Ebbie v3.0.8

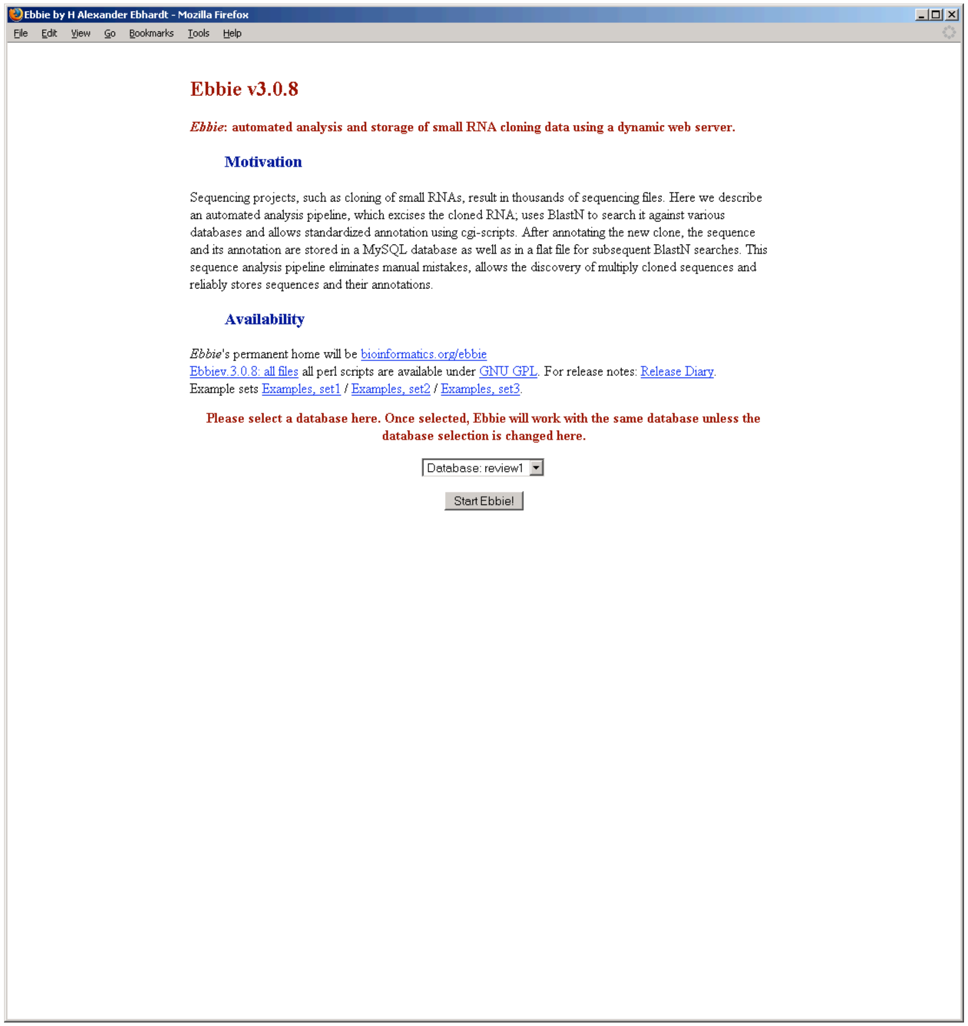
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

by H Alexander Ebhardt

Tutorial

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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***Ebbie*: automated analysis and storage of small RNA cloning data using a dynamic web server.**

Motivation

Sequencing projects, such as cloning of small RNAs, result in thousands of sequencing files. Here we describe an automated analysis pipeline, which excises the cloned RNA; uses BlastN to search it against various databases and allows standardized annotation using cgi-scripts. After annotating the new clone, the sequence and its annotation are stored in a MySQL database as well as in a flat file for subsequent BlastN searches. This sequence analysis pipeline eliminates manual mistakes, allows the discovery of multiply cloned sequences and reliably stores sequences and their annotations.

Availability

Ebbie's permanent home will be bioinformatics.org/ebbie
Ebbiev.3.0.8: all files all perl scripts are available under GNU GPL. For release notes: Release Diary.
Example sets Examples, set1 / Examples, set2 / Examples, set3.

Please select a database here. Once selected, Ebbie will work with the same database unless the database selection is changed here.

Front page of *Ebbie*.

- Links to
  - http://bioinformatics.org/ebbie
  - All files, GNU GPL, Release Diary
  - Example files

  can be found.
- Choose database here; *Ebbie* will use this database until it is changed on the front page.

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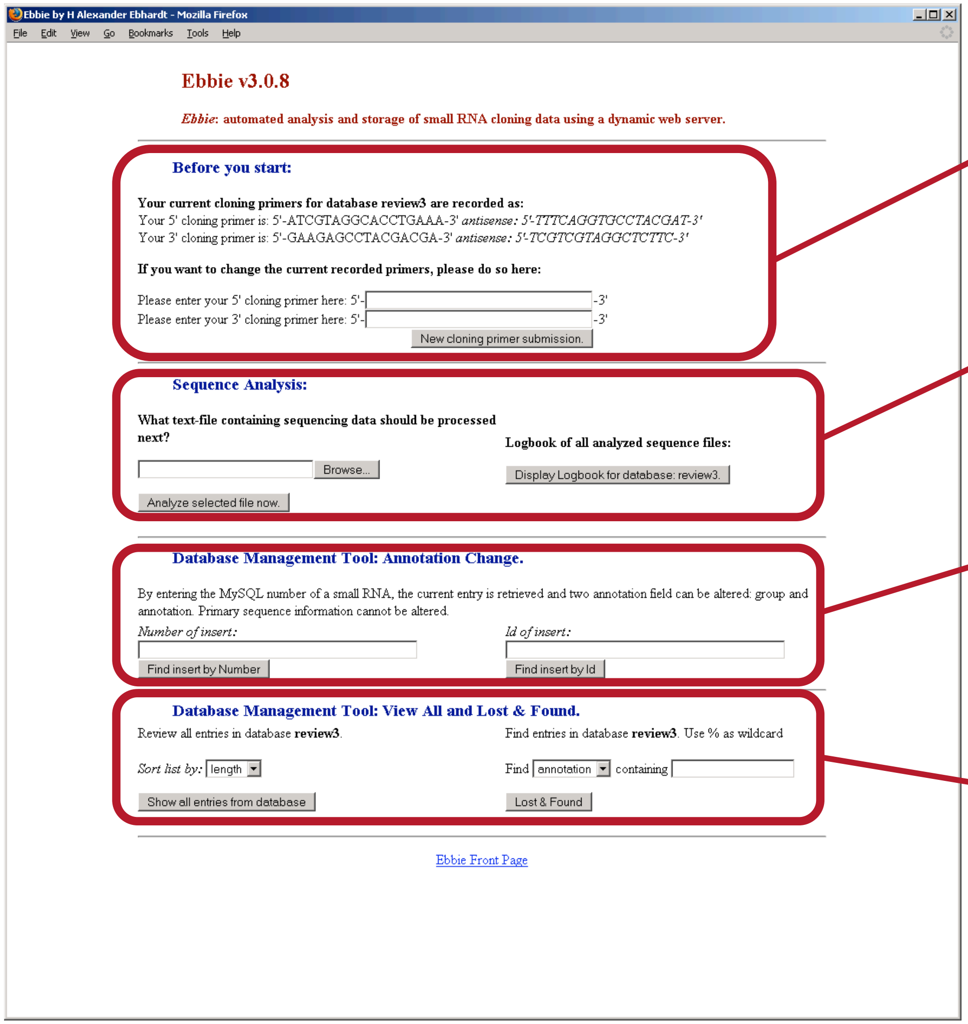
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

Before you start:

Your current cloning primers for database review3 are recorded as:
Your 5' cloning primer is: 5'-ATCGTAGGCACCTGAAA-3' *antisense: 5'-TTTCAGGTGCCTACGAT-3'*
Your 3' cloning primer is: 5'-GAAGAGCCTACGACGA-3' *antisense: 5'-TCGTCGTAGGCTCTTC-3'*

If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'- -3'
Please enter your 3' cloning primer here: 5'- -3'

Sequence Analysis:

What text-file containing sequencing data should be processed next?

Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Find entries in database **review3**. Use % as wildcard

Sort list by: length

Find annotation containing

Main page of *Ebbie*.

- Before you start:
  - Reports 5′- and 3′-cloning primers (5-CP, 3-CP).
  - New primer pairs can be entered.
  - Only ACGT are valid characters.
- Sequence Analysis:
  - Selection of a text file containing DNA sequencing data.
  - Logbook of all files analyzed.
- Database Management Tool: Annotation Change
  - Search for insert by its database number (e.g. cloned small RNA).
  - Copy/Paste Id of insert (e.g. from BlastN search result).
- Database Management Tool: View All and Lost & Found
  - Review all entries, order by id, length, number or group.
  - Find entries by using wild card characters.

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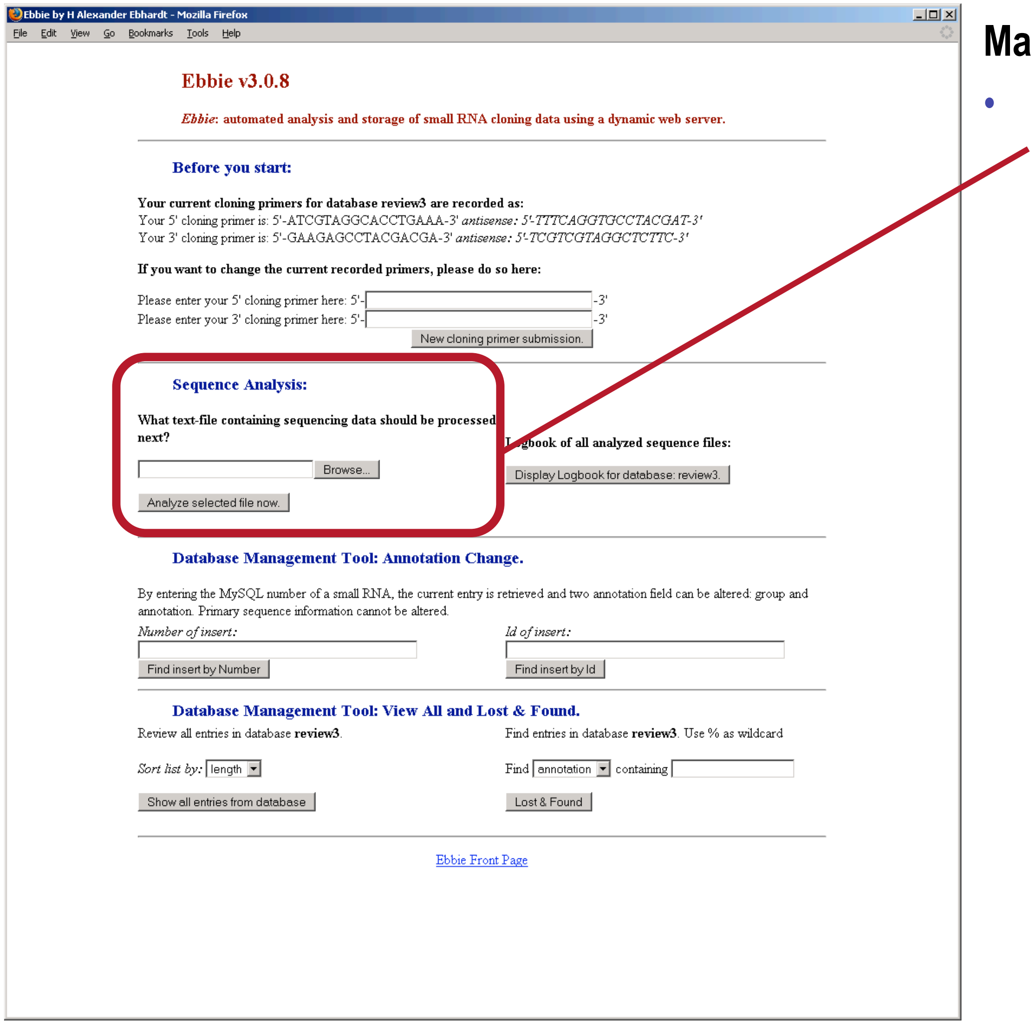
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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If you want to change the current recorded primers, please do so here:

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Please enter your 3' cloning primer here: 5'- -3'

Sequence Analysis:

What text-file containing sequencing data should be processed next?

Logbook of all analyzed sequence files:

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By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

Find annotation containing

Ebbie Front Page

Main page of *Ebbie*.

- Sequence Analysis:
  - Uploading text file containing DNA sequencing data.

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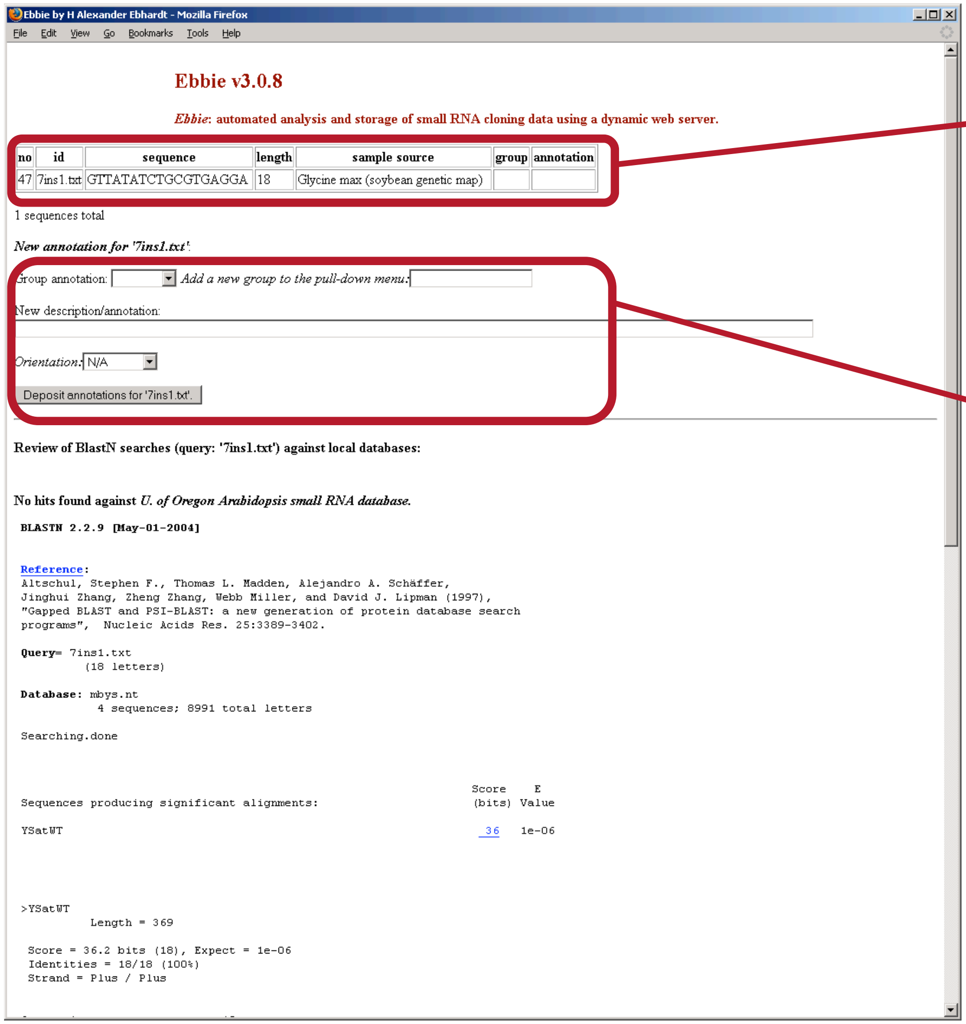
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

no	id	sequence	length	sample source	group	annotation
47	7ins1.txt	GTTATATCTGCGTGAGGA	18	Glycine max (soybean genetic map)		

1 sequences total

New annotation for '7ins1.txt':

Group annotation: *Add a new group to the pull-down menu:*

New description/annotation:

Orientation: N/A

Review of BlastN searches (query: '7ins1.txt') against local databases:

No hits found against *U. of Oregon Arabidopsis small RNA database.*

```
BLASTN 2.2.9 [May-01-2004]


Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.

Query= 7ins1.txt
         (18 letters)

Database: mbys.nt
           4 sequences; 8991 total letters

Searching.done



                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

YSatWT                                                             36   1e-06




>YSatWT
          Length = 369

 Score = 36.2 bits (18), Expect = 1e-06
 Identities = 18/18 (100%)
 Strand = Plus / Plus
```

***Ebbie*: analyzing single insert**

- Automatically deposits insert into MySQL:
  - Number (no, automatically)
  - Id (from filename)
  - Insert (nt sequence)
  - Length (counted insert)
  - Sample source (inferred from filename)
- Annotation fields:
  - Group annotation
    - Pull down menu
    - Add to pull down menu in text field
  - Additional comments
  - Orientation (N/A, sense, antisense)
- BlastN search results
  - Searches *Ebbie* associated databases.
    - If negative search result, then only 1 line displayed.
    - Otherwise, whole BlastN search result printed.
- Press 'Deposit annotation for … ' to update insert annotation and add to BlastN database.

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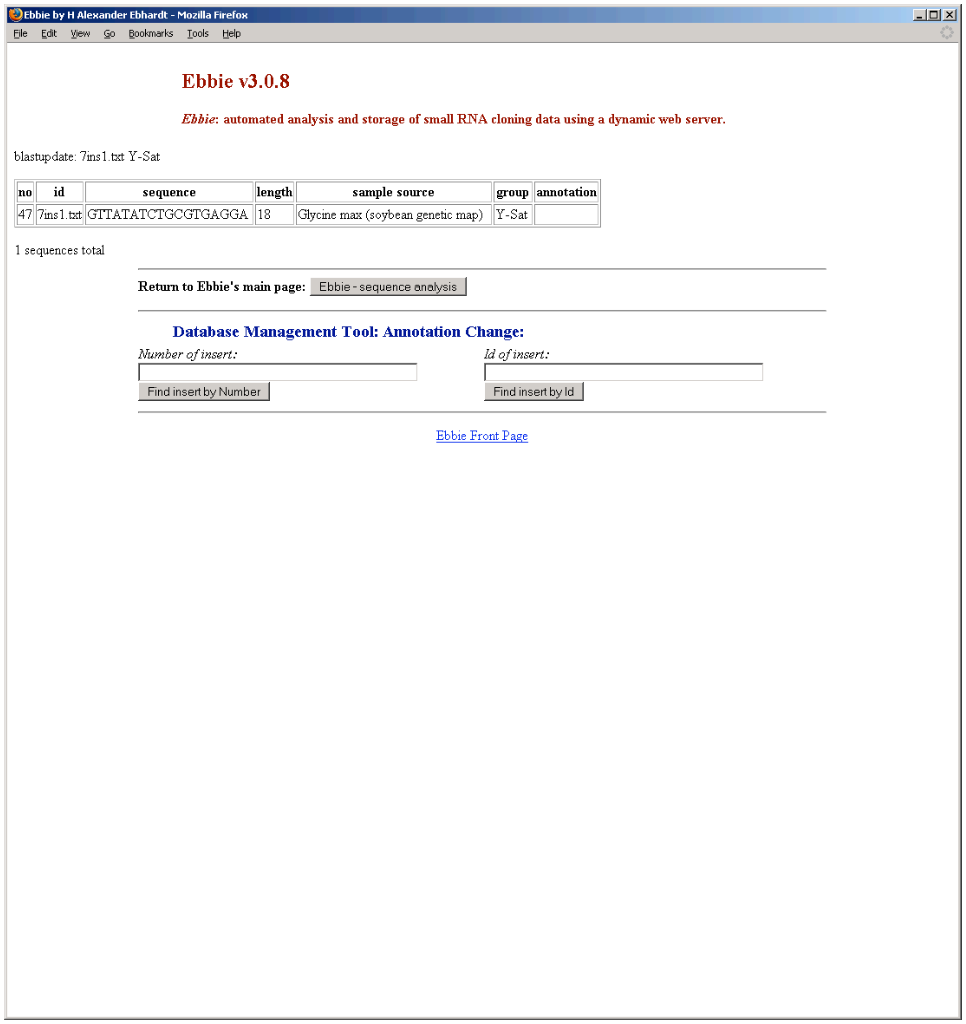
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

blastupdate: 7ins1.txt Y-Sat

no	id	sequence	length	sample source	group	annotation
47	7ins1.txt	GTTATATCTGCGTGAGGA	18	Glycine max (soybean genetic map)	Y-Sat	

1 sequences total

Return to Ebbie's main page: Ebbie - sequence analysis

Database Management Tool: Annotation Change:

Number of insert:

Find insert by Number

Id of insert:

Find insert by Id

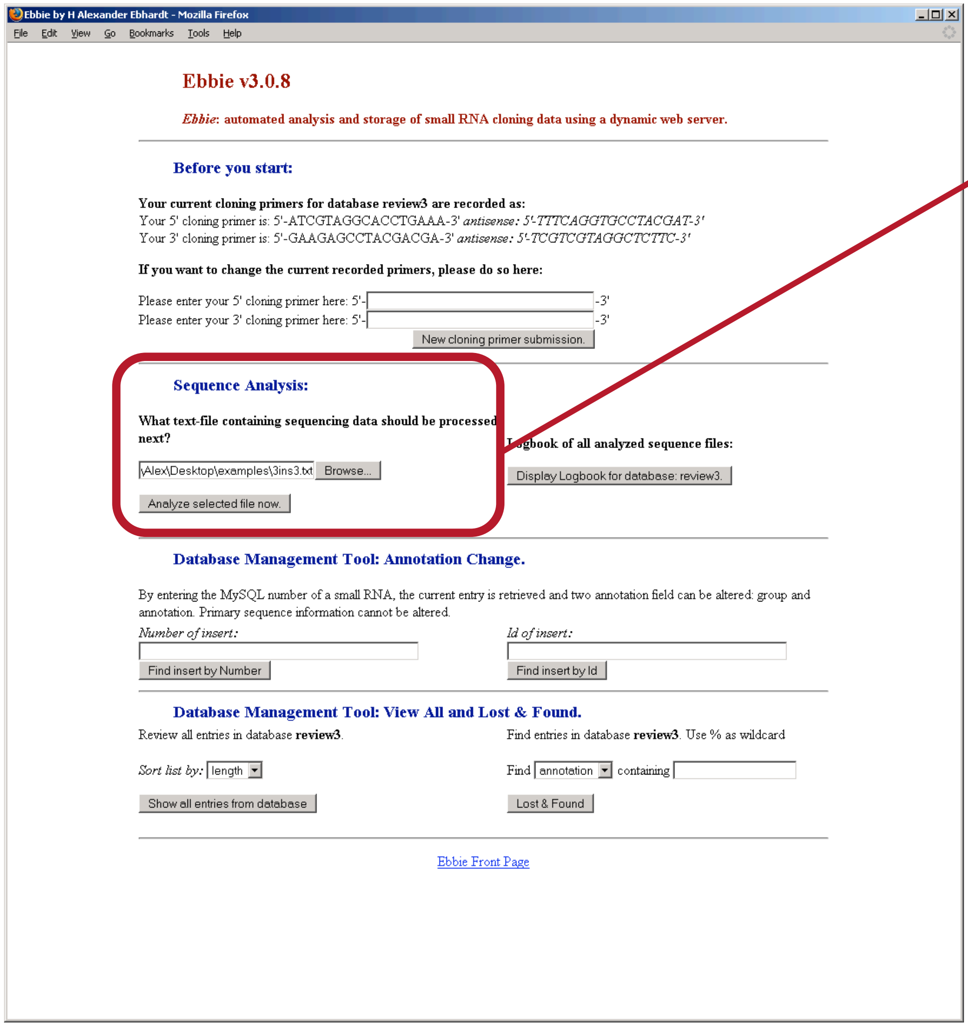
Ebbie Front Page

Ebbie: depositing single insert

  - BlastN id and group displayed.
  - MySQL entry displayed.
- Return to *Ebbie*'s Main Page for more sequence analysis
- OR: enter number or id of insert for Database Management Tool: Annotation Change

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

Before you start:

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Your 3' cloning primer is: 5'-GAAGAGCCTACGACGA-3' *antisense: 5'-TCGTCGTAGGCTCTTC-3'*

If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'- -3'
Please enter your 3' cloning primer here: 5'- -3'

Sequence Analysis:

What text-file containing sequencing data should be processed next?

\Alex\Desktop\examples\3ins3.txt

Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

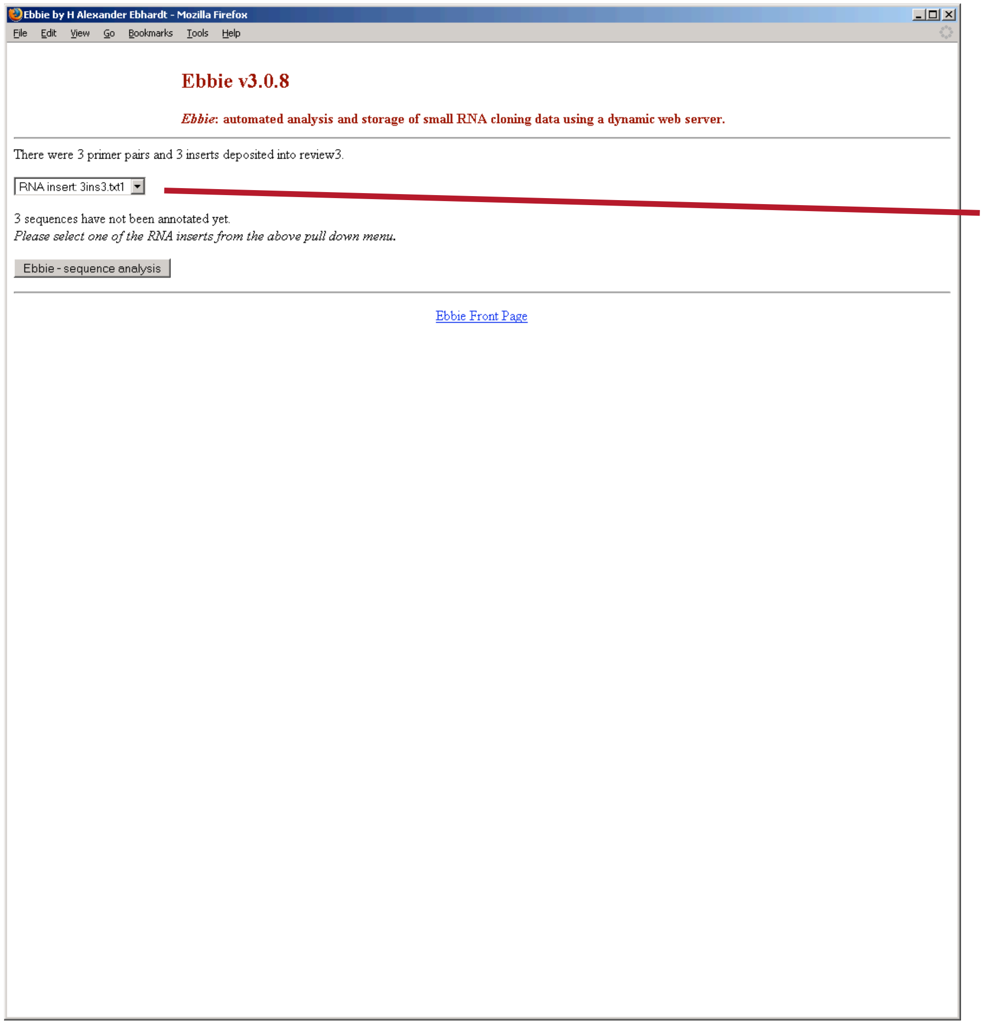
Find annotation containing

Main page of *Ebbie*: Multiple Inserts.

- Sequence Analysis:
  - Uploading text file containing DNA sequencing data

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

There were 3 primer pairs and 3 inserts deposited into review3.

RNA insert: 3ins3.txt1

3 sequences have not been annotated yet.
Please select one of the RNA inserts from the above pull down menu.

Ebbie: Multiple Inserts.

- Program indicates how many primer pairs and how many inserts were found.
- From pull down menu, choose an insert.
- Press: ‘Ebbie – sequence analysis submit button to start analysis of the insert.

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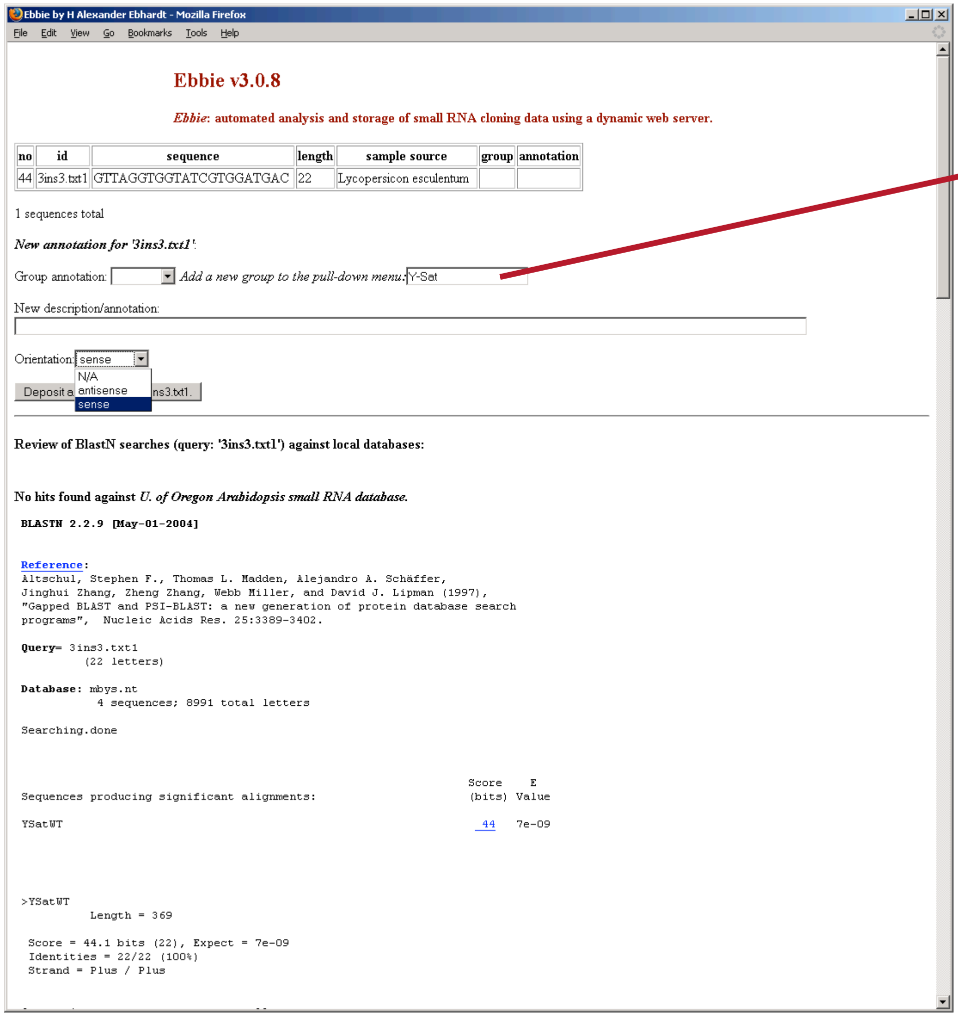
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

no	id	sequence	length	sample source	group	annotation
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum		

1 sequences total

New annotation for '3ins3.txt1':

Group annotation: *Add a new group to the pull-down menu:* Y-Sat

New description/annotation:

Orientation: sense

Review of BlastN searches (query: '3ins3.txt1') against local databases:

No hits found against *U. of Oregon Arabidopsis small RNA database.*

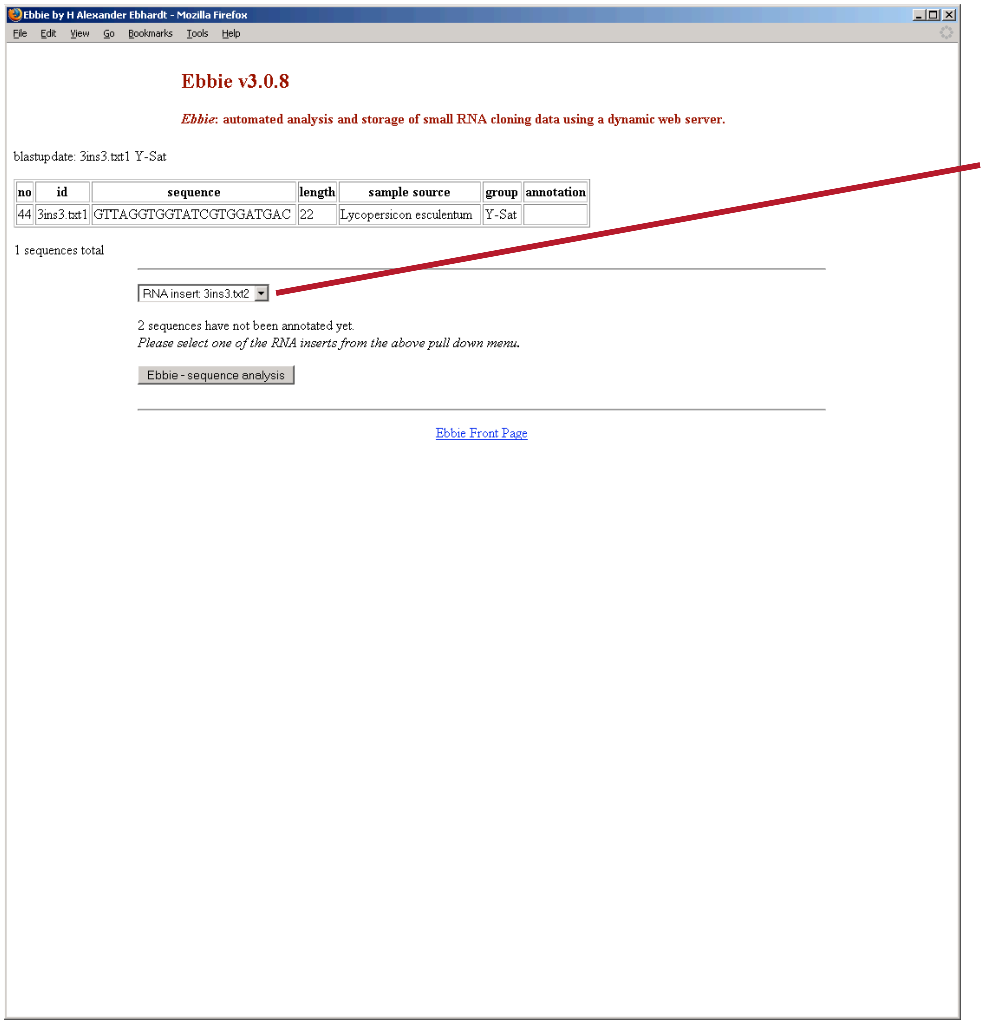
```
BLASTN 2.2.9 [May-01-2004]


Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.

Query= 3ins3.txt1
         (22 letters)

Database: mbys.nt
           4 sequences; 8991 total letters

Searching.done



                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

YSatWT                                                             44   7e-09




>YSatWT
          Length = 369

 Score = 44.1 bits (22), Expect = 7e-09
 Identities = 22/22 (100%)
 Strand = Plus / Plus
```

***Ebbie:* Multiple Inserts.**

- BlastN analysis of insert
- Group annotation: added ‘Y-Sat’ to text menu.
- Deposit annotation…

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

blastupdate: 3ins3.txt1 Y-Sat

no	id	sequence	length	sample source	group	annotation
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum	Y-Sat	

1 sequences total

RNA insert: 3ins3.txt2

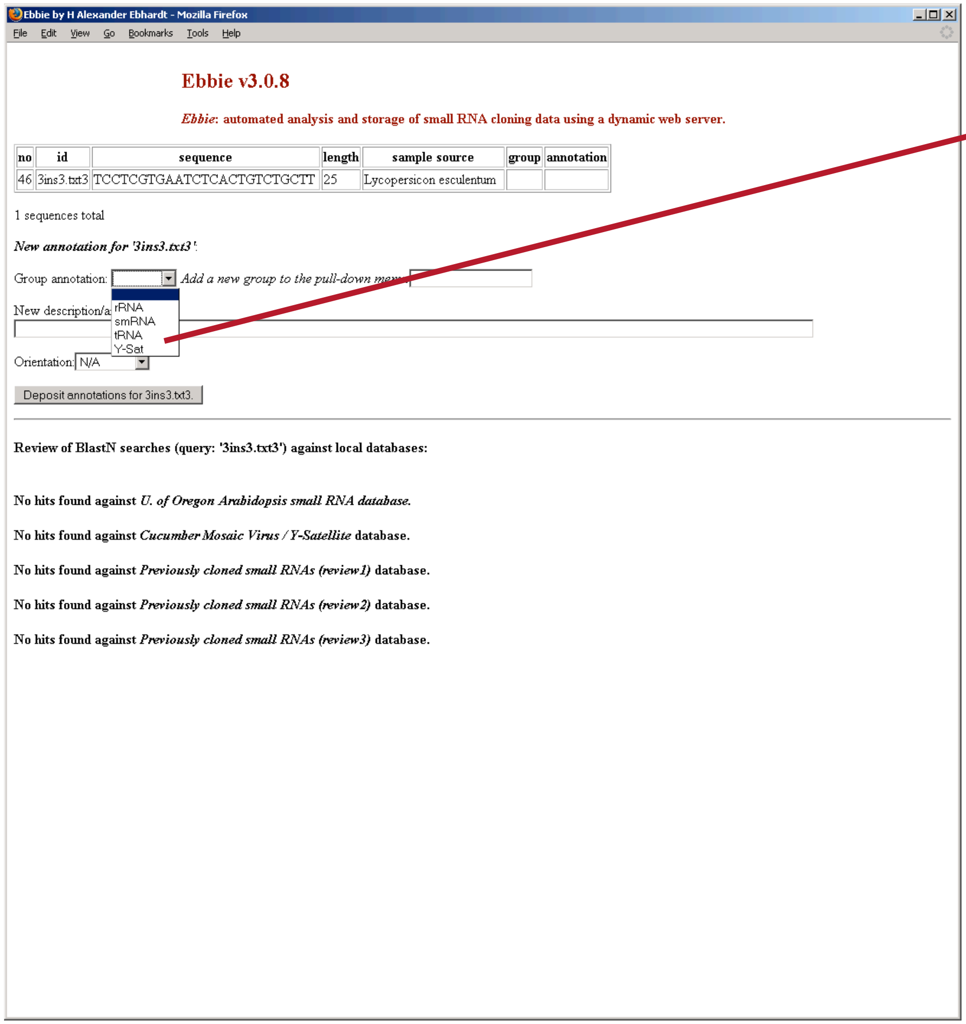
2 sequences have not been annotated yet.
Please select one of the RNA inserts from the above pull down menu.

Ebbie: Multiple Inserts.

- MySQL entry of previous insert depicted.
- Choose next insert from pull down menu (number of inserts remaining is shown below).
- Submit sequence.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

no	id	sequence	length	sample source	group	annotation
46	3ins3.txt3	TCCTCGTGAATCTCACTGTCTGCTT	25	Lycopersicon esculentum		

1 sequences total

New annotation for '3ins3.txt3':

Group annotation: *Add a new group to the pull-down menu*

New description/a

Orientation: N/A

Review of BlastN searches (query: '3ins3.txt3') against local databases:

No hits found against *U. of Oregon Arabidopsis small RNA database.*

No hits found against *Cucumber Mosaic Virus / Y-Satellite* database.

No hits found against *Previously cloned small RNAs (review1)* database.

No hits found against *Previously cloned small RNAs (review2)* database.

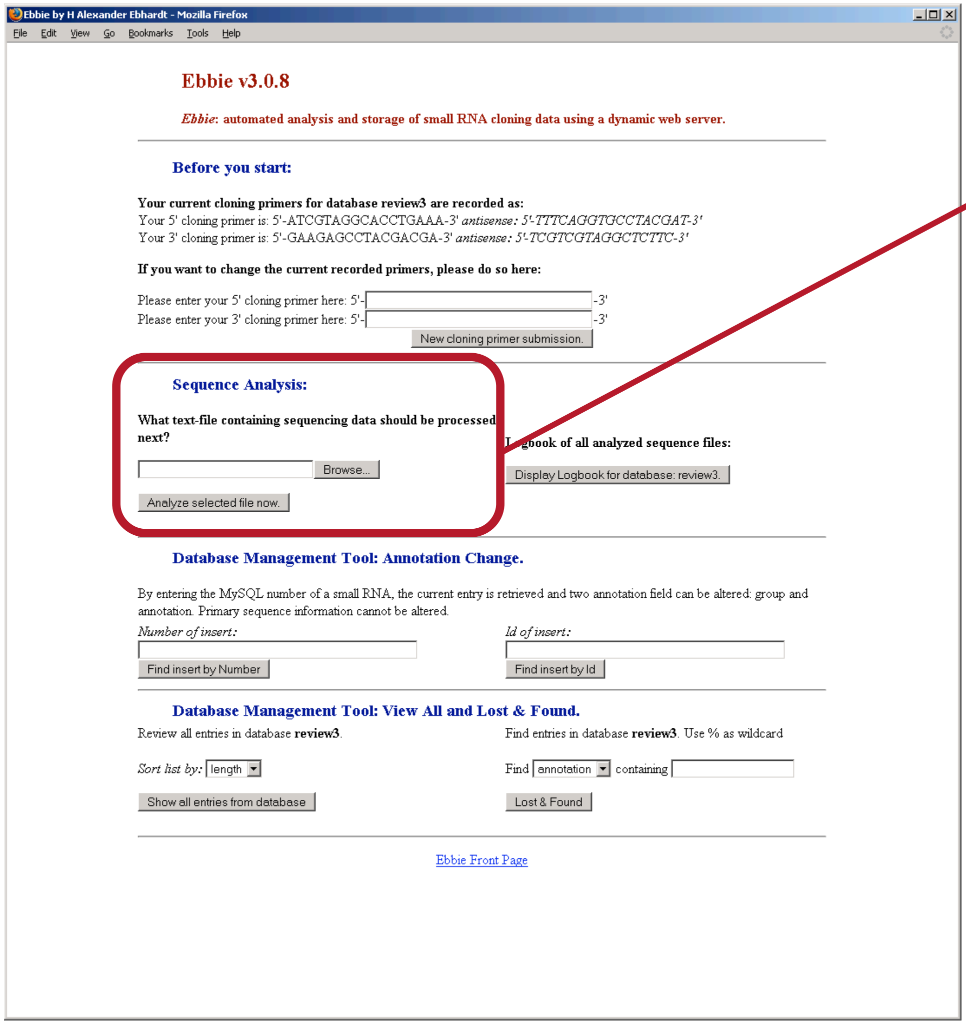
No hits found against *Previously cloned small RNAs (review3)* database.

Ebbie: Multiple Inserts.

- ‘Y-Sat’ appears in alphabetical order (not case sensitive) in group pull down menu.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

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Before you start:

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Your 3' cloning primer is: 5'-GAAGAGCCTACGACGA-3' *antisense: 5'-TCGTCGTAGGCTCTTC-3'*

If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'-______-3'
Please enter your 3' cloning primer here: 5'-______-3'

Sequence Analysis:

What text-file containing sequencing data should be processed next?

Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Find entries in database **review3**. Use % as wildcard

Sort list by: length

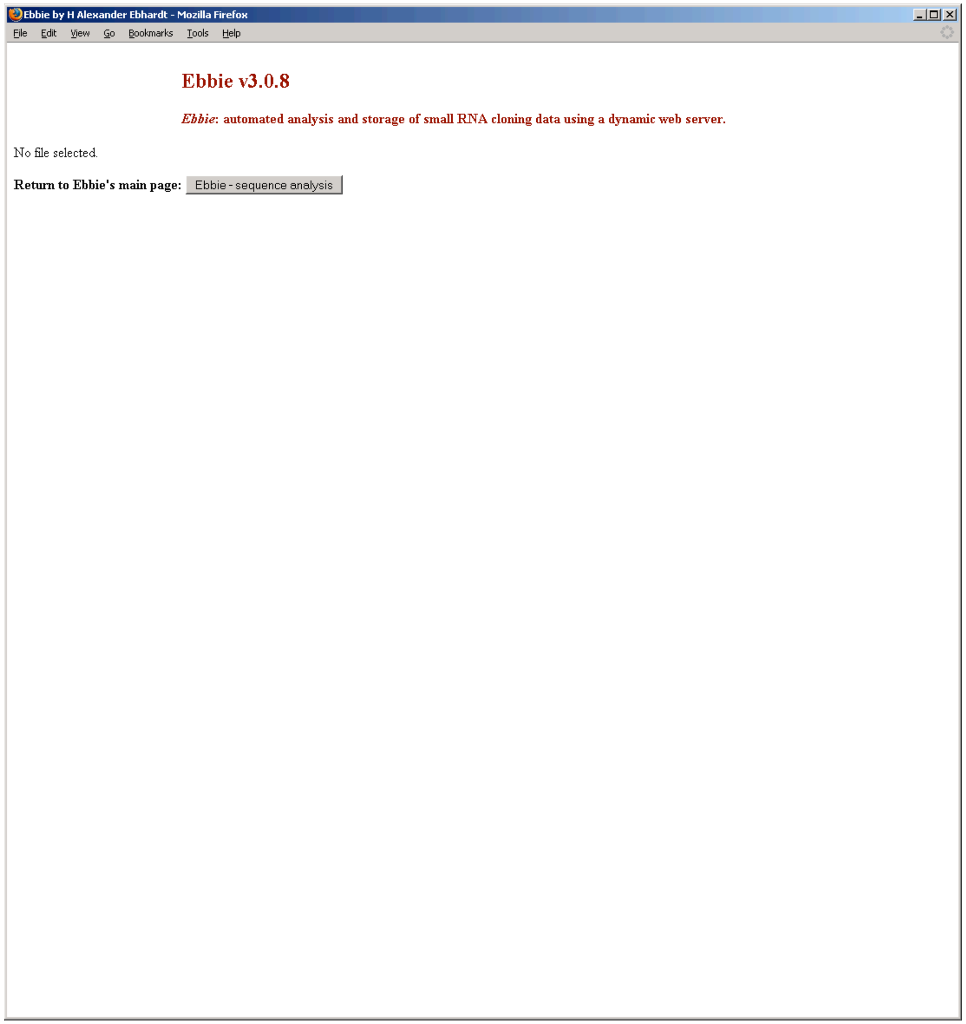
Find annotation containing

Ebbie: error messages:

- Sequence Analysis:
  - Uploading text file containing DNA sequencing data, error messages.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

No file selected.

Return to Ebbie's main page: Ebbie - sequence analysis

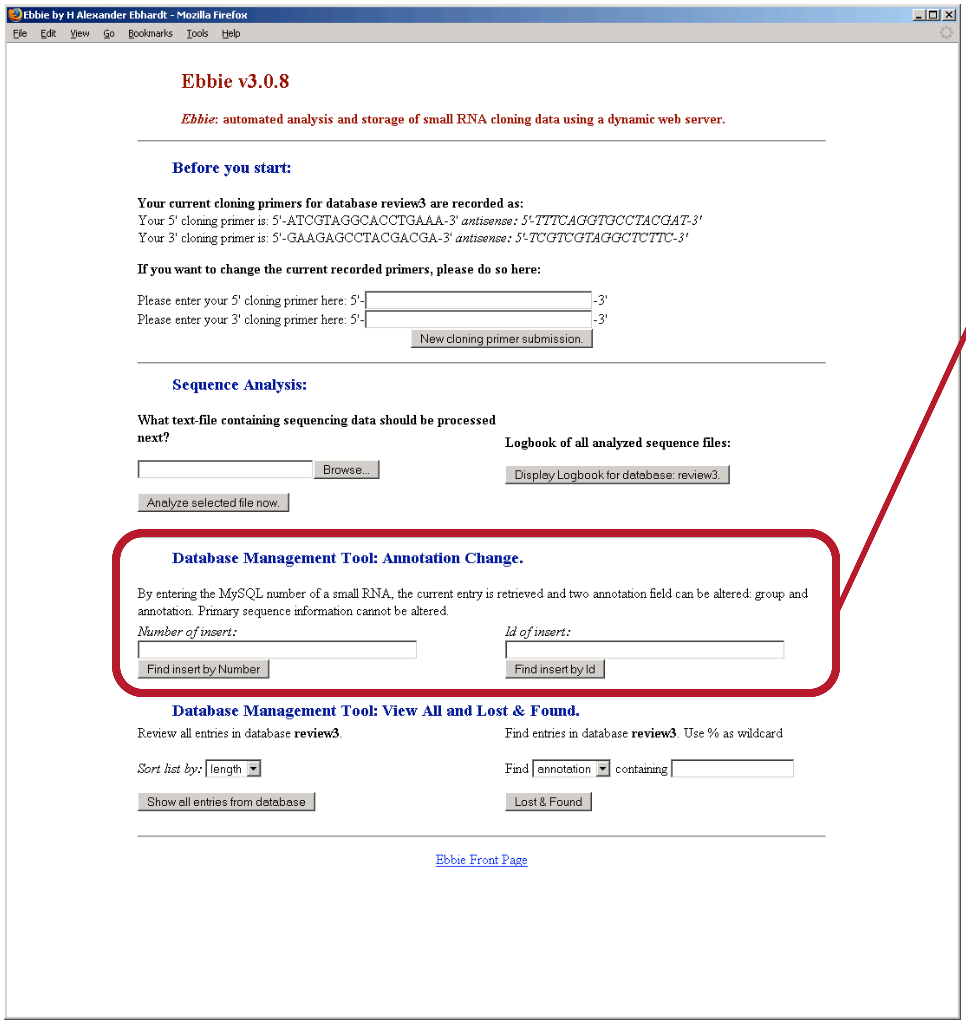
Ebbie: error messages:

- No file selected
- Filename already exists in database
- Uneven number of 5′- and 3′-cloning primer (logged*)
- No insert (logged*).

* logged: filename, date and comment will appear in logboook.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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If you want to change the current recorded primers, please do so here:

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Sequence Analysis:

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Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

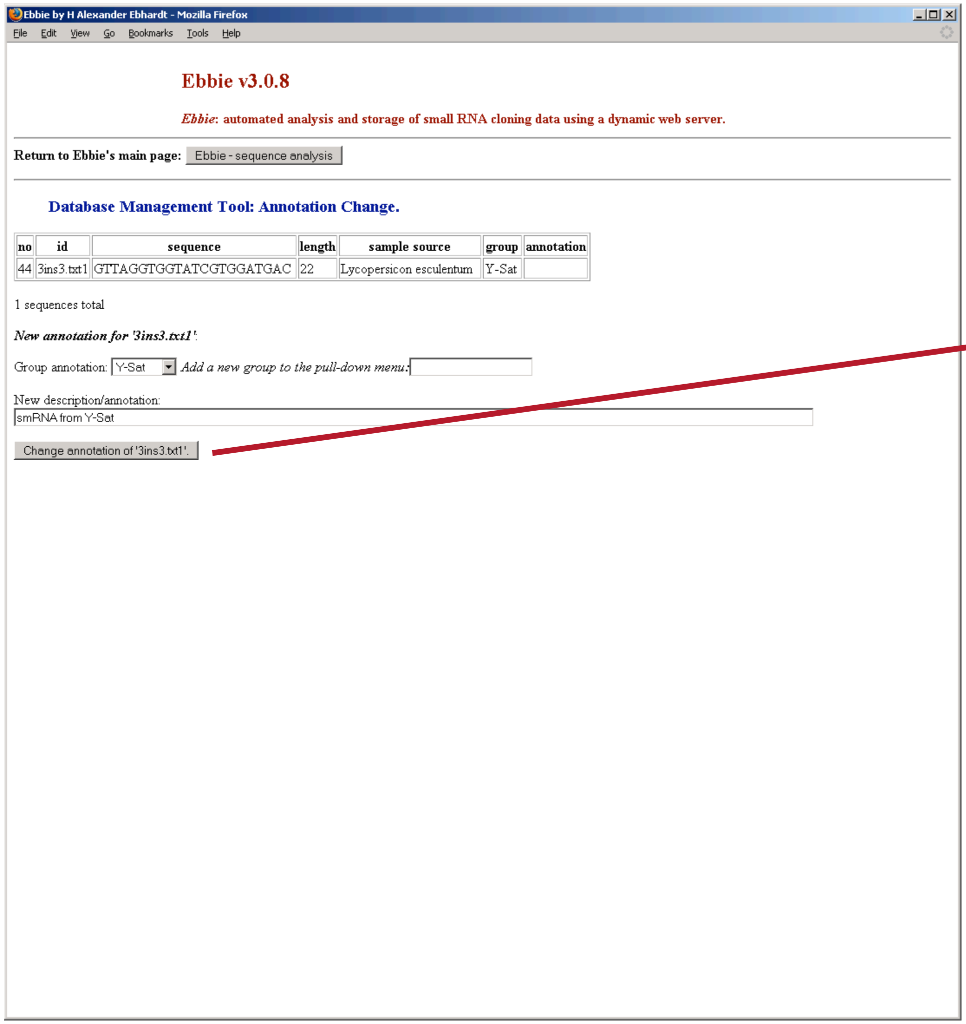
Find annotation containing

Ebbie: Database Management Tool: Annotation Change.

- Enter number or id of insert to update annotation.
  - For example, insert ‘44’ into *Number of insert.*

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

***Ebbie*: automated analysis and storage of small RNA cloning data using a dynamic web server.**

Return to Ebbie's main page: Ebbie - sequence analysis

Database Management Tool: Annotation Change.

no	id	sequence	length	sample source	group	annotation
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum	Y-Sat	

1 sequences total

New annotation for '3ins3.txt1':

Group annotation: Y-Sat *Add a new group to the pull-down menu:*

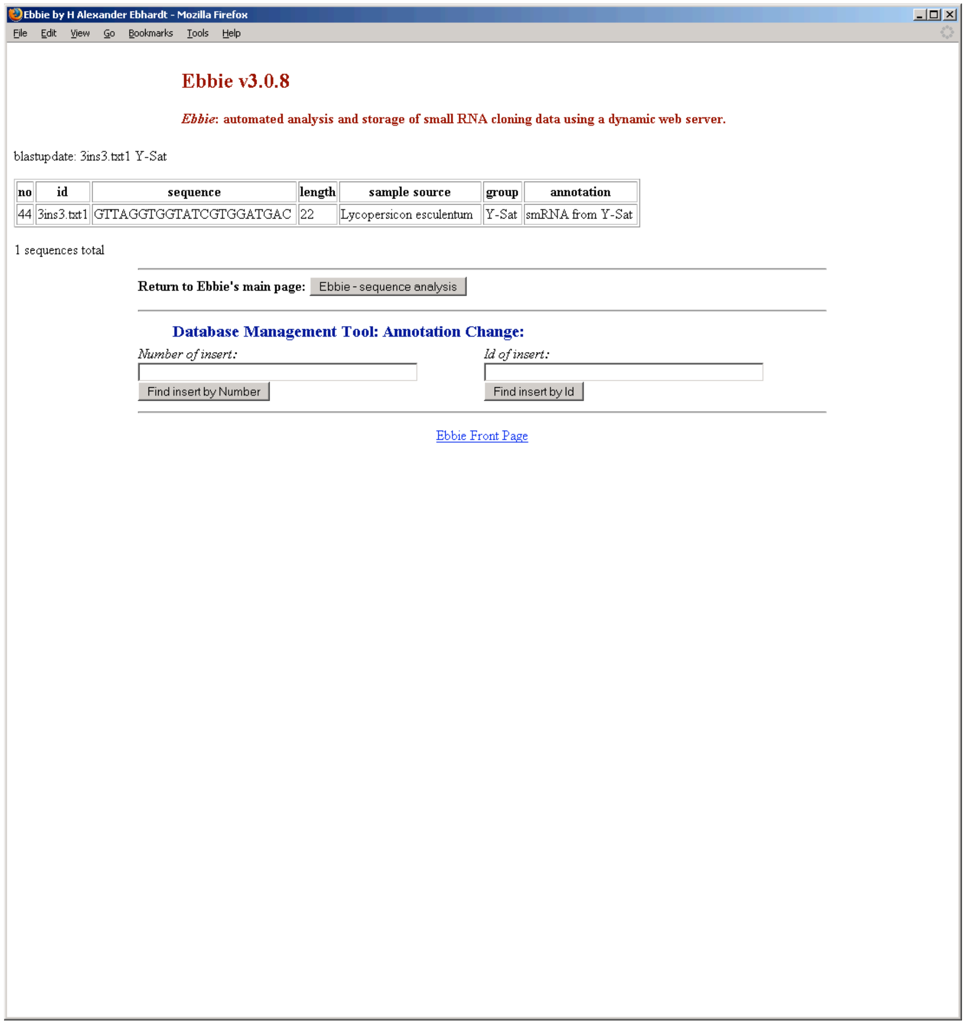
New description/annotation:
smRNA from Y-Sat

Ebbie: Database Management Tool: Annotation Change.

- Database entry retrieved.
- Change group annotation.
- Update additional annotation.
- Submit updated information.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

blastupdate: 3ins3.txt1 Y-Sat

no	id	sequence	length	sample source	group	annotation
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum	Y-Sat	smRNA from Y-Sat

1 sequences total

Return to Ebbie's main page: Ebbie - sequence analysis

Database Management Tool: Annotation Change:

Number of insert:

Find insert by Number

Id of insert:

Find insert by Id

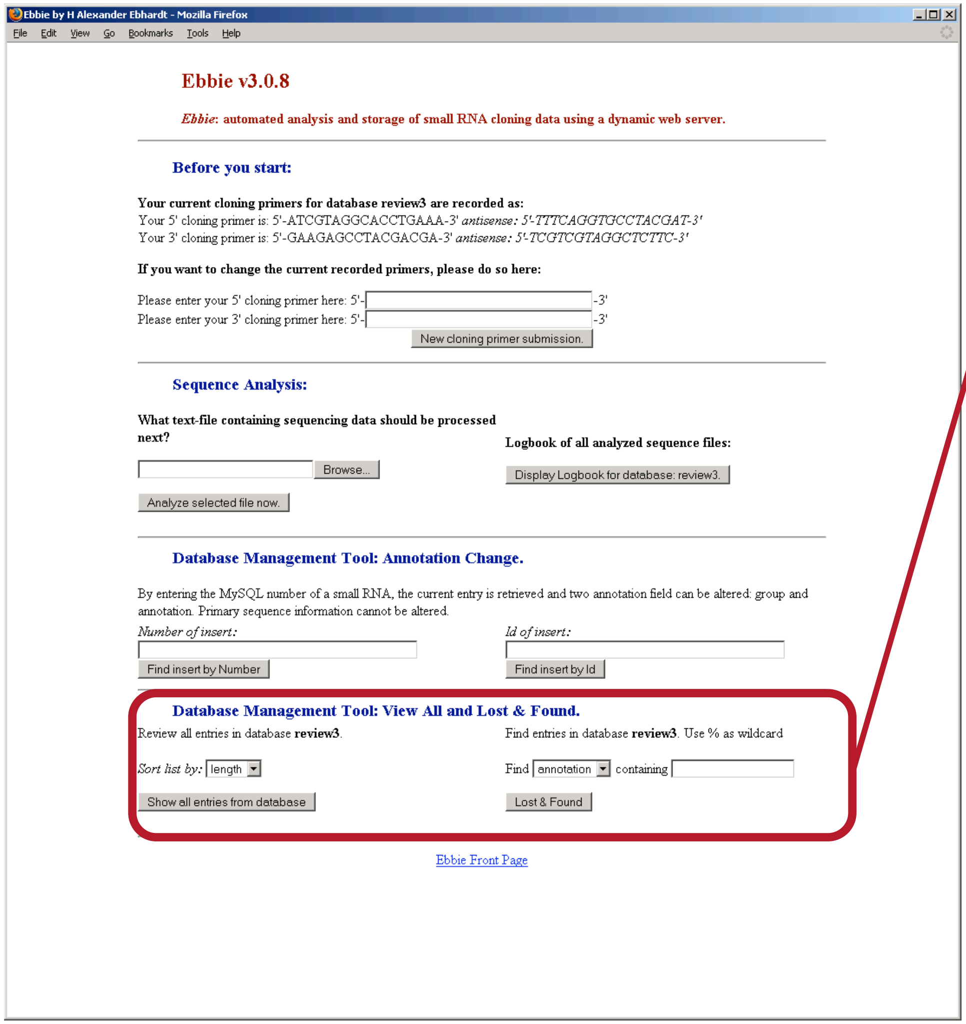
Ebbie Front Page

Ebbie: Database Management Tool: Annotation Change.

- Updated information appears.
  - Go to *Ebbie*'s Main page.
  - Or enter id or number for annotation change tool.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'- -3'
Please enter your 3' cloning primer here: 5'- -3'

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What text-file containing sequencing data should be processed next?

Logbook of all analyzed sequence files:

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By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

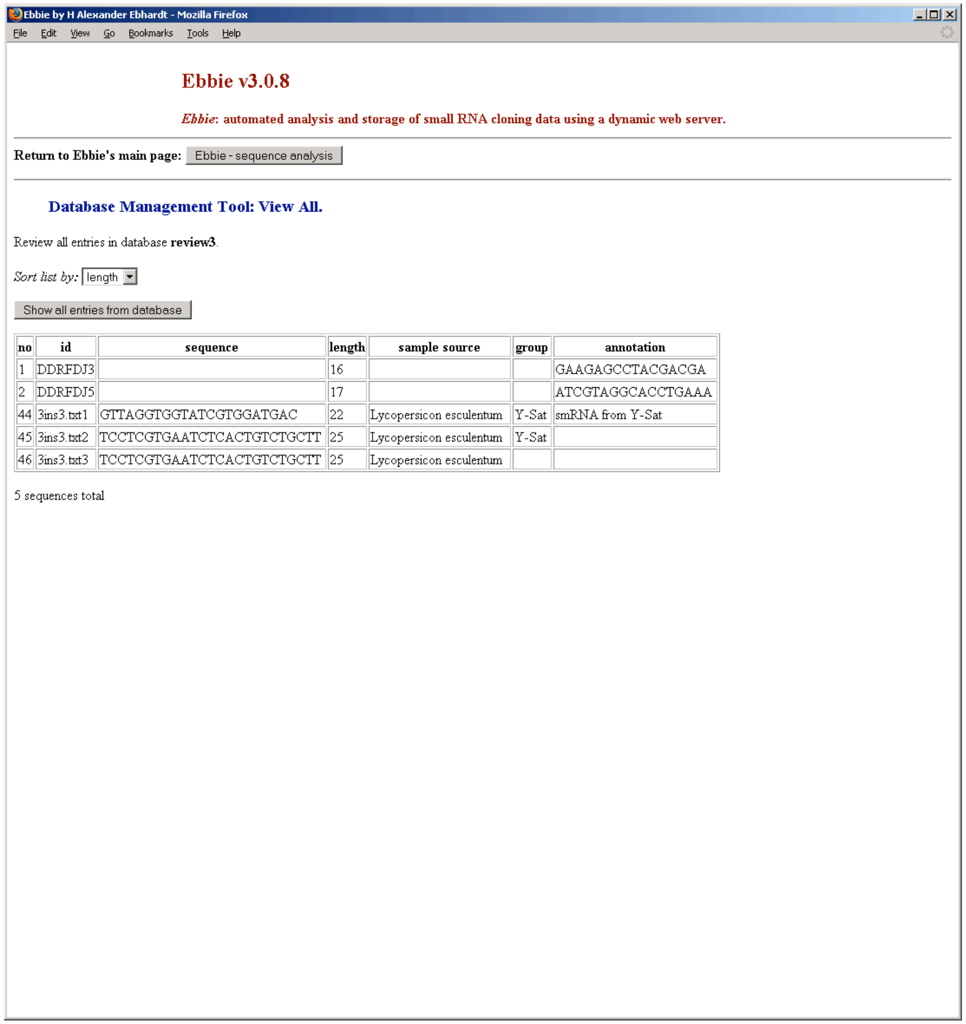
Find annotation containing

Main page of *Ebbie*.

- Database Management Tool: View All and Lost & Found
  - Review all entries, order by id, length, number or group.
  - Select a parameter and enter into text field a query using '%' as wild card character.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

Return to Ebbie's main page: Ebbie - sequence analysis

Database Management Tool: View All.

Review all entries in database **review3**.

Sort list by: length

Show all entries from database

no	id	sequence	length	sample source	group	annotation
1	DDRFDJ3		16			GAAGAGCCTACGACGA
2	DDRFDJ5		17			ATCGTAGGCACCTGAAA
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum	Y-Sat	smRNA from Y-Sat
45	3ins3.txt2	TCCTCGTGAATCTCACTGTCTGCTT	25	Lycopersicon esculentum	Y-Sat	
46	3ins3.txt3	TCCTCGTGAATCTCACTGTCTGCTT	25	Lycopersicon esculentum		

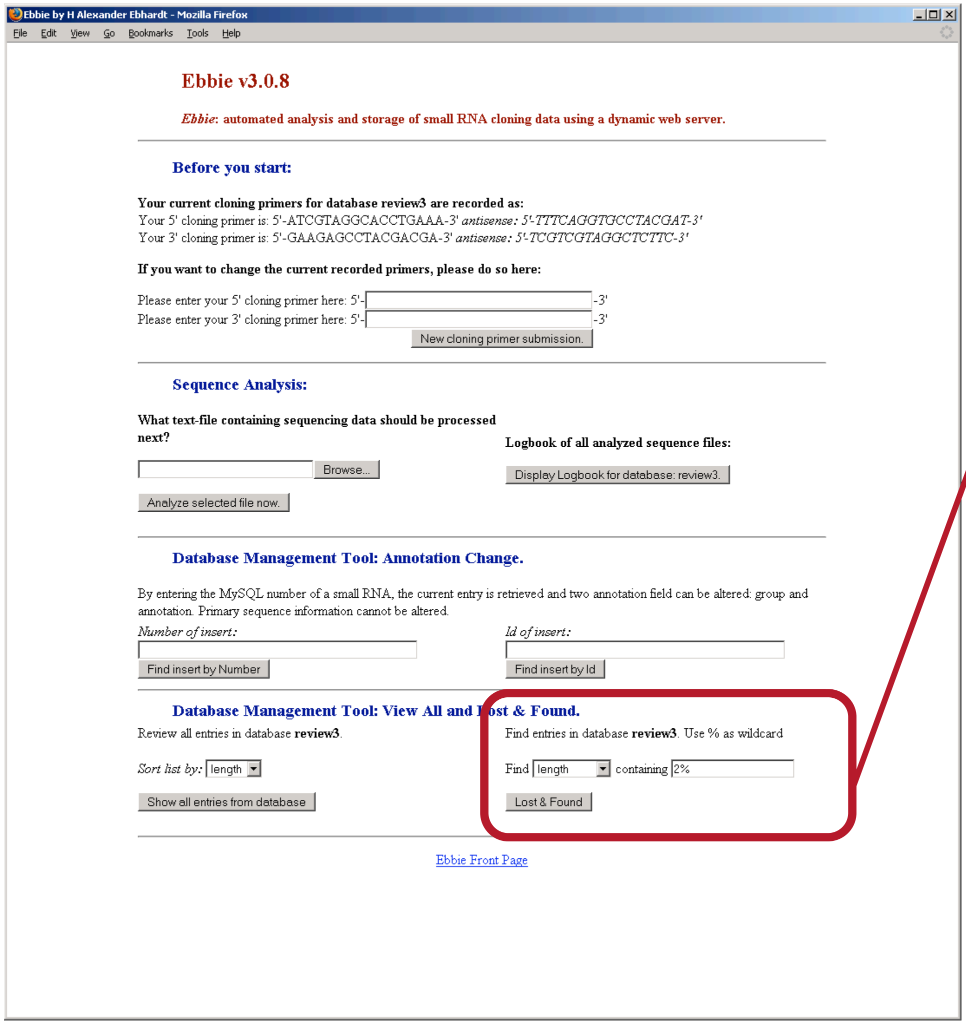
5 sequences total

Ebbie: **Database Management Tool: View All:**

- Sort by number, id, length and group.
- Displays all MySQL database entries.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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Your 3' cloning primer is: 5'-GAAGAGCCTACGACGA-3' *antisense: 5'-TCGTCGTAGGCTCTTC-3'*

If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'- -3'
Please enter your 3' cloning primer here: 5'- -3'

Sequence Analysis:

What text-file containing sequencing data should be processed next?

Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

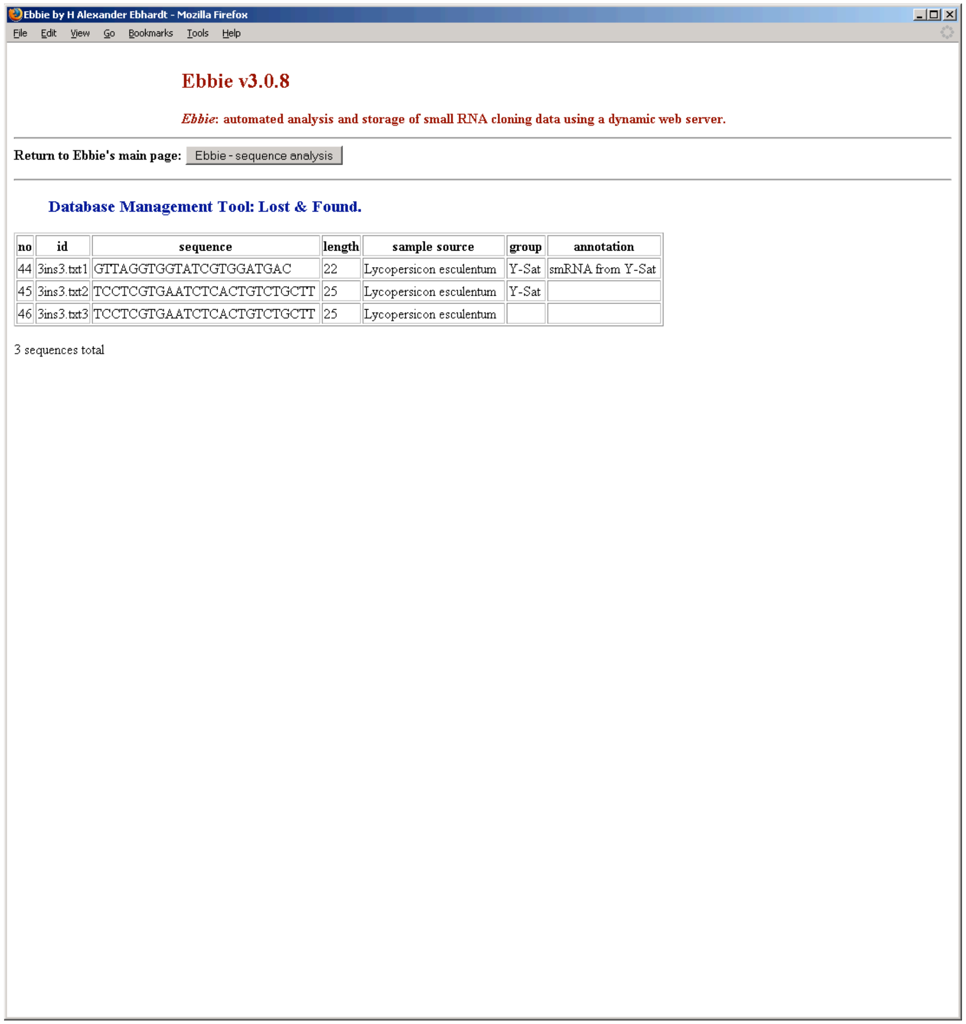
Find length containing 2%

Ebbie: Database Management Tool: Lost & Found:

- Enter query, e.g. length ‘2%’.

Ebbie v3.0.8

Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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Return to Ebbie's main page:

Database Management Tool: Lost & Found.

no	id	sequence	length	sample source	group	annotation
44	3ins3.txt1	GTTAGGTGGTATCGTGGATGAC	22	Lycopersicon esculentum	Y-Sat	smRNA from Y-Sat
45	3ins3.txt2	TCCTCGTGAATCTCACTGTCTGCTT	25	Lycopersicon esculentum	Y-Sat	
46	3ins3.txt3	TCCTCGTGAATCTCACTGTCTGCTT	25	Lycopersicon esculentum		

3 sequences total

Ebbie: Database Management Tool: Lost & Found:

- Displays all MySQL database entries matching the query.

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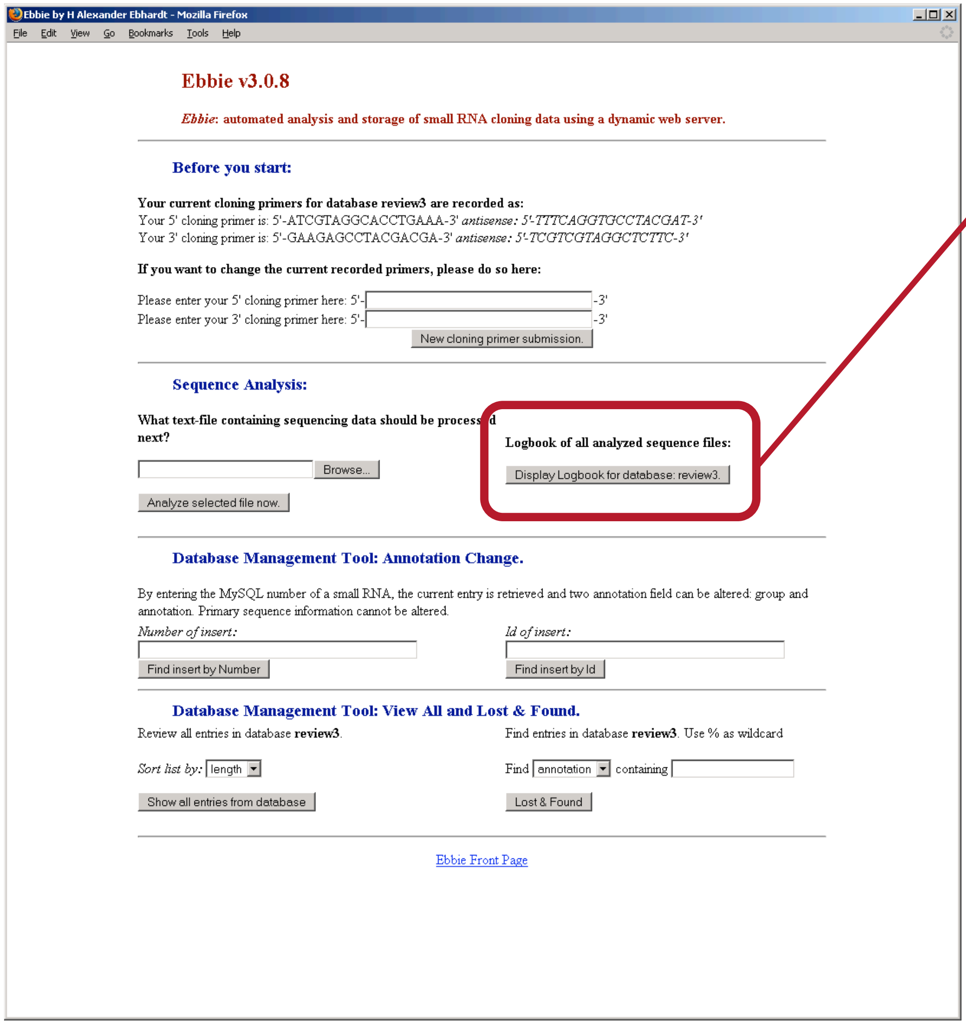

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Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.

Before you start:

Your current cloning primers for database review3 are recorded as:
Your 5' cloning primer is: 5'-ATCGTAGGCACCTGAAA-3' *antisense: 5'-TTTCAGGTGCCTACGAT-3'*
Your 3' cloning primer is: 5'-GAAGAGCCTACGACGA-3' *antisense: 5'-TCGTCGTAGGCTCTTC-3'*

If you want to change the current recorded primers, please do so here:

Please enter your 5' cloning primer here: 5'- -3'
Please enter your 3' cloning primer here: 5'- -3'

Sequence Analysis:

What text-file containing sequencing data should be process d next?

Logbook of all analyzed sequence files:

Database Management Tool: Annotation Change.

By entering the MySQL number of a small RNA, the current entry is retrieved and two annotation field can be altered: group and annotation. Primary sequence information cannot be altered.

Number of insert:

Id of insert:

Database Management Tool: View All and Lost & Found.

Review all entries in database **review3**.

Sort list by: length

Find entries in database **review3**. Use % as wildcard

Find annotation containing

Main page of *Ebbie*.

- Sequence Analysis:
  - Logbook of all files analyzed.

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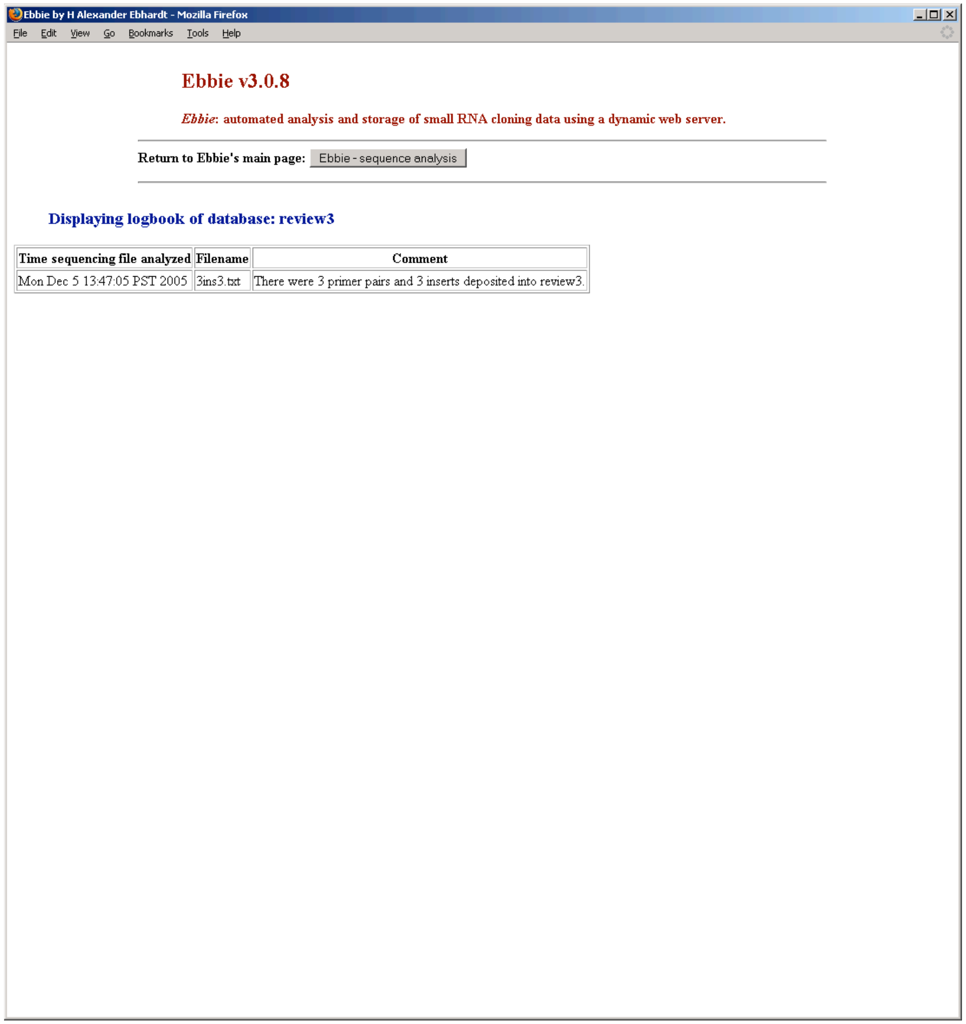
Ebbie: automated analysis and storage of small RNA cloning data using a dynamic web server.



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Return to Ebbie's main page:

Displaying logbook of database: review3

Time sequencing file analyzed	Filename	Comment
Mon Dec 5 13:47:05 PST 2005	3ins3.txt	There were 3 primer pairs and 3 inserts deposited into review3.

Ebbie: Logbook

- Displays all files analyzed.
- Table: date (system time), filename and comment.
- Comments are:
  - Single insert found.
  - Number of 5’- and 3’- cloning primers uneven!
  - There were *x* primer pairs and *y* inserts deposited into *z* (where *x* is the number of inserts / smRNAs found, *y* the number of primer pairs and *z* the database).
  - Single insert found.

End of Tutorial.