Restriction Enzyme Patterns and RE Finder tools

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Introduction

- Molecular Scissors. Restriction enzyme, restriction endonuclease, REase, ENase or restrictase is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.
- Restriction endonucleases are named from the fact that they stop bacteriophages from multiplying by recognizing and cutting DNA at specified locations
- In 1978, the Nobel Prize in Physiology or Medicine was awarded jointly to Werner Arber, who predicted the existence of restriction enzymes, Hamilton Smith, who discovered the first Type II restriction enzyme, and Nathans, who demonstrated how to use the restriction enzymes to analyze viral DNA

- Enzymes that are part of the restriction-modification systems. They catalyze the endonucleolytic cleavage of DNA sequences which lack the species-specific methylation pattern in the host cell's DNA. Cleavage yields random or specific double-stranded fragments with terminal 5'-phosphates.
- 1st discovered HindII- naming of RE**.** The first letter of the restriction endonuclease name comes from the genus and the second two letters come from the species of the prokaryotic cell from which they are isolated. For example: EcoRI comes from Escherichia coli RY 13.

Types of RE

	Type I	Type II	Type III	Type IV
Nuclease structure	Multimer; heterotrimer	Homodimer	Homodimer	
Recognition site pattern	Two sites, in any orientation	Small (4–8 bp); usually palindromic	Two sites, in head-to-head orientation; non-palindromic	Weak specificity
Cleavage site	Variable distance from recognition site; non-specific cleavage	Cleavage within (Type IIP) or outside (Type IIS) the recognition site	Cleavage of one strand (nicking activity) 24–25 bp from recognition site	Methylated only
Cofactor	ATP, Mg^{2+}, SAM	Mg^{2+}	ATP, Mg^{2+}, SAM	ATP, GTP

https://www.snapgene.com/guides/restriction-enzymes

Advantages of using RE

- Type II enzyme is that it cleaves specifically within or close to its recognition site and that it does not require ATP hydrolysis for its nucleolytic activity.
- Molecular Biology tool- cutting specific sites of DNA
- Restriction mapping is a method used to map an unknown segment of DNA by breaking it into pieces and then identifying the locations of the breakpoints. This method relies upon the use of proteins called restriction enzymes

Restriction enzyme cutting patterns

- Palindrome Sequence: recognized by RE
  - Mirror like palindrome: same forward and backward on single DNA strand GTAATG
  - Inverted Repeat Palindrome: sequence that read the same forward and backwards GTATAC being complimentary to CATATG

- Many restriction enzymes make staggered cuts, producing ends with single-stranded DNA overhangs. However, some produce blunt ends
- 4 or 6 base pair sequences

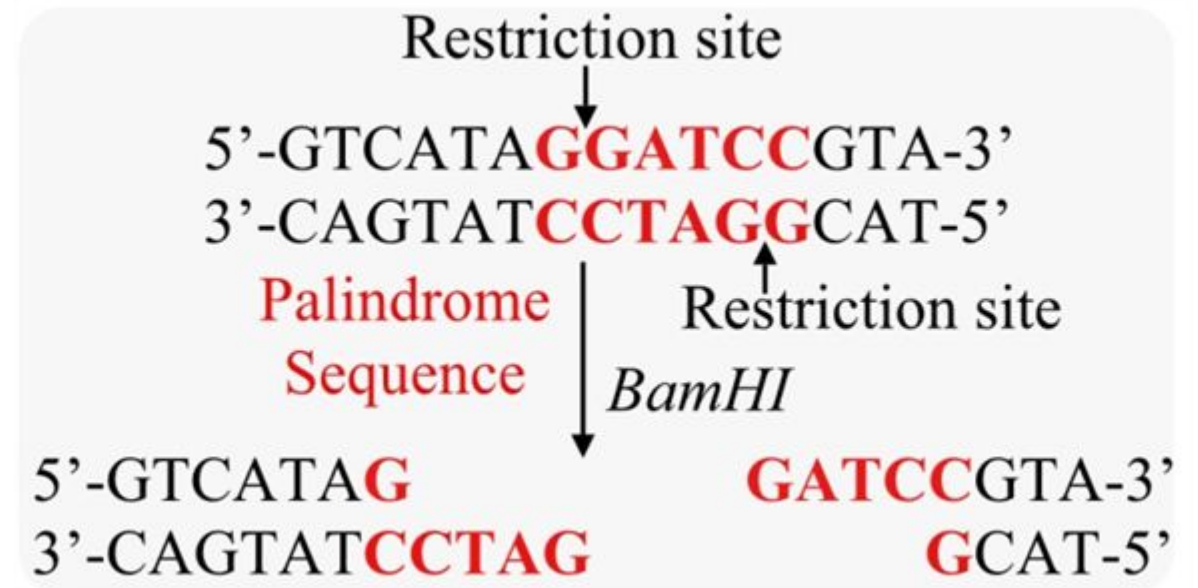


Table 1. Examples of Recognition Sequences of Type I, II, and III Restriction Enzymes

	Recognition Sequences	Comments
Type I Enzymes		
*Eco*KI	AACNNNNNNGTGC	type IA family, prototype
*Eco*AI	GAGNNNNNNNGTCA	type IB family, prototype
*Eco*R124I	GAANNNNNNRTCG	type IC family, prototype
*Sty*SBLI	CGANNNNNNTACC	type ID family, prototype
Type II Enzymes		
*Sau*3AI	GATC	4 nucleotide palindrome
*Eco*RI	GAATTC	6 nucleotide palindrome
*Not*I	GCGGCCGC	8 nucleotide palindrome
*Sap*I	GCTCTTC	7 nucleotide non-palindrome
*Bcg*I	CGANNNNNNTGC	interrupted non-palindrome
Type III Enzymes		
*Eco*P1	AGACC.........GGTCT	inverted 5 nucleotide pair
*Eco*P15I	CAGCAG........CTGCTG	inverted 6 nucleotide pair

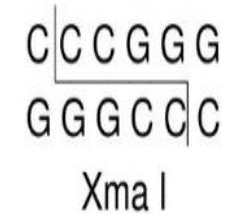
Ellrott, Kyle, Julie K. A. Kasarjian, Tao Jiang and Junichi Ryu. "Restriction enzyme recognition sequence search program." *BioTechniques* 33 6 (2002): 1322-6 .

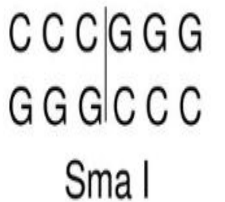
Isoschizomers

- Isoschizomers are restriction enzymes that have the same recognition sequence and cleave the DNA at the same positions. These restriction enzymes have the same specificity.
- The first discovered restriction enzyme that recognizes a given sequence is known as a prototype, while all subsequently identified restriction enzymes that recognize that same sequence are called isoschizomers.
- However, isoschizomers may differ in site preferences, reaction conditions, methylation sensitivity, and star activity. Isoschizomers are isolated from different strains of bacteria. Therefore, they may require different reaction conditions
- This particular feature of isoschizomers helps identification of methylation state of the restriction site while isolating it from a bacterial state.

Neoschizomers

- Neoschizomers are restriction enzymes that have the same recognition sequence but cleave DNA at different positions.
- The neoschizomers are a subset of isoschizomers. Well-known examples for neoschizomers are SmaI (5’-CCC↓GGG-3’) and XmaI (5’-C↓CCGGG-3’); both recognize 5’-CCCGGG-3’ sequence but cleave them at a different position.
- Thus, these two restriction enzymes generate different types of ends. In this case, SmaI produces blunt ends, and XmaI produces 5’ protruding ends.





Isocaudomers

- **Isocaudomers** are pairs of restriction enzymes that have slightly different recognition sequences but upon cleavage generate identical termini.
- These sequences can be ligated to one another, but then form an asymmetrical sequence that cannot be cleaved by a restriction enzyme.

```
Mbo I
   N*GATC N
   N CTAG*N
BamH I
   G*GATC C
   C CTAG*G
```

Restriction Enzyme Database

- REBASE is a database of information about restriction enzymes and DNA methyltransferases. REBASE contains an extensive set of references, sites of recognition and cleavage, sequences and structures. It also contains information on the commercial availability of each enzyme.

http://rebase.neb.com/rebase/rebase.html

RESTRICTION ENDONUCLEASE DIGESTION

BACKGROUND INFORMATION: General review *(Promega)*, General review *(P. McClean)*; Gene Infinity (good meta source)

SITES: Though I favour Webcutter 2.0 and WatCut for their simplicity all of these sites are well worthwhile visiting.

- Webcutter 2.0 *(U.S.A.)*
- WatCut *(Michael Palmer, University of Waterloo, Canada)* - provides restriction analysis coupled with where the sites are located within genes.
- Restriction Site Analysis - *(University of Massachusetts Medical School, U.S,A.)* uses H. Mangalam's TACG2 program. Provides one with considerable choice of enzymes and output format, including pseudo gel maps.

- Restriction Enzyme Picker *(G. Rocap & E. Collins, School of Oceanography, University of Washington, U.S.A.)* - finds sets of 4 commercially available restriction endonucleases which together uniquely differentiate designated sequence groups from a supplied FASTA format sequence file for use in T-RFLP.

- NEBcutter *(New England Biolabs, U.S.A.)* - provides opportunities to upload local files, choose from common vector sequences or enter GenBank accession numbers. Also includes ability to map sites in genes. After you have the restriction map for this sequence you might want to consult the New England Biolabs (U.S.A.) site: The Restriction Enzyme Database for specifics on each restriction endonuclease and its availability.

- Other restriction sites include Restriction enzyme digest of DNA, RestrictionMapper, Restriction Map, and Restriction Digest.

- Restriction Analyzer *(Vladimír Cermák, molbiotools.com)* - carry out in silico restriction analysis online. Quickly find absent and unique sites. Tabular and graphical output. Analyze restriction fragments. Simulate a gel electrophoresis.

- Restriction Comparator *(Vladimír Cermák, molbiotools.com)* - Carry out parallel in silico restriction analysis online. Compare two sequences side by side. Find distinguishing restriction sites. Visualize restriction patterns.

- WebDSV *(Vladimír Cermák, molbiotools.com)* - is a basic molecular biology app to create, edit and analyze DNA sequences, mark and visualize sequence features, and generate plasmid maps. With WebDSV you can analyze restriction sites, perform in silico molecular cloning, and design PCR primers.

- *In silico* restriction digest of complete genomes *(University of the Basque Country, Spain)* - allows *in silico* digestion of over 300 prokaryotic genomes and simulated pulsed-field gel electrophoretic separation of the fragments.

- Computation of size of DNA and Protein Fragments from Their Electrophoretic Mobility (Reference: Raghava, G. P. S. 2001. Biotech Software and Internet Report **2**:198-200).

- Sequence Extractor *(Paul Stothard)* - generates a clickable restriction map and PCR primer map of a DNA sequence (accepted formats are: raw, GenBank, EMBL, and FASTA) offering a great deal of control on output. Protein translations and intron/exon boundaries are also shown. Use Sequence Extractor to build DNA constructs *in silico*.

https://molbiol-tools.ca/Restriction_endonuclease.htm

nc3.neb.com/NEBcutter/

YouTube to Mp3 C... Sci-Hub: removing... 10.3 Genomics and... A Global School For... YouTube to mp3 Co... Bookmarks Svadhishthana Cha... Yoga for Hip Pain a... Every Pranayam De... Common Yoga Prot... bmn,/'1 Home - URKUND

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WELCOME GUEST, SIGN IN OR SIGN UP FEEDBACK ? HELP

Open Recent Project

Projects will be automatically deleted 7 day(s) after they were last accessed.

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Enter a DNA sequence, or select from other options, to identify cut sites. Once you submit a sequence, you may choose to customize your digest.

1. Input or choose sequence.

Text | File | GenBank | Plasmid Vector | Viral & Phage

Type or paste sequence

2. Set preferences.

Circular | Additional Preferences (enzymes, oligos, etc)

3. Name project (optional).

Enter project name

Submit

Looking for NEBcutter v2? It's still available at https://nc2.neb.com, but is no longer being updated.

ABOUT THIS TOOL HISTORY ALL TOOLS TECH SUPPORT

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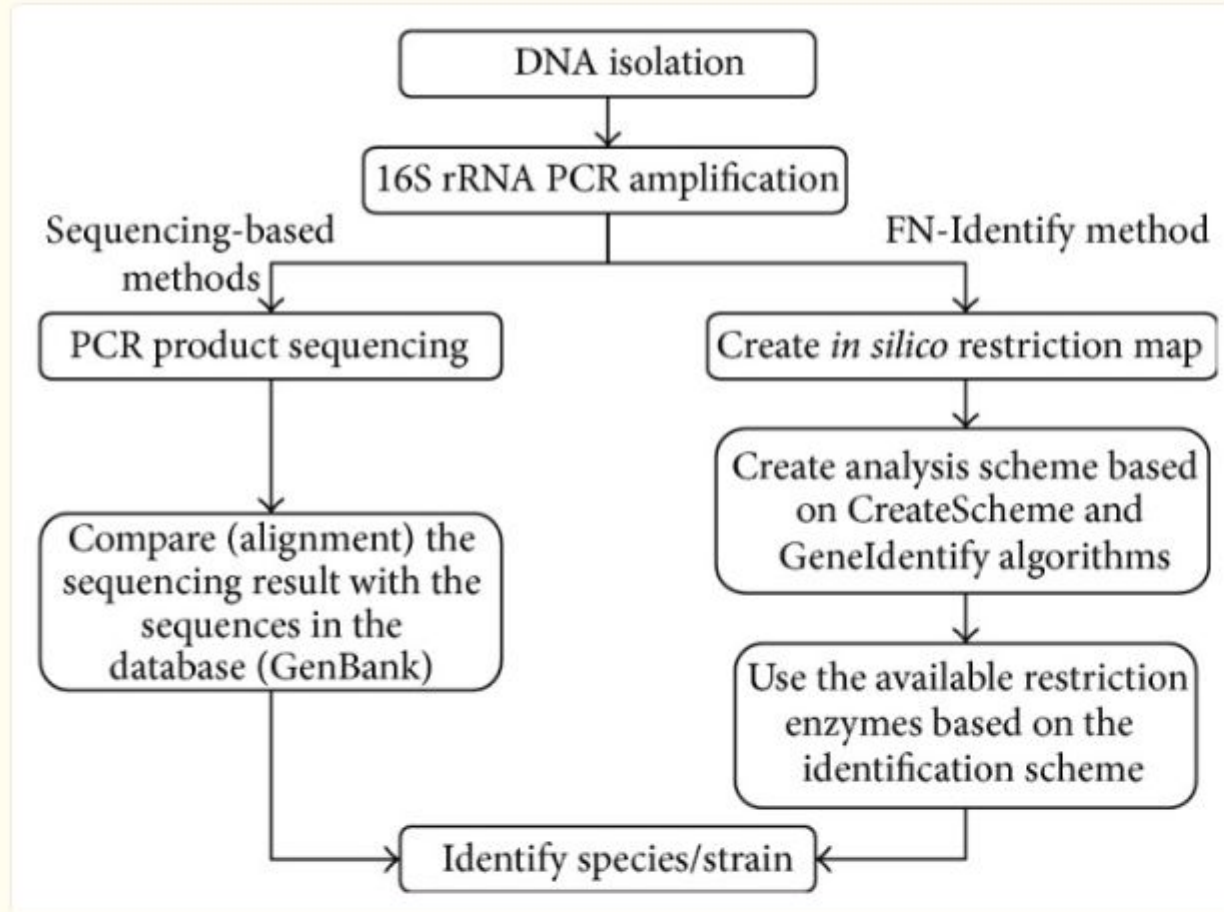

Figure 1

Comparison between sequencing-based identification approach and FN-Identify proposed approach.

Awad M, Ouda O, El-Refy A, El-Feky FA, Mosa KA, Helmy M. FN-Identify: Novel Restriction Enzymes-Based Method for Bacterial Identification in Absence of Genome Sequencing. Adv Bioinformatics. 2015;2015:303605. doi: 10.1155/2015/303605.

Assignment