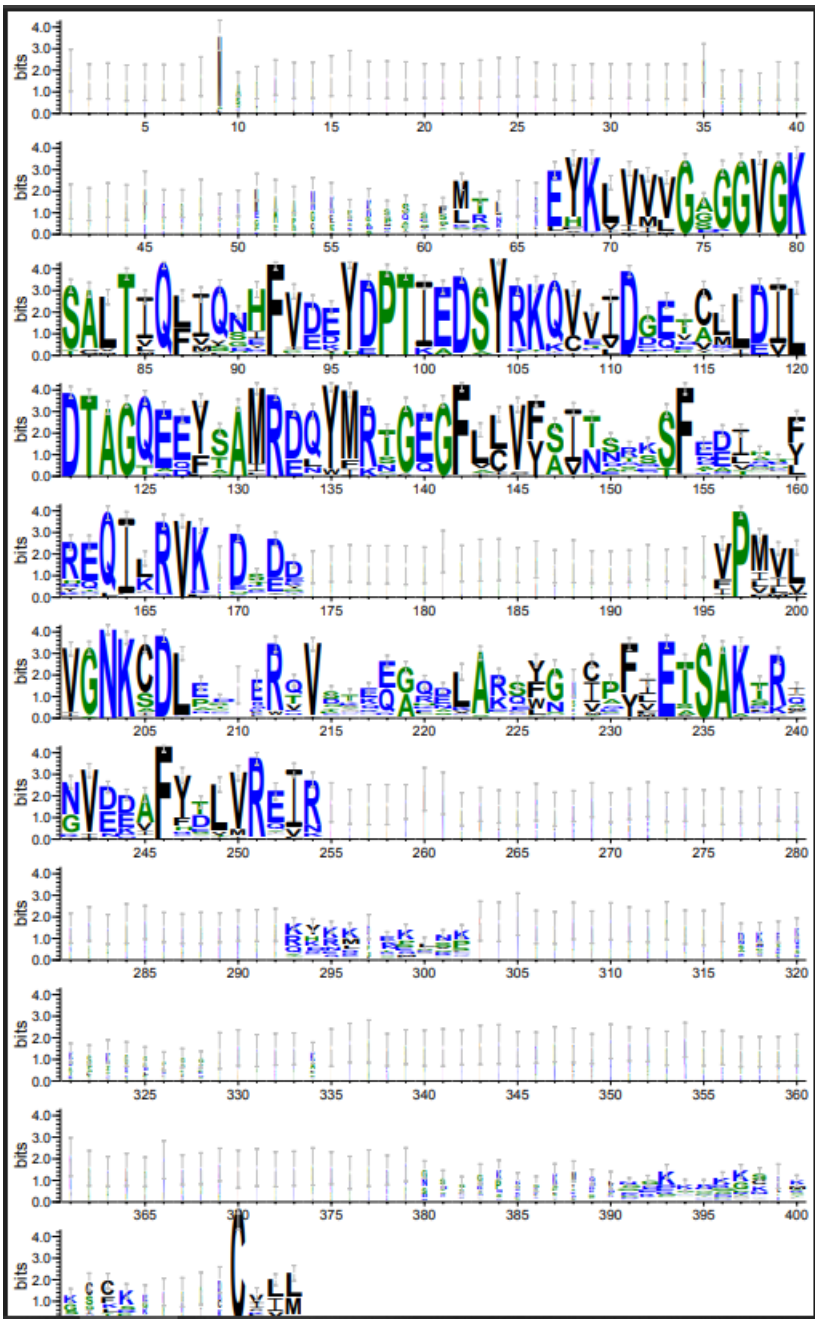
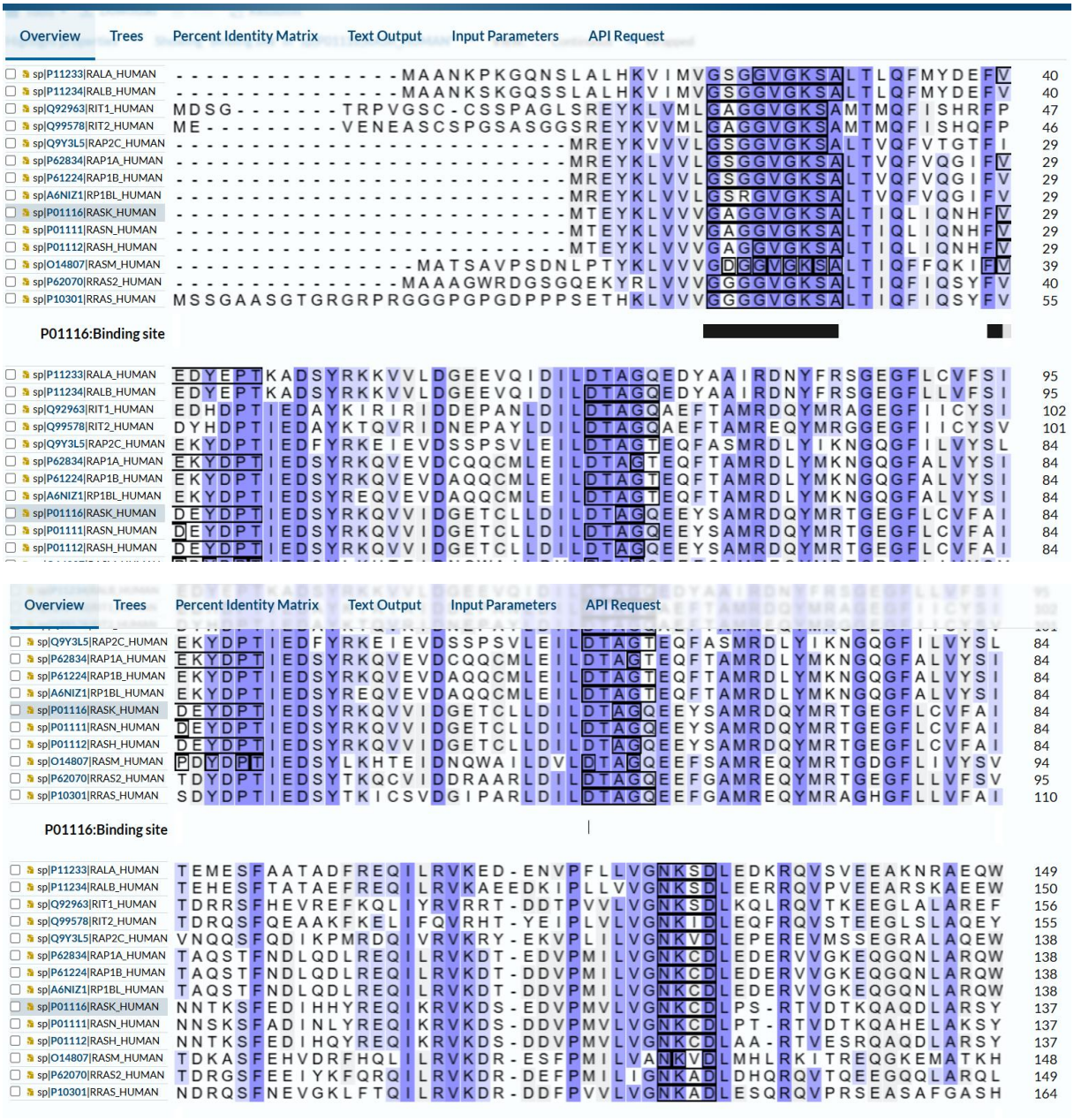


4. V zbirki Swiss-Prot zdaj poišči človeške proteine, ki vsebujejo RAS domeno (vsaj 10 takih proteinov). Glede na najdene proteine oceni selektivnost zdravila PROTAC za tarčenje KRAS v človeških celicah in možnost potencialne razgradnje napačnih proteinov.

Postopek je podoben kot pri prejšnji nalogi, le da zdaj pri iskanju z blastp pod organizem vpišeš človeka, s čimer se omejiš le na iskanje človeških proteinov. Iz poravnave je vidno, da je vezavno mesto, ki ga tvorita 59 in 60 aminokislinski ostanek v človeškem KRAS, ohranjeno pri vseh izbranih človeških proteinih z RAS domeno. Zelo dobro so ohranjeni tudi aminokislinski ostanki od 10 do 18 (številčenje po človeškem KRAS). Ohranjeni so tudi ostanki od 116 do 119, z izjemo cisteina na 118 mestu. Ostanki 29-35 so najslabše ohranjeni.

Prisotnost določenih ohranjenih sekvenc ostankov dopušča možnost vezave PROTAC na napačno tarčo in tako razgradnjo napačnih proteinov. Potrebno pa je imeti v mislih, da je tudi pomembno, kateri aminokislinski ostanek manjka. Če manjka npr. cistein, ki je zaradi svoje nukleofilnosti pogosto za vezavo ključnega pomena, je vendarle drugače, kot če manjka ostanek, ki za vezavo nima ključnega pomena (npr. običajno glicin).

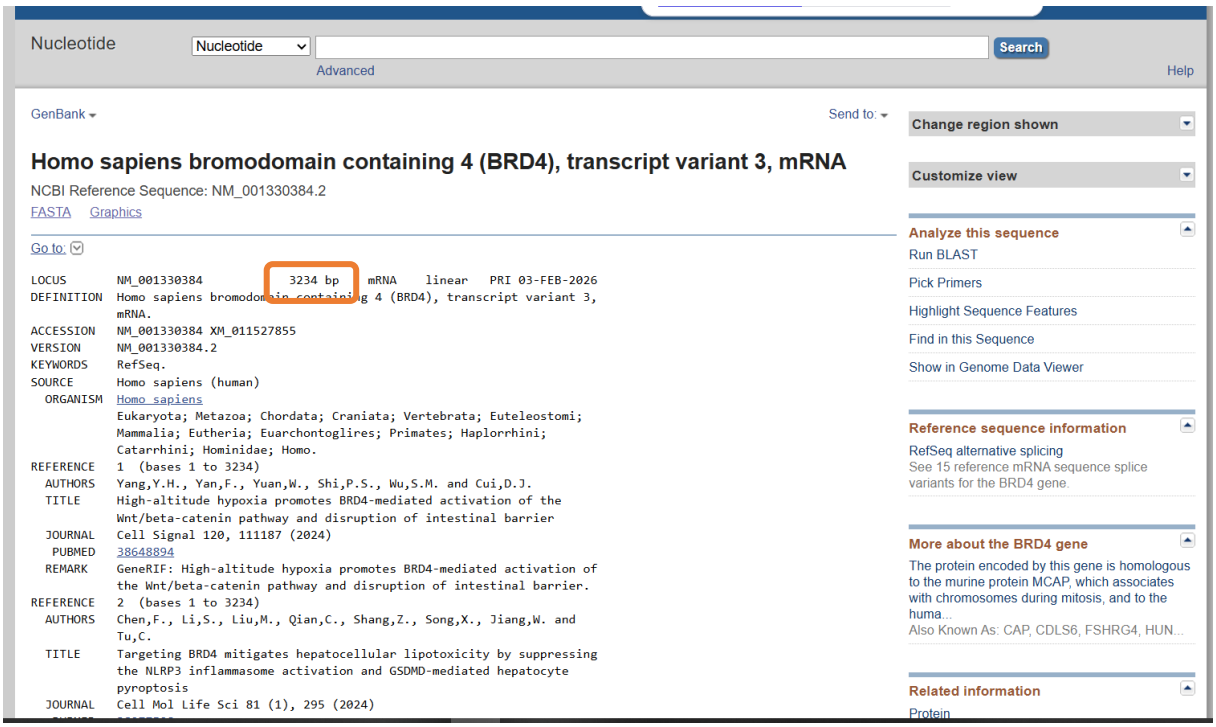


Primerjava KRAS z drugimi PROTAC tarčami

Poleg proteina KRAS so pomembne tarče za razvoj PROTAC terapij tudi BET proteini, med katerimi je posebej pomemben BRD4 (bromodomain containing 4).

1. Kako dolga je mRNA, ki kodira človeški protein BRD4 (glej varianto transkripta 3)?

3234 bp



2. Koliko eksonov vsebuje njegova mRNA (glej varianto transkripta 3)?

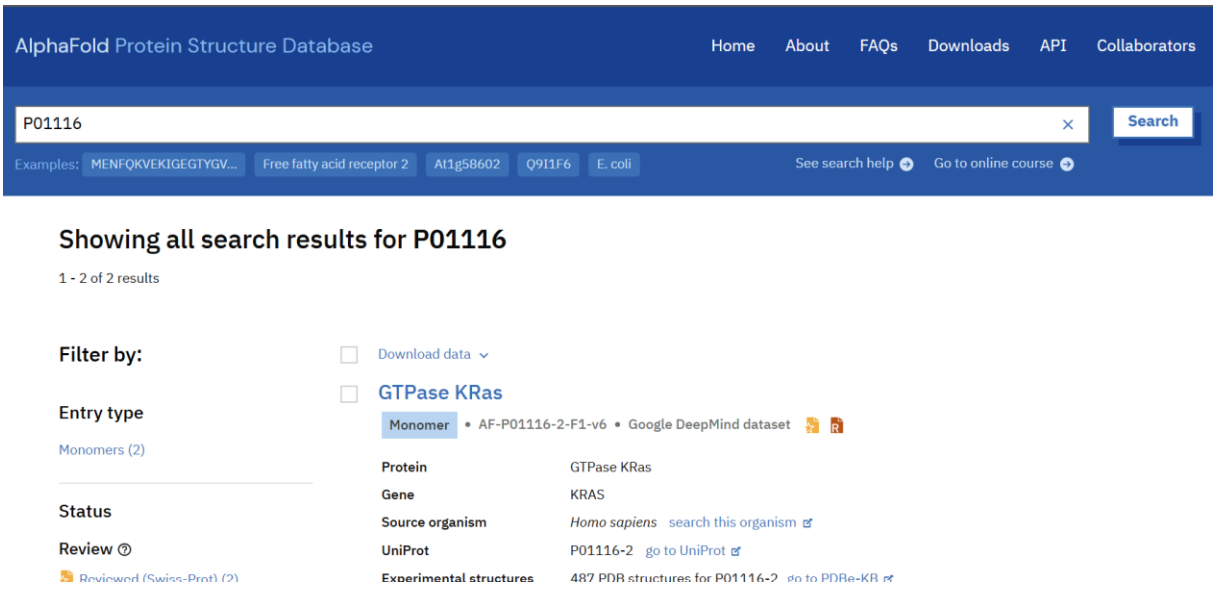
Informacijo najdeš v zgornjem zadetku pod razdelkom Features, kjer prešteješ eksone. Vseh eksonov je 12.

Ker se PROTAC molekula na tarčni protein veže preko liganda, ki prepozna specifično vezavno mesto na proteinu, dostopnost in struktura teh mest pomembno vplivata na uspešnost degradacije proteina. Zato primerjajte proteina KRAS in BRD4 ter ocenite, kateri predstavlja primernejšo tarčo za razvoj PROTAC terapij glede na dostopnost in definiranost vezavnih mest za ligand.

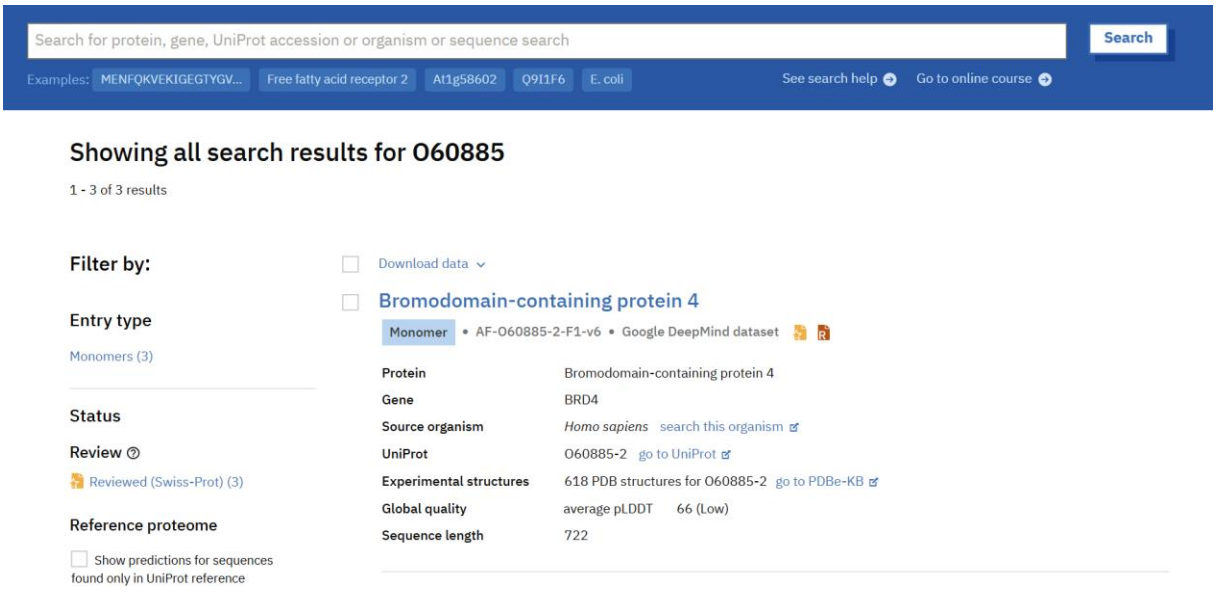
* V AlphaFold Protein Structure Database poiščite strukturi obeh proteinov (povezavi najdete v UniProt).

* Pri iskanju modela strukture proteina KRAS boš našel dva modela, za protein BRD4 pa tri modele. Za KRAS uporabi oba modela in zanju v Chimeri X naredi superpozicijo, za BRD4 pa le prvi model. S pomočjo UniProt-a ustrezno identificiraj vezavna mesta obeh proteinov in jih v ChimeriX označi z drugo barvo.

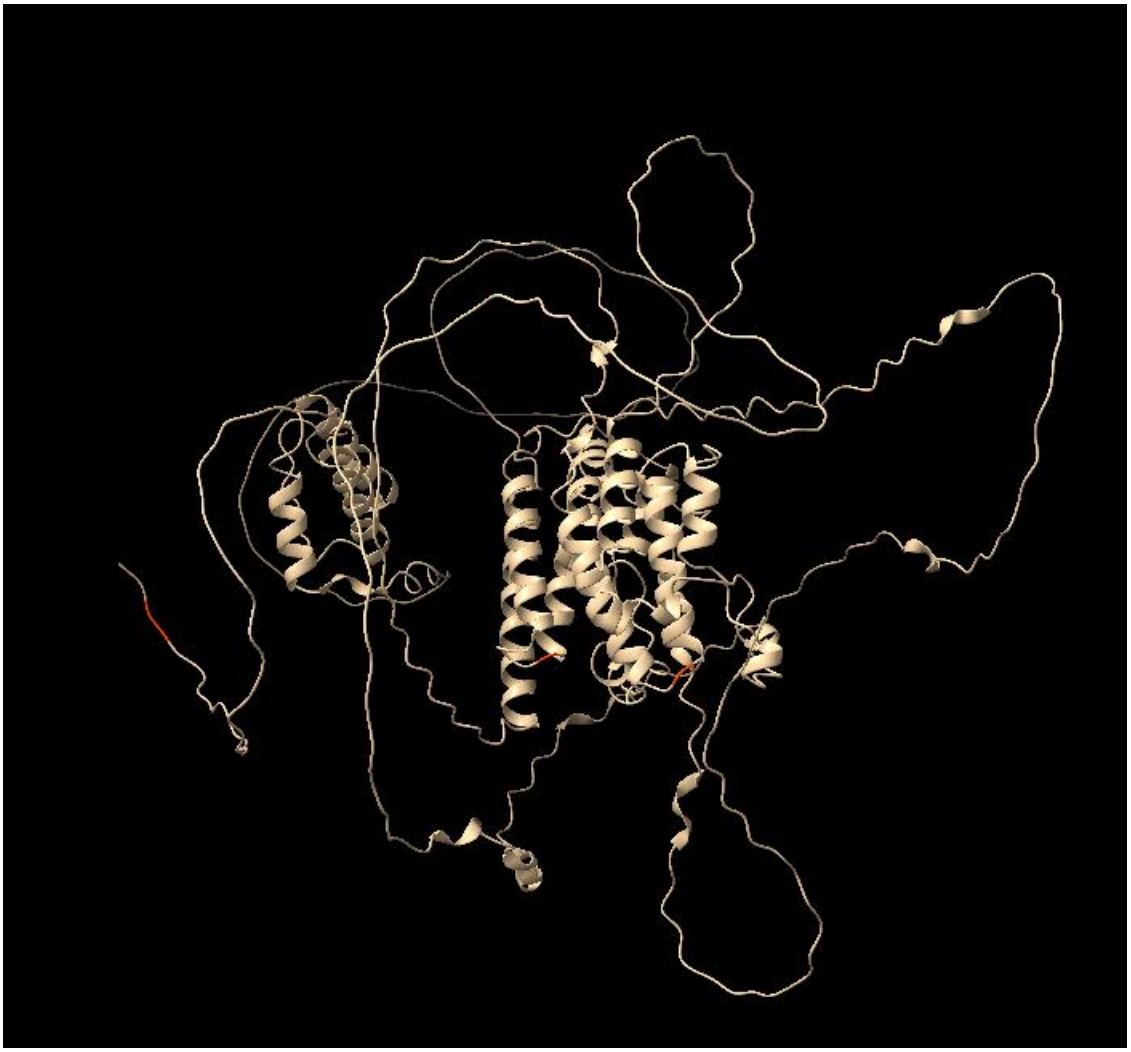
KRAS



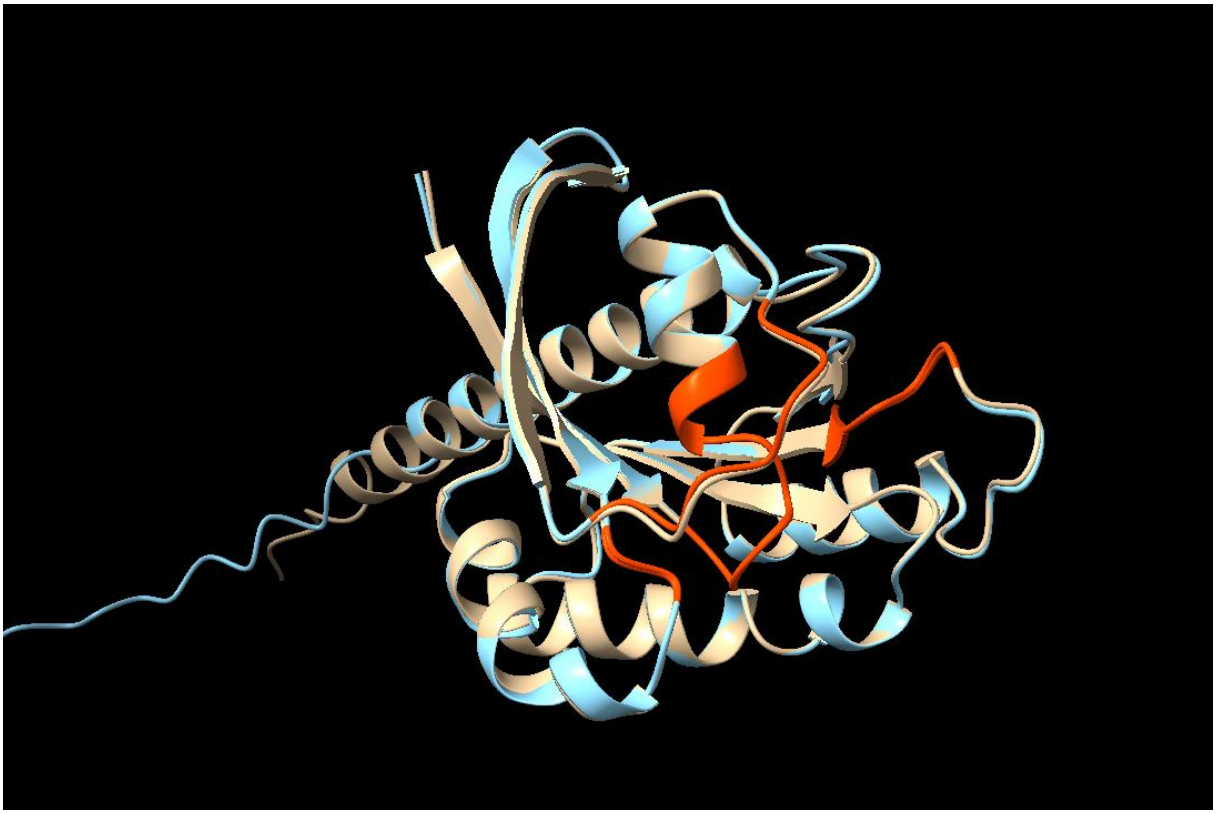
BRD4



ChimeraX – BRD4



ChimeraX – KRAS



1. Na podlagi primerjave same definiranosti vezavnih mest in možnosti selektivne vezave liganda, predvidi, kateri protein predstavlja primernejšo PROTAC tarčo. Pomagaš si lahko tudi z UniProt-om (preveri domene BRD4).

Protein KRAS ima plitve in dinamične interakcijske površine ter slabo definirana vezavna mesta za vezavo ligandov, zaradi česar predstavlja zahtevno tarčo PROTAC terapij. Nasprotno protein BRD4 vsebuje dobro definirane bromodomene (podatek v UniProtu pod Family and Domains), sama vezava ligandov na BRD4 pa je bolj specifična in stabilna, zaradi česar slednji predstavlja primernejšo PROTAC tarčo.