Virtual Restriction Analysis

Among the most important tools in gene technology or "synthetic biology"[1] are restriction endonucleases: enzymes which specifically recognize and cleave DNA sequence patterns. By far the most relevant restriction enzymes are of type II and recognize tetra-, penta-, and hexanucleotide sequences with twofold symmetry (palindromic structure), such as of *Bacillus amyloliquefaciens H* (BamHI; for nomenclature see ref. [2]): 5′-G/GATCC-3′. BamHI hydrolyzes double-stranded DNA at the first 5′-G of each strand, and yields a 3′-hydroxy terminus and a 5′-terminal phosphate. Due to the cleavage offset, terminal "overhangs" (cohesive or sticky ends) are generated. Some other endonucleases, however, produce plain termini (blunt ends). Currently, about 3000 different restriction endonucleases are known, several hundred of which are commercially available.

The generation of DNA fragments is essential for the production of new genetic combinations which are introduced into a new, nonnatural context where they are reproduced. In order to plan this process, the nucleotide sequences of fragments and vectors (plasmids, "gene shuttles") must be searched for the recognition sequences of restriction endonucleases. Beyond doubt, this task is an awful bore—and better solved by a computer: It searches faster and more reliably. Already before the era of Windows, software was available for the restriction analysis of DNA fragments and plasmids, among these were programs such as Map and Mapsort from the high-performance GCG Wisconsin Package, which only few institutions can afford. Meanwhile, a lot of comprehensive (and expensive) Windows programs are available for restriction mapping, but there are also some web-based programs that are perfect for low budgets. One of these is Restriction Mapper by molecular biologist Peter Blaiklock, a software that is straightforward, intuitively ascertainable, and now available in a third, revised version (Figure 1).

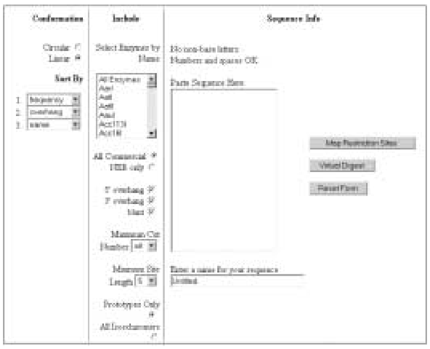
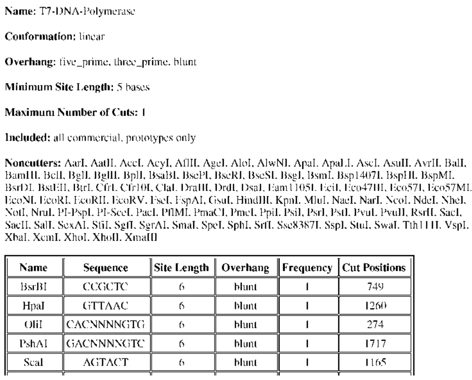


Figure 1. Restriction Mapper home page (excerpt).

By copying and pasting, users can transfer single-stranded DNA sequences of any format (text, GCG, GenBank, etc.) to this web site where they are searched for cleavage patterns of all commercially available restriction enzymes. The program supports

- distinction between linear and circular DNA (important for finding cleavage sites within plamids),
- selective choice of enzymes: prototypes, or isoschizomers (enzymes with identical recognition sites),
- limitation to maximum cut numbers (for many purposes, "single cutters" are preferred), and
- sorting and filtering the analysis output by number or length of restriction sites, by type of produced overhang, or simply by alphabet (Figure 2).

Enzymes listed in the output table are conveniently linked to New England Biolab's database REBASE which stores information on all known restriction enzymes, for example their recognition sites, dependence on methylation patterns, availability, and references. Choosing the coding sequence of T7 DNA polymerase as an example, as well as identical mapping conditions, one obtains largely identical (85 %) results with Restriction mapper and Map (see above): "Dinosaur" Map finds 20 recognition sites, Restriction Mapper locates 17 and fails to find the patterns of BlpI, FspI, and TaqII, although the first two of these are commercially available. In four cases, Map yields isoschizomers of enzymes found by Restriction Mapper; these specifications, however, are easily interconvertible by using the catalogs of common suppliers.



Name: T7-DNA-Polymerase

Conformation: linear

Overhang: five_prime, three_prime, blunt

Minimum Site Length: 5 bases

Maximum Number of Cuts: 1

Included: all commercial, prototypes only

Noncutters: AarI, AatII, AccI, AcyI, AflII, AgeI, AloI, AlwNI, ApaI, ApaLI, AscI, AsuII, AvrII, BalI, BamHI, BclI, BglI, BglII, BplI, BsaBI, BsePI, BseRI, BseSI, BsgI, BsmI, Bsp1407I, BspHI, BspMI, BsrDI, BstEII, BtrI, CfrI, Cfr10I, ClaI, DraIII, DrdI, DsaI, Eam1105I, EciI, Eco47III, Eco57I, Eco57MI, EcoNI, EcoRI, EcoRII, EcoRV, FseI, FspAI, GsuI, HindIII, KpnI, MluI, NaeI, NarI, NcoI, NdeI, NheI, NotI, NruI, PI-PspI, PI-SceI, PacI, PflMI, PmaCI, PmeI, PpiI, PsiI, PsrI, PstI, PvuI, PvuII, RsrII, SacI, SacII, SalI, SexAI, SfiI, SgfI, SgrAI, SmaI, SpeI, SphI, SrfI, Sse8387I, SspI, StuI, SwaI, Tth111I, VspI, XbaI, XcmI, XhoI, XhoII, XmaIII

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BsrBI	CCGCTC	6	blunt	1	749
HpaI	GTTAAC	6	blunt	1	1260
OliI	CACNNNNGTG	6	blunt	1	274
PshAI	GACNNNNGTC	6	blunt	1	1717
ScaI	AGTACT	6	blunt	1	1165

Figure 2. Typical result table.

Suggest a web site or submit a review:
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Restriction Mapper also provides a "virtual digest" function that simulates the simultaneous cleavage of a chosen sequence with multiple enzymes: The output is a modified sequence text. It comprises the resulting fragments cut according to the specifications, and is sorted by length (similar to the band pattern of a gel electrophoresis).

A short and concise help function, some FAQs, and a number of links to interesting web sites add to the good overall impression. The potential user can, however, safely ignore the dilution calculator. Neither is this function essential in the context of restriction analysis, nor is there an obvious use. Trust your first-year stoichiometry class instead…

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[1] H. Beyer, W. Walter, W. Francke, *Lehrbuch der Organischen Chemie*, S. Hirzel Verlag, Stuttgart, **1998**.
[2] E.-L. Winnacker, *Gene und Klone*, Verlag Chemie, Weinheim, **1990**.

For further information visit:
http:http://www.restrictionmapper.org/
or contact
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