Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA

Nitrogen-fixing root nodules on legumes result from two developmental processes, bacterial infection and nodule organogenesis. To balance symbiosis and plant growth, legume hosts restrict nodule numbers through an inducible autoregulatory process. Here, we present a mechanism where repression of a negative regulator ensures symbiotic susceptibility of uninfected roots of the host Lotus japonicus. We show that microRNA miR2111 undergoes shoot-toroot translocation to control rhizobial infection through posttranscriptional regulation of the symbiosis suppressor TOO MUCH LOVE in roots. miR2111 maintains a susceptible default status in uninfected hosts and functions as an activator of symbiosis downstream of LOTUS HISTIDINE KINASE1mediated cytokinin perception in roots and HYPERNODULATION ABERRANT ROOT FORMATION1, a shoot factor in autoregulation. The miR2111-TML node ensures activation of feedback regulation to balance infection and nodulation events.

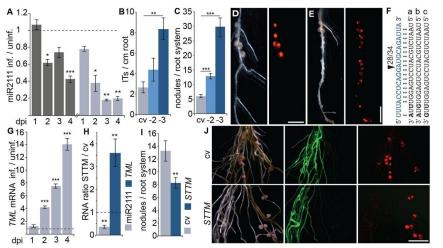


Fig. 1 miR2111 regulates *TML* posttranscriptionally. (A) miR2111 abundance in Lotus leaves (dark bars) and roots (light bars) at 1-4 days postinfection (dpi) with *M. loti*. (**B**) Infection thread (IT: 10 dpi) and (C) nodule numbers (21 dpi) in pUBQ1:MIR2111-2 (-2) and pUBQ1:MIR2111-3 (-3) compared to control (cv) roots. (D and E) Nodulation in control (D) and pUBQ1:MIR2111-3 (E) roots (21 dpi). Righthand panels visualize M. loti DsRED in nodules. Scale bars, 2 mm. (F) miR2111 directs TML cleavage. Bold font marks polymorphisms between miR2111 isoforms a-c (black). Numbers: degradome 5' ends at arrowhead/total within TML target region (blue). (G) TML mRNA in M. loti-infected roots (1-4 dpi). (H to J) miR2111STTM (STTM) expression reduced miR2111, increased TML (H), and reduced nodulation [(I) and (J)] compared to control roots (cv). (I) n = 23/26 (STTM/cv). Green fluorescence [(J), center] shows co-transformation, red [(J), right] nodules with M. loti DsRED. Scale bar, 5 mm. Transgenic roots [(B) to (E) and (H) to (J)] were A. rhizogenes-induced. [(A), (G), and (H)] gRT-PCR analyses. RNA levels are relative to two reference genes. Error bars: SEM of at least three biological replicates. Student's t test P values: * $P \le$ 0.05; ** $P \le 0.01$; *** $P \le 0.001$. (I) Results represent one biological replicate (P = 0.006) and were similar in a second (P = 0.001).

http://science.sciencemag.org/content/early/2018/08/29/science.aat6907

Announcements

- Phytobacteriology in the News! Week 6 <u>LINK</u>
- Reviewing assignments, how is it going?
- Reading for next week (published last week) <u>LINK</u>

Phevamine A, a small molecule that suppresses plant immune responses



Erinn M. O'Neill, Tatiana S. Mucyn, Jon B. Patteson, Omri M. Finkel, Eui-Hwan Chung, Joshua A. Baccile, Elisabetta Massolo, Frank C. Schroeder, Jeffery L. Dangl, and Bo Li

PNAS published ahead of print September 20, 2018 https://doi.org/10.1073/pnas.1803779115

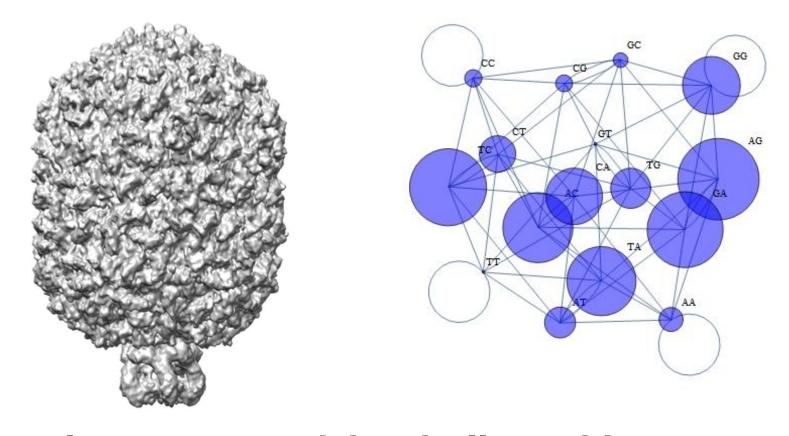
Edited by Sheng Yang He, Michigan State University, East Lansing, MI, and approved August 10, 2018 (received for review March 3, 2018)

Article

Figures & SI

Info & Metrics





Guest lecturer: Dr. Richard Allen White III

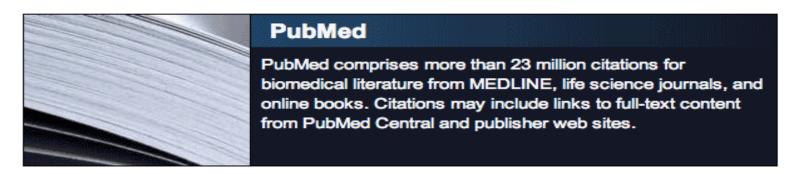
Post-doc: Friesen lab

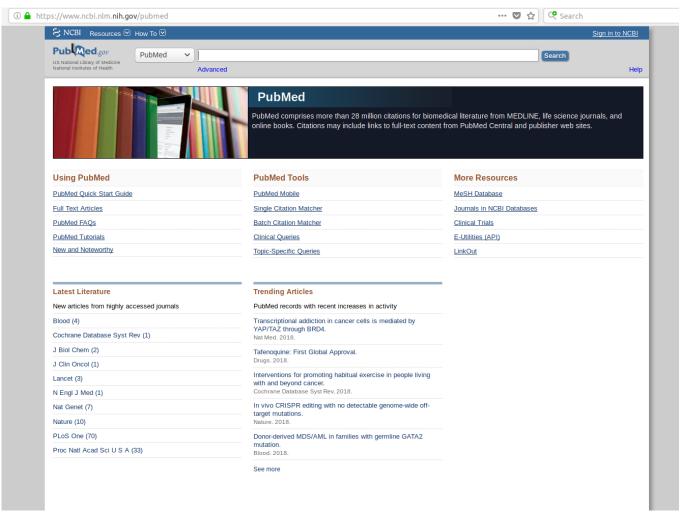
Learning Objectives

- 1. NCBI/pubmed walk through
- 2. BLAST tutorial
- 3. Global vs. local alignment
- 4. Multiple sequence alignment
- 5. Quick tree building in BLAST

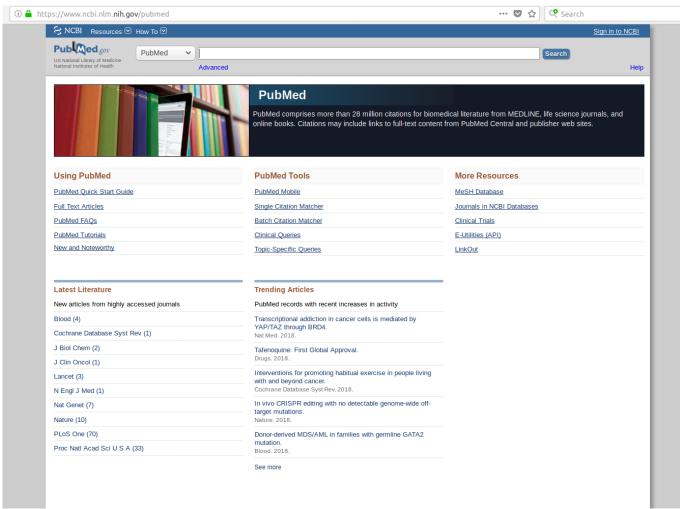
Go to www.pubmed.com



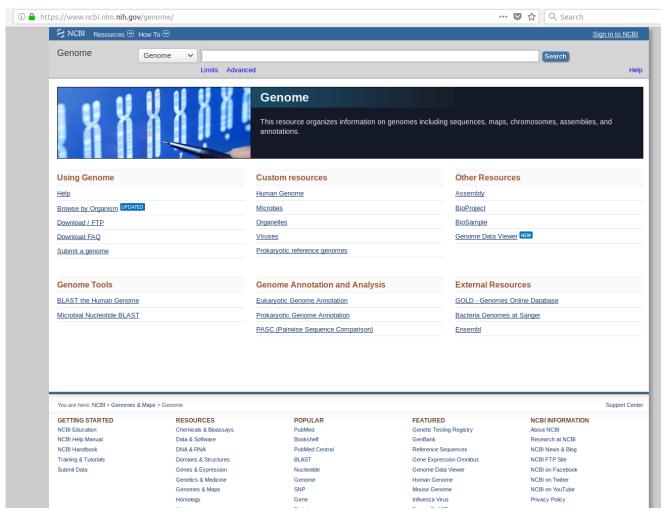




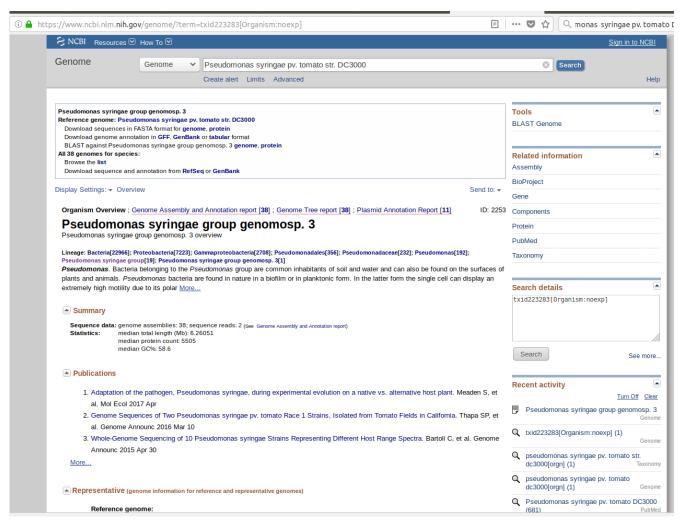
Type Phevamine A



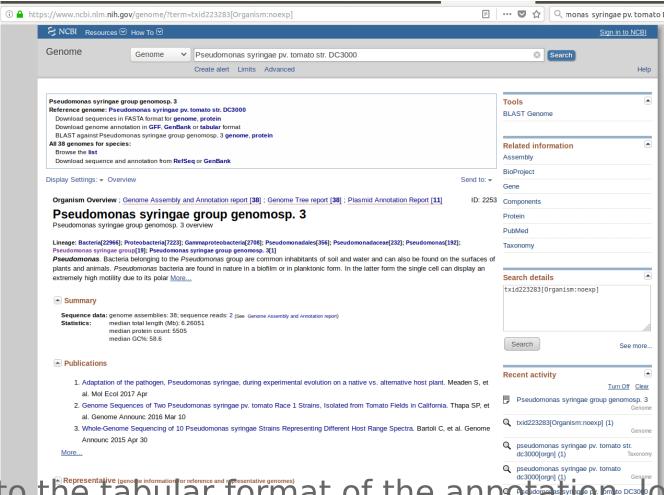
Type - Pseudomonas syringae pv. Tomato DC



Type - Pseudomonas syringae pv. Tomato DC



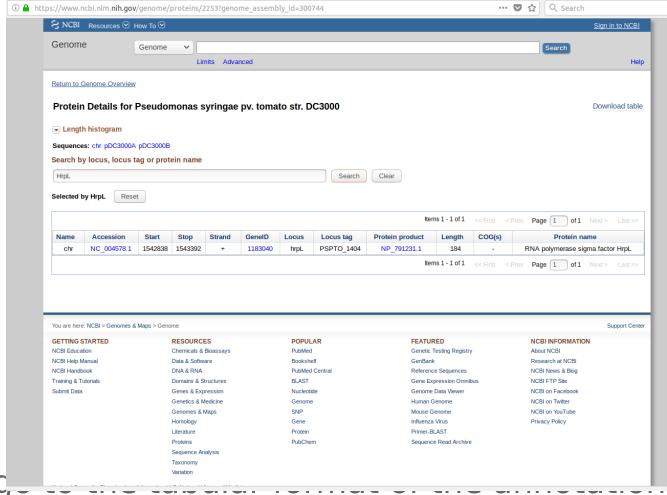
Type - download the gff, contig fasta, and tell me the genome size and number of genes on the main chromosom



Type - go to the Labular format of the annotative genoment of the annotative genoment of the find it

Download the gene as fasta format (as a protein, save the sequence).

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Type - g., look for F find it

Download the gene as fasta format (as a protein, save the sequence).

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(1)From protein tab, Type NP_791231.1, NC_004578.1

What do you get?

(2) From www.pubmed.com (main page), Type NP_791231.1, NC_004578.1

What do you get?

What are these numbers called?

Basic Local Alignment Search Tool -

J. Mol. Biol. 1990 Oct 5;215(3):403-10 —the primary reference for the BLAST algorithm.

Basic Local Alignment Search Tool

Stephen F. Altschul¹, Warren Gish¹, Webb Miller² Eugene W. Myers³ and David J. Lipman¹

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(Received 26 February 1990; accepted 15 May 1990)

A new approach to rapid sequence comparison, basic local alignment search tool (BLAST), directly approximates alignments that optimize a measure of local similarity, the maximal segment pair (MSP) score. Recent mathematical results on the stochastic properties of MSP scores allow an analysis of the performance of this method as well as the statistical significance of alignments it generates. The basic algorithm is simple and robust; it can be implemented in a number of ways and applied in a variety of contexts including straight-forward DNA and protein sequence database searches, motif searches, gene identification searches, and in the analysis of multiple regions of similarity in long DNA sequences. In addition to its flexibility and tractability to mathematical analysis, BLAST is an order of magnitude faster than existing sequence comparison tools of comparable sensitivity.

Most important and first major computational biology tool ever created...
Cited over 50,000 times

1. Introduction

The discovery of sequence homology to a known protein or family of proteins often provides the first clues about the function of a newly sequenced gene. As the DNA and amino acid sequence databases continue to grow in size they become increasingly useful in the analysis of newly sequenced genes and proteins because of the greater chance of finding such homologies. There are a number of software tools for

(Coulson et al., 1987).

Rapid heuristic algorithms that attempt to approximate the above methods have been developed (Waterman, 1984), allowing large databases to be searched on commonly available computers. In many heuristic methods the measure of similarity is not explicitly defined as a minimal cost set of mutations, but instead is implicit in the algorithm itself. For example, the FASTP program (Lipman & Pearson, 1985; Pearson & Lipman, 1988) first finds locally similar regions between two sequences

Basic Local Alignment Search Tool

Query sequence: R P P Q G L F

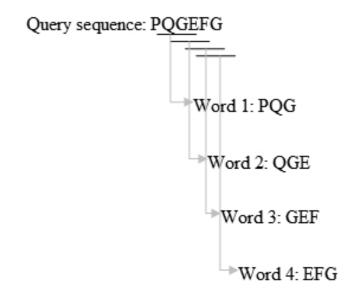
Database sequence: D P P E G V V

Exact match is scanned.

Score: -2 7 7 2 6 1 -1

HSP

Optimal accumulated score = 7+7+2+6+1 = 23



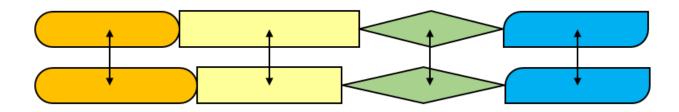
Basic Local Alignment Search Tool - types of sequence analysis

- Nucleotide Blast (MegaBlast, BlastN, nuc-nuc, db-query)
- 2. BlastX (translated nucleotide query to protein database)
- 3. tBlastN (protein query to translated nucleotide database)
- 4. tBlastX (translated nucleotide query and database)
- 5. Blastp (protein query and database)

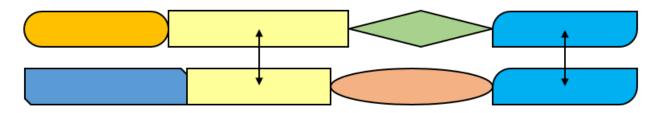
Alternatives to BLAST

-Pre-blast-

Smith-Waterman algorithm (Local alignment)
Needleman-Wunsch algorithm (Global alignment)



Global Alignment



Local Alignment

Alternatives to BLAST

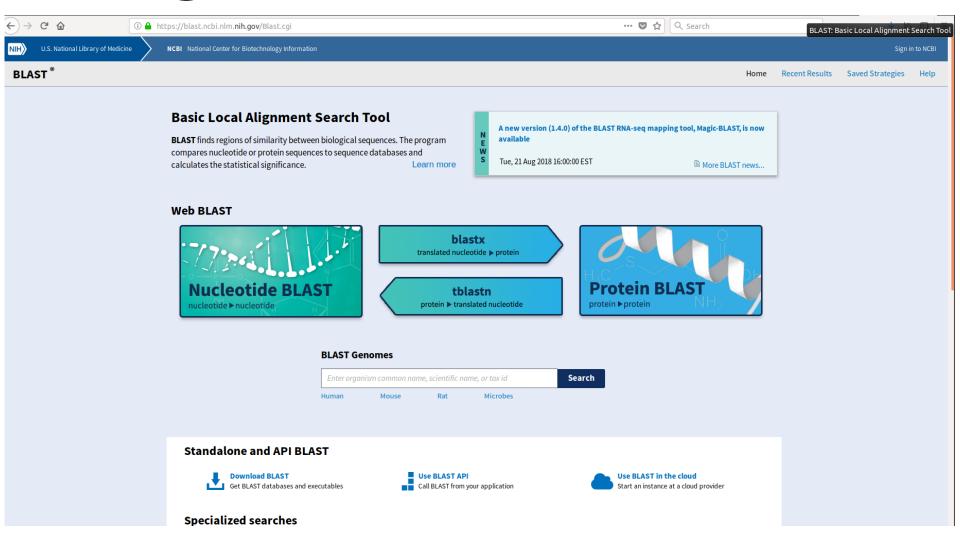
-Pre-blast-Smith-Waterman algorithm (Local alignment) Needleman-Wunsch algorithm (Global alignment)

Basic differences between SW and NW

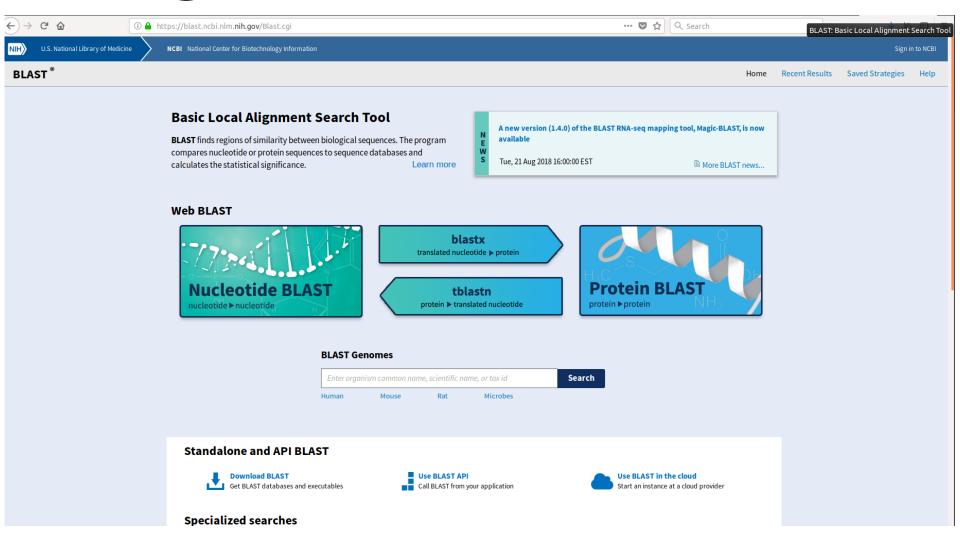
	Smith-Waterman algorithm	Needleman-Wunsch algorithm
Initialization	First row and first column are set to 0	First row and first column are subject to gap penalty
Scoring	Negative score is set to 0	Score can be negative
Traceback	Begin with the highest score, end when 0 is encountered	Begin with the cell at the lower right of the matrix, end at top left cell

Alternatives to BLAST

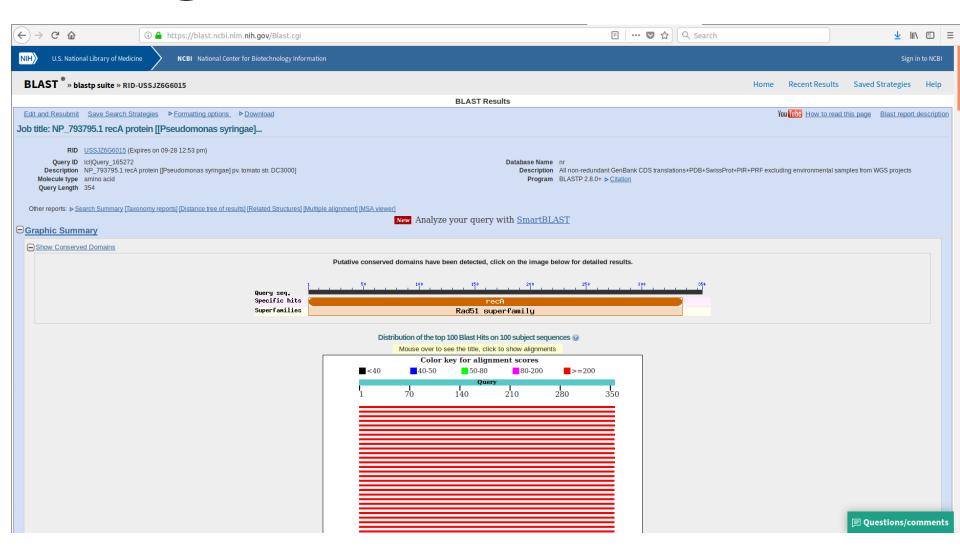
- -Post-blast-
- 1. BLAT (Blast-like alignment tool)
- 2. PatternHunter
- 3. LAST (Local alignment search tool)
- 4. KLAST
- 5. Sword (awesome, both fast SW and NW)
- 6. USEARCH
- 7. MMseq2
- 8. DIAMOND
- 9. Bowtie2, BWA
- 10.Hmmer (based on HMM)



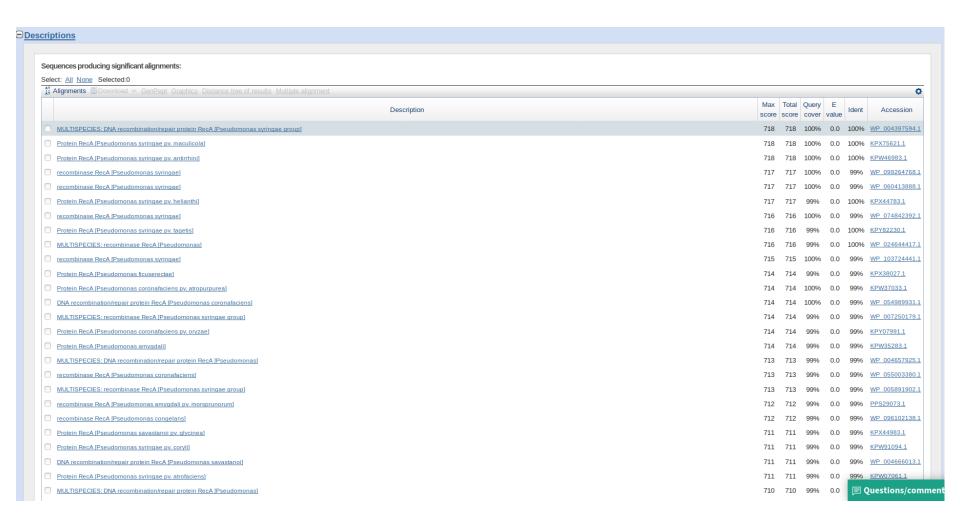
Go to website - https://www.ncbi.nlm.nih.gov/BLAST/

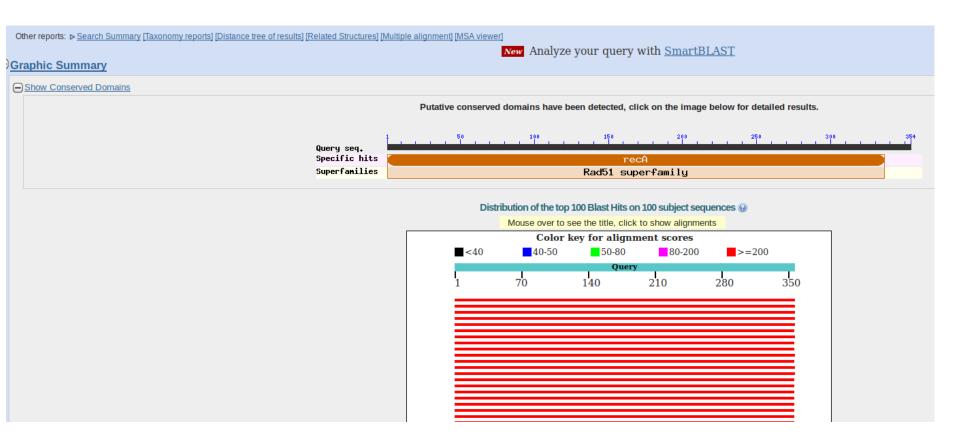


Complete a Blastp on your HrpL and RecA sequences

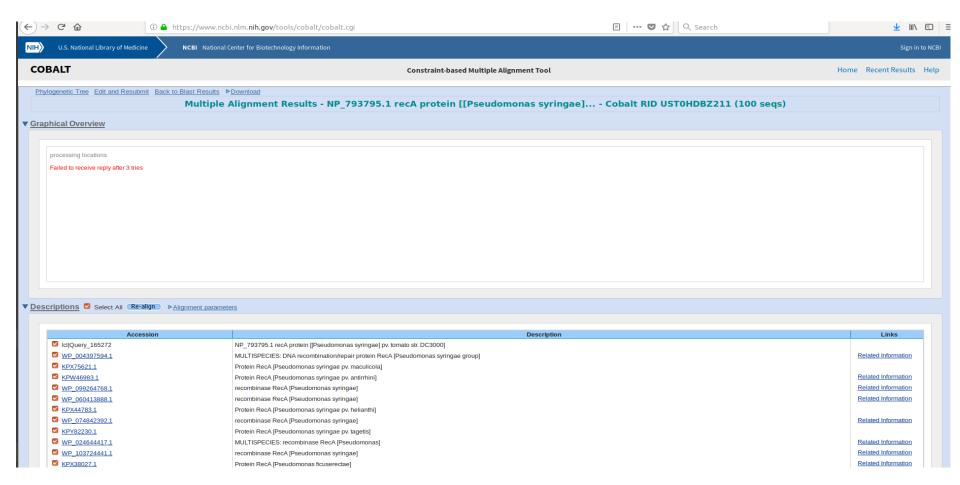


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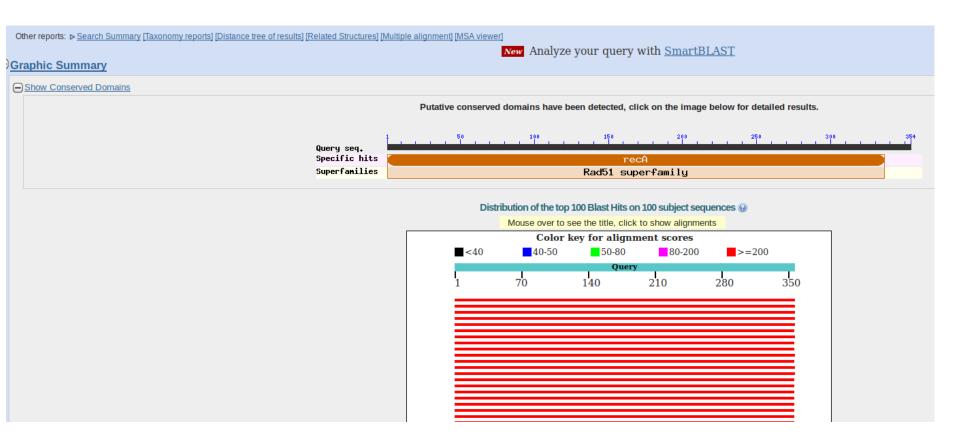




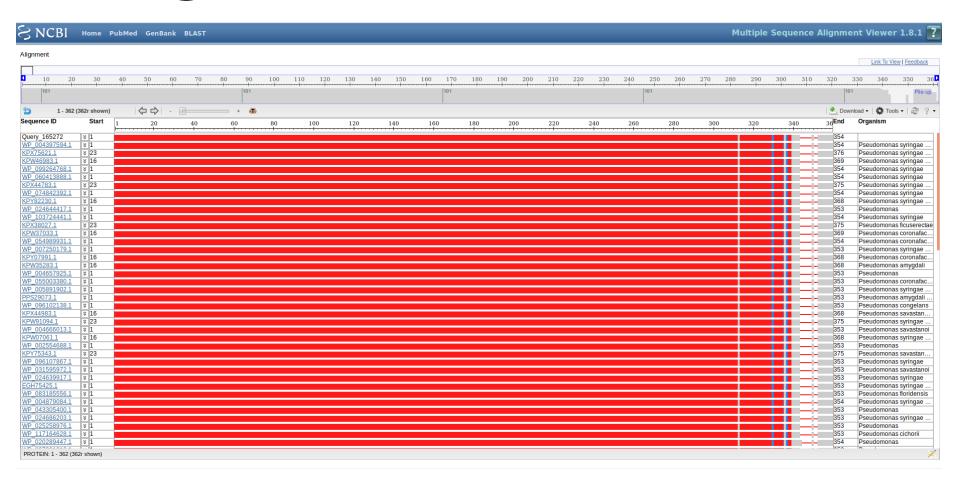
Click the Multiple alignment link, will turn on COBALT

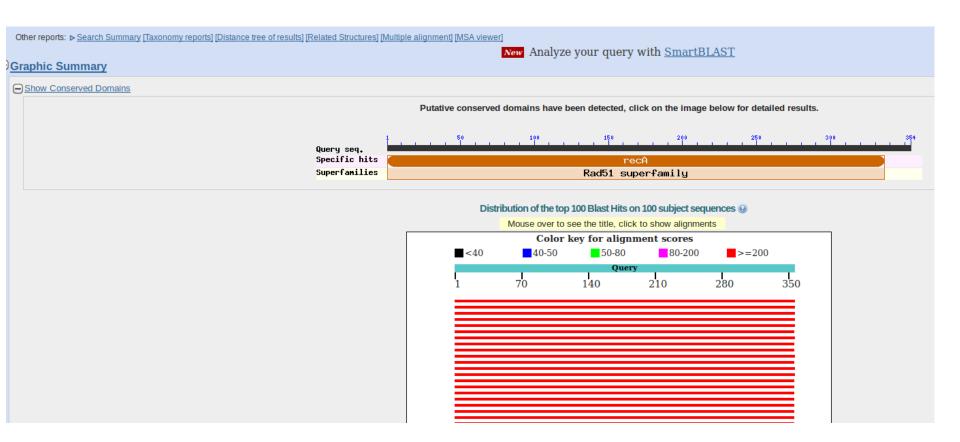


Click the Multiple alignment link, will turn on COBALT



Click the MSA viewer





Click the Distance tree results

