# **Restriction Analysis**

From this chapter you can learn how to search for restriction sites on a DNA sequence.

The restriction sites found are stored as automatic annotations. This means that if the automatic annotations highlighting is enabled then the restiction sites are searched and highlighted for each nucleotide sequence opened. Refer Automatic Annotations Highlighting to learn more.

Open a DNA sequence in and click the following button on the Sequence View toolbar:

🗄 🗖 🎦 🗒 🖓 🖾 Da Da Da Da Da Da Da 🏠	Go 🖉 1:1 899 5W 💌 🚍 🚮 🕅 🚓 Tr 🔬	🗶 🕢 Hi Hi 🔍 🗉 🗶 🥪 👾 🔿 💛 🤝

The Find restriction sites dialog appears:

• • •		Find Restriction Sites			
Name filter				Open enzymes	
Name ^ Acces	ision Type Sequence	Organizm / Details Aanl Axyl Baal Bugl	Suppliers	Export enzymes	
<ul> <li>C (1, 22)</li> <li>D (1, 9)</li> </ul>		Cac8I CviQI Ddel DseDI		Select All	
✓ E (2, 38) Eael RB00	0800 R2 YGGCCR	Eael Esp3l Enterobacter aerogenes	Takara Bio Inc.; N	Select None	
Eagl RB00	J802 R2 CGGCCG	Enterobacter agglomerans	New England Biola	Select by length	
Eam11051 RB00	0805 R2 CTCTTC 0806 R2 GACNNNNN 0807 R2 CTCTTC	Enterobacter amnigenus RFL1105 Enterobacter aerogenes	Thermo Fisher Sci New England Biola	Invert selection	
Ecil RB00	0817 R2 GGCGGA	Escherichia coli	New England Biola	Load selection	
Checked enzymes		Selected enzyme info		Save selection	
BamHI,BgIII,Clal,Dral,EcoRI,Eco	RV,HindIII,Pstl,Sall,Smal,Xmal	Eam1104]: type 2 restriction enzyme 3' CTCTTC(N)1' 5' 5' GAGAAG(N)1 (N)14 3'		REBASE Info	
Result count filter		1 😓 Maximum hits:		2 🗘	
Enzyme table filter					
Suppliers	15 suppliers			one Invert	
Recognition sequence length	1 🖸 to 9	+ 🖸			
Overhang type	Any	0			
Keep only Palindromic Uninterrupted Nondegenerate					
Search region					
Region Whole sequence		•	1 -	199950	
		Exclude	1-	199950	
Total number of enzymes: 656, s	selected 11				
Help				Cancel OK	

## The tree of restriction enzymes

Restriction enzymes are collected in a tree view. Enzymes are grouped by their names first letter here. Click on the > icon of a group (or just double click on the entire line) to show the whole group. Check the checkbox to include enzyme to the search list and uncheck to exclude. Use **Name filter** to find certain enzyme by its name.

### **Checked enzymes**

Here you may see the all checked enzymes. This enzymes will be searched after clicking OK.

## Selected enzyme info

Some information about the enzyme selected. It includes name, link to the REBASE database, the enzyme type detailed information, number of enzymes in the current sequence and sites and cuts location.

## Control buttons (right side)

- Open enzymes open file with enzymes in the bairoch format. It is required if you want to update enzymes database or open a database with some limited amount of enzymes. See Using Custom File with Enzymes for details.
- Export enzymes export selected enzymes to the separate file (using the bairoch format).
- Select all check all enzymes in the tree.
- Select none uncheck all enzymes in the tree.
- Select by length check enzymes only if its recognition site has length more than you set in the dialog window appeared.
- Invert selection switch checked and unchecked enzymes.
- Load selection load selected enzymes from the file. This file should contain comma-separated enzyme name list, for example: <u>BamHI,BgIII,ClaI,</u> <u>DraI,EcoRV,HindIII,PstI,SalI,SmaI,XmaI</u>
- Save selection save checked enzymes to the separate file using the format, described above.
- REBASE info open the REBASE database page of the selected enzyme.

## The filter of the results number

Show enzyme only if there are not less than "Minimum hints" and not more than "Maximum hints" values.

## Enzyme table filter

Show/hide enzymes depending on certain parameters:

Suppliers - check/uncheck suppliers, whose enzymes will be shown. By default, enzymes with undefined commercial suppliers are not shown.
 Recognition sequence length - show only enzymes, whose recognition sequence length is in bounds. <u>NOTE:</u> N bases are not included in recognition site. That means, that if enzyme has the following sequence:

#### C C N N N N N N N G G

its recognition site length will be four, not eleven.

Overhang type - show only enzymes with certain overhang type. The following options are presented:

<u>Any</u> - show all enzymes.

• No overhang - site has no cuts at all. Example:

3' CTCGAG 5'

5' GAGCTC 3'

• 5' - the cut of the forward strand is to the **left** of the cut of the reverse-complementary strand. *Example*:

3' C CT N A G G 5' 5' G G A N TC C 3'

• <u>3'</u> - the cut of the forward strand is to the **right** of the cut of the reverse-complementary strand. *Example:* 

3' C G A TC G 5' 5' G CT A G C 3'

- <u>Blunt</u> cuts are located in the middle of the site.
- Sticky cuts are locate anywhere but the middle of the site. It is 5' and 3' both.
- Nondegenerate sticky the same as Sticky, but the overhang between cuts has only A, C, G or T (no extended) symbols.
- Blunt or sticky Blunt + Sticky.
- Blunt or nondegenerate sticky Blunt + Nondegenerate sticky.
- Keep only show most interesting enzymes only:
  - Palindromic forward and reverse-complementary strands are equal.
  - <u>Uninterrupted</u> no internal **N** bases.
  - Nondegenerate no extended DNA alphabet symbols (only A, C, G, T and N).

## Search region

The region to search enzymes in. You may also Exclude some region and not to search in it.

The information about enzymes was obtained from the REBASE database. For each enzyme in the list a brief description is available (the accession ID in the database, the recognition sequence, etc.). If you're online you can get more detailed information about an enzyme selected by clicking the *REBASE Info* button.

- Selecting Restriction Enzymes
- Using Custom File with Enzymes
- Filtering by Number of Hits
- Excluding Region
- Circular Molecule
- Results