



Общая характеристика векторов, используемых в генетических технологиях. Классификация. Основные свойства.

Спецпрактикум по биохимической генетике - Лекция 3
Старший преподаватель: PhD, Сметенов Изат Темиргалиевич
Кафедра молекулярной биологии и генетики



ЦЕЛЬ ЛЕКЦИИ

Изучить особенности и классификацию векторов, используемых для переноса генетического материала в клетку, а также их основные свойства. Изучить компьютерные программы для анализа нуклеотидных и аминокислотных последовательностей с целью повышения эффективности молекулярно-биологических исследований.

Задачи

- ✓ Дать определение и общую характеристику векторов.
- ✓ Рассмотреть классификацию векторов по типу и функции.
- ✓ Изучить ключевые свойства, необходимые для эффективного переноса и экспрессии генов.
- ✓ Понять применение векторов в генетических технологиях.
- ✓ Изучить программы по работе с векторами.

Ключевые термины

Вектор, плаزمид, бактериофаг, вирусный вектор, интеграция, репликация, селекционный маркер, ген интереса, клонирование, экспрессия, трансформация, трансфекция, перенос ДНК, SnapGene, аминокислотные последовательности, визуализация последовательностей

1) Общая характеристика векторов

- ✓ Векторы — это **ДНК-молекулы, используемые для переноса и экспрессии генетического материала в клетках-хозяевах.**
- ✓ Основное назначение: **клонирование, экспрессия и анализ генов.**

2) Классификация векторов

- ✓ **Плазмиды:** небольшие кольцевые ДНК, автономно реплицирующиеся в бактериях.
- ✓ **Вирусные векторы:** бактериофаги, ретровирусы, аденовирусы; могут обеспечивать интеграцию или временную экспрессию генов.
- ✓ **Космиды, бактериальные и еукариотические искусственные хромосомы (BAC, YAC):** для клонирования крупных фрагментов ДНК.
- ✓ **Эукариотические экспрессионные векторы:** обеспечивают транскрипцию и трансляцию генов в клетках животных или растений.

3) Основные свойства векторов

- ✓ **Селективность и наличие маркеров:** антибиотикоустойчивость, метаболические гены.
- ✓ **Репликация в клетке-хозяине:** автономная или интегративная.
- ✓ **Наличие сайтов рестрикции** для внедрения генов.
- ✓ **Стабильность и совместимость с хост-клеткой.**
- ✓ **Возможность экспрессии гена в нужной системе:** бактерии, дрожжи, растения или животные клетки.



ОСНОВНЫЕ ВОПРОСЫ

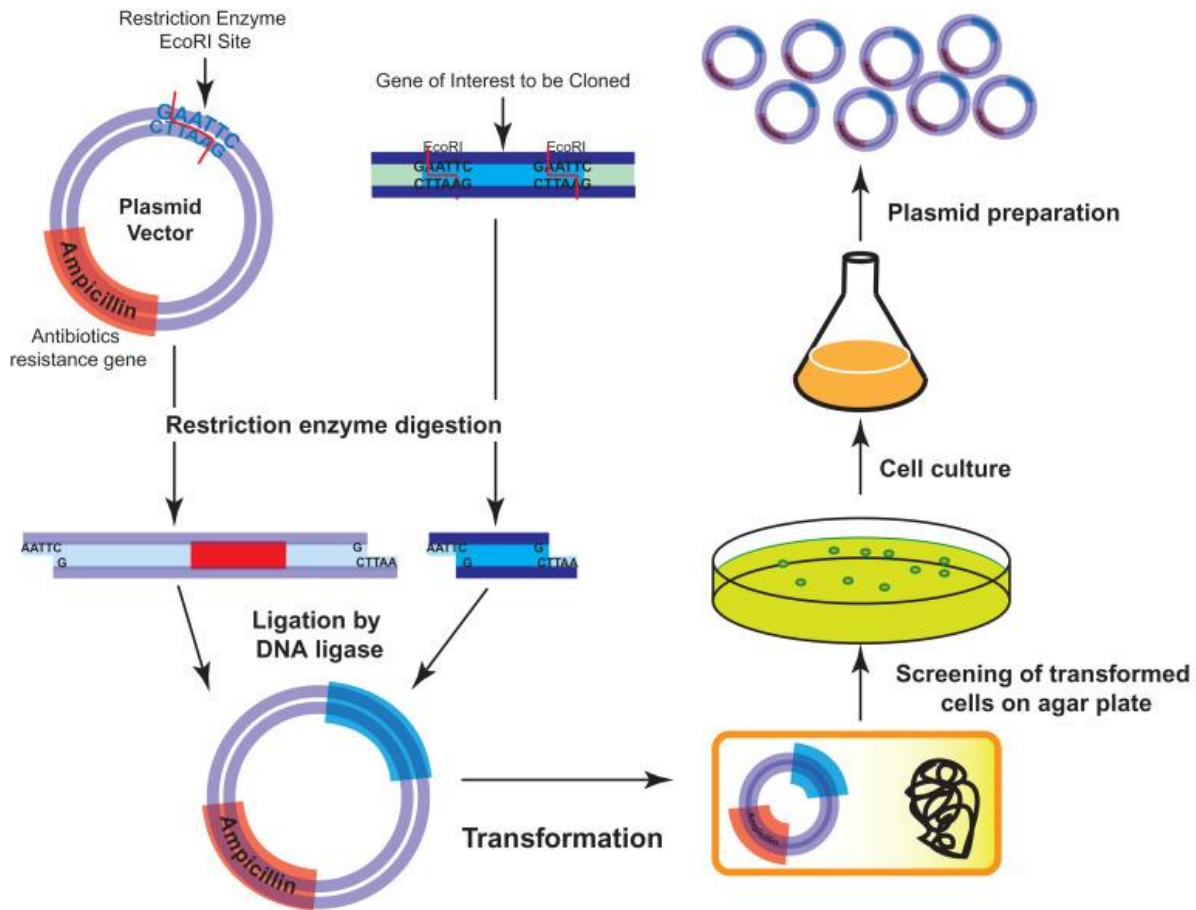
- 1) Что такое вектор и какую роль он играет в генетических технологиях?
- 2) Как классифицируются векторы по типу и назначению?
- 3) Какие основные свойства делают вектор эффективным для переноса и экспрессии генов?
- 4) Чем отличаются плазмидные векторы от вирусных?
- 5) Какие векторы используют для клонирования крупных фрагментов ДНК?
- 6) Почему важны селекционные маркеры и сайты рестрикции?
- 7) Как выбирается подходящий вектор для бактерий, дрожжей или клеток животных?
- 8) Как работать в SnapGene и для чего он нужен?

Классификация векторов

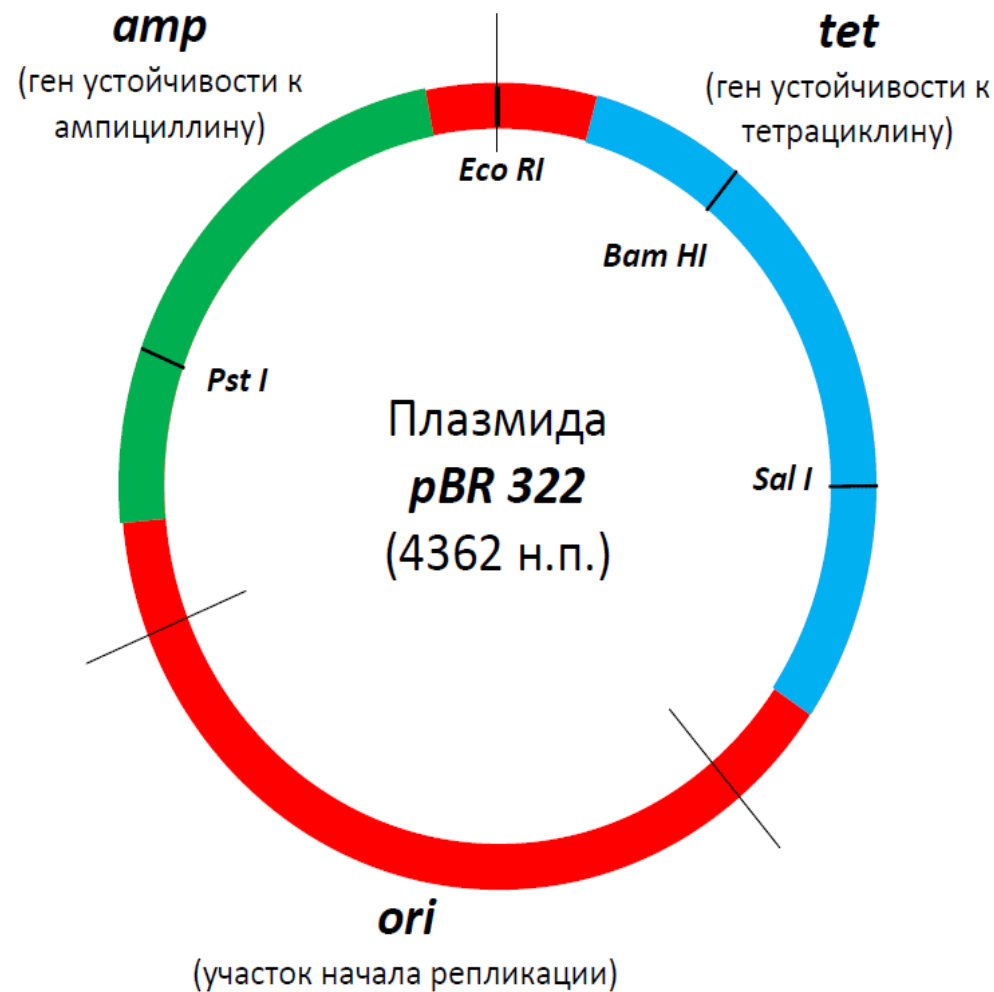
Векторы могут быть разделены на различные группы в зависимости от цели процесса и типа частиц, используемых в процессе. Ниже приведены наиболее часто изучаемые группы векторов, которые используются для различных целей.

1. Клонировующие векторы (плазмиды, космиды, бактериофаг векторы, ВАС, YAC, HAC)
2. Вирулентные векторы
3. Вектор экспрессии
4. Вектор шатлы
5. Векторы секреции

Плазмиды



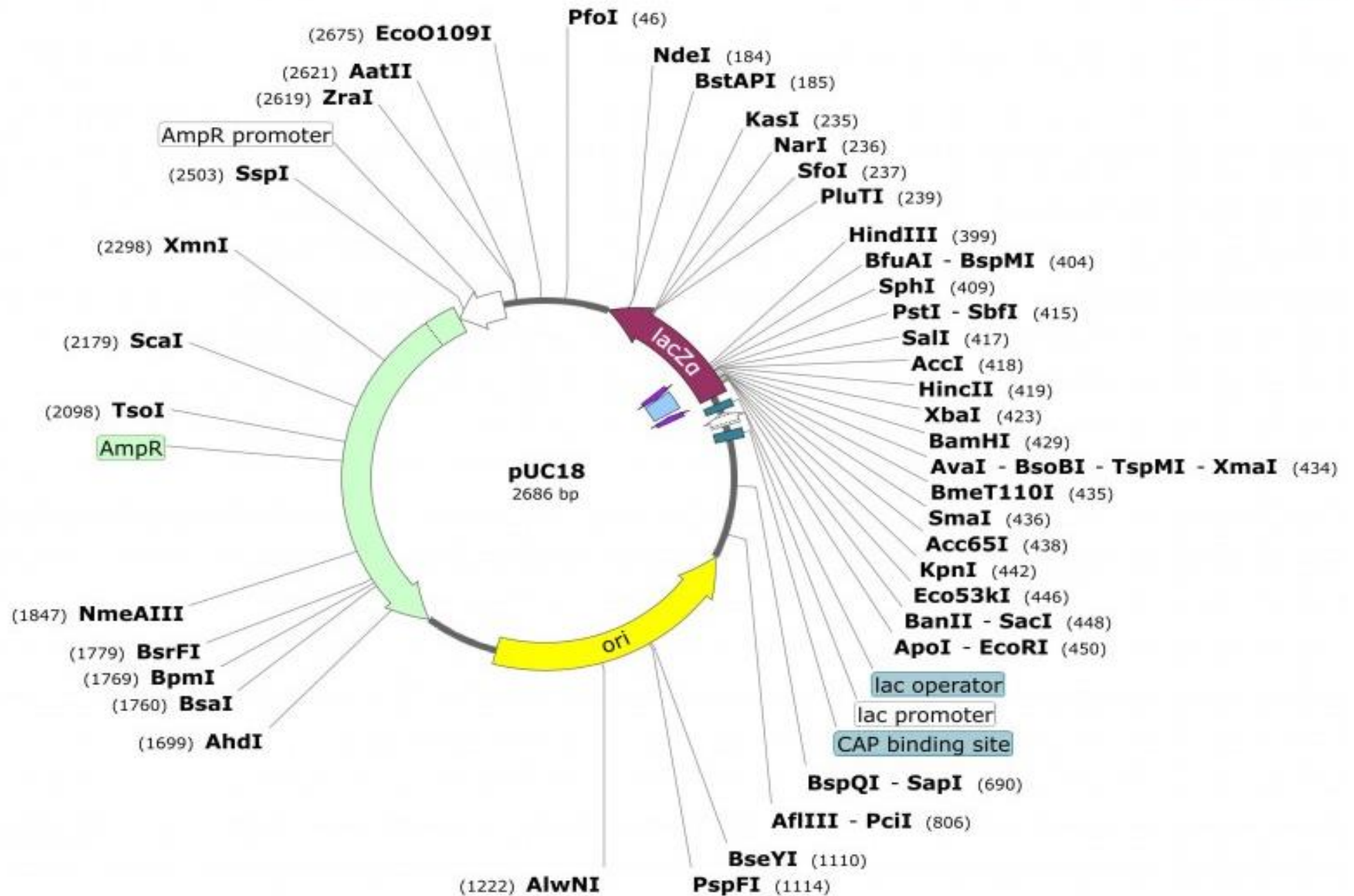
Плазмиды, факторы наследственности, расположенные в клетках вне хромосом. К П. относят генетические факторы клеточных органелл (митохондрий, пластид и др.) и генетические факторы, не являющиеся обязательными компонентами клеток. Из последних более изучены так называемый каппа-фактор у парамеций, продуцирующего антибиотическое вещество парамеции, фактор чувствительности к CO_2 и агент, обуславливающий бессамцовость у дрозофил, а также ряд бактериальных П. У бактерий П. могут контролировать устойчивость к лекарственным веществам, синтез бактерицинов, энтеротоксина, гемолизина и некоторых антигенов. П., называемые половыми факторами, определяют половую дифференциацию у бактерий. Показано, что многие П. состоят из кольцевых молекул двухнитевой ДНК с молекулярной массой 10^{-6} — 10^{-8} дальтон.

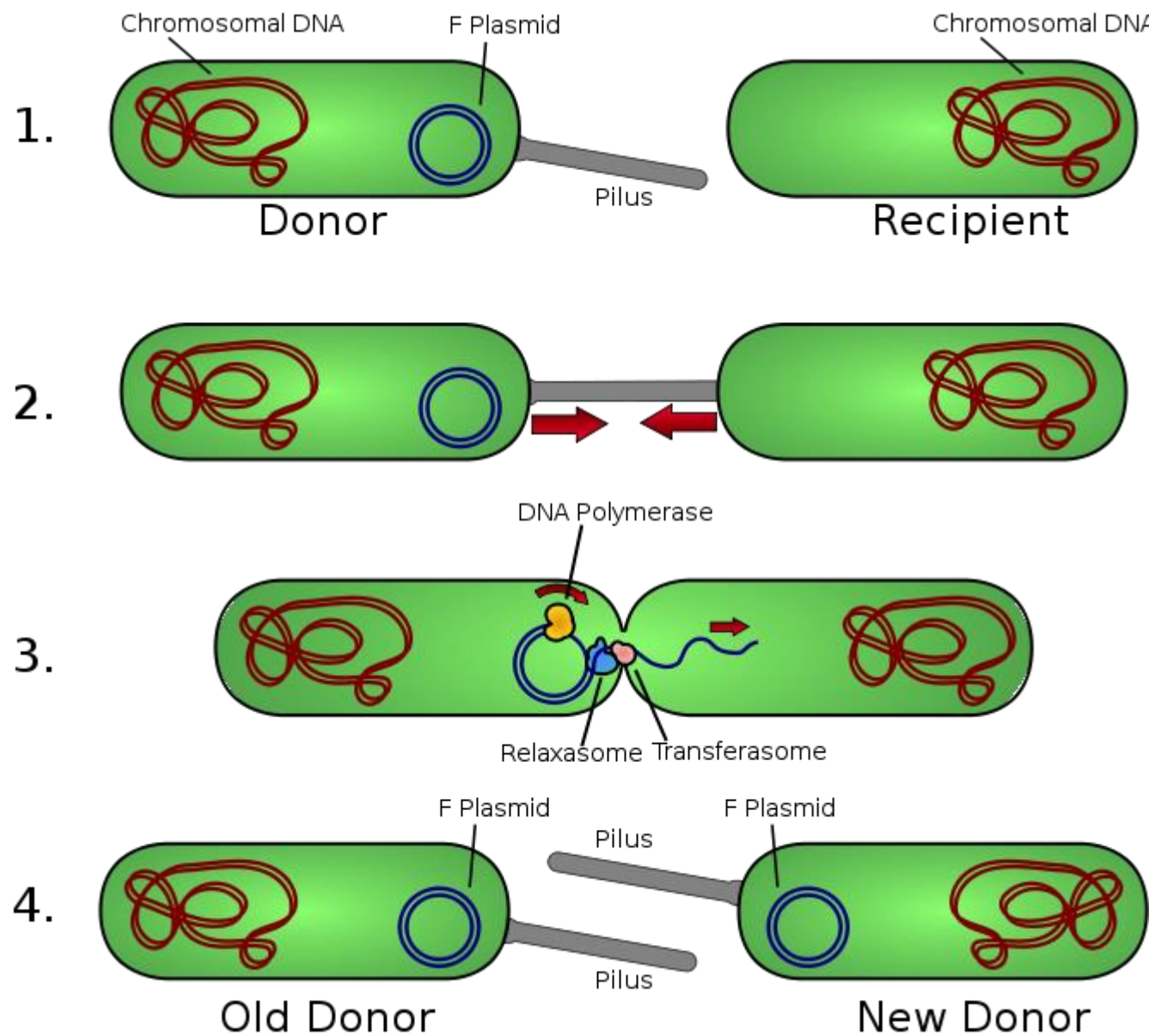


Буква "р" указывает на то, что это действительно плазмида

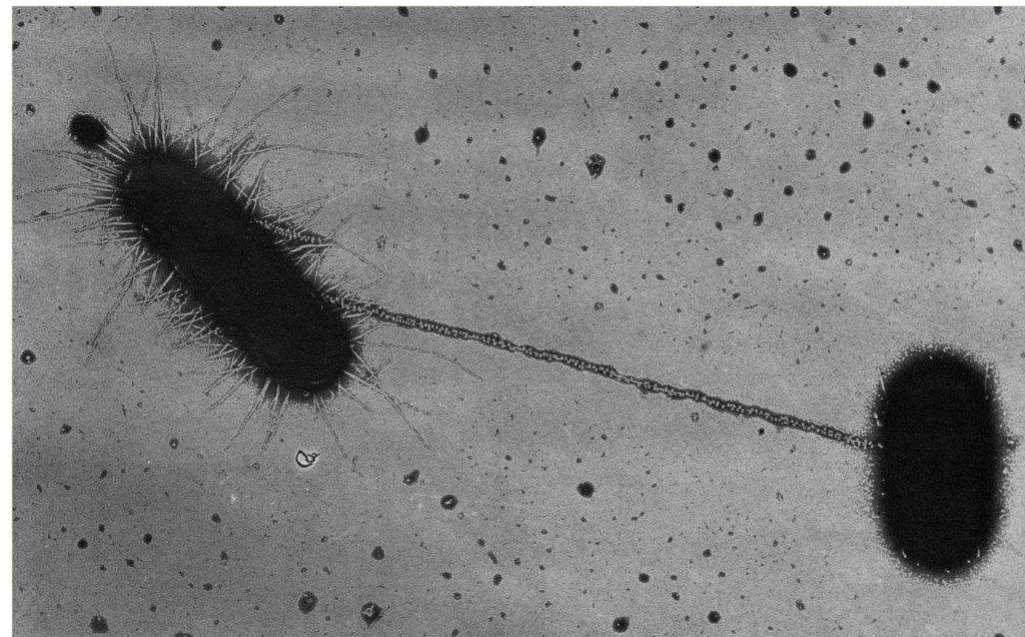
Буква "BR" обозначает лабораторию, в которой первоначально был создан вектор. Буква "BR" обозначает Боливара и Родригеса, двух исследователей, разработавших pBR322.

"322" отличает эту плазмиду от других, разработанных в той же лаборатории.





Конъюгация у бактерий



Виды бактериальных плазмид

Обычно это кольцевая ДНК, в основном независимая от хромосомы хозяина, часто встречающаяся в бактериальных и некоторых других типах клеток. Природные плазмиды обычно реплицируются независимо от бактериальной хромосомы. Плазмиды способны содержать ген размером до 10 кбайт. В бактериях обнаружено множество различных типов плазмид. Наиболее полезная классификация природных плазмид основана на основных характеристиках, кодируемых генами плазмид. Согласно этой классификации, пять основных типов плазмид представлены ниже:

- а. **F - Плазмиды** фертильности несут только гены *tra* и не обладают никакими характеристиками, кроме способности способствовать конъюгативному переносу плазмид. например, плазида F из *E.coli*
- б. **R-плазмиды.** Плазмиды устойчивости содержат гены, придающие бактерии-хозяину устойчивость к одному или нескольким антибактериальным препаратам, таким как хлорамфеникол, ампициллин и т.д., Этот тип плазмид в основном используется в технологии рекомбинантной ДНК.
- в. **Col Плазмиды.** Эти плазмиды кодируют колицины (белки), которые убивают рост других бактерий, например, ColE1 из *E.coli*.
- г. **плазмиды, способные к разложению.** Эти плазмиды позволяют бактерии-хозяину усваивать необычные молекулы, такие как толуол и салициловая кислота.
- д. **Плазмиды вирулентности.** Они придают бактериям-хозяевам патогенность, например, плазмиды Ti *Agrobacterium tumefaciens*, которые вызывают коронованную желтуху у двудольных растений.

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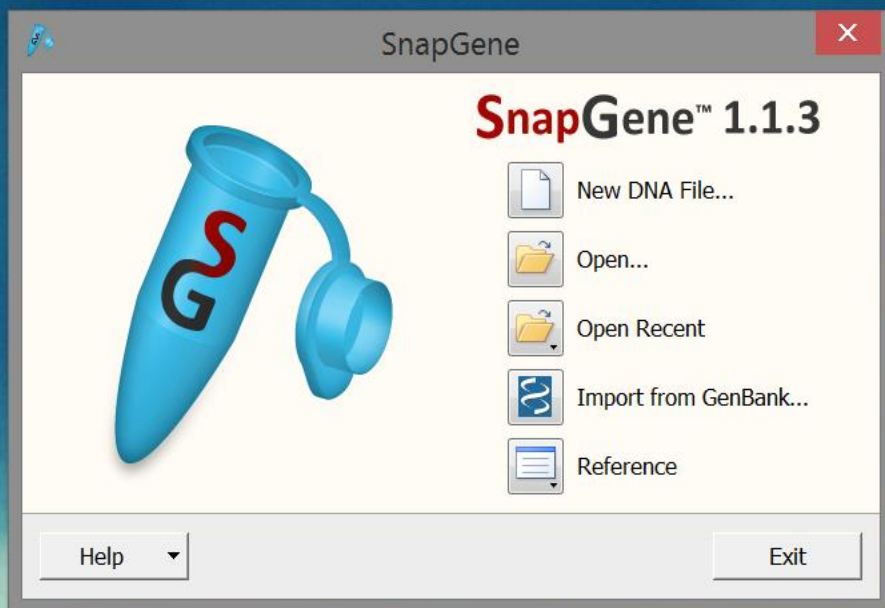
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Expression vectors supplied by centers for structural genomics

TA and GC Cloning Vectors

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295 Sequences

Individual Sequences & Maps

BlueScribe	pEASY-Blunt Simple	pICoZ	pSMART HCAmp
CAG promoter	(linearized)	pInvRecA	pSMART HCAmp
chicken β-actin	pEASY-Blunt Zero	pJAZZ-OC	(linearized)
promoter	(linearized)	pJAZZ-OK	pSMART HCKan
CLIP-tag	pEASY-T1 (linearized)	pJET1.2	pSMART HCKan
CMV promoter	pEASY-T1 Simple	pJET1.2/blunt	(linearized)
GAL1 promoter	(linearized)	pJW168	pSMART LCamp
GST	pEASY-T3 (linearized)	pKF 18k-2	pSMART LCamp
HaloTag	pEASY-T5 Zero	pKF 19k-2	(linearized)
HSV TK promoter	(linearized)	pKOV	pSMART LCKan
lacZ	pEX-1	pLATE11 (linearized)	pSMART LCKan
lac operator	pEX-A	pLATE31 (linearized)	(linearized)
lac promoter	pEX-C-GST	pLATE51 (linearized)	pSP64 Poly(A)
lac UV5 promoter	pEX-C-His	pLATE52 (linearized)	pSP72
lambda	pEX-K	pLEXX-AK	pSP73
LITMUS28	pEX-N-GST	pLPS-B (linearized)	pSpark Done
LITMUS28i	pEX-N-His	pMAL-c5E	(linearized)
LITMUS29	pEX-N-His-GST	pMAL-c5G	pSpark III (linearized)

Plasmid Maps and Sequence xBasic Cloning Vectors x

pET & Duet Vectors (Novage x

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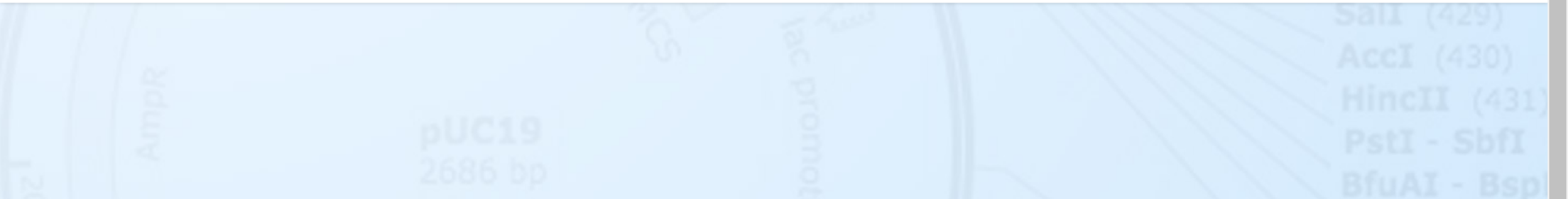


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pcDNA3.1(-)	pFN19A HaloTag T7	pPCR-Script Cam	pTXB1
pCold I	SP6	SK(+)	pTYB21
pCold II	pFN19K HaloTag T7	pPTR I	pTZ19R
pCold III	SP6	pPTR II	pTZ19U
pCold IV	pFN29A His6HaloTag	pRACE (linearized)	pTZ57R
pCold TF	T7	pRANGER-BTB-1	pUAST
pCR-XL-2-TOPO	pFN29K His6HaloTag	pRANGER-BTB-2	pUC18
pCR-XL-2-TOPO	T7	pRANGER-BTB-3	pUC19
(linearized)	pGEM-3Z	pRANGER-BTB-5	pUC19L
pDD	pGEM-3Zf(+)	pRHA-67	pUC57
pDNR-Dual	pGEM-3Zf(-)	pRHA-109	pUC57-Kan
pDNR-LIB	pGEM-4Z	pRHA-113	pUC57-Simple
pE-SUMOpro3 Amp	pGEM-5Zf(+)	pRSET A	pUC118
pE-SUMOpro3 Kan	pGEM-7Zf(+)	pRSET B	pUC119
pE-SUMOpro Amp	pGEM-7Zf(-)	pRSET C	pUCmu
pE-SUMOpro Kan	pGEM-9Zf(-)	pSB1C3	pVLT31
pE-SUMOstar Amp	pGEM-11Zf(+)	pSC101	pVLT33
pE-SUMOstar Kan	pGEM-11Zf(-)	pSEAP2-Basic	pVLT35
pEASY-Blunt3	pGEM-T	pSF-Core	pYES1L
(linearized)	pGEM-T (linearized)	pSMART BAC v2.0	pZErO-2
pEASY-Blunt	pGEM-T Easy	pSMART BAC v2.0	SNAP-tag
(linearized)	pGEM-T Easy	(BamHI	SP6 promoter
pEASY-Blunt E1	(linearized)	linearized)	SV40 promoter
(linearized)	pGGA	pSMART BAC v2.0	T3 promoter
pEASY-Blunt E2	PGK promoter	(EcoRI linearized)	T5 promoter
		pSMART BAC v2.0	T7 promoter

https://www.snapgene.com/resources/plasmid-files/?set=basic_cloning_vectors&plasmid=pUC19

1



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pUC19

Standard E. coli vector with a multiple cloning site (MCS) for DNA cloning. The MCS is reversed in [pUC18](#).

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pUC19.dna

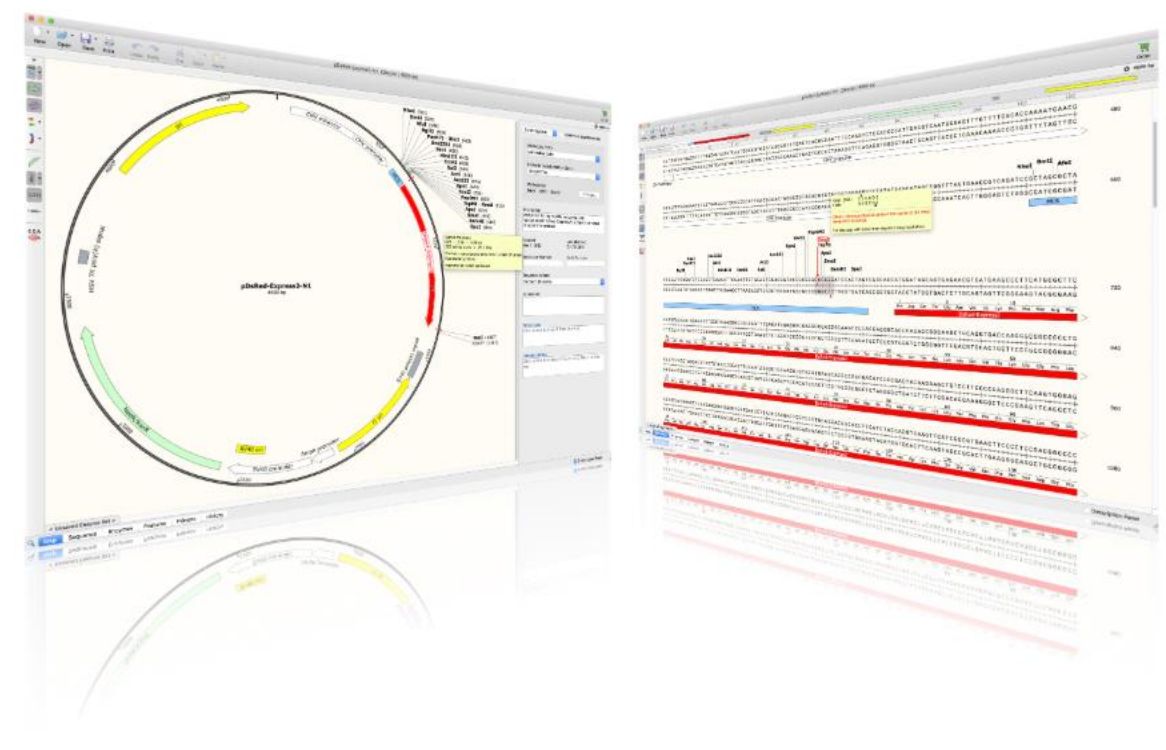
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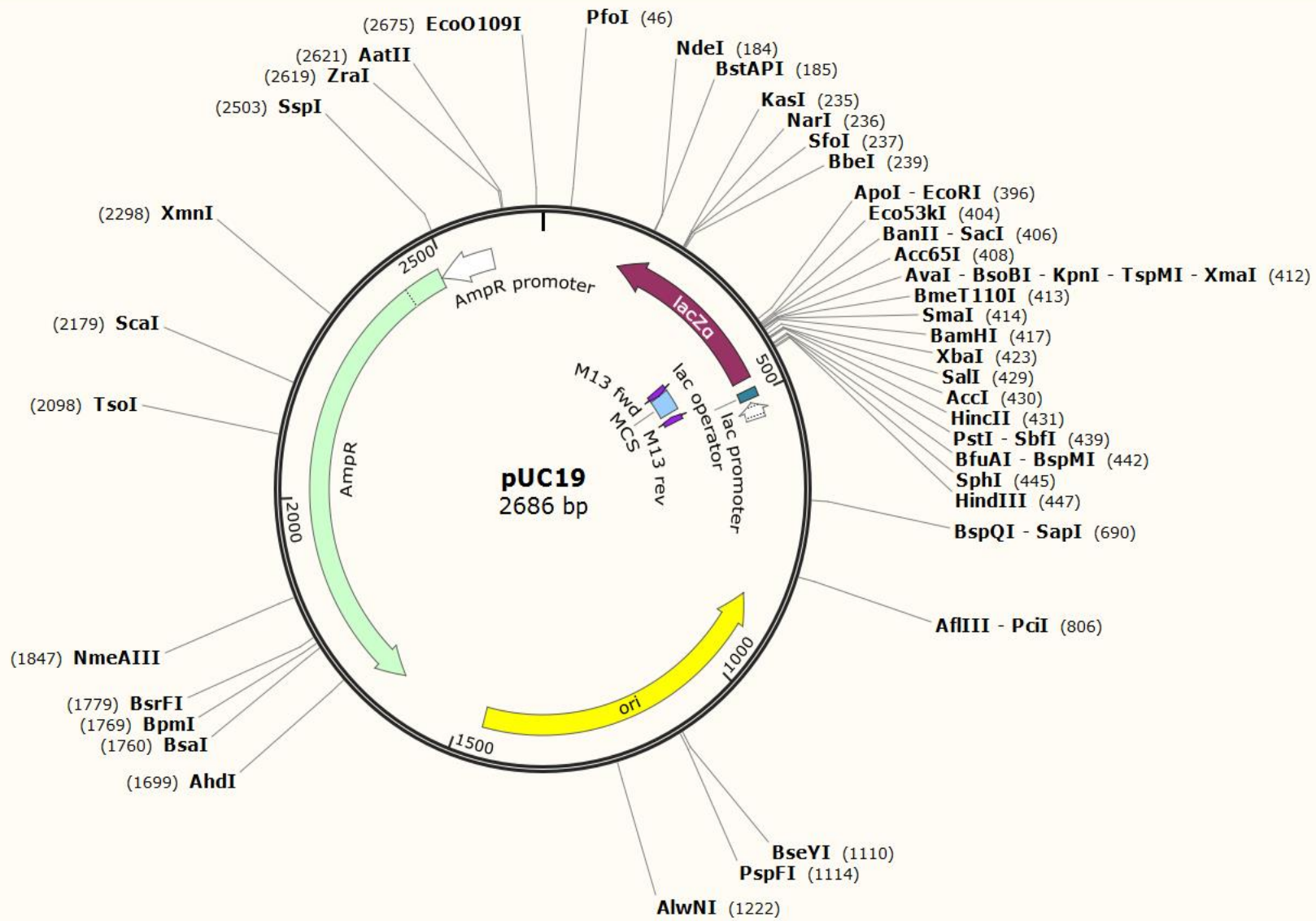
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2686 bp



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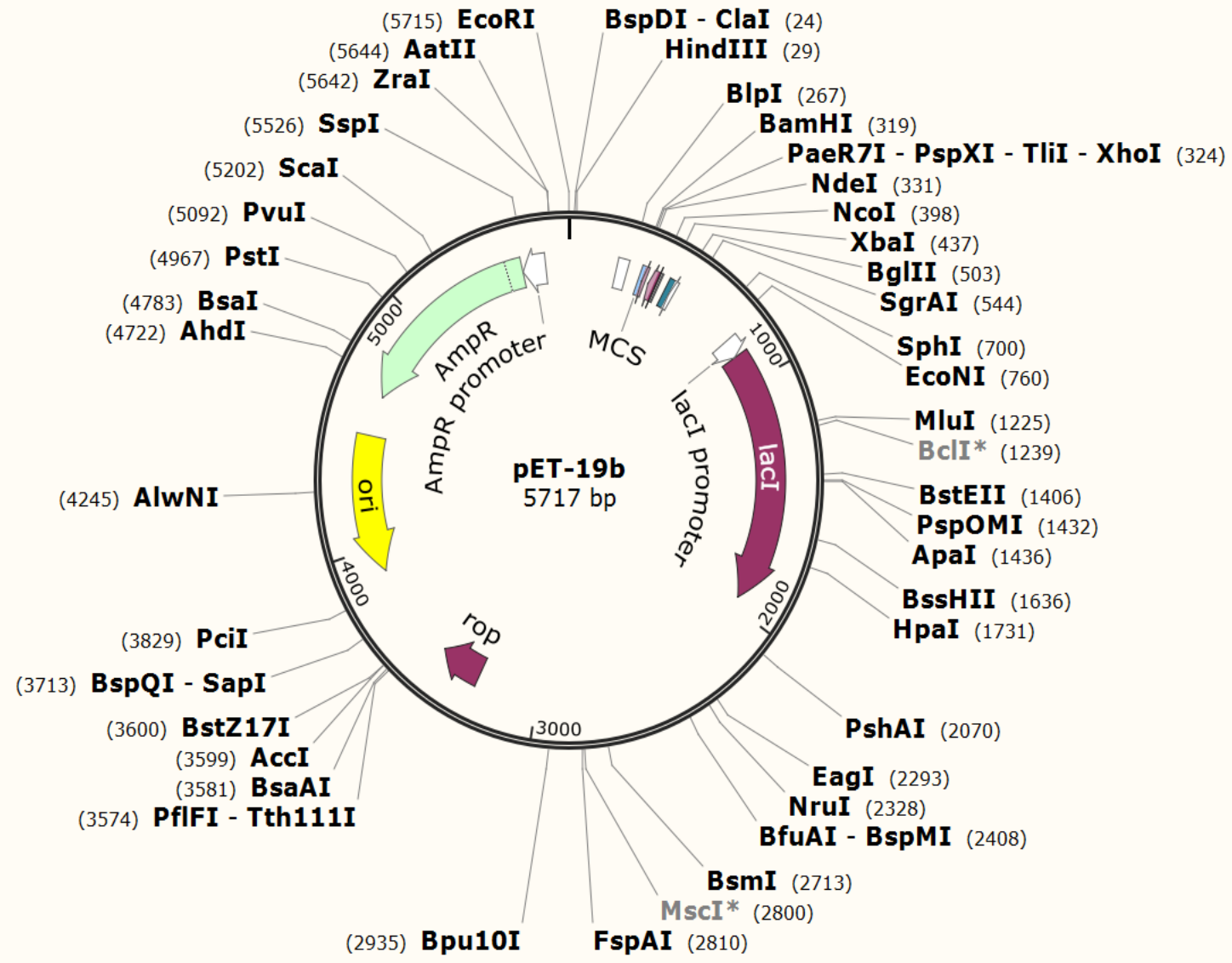
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5717 bp



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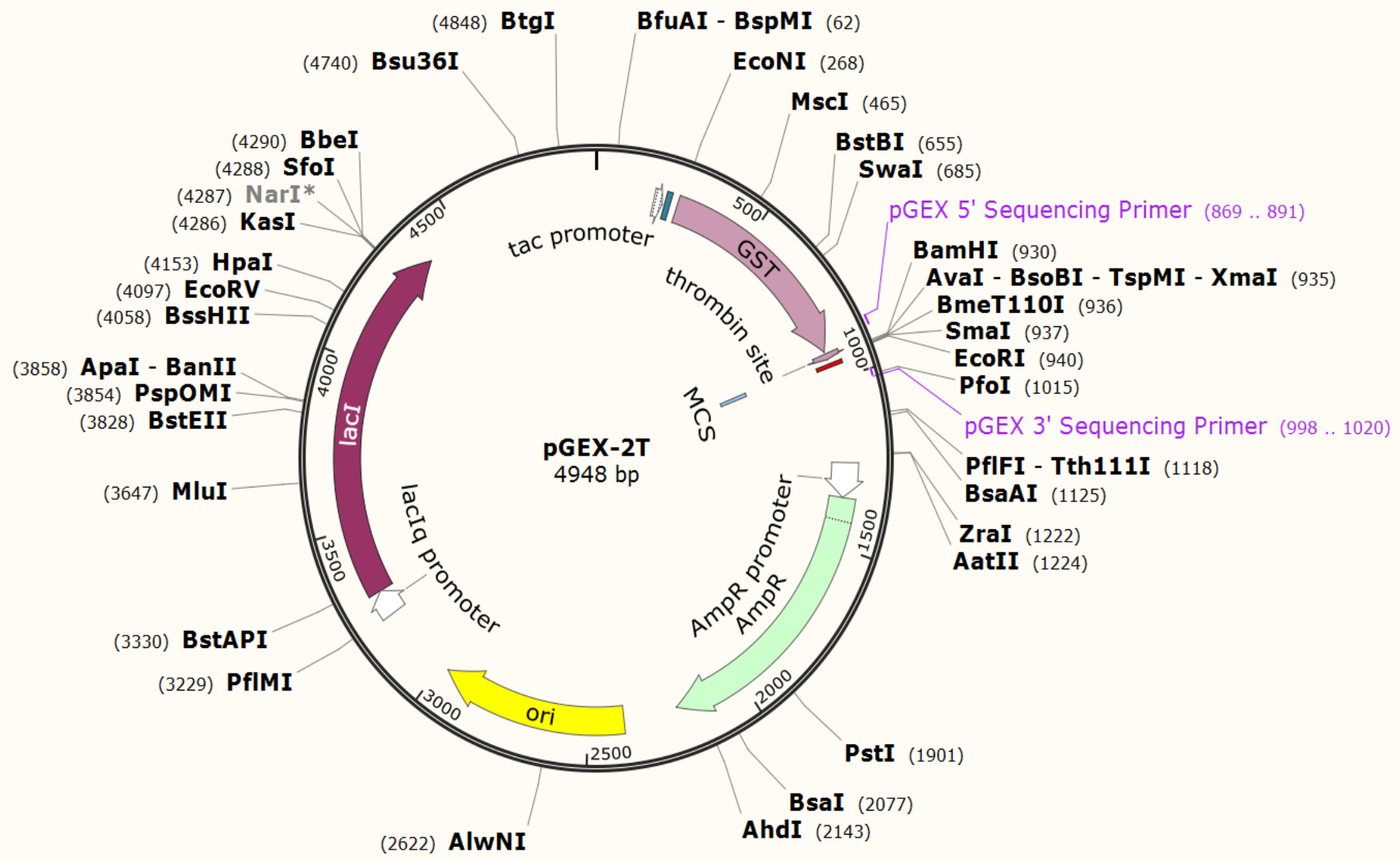
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4948 bp



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Commonly used TA and GC cloning vectors

Luciferase Vectors

Commonly used luciferase plasmids

TOPO Cloning Vectors

Commonly used TOPO sequences including blunt, directional, and TOPO TA cloning vectors

Lucigen Vectors

Cloning, expression, and tagging vectors

Viral Expression & Packaging Vectors

Retroviral, lentiviral, and adenoviral vectors from Clontech, Invitrogen, and others

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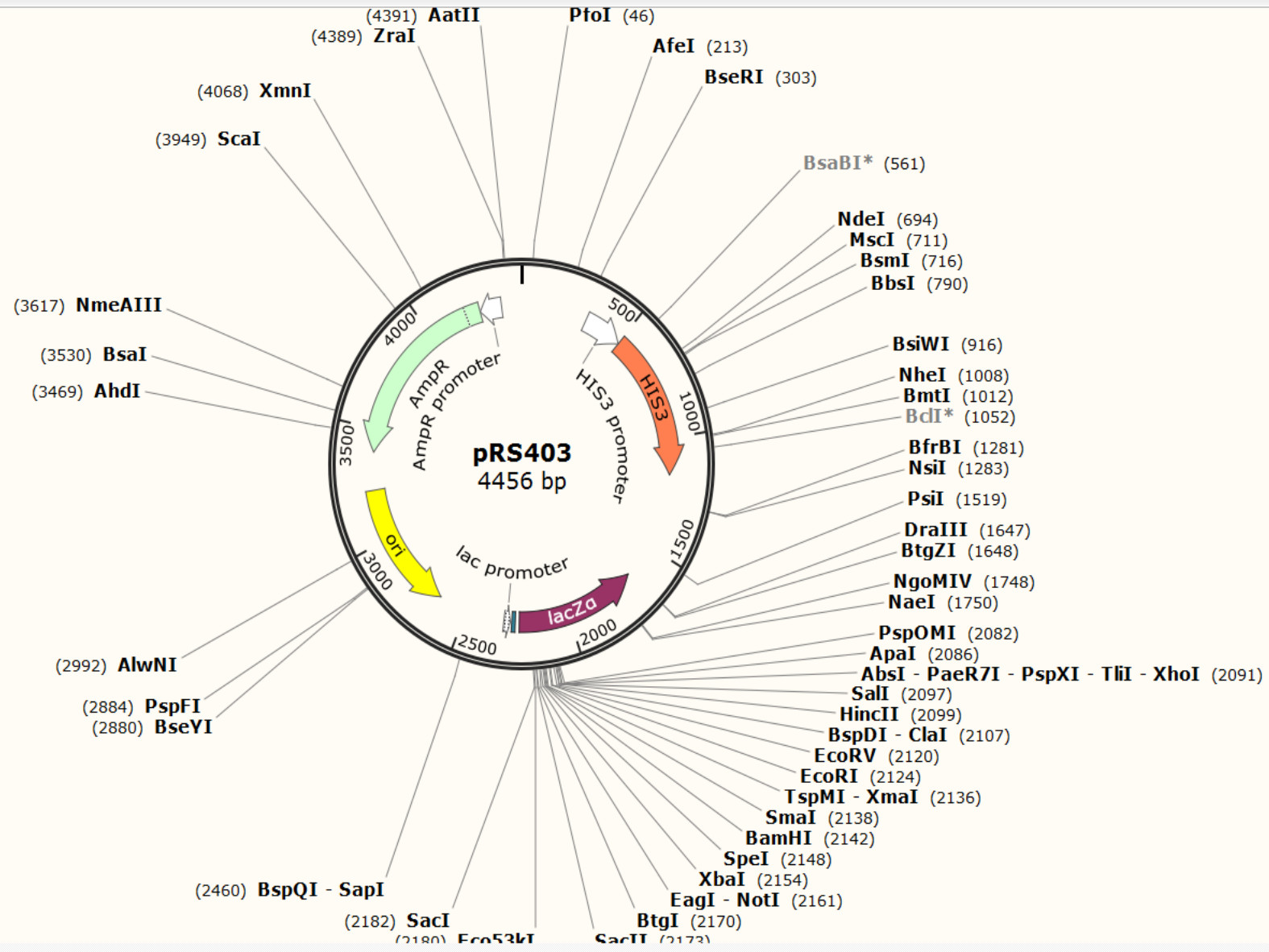
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4456 bp

6 features are not



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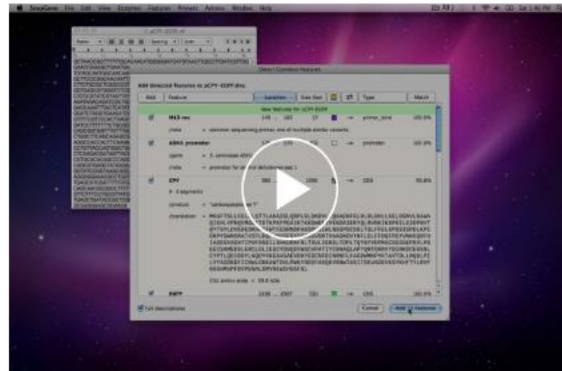
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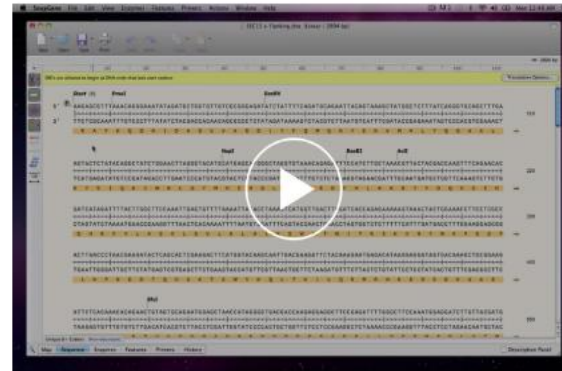
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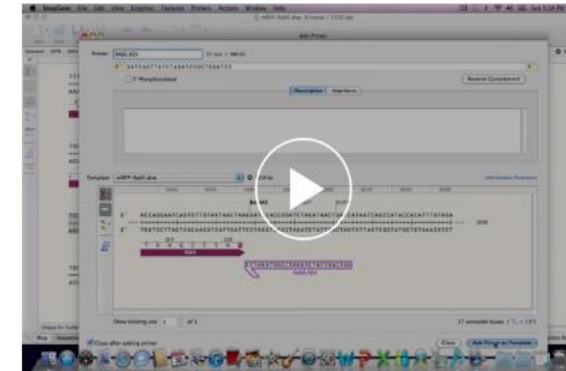
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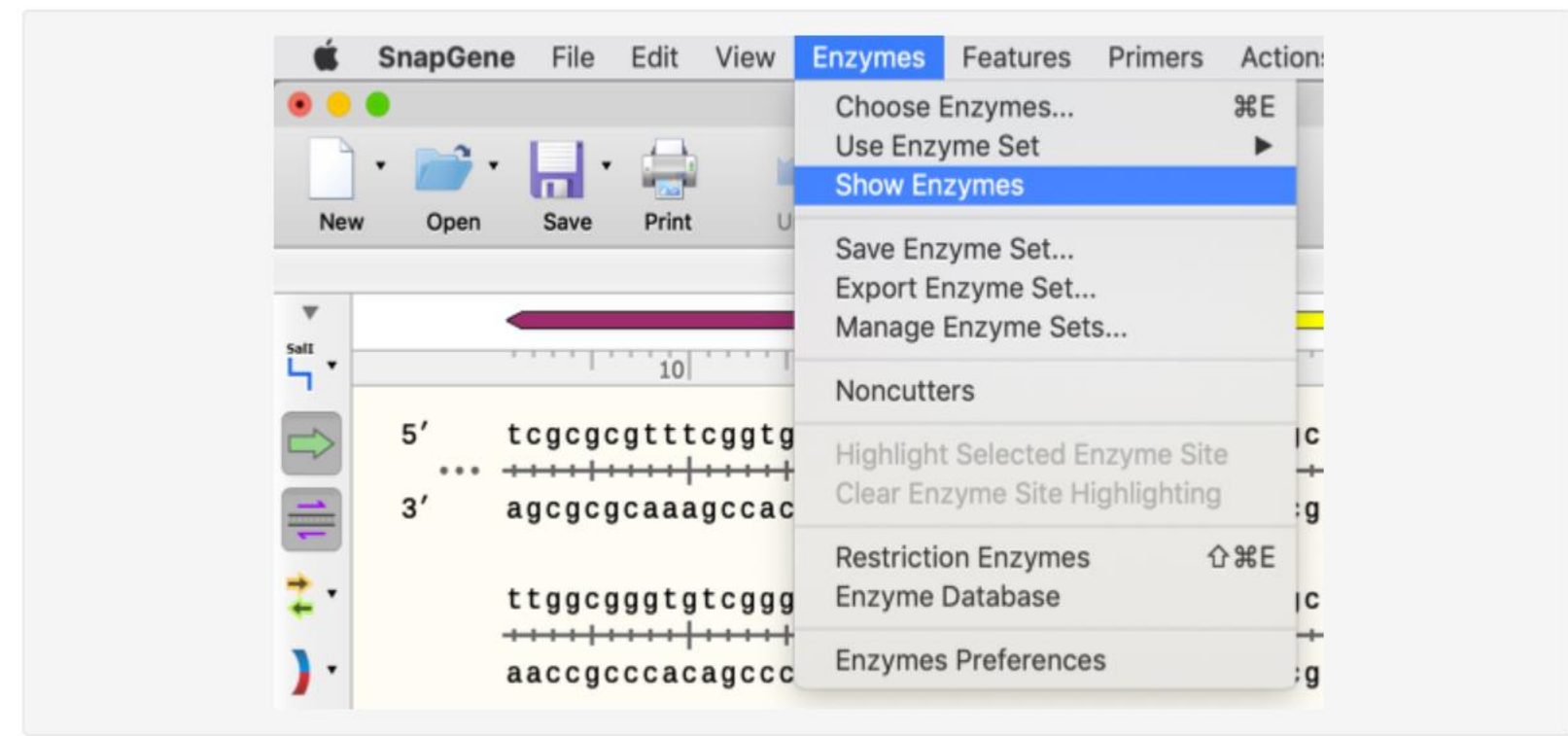
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RESEARCH

RESEARCH ARTICLE

CORONAVIRUS

Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2

Renhong Yan^{1,2}, Yuanyuan Zhang^{1,2*}, Yaning Li^{3*}, Lu Xia^{1,2}, Yingying Guo^{1,2}, Qiang Zhou^{1,2†}

Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for severe acute respiratory syndrome-coronavirus (SARS-CoV) and the new coronavirus (SARS-CoV-2) that is causing the serious coronavirus disease 2019 (COVID-19) epidemic. Here, we present cryo-electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B⁰AT1 with or without the receptor binding domain (RBD) of the surface spike glycoprotein (S protein) of SARS-CoV-2, both at an overall resolution of 2.9 angstroms, with a local resolution of 3.5 angstroms at the ACE2-RBD interface. The ACE2-B⁰AT1 complex is assembled as a dimer of heterodimers, with the collectrin-like domain of ACE2 mediating homodimerization. The RBD is recognized by the extracellular peptidase domain of ACE2 mainly through polar residues. These findings provide important insights into the molecular basis for coronavirus recognition and infection.

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is a positive-

SARS-CoV-2 S protein binds to the PD of ACE2 with a dissociation constant (K_d) of ~15 nM (14).

The membrane trafficking mechanism for ACE2 and B⁰AT1 is similar to that of the LAT1-4F2hc complex, a large neutral-amino acid transporter complex that requires 4F2hc for its plasma membrane localization (31). Our structure of LAT1-4F2hc shows that the cargo LAT1 and chaperone 4F2hc interact through both extra-cellular and transmembrane domains (32). We reasoned that the structure of full-length ACE2 may be revealed in the presence of B⁰AT1.

Here, we report cryo-electron microscopy (cryo-EM) structures of the full-length human ACE2-B⁰AT1 complex at an overall resolution of 2.9 Å and a complex between the RBD of SARS-CoV-2 and the ACE2-B⁰AT1 complex, also with an overall resolution of 2.9 Å and with 3.5-Å local resolution at the ACE2-RBD interface. The ACE2-B⁰AT1 complex exists as a dimer of heterodimers. Structural alignment of the RBD-ACE2-B⁰AT1 ternary complex with the S protein of SARS-CoV-2 suggests that two S protein trimers can simultaneously bind to an ACE2 homodimer.

Structural determination of the

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Ангиотензин-превращающий фермент 2 (ACE2) является клеточным рецептором для тяжелого острого респираторного синдрома - коронавируса (SARS-CoV) и нового коронавируса (SARS-CoV-2), который вызывает серьезную эпидемию коронавирусной болезни 2019 (COVID-19).

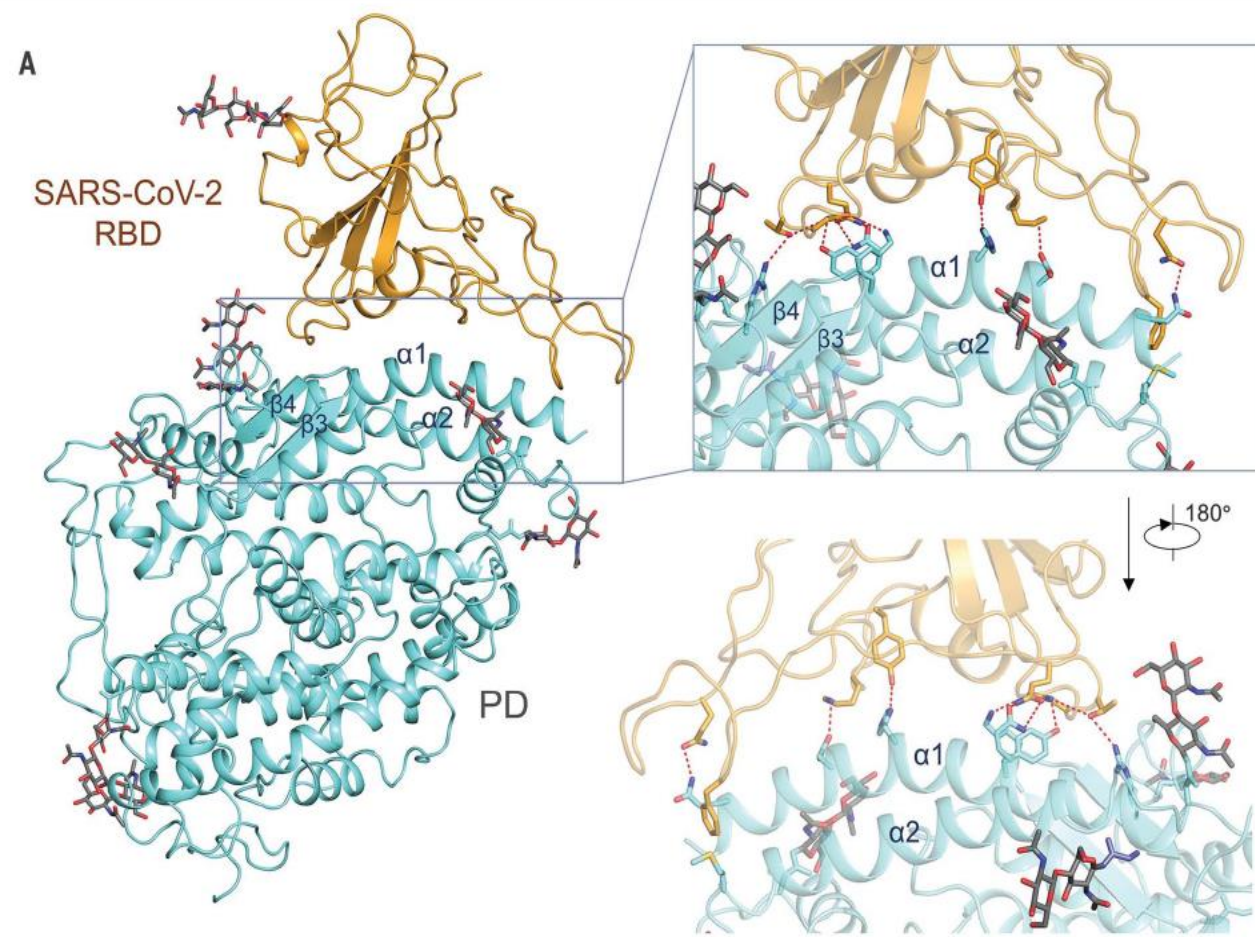


Fig. 4. Interactions between SARS-CoV-2-RBD and ACE2. (A) The PD of ACE2 mainly engages the $\alpha 1$ helix in the recognition of the RBD. The $\alpha 2$ helix and the linker between $\beta 3$ and $\beta 4$ also contribute to the interaction. Only one RBD-ACE2 is shown. (B to D) Detailed analysis of the interface between SARS-CoV-2-RBD and ACE2. Polar interactions are indicated by red dashed lines. NAG, *N*-acetylglucosamine.

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Materials and Methods

Protein preparation

The cDNAs for full-length human B⁰AT1 (accession number: NM_001003841) and ACE2 (accession number: NM_001371415) were subcloned into pCAG respectively. An N-terminal FLAG tag was fused to B⁰AT1, and one Strep tag was fused after the N-terminal signal peptide of ACE2 using a standard two-step PCR.

HEK 293F cells (Invitrogen) were cultured in SMM 293T-II medium (Sino Biological Inc.) at 37 °C under 5% CO₂ in a Multitron-Pro shaker (Infors, 130 rpm). To co-express B⁰AT1 and ACE2, the cells were transiently transfected with the plasmids and polyethylenimines (PEIs) (Polysciences) when the cell density reached approximately 2.0×10^6 /ml. For transfection one liter of cell culture, about 0.75 mg plasmids for B⁰AT1 and 0.75 mg plasmids for ACE2 were premixed with 3 mg PEIs in 50 ml of fresh medium for 15 mins before adding to cell culture. The transfected cells were cultured for 48-60 hours before harvesting.

For purification of the B⁰AT1 and ACE2 complex, the cells were collected in a buffer containing 25 mM Tris, pH 8.0, 150 mM NaCl, and three protease inhibitors ,

aprotinin (1.3 µg/ml, AMRESCO), pepstatin (0.7 µg/ml, AMRESCO), and leupeptin

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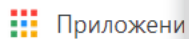
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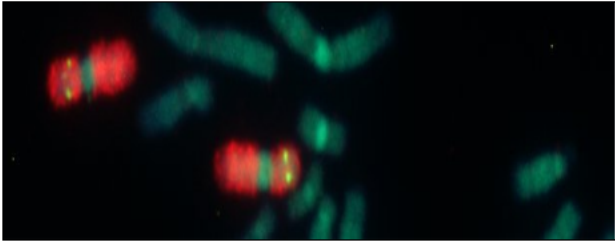
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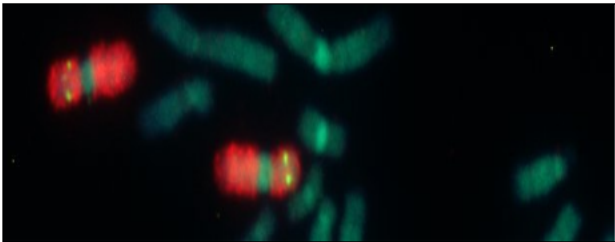
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ACE2 angiotensin I converting enzyme 2 [*Homo sapiens* (human)]

Gene ID: 59272, updated on 22-Mar-2020

Summary

Official Symbol ACE2 provided by HGNC
Official Full Name angiotensin I converting enzyme 2 provided by HGNC
Primary source HGNC:HGNC:13557
See related Ensembl:ENSG00000130234 MIM:300335
Gene type protein coding
RefSeq status REVIEWED
Organism *Homo sapiens*
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as ACEH
Summary The protein encoded by this gene belongs to the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases and has considerable homology to human angiotensin 1 converting enzyme. This secreted protein catalyzes the cleavage of angiotensin I into angiotensin 1-9, and angiotensin II into the vasodilator angiotensin 1-7. The organ- and cell-specific expression of this gene suggests that it may play a role in the regulation of cardiovascular and renal function, as well as fertility. In addition, the encoded protein is a functional receptor for the spike glycoprotein of the human coronavirus HCoV-NL63 and the human severe acute respiratory syndrome coronaviruses, SARS-CoV and SARS-CoV-2 (COVID-19 virus). [provided by RefSeq, Mar 2020]
Expression Biased expression in small intestine (RPKM 93.7), duodenum (RPKM 69.0) and 5 other tissues [See more](#)
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Genomic context

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Markers, Clone Names, Homology, Gene Ontology
- General protein information
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- Additional links
Locus-specific Databases

Genome Browsers

Genome Data Viewer

Organism Homo sapiens

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo

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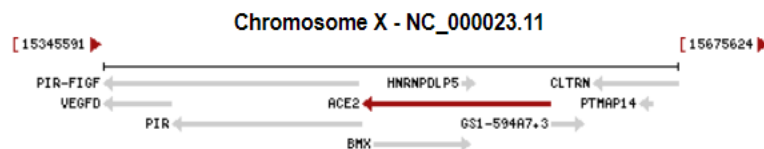
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Location: Xp22.2

[See ACE2 in Genome Data Viewer](#)

Exon count: 21

Annotation release	Status	Assembly	Chr	Location
109.20200228	current	GRCh38.p13 (GCF_000001405.39)	X	NC_000023.11 (15494520..15602158, complement)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	X	NC_000023.10 (15579156..15620192, complement)

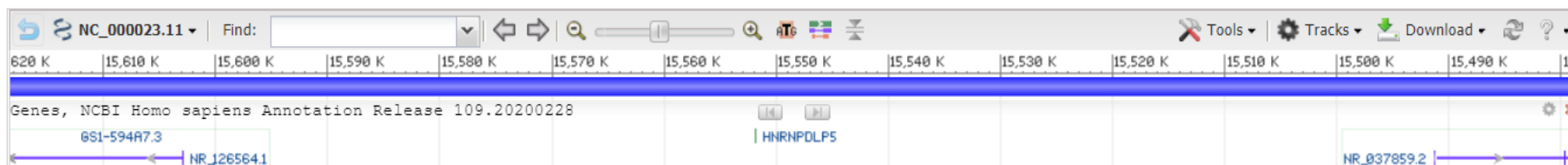


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Genomic Sequence: NC_000023.11 Chromosome X Reference GRCh38.p13 Primary Assembly ▼

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General protein information

NCBI Reference Sequences (RefSeq)

Related sequences

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Variation Viewer (GRCh37.p13)

Variation Viewer (GRCh38)

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3D structures

BioAssay by Target (List)

BioAssay by Target (Summary)

BioAssay, by Gene target

BioAssays, RNAi Target, Active

BioAssays	RNAi Target	Tested
Cell Viability	Yes	Yes
Cell Proliferation	Yes	Yes
Cell Apoptosis	Yes	Yes
Cell Differentiation	Yes	Yes
Cell Migration	Yes	Yes
Cell Death	Yes	Yes
Cell Growth	Yes	Yes
Cell Survival	Yes	Yes
Cell Function	Yes	Yes
Cell Signaling	Yes	Yes
Cell Metabolism	Yes	Yes
Cell Homeostasis	Yes	Yes
Cell Communication	Yes	Yes
Cell Development	Yes	Yes
Cell Maintenance	Yes	Yes
Cell Repair	Yes	Yes
Cell Regeneration	Yes	Yes
Cell Adaptation	Yes	Yes
Cell Response	Yes	Yes
Cell Behavior	Yes	Yes
Cell Interaction	Yes	Yes
Cell Motility	Yes	Yes
Cell Adhesion	Yes	Yes
Cell Attachment	Yes	Yes
Cell Detachment	Yes	Yes
Cell Fusion	Yes	Yes
Cell Division	Yes	Yes
Cell Replication	Yes	Yes
Cell Transcription	Yes	Yes
Cell Translation	Yes	Yes
Cell Translation Initiation	Yes	Yes
Cell Translation Elongation	Yes	Yes
Cell Translation Termination	Yes	Yes
Cell Translation Regulation	Yes	Yes
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Cell Translation Duration	Yes	Yes
Cell Translation Frequency	Yes	Yes
Cell Translation Rate	Yes	Yes
Cell Translation Efficiency	Yes	Yes

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Organism Homo sapiens

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo

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Location: Xp22.2

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Exon count: 21

Chromosome X - NC_000023.11

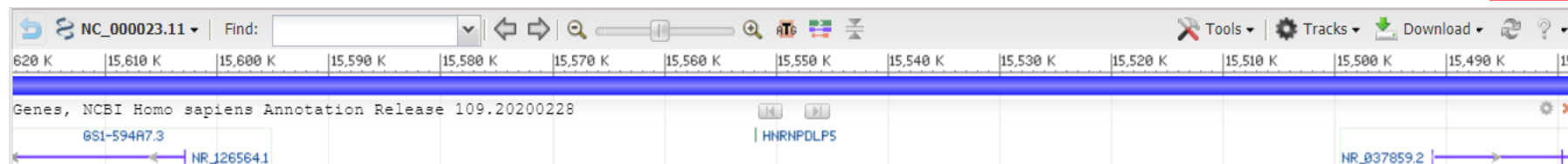
Genomic map of Chromosome X (NC_000023.11) showing gene locations and orientations. The map spans from 15345591 to 15675624. Genes shown include PIR-FIGF, VEGFD, PIR, ACE2, HNRNPDLPS, CLTRN, GS1-594A7.3, and PTMRP14. The ACE2 gene is highlighted in red.

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Genomic Sequence: NC_000023.11 Chromosome X Reference GRCh38.p13 Primary Assembly ▼

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NCBI Reference Sequence: NC_000023.11

FASTA

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LOCUS NC_000023 107639 bp DNA linear CON 02-MAR-2020

DEFINITION Homo sapiens chromosome X, GRCh38.p13 Primary Assembly.

ACCESSION [NC_000023](#) REGION: complement(15494520..15602158)

VERSION NC 000023.11

DBLINK BioProject: [PRJNA168](#)

Assembly: GCF_000001405.39

KEYWORDS RefSeq.

SOURCE Homo sapiens (human)

ORGANISM *Homo sapiens*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 107639)

AUTHORS Ross,M.T., Grafham,D.V., Coffey,A.J., Scherer,S., McLay,K., Muzny,D., Platzer,M., Howell,G.R., Burrows,C., Bird,C.P., Frankish,A., Lovell,F.L., Howe,K.L., Ashurst,J.L., Fulton,R.S., Sudbrak,R., Wen,G., Jones,M.C., Hurler,M.E., Andrews,T.D., Scott,C.E., Searle,S., Ramser,J., Whittaker,A., Deadman,R., Carter,N.P., Hunt,S.E., Chen,R., Cree,A., Gunaratne,P., Havlak,P., Hodgson,A., Metzker,M.L., Richards,S., Scott,G., Steffen,D., Sodergren,E., Wheeler,D.A., Worley,K.C., Ainscough,R., Ambrose,K.D., Ansari-Lari,M.A., Aradhya,S., Ashwell,R.I., Babbage,A.K., Bagguley,C.L., Ballabio,A., Banerjee,R., Barker,G.E., Barlow,K.F., Barrett,I.P., Bates,K.N., Beare,D.M., Beasley,H., Beasley,O., Beck,A., Bethel,G., Blechschmidt,K., Brady,N., Bray-Allen,S., Bridgeman,A.M., Brown,A.J., Brown,M.J., Bonnin,D., Brunfild,E.A., Buhay,C., Burch,P., Bunford,D., Burgess,J.

☐ Whole sequence (abbreviated view)

☒ Selected region

from: 15494520 to: 15602158

Update View

Customize view

☐ Abbreviated view☒ Customize

Basic Features

☐ All features

☒ Gene, RNA, and CDS features only

Display options

☒ Show sequence☒ Show reverse complement

Update View

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

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FEATURES

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ace2

1/12

^

v

X

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search

Advanced Help

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

GenBank Send to

Change region shown

Homo sapiens angiotensin I converting enzyme 2 (ACE2), transcript variant 2, mRNA

NCBI Reference Sequence: NM_021804.3
[FASTA](#) [Graphics](#)

Go to

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ORGANISM [Homo sapiens](#)
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REFERENCE 1 (bases 1 to 3596)
AUTHORS Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF and Shi ZL.
TITLE A pneumonia outbreak associated with a new coronavirus of probable bat origin
JOURNAL Nature 579 (7798), 270-273 (2020)
PUBMED [32015507](#)
REFERENCE 2 (bases 1 to 3596)
AUTHORS Letko M, Marzi A and Munster V.
TITLE

Customize view

Analyze this sequence

- Run BLAST
- Pick Primers
- Highlight Sequence Features
- Find in this Sequence
- Show in Genome Data Viewer

Articles about the ACE2 gene

- Functional assessment of cell entry and receptor usage for SARS-CoV-2 and [Nat Microbiol. 2020]
 - High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral [Int J Oral Sci. 2020]
 - A pneumonia outbreak associated with a new coronavirus of probable bat origin. [Nature. 2020]
- See all...

Pathways for the ACE2 gene

- Metabolism of Angiotensinogen to Angiotensins

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Этот
компьютер



Новая папка



Корзина



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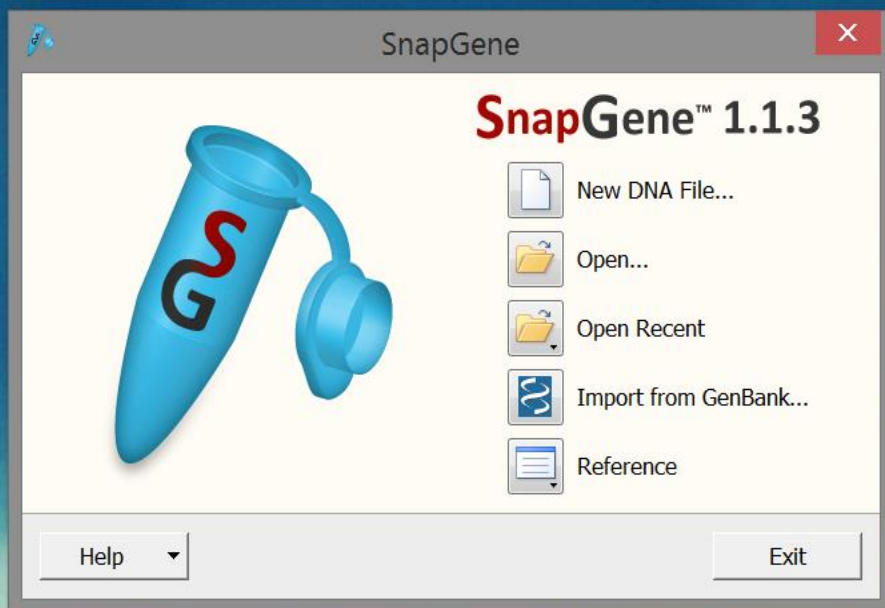
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Иманали И...



жанар
перевод



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Новая папка

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Темірғали Иманали И...

жанар перевод

4x_1172_pl...

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☐ Reverse complement

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☒ Linear☐ Circular

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OK

Cancel



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☐ Reverse complement

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Create a sequence of 1000 N's

Topology:
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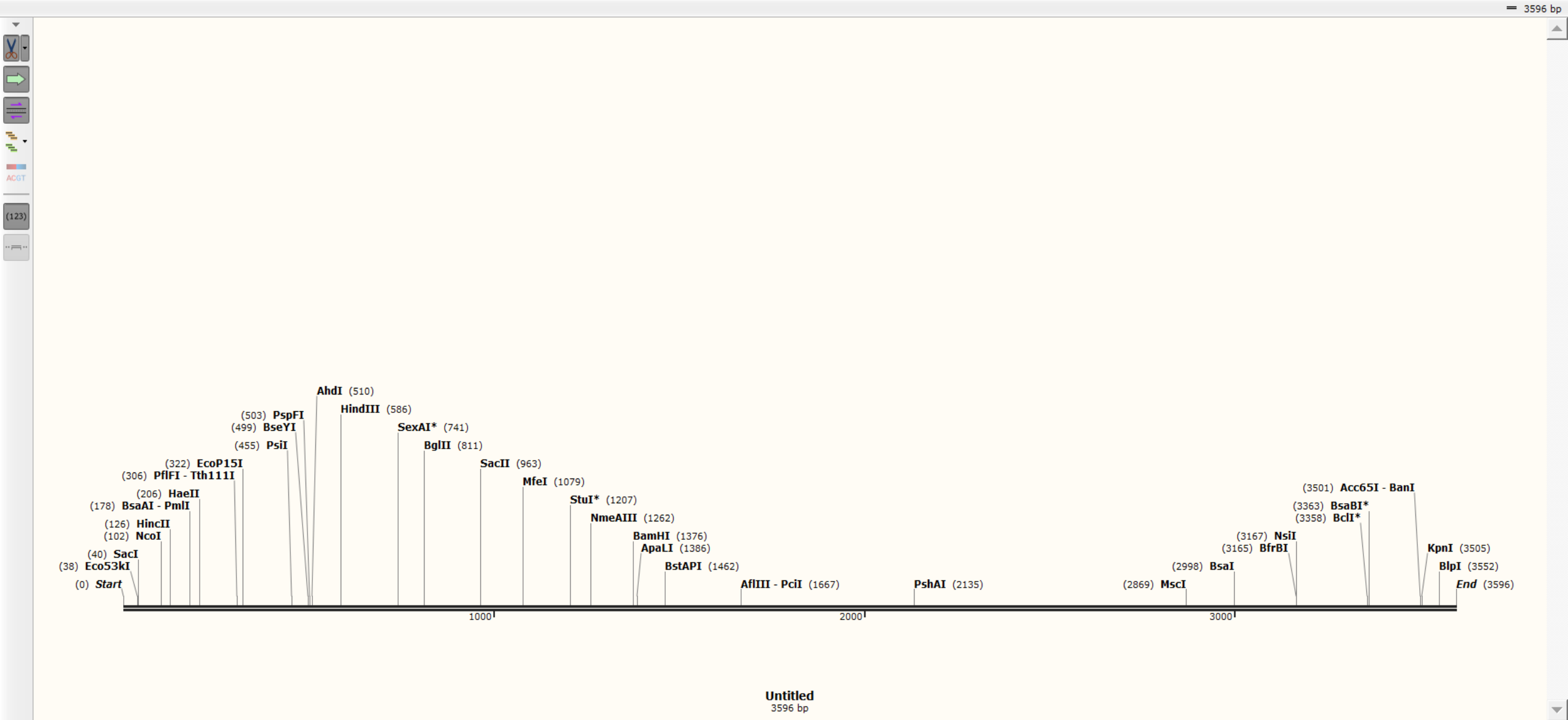
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OK

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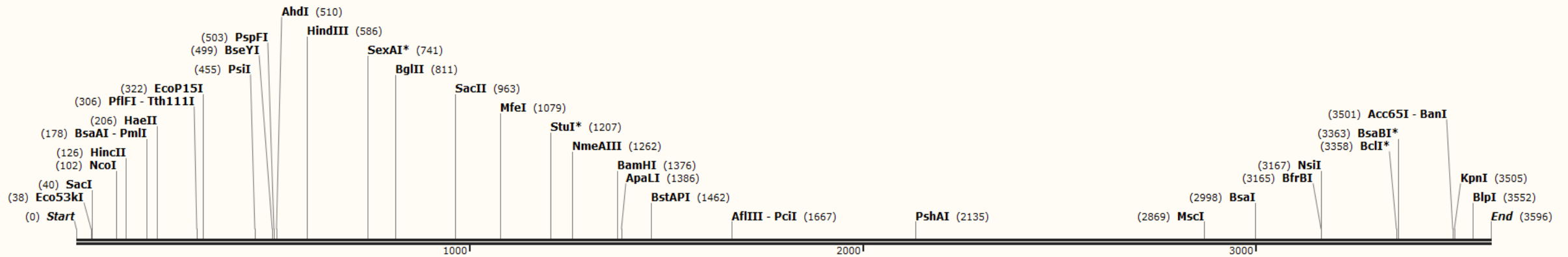
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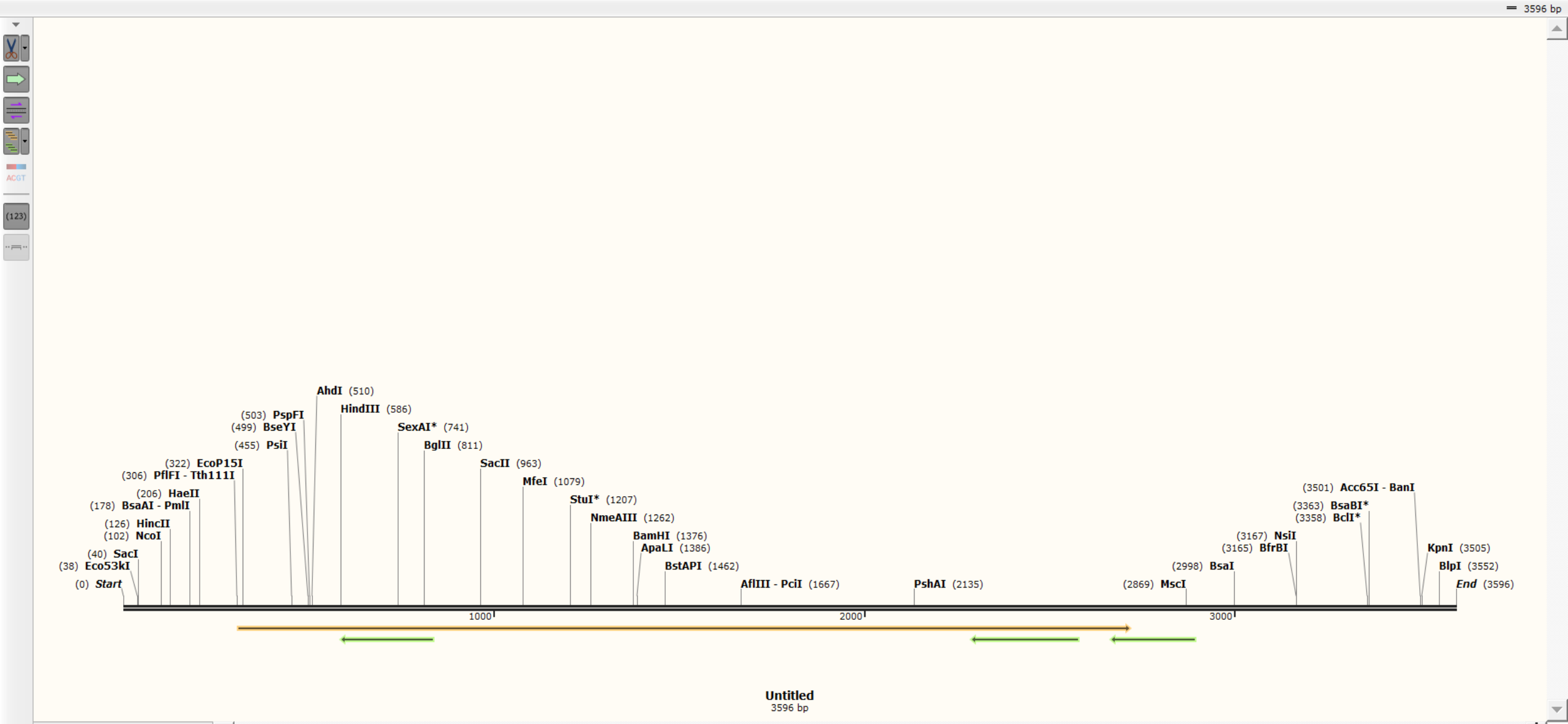


Show translations



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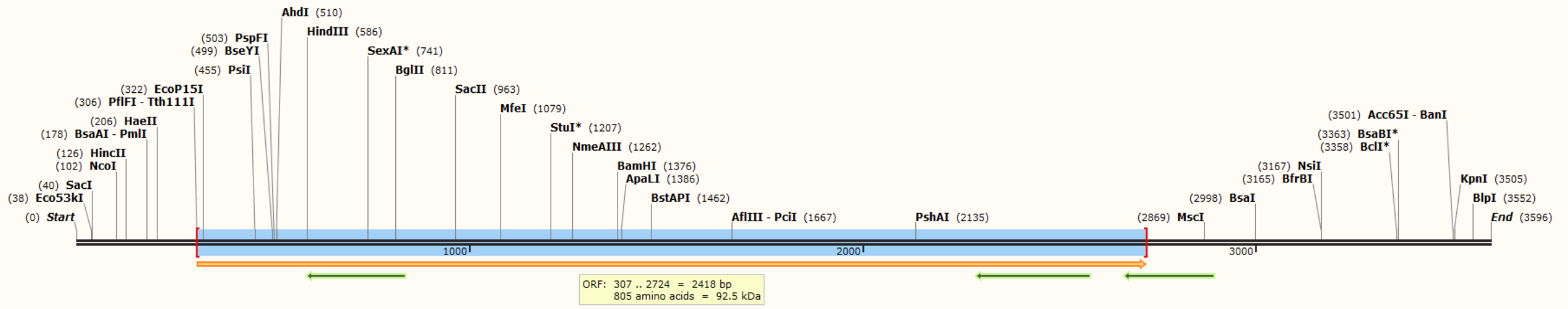
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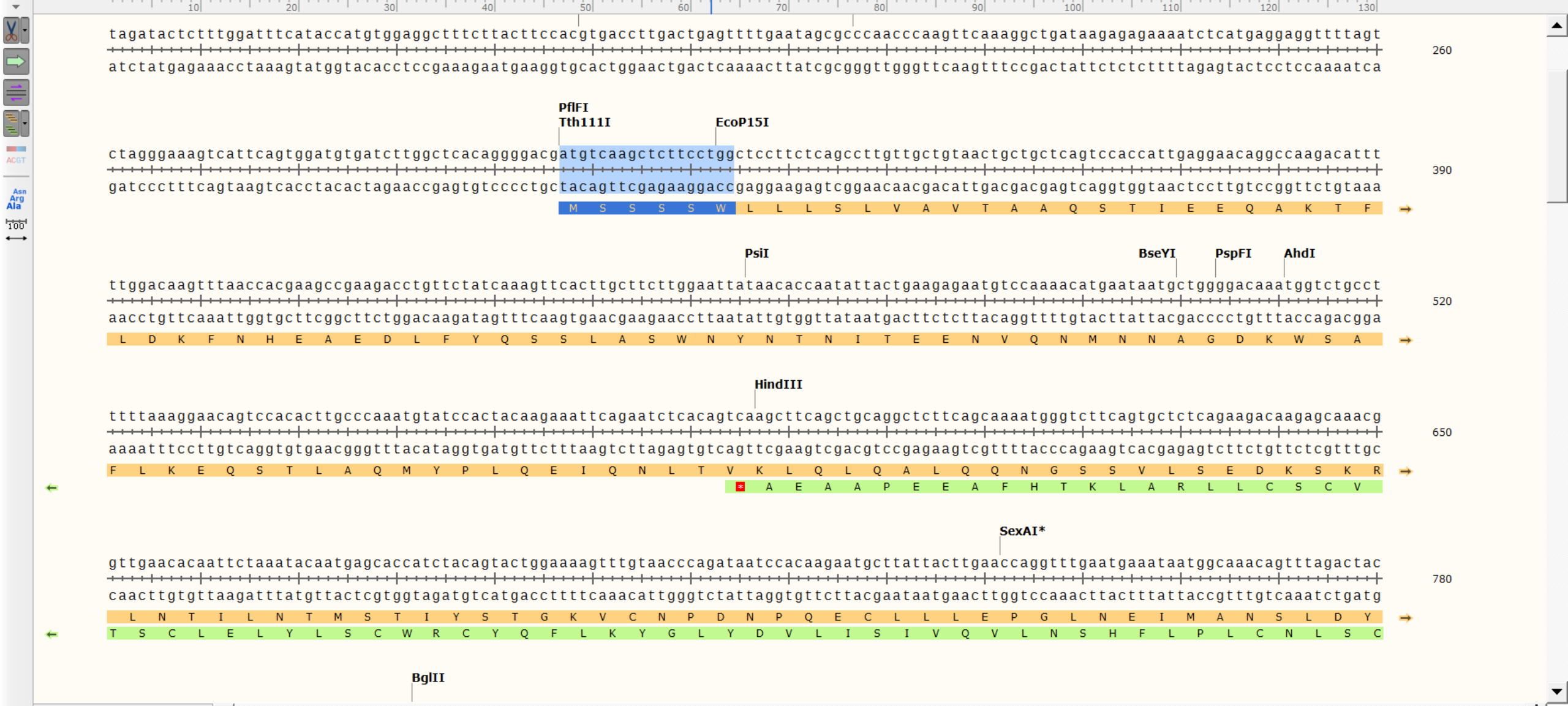
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Selected: 6 codons (307 .. 324 = 18 bp)

3596 bp



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DDVQTSF"
sig_peptide 307..357



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angiotensin-converting enzyme 2 precursor [Homo sapiens]

NCBI Reference Sequence: NP_068576.1

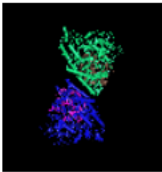
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LOCUS NP_068576 805 aa linear PRI 22-MAR-2020
DEFINITION angiotensin-converting enzyme 2 precursor [Homo sapiens].
ACCESSION NP_068576 XP_005274628
VERSION NP_068576.1
DBSOURCE REFSEQ: accession [NM_021804.3](#)
KEYWORDS RefSeq.
SOURCE Homo sapiens (human)
ORGANISM [Homo sapiens](#)
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
Catarrhini; Hominidae; Homo.
REFERENCE 1 (residues 1 to 805)
AUTHORS Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF and Shi ZL.
TITLE A pneumonia outbreak associated with a new coronavirus of probable bat origin
JOURNAL Nature 579 (7798), 270-273 (2020)
PUBMED [32015507](#)
REFERENCE 2 (residues 1 to 805)
AUTHORS Letko M, Marzi A and Munster V.
TITLE Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses

- Analyze this sequence
- Run BLAST
- Identify Conserved Domains
- Highlight Sequence Features
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- Show in Genome Data Viewer

Protein 3D Structure



ACE2-B0AT1 complex, open conformation
PDB: 6M1D
Source: Homo sapiens
Method: Electron Microscopy
Resolution: 4.5 Å

See all 16 structures...

Articles about the ACE2 gene

Functional assessment of cell entry and receptor

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//
```

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Protein

angiotensin-converting enzyme 2 precursor [Homo sapiens]

NCBI Reference Sequence: NP_068576.1

Identical Proteins FASTA Graphics

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LOCUS NP_068576 805 aa linear PRI

DEFINITION angiotensin-converting enzyme 2 precursor [Homo sapiens]

ACCESSION NP_068576 XP_005274628

VERSION NP_068576.1

DBSOURCE REFSEQ: accession [NM_021804.3](#)

KEYWORDS RefSeq.

SOURCE Homo sapiens (human)

ORGANISM [Homo sapiens](#)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.

REFERENCE 1 (residues 1 to 805)

AUTHORS Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF and Shi ZL.

TITLE A pneumonia outbreak associated with a new coronavirus of probable bat origin

JOURNAL Nature 579 (7798), 270-273 (2020)

PUBMED [32015507](#)

REFERENCE 2 (residues 1 to 805)

AUTHORS Letko M, Marzi A and Munster V.

View conserved domains detected in this protein sequence using CD-search.

Analyze this sequence

Run BLAST

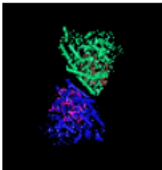
Identify Conserved Domains

Highlight Sequence Features

Find in this Sequence

Show in Genome Data Viewer

Protein 3D Structure



ACE2-B0AT1 complex, open conformation
PDB: 6M1D
Source: Homo sapiens
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Resolution: 4.5 Å

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Functional assessment of cell entry and receptor

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

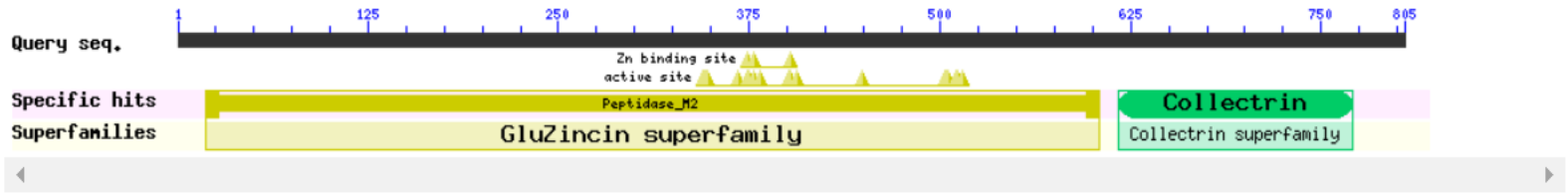
Conserved domains on [gi|11225609|ref|NP_068576|] View Concise Results

angiotensin-converting enzyme 2 precursor [Homo sapiens]

Protein Classification

angiotensin-converting enzyme 2 (domain architecture ID 11117526)
angiotensin-converting enzyme 2 is a carboxypeptidase which converts angiotensin I to angiotensin 1-9, and angiotensin II to the vasodilator angiotensin 1-7

Graphical summary ☐ Zoom to residue level show extra options



List of domain hits

	Name	Accession	Description	Interval	E-value
[+]	Peptidase_M2	pfam01401	Angiotensin-converting enzyme; Members of this family are dipeptidyl carboxydiptidases ...	19-605	0e+00
[+]	Collectrin	pfam16959	Renal amino acid transporter; Collectrin is a single-pass transmembrane protein that is ...	617-770	5.51e-93

References:

- Marchler-Bauer A et al. (2017), "CDD/SPARCLE: functional classification of proteins via subfamily domain architectures.", **Nucleic Acids Res.**45(D)200-3.
- Marchler-Bauer A et al. (2015), "CDD: NCBI's conserved domain database.", **Nucleic Acids Res.**43(D)222-6.
- Marchler-Bauer A et al. (2011), "CDD: a Conserved Domain Database for the functional annotation of proteins.", **Nucleic Acids Res.**39(D)225-9.
- Marchler-Bauer A, Bryant SH (2004), "CD-Search: protein domain annotations on the fly.", **Nucleic Acids Res.**32(W)327-331.



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260

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390

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Serine (19)
ORF: 307 .. 2724 = 2418 bp
805 amino acids = 92.5 kDa

PsiI

PspFI

AhdI

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520

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SexAI*

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780

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BglII

Unique 6+ Cutters (Nonredundant)

Untitled.dna* (Linear / 3596 bp)

FileEditViewEnzymesFeaturesPrimersActionsWindowHelp

NewOpenSavePrintUndoRedoCopyPaste

Selected: 1 codon (2119 .. 2121 = 3 bp)3596 bp

100

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PshAI

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F T W L K D Q N K N S F V G W S T D W S P Y A D Q S I K V R I S L K S A L G D K A Y E W

Glycine (605)

ORF: 307 .. 2724 = 2418 bp

805 amino acids = 92.5 kDa

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N D N E M Y L F R S S V A Y A M R Q Y F L K V K N Q M I L F G E E D V R V A N L K P R

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Unique 6+ Cutters (Nonredundant)

MapSequenceEnzymesFeaturesPrimersHistory

Description Pa



Selected: 587 codons (361 .. 2121 = 1761 bp)

3596 bp



100

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F L K E Q S T L A Q M Y P L Q E I Q N L T V K L Q L Q A L Q Q N G S S V L S E D K S K R
A E A A P E E A F H T K L A R L L C S C V
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L N T I L N T M S T I Y S T G K V C N P D N P Q E C L L L E P G L N E I M A N S L D Y
T S C L E L Y L S C W R C Y Q F L K Y G L Y D V L I S I V Q V L N S H F L P L C N L S C
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PfFI
Tth111I

EcoP15I

PsiI

BseYI

PspFI

AhdI

HindIII

SexAI*

BglII

Unique 6+ Cutters (Nonredundant)





Selected: 587 codons (361 .. 2121 = 1761 bp)

3596 bp



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M S S S S W L L L S L V A V T A A Q S T I E E Q A K T F
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L D K F N H E A E D L F Y Q S S L A S W N Y N T N I T E E N V Q N M N N A G D K W S A
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F L K E Q S T L A Q M Y P L Q E I Q N L T V K L Q L Q A L Q Q N G S S V L S E D K S K R
A E A A P E E A F H T K L A R L L C S C V
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L N T I L N T M S T I Y S T G K V C N P D N P Q E C L L L E P G L N E I M A N S L D Y
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PfFI
Tth111I

EcoP15I

PsiI

BseYI

PspFI

AhdI

HindIII

SexAI*

BglII

Unique 6+ Cutters (Nonredundant)

FileEditViewEnzymesFeaturesPrimersActionsWindowHelp

NewOpenSavePrintUndoRedoCopyPaste

Selected: 587 codons (361 .. 2121 = 1761 bp)

3596 bp

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ttggacaagttaaaccacgaagccgaagacctgt
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aatgagaggctctgggcttgggaaagctggagatctgaggtcggcaagcagctgaggccattatatgaagagtatgtggtcttgaaaaatgagatggcaagagcaaatacattatgaggactatggggatt

3612121

1761 bp / 1 segment

Segment	Location	Size (bp)	Color
1	Feature 1361 .. 2121	1761	

/note =

OKCancel

cataggaggaacaggccaagacattt
gtaactccttgtccgggttctgtaaa

I E E Q A K T F

BseYIPspFI AhdI

aatgctggggacaaatgggtctgcct
ttacgaccctgtttaccagacgga

N A G D K W S A

tgctctcagaagacaagagcaaacg
acgagagtcttctgttctcgtttgc

V L S E D K S K R

A R L L C S C V

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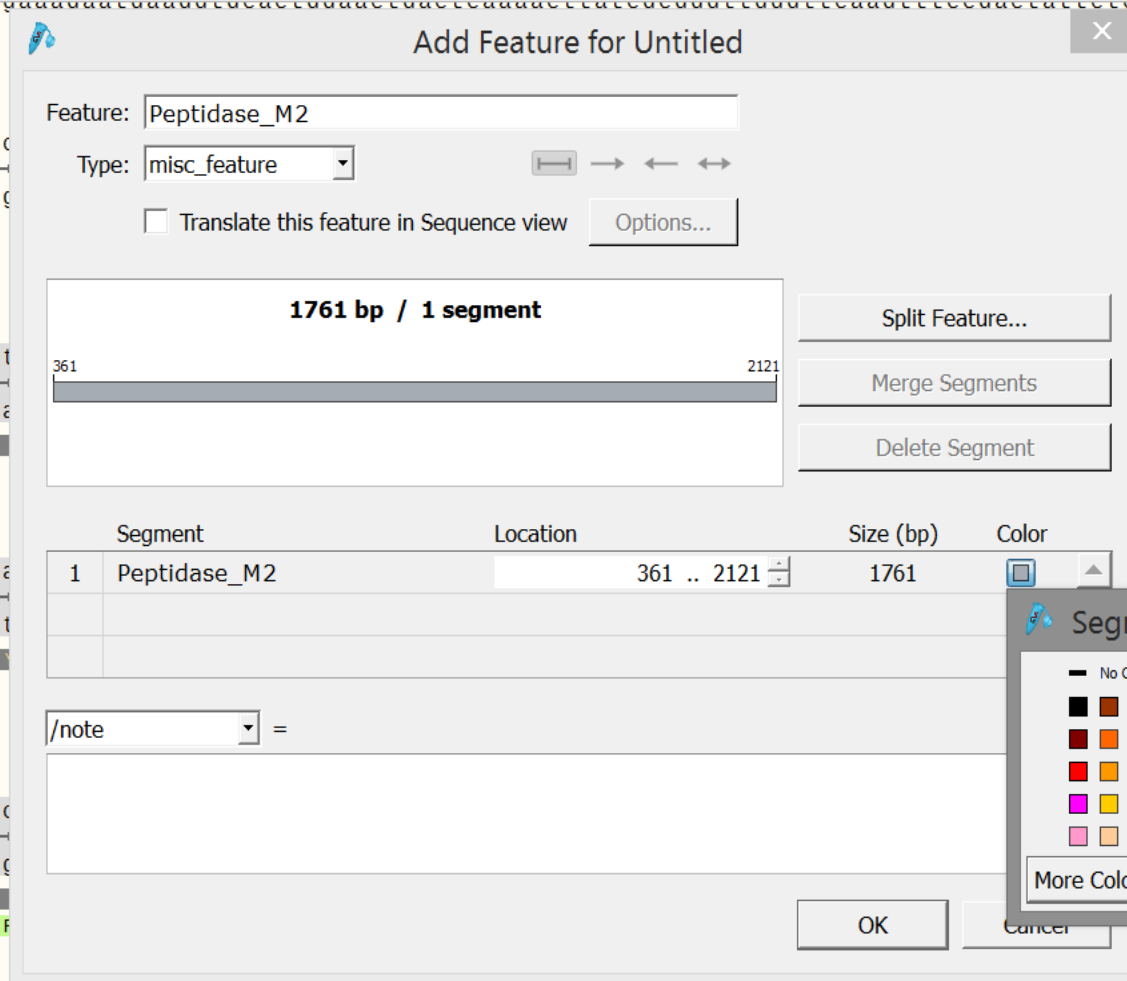
F L P L C N L S C

Unique 6+ Cutters (Nonredundant)

MapSequenceEnzymesFeaturesPrimersHistory

Description Pa

= 3596 bp



☐ Description Par

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

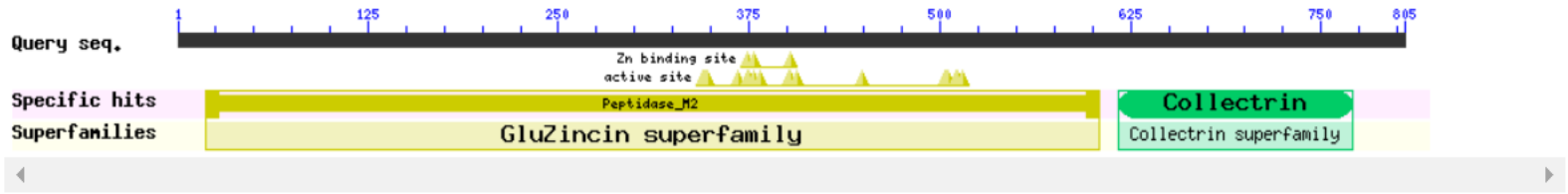
Conserved domains on [gi|11225609|ref|NP_068576|] View Concise Results

angiotensin-converting enzyme 2 precursor [Homo sapiens]

Protein Classification

angiotensin-converting enzyme 2 (domain architecture ID 11117526)
angiotensin-converting enzyme 2 is a carboxypeptidase which converts angiotensin I to angiotensin 1-9, and angiotensin II to the vasodilator angiotensin 1-7

Graphical summary ☐ Zoom to residue level show extra options



Search for similar domain architectures Refine search

List of domain hits

	Name	Accession	Description	Interval	E-value
[+]	Peptidase_M2	pfam01401	Angiotensin-converting enzyme; Members of this family are dipeptidyl carboxydiptidases ...	19-605	0e+00
[+]	Collectrin	pfam16959	Renal amino acid transporter; Collectrin is a single-pass transmembrane protein that is ...	617-770	5.51e-93

References:

- Marchler-Bauer A et al. (2017), "CDD/SPARCLE: functional classification of proteins via subfamily domain architectures.", **Nucleic Acids Res.**45(D)200-3.
- Marchler-Bauer A et al. (2015), "CDD: NCBI's conserved domain database.", **Nucleic Acids Res.**43(D)222-6.
- Marchler-Bauer A et al. (2011), "CDD: a Conserved Domain Database for the functional annotation of proteins.", **Nucleic Acids Res.**39(D)225-9.
- Marchler-Bauer A, Bryant SH (2004), "CD-Search: protein domain annotations on the fly.", **Nucleic Acids Res.**32(W)327-331.

Untitled.dna* (Linear / 3596 bp)

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Selected: 154 codons (2155 .. 2616 = 462 bp)

3596 bp

100%

100

←

→

EAGQLFNMLRLGLKSEPTWTLALENVVGAKNMNVRLNLNYFEP

→

←

→

PshAI

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Peptidase_M2

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RD

CLLFYDLIGFCQH

2210

2340

2470

2600

2730

Unique 6+ Cutters (Nonredundant)

MapSequenceEnzymesFeaturesPrimersHistory

Description Pa

Untitled.dna* (Linear / 3596 bp)

FileEditViewEnzymesFeaturesPrimersActionsWindowHelp

NewOpenSavePrintUndoRedoCopyPaste

3596 bp

102030405060708090100110120130

PshAI

2210

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Peptidase_M2Collectrin

F T W L K D Q N K N S F V G W S T D W S P Y A D Q S I K V R I S L K S A L G D K A Y E W

2340

gaacgacaatgaaatgtacctgttccgatcatctgttgcataatgctatgaggcagttacttttaaaagtaaaaaatcagatgattcttttggggaggaggatgtgcgagtggtctaatttgaaaccaaga

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Collectrin

N D N E M Y L F R S S V A Y A M R Q Y F L K V K N Q M I L F G E E D V R V A N L K P R

I I R K P S S S T R T A L K F G L

2470

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Collectrin

I S F N F F V T A P K N V S D I I P R T E V E K A I R M S R S R I N D A F R L N D N S

I E K L K K T V A G L F T D S I M G L V S T S F A M L I D R L R I L S A K R R F S L L R

2600

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Collectrin

L E F L G I Q P T L G P P N Q P P V S I W L I V F G V V M G V I V V G I V I L I F T G I

S N R P I C G V S P G G L W G G T E M H S I T K P T T I P T I T T P M T M

2730

cagagatcggaagaagaaaaataaagcaagaagtggagaaaaatccttatgcctccatcgatattagcaaaggagaaaaataatccaggattccaaaacactgatgatgttcagacctccttttagaaaaat

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Collectrin

Unique 6+ Cutters (Nonredundant)

MapSequenceEnzymesFeaturesPrimersHistory

Description Pa

FileEditViewEnzymesFeaturesPrimersActionsWindowHelp

NewOpenSavePrintUndoRedoCopyPaste

3596 bp

2080

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Peptidase_M2

2210

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Peptidase_M2Collectrin

PshAI

2340

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Collectrin

2470

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Collectrin

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Collectrin

2860

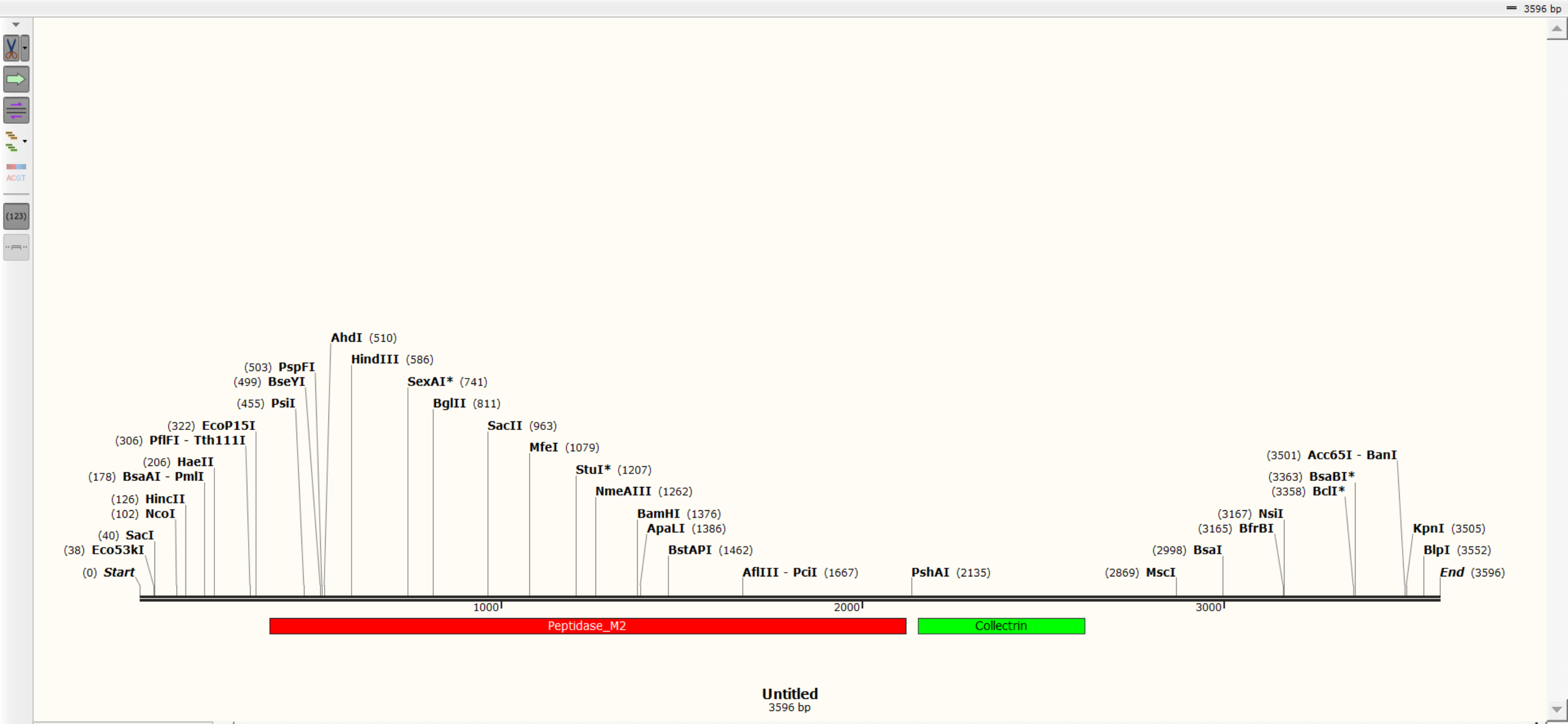
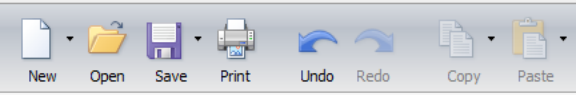
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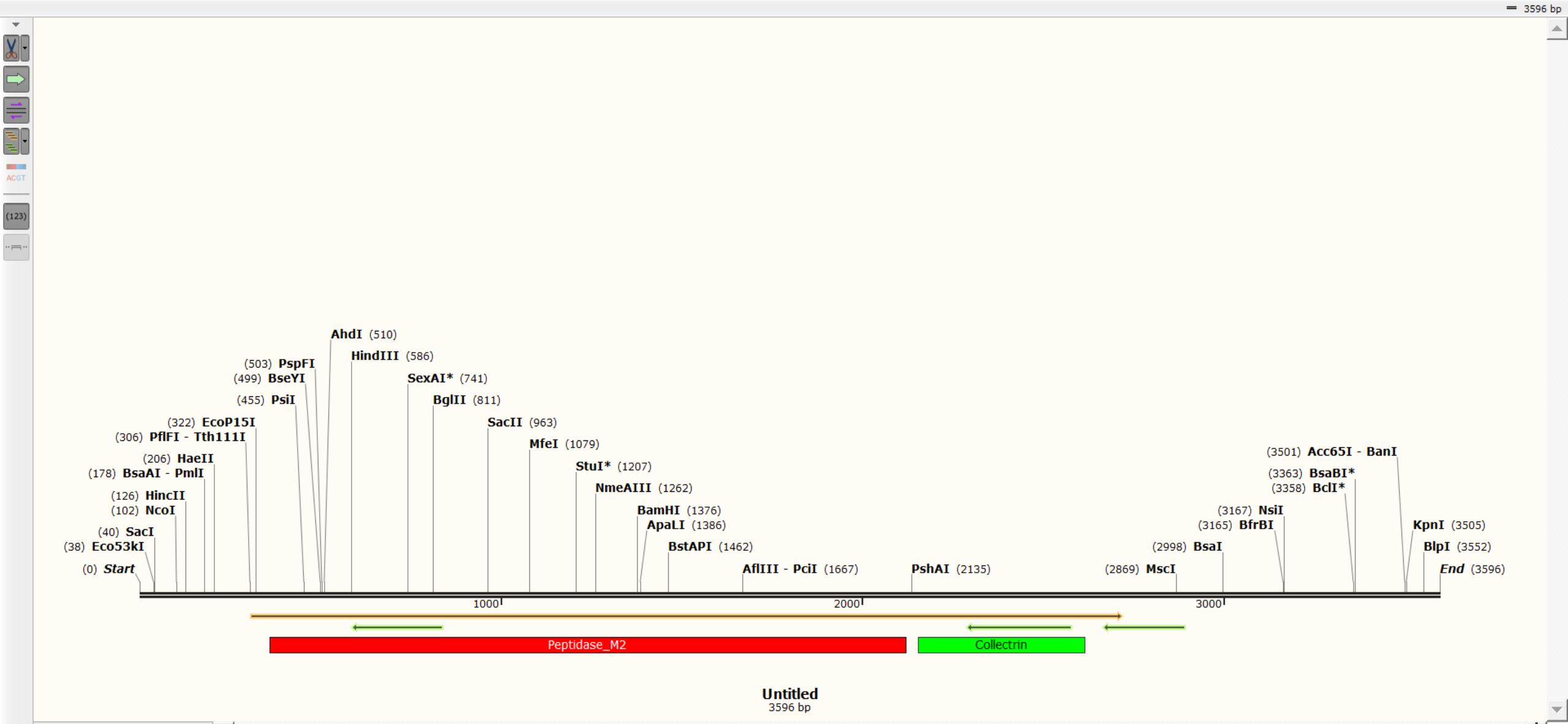
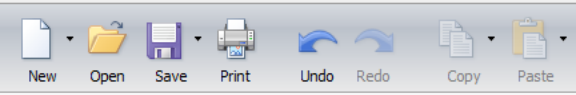
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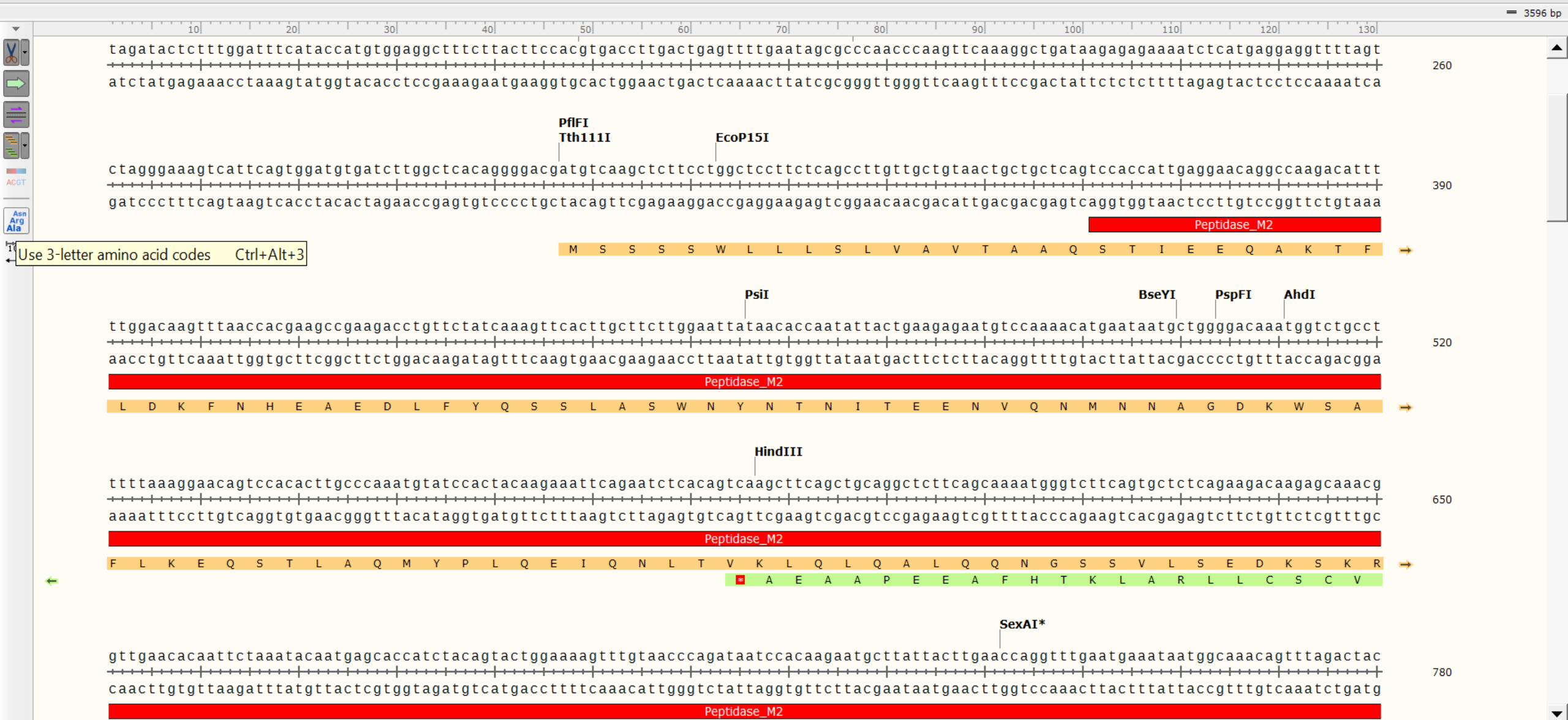
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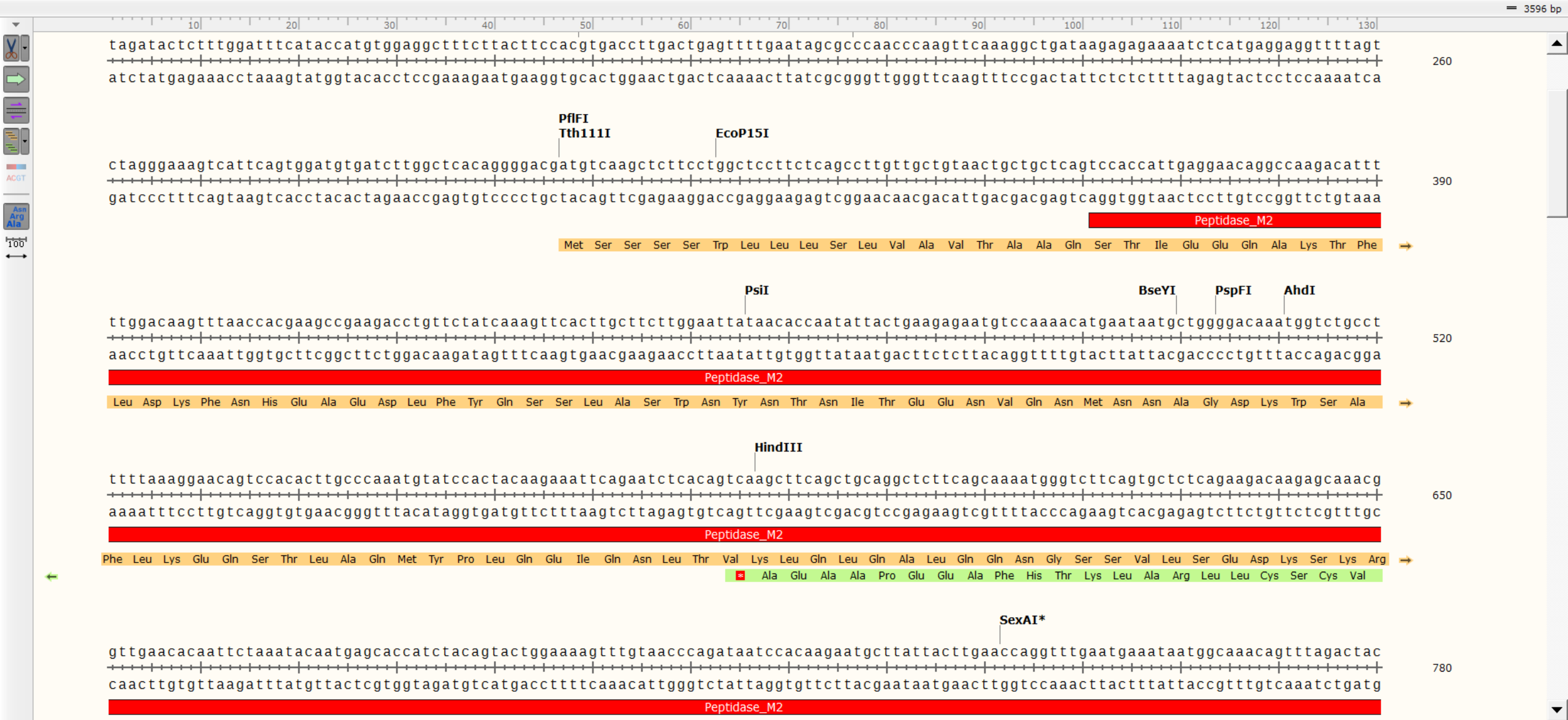
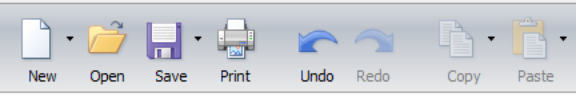
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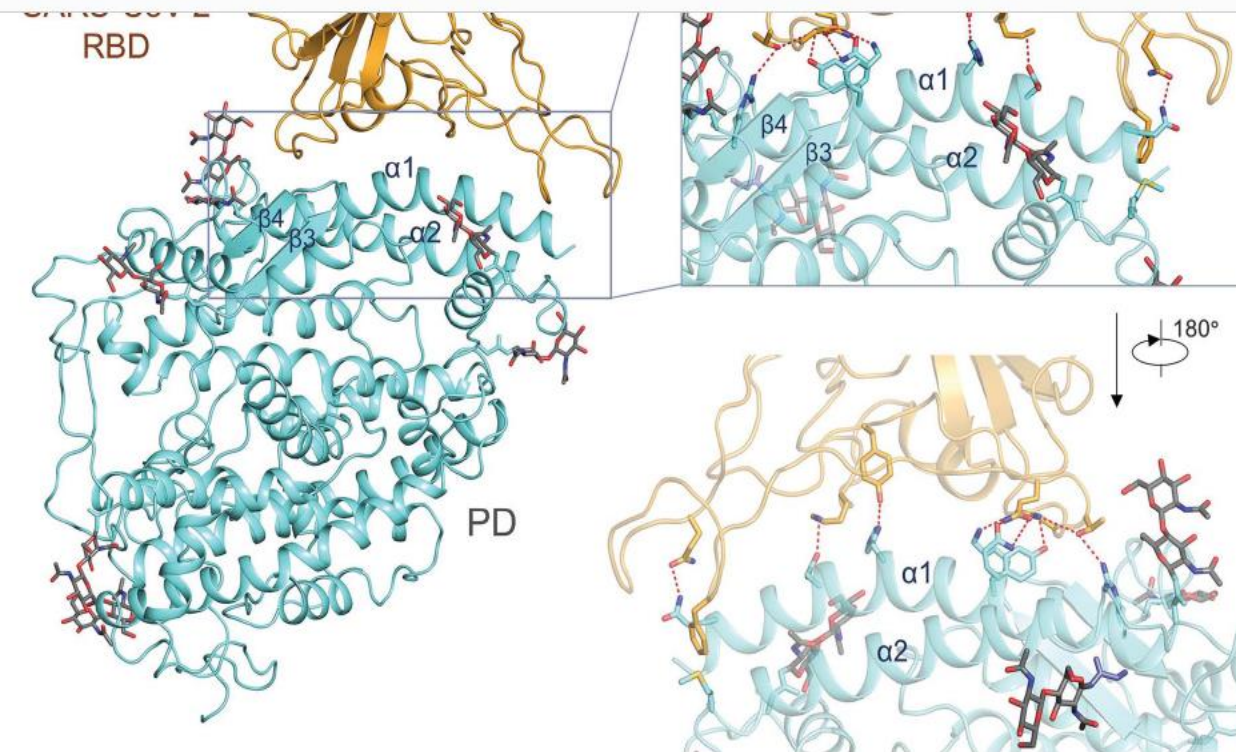
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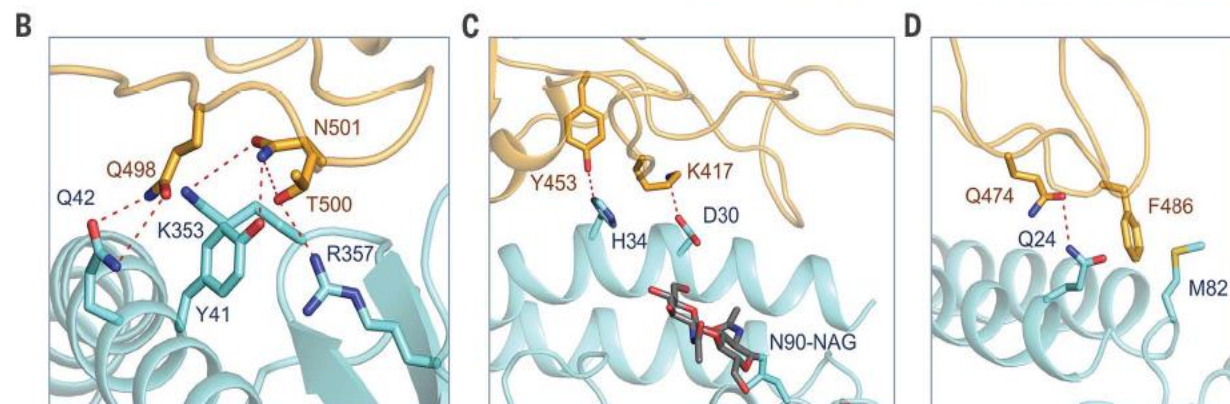






RBD. The $\alpha 2$ helix and the linker between $\beta 3$ and $\beta 4$ also contribute to the interaction. Only one RBD-ACE2 is shown. (B to D) Detailed analysis of the interface between SARS-CoV-2-RBD and ACE2. Polar interactions are indicated by red dashed lines. NAG, *N*-acetylglucosamine.

science.sciencemag.org/ on March 30, 2020

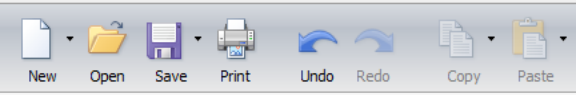


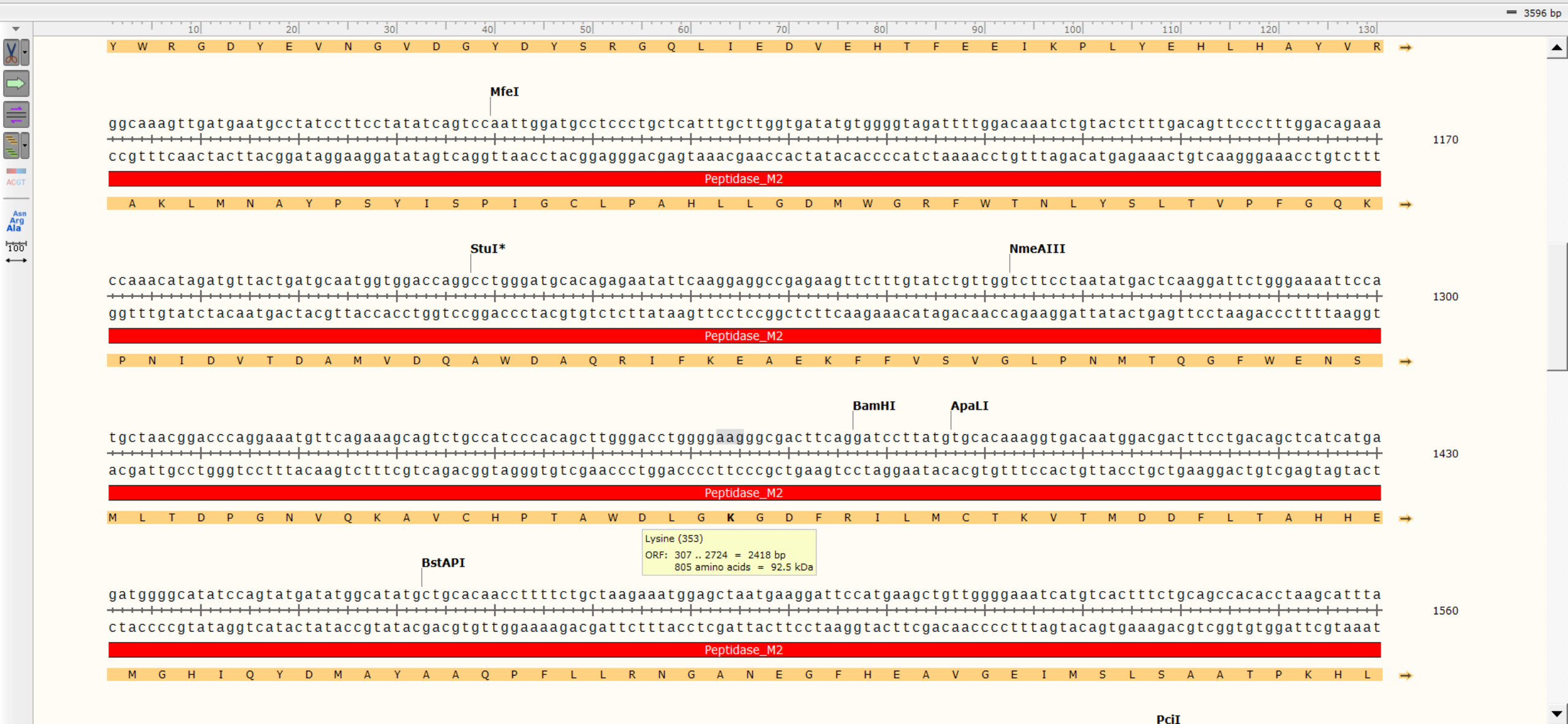
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Бесплатная пробная версия





Lysine (353)
ORF: 307 .. 2724 = 2418 bp
805 amino acids = 92.5 kDa



Selected: active (1363 .. 1365 = 3 bp)

3596 bp

Y

W

R

G

D

Y

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Y

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Y

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Peptidase_M2

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Peptidase_M2

M

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G

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L

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A

H

H

E

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Peptidase_M2

M

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H

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Q

Y

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A

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F

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G

A

N

E

G

F

H

E

A

V

G

E

I

M

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T

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K

H

L

Unique 6+ Cutters (Nonredundant)

Nucleotide

Nucleotide [v]

SaRC-Cov-2

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Homo sapiens chromosome X, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC_000023.11

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LOCUS NC_000023 107639 bp DNA linear CON 02-MAR-2020
DEFINITION Homo sapiens chromosome X, GRCh38.p13 Primary Assembly.
ACCESSION [NC_000023](#) REGION: complement(15494520..15602158)
VERSION NC_000023.11
DBLINK BioProject: [PRJNA168](#)
Assembly: [GCF_000001405.39](#)
KEYWORDS RefSeq.
SOURCE Homo sapiens (human)
ORGANISM [Homo sapiens](#)
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 107639)
AUTHORS Ross,M.T., Grafham,D.V., Coffey,A.J., Scherer,S., McLay,K.,
Muzny,D., Platzer,M., Howell,G.R., Burrows,C., Bird,C.P.,
Frankish,A., Lovell,F.L., Howe,K.L., Ashurst,J.L., Fulton,R.S.,
Sudbrak,R., Wen,G., Jones,M.C., Hurles,M.E., Andrews,T.D.,
Scott,C.E., Searle,S., Ramser,J., Whittaker,A., Deadman,R.,
Carter,N.P., Hunt,S.E., Chen,R., Cree,A., Gunaratne,P., Havlak,P.,
Hodgson,A., Metzker,M.L., Richards,S., Scott,G., Steffen,D.,
Sodergren,E., Wheeler,D.A., Worley,K.C., Ainscough,R.,
Ambrose,K.D., Ansari-Lari,M.A., Aradhya,S., Ashwell,R.I.,
Babbage,A.K., Bagguley,C.L., Ballabio,A., Banerjee,R., Barker,G.E.,
Barlow,K.F., Barrett,I.P., Bates,K.N., Beare,D.M., Beasley,H.,
Beasley,O., Beck,A., Bethel,G., Blechschmidt,K., Brady,N.,
Bray-Allen,S., Bridgeman,A.M., Brown,A.J., Brown,M.J., Bonnin,D.,
Bunford,D., Burch,C., Burch,P., Burch,D., Burch,D., Burch,D.,

Change region shown

☐ Whole sequence (abbreviated view)☒ Selected region

from: 15494520 to: 15602158

Update View

Customize view

☐ Abbreviated view☒ Customize

Basic Features

☐ All features☒ Gene, RNA, and CDS features only

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☒ Show sequence☒ Show reverse complement

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Full Report

S surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]

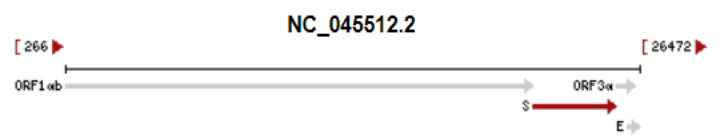
Gene ID: 43740568, updated on 31-Mar-2020

Summary

Gene symbol	S
Gene description	surface glycoprotein
Locus tag	GU280_gp02
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Severe acute respiratory syndrome coronavirus 2 (isolate: Wuhan-Hu-1, nat-host: Homo sapiens)
Lineage	Viruses; Riboviria; Nidovirales; Coronidovirineae; Coronaviridae; Orthocoronavirinae; Betacoronavirus; Sarbecovirus
Also known as	spike glycoprotein

Genomic context

Sequence: NC_045512.2 (21563..25384)



Genomic regions, transcripts, and products

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Related information

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Conserved domains on [gi|1796318598|ref|YP_009724390.1|]

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surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]

Graphical summary

☐ Zoom to residue level

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Query seq.

Superfamilies

1250

25050075010001273

Spike_rec_bindCorona_S2

Search for similar domain architectures

Refine search

List of domain hits

	Name	Accession	Description	Interval	E-value
[+]	Corona_S2 super family	cl20218	Coronavirus S2 glycoprotein; The coronavirus spike glycoprotein forms the characteristic ...	662-1270	0e+00
[+]	Spike_rec_bind	pfam09408	Spike receptor binding domain. Spike is an envelope glycoprotein which aids viral entry into ...	330-583	4.39e-79

References:

Marchler-Bauer A et al. (2017), "CDD/SPARCLE: functional classification of proteins via subfamily domain architectures.", **Nucleic Acids Res.**45(D)200-3.

Marchler-Bauer A et al. (2015), "CDD: NCBI's conserved domain database.", **Nucleic Acids Res.**43(D)222-6.

Marchler-Bauer A et al. (2011), "CDD: a Conserved Domain Database for the functional annotation of proteins.", **Nucleic Acids Res.**39(D)225-9.

Marchler-Bauer A, Bryant SH (2004), "CD-Search: protein domain annotations on the fly.", **Nucleic Acids Res.**32(W)327-331.

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1) Найдите последовательность и скажите **NCBI Reference Sequence id NUMBER**: для Mycobacterium virus D29 - gp10 гена.

2) Найдите домен данного белка и укажите его **первую и последнюю аминокислоты**.

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