BLASTing Through the Kingdom of Life

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BLASTing Through the Kingdom of Life

Learning Objectives

- Learn about sequence alignment.
  - What is sequence alignment?
  - What is biological sequence alignment?
  - Why is biological sequence alignment important?
- Learn how to use BLAST for biological sequence alignment.

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What is a Sequence Alignment?

- Arrange two or more words (sequences of letters) so that you can identify regions of similarity between them.
- For the current discusion, the alphabet (set of letters) is the standard English alphabet (size = 26).
- Consider these four words: bat, hat, batter, hatter.
- How similar are bat and hat?
- Whic word is more similar to bat: hat or batter? What about hatter? Why?
- Which pair of words is more similar to each other: (bat, hat) or (batter, hatter)? Why?

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Sequence Alignments → Longest Common Subsequence

- Let's be a bit more formal about determining similarity.
- For a particular alignment, we will score them as follows:
  - Perfect match = 1 (A – A, B – B, etc.)
  - Mismatch = 0 (A – B, B – A, etc.)
- Using this scoring scheme, we'll find the longest common subsequence in each word pair and score them to determine the most similar sequences.

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Sequence Alignments → Longest Common Subsequence Examples

Alignment 1

```
BAT
 ||
HAT
```

Score = 2

Alignment 2

```
HAT
 ||
BATTER
```

Score = 2

Alignment 3

```
BAT
 ||
HATTER
```

Score = 2

Alignment 4

```
BAT
|||
BATTER
```

Score = 3

Alignment 5

```
HAT
|||
HATTER
```

Score = 3

Alignment 6

```
BATTER
 |||||
HATTER
```

Score = 5

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Sequence Alignments → Gapped Alignments

- Consider these three sequences:
  1. THE CATS IN THE HAT
  2. THE CAT IN THE HAT
  3. THE CAT IS A HAT
- Which phrase (2 or 3) is the most similar to phrase 1?

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Sequence Alignments → Gapped Alignments

- Again, let's be a bit more formal about determining similarity.
- For a particular alignment, we will score it as follows:
  - Perfect match = 1 (A – A, B – B, etc.)
  - Mismatch = 0 (A – B, B – A, etc.)
  - Gap open = -1
  - Gap extension = 0
- Using this scoring scheme, we'll find the longest gapped subsequence in each word pair and score them to determine the most similar sequences.

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Sequence Alignments → Gapped Alignment Examples

Alignment 1

```
THECATSINTHEHAT
|||||| 
THECATINTHEHAT
```

Score = 6

Alignment 2

```
THECATSINTHEHAT
|||||| |||||||||
THECAT-INTHEHAT
```

Score = 14 – 1 = 13

Alignment 3

```
THECATSINTHEHAT
|||||| 
THECATISAHAT
```

Score = 6

Alignment 4

```
THECATSINTHEHAT
|||||||     |||
THECAT---ISAHAT
```

Score = 6 - 1 + 3 = 8

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Sequence Alignments → Global vs. Local Alignment

- Global alignment → find the single best alignment across the entire length of both sequences.
- Local alignment → find one or more highly similar local regions between both sequences.

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What is a Biological Sequence Alignment?

- Arrange two or more molecular sequences (DNA, RNA, protein) to identify regions of similarity between them.
  - Matches, mismatches and gaps.
  - Pairwise vs. multiple sequence alignment (MSA)
- We'll focus on DNA.
- DNA is a sequence of four possible nucleotides: adenine, guanine, cytosine and thymine.
  - DNA alphabet (letters) = {A, C, G, T}
- Much smaller than English alphabet.

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Biological Sequence Alignment Example

- How would you globally align these two sequences?
  - Sequence 1 → ACTG
  - Sequence 2 → ACGTG

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Biological Sequence Alignment Example

Alignment 1

```
ACTG-
 | |
ACGTG
```

Alignment 2

```
-ACTG
   | |
ACGTG
```

Alignment 3

```
AC-TG
|| ||
ACGTG
```

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Why are Biological Sequence Alignments Important?

- The more similar two molecular sequences are, the more likely that the molecules are also similar in structure, function or evolutionary history.
- DNA sequences
  - Mismatch = point mutation
  - Gap = insertion or deletion (indel)
- When developing primers for a PCR reaction (you'll learn about this later), need to make sure that the primer is unique to the organism's genome.

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Why Do We Need Computers and Algorithms to Find Alignments?

- Why not find all biological sequence alignments manually?
- How many times can you find the query sequence ATCGGCCATTAC in the following target sequence? Is it there at all? If so, is it unique?

```
ATCACTGTAGTAGTAGCTGGAAAGAGAAATCTGTGACTCCAATTAGCCAGTTCCTGCAGACCTTGTGAGGACTAG
AGGAAGAATGCTCCTGGCTGTTTTGTACTGCCTGCTGTGGAGTTTCCAGACCTCCGCTGGCCATTTCCCTAGAGC
CTGTGTCTCCTCTAAGAACCTGATGGAGAAGGAATGCTGTCCACCGTGGAGCGGGGACAGGAGTCCCTGTGGCCA
GCTTTCAGGCAGAGGTTCCTGTCAGAATATCCTTCTGTCCAATGCACCACTTGGGCCTCAATTTCCCTTCACAGG
GGTGGATGACCGGGAGTCGTGGCCTTCCGTCTTTTATAATAGGACCTGCCAGTGCTCTGGCAACTTCATGGGATT
CAACTGTGGAAACTGCAAGTTTGGCTTTTGGGGACCAAACTGCACAGAGAGACGACTCTTGGTGAGAAGAAACAT
CTTCGATTTGAGTGCCCCAGAGAAGGACAAATTTTTTGCCTACCTCACTTTAGCAAAGCATACCATCAGCTCAGA
CTATGTCATCCCCATAGGGACCATTGGCCAAATGAAAAATGGATCAACACCCATGTTTAACGACATCAATATTTA
TGACCTCTTTGTCTGGATGCATTATTATGTGTCAATGGATGCACTGCTTGGGGGATCTGAAATCTGGAGAGACAT
TGATTTTGCCCATGAAGCACCAGCTTTTCTGCCTTGGCATAGACTCTTCTTGTTGCGGTGGGAACAAGAAATCCA
GAAGCTGACAGGAGATGAAAACTTCACTATTCCATATTGGGACTGGCGGGATGCAGAAAAGTGTGACATTTGCAC
AGATGAGTACATGGG
```

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What is BLAST?

- Basic Local Alignment Search Tool
- Very fast tool for finding local regions of similarity between sequences.
  - Given a query sequence, search one or more target databases for matches.
- Target databases are extremely large; millions of sequences.
  - nt = non-redundant nucleotide sequence database
  - nr = non-redundant protein sequence database

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Interpreting BLAST Results

- For a given query sequence, you will receive a list of matching target sequences with corresponding alignments.
  - The query sequence can match more than one region for a target sequence (local alignments).
- Each alignment will have an E-value; statistical significance of alignment.
  - Number of sequence matches you would expect to find in a target database composed entirely of random sequences.

Interpreting BLAST Results

- Low E-value means that the match is not random.
- What does an E-value = 4 mean?
- A couple observations about E-values to remember:
  - Greater chance of finding a random match in a larger target database (larger E-value).
  - Greater chance of finding a random match for a shorter query sequence (larger E-value).

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Hands-On Tutorial

- Go to http://blast.ncbi.nlm.nih.gov/Blast.cgi

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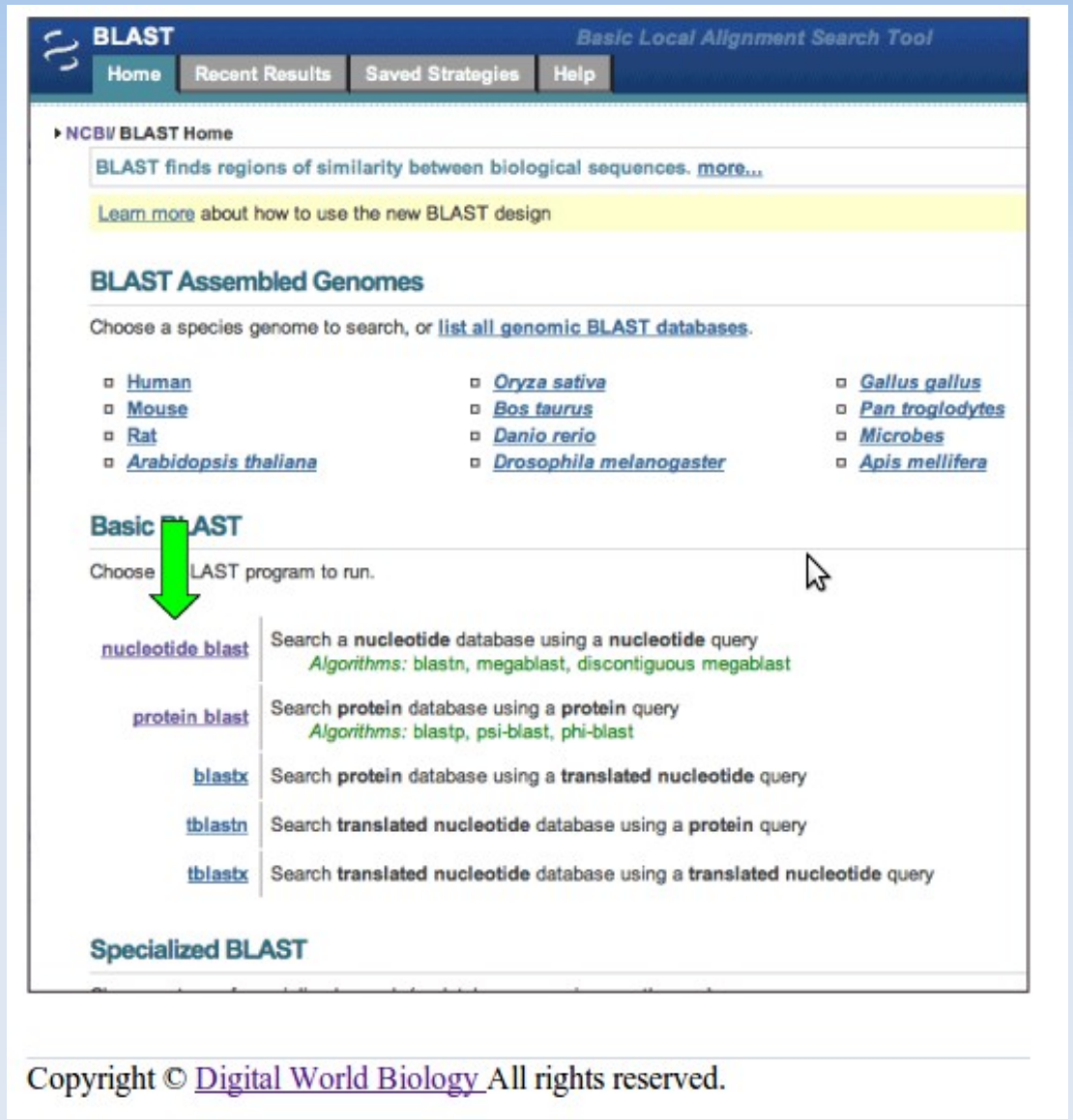
Hands-On Tutorial



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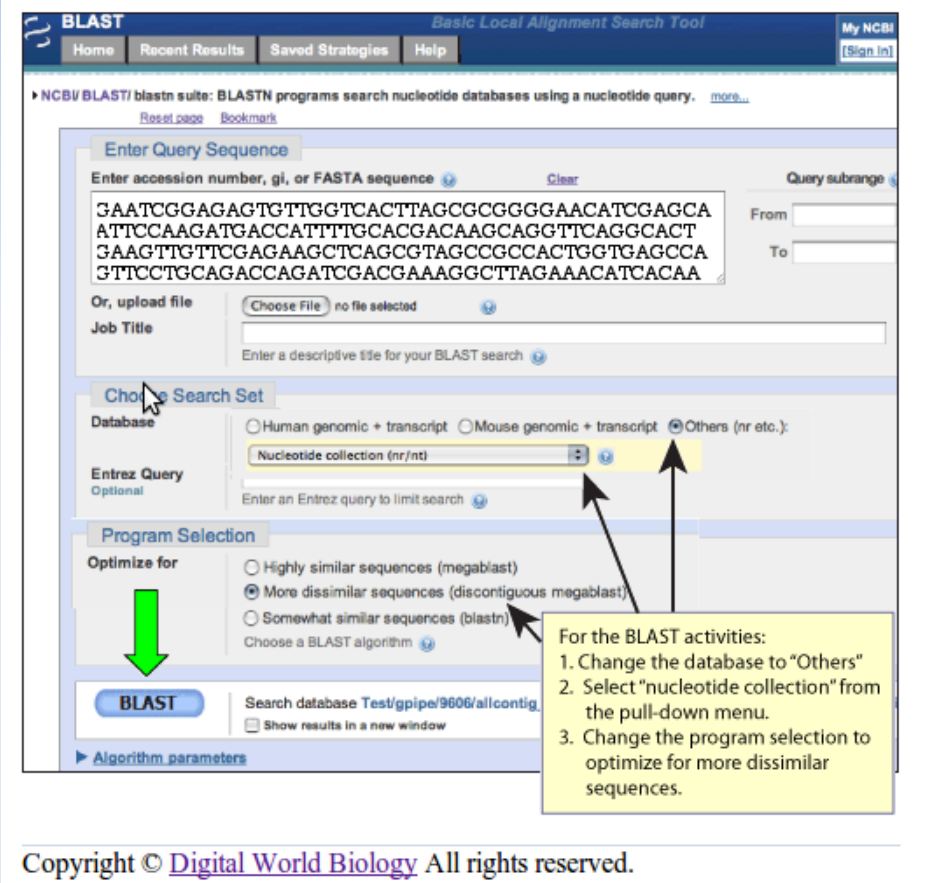
Hands-On Tutorial

- Copy the first query sequence (> Example) from ***http://www.digitalworldbiology.com/BLAST/62000sequences.html***
- Paste target sequence into textbox on BLAST start screen.
- Additional step:
  - Algorithm parameters → Set max target sequences to 500.



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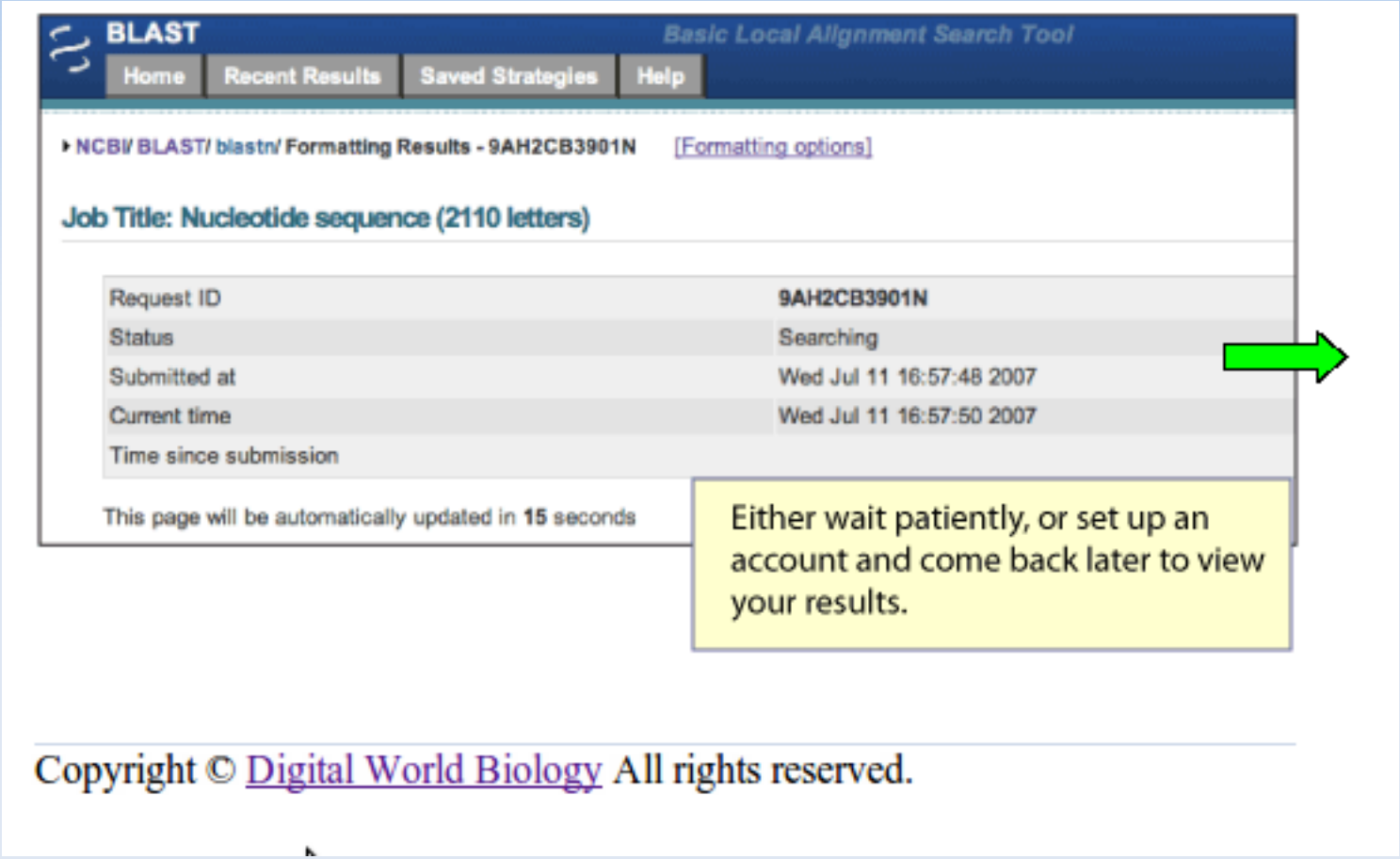
Hands-On Tutorial



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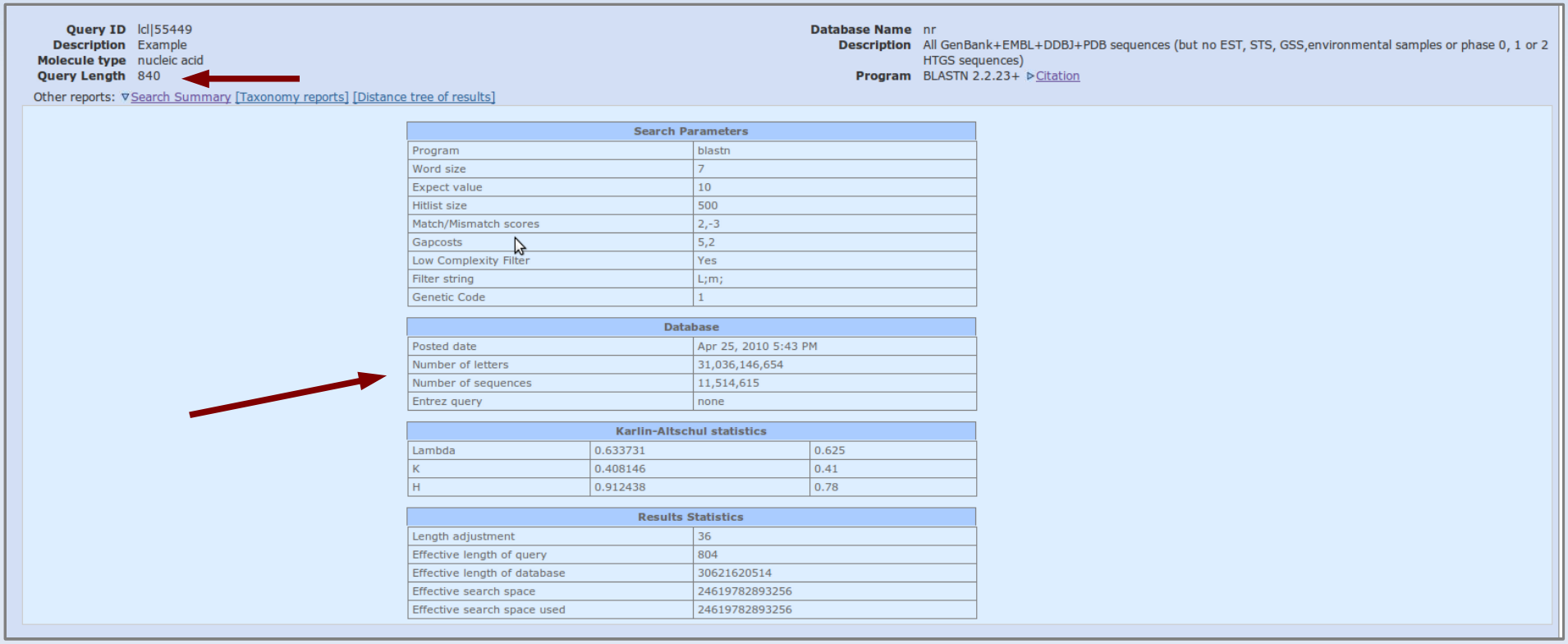
Hands-On Tutorial

- How many sequences are in the database?
- How many letters are in the database?
- How long is the query sequence?



Query ID lcl|55449
Description Example
Molecule type nucleic acid
Query Length 840

Database Name nr
Description All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS,environmental samples or phase 0, 1 or 2 HTGS sequences)
Program BLASTN 2.2.23+ ▹Citation

Other reports: ▿Search Summary [Taxonomy reports] [Distance tree of results]

Search Parameters	
Program	blastn
Word size	7
Expect value	10
Hitlist size	500
Match/Mismatch scores	2,-3
Gapcosts	5,2
Low Complexity Filter	Yes
Filter string	L;m;
Genetic Code	1

Database	
Posted date	Apr 25, 2010 5:43 PM
Number of letters	31,036,146,654
Number of sequences	11,514,615
Entrez query	none

Karlin-Altschul statistics		
Lambda	0.633731	0.625
K	0.408146	0.41
H	0.912438	0.78

Results Statistics	
Length adjustment	36
Effective length of query	804
Effective length of database	30621620514
Effective search space	24619782893256
Effective search space used	24619782893256

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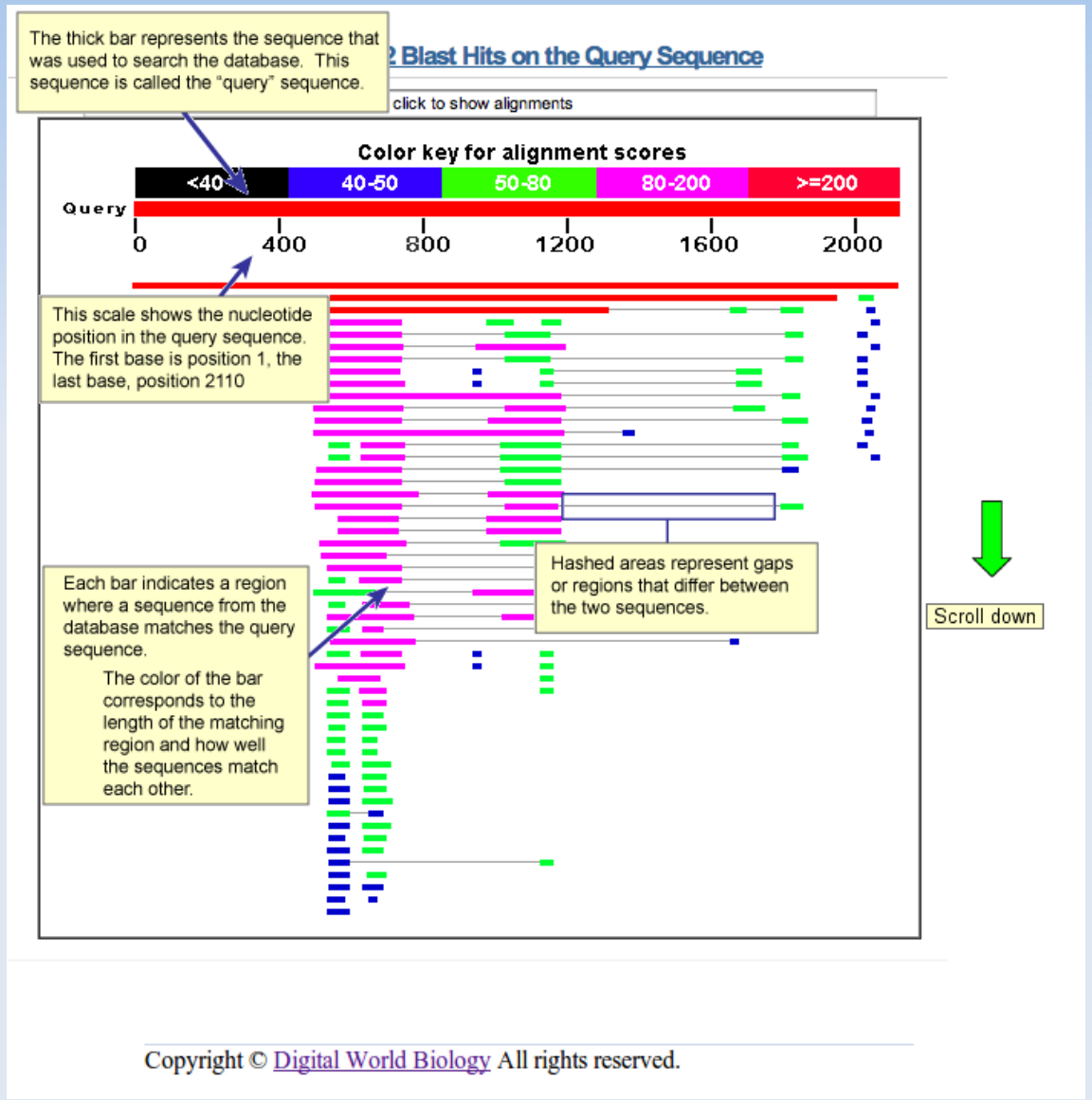
Hands-On Tutorial



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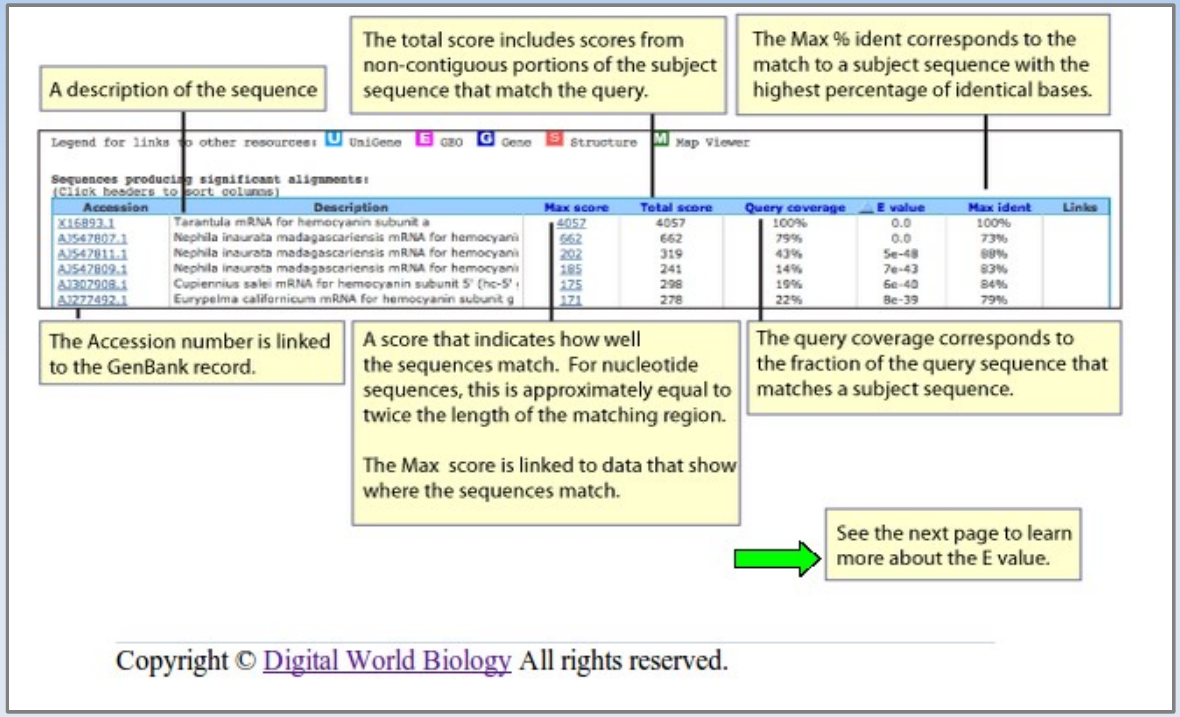
Hands-On Tutorial

- What is the most likely identity of your sequence? What is its accession number?
- What data supports this conclusion?
- What organism is the source of the target sequence?
- Approximately how many alignments have an E-value less than 0.01?
- For which other organisms are there matches?



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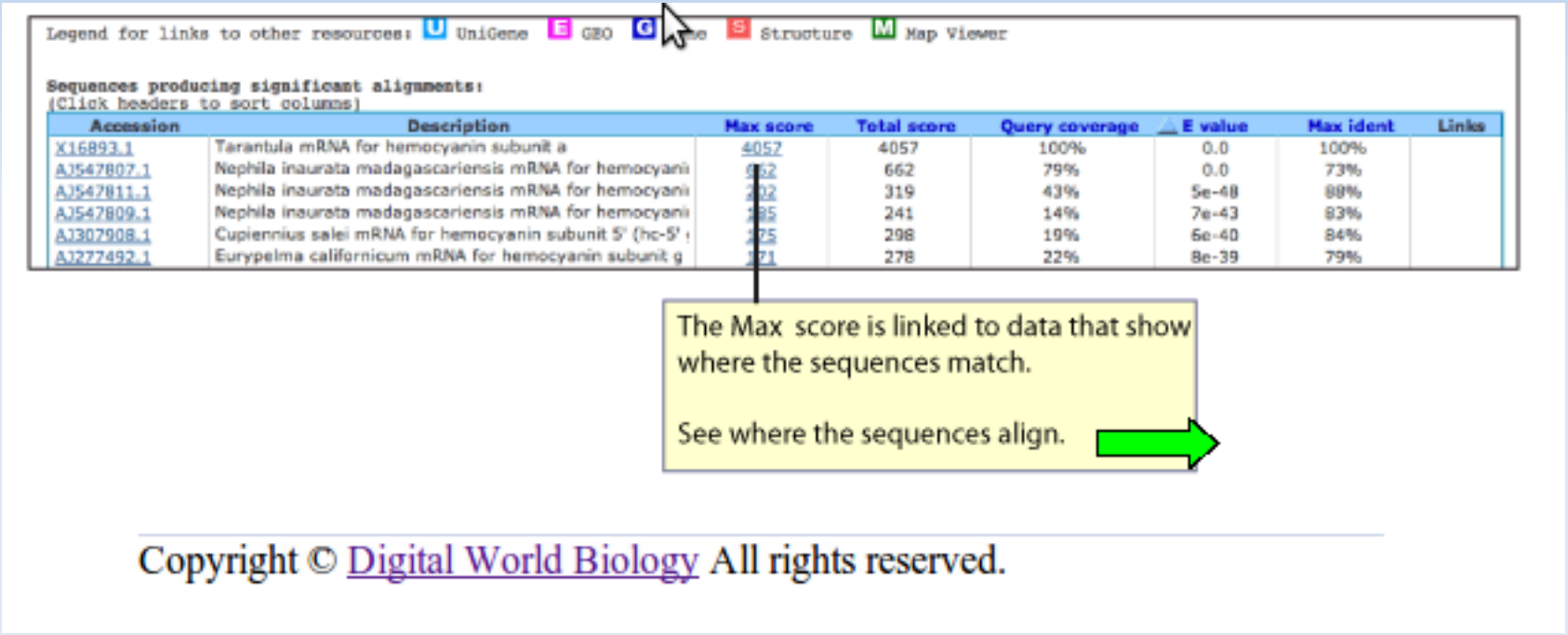
Hands-On Tutorial



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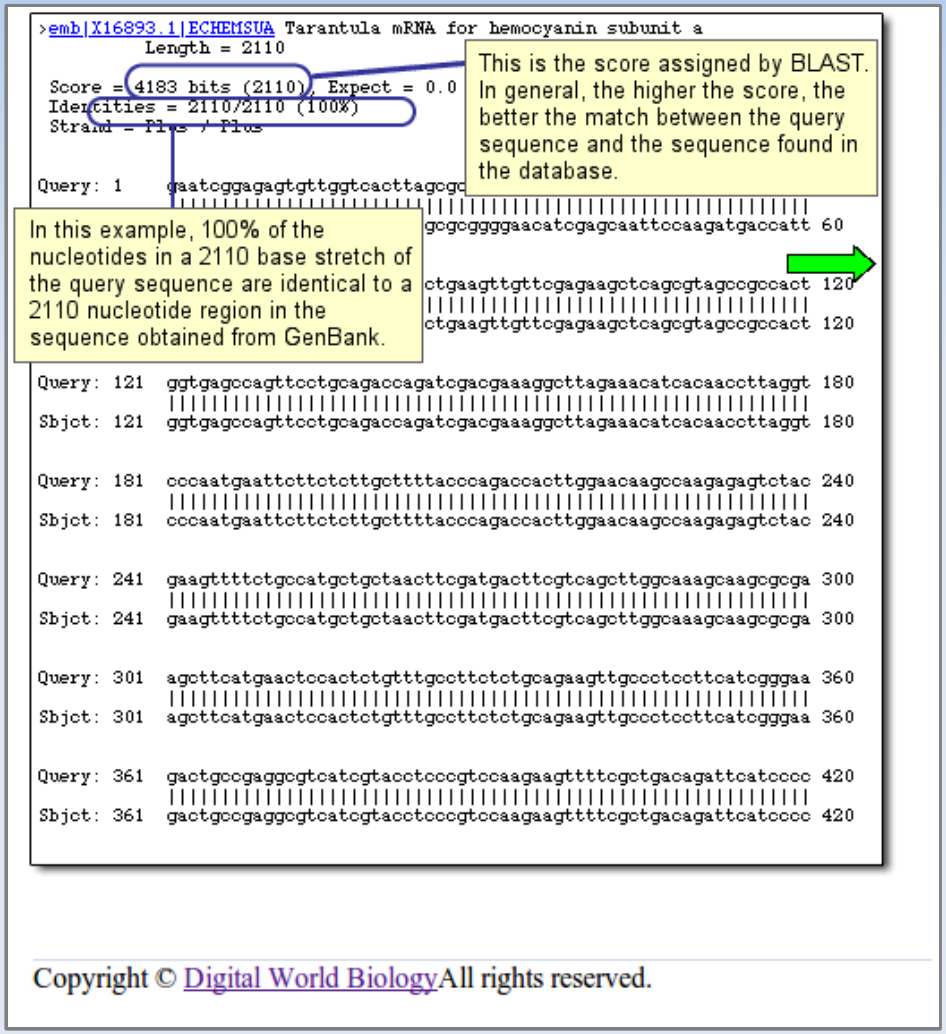
Hands-On Tutorial

- How many identical nucleotides are there for your sequence?



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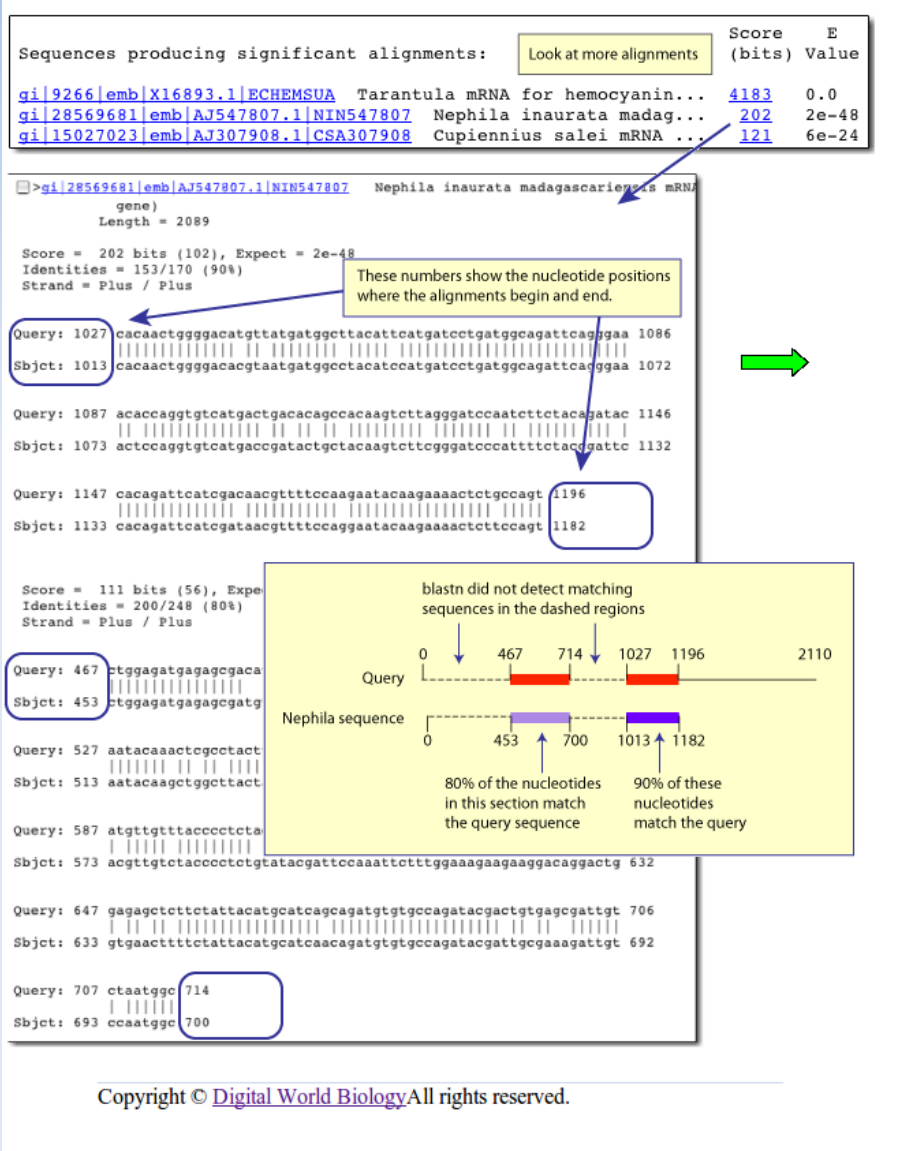
Hands-On Tutorial



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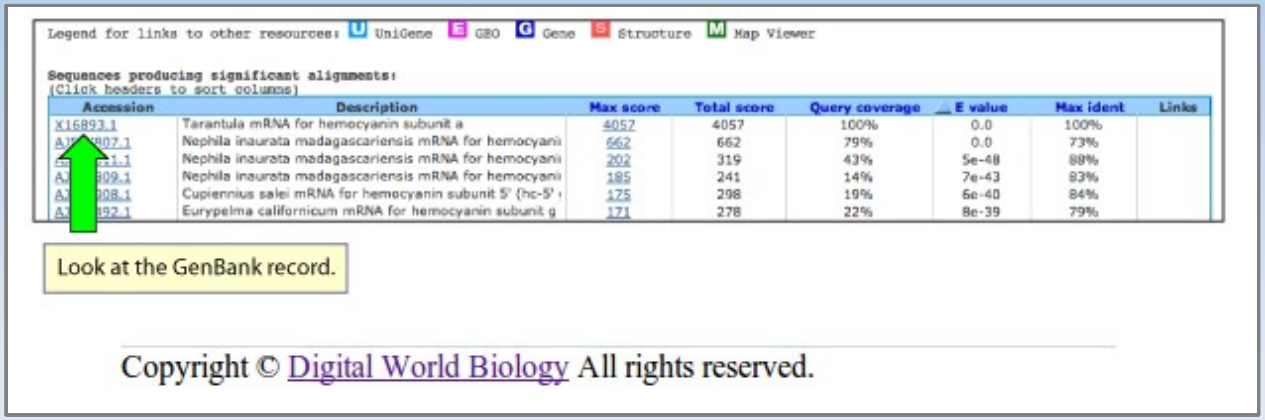
Hands-On Tutorial

- Find the sequence ”NM_000372.4”
- Is your sequence expressed?
- How do you know?
- How is this sequence related to the first alignment in the results?



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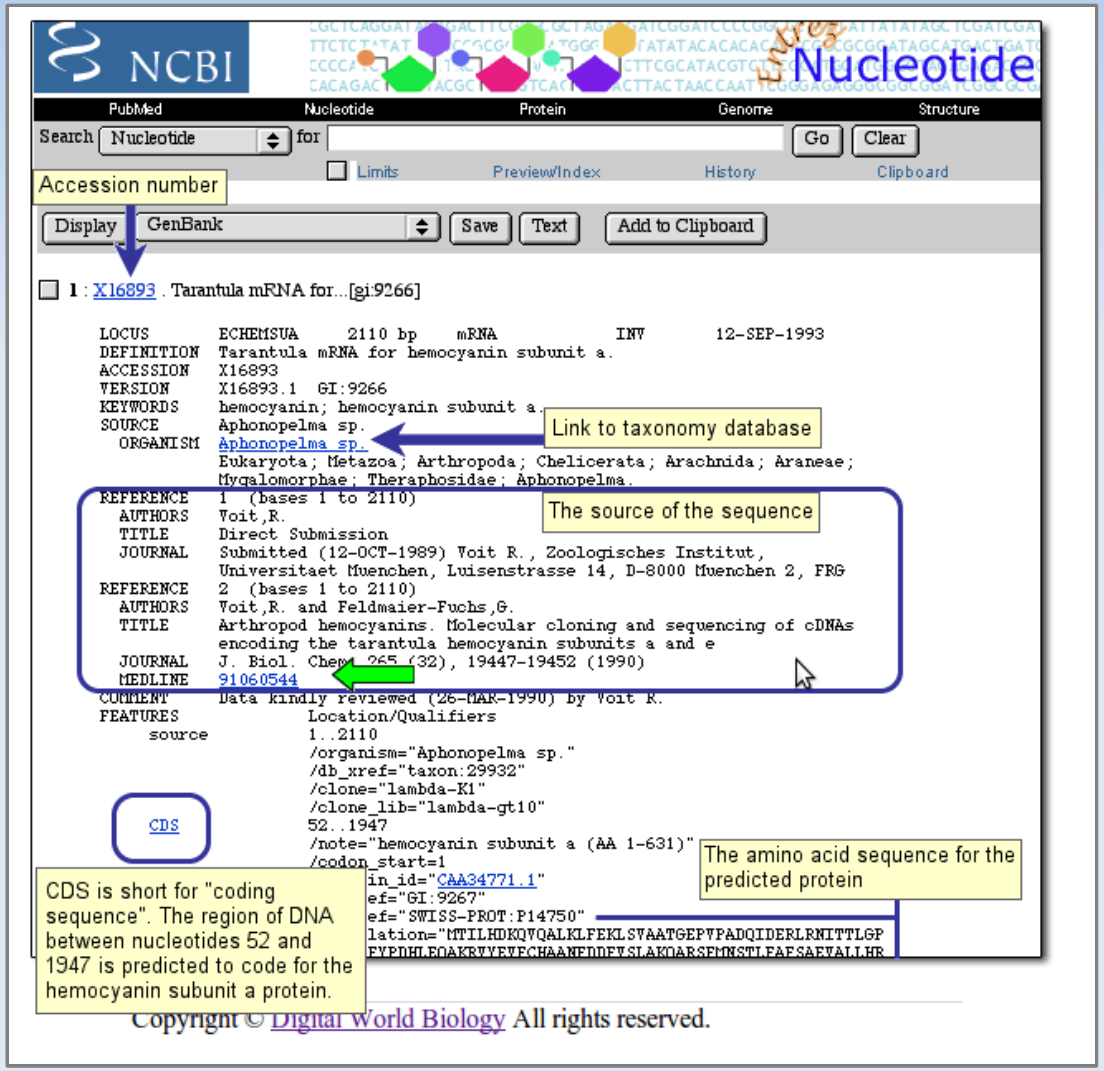
Hands-On Tutorial

- On which chromosome is the matching target sequence located?



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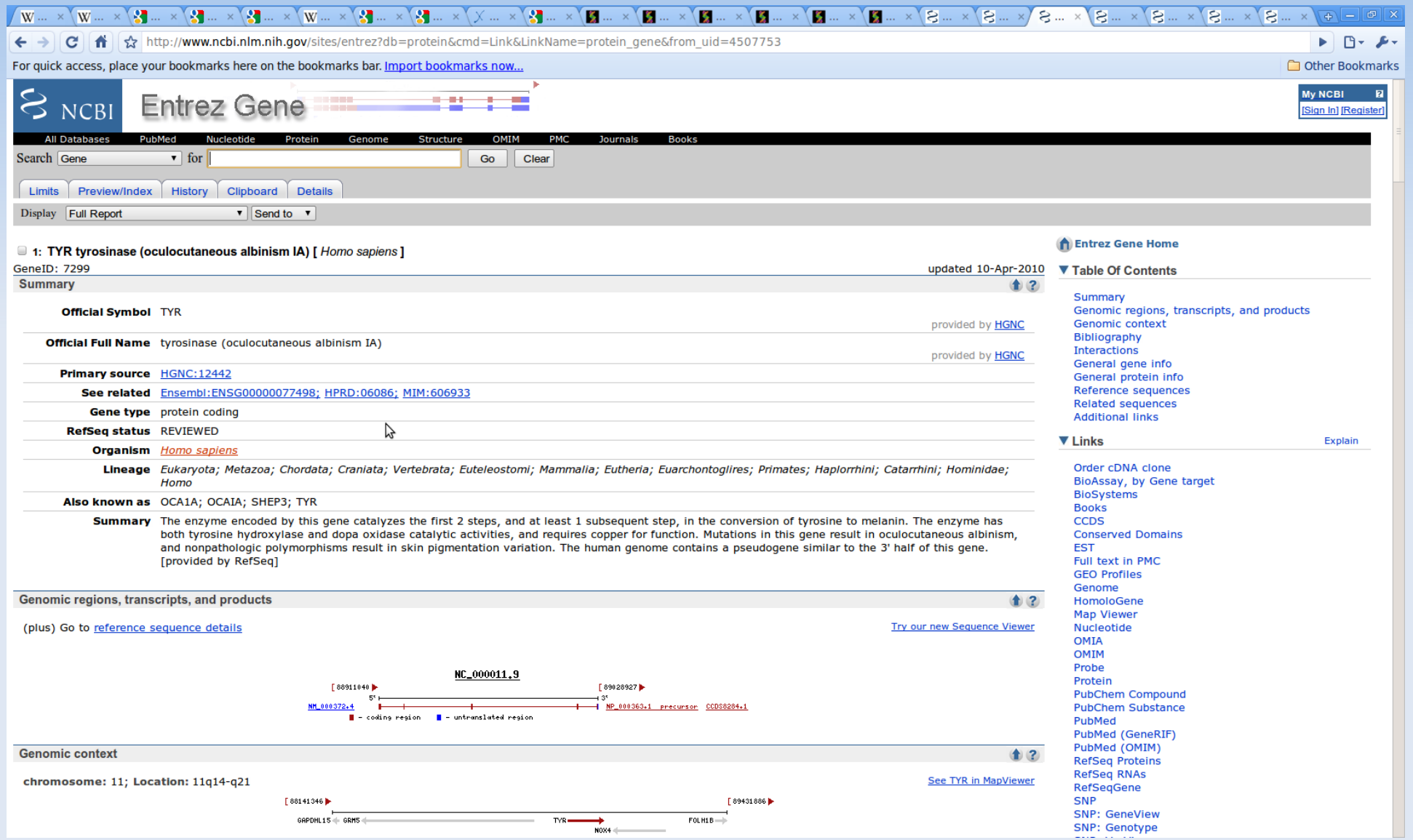
Hands-On Tutorial



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Hands-On Tutorial