Enhancing Sequence Search Efficiency by Using STNext®

Jim Brown (FIZ), John Kratunis (CAS)

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Agenda

- Database Overview
- Steps for a CAS Registry BLAST® Sequence Search
- Create a BLAST Alignment Report
- Substructure Search on a Short Sequence
- Step-by-step through a multi-database BLAST search
- Post-processing of a multi-step BLAST search
- Overview of results
- Summary

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Substance databases containing sequence information

- CAS REGISTRY®
  - Contains >71M biosequences
- DGENE (Derwent GENESEQ™)
  - Contains >46M DWPI biosequences
- GENBANK®
  - Contains >230M sequences
- PCTGEN
  - Contains >16M WIPO biosequences
- USGENE®
  - Contains >61M USPTO biosequences

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CAS REGISTRY supports searching sequences

- NCBI BLAST similarity searching on STNext
  - Via the BLAST Module for REGISTRY/Caplus℠
  - Available via the command line for USGENE, PCTGEN, and DGENE
- Structure search for shorter sequences
- Text-based searching
  - Sequence code match (motif): exact, family and subsequence
  - Name fragments
  - Chemical modifications

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Steps for a REGISTRY BLAST Sequence Search

1. Download the stand-alone BLAST module
2. Logon to BLAST module and complete the BLAST search
3. View Results and Select desired sequences
4. Get STN® Data Script (.scb) and alignment data (.xss)
5. Import into scripts folder on STNext
6. Run script to get sequence records in REGISTRY
7. Cross-file search into CAplus℠
8. Display BIB ABS HITRN
9. Create BLAST alignment report from Transcripts folder

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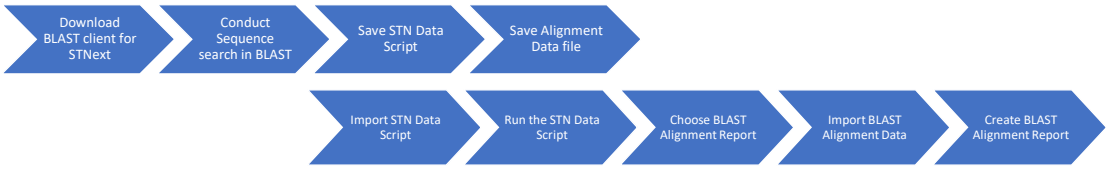
CAS Registry BLAST Searching: Example

Search Question:

Locate references that report protein sequences similar to CAS RN 642514-19-0.



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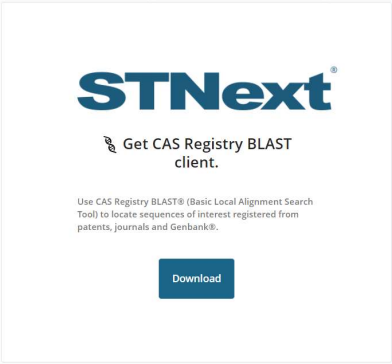
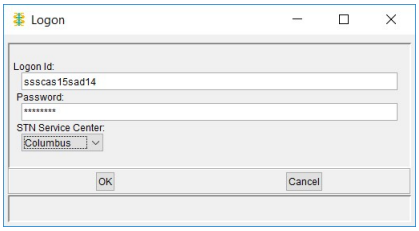
NCBI BLAST for CAS REGISTRY / CAplus

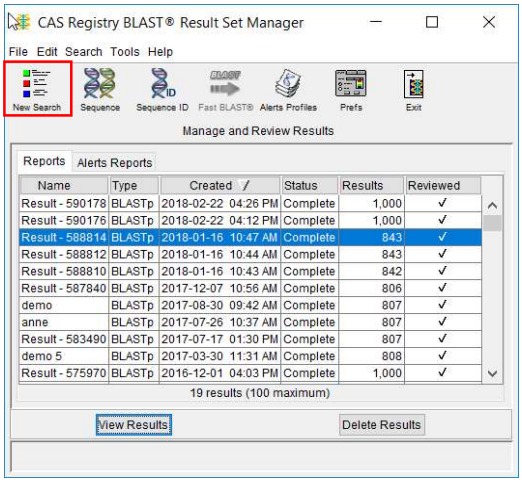
- To download the separate BLAST Module:
  - Login to STNext at the following site https://next.stn.org/stn/downloads/blast-download.html
  - Click to download



Launch CAS Registry BLAST and Logon



CAS Registry BLAST Result Set Manager



The Result Set Manager is the starting point
- To begin a new sequence search
- To review results of previous sequence searches

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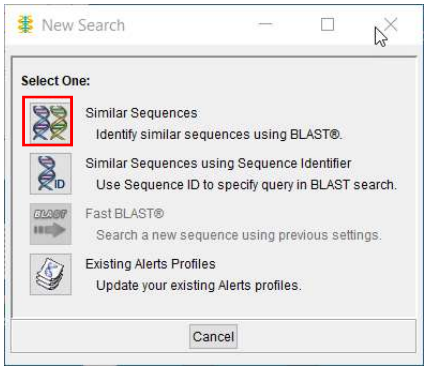
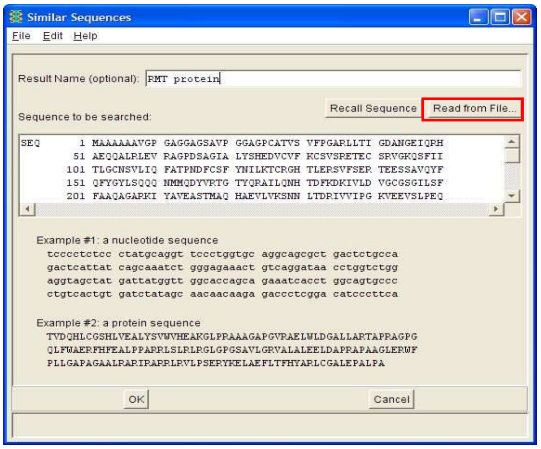
Select search option and input the search query Read from File Option (for text files)





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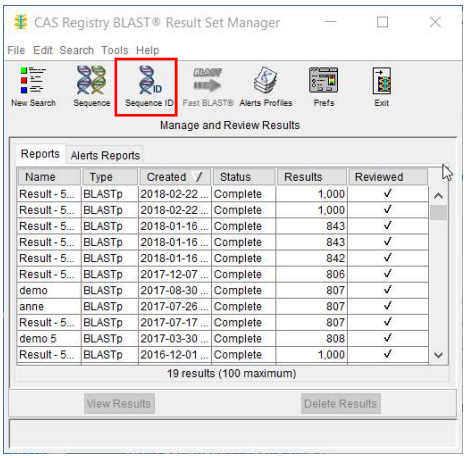
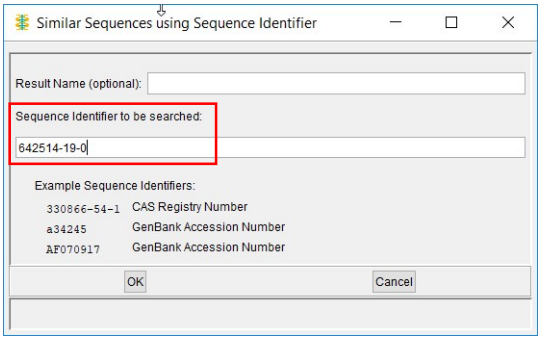
Alternatively: Search via the Sequence Identifier





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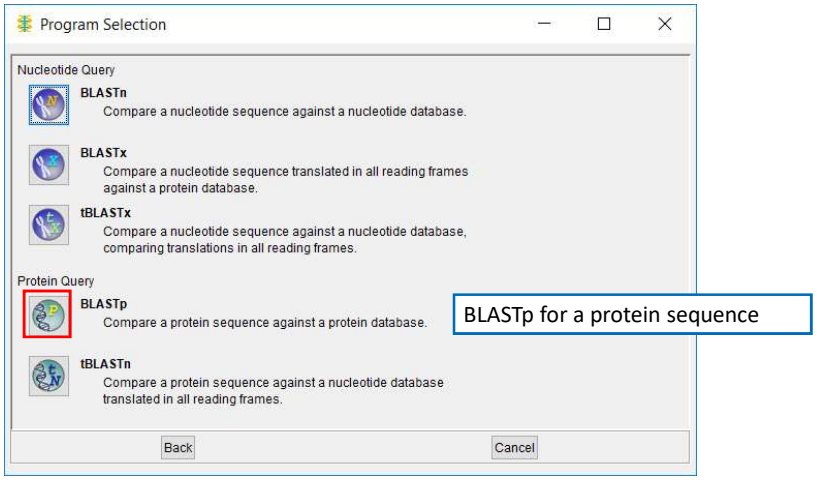
Select the BLAST program



BLASTp for a protein sequence

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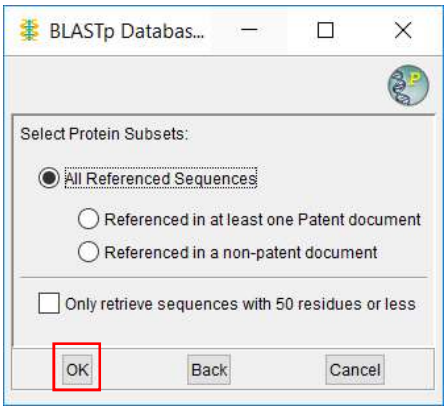
Select the segment of the database to search



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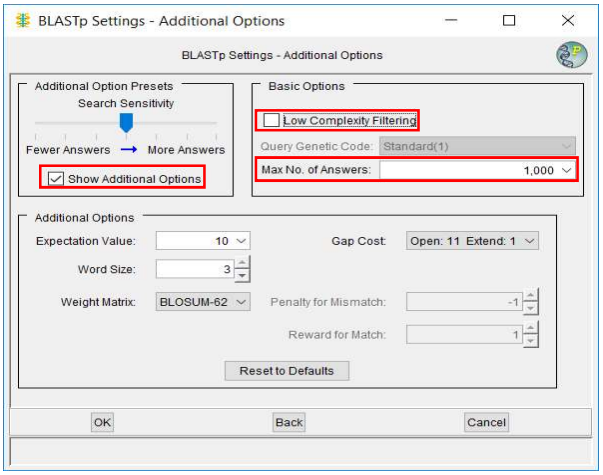
Verify BLAST settings; Launch the search



Recommended settings for patent searches:

- Low Complexity Filtering – unchecked
- Max No. of Answers - 1000

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NCBI recommended settings* for searching small sequence queries

Peptide sequences

- E-value: 20,000
- Word size: 2
- Matrix: PAM-30
- Gap cost: 9 and 1

Nucleotide sequences

- E-value: 1,000
- Word size: 7
- Matrix: Leave as is
- Gap cost: n/a

NCBI definition of "small" is less than 16 peptides, or less than 20 nucleotides.

* http://www.ncbi.nlm.nih.gov/blast/Why.shtml

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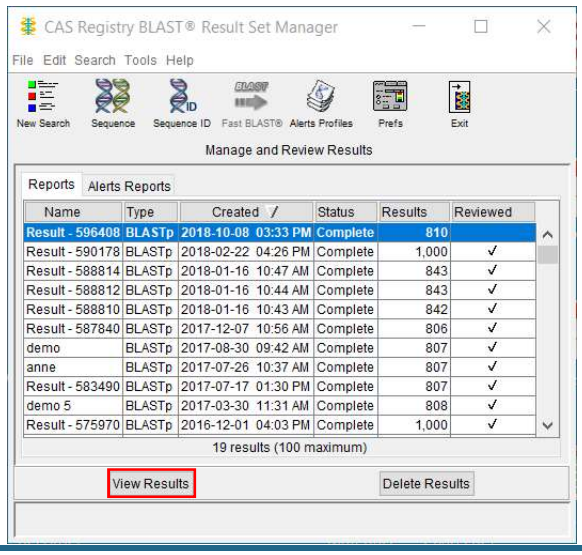
View results

Highlight the result set to be viewed, and click on View Results.



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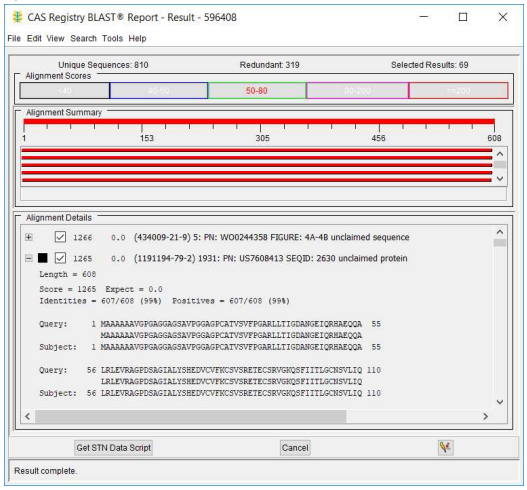
Evaluate the alignment report

Click on + to show details including sequence length, score, and percent identity.



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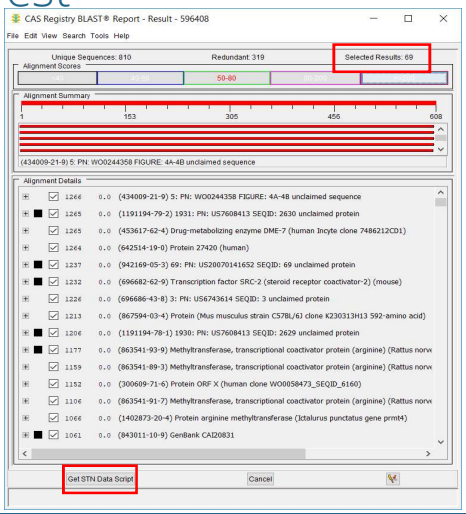
Select sequences of interest

Sequences can be selected:
- In groups, using a bar in the Alignment Scores
- Individually, by selecting the check box

To transfer the sequence data to STN, click the Get STN Data Script button.



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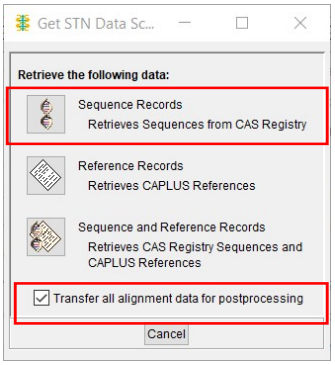
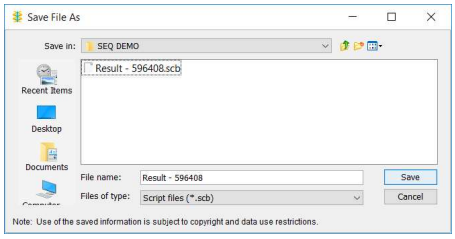
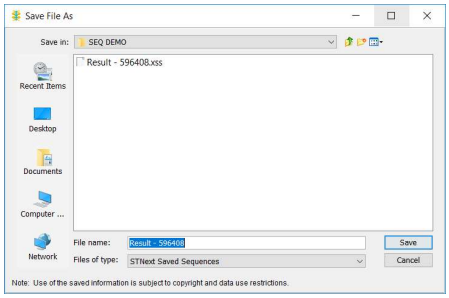
Get STN Data (.scb file) and Save Alignments (.xss file)







Alignment data needs to be transferred for post-processing.

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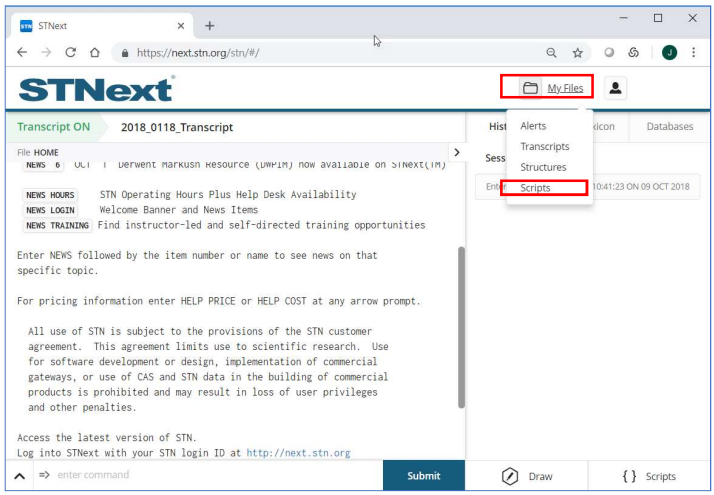
Logon to STNext

Select “Scripts” from the “My Files” menu



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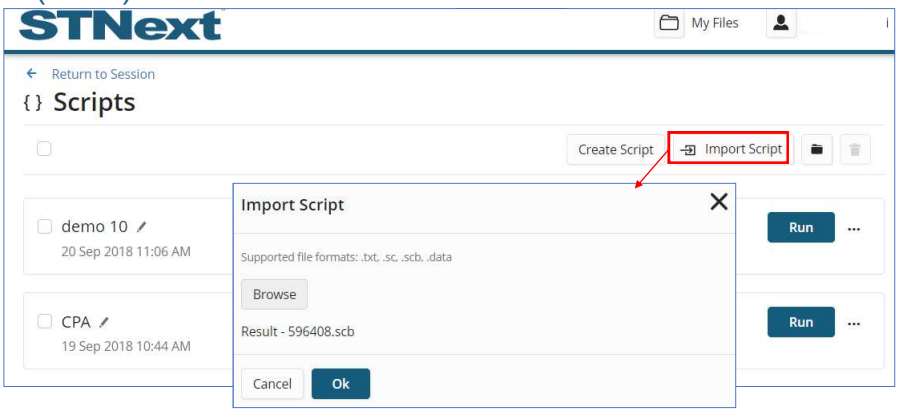
Select Import Script and Browse to find Sequence Data Script (.scb)



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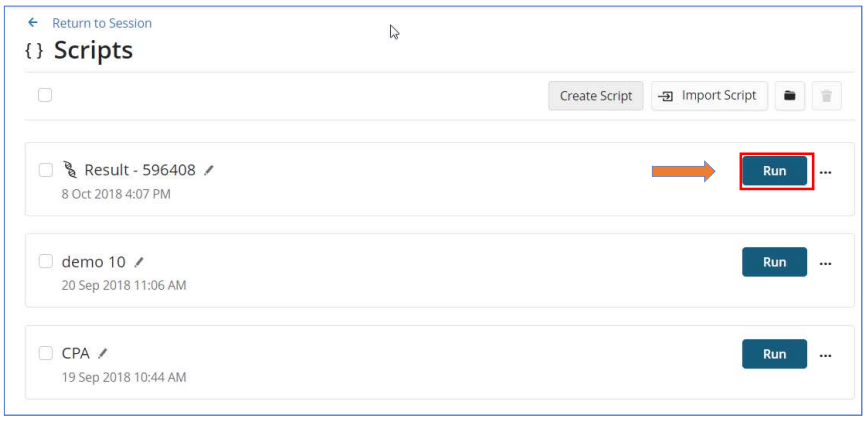
Select RUN; the script will find sequences in REGISTRY



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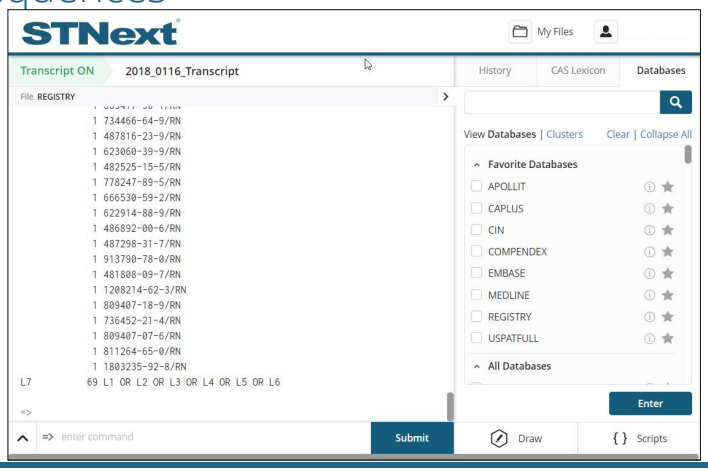
Search retrieves the CAS Registry Numbers® of the selected sequences



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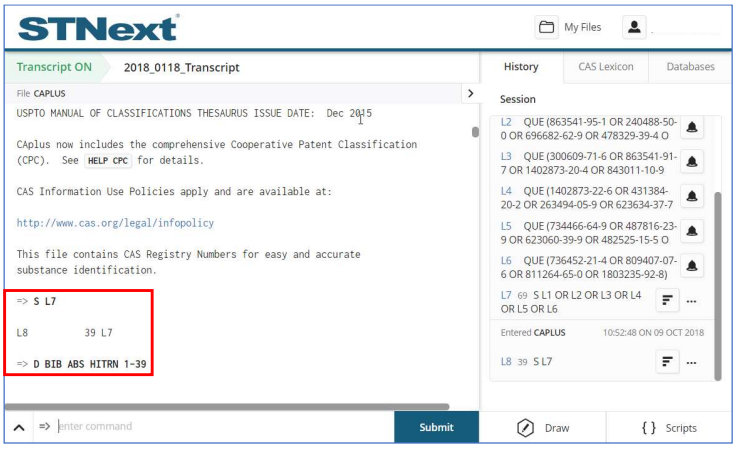
Search last L-number in CAplus and display references using the BIB ABS HITRN formats



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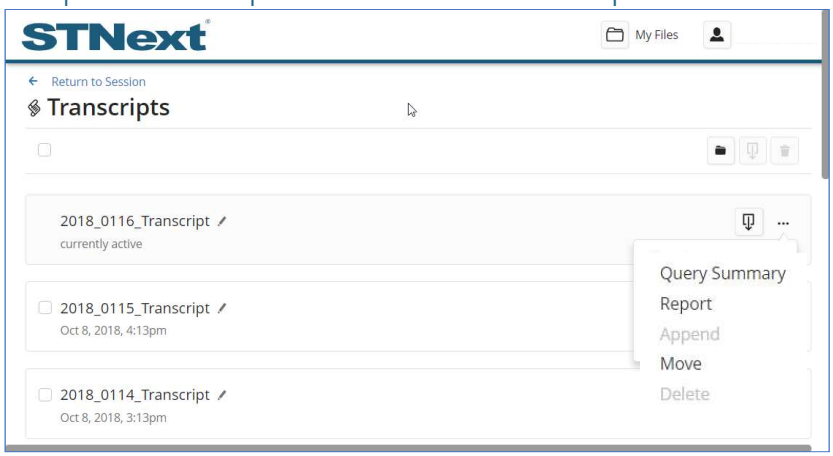
Create BLAST Alignment Report
Step 1: Request a Report in the Transcripts Menu



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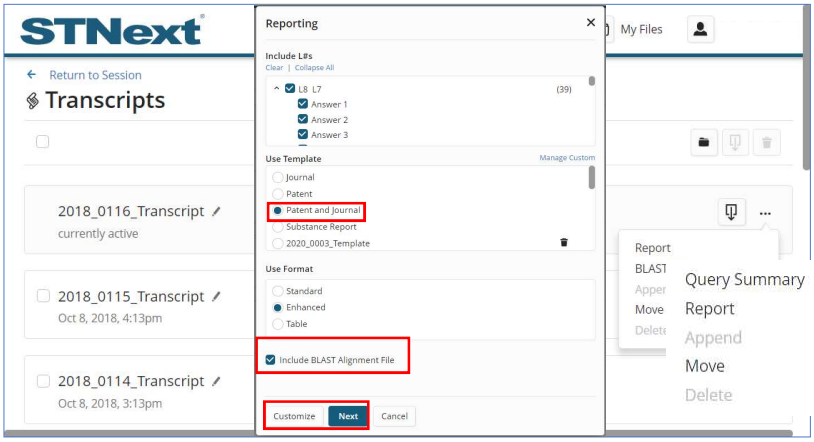
Step 2: Select the L#, Template, Format, and check the "Include BLAST Assignment File" option



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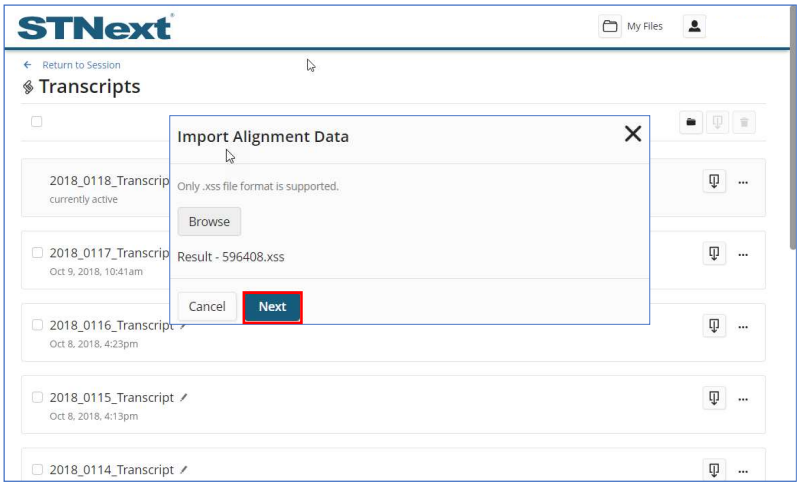
Step 3: Import Alignment Data (.xss file)



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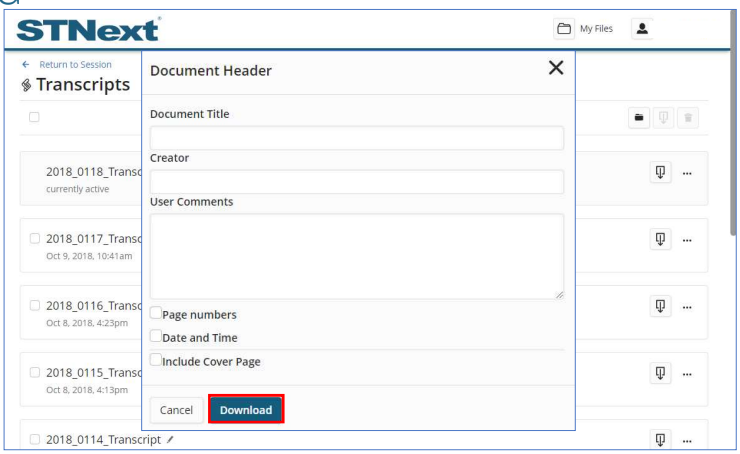
Step 4: Add Document Header Options, and Download



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The BLAST alignment report contains the bibliographic record and sequence alignment

L8 Patent | English | 4/39

Crystal structure of human CARM1 methyltransferase for use in identification of modulators of CARM1 activity and rational drug design

PatentPak PDF | Full-text

Accession Number: 2008:1278320 CAPLUS
Inventor Name: Foreman, Kenneth William; Shaaban, Salam; Park, Frances E.; Sauder, Micha
Patent Assignee: OSI Pharmaceuticals, Inc., USA; SGX Pharmaceuticals, Inc.
Document Number: *149:488720*
Family Accession Number Count: *1*
Source: *PCT Int. Appl., 324pp.*
CODEN: PIXXD2
PatentPak Information:

Patent No.	Kind	Date	Language	Patent
WO 2008128050	A2	20081023	English	PDF

Patent Information:

Patent No.	Kind	Date	Application No.
WO 2008128050	A2	20081023	WO 2008-US60043
WO 2008128050	A3	20090226	
US 20080312298	A1	20081218	US 2008-101631

Index Terms and Role:
1072557-06-2D, complex with S-adenosyl-L-homocysteine 1072557-07-3D, complex with S-adenosyl-L-homocysteine
1072557-07-3
BLAST® Alignment Data

```
Length = 353    Score = 745    Expect = 0.0


 Score = 745  Expect = 0.0
 Identities = 353/353 (100%)  Positives = 353/353 (100%)

 Query:   128 RGHTLERSVFSERTEESSAVQYFQFYGYLSQQQNMMQDYVRTGTYQRAILQNHTD 182
              RGHTLERSVFSERTEESSAVQYFQFYGYLSQQQNMMQDYVRTGTYQRAILQNHTD
 Subject:   1 RGHTLERSVFSERTEESSAVQYFQFYGYLSQQQNMMQDYVRTGTYQRAILQNHTD  55

 Query:   183 FKDKIVLDVGCGSGILSFFAAQAGARKIYAVEASTMAQHAEVLVKSNNLTDRIVV 237
              FKDKIVLDVGCGSGILSFFAAQAGARKIYAVEASTMAQHAEVLVKSNNLTDRIVV
 Subject:  56 FKDKIVLDVGCGSGILSFFAAQAGARKIYAVEASTMAQHAEVLVKSNNLTDRIVV 110

 Query:   238 IPGKVEEVSLPEQVDIIISEPMGYMLFNERMLESYLHAKKYLKPSGNMFPTIGDV 292
              IPGKVEEVSLPEQVDIIISEPMGYMLFNERMLESYLHAKKYLKPSGNMFPTIGDV
 Subject: 111 IPGKVEEVSLPEQVDIIISEPMGYMLFNERMLESYLHAKKYLKPSGNMFPTIGDV 165

 Query:   293 HLAPFTDEQLYMEQFTKANFWYQPSFHGVDLSALRGAAVDEYFRQPVVDTFDIRI 347
              HLAPFTDEQLYMEQFTKANFWYQPSFHGVDLSALRGAAVDEYFRQPVVDTFDIRI
 Subject: 166 HLAPFTDEQLYMEQFTKANFWYQPSFHGVDLSALRGAAVDEYFRQPVVDTFDIRI 220
```

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Substructure search on a short sequence

- Another search option, distinct from percent match or sequence match
- Many short sequences are in REGISTRY as drawn structures
- You can search a known sequence to find overlapping matches
- Use the drawing tool to indicate specific variations in a known sequence that you'd like to search

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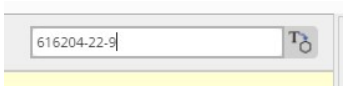
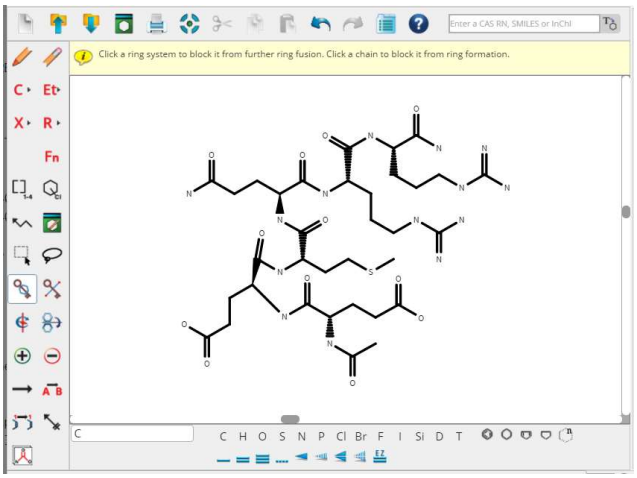
Example: Argireline (616204-22-9), a topical mimetic of Botox



The "text to structure" tool converts CAS Registry Numbers, SMILES and InChi strings into structures



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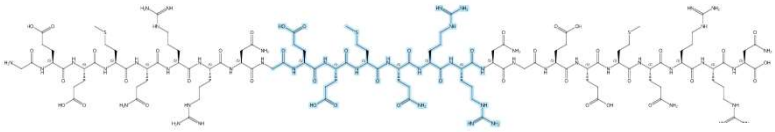
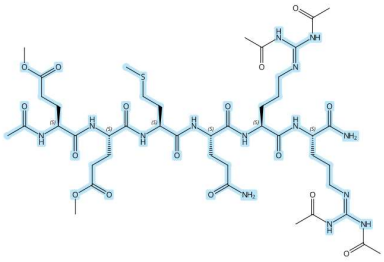
A substructure search finds overlapping short sequences



Absolute stereochemistry shown



Absolute stereochemistry shown

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Sequence searchable databases on STN

- DGENE (Derwent GENESEQ™)*
- USGENE
- PCTGEN
- CAS REGISTRY *

*Sequences intellectually derived by indexers
(include unique sequences not disclosed in formal listings)

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DGENE (Derwent Geneseq)

- Value-added patent sequence data produced by Clarivate Analytics
  - Enhanced titles from DWPI
  - Concise one-line description of the sequence
  - Keyword indexing and abstract focused on sequence
  - Abstract providing information on sequence and context
  - Additionally: feature table (FEAT), patent sequence location (PSL),…
- Sequences from 1981 of the basic patents of the Derwent World Patents Index®, covering 47 patent-issuing authorities
- Nucleotides of 10 or more bases, amino acid sequences of 4 or more residues and primers and probes of any length
- Sequences intellectually derived by indexers
- Legal status data from INPADOCDB (D LS or LS2) directly displayable

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USGENE

- Protein (>3 aa) and nucleic acid (>9 nt) sequences from 1981 to date
- All available peptide and nucleic acid sequences from published applications and issued patents of USPTO
- USPTO consolidates four sources (/SSO)
- Bibliographic details including publication and priority details, assignee and inventor names
- Sequence details including one-line description, organism name, length, molecule type, sequence sourcse, feature table and patent sequence location (PSL) from 2005 onwards
- Original title, abstract and claims text (ECLM searchable)
- Updated weekly, within 3 days of publication

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PCTGEN

- All peptide and nucleic acid sequences electronically submitted to WIPO, 2001 to present
- Bibliographic details including publication and application details, assignee and inventor names
- Sequence details include molecule type, organism, sequence length, feature table
- Original published application title
- Records created from image format sequence listings are clearly marked („…created by using OCR…“)
- Updated weekly, within 1 day of publication

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CAS REGISTRY

- Produced by CAS
- Sequences from over 3,000 life science journals and 63 patent authorities, including WO US EP JP DE GB FR RU CA IN KR CN
- Sequence details include sequence type, sequence length, nucleic acid type, 1 and 3 letter amino acid code
- Unique sequence types (e.g. cyclic peptides, peptide-metal complexes,…)
- Sequences linked to value-added CAplus records by RNs

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Step-by-step through a multi-database BLAST search

Search example:

Find all patents disclosing the gene *CBP1* from the soil bacterium *Serratia marcescens*. CBP21 has been described in degradation of crystalline cellulose and chitin.

NAME: *Chitin-binding protein precursor CBP1*
GENE MODEL: *AY665558*
ORGANISM: *Serratia marcescens strain BJL200*
SEQUENCE TYPE: coding sequence (CDS)
SEQUENCE LENGTH: 594 bp
SEQUENCE: ATGAACAAAACTTCCCGTACCCTGCTCTCTCTGGGCCTGCTGAGCGCGGCCATGTTCGGCGTTTCGCAACA
GGCGAATGCCCACGGTTATGTCGAATCGCCGGCCAGCCGCGCCTATCAGTGCAAACTGCAGCTCAACACG
CAGTGCGGCAGCGTGCAGTACGAACCGCAGAGCGTCGAGGGCCTGAAAGGCTTCCCGCAGGCCGGCCCG
GCTGACGGCCATATCGCCAGCGCCGACAAGTCCACCTTCTTCGAACTGGATCAGCAAACGCCGACGCGCT
GGAACAAGCTCAACCTGAAAACCGGTCCGAACTCCTTTACCTGGAAGCTGACCGCGCGTCACAGCACCA…

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Two different procedures for sequence searching

- DGENE (Derwent Geneseq)
- USGENE
- PCTGEN

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- CAS REGISTRY

CAS REGISTRY BLAST

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Sequence searching in DGENE/USGENE/PCTGEN

DGENE/USGENE/PCTGEN

(0) FIL DGENE
(1) Import sequence in STNext
(2) Validate sequence in Biosequence Editor
(3) Upload sequence in sequence-database
(4) Verify if uploaded sequence is correct
(5) Run BLAST search (and decide how many answers to keep)
(6) Review search (e.g. D TRIAL ALIGN)
(7) Run BLAST in USGENE and PCTGEN
(8) Merge answer sets
(9) Sort results (SCORE, IDENT)
(10) Family sorting and display

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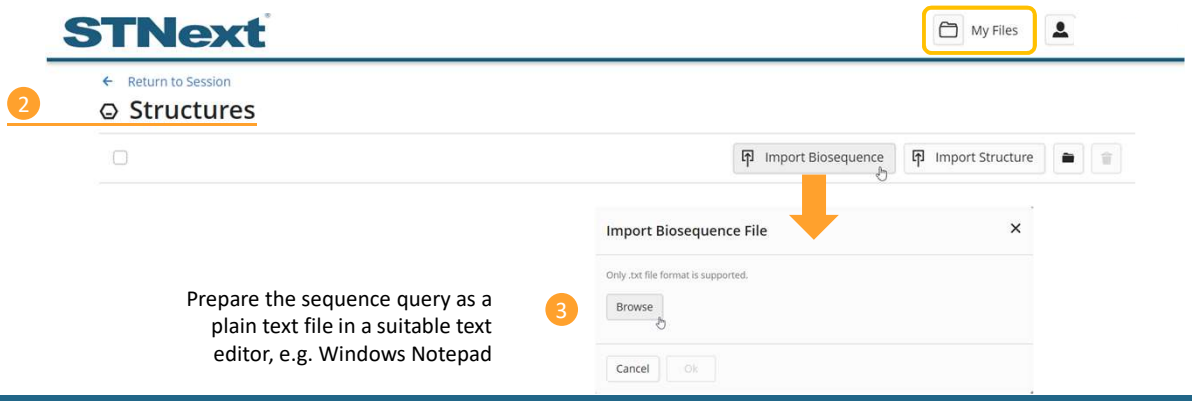
Import sequence for search in DGENE, USGENE and PCTGEN

1 => FIL DGENE



Prepare the sequence query as a plain text file in a suitable text editor, e.g. Windows Notepad

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Supported sequence formats

```
MSSPSLKWCF TLNYSSAAER ENFLSLLKEE DVHYAVVGDE VAPATGQKHL
QGYLSLKKRI RLGGLKKKYG SRAHWEIARG TDEENSKYCS KGTLILELGF
PVVNGSNKRK ISEMVARSPD RMKIEQPEIF HRYQSVNKLK KFKEEFVHPC
LDSPWQIQLT EAIDEEPDDR SIIWVYGPYG NEGKSTYAKS LIKKDWFYTR
```

plain text format

```
>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLVEW
WGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTF
```

FASTA format

```
  1 acaagatgcc attgtccccc ggcctcctgc tgctgctgct
 41 ctccggggcc acggccaccg ctgccctgcc cctggagggt
 81 ggccccaccg gccgagacag cgagcatatg caggaagcgg
121 caggaataag gaaaagcagc ctcctgactt tcctcgcttg
```

GENBANK format

```
acaagatgcc attgtccccc ggcctcctgc tgctgctgct       40
ctccggggcc acggccaccg ctgccctgcc cctggagggt       80
ggccccaccg gccgagacag cgagcatatg caggaagcgg       120
caggaataag gaaaagcagc ctcctgactt tcctcgcttg       160
```

EMBL format

Save as .TXT-file

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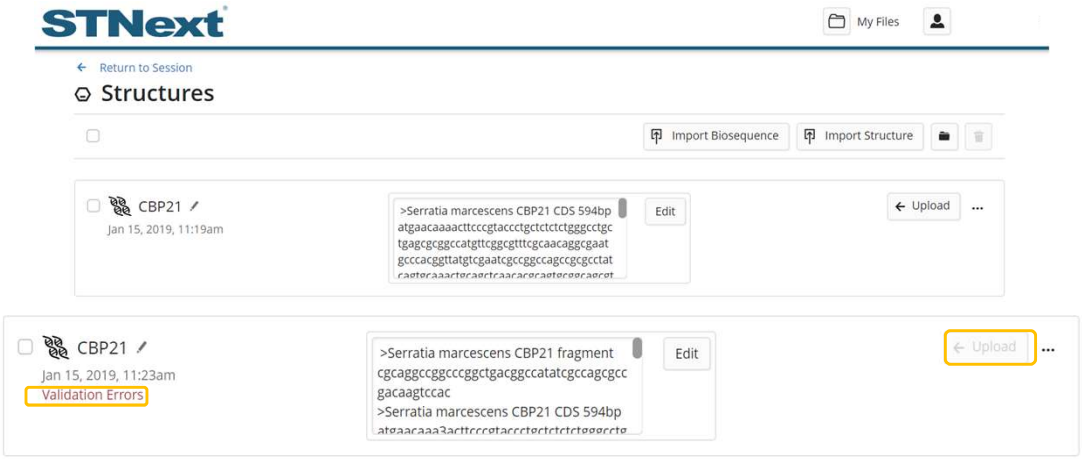
Sequences are stored under My Files/Structures



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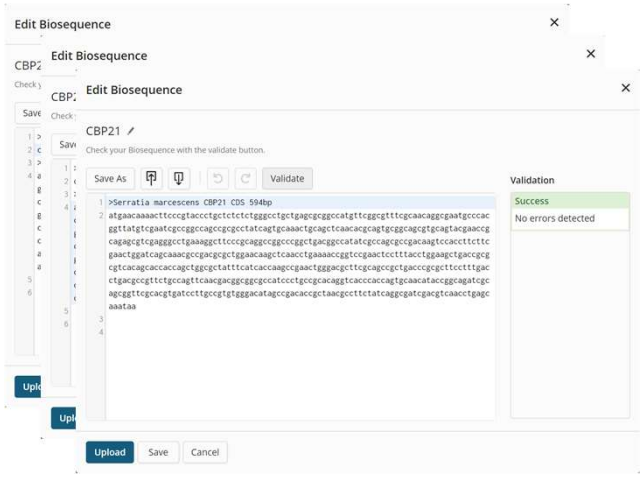
Validate sequence in Biosequence editor



- Multiple sequences detected
- Validation errors,
  e.g. invalid characters

(see STNext Help for examples)

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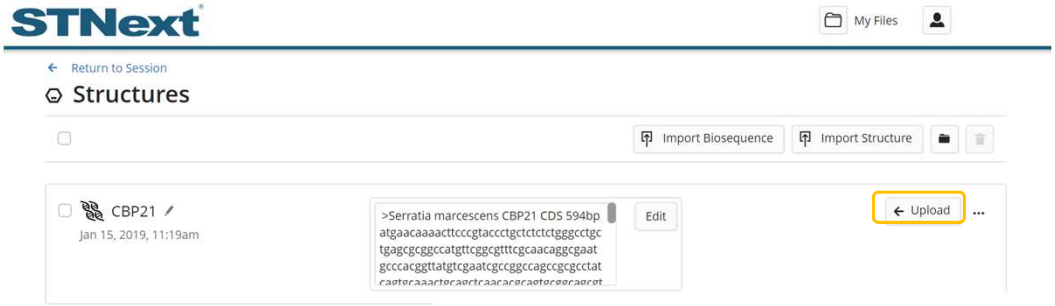
Upload sequence to STNext



Before Upload:
Enter DGENE, USGENE or PCTGEN

```
=> FIL DGENE
...

=>
Uploading sequence file: CBP21

UPLOAD SUCCESSFULLY COMPLETED
L1    GENERATED
```

A sequence query L-number is **automatically** generated

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Use D LQUE to verify your sequence query

```
=> D L1 LQUE

L1    ANSWER 1  DGENE  COPYRIGHT 2019 CLARIVATE ANALYTICS on STN
LQUE  atgaacaaaacttcccgtaccctgctctctctgggcctgctgagcgcggccatgttcggcgtttcg
      caacaggcgaatgcccacggttatgtcgaatcgccggccagccgcgcctatcagtgcaaactgcag
      ctcaacacgcagtgcggcagcgtgcagtacgaaccgcagagcgtcgagggcctgaaaggcttcccg
      caggccggcccggctgacggccatatcgccagcgccgacaagtccaccttcttcgaactggatcag
      caaacgccgacgcgctggaacaagctcaacctgaaaaccggtccgaactcctttacctggaagctg
      accgcgcgtcacagcaccaccagctggcgctatttcatcaccaagccgaactgggacgcttcgcag
      ccgctgacccgcgcttcctttgacctgacgccgttctgccagttcaacgacggcggcgccatccct
      gccgcacaggtcacccaccagtgcaacataccggcagatcgcagcggttcgcacgtgatccttgcc
      gtgtgggacatagccgacaccgctaacgccttctatcaggcgatcgacgtcaacctgagcaaataa
```

Verify sequence
Any spaces, numbers or headers in the query are stripped out during Upload. Uploaded sequence queries may be up to 10,000 characters in length for BLAST search.

The sequence query is now ready for searching directly in DGENE, PCTGEN and USGENE using the L-number (regardless of database used for upload).

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The RUN BLAST command

```
=> RUN BLAST L1 /SQN -F F BATCH
```

BATCH mode: Enter an email address to receive a notification when search is complete. Use RUN GETBATCH to check on status or to receive results.

The **low complexity filter** can eliminate biologically uninteresting segments that have low compositional complexity. The filter is set to F (False, off) by /SQN –F F (recommended for patent sequence search). The default setting is T (True, on).

Protein search:	RUN BLAST L1 /**SQP**
Nucleotide search:	RUN BLAST L1 /**SQN**
Translated search:	RUN BLAST L1 /**TSQN**

BLAST	NCBI BLAST for advanced similarity searching (this seminar)
GETSIM	FASTA based search algorithm; comparison of entire sequence length; more computational time required – use BATCH mode
GETSEQ	Sequence Code Match (SCM) for simple sequence queries to find exact sequences or with controlled variations

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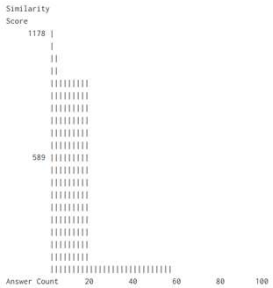
Run BLAST in DGENE

```
=> RUN BLAST L1 /SQN -F F
...

  56 ANSWERS FOUND BELOW EXPECTATION VALUE OF 10.0

      QUERY SELF SCORE VALUE IS  1178
      BEST ANSWER SCORE VALUE IS 1178
```



Query Self Score: ideal score for a perfect answer match.

Best Answer Score: in this example, there is at least one perfect answer match to the query.

The graphic representation gives a count of hit sequences (x-axis) and similarity score (y-axis). The graph provides a visual idea about the proportion of similar and not so similar sequences in the answer set.

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Decide how many answers to keep

```
ENTER EITHER THE NUMBER OF ANSWERS YOU WISH TO KEEP
 OR ENTER MINIMUM PERCENT OF SELF SCORE FOLLOWED BY %
 (BEST ANSWER PERCENTAGE OF SELF SCORE IS 100%)
ENTER (ALL) OR ? :  ALL

L2     RUN STATEMENT CREATED
L2           56 ATGAACAAAACTTCCCGTACCCTGCTCTCTCTGGGCCTGCTGAGCGCGGC
                CATGTTCGGCGTTTCGCAACAGGCGAATGCCCACGGTTATGTCGAATCGC
                CGGCCAGCCGCGCCTATCAGTGCAAACTGCAGCTCAACACGCAGTGCGGC
                AGCGTGCAGTACGAACCGCAGAGCGTCGAGGGCCTGAAAGGCTTCCCGCA
                GGCCGGCCCGGCTGACGGCCATATCGCCAGCGCCGACAAGTCCACCTTCT
                TCGAACTGGATCAGCAAACGCCGACGCGCTGGAACAAGCTCAACCTGAAA
                ACCGGTCCGAACTCCTTTACCTGGAAGCTGACCGCGCGTCACAGCACCAC
                CAGCTGGCGCTATTTCATCACCAAGCCGAACTGGGACGCTTCGCAGCCGC
                TGACCCGCGCTTCCTTTGACCTGACGCCGTTCTGCCAGTTCAACGACGGC
                GGCGCCATCCCTGCCGCACAGGTCACCCACCAGTGCAACATACCGGCAGA
                TCGCAGCGGTTCGCACGTGATCCTTGCCGTGTGGGACATAGCCGACACCG
                CTAACGCCTTCTATCAGGCGATCGACGTCAACCTGAGCAAATAA/SQN.-
                F F

Answer set arranged by accession number; to sort by descending
similarity score, enter at an arrow prompt (=>) "sor score d".
```

In this example all results are kept. Preselect by setting cut off, e.g. 80% of the *Query Self Score* (0.8 x 1178 = 942) can be used to select out the most relevant results. For this, type "80%" or "942".

For reviewing the DGENE results, sort before displaying (see next slide). Alternatively, refine search (e.g. priority date) and then sort and review.

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Review DGENE records

```
=> SOR L2 SCORE D
...

=> D TRIAL SCORE ALIGN 1-20

AN    BFE50692  DNA       DGENE
TI    Enzymatically degrading polysaccharide, comprises contacting
      polysaccharide with lytic polysaccharide monooxygenase, where the
      degradation is carried out in reaction in presence of reducing agent
      and hydrogen peroxide or means generating it.
DESC  Serratia marcescens LPMO10A coding DNA, SEQ ID 1.
KW    Chitin-binding protein; LPMO10A gene; Lytic polysaccharide
      monooxygenase 10A; Lytic polysaccharide monooxygenase auxiliary
      activity family 10A; alcohol; biofuel; degradation; ds; ethanol;
      fermentation; gene; polysaccharide.
SQL   594
SCORE 1178      100% of query self score 1178
BLASTALIGN
   Query  = 594 letters
   Length = 594
   Score  = 1178 bits (594), Expect = 0.0
   Identities = 594/594 (100%)
   Strand = Plus / Plus

  Query: 1   atgaacaaaacttcccgtaccctgctctctctgggcctgctgagcgcggccatgttcggc
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct: 1   atgaacaaaacttcccgtaccctgctctctctgggcctgctgagcgcggccatgttcggc...
```

The TRIAL display format is a free-of-charge display format. Based on the graphic representation of the similarity score, 20 records are selected for display.

The SCORE display field includes the percentage of the Query Self Score.

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Repeat sequence search in PCTGEN and USGENE

```
=> FIL USGENE
=> RUN BLAST L1 /SQN -F F

L3    RUN STATEMENT CREATED
L3            65 ATGAACAAAACTTCCCGTACCCTGCTCTCTCTGGGCCTGCTGAGCGCGGC
              CATGTTCGGCGTTTCGCAACAGGCGAATGCCCACGGTTATGTCGAATCGC
              ...


=> FIL PCTGEN
=> RUN BLAST L1 /SQN -F F

L4    RUN STATEMENT CREATED
L4            11 ATGAACAAAACTTCCCGTACCCTGCTCTCTCTGGGCCTGCTGAGCGCGGC
              CATGTTCGGCGTTTCGCAACAGGCGAATGCCCACGGTTATGTCGAATCGC
              ...
```

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Sequence searching in DGENE/USGENE/PCTGEN

DGENE/USGENE/PCTGEN

(0) FIL DGENE
- (1) Import sequence in STNext
- (2) Validate sequence in Biosequence Editor
- (3) Upload sequence in sequence-database
- (4) Verify if uploaded sequence is correct
- (5) Run BLAST search (and decide how many answers to keep)
- (6) Review search (e.g. D TRIAL ALIGN)
- (7) Run BLAST in USGENE and PCTGEN
- (8) Merge answer sets
- (9) Sort results (SCORE, IDENT) (> L6)
- (10) Family sorting and display (>L7)

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Merge sequence results from DGENE, USGENE and PCTGEN

```
=> SET DUPORDER FILE

SET COMMAND COMPLETED

=> DUP IDE L2 L3 L4

FILE 'DGENE' ENTERED AT 13:06:37 ON 17 JAN 2019
COPYRIGHT (C) 2019 CLARIVATE ANALYTICS
FILE 'USGENE' ENTERED AT 13:06:37 ON 17 JAN 2019
COPYRIGHT (C) 2019 SEQUENCEBASE CORP
FILE 'PCTGEN' ENTERED AT 13:06:37 ON 17 JAN 2019
COPYRIGHT (C) 2019 WIPO

PROCESSING COMPLETED FOR L2
PROCESSING COMPLETED FOR L3
PROCESSING COMPLETED FOR L4
L5          132 DUP IDE L2 L3 L4 (INCLUDES 0 SETS OF DUPLICATES)
                ANSWERS '1-56' FROM FILE DGENE
                ANSWERS '57-121' FROM FILE USGENE
                ANSWERS '122-132' FROM FILE PCTGEN

=> SOR L5 SCORE D IDENT D

PROCESSING COMPLETED FOR L6
L6          132 SOR L5 SCORE D IDENT D
```

SET DUPORDER FILE first to retrieve answers according file entries. By default, the answers are arranged in reverse chronological order.

DUPLICATE IDENTIFY (DUP IDE) is used here to create a single multi-file L-number in preferred file order

The multi-file answer set is sorted by descending similarity score and identity. Alternatively, the answer set can be sorted by BLAST identity (SOR IDENT D) or similarity score (SOR SCORE D) alone. (D…Descending)

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Optional: Review results with an alignment (1)

```
=> D L6 BIB SCORE ALIGN

L6    ANSWER 1 OF 132  DGENE  COPYRIGHT 2019 CLARIVATE ANALYTICS on STN
AN    BFE50692  DNA       DGENE Full-text
TI    Enzymatically degrading polysaccharide, comprises contacting
      polysaccharide with lytic polysaccharide monooxygenase, where the
      degradation is carried out in reaction in presence of reducing
agent
      and hydrogen peroxide or means generating it.
IN    Bissaro B; Eijsink V; Vaaje-Kolstad G
PA    (UYNO-N)    UNIV NORWEGIAN LIFE SCI.
      (INRG)      INRA INST NAT RECH AGRONOMIQUE.
PI    WO 2018060498   A1 20180405              135
AI    WO 2017-EP74904      20170929
PRAI  GB 2016-16707        20160930
      GB 2017-5056         20170329
PSL   Disclosure; SEQ ID NO 1
DT    Patent
LA    English
OS    2018-26087T [28]
CR    P-PSDB: BFE50693
      GENBANK: AY665558.1
      NCBI: gi52854326
DESC  Serratia marcescens LPMO10A coding DNA, SEQ ID 1.
```

AN (Accession number) and MTY (molecule type), here DNA

Enhanced title from DWPI

(PACO) Patent Assignee Code

PSL (Patent sequence location): claim, disclosure or example

OS (Other source): accession number of corresponding DWPI record

CR: Internal cross reference (e.g. BFE50693 was disclosed as the protein encoded by BFE50694) and external cross references

DESC: Concise, one-line description

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Optional: Review results with an alignment (1)

```
...
SCORE 1178      100% of query self score 1178
BLASTALIGN
   Query  = 594 letters
   Length = 594
   Score  = 1178 bits (594), Expect = 0.0
   Identities = 594/594 (100%)
   Strand = Plus / Plus

  Query: 1   atgaacaaaacttcccgtaccctgctctctctgggcctgctgagcgcggccatgttcggc
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct: 1   atgaacaaaacttcccgtaccctgctctctctgggcctgctgagcgcggccatgttcggc

  Query: 61  gtttcgcaacaggcgaatgcccacggttatgtcgaatcgccggccagccgcgcctatcag
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct: 61  gtttcgcaacaggcgaatgcccacggttatgtcgaatcgccggccagccgcgcctatcag

  Query: 121 tgcaaactgcagctcaacacgcagtgcggcagcgtgcagtacgaaccgcagagcgtcgag
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct: 121 tgcaaactgcagctcaacacgcagtgcggcagcgtgcagtacgaaccgcagagcgtcgag

  Query: 181 ggcctgaaaggcttcccgcaggccggcccggctgacggccatatcgccagcgccgacaag
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct: 181 ggcctgaaaggcttcccgcaggccggcccggctgacggccatatcgccagcgccgacaag
```

Query Self Score and percentage

BLAST Percent Identity (IDENT)

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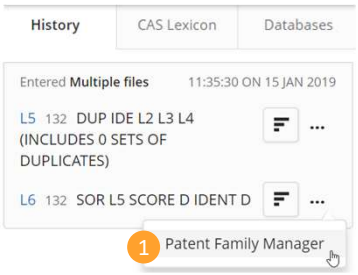
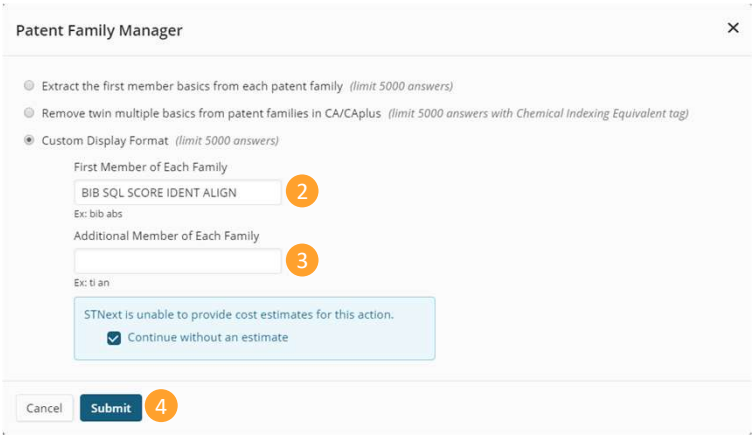
Use the Patent Family Manager to display the results





2 The first record of each family will be displayed.

3 Optional: Choose different display format for other family members, e.g. TRIAL SCORE ALIGN

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The Patent Family Manager organizes results in extended patent families

```
=> FSORT L6
...
L7          132 FSO L6

             16 Multi-record Families   Answers 1-131
                  Family 1              Answers 1-2
                  Family 2              Answers 3-10
                  Family 3              Answers 11-15
                  Family 4              Answers 16-28
                 ...
             1 Individual Record       Answer 132
              0 Non-patent Records

=> DIS L7 PFAM=1 1 BIB SQL SCORE IDENT ALIGN

L7    ANSWER 1 OF 132  DGENE  COPYRIGHT 2019 CLARIVATE ANALYTICS on STN
      FAMILY1
AN    BFE50692  DNA       DGENE Full-text
TI    Enzymatically degrading polysaccharide, comprises contacting
      polysaccharide with lytic polysaccharide monooxygenase, where the
      degradation is carried out in reaction in presence of reducing agent
      and hydrogen peroxide or means generating it.
IN    Bissaro B; Eijsink V; Vaaje-Kolstad G
```

The Patent Family Manager starts automatically with an FSORT, which means all patent records will be sorted in extended patent families.

The user does not have to type in these commands!

16 extended patent families and 1 individual record.

The first record of each family will be displayed. This record (answer 1) is from Family 1 containing 2 records.

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Expectation Value (-E)

Expectation value (E value) is the statistical significance threshold for reporting matches against a sequence database. The E value can be any positive number, and the default value is 10. This means that 10 matches may be expected to be found merely by chance. In general E value is lowered to make the search more precise and raised to retrieve more answers.

Word Size (-W)

Word Size is the length of the character string fragments of a sequence query which are used as the basis for a BLAST search. For SQN the default is 11 and the range 7-23. For all other BLAST searches the default is 3 and the range 2-3. For short search queries, reducing the default word size can give improved search results.

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Low Complexity Filtering (on by default) (-F)

The low complexity filter can eliminate biologically uninteresting segments that have low compositional complexity and are statistically significant, as determined by specific programs for peptide or nucleotide sequences in nature. Filtering is applied to the query sequence and is indicated by a series of Xs for peptide sequences and Ns for nucleotide sequences. Low Complexity Filtering can be turned off (i.e., set to F - false).

Peptide similarity matrices (-M)

For peptide based searches SQP and TSQN the advanced options provide additional scoring matrices to the default BLOSUM62

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NCBI guidelines for using Advanced Settings for peptide sequence searching

Query Length	Matrix	Gap costs
<35	PAM30	(9,1)
35-50	PAM70	(10,1)
50-85	BLOSUM80	(10,1)
>85	BLOSUM62	(11,1) (BLAST default)

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BLAST search with different parameters

Search example:

Find all patents disclosing the sequence –

iskdgst

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BLAST search in DGENE with default settings

STNext

Transcript ON | Changing BLAST values

File DGENE

```
=> run blast iskdgst/sqp -f f
...
BLAST Version 2.2.20
```

```
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 44 (21.6 bits)
S2: 68 (30.8 bits)
Total Execution Time: 2.5974

 NO ANSWERS FOUND BELOW EXPECTATION VALUE OF 10.0
```

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BLAST search in DGENE with modified parameters

```
=> run blast iskdgst/sqp -f f -m pam30 -e 10000 -w 2


BLAST Version 2.2.20
```

```
X2: 35 (14.8 bits)
X3: 58 (24.6 bits)
S1: 39 (21.7 bits)
S2: 42 (21.0 bits)
Total Execution Time: 2.66294

 114 ANSWERS FOUND BELOW EXPECTATION VALUE OF 10000.0

     QUERY SELF SCORE VALUE IS    24
     BEST ANSWER SCORE VALUE IS   24

Similarity
Score
       24 ||||
          ||||
          ||||||||||||||||||||||||||||||||||||||||
          ||||||||||||||||||||||||||||||||||||||||
          ||||||||||||||||||||||||||||||||||||||||
          ||||||||||||||||||||||||||||||||||||||||
```

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Refine with keyword search

```
          ||||||||||||||||||||||||||||||||||||||||
Answer Count      30        60        90       120       150

ENTER EITHER THE NUMBER OF ANSWERS YOU WISH TO KEEP
 OR ENTER MINIMUM PERCENT OF SELF SCORE FOLLOWED BY %
 (BEST ANSWER PERCENTAGE OF SELF SCORE IS 100%)
ENTER (ALL) OR ? :all

L3    RUN STATEMENT CREATED
L3         114 ISKDGST/SQP.-F F -M PAM30 -E 10000 -W 2

Answer set arranged by accession number; to sort by descending
similarity score, enter at an arrow prompt (=>) "sor score d".

=> s l3 and (cdr or compliment?(w)determin?(w)region?)

        475603 CDR
        171374 CDRS
        614631 CDR
                 (CDR OR CDRS)
         17319 COMPLIMENT?
      11454900 DETERMIN?
      10040097 REGION?
          3627 COMPLIMENT?(W)DETERMIN?(W)REGION?
L4          12 L3 AND (CDR OR COMPLIMENT?(W)DETERMIN?(W)REGION?)
```

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SORT and DISPLAY

```
=> sor score d ident d

PROCESSING COMPLETED FOR L4
L5          12 SOR L4 SCORE D IDENT D

=> d kwic align score 1-3
```

```
L5    ANSWER 1 OF 12  DGENE  COPYRIGHT 2020 CLARIVATE ANALYTICS on STN
AB    The present invention relates to a novel dual binding moiety comprising a
      non-complementarity determining region (CDR) loop and a cleavable
      linker, where the moiety is capable of interacting with a domain by
      specific  binding or by..  .  .
BLASTALIGN
   Query  = 7 letters
   Length = 117
   Score  = 23.5 bits (48), Expect = 7e-05
   Identities = 7/7 (100%), Positives = 7/7 (100%)
  Query: 1  ISKDGST 7
            ISKDGST
  Sbjct: 51 ISKDGST 57
SCORE 24      100% of query self score 24
```

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Summary

- STNext offers excellent combination of value-added and first level sequence data
- DGENE and REGISTRY are the “industry-standard” databases
- STNext ideally supports data cross over to other databases and combine data from several databases in one report
- DGENE, REGISTRY, USGENE and PCTGEN are all required for a comprehensive search

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helpdesk@fiz-karlsruhe.de
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