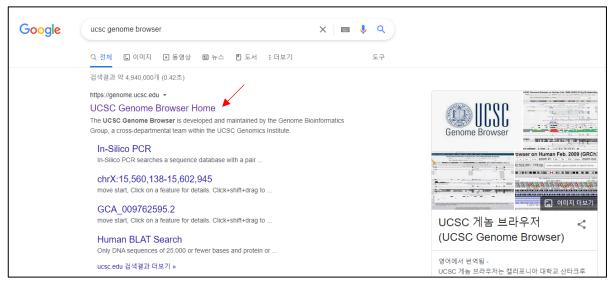
# **Molecular Biology Laboratory**

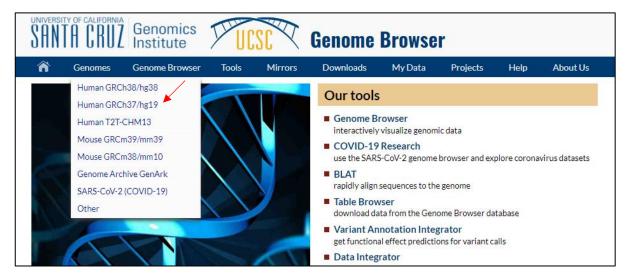
Bioinformatics and Genomics Lab.

## Week1. DNA / RNA Sequence Alignment (BLAST) & Genome Browser

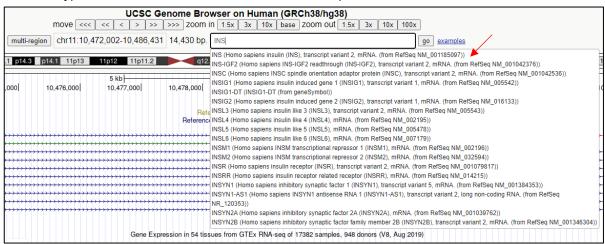
- 1. Practice how to use UCSC Genome Browser
- Access to the UCSC Genome Browser and find human insulin gene
  - Search "UCSC Genome Browser" in google and access to genome browser.



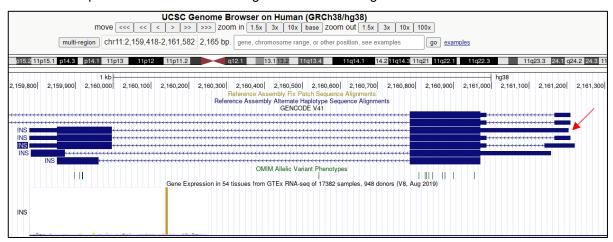
➤ Hover the mouse pointer to "Genomes" and select genome version. We will use "Human GRC38/hg38" in our practical exercise.



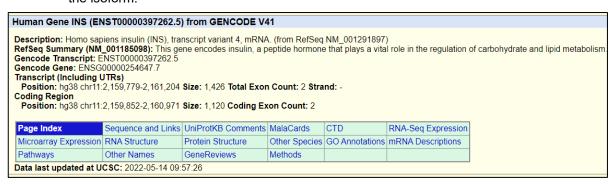
Type "INS" search window and select "Homo sapiens insulin".



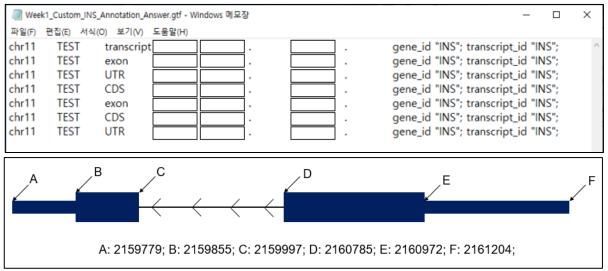
Compare Isoforms of the insulin gene and look through other information.



- Make custom gene annotation and visualize
  - ➤ Click the marked insulin isoform, "ENST00000397262.5", and look through the information of the isoform.

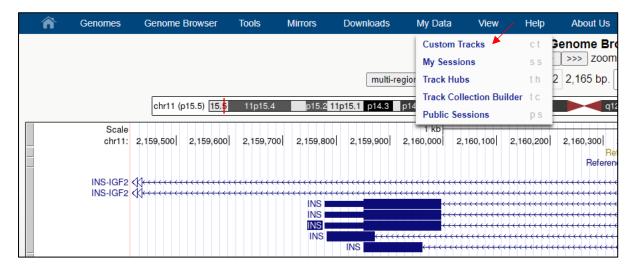


- Open notepad and save it as "Custom\_INS\_Annotation.gtf".
- Fill the blank with reference to the below information.

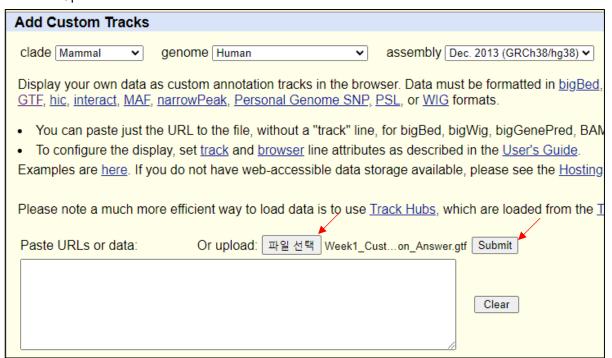


#### Cautions

- ✓ Each row is arranged in ascending order based on the start position.
- ✓ There is a difference of 1 between the end position of UTR and the end position of CDS.
- ✓ Each column is separated by a tab, but gene id and transcript id are written in one column and they are separated by space.
- For uploading custom a annotation file to the genome browser, hover the mouse on "My Data" and click "Custom Tracks".



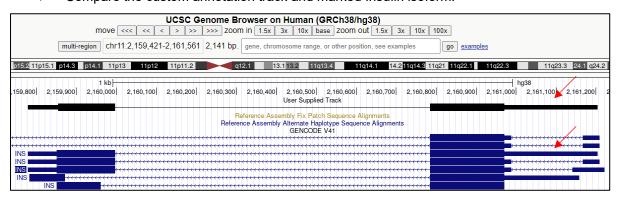
Upload the custom annotation file and click "Submit".



After uploading, click "go" to visualize the custom annotation track.

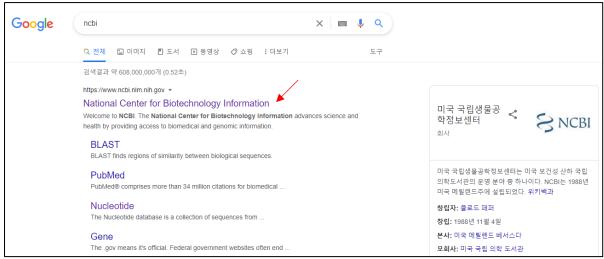


> Compare the custom annotation track and marked Insulin isoform.

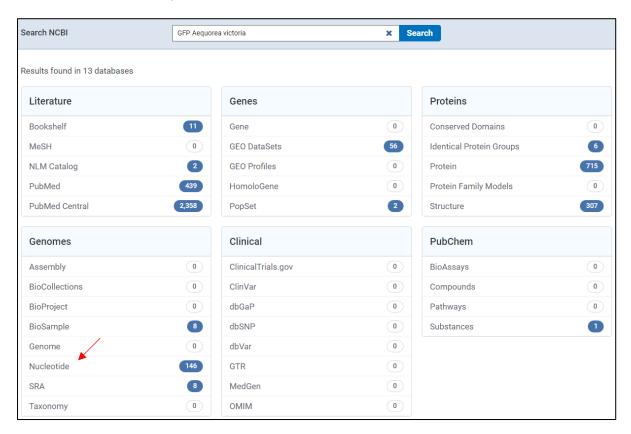


### 2. Practice how to get sequence data and use BLASTN

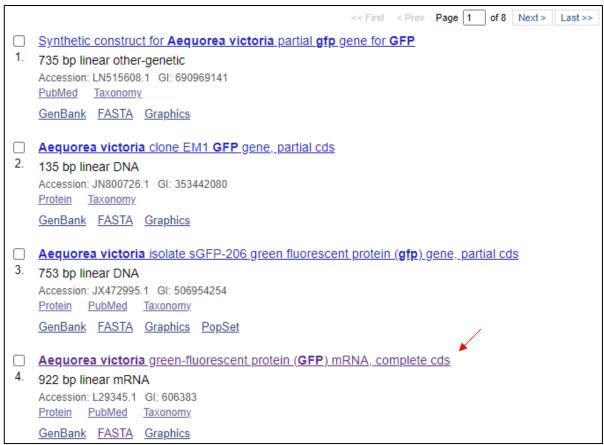
- Find GFP gene sequence and align the sequence
  - Search "NCBI" in google and access NCBI.



Search "GFP Aequorea victoria" and click "Nucleotide".



Select one from the list. We will use 4<sup>th</sup> one, "Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds" with 922 bp linear mRNA in a practical exercise.

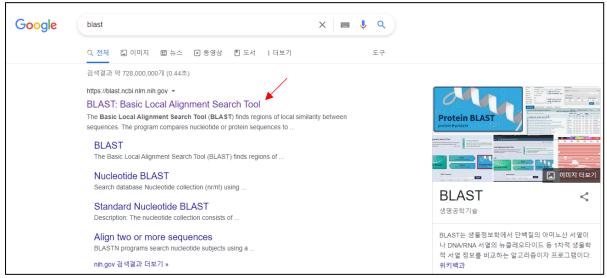


Click "FASTA" to get the gene sequence of GFP.

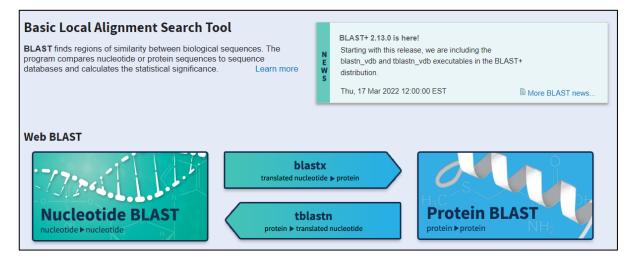
```
Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds
GenBank: L29345.1
FASTA Graphics
Go to: ✓
                                   922 bp
                                            mRNA
                                                   linear INV 30-DEC-1994
DEFINITION Aequorea victoria green-fluorescent protein (GFP) mRNA, complete
           cds.
ACCESSION
           L29345
VERSION
           L29345.1
KEYWORDS:
           aequorin; bioluminescence; energy acceptor protein;
           green-fluorescent protein.
           Aequorea victoria
 ORGANISM <u>Aequorea victoria</u>
           Eukaryota; Metazoa; Cnidaria; Hydrozoa; Hydroidolina; Leptothecata;
           Aequoreidae; Aequorea.
          1 (bases 1 to 922)
REFERENCE
 AUTHORS Inouye, S. and Tsuji, F.I.
           Aequorea green fluorescent protein. Expression of the gene and
 TITLE
           fluorescence characteristics of the recombinant protein
 JOURNAL
           FEBS Lett. 341 (2-3), 277-280 (1994)
  PUBMED
           8137953
REFERENCE
           2 (bases 1 to 922)
 AUTHORS
           Inouye,S. and Tsuji,F.I.
           Evidence for redox forms of the Aequorea green fluorescent protein
 TITLE
 JOURNAL
          FEBS Lett. 351 (2), 211-214 (1994)
  PUBMED
           8082767
```

#### Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds GenBank: L29345.1 GenBank Graphics >L29345.1 Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTT GTTGAATTAGATGCCGATGTTAATGGGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACAT ACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGGAAGCTACCTGTTCCATGGCCAACACTTGTCAC TACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAG AGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTACAAAGATGACGGGAACTACAAGACAC GTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGA AGATGGAAACATTCTTGGACACAAAATGGAATACAACTATAACTCACATAATGTATACATCATGGCAGAC AAACCAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTAAAGATGGAAGCGTTCAATTAG CAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTC CACACAATCTGCCCTTTCCAAAGATCCCAACGAAAAGAGAGATCACATGATCCTTCTTGAGTTTGTAACA AGATTCATTAAAATTTTATGAATAATTTATTGATGTTATTAATAGGGGCTATTTTCTTATTAAATAGGCT ACTGGAGTGTAT

Search "BLAST" in google and access BLAST.



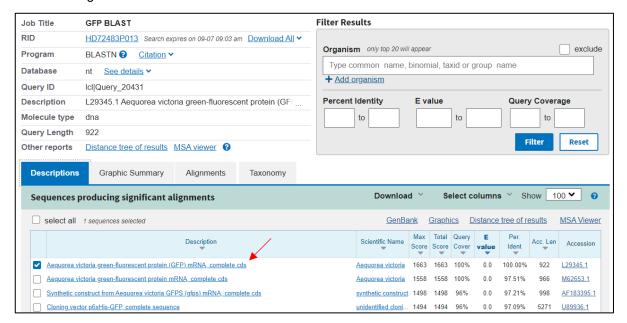
> Click "Nucleotide BLAST" (BLASTN).

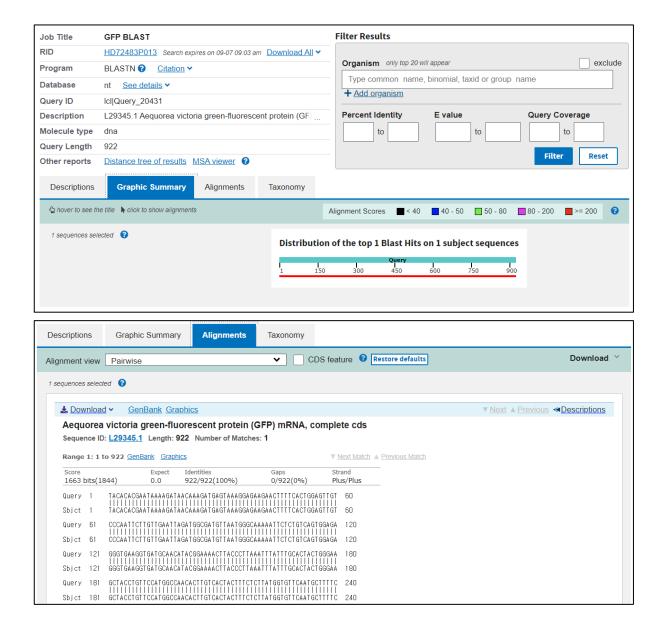


Copy and paste the GFP sequence to the query sequence box, and set the job title. When pasting the sequence, we must paste the header part too. There are many options, but we will use the default setting in the practical experiment. After then, select the program "BLASTN" and click "BLAST" for alignment.

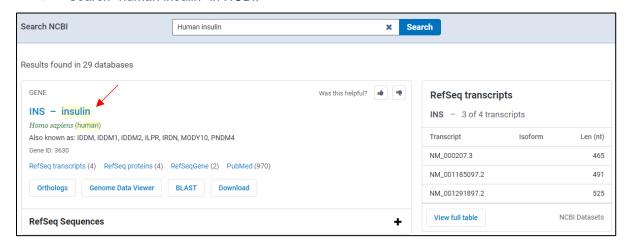
BLAST® >> blasts   b		
BLASTN programs search nucleotide databases using a nucleotide quer  Enter Query Sequence  Enter accession number(s), gl(s), or FASTA sequence(s) ⓒ clear  1_23345_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_23345_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_23345_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_2345_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_245_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_245_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_245_1 Aeguorea victoria green-fluorescent protein (GFP) mRNA compilete data 1_245_1 Aeguorea victoria green-fluorea victoria protein green-		BLAST® » blastn suite
Enter Query Sequence Enter accession number(s), gi(s), or FASTA sequence(s) ② clear  > 129345.1 Aeguorea victoria green-fluorescent protein (GEP) mRNA, compilete \$\frac{3}{2}\$ AAGACACAAGATAACAAGATAACAAGATAAGATAAGAAGAGAGAAGAACATITICAC TOR, upload file  Job Title  GFP BLAST Enter a descriptive title for your BLAST search ②  Align two or more sequences ③  Choose Search Set  Database  ③ Standard databases (nr etc.): \( \) rRNA/ITS databases \( \) Genomic + transcript databases \( \) Betacoronavirus  Nucleotide collection (nr/nt)  Organism Optional  Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ②  Exclude Optional Limit to Optional Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ②  Exclude Optional Limit to Optional Enter Cuery Optional Enter an Enterz query to limit search ②  Program Selection  Optimize for  Highly similar sequences (megablast) ⑤ somewhat similar sequences (discontiguous megablast) ⑥ somewhat similar sequences (discontiguous megablast) ⑥ somewhat similar sequences (discontiguous megablast) ⑥ somewhat similar sequences (discontiguous megablast) Chopse a BLAST algorithm ②	<b>blastn</b> b	
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Choose Search Set	Enter Query	Sequence
TACACACGATAAAAGATAACAAAGATGAGTAAAGGAGAAAGAA	Enter accession	number(s), gi(s), or FA STA sequence(s) 😯 Clear 🗡 Query subrange 😯
TGGAGTTGTCCCATTCTT  Or, upload file  Job Title  GFP BLAST  Enter a descriptive title for your BLAST search ②  Align two or more sequences ③  Choose Search Set  Database  ③ Standard databases (nr etc.): ☐ rRNA/ITS databases ⑤ Genomic + transcript databases ⑥ Betacoronavirus  Nucleotide collection (nr/int)  Organism Optional Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ②  Exclude Optional Limit to Optional Enter Query Optional Entrez Query Optional Enter are Entrez query to limit search ③  Program Selection  Optimize for Highly similar sequences (megablast)  More dissimilar sequences (discontiguous megablast) ⑤ Somewhat similar sequences (blastin)  Choose a BLAST algorithm ②	complete cds	From
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Enter a descriptive title for your BLAST search  Align two or more sequences  Set  Choose Search Set  Database  Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases   Nucleotide collection (nr/nt)  V  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 Limit to Optional Entrez Query to limit search  More dissimilar sequences (megablast) More dissimilar sequences (discontiguous megablast) Somewhat similar sequences (blastn) Choose a BLAST algorithm  Choose a Choose a BLAST algorithm  Choose a Choose a BLAST algorithm  Choos		파일 선택 선택된 파일 없음
Align two or more sequences   Choose Search Set  Database  Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronavirus  Nucleotide collection (nr/nt)  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 Limit to Sequences from type material Optional Enter an Entrez query to limit search   Program Selection  Optimize for Highly similar sequences (discontiguous negablast) More dissimilar sequences (blastn) Choose a BLAST algorithm   Choose a BLAST algorithm   Organism  Enter organism name or id—completions will be suggested exclude add organism  Exclude Optional  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Exclude Optional  Limit to Sequences (binding organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Exclude Optional  Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  Exclude Optional  Enter organism name or id—completions will be suggested exclude exclude Add organism  Exclude Optional  Enter organism name or id—completions will be suggested exclude exclude exclude exclude exclude exclude ex	Job Title	
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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 Limit to Optional Entrez Query Optional  Enter an Entrez query to limit search  Optimize for  Highly similar sequences (discontiguous megablast)  Somewhat similar sequences (blastn)  Choose a BLAST algorithm   Nucleotide collection (nr/nt)  Enter organism name or id—completions will be suggested exclude Add organism  exclude Optional  Enter organism name or id—completions will be suggested exclude Add organism  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  Limit to Optional  Entrez Query Optional  Optional  Program Selection  Optimize for  Highly similar sequences (discontiguous megablast)  Somewhat similar sequences (blastn)	Database	Standard databases (nr etc.): O rRNA/ITS databases O Genomic + transcript databases O Betacoronavirus
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  Limit to Optional  Entrez Query Optional  Entrez Query Optional  Finter an Entrez query to limit search   Program Selection  Optimize for Highly similar sequences (discontiguous megablast)  Somewhat similar sequences (blastn)  Chopse a BLAST algorithm   Enter organism name or id—completions will be suggested exclude Add organism  Enter organism name or id—completions will be suggested exclude Add organism  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   Sequences from type material  Vou Tithe Create custom database  Enter an Entrez query to limit search   Optimize for Highly similar sequences (discontiguous megablast)  Optimize for Highly similar sequences (discontiguous megablast)  Chopse a BLAST algorithm   Chopse a BLAST algorithm   Optimize for Highly similar sequences (blastn)		Nucleotide collection (nr/nt)
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Optimize for  Highly similar sequences (megablast)  More dissimilar sequences (discontiguous megablast)  Somewhat similar sequences (blastn)  Chopse a BLAST algorithm ?	Optional	Enter an Entrez query to limit search
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BLAST Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar sequences)		Choose a BLAST algorithm 🚱
BLAST Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar sequences)		
Show results in a new window	BLAST	

We can see the alignment result. There are many similar sequences in the database, but the first one is the best-matched sequence. Select it and see "Graphic Summary" and "Alignments".





- Find insulin gene sequences of human and pig, and compare the two sequences
  - Search "human insulin" in NCBI.



Go below and click "FASTA" to get the gene sequence of INS.



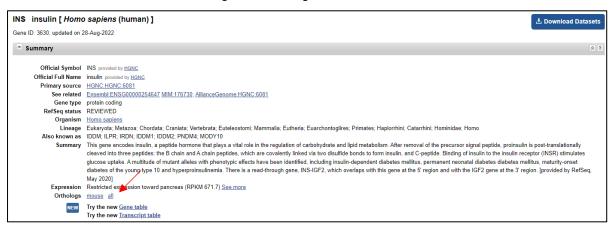
# Homo sapiens chromosome 11, GRCh38.p14 Primary Assembly

NCBI Reference Sequence: NC\_000011.10

#### GenBank Graphics

>NC\_000011.10:c2161209-2159779 Homo sapiens chromosome 11, GRCh38.p14 Primary Assembly AGCCCTCCAGGACAGGCTGCATCAGAAGAGGCCATCAAGCAGGTCTGTTCCAAGGGCCTTTGCGTCAGGT GGGCTCAGGATTCCAGGGTGGCTGGACCCCAGGCCCCAGCTCTGCAGCAGGAGGACGTGGCTGGGCTCG CTGTCTCCCAGATCACTGTCCTTCTGCCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTG GCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAG CTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCGGGGAGGCAGAGGACCT GCAGGGTGAGCCAACTGCCCATTGCTGCCCCTGGCCGCCCCAGCCACCCCCTGCTCCTGGCGCTCCCAC CCAGCATGGGCAGAAGGGGGCAGGAGGCTGCCACCCAGCAGGGGGTCAGGTGCACTTTTTTAAAAAGAAG TTCTCTTGGTCACGTCCTAAAAGTGACCAGCTCCCTGTGGCCCAGTCAGAATCTCAGCCTGAGGACGGTG TGCCCCGCAGCCCATTTCTCCACCCTCATTTGATGACCGCAGATTCAAGTGTTTTGTTAAGTAAAGTCCT GGGCGTGGCTGCCTGAGTGGGCCAGACCCCTGTCGCCAGGCCTCACGGCAGCTCCATAGTCAGGAG ATGGGGAAGATGCTGGGGACAGGCCCTGGGGAGAAGTACTGGGATCACCTGTTCAGGCTCCCACTGTGAC GCTGCCCGGGGCGGGGAAGGAGGTGGGACATGTGGGCGTTGGGGCCTGTAGGTCCACACCCAGTGTGG AGGCGGGCACTGTGTCTCCCTGACTGTGTCCTCCTGTGTCCCTCTGCCTCGCCGCTGTTCCGGAACCTGC CCTTGGCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT CTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCCCCCACACCCGCCGCCTCCTGCACC GAGAGAGATGGAATAAAGCCCTTGAACCAGC

Click "all" to see the orthologs of the INS gene.



Click "INS" of pig and get gene sequence in the same way.

Species	Gene	Architecture	. aa	$\stackrel{\triangle}{\forall}$
Homo sapiens human	INS insulin	—	110	~
Mus musculus house mouse	Ins2 insulin II		110	~
Rattus norvegicus Norway rat	Ins2 insulin 2		119	~
Bos taurus cattle	INS insulin		221	~
Sus scrofa pig	INS insulin		175	~
Gallus gallus chicken	INS insulin	—	107	~

NCBI Reference Sequence: NC\_010444.4

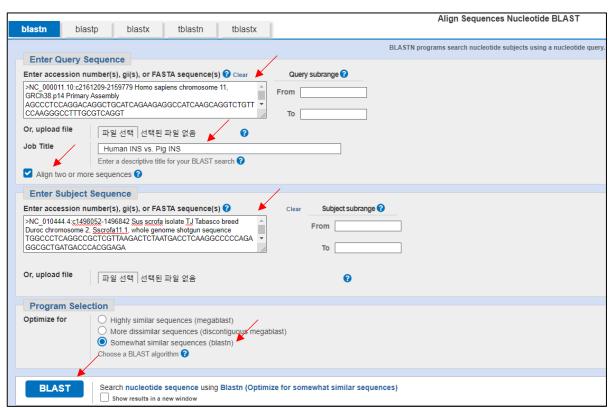
GenBank Graphics

>NC\_010444.4:c1498052-1496842 Sus scrofa isolate TJ Tabasco breed Duroc chromosome 2,

Sscrofall.1, whole genome shotgun sequence

TGGCCCTCAGGCCGCTCGTTAAGACTCTAATGACCTCAAGGCCCCCAGAGGCGCTGATGACCCACGGAGA TGATCCCGCAGGCCTGGCAGCAGGGAAATGATCCAGAAAGTGCCACCTCAGCCCCCAGCCATCTGCCACC CAGCCCTCTGGGACCAGCTGCGTTCCCAGGCCGCCGGCAAGCAGGTCTGTCCCCCTGGGCTCCCGTCAGC TGGGTCTGGGCTGTCCTGCTGGGGCCAGGGCATCTCGGCAGGAGGACGTGGGCTCCTCTCTCGGAGCCCT CACCCCCGCCATGGCCCTGTGGACGCGCCTCCTGCCCCTGCTGGCCCTGCTGGCCCTCTGGGCCCCCGC CCCGGCCCAGGCCTTCGTGAACCAGCACCTGTGCGGCTCCCACCTGGTGGAGGCGCTGTACCTGGTGTGC GGGGAGCGCGCTTCTTCTACACGCCCAAGGCCCGTCGGGAGGCGGAGAACCCTCAGGGTGAGCCGAGGG GGCGTCCCGGGAGCGGTCGGGGGAGTTTTTAAGGAGGAAATTGGTAAAAGTGACCAACTCCCTGGGAGCT GCGGCTCCAGGGAGGAATCTTACGGAGTCAAGGCCCGGGTGCCGCTGGTCTCCGAGTGACATGGCCGTGG TGTCCCGTCTGCCGGCCCACATGCCCGTGGGAGATGCCCCATCCCCCTGGGAGGGGGCCCCGTGCCGGGC AGGCAGCGGGAGGCCCAGGACCGGCGGCTGCTGCGGCTTCCACTCCAGGGTGGGCGGGGTGGGGGGTGGC TGTCTCTGTGTGACCGGCTCTCCCCGCAGCAGGTGCCGTGGAGCTGGGCGGAGGCCTGGGCGGCCTGCAG GCCCTGGCGCTGGAGGGGCCCCCGCAGAAGCGTGGCATCGTGGAGCAGTGCTGCACCAGCATCTGTTCCC TCTACCAGCTGGAGAACTACTGCAACTAGGCCGCCCCTGAGGGCGCCTGCTGCTCCCCGCACCCCAAAAC CCAATAAAGTCCTGAATGAGC

Go to BLASTN and check the "Align two or more sequences". Paste the human sequence in the query sequence and paste the pig's sequence in the subject sequence. After then, select "blastn" for the program, and click "BLAST".



- We can see how similar human's and pig's insulin gene sequence from the result.

