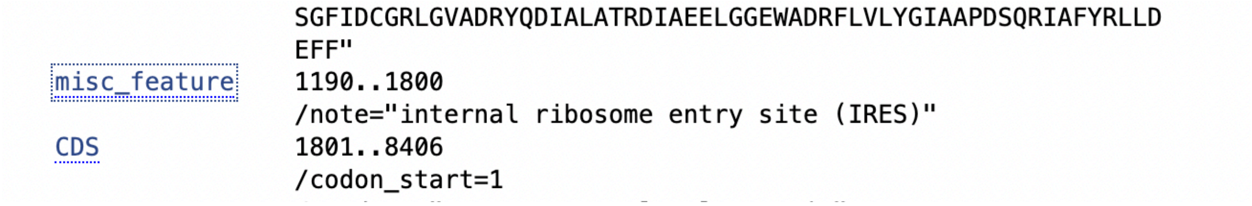
Rešitve »Kaj se lahko iz divje zabave nauči nemoralni biokemik?«

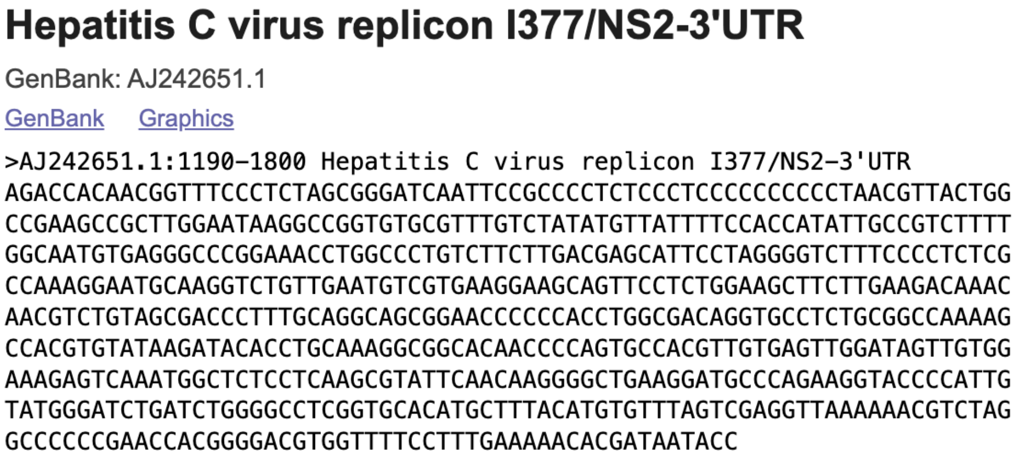
1.) Izvedba PCR reakcije:

- podan link, kjer je zapis za *internal ribosome entry site (IRES),* ki ga moramo najti v mRNA zapisu



```
                     SGFIDCGRLGVADRYQDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLD
                     EFF"
     misc_feature    1190..1800
                     /note="internal ribosome entry site (IRES)"
     CDS             1801..8406
                     /codon_start=1
```

- poiščemo zapisa v FASTA formatu za IRES

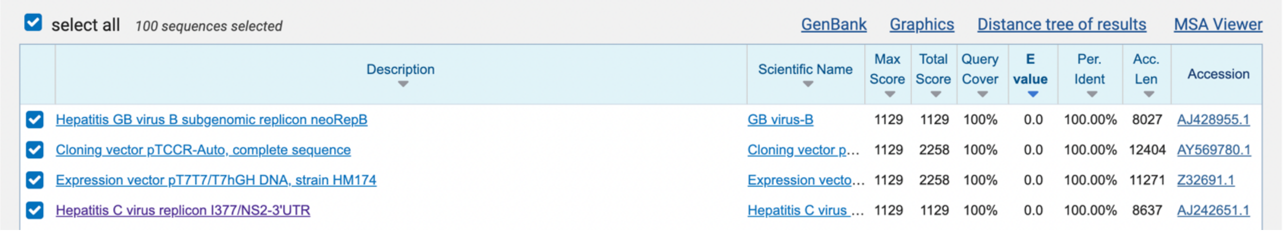


Hepatitis C virus replicon I377/NS2-3'UTR

GenBank: AJ242651.1

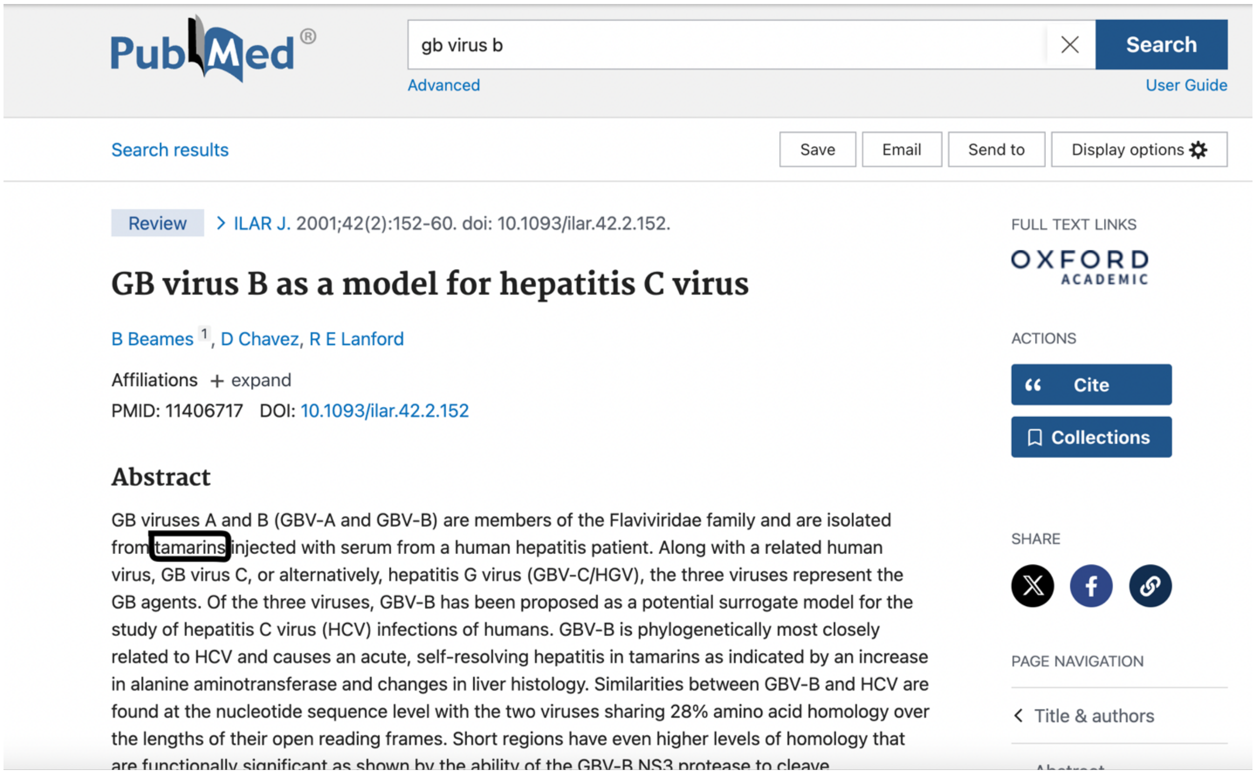
```
>AJ242651.1:1190-1800 Hepatitis C virus replicon I377/NS2-3'UTR
AGACCACAACGGTTTCCCTCTAGCGGGATCAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGG
CCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTT
GGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCG
CCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAAC
AACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAG
CCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGG
AAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTG
TATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAG
GCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACC
```

- virus hepatitis C je patogen za ljudi, zato moramo najti enako zaporedje v virusih, ki niso patogeni za ljudi
- uporaba BLASTn, za iskanje podobnih zaporedij (oziroma enakih) v drugih organizmih, uporabimo najbolj podobnega, torej virus hepatitis GB virus B



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Hepatitis GB virus B subgenomic replicon neoRepB	GB virus-B	1129	1129	100%	0.0	100.00%	8027	AJ428955.1
☑	Cloning vector pTCCR-Auto, complete sequence	Cloning vector p...	1129	2258	100%	0.0	100.00%	12404	AY569780.1
☑	Expression vector pT7T7/T7hGH DNA, strain HM174	Expression vecto...	1129	2258	100%	0.0	100.00%	11271	Z32691.1
☑	Hepatitis C virus replicon I377/NS2-3'UTR	Hepatitis C virus ...	1129	1129	100%	0.0	100.00%	8637	AJ242651.1

- preverimo patogenost Hepatitisa GB virus B v ljudeh in poiščemo primerni organizm (celice), ki bi jih z virusom okužili (PubMed)



Review > ILAR J. 2001;42(2):152-60. doi: 10.1093/ilar.42.2.152.

GB virus B as a model for hepatitis C virus

B Beames [1], D Chavez, R E Lanford

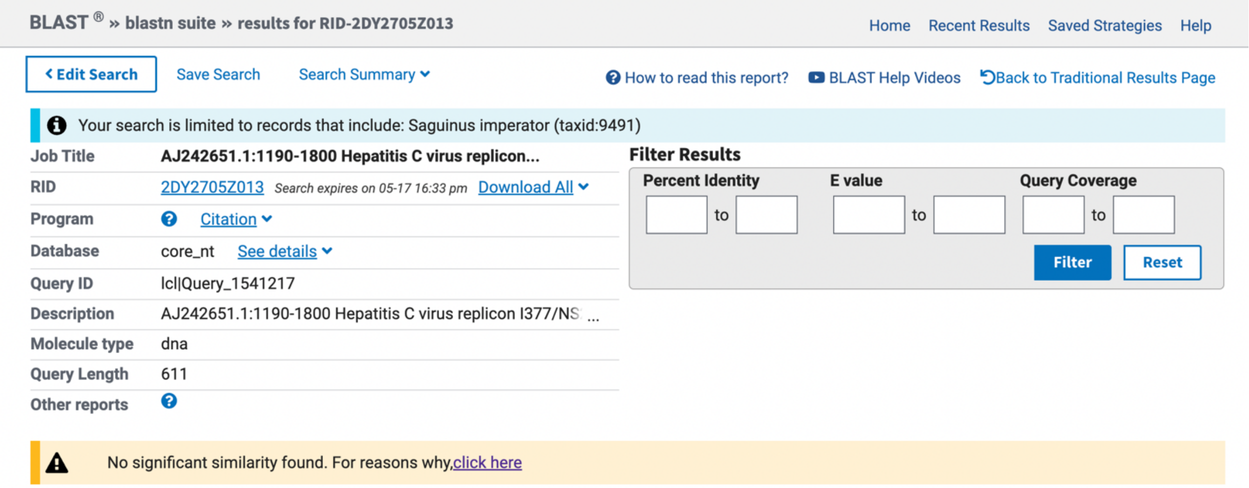
Affiliations + expand

PMID: 11406717 DOI: 10.1093/ilar.42.2.152

Abstract

GB viruses A and B (GBV-A and GBV-B) are members of the Flaviviridae family and are isolated from tamarins injected with serum from a human hepatitis patient. Along with a related human virus, GB virus C, or alternatively, hepatitis G virus (GBV-C/HGV), the three viruses represent the GB agents. Of the three viruses, GBV-B has been proposed as a potential surrogate model for the study of hepatitis C virus (HCV) infections of humans. GBV-B is phylogenetically most closely related to HCV and causes an acute, self-resolving hepatitis in tamarins as indicated by an increase in alanine aminotransferase and changes in liver histology. Similarities between GBV-B and HCV are found at the nucleotide sequence level with the two viruses sharing 28% amino acid homology over the lengths of their open reading frames. Short regions have even higher levels of homology that are functionally significant as shown by the ability of the GBV-B NS3 protease to cleave

- preverimo, če je enako zaporedje tudi v opicah tamarinkah, da ne bi motilo našega PCR

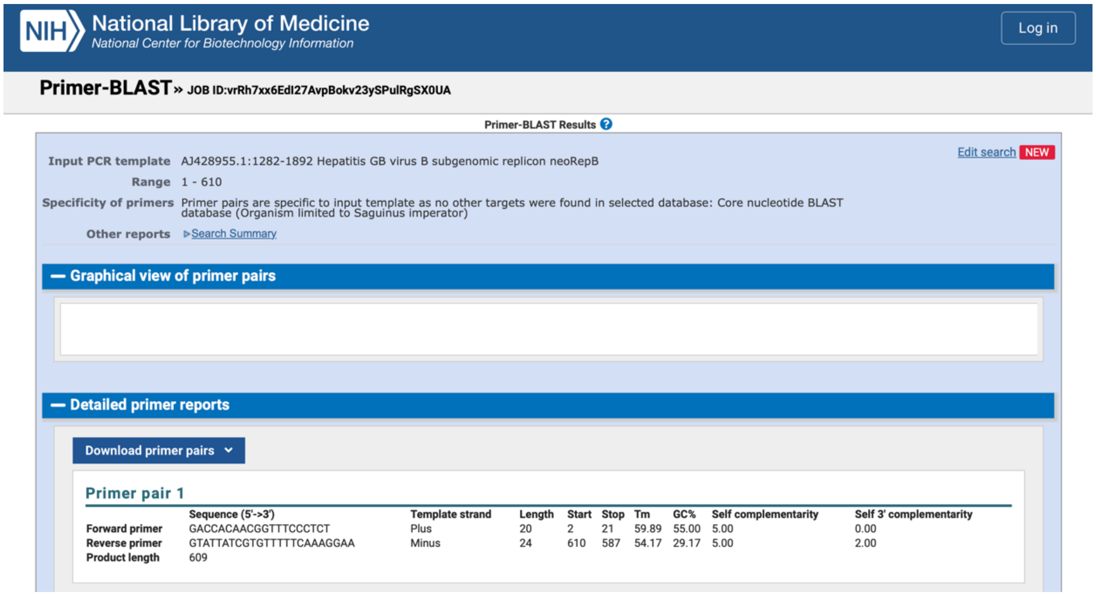


Your search is limited to records that include: Saguinus imperator (taxid:9491)

Job Title	AJ242651.1:1190-1800 Hepatitis C virus replicon...
RID	2DY2705Z013 Search expires on 05-17 16:33 pm Download All
Program	Citation
Database	core_nt See details
Query ID	lcl|Query_1541217
Description	AJ242651.1:1190-1800 Hepatitis C virus replicon I377/NS ...
Molecule type	dna
Query Length	611
Other reports	

No significant similarity found. For reasons why, click here

- vidimo, da je te opice ne vsebujejo nobenega enakega oziroma zelo podobnega zaporedje
- ugotovimo, katere začetne oligonukleotide bi potrebovali za izvedbo PCR (Primer-BLAST)



Primer-BLAST » **JOB ID:vrRh7xx6EdI27AvpBokv23ySPuIRgSX0UA**

Primer-BLAST Results

Input PCR template AJ428955.1:1282-1892 Hepatitis GB virus B subgenomic replicon neoRepB
Range 1 - 610
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Core nucleotide BLAST database (Organism limited to Saguinus imperator)
Other reports Search Summary

— Graphical view of primer pairs

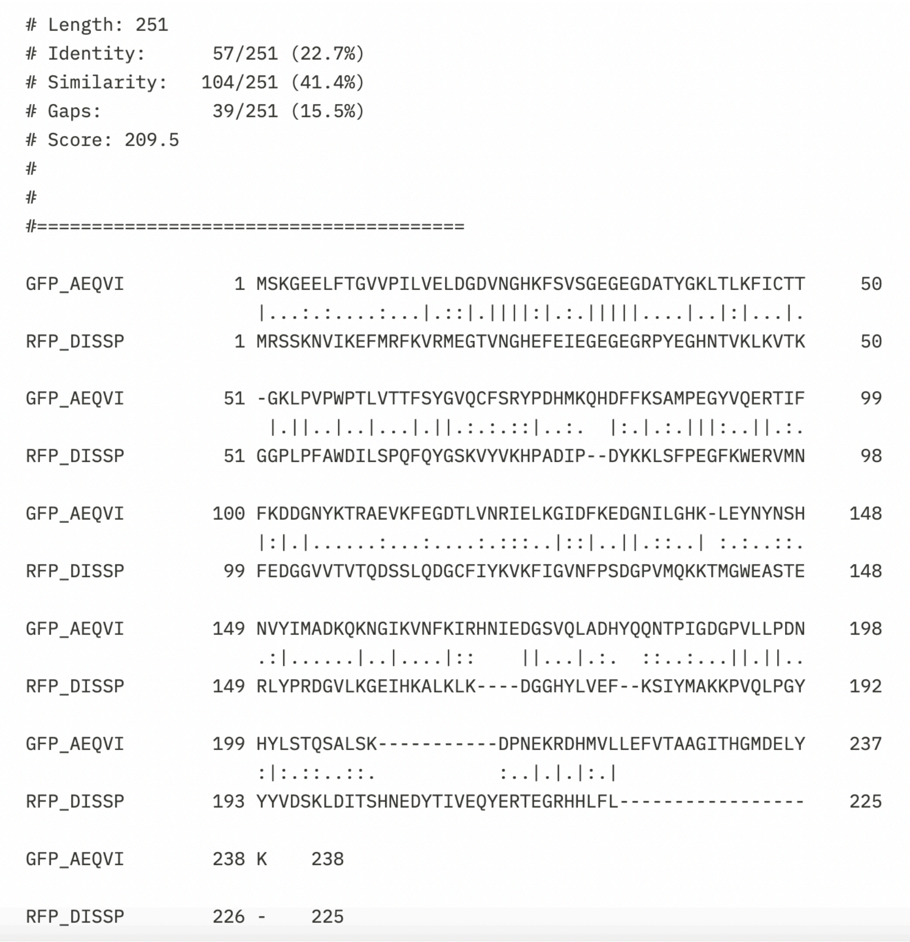
— Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GACCACAACGGTTTCCCTCT	Plus	20	2	21	59.89	55.00	5.00	0.00
Reverse primer	GTATTATCGTGTTTTTCAAAGGAA	Minus	24	610	587	54.17	29.17	5.00	2.00
Product length	609								

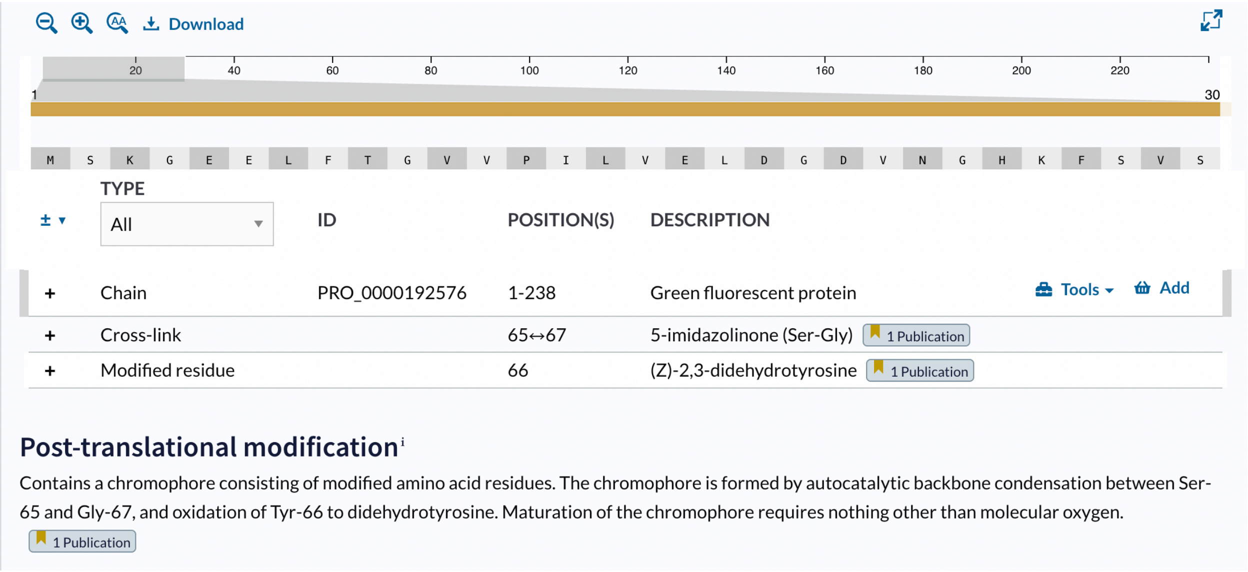
2.) priprava mRNA konstrukta

- ugotovimo, da bi za zaznavenje izražanja genov zaradi vezave ribosoma na m7G kapo in zaradi vezave na interno ribosomsko vstopno mesto lahko uporabili fluorescenčne proteine (npr. zeleni fluorescenčni protein in rdeči fluorescenčni protein)
- preverimo, če so fluorescenčni proteini homologi
- poiščemo zaporedji dveh proteinov (npr. GFP in RFP) na UniProt



```
# Length: 251
# Identity:      57/251 (22.7%)
# Similarity:   104/251 (41.4%)
# Gaps:          39/251 (15.5%)
# Score: 209.5
#
#
#=======================================

GFP_AEQVI          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTT     50
                     |...:.:....:...|.::|.||||:|.:.|||||....|..|:|...|.
RFP_DISSP          1 MRSSKNVIKEFMRFKVRMEGTVNGHEFEIEGEGEGRPYEGHNTVKLKVTK     50

GFP_AEQVI         51 -GKLPVPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF     99
                      |.||..|..|...|.||.:.:.::|..:.  |:.|.:.|||:..||.:.
RFP_DISSP         51 GGPLPFAWDILSPQFQYGSKVYVKHPADIP--DYKKLSFPEGFKWERVMN     98

GFP_AEQVI        100 FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK-LEYNYNSH    148
                     |:|.|......:...:....:.:::..|::|..||.::..| :.:..::.
RFP_DISSP         99 FEDGGVVTVTQDSSLQDGCFIYKVKFIGVNFPSDGPVMQKKTMGWEASTE    148

GFP_AEQVI        149 NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDN    198
                     .:|......|..|....|::    ||...|.:.  ::..:...||.||..
RFP_DISSP        149 RLYPRDGVLKGEIHKALKLK----DGGHYLVEF--KSIYMAKKPVQLPGY    192

GFP_AEQVI        199 HYLSTQSALSK-----------DPNEKRDHMVLLEFVTAAGITHGMDELY    237
                     :|:.::..::.           :..|.|.|:.|
RFP_DISSP        193 YYVDSKLDITSHNEDYTIVEQYERTEGRHHLFL-----------------    225

GFP_AEQVI        238 K    238

RFP_DISSP        226 -    225
```

- z globalno poravnavo ugotovimo, da sta si proteina homologna in tako primerna za vključitev v naš konstrukt
- zanima nas, v katerih celicah lahko opravljamo transkripcijo in translacijo tega konstrukta, torej nas zanima, če imata naša proteina dodatne posttranslacijske modifikacije, kar bi onemogočalo izražanje v bakterijskih celicah
- to preverimo v UniProt

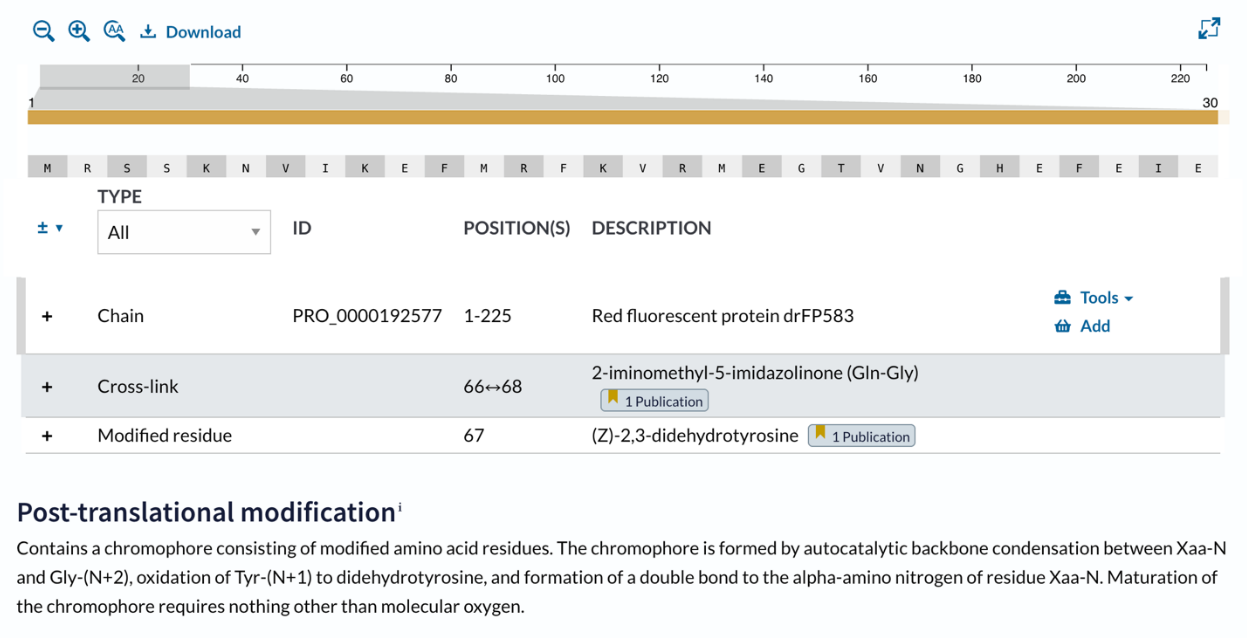


TYPE All	ID	POSITION(S)	DESCRIPTION
Chain	PRO_0000192576	1-238	Green fluorescent protein
Cross-link		65↔67	5-imidazolinone (Ser-Gly) 1 Publication
Modified residue		66	(Z)-2,3-didehydrotyrosine 1 Publication

Post-translational modification

Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Ser-65 and Gly-67, and oxidation of Tyr-66 to didehydrotyrosine. Maturation of the chromophore requires nothing other than molecular oxygen.

1 Publication



TYPE All	ID	POSITION(S)	DESCRIPTION
Chain	PRO_0000192577	1-225	Red fluorescent protein drFP583
Cross-link		66↔68	2-iminomethyl-5-imidazolinone (Gln-Gly) 1 Publication
Modified residue		67	(Z)-2,3-didehydrotyrosine 1 Publication

Post-translational modification

Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Xaa-N and Gly-(N+2), oxidation of Tyr-(N+1) to didehydrotyrosine, and formation of a double bond to the alpha-amino nitrogen of residue Xaa-N. Maturation of the chromophore requires nothing other than molecular oxygen.

- ker vidimo, da proteina nimata posebnih posttranslacijskih modifikacij, ju lahko izražamo tudi v bakterijskih celicah