

Welcome to the Gramene BLAST Tutorial

- This tutorial will show you how to conduct a BLAST search.
- With BLAST you may:
 - Search for sequence similarity matches in the Gramene database - ideal for cross-species comparisons.
 - Select the best target database for your search.
 - Choose the best algorithm for your search.
 - Fine-tune search parameters.

Tutorial Tips



If you are viewing this tutorial with Adobe Acrobat Reader, click the "bookmarks" on the left hand side of the Reader for easier navigation.

Note! Although we continually work to make Gramene compatible with all browsers, there are problems with some browser versions. If you're having difficulty viewing Gramene, try using a different browser. Please report any problems with browsers through Gramene Feedback.

Gramene Home Page

www.gramene.org

The screenshot shows the Gramene Home Page with a green header. The header includes the Gramene logo, the tagline 'A Resource for Comparative Grass Genomics', the version 'V24 (March 2007)', and a navigation menu with links: Search, Genomes, Species, Download, Resources, About, Help, and Feedback. A left sidebar contains a 'Quick Search' box, a 'Have Questions...?' section with links to tutorials, feedback, and FAQ, and a 'Gramene Tip' section. The main content area is divided into 'Quick Start' and 'Featured News' sections. A blue callout bubble points to the 'Sequences-BLAST' link in the left sidebar, with the text 'Click here to open BLAST Home Page'.

GRAMENE A Resource for Comparative Grass Genomics V24 (March 2007)

Search Genomes Species Download Resources About Help Feedback

Quick Search

All Available

Search

Search a single module or all available modules plus online documentation.

Diversity, Pathways, BLAST and Mart not available in this search.

Have Questions...?

- Gramene now has [tutorials](#) for every module, also recommended for experienced users.
- Ask questions through [Feedback](#) or [Email](#).
- See [FAQ](#) for questions and answers.

Gramene Tip:

You can contact Gramene either by e-mailing us at gramene@gramene.org or by sending a question to the curators and developers at Gramene on any page by selecting the Feedback button in the top right hand corner.

- [Browse All Tips](#)

Quick Start

sequenced genomes for [Rice](#), [Maize](#) & [Arabidopsis](#); Look for [rice/maize synteny](#); [GrameneMart](#); Search for sequence alignment with [BLAST](#); search by [Gene](#)

[PFam](#) or [ProSite](#) or Browse by Gene Ontology using [GO Slim](#).

or physical maps for [Rice](#), [Wild Rice](#), [Maize](#), [Wheat](#), [Barley](#), [Oats](#), [Sorghum](#), and the Comparative Map Viewer ([CMap](#)) to compare maps of different types and

Genetic markers (RFLPs, SSRs, etc.), DNA Probes (Primers, Overgos, etc.), [Genes](#), [FPContigs](#), etc.), and Sequences (GSSs, ESTs, etc.); Use the Simple Sequence [Pool \(SSRIT\)](#); or search by species, including [Rice \(Oryza sativa\)](#), [Maize](#), [Sorghum](#)

Sequences-BLAST

TRAITS: Search the [Genes](#) or [QTL](#) database for important phenotype-related loci such as [Rice Genes](#), [Rice QTL](#), [Maize QTL](#). Don't forget to explore traits in [Ontologies](#).

GENETIC DIVERSITY: Search for SNP and SSR allelic variation on loci of [rice](#), [maize](#), and [wheat](#) germplasms.

BIOCHEMICAL PATHWAYS: Search for ALL the rice pathways on [starch biosynthesis](#) or get an overview of the metabolic datasets.

LITERATURE: Search for [Rice](#) literature of interest.

SUBMISSIONS: Submit your [Rice](#) data to the [Gramene](#) database.

Featured News

- [NEW](#) March 2007, V 24 release notes.
- [NEW](#) [Gramene](#) Jan/Feb Newsletter
- [Rice News Worldwide](#) from IRRI

Visit with us at

- March 15-18, 2007. [CSHL Plant Genome meeting](#)
- March 22-25, 2007. [Maize Genetics Meeting](#)
- April 16-20, 2007. [ITMI](#)
- May 8-12, 2007. [Biology of Genomes](#)
- July 7-11, 2007. [ASPB](#)

[View Previous Gramene Presentations](#)

[Calendar](#)

Gramene is a curated, open-source, web-accessible data resource for comparative genome analysis in the grasses. Our goal is to facilitate the study of cross-species homology relationships using information derived from public projects involved in genomic and EST sequencing, protein structure and function analysis, genetic and physical mapping, interpretation of biochemical pathways, gene and QTL localization and descriptions of phenotypic characters and mutations.

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BLAST Home Page

Sequence Information:
Enter a sequence that you
have and are trying to
locate, or are trying to
find a similar sequence
for

Search Information:
where would you like to
search for similar
sequences, using which
tool?

TBLASTX compares a
nucleotide query sequence
translated in all reading
frames against a protein
sequence database

The screenshot shows the BLAST Home Page interface. At the top, there are navigation buttons: 'new', 'SETUP' (highlighted), 'CONFIG', 'RESULTS', and 'DISPLAY'. On the right side, there are links for 'refresh', 'FAQ', 'Online Help', and 'Tutorial'. Below these is a 'Summary' section with links for 'setup', 'configure', and 'results', each with a status indicator 'Not yet initialised'.

The main content area is divided into three sections:

- Enter the Query Sequence:** This section has three options:
 - Either Paste sequences (max 10) in FASTA or plain text:** A large text input field.
 - Or Upload a file containing one or more FASTA sequences:** A file upload button labeled 'Browse...'.
 - Or Enter an existing ticket ID:** A text input field and a 'Retrieve' button.Below these options are radio buttons for 'dna queries' (selected) and 'peptide queries'.
- Select the databases to search against:** This section includes a 'Select species:' dropdown menu with options 'Millet', 'Poaceae', and 'Rice' (selected). Below this are radio buttons for 'dna database' (selected) and 'peptide database'. To the right of these are two dropdown menus: 'Genomic sequence' and 'Peptides (Fgenesh gene models)'.
- Select the Search Tool:** A dropdown menu with options 'BLASTN' (selected) and 'TBLASTX'.

At the bottom of the form, there is a 'Search sensitivity:' dropdown menu with 'None' selected, and a 'RUN' button. A 'configure' button is also present next to the 'RUN' button.

Annotations in blue speech bubbles provide additional information:

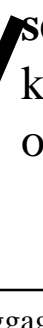
- A bubble pointing to the 'dna queries' radio button states: 'DNA codes contain: ACTG' and 'Peptide codes contain: GALMFWKQESPVICYHRNDT'.
- A bubble pointing to the 'BLASTN' option in the 'Select the Search Tool' dropdown states: 'BLASTN compares a nucleotide (dna) query sequence against a nucleotide sequence database'.

Example

BLAST - The Basic Local Alignment Search Tool – is used when searching for related (similar) sequences.

Problem: Finding a Nucleotide Match in the Rice Genome

You have an RFLP (restriction fragment length polymorphism) genetic marker with a **known hybridization sequence**, and want to know where it is located on the rice genome



```
1  ggcatccatg gcgccaagg cggagaagaa gccggcggcg aagaagccg cggaggagga
61  gcccgcggcg gagaaggccg agaaggcctg gcggggaaga agccaaggc ggagaagcgt
121 ctccccgccg gcaaggccga gaagagcagc ggcgagggga agaaggcggg gcggaagaag
181 gcgaagaaga gcgtcgagac ctacaagatc tacatctca aggtgctcaa gcaggtccac
241 cccgacatcg gcatctctc caaggccatg tccatcatga actcctcat caacgacatc
301 ttcgagaagc tcgccgggga gtccgccaag ctgcgcgct acaacaagaa gccaccatc
361 aactnacggg agatccagac ctncgtccgc cttgtc
```

Step 1: Enter the Sequence

The screenshot shows a web interface with a navigation bar at the top containing buttons: **new**, **SETUP** (highlighted in red), **CONFIG**, **RESULTS**, and **DISPLAY**. On the right side, there are buttons for **refresh**, **FAQ** (highlighted in red), **Online Help**, and **Tutorial** (highlighted in red). The main content area is titled "Enter the Query Sequence" and contains three options:

- Either Paste sequences (max 10) in FASTA or plain text:** A text area containing a sample FASTA sequence:

```
ggcatccatg ggcaccaagg cggagaagaa gccggcggcg aagaagcccg  
gcccggggcg gagaaggccg agaaggcctg gccgggaaga agcccaaggc  
ctccccgccg gcaaggccga gaagagcagc gccgagggga agaaggccgg  
gcgaagaaga gcgtcgagac ctacaagatc tacatcttca aggtgctca
```
- Or Upload a file containing one or more FASTA sequences:** A text input field followed by a **Browse...** button.
- Or Enter an existing ticket ID:** A text input field.

At the bottom left, there are two radio buttons: ☒ **dna queries** and ☐ **peptide queries**.

Callouts provide additional instructions:

- 1. Paste in the sequence** (points to the text area)
- Browse FAQ** (points to the FAQ button)
- Click for Help** (points to the Online Help button)
- Alternatively, save a sequence to a file and use this box to upload it.** (points to the file upload section)
- 1a. Select "dna queries" because this is a nucleotide sequence** (points to the dna queries radio button)
- You can search with up to 10 sequences at a time. Simply format them using FASTA format.** (points to the text area)

Step 2: Choose Target Database

2b. Select your database. For this example, choose “dna database”

2a. Select your target organism(s), in this case “Rice”

the databases to search against

Select species: Poaceae
Rice
Rice_alta

Use 'ctrl' key to select multiple species

☒ dna database
☐ peptide database

Genomic sequence
Peptides (Fgenesh gene models)

Select the Search Tool

BLASTN
TBLASTX

configure ▶ RUN ▶

Search sensitivity: Near-exact

Opt... h parameters to find the following alignments

2c. Select a specific database. In this case, “Genomic Sequence”

2e. Select your search sensitivity

2f. Click RUN

Search tool options will change according to species and database.

2d. Select your search tool, “BLASTN” for this example

Used To examine or alter the optimized default parameters (see slide 16)

Use BLASTN for simple nucleotide against nucleotide searches

Use BLASTP for simple protein versus protein searches

Use TBLASTN for a protein query versus a DNA library search

Use BLASTX for DNA query versus protein library

Use TBLASTX for a translated DNA query against a translated DNA database

BLAST Queries

The screenshot shows the Gramene BlastSearch (BlastView) interface in a SmartFox Internet Browser. The browser's address bar displays the URL http://www.gramene.org/Multi/blastview/BLA_laO45mV6D, which is circled and labeled "Ticket ID". The page features a green header with the "GRAMENE Multi" logo and navigation links: Search, Genomes, Download, Resources, About, and Help. A search bar with the placeholder "Find anything" and a "Search" button is located on the right. Below the header, a navigation bar contains buttons for "new", "SETUP", "CONFIG", "RESULTS", and "DISPLAY". The main content area displays "Displaying unnamed sequence alignments vs Rice LATESTGP database" and "Showing top 100 alignments of 167, sorted by Raw Score". A dropdown menu is open, showing options: E-value, Alignment Length, % Identity, P-value, and Raw Score. A callout points to the "refresh" button next to the dropdown, stating "To sort results select an option and refresh". Another callout points to the "refresh" button in the top right corner, stating "Use Feedback to make enquiries to Gramene Staff". A third callout points to the "Alignment Locations vs. Karyotype (click arrow to hide)" link, stating "Click Arrows to hide or reveal results sections." A large blue box at the bottom contains the text: "Results are stored on Gramene's server for one week, so that they can be accessed later with the **Ticket ID** or a bookmark to the results page."

Ticket ID

Use Feedback to make enquiries to Gramene Staff

Click Arrows to hide or reveal results sections.

To sort results select an option and refresh

Results are stored on Gramene's server for one week, so that they can be accessed later with the **Ticket ID** or a bookmark to the results page.

Step 3: Results - Genomic Context

Click "CONFIG" if you want to change parameters & try again (slides 16 - 17).

Displaying unnamed sequence alignments vs Rice LATESTGP database

Showing top 100 alignments of 167, sorted by Raw Score

refresh

Alignment Locations vs. Karyotype (click arrow to ...)

Alignment vs AP002522

Alignment...
Alignment...
Query Sequence...
Genomic Sequence...
ContigView...
Raw Score: 343
PercentID: 96.69
Length: 393
P-value: 3.2e-196
E-value: 3.2e-196
Alignment...
Query Sequence...
Query Sequence...
Genomic Sequence...
Genomic Sequence...
ContigView...
Raw Score: 339
PercentID: 96.44
Length: 393
P-value: 4.7e-193
E-value: 4.7e-193
Alignment...
Query Sequence...
Query Sequence...
Genomic Sequence...
Genomic Sequence...
ContigView...
Raw Score: 334
PercentID: 96.17
Length: 392
P-value: 9.0e-190

Identifies number of results

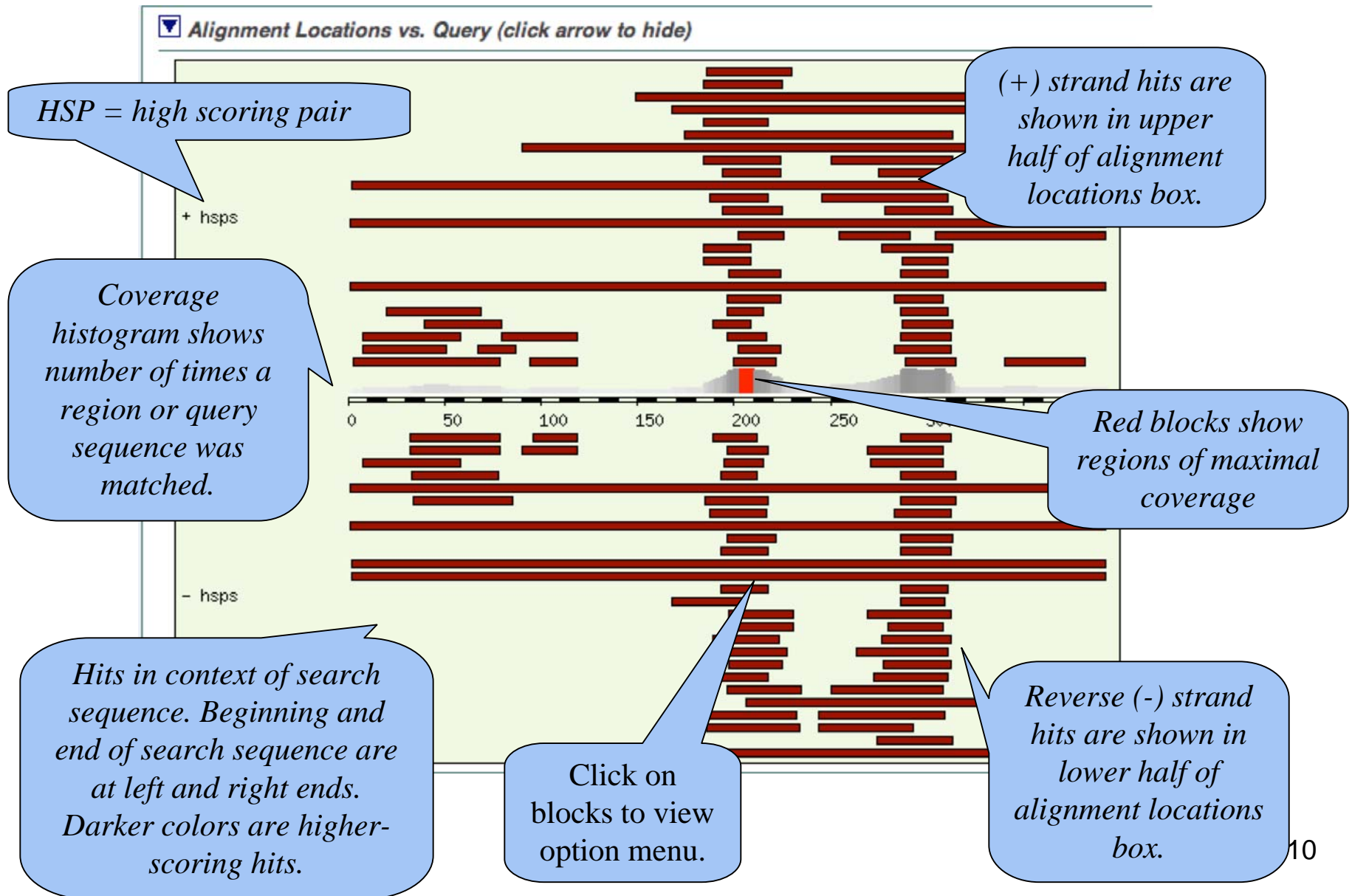
Click on pointers to view option menu.

Hits are in genomic context. Darker triangles indicate higher scoring hits. The best hit is in a box.

Summary

- setup
 - Rice
 - Genomic sequence
 - BLASTN
 - Low sensitivity
- configure
 - E: 10
 - B: 100
 - filter: dust
 - W: 15
 - M: 1
 - N: -3
 - Q: 3
 - R: 3
- results
- display
 - Not yet initialised

Step 3 Results - Search Sequence



Step 3: Results – Alignment Summary

Alignment Summary (click arrow to hide)

Select rows to include in
(Use the 'ctrl' key to select

Query
Name
Start
End
Ori

Subject
off
Name
Start
End

5. Click the [C] for “**contig**”
view of the genome (see
Genome Browser Tutorial).

Sort By
>Score
<E-val
>E-val
<P-val

Links

Links	Query			Chromosome			Stats				
	Start	End	Ori	Name	Start	End	Chr	Score	E-val	%ID	Length
[A] [S] [G] [C]	1	392	-	Chr:1	2817113	2817505	+	379	1.1e-217	98.98	393
[A] [S] [G] [C]	1	392	-	Chr:1	2834924	2835316	+	343	3.2e-196	96.69	393
[A] [S] [G] [C]	1	392	+	Chr:1	2671274	2671666	+	339	4.7e-193	96.44	393
[A] [S] [G] [C]	2	392	-	Chr:1	2854476	2854867	+	334	9.0e-190	96.17	392
[A] [S] [G] [C]	2	392	-	Chr:1	2841537	2841928	+	330	9.0e-190	96.17	392
[A] [S] [G] [C]	392	+	Chr:1	2683597	2683989	+	392	6.8e-188	95.93	393	
[A] [S] [G] [C]	392	+	Chr:1	2668984	2669381	+	397	6.8e-188	95.93	393	
[A] [S] [G] [C]	392	+	Chr:5	28350554	2835446	+	90	3.8e-155	100.00	77	

4. Click the [A] link of the best (first)
hit to **view the alignment**. (Slide 12)

Select from menus to
configure results

Click these links to view:

[A] alignment
[S] query sequence
[G] target (genome) sequence
[C] on genome (“ContigView”)

3/22/07

*Shortcut: You can also click
on the best hit in the genome
graphic (from Slide 9) to open a
menu for that alignment.*

Alignment Locations vs. Karyotype (click arrow to hide)

Alignment vs AP003045

Alignment...

Query Sequence...

Genomic Sequence...

ContigView...

Raw Score: 379

PercentID: 98.98

Length: 393

P-value: 1.1e-217

E-value: 1.1e-217

Alignment...

Query Sequence...

Genomic Sequence...

ContigView...

Raw Score: 343

PercentID: 96.69

Length: 393

P-value: 3.2e-196

E-value: 3.2e-196

Alignment...

Query Sequence...

Alignment Summary (click arrow to hide)

Select rows to include in table, and type of sort
(Use the 'ctrl' key to select multiples)

Query
off
Name
Start

Subject
off
Name
Start

Chromosome
off
Name
Start

Clone
off
Name
Start

Stats
off
Score
E-val

Sort By
>Clone
<Score
>Score

Links	Query	Chromosome	Start	Stats	E-val
	Start	Name		Score	
[A][S][G][C]	1	Chr:1	2817113	379	1.1e-217
[A][S][G][C]	1	Chr:1	2834924	343	3.2e-196
[A][S][G][C]	1	Chr:1	2671274	339	4.6e-193
[A][S][G][C]	2	Chr:1	2841537	334	8.9e-190
[A][S][G][C]	2	Chr:1	2854476	334	8.9e-190
[A][S][G][C]	1	Chr:1	2683597	331	6.8e-188
[A][S][G][C]	2	Chr:1	2668984	300	6.8e-169
[A][S][G][C]	90	Chr:5	28350554	216	3.8e-155

Step 4: Review Alignment

```
Query location      : unnamed      1 to      392 (-)
Database location   : AP002522    124988 to 125380 (+)
Genomic location    : 1            2817113 to 2817505 (+)
```

Alignment score : 379
E-value : 1.1e-217
Alignment length : 393
Percentage identity: 98.98

This subject looks like a pretty good match for the query.

*The sequence
you searched.*

*The result you
are viewing.*

Query: 392 AGGCGGACGNAGGTCTGGATCTCCCGTNAGTTGATGGTGGGCTTCTTGTTGTAG
|||||
Sbjct: 124988 AGGCGGACGAGGTCTGGATCTCCCGTGAGGTGATGGTGGGCTTCTTGTTGTAGCGCGCG 125047

Query: 332 AGCTTGGCGGACTCCCCGGCGAGCTTCTCGAAGATGTCGTTGATGAAGGAGTTCATGATG 273
 |||
 Subject: 125048 AGCTTGGCGGACTCCCCGGCGAGCTTCTCGAAGATGTCGTTGATGAAGGAGTTCATGATG 125107

212 GACATGGCCCTGGAGGAGATGCCGATGTCGGGGTGGACCTGCTTGAGCACCTTGAAGATG 213
 |||||
 Sbjct: 125108 GACATGGCCCTGGAGGAGATGCCGATGTCGGGGTGGACCTGCTTGAGCACCTTGAAGATG 125167

```
Query:      212 TAGATCTTGTAGGTCTCGACGCTCTTCTT
            |||
Sbjct: 125168 TAGATCTTGTAGGTCTCGACGCTCTTCTT
```

Note that the long sequences continue to the next line, like reading a sentence

Query: 152 CCGCTGCTCTTCTCGGCCTTGCCGGCGGG
Sbjct: 125228 CCGCTGCTCTTCTCGGCCTTGCCGGCGGGGAGACGCTTCTCCGCCTTGGGCTTCTTCCCC 125287

Query: 92 GCCAGG-CCTTCTCGGCCTTCTCCGCCGCGGGCTCCTCCTCCGCGGGCTTCTTCGCCGCC 34
 |||||
 Sbjct: 125288 GCCAGGGCCTTCTCGGCCTTCTCCGCCGCGGGCTCCTCCTCCGCGGGCTTCTTCGCCGCC 125347

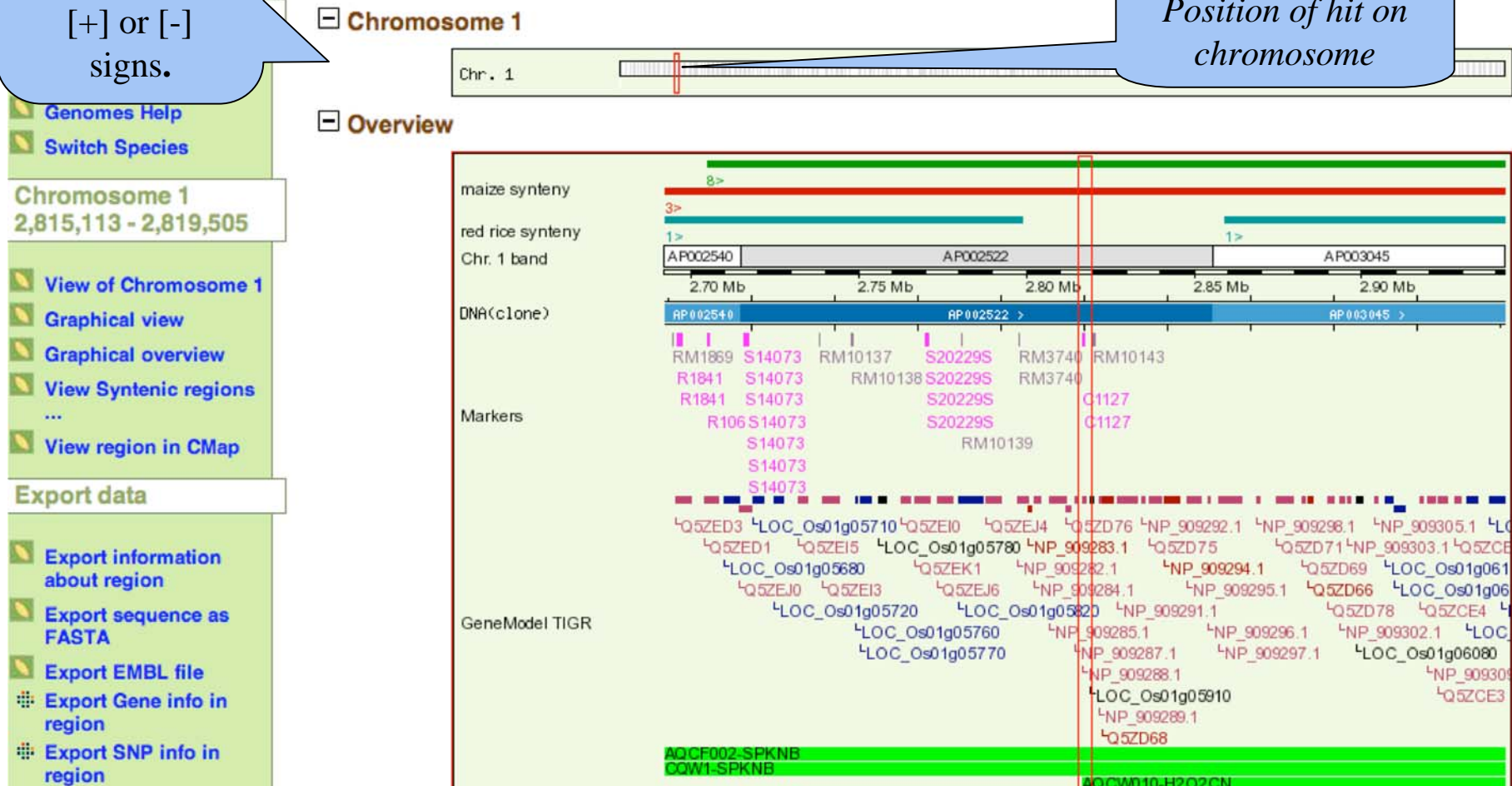
```
Query:      33 GGCTTCTTCTCCGCCTTGGGCGCCATGGATGCC 1
             |||
Sbjct: 125348 GGCTTCTTCTCCGCCTTGGGCGCCATGGATGCC 125380
```

Note the lines identifying matches, and the absence of a line if it doesn't match.

**Show or Hide
tables by
clicking on the
[+] or [-]
signs.**

Step 5: Review Genome with Genome Browser

Position of hit on chromosome



For more information see [Genome Browser Tutorial](#) and [Contig View help](#).

Step 5: Review Best Hit on Genome

Chromosome 1

Chr. 1

Overview

Detailed view

Features ▾ ESTs ▾ GSSs ▾ FSTs ▾ Arrays ▾ Markers ▾ DAS Sources ▾ Repeats ▾ Decorations ▾ Image size ▾

Jump to region 1 : 2815113 - 2819505 Refresh

Zoom

Chr. 1

Length

DNA(clone)

Blast hits

GeneModel TIGR

Rice_EST

Forward strand

4.39 Kb

AP002522

< NP_909287.1 tigr_gene

< NP_909288.1 tigr_gene

< LOC tigr_ge

Note that the overview has been hidden (from previous slide).

Use menu to select features displayed

Position of hit shown at high magnification.

Collapse or Expand tables by clicking on the [+] or [-] signs.

This is the most probable position of RFLP. You should correlate with genetic position to be sure.

For more information see Genome Browser Tutorial and Contig View help.

Step 6: Adjusting Settings

Select the databases to search against

Select species:
Use 'ctrl' key to select multiple species

Poaceae
Rice
Rice_alta

☒ dna database
☐ peptide database

Genomic sequence
Peptides (Fgenesh gene models)

Select the Search Tool

BLASTN
TBLASTX

configure ▶ RUN ▶

Search sensitivity:
Optimise search parameters to find the following alignments

Near-exact matches

During the search (Slide 7)
Instead of hitting RUN,
press **configure**.

Step 6: Adjust Settings - Configure

new SETUP CONFIG RESULTS DISPLAY refresh Online Help FAQ Tutorial

Run Search

Configuration for BLASTN

E-value cutoff: 0.0001, 0.001, 0.01, 0.1, 1, **✓ 10**, 100, 1000, 10000, 100000, dust

Maximum E-value for reported alignments

Number of database hits to report

Used to filter query sequence

-sort_by

Sort option for database hits

Statistics

Statistics option for calculation of alignment score

Word size for seeding alignments

-wink

Step-size for sliding-window used to seed alignments

One-hit seeding. (One-hit)

Character

and remaining gap characters

-nogap

Turns off gapped alignments

-X

Alignment extension cutoff

Additional

Other options (not validated)

Summary

- BLASTN
- Low sensitivity
- configure
- results
- display

See *help* for
“*config*” assistance

6b. Click “**RUN**”.

Identifies version of
BLAST selected

Each option has listed
it's explanation

Tune search by adjusting
desired BLASTN
parameters. Parameter
set will depend on the
version of BLAST
selected in previous step.

6a. Change E-value cutoff to
0.001 to increase stringency
of search.

Step 7: Refined Results

The screenshot displays a web-based BLAST interface. At the top, navigation tabs include 'NEW', 'SETUP', 'CONFIG', 'RESULTS', and 'DISPLAY'. The 'RESULTS' tab is active, showing 'Displaying unnamed sequence alignments vs Rice LATESTGP database'. Below this, a dropdown menu indicates 'Showing top 100 alignments of 167, sorted by Raw Score'. A 'refresh' button is present. The main content area is divided into two sections: 'Alignment Locations vs. Karyotype (click arrow to hide)' and 'Alignment Locations vs. Karyotype'. The first section shows a karyotype with 10 chromosomes, with red arrows indicating alignment locations. The second section shows a karyotype with 12 chromosomes, with red arrows indicating alignment locations. A blue speech bubble points to the second karyotype, stating: 'Notice there are fewer low-scoring hits after doing a config.' The right panel contains a 'Summary' section with links for 'refresh', 'Online Help', 'FAQ', and 'Tutorial'. Below this, a 'setup' section lists 'Rice' and 'Genomic'. A 'configure' section lists search parameters: '-E: 0.0001', '-B: 100', '-filter: dust', '-W: 15', '-M: 1', '-N: -1', '-Q: 3', and '-R: 3'. A 'results' section and a 'display' section are also visible. A blue speech bubble points to the 'configure' section, stating: 'The current search parameters are shown in the right panel.'

Displaying unnamed sequence alignments vs Rice LATESTGP database
Showing top 100 alignments of 167, sorted by Raw Score

Alignment Locations vs. Karyotype (click arrow to hide)

Alignment Locations vs. Karyotype

Notice there are fewer low-scoring hits after doing a config.

The current search parameters are shown in the right panel.

- Rice
- Genomic sequence
- BLASTN
- Custom sensitivity

configure

- -E: 0.0001
- -B: 100
- -filter: dust
- -W: 15
- -M: 1
- -N: -1
- -Q: 3
- -R: 3

results

display

More BLAST Information

Web sites that provide more BLAST information and advice on setting parameters include:

- the WU-BLAST web site <http://blast.wustl.edu/>
- EBI 2can introductions for protein or nucleotide BLAST, <http://www.ebi.ac.uk/2can/>
- NCBI-BLAST (*a good tutorial although focused on a slightly different implementation.*)
<http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html>

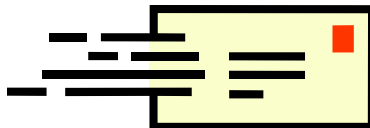
A book on the BLAST family of sequence similarity search algorithms (Joseph Bedell, Ian Korf and Mark Yandell, **BLAST**, O'Reilly & Associates, 2003, ISBN: 0-596-00299-8) provides profound theoretical background, as well as a protocol section covering common practical search problems. The pre-defined optimized parameter sets are based on recommendations in this book.
(<http://www.oreilly.com/catalog/blast/>)

Contact Gramene



Use the feedback button, located at the top of every page, to provide **feedback** or to **ask questions** about Gramene or your search needs.

or



Email the Gramene list at gramene@gramene.org