Roche Applied Science

Restriction Enzymes FAQS and Ordering Guide

A Tradition of Premium Quality and Scientific Support

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Restriction Enzymes from Roche Applied Science – A Tradition of Premium Quality and Scientific Support

Roche Applied Science introduced the first restriction enzymes in 1976. Since then many researchers have chosen to apply our enzymes in their everyday work and have relied on the quality and consistency we provide. The key to our success lies in:

Guaranteed stability

Use an enzyme whose purity and full activity are guaranteed for up to 24 months. Roche Applied Science gives you the security of a expiration date for each enzyme – and guarantees 100% activity through that date.

Selection

With more than 115 restriction enzymes now available, Roche Applied Science provides restriction enzymes that span a wide range of recognition sequences, including some which cannot be ordered from any other supplier. Choices range from rare cutters for genomic mapping to standard enzymes thus reagents and kits for upstream and downstream applications are offered by RAS.

Purity and function testing

Obtain the results you expect by using enzymes tested for endonuclease, exonuclease and phosphatase activity (Figure 1), as well as with the ability to recut DNA after subsequent ligation.

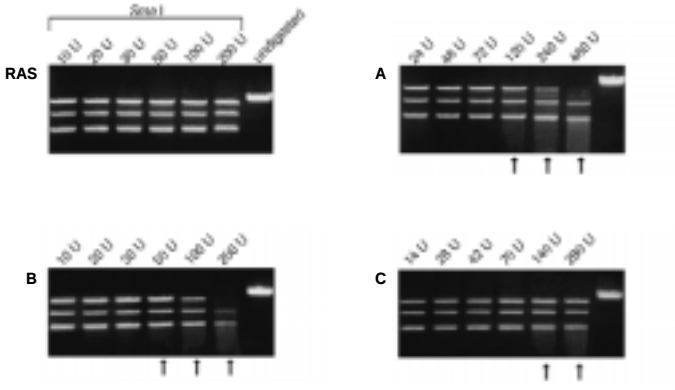

Figure 1: Absence of exonuclease contamination. One microgram of DNA was incubated for 4 hours with various amounts of *Sma* I from different suppliers to test for exonuclease contamination. Only the restriction enzyme from Roche Applied Science (RAS) showed absolutely no exonuclease contamination. Exonuclease activity may lead to the subsequent cloning of incomplete DNA.

Guaranteed cutting activity

Achieve complete digestion with just one unit of a Roche Applied Science restriction enzyme (Figure 2). Use an enzyme whose activity is 100% guaranteed when used in the appropriate SuRE/Cut Buffer.

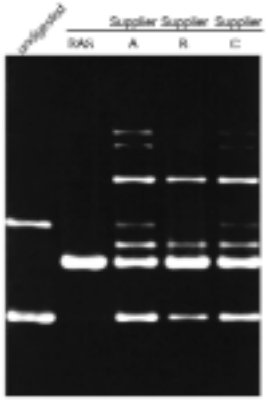

Figure 2: Complete digestion in one hour. pUCBM21 DNA was cut with 1 unit *Bam*H I from different suppliers (each enzyme in its complimentary buffer) for 1 hour. Only the restriction enzyme from Roche Applied Science (RAS) completely cut the DNA.

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Buffers

Perform restriction digests with more than 115 restriction enzymes, using only five optimized SuRE/Cut Buffers. The SuRE/Cut Buffer system takes the guesswork out of double digests. Roche Applied Science includes a complementary vial of SuRE/Cut Buffer with each restriction enzyme, and guarantees 100% activity with each corresponding enzyme.

Convenient dual concentration

Choose from the most extensive line of high and low concentration restriction enzymes available.

Service

Expect customer service that is committed to filling and shipping each order the same day it's received; plus technical service scientists with the expertise to answer questions on any product in Roche Applied Science's complete product line.

Beside our tradition of premium quality, Roche Applied Science is also known as the information provider in terms of comprehensive reference material and a committed product support. To follow our reputation, we have prepared Frequently Asked Questions in short FAQs about restriction enzymes. They also include questions focused on troubleshooting.

The FAQs included in this short manual are organized in the following way:

- Basic Information
- Information provided by Roche Applied Science
- Standard Digest
- Double Digest
- Genomic Digest
- DNA Methylation
- Troubleshooting

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Reference materials available are,

- The additional information available in the **Biochemicals Catalog.**

- The comprehensive **Restriction Enzyme Poster** well known as the information source for commercially available restriction enzymes and their recognition sequence.

- The additional information available in Lab FAQS-Find a Quick Solution (2nd edition).

- The handy **Laminated Buffer Chart** containing all the information needed for quick selection of optimal buffer and reaction conditions for your restriction enzymes.

- The convenient ***RE Finder Program*** located on our Bench Mate website, **http://www.roche-applied-science.com/benchmate/** helps you to identify the enzymes that will cut your DNA sequence, and displays the names and recognition sequences of enzymes and isoschizomers. Thus detailed information (pack inserts) of these respective restriction enzymes are linked to your search result.

- Also note the ***Biochemica Newsletter*** articles available (also on our website):

  - Restriction Enzymes from Roche Applied Science – A Tradition of Premium Quality
    Roche Applied Science Biochemica 2002; 4:31
  - Restriction Enzymes Carrying an ATG Sequence in the Recognition Site
    Roche Applied Science Biochemica 2002; 4:32
  - Activity of Restriction Enzymes in a PCR Mix
    Roche Applied Science Biochemica 1997; 3:25

- And last but not least, visit our Online Technical Support at

http://www.roche-applied-science.com/support

for further product information.

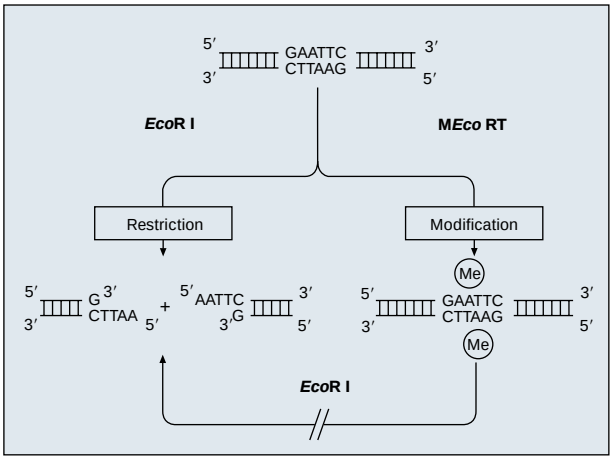
Content

Frequently Asked Questions - Topics

1 What are restriction enzymes, and what is their biological role?

Restriction endonucleases are *endo-deoxyribonucleases* that recognize specific DNA sequences, and digest double-stranded DNA by cleaving two phosphodiester bonds (one within each strand of the duplex DNA).

Restriction enzymes form part of the restriction-modification system of bacterial cells that provides protection against invasion of the cell by foreign DNA - in particular, bacteriophage DNA. Protection against self-digestion is achieved by the presence of specific DNA methyltransferases, which transfer methyl groups to adenine or cytosine residues to produce N^6-methyladenine or 5-methylcytosine. Unmodified foreign DNA entering the cell is degraded by the host restriction-modification system (see figure below).



2 What types of restriction enzymes exist, and which are used in molecular biology applications?

All restriction endonucleases and their corresponding DNA modification methyltransferases have been classified into three classes - I, II, and III - according to their gene and protein structure, cofactor dependence, and specificity of binding and cleavage.

Class I enzymes exhibit both restriction and DNA modification activities that are located on different subunits of multifunctional enzyme complexes. They require Mg^{2+} ions, ATP, and S-adenosylmethionine (SAM) as cofactors. These enzymes cleave DNA at nonspecific sites, usually 100 to 1000 bp downstream of their recognition sequence.

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What types of restriction enzymes exist, and which are used in molecular biology applications? *(continued)*

Class II restriction enzymes and their corresponding modification methyltransferases act as separate proteins. The enzymes in this class are *site-specific*, and hydrolyze specific phosphodiester bonds in both strands of the DNA, within or in close proximity to their recognition sequence. They require only Mg^{2+} ions as cofactors.

Class III enzymes, like Class I enzymes, combine restriction and modification activities in a single enzyme complex composed of different subunits. These enzymes recognize specific sequences, but cleave 25 to 27 base pairs in a 3´ direction outside of the recognition sequence. They require Mg^{2+} ions for activity, but lack both the ATPase activity of Class I enzymes and their absolute requirement for SAM.

Due to their absolute sequence specificity, **Class II restriction endonucleases are generally used as key reagents for a variety of applications in molecular biology and recombinant DNA techniques**, including genomic mapping, restriction fragment length polymorphism (RFLP) analysis, DNA sequencing, and cloning. In this respect, they can be considered to be the "work horses" of molecular biology.

3 Which cleavage sites are recognized by Class II restriction endonucleases?

Class II restriction endonucleases recognize short nucleotide sequences, and cleave double-stranded DNA at specific sites within or adjacent to those sequences. The length of these recognition sequences varies considerably. For example, the enzyme *Not* I recognizes an 8-bp sequence, while the enzyme *Sau*3A I recognizes only a 4-bp sequence. The length of the recognition sequence indicates how frequently the enzyme will cut, on average, in a random sequence of DNA. Enzymes with an 8-bp recognition site will cut every 4^8 or 65,536 bp; a 4-bp recognition site will occur every 256 bp. Enzymes with long recognition sequences are therefore also called "rare cutters" and are ideal tools for mapping eukaryotic genomic DNA.

The majority of enzymes have a *palindromic* recognition sequence characterized by a common structural property: a twofold axis of rotational (dyad) symmetry (*i.e.*, a DNA sequence which is the same when complementary strands are read in opposite directions). An example is the recognition sequence of the enzyme *Swa* I:

5'-ATTTAAAT-3'

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Which cleavage sites are recognized by Class II restriction endonucleases? *(continued)*

Cleavage sites can be unambiguous or ambiguous. The enzyme *Bam*H I recognizes unambiguously only the single defined sequence GGATCC. In contrast, *Hin*f I recognizes the ambiguous 5-bp sequence GA**N**TC, with „N“ representing any nucleotide. Other possible ambiguities are sequences that contain one of the pyrimidines or one of the purines; *e.g.*, in the recognition site of *Xho* II:

5´ [(A/G) GATC (G/T)] 3´

Xho II is also a good example of an enzyme with another characteristic to be considered when talking about recognition sites: the recognition site for one enzyme may include the restriction site for another. Due to its ambiguous sequence, one of the four possible *Xho* II sites will also be a recognition site for *Bam*H I (GGATCC), and all four will be cut by *Sau*3A I (GATC).

A subgroup of Class II restriction enzymes, called “Class IIS”, does not have palindromic recognition sequences and does not cleave within the recognition site itself. The enzymes in this subgroup cleave at a certain, but precise distance from their recognition sequence, *e.g.*, *Bpu*A I:

5´ GAAAGACNN ↓ 3´
3´ CTTTCTGNNNNNN ↓ 3´

4 What types of DNA ends are generated by restriction enzyme cleavage?

Class II restriction enzymes generate three types of DNA ends, all possessing 5´-phosphate and 3´-hydroxyl groups:

- **Cohesive 5´ ends**

  For example, ends generated by Hind III:

  5´-AAGCTT-3´ → A + 5´-AGCTT
  3´-TTCGAA-5´ TTCGA-5´ A

- **Cohesive 3´ ends**

  For example, ends generated by Kpn I:

  5´-GGTACC-3´ → GGTAC-3´ + C
  3´-CCATGG-5´ C 3´-CATGG

- **Blunt ends**

  For example, ends generated by Pvu II:

  5´-CAGCTG-3´ → CAG-3´ + 5´-CTG
  3´-GTCGAC-5´ GTC-5´ 3´-GAC

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What types of DNA ends are generated by restriction enzyme cleavage? *(continued)*

In contrast to blunt ends, protruding 5´ and 3´ ends are also called “sticky” or “overhanging” ends.

Note: In the Roche Applied Science catalog and the “Recognition Sequences of Restriction Endonucleases” poster, the type of DNA end generated by a restriction enzyme is indicated by the position of a downward arrow:

- A ↓ AGCTT represents an overhanging 5´ end
- GGTAC ↓ C represents an overhanging 3´ end
- CAG ↓ CTG represents a blunt end

Restriction enzymes that produce the same single-strand fragment ends form enzyme families. Although the individual enzymes have different recognition sequences, their overhanging ends are complementary so that their cleavage fragments can be combined (ligated) with fragments produced by any other member of the same family. An example of such a group of enzymes is the GATC family, whose members include *Bam*H I, *Bcl* I, *Bgl* II, *Sau*3A, and *Xho* II.

5 What are isoschizomers?

Restriction enzymes that are isolated from different organisms and recognize identical sequences are called isoschizomers. Isoschizomeric enzymes that possess different cleavage sites in a particular recognition sequence are called neoschizomers (*e.g.*, *Sma* I [CCC ↓ GGG] and *Xma*C I [C ↓ CCGGG]).

Because isoschizomers are isolated from different bacterial species or strains, they often have different stabilities or require different optimum reaction conditions. They may also possess different sensitivities to methylation, which might be helpful when methylation sensitivity can interfere with digestion and cloning steps.

6 How is the name of a restriction enzyme defined?

The first three letters refer to the organism from which the restriction enzyme was originally isolated, the fourth letter (if present) refers to the strain, and the Roman numerals serve as indices if the same organism contains several different restriction enzymes.

*Bam*H I: **B**acillus **am**yloliquefaciens, strain **H**, enzyme **I**

7 What is star activity, and when does a restriction enzyme display it?

Under nonstandard conditions, a restriction enzyme cleaves at sites that are similar, but not identical, to its normal recognition sequence. Such altered cutting is called “star” or “relaxed” activity.

*Eco*R I is a well-known example of an enzyme that exhibits star activity. Its normal specific recognition site is G ↓ AATTC; however, under nonstandard conditions the recognition site changes to N ↓ AATTN. This sequence will occur with a much higher frequency than the normal one. If the reaction conditions become even more nonstandard, the recognition sequence changes to Pu ↓ PuATPyPy, resulting in almost completely nonspecific digestion of DNA.

Nonstandard conditions include:

- High pH (>8.0) or low ionic strength
- Glycerol concentrations >5% (important, because enzymes are usually delivered as concentrated stock in 50% glycerol)
- Extremely high concentration of enzyme (>100 U/µg of DNA)
- Prolonged incubation time with enzyme
- Presence of organic solvents in the reaction (*e.g.*, phenol, chloroform, ethanol, DMSO)
- Incorrect cofactor (*i.e.*, Mn^{2+}, Hg^{2+}, or Co^{2+} instead of Mg^{2+})

It was previously thought that star activity was only exhibited by certain enzymes; however, it may be a general characteristic of all restriction enzymes. Information about star activity is provided in the pack insert of the respective enzyme.

To avoid star activity, always use the optimal buffer system and enzyme amount recommended in the pack insert. Make sure that the DNA preparation is free of organic solvents and contaminating salts.

1 I am using restriction enzymes for the first time - what do I need to be aware of?

Mix the restriction enzyme solutions before using them; the 50% glycerol solution is viscous, so fairly vigorous mixing is required (mix by pipetting - do not vortex). Mixing is especially important the first time a new vial is used.

Choose which enzyme to use by considering:

- Substrate used
- Site preference
- Methylation
- Ease of use
- Star activity

2 What experimental setup do you recommend for a basic restriction enzyme digest?

A standard experiment is set up as indicated under „Typical experiment“ in each insert supplied with the respective enzyme *e.g.*,

Component	Final concentration
DNA	1 µg
Respective 10 x SuRE/Cut Buffer	2.5 µl
Sterile redist. water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at 37°C for one hour.

3 Which reagents do you recommend for the purification of DNA?

To purify your substrate we recommend to use,

- the **High Pure PCR Template Preparation Kit**† (Cat. No. 11 796 828 001), the **DNA Isolation Kit for Mammalian Blood**†(Cat. No. 11 667 327 001) or the **DNA Isolation Kit for Cells and Tissues**† (Cat. No. 11 814 770 001) when using genomic DNA.
- the **Genopure Plasmid Midi** or **Maxi Kit** (Cat. Nos. 03 143 414 001, 03 143 422 001) when using plasmid DNA.

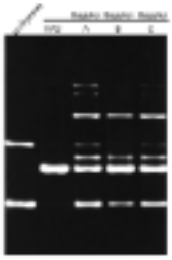
4 What information about quality control is provided by Roche Applied Science?

Information about quality control and heat inactivation is provided in the pack insert of the respective enzyme. To see an example, view the pack insert for *Sma* I at

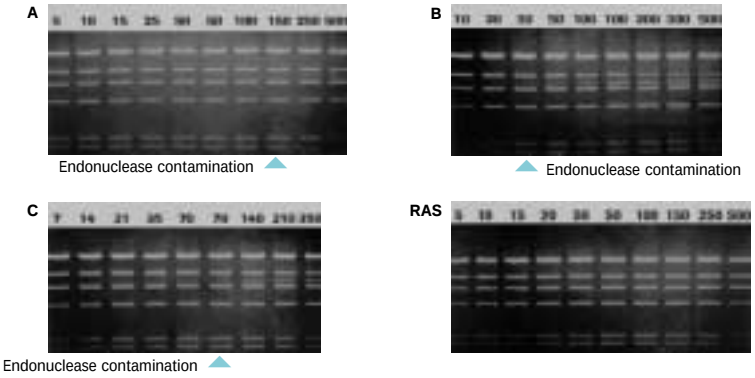
http://www.roche-applied-science.com/pack-insert/0220566a.pdf

A typical quality control assay consists of the following tests:

- **Activity assay**
  - Rigorous definition of unit activity ensures complete and specific cutting
  - Allows economical usage

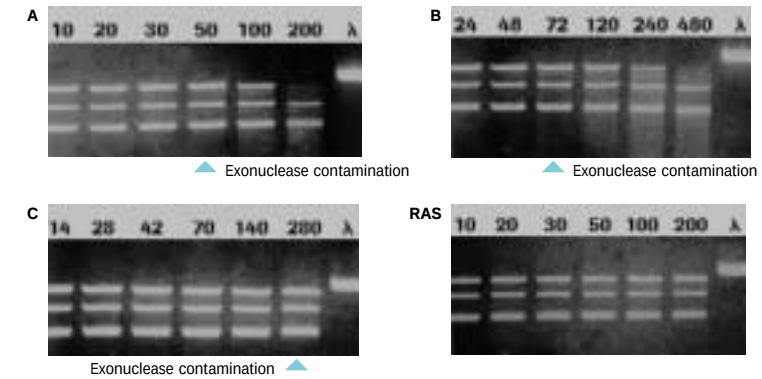


- **Absence of nonspecific endonuclease activities**
  - Ensures reliable and consistent results: no partial digestions, no nonspecific sites



1µg bacteriophage λ DNA was incubated for 16 h at 37°C with an excess (10 to 500 units) of *Hin*d III from different suppliers. Buffer conditions were those as recommended by each supplier. The numbers indicate the amount of units that were used. The arrows indicate endonuclease contamination as seen by the appearance of a smear or undefinded fragments on the gel.

- **Absence of exonuclease and phosphatase activity**
  - Ensures intact ends for correct and efficient ligations



1µg bacteriophage λ DNA was incubated for 16 h at 37°C with an excess (10 to 500 units) of *Sma* I from different suppliers. Buffer conditions were those as recommended by each supplier. The numbers indicate the amounts of units that were used. The arrows indicate a downward smear that is typically caused by exonuclease or phosphatase contamination.

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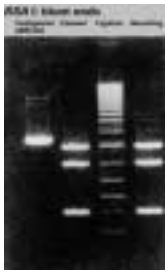
What information about quality control is provided by Roche Applied Science? *(continued)*

- **Ligation and recutting assay**
  - Ensures that the correct restriction sequence is preserved



See the restriction enzyme's **data label** for lot-specific values.

5 How is one unit of restriction enzyme defined?

The catalytic activity of the restriction endonucleases available from Roche Applied Science is based on the determination of the minimum amount of enzyme required for the generation of the enzyme-specific final fragment pattern of a given substrate DNA (Phage-λ DNA in most cases).

One unit is the enzyme activity that completely cleaves 1 µg of substrate DNA suspended in 50 µl of the recommended reaction buffer in 60 minutes at the appropriate temperature under optimal assay conditions as stated for each restriction endonuclease.

For enzyme-specific parameters, refer to the respective pack insert.

Note: This unit definition is not based on classic enzyme kinetics. A unit defined by this method measures enzyme activity by an endpoint determination, not via the classical initial rate term. The molar concentration of the enzyme is in excess. Complete digestion is confirmed on an ethidium bromide-stained gel by visualization of the pattern of cleaved DNA fragments resolved by electrophoresis.

6 How stable is a restriction enzyme?

All restriction enzymes are supplied with control data indicated on the label. Roche Applied Science guarantees 100% activity until the enzyme's respective expiration date. The majority of restriction enzymes are guaranteed to remain stable for 18 months (a few for 12 months) from the date of manufacture, if properly stored. Refer to the pack insert for correct storage conditions, and to the data label on the enzyme's container for the lot-specific expiration date.

Important: Restriction enzymes are, in general, heat labile and therefore should be kept at -15 to -20°C. For brief periods of time, the enzymes can be kept on ice or in a small freezer box.

7 After delivery, the shipping package containing the enzyme remained on the lab bench for two days. Is the enzyme still active?

Our restriction enzymes are always shipped on dry ice. When the enzyme arrives, there should be dry ice remaining in the shipping package. If the enzyme container still feels cold to the touch, the enzyme, in most cases, should still be completely active.

8 Which restriction enzymes have sufficient activity to be used directly in a PCR mix?

In many cases, our restriction enzymes are fully active in a PCR mix, and therefore are suitable for direct use in the appropriate restriction analysis. Restriction endonuclease activity is influenced by the buffer used for PCR as well as the enzyme's ability to cleave in the presence of primers.

The activity of a restriction enzyme in PCR buffer is indicated in its corresponding pack insert. An overview table, containing information on the relative activity of our restriction enzymes in a standard PCR mix is provided below.

Enzyme activity in an standard PCR Mix
(10 mM Tris/HCl, ph 8.3 at 20°C, 50 mM KCL, 1.5 mM $MgCl_2$

Restriction Enzyme	Recommended SuRE/Cut Buffer	Relative Activity (%) in PCR Mix	Restriction Enzyme	Recommended SuRE/Cut Buffer	Relative Activity (%) in PCR Mix
Acc I	A	<5	*Ksp* I	L	0
Alu I	A	100	*Mam* I	H	20
Apa I	A	100	*Mlu* I	H	<5
*Asp*700 I	B	10	*Mvn* I	M	40
*Asp*718 I	B	100	*Nae* I	A	0
Ava I	B	20	*Nco* I	H	50
Ava II	A	<5	*Not* I	H	0
Avi II	H	30	*Nru* I	B	75
*Bam*H I	B	100	*Pst* I	H	90
*Bbr*P I	B	100	*Pvu* I	H	<5
Bfr I	M	100	*Pvu* II	M	100
Bgl II	M	0	*Rsa* I	L	100
Cla I	H	100	*Sac* I	A	100
Dpn I	A	100	*Sal* I	H	0
Dra I	M	100	*Sau*3A I	A	100
*Ecl*X I	B	0	*Sca* I	H	<5
*Eco*47 III	H	0	*Sma* I	A	100
*Eco*R I	H	50	*Sna*B I	M	50
*Eco*R V	B	10	*Sph* I	M	<5
Hae III	M	100	*Ssp* I	H	0
*Hin*d II	M	100	*Stu* I	B	30
*Hin*d III	B	10	*Sty* I	H	<5
*Hin*f I	H	50	*Tag* I	B	100
Hpa I	A	100	*Xba* I	H	60
Kpn I	L	50	*Xho* I	H	<5

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Which restriction enzymes have sufficient activity to be used directly in a PCR mix? *(continued)*

For cloning applications, the direct digestion of the amplified fragment in the PCR mixture without purification is not recommended. Taq DNA Polymerase may still be active and this will result in polishing of 5´ sticky ends and the addition of an extra dA residue to the blunt ends of restriction fragments.

9 Is digestion of hapten-labeled DNA fragments possible?

In principle, a DIG label will interfere with the restriction enzyme's activity if the recognition sequence of the enzyme contains a DIG- or Biotin-dUTP.

The probability of interference also depends on the labeling ratio of DIG-dUTP to dTTP. For example, a labeling ratio of 1:3 (required to synthesize highly specific hybridization probes, for example, when using the **PCR DIG Probe Synthesis Kit**‡, Cat. No. 11 636 090 001) will lead to one DIG moiety in every stretch of 20-25 nucleotides.

The lower the labeling ratio (*e.g.*, 1:10 or 1:20 in the **PCR DIG Labeling Mix**†, Cat. No. 11 585 550 001; please note that this kit is not suited for the generation of hybridization probes), the higher the probability that the recognition sequence will not be affected.

10 Is a dU-containing substrate digested by some common restriction enzymes?

A dU-containing substrate is readily digested by restriction enzymes (*e.g.*, *Eco*R I und *Bam*H I), while others show reduced activity (*e.g.*, *Hpa* I, *Hin*d I, *Hin*d III) on these substrates.

11 I want to design a hybridization probe that can be cleaved by a restriction enzyme. What is the minimum sequence length 5´ and 3´ adjacent to the recognition sequence that is required to ensure sufficient cleavage? Will the enzyme work if the recognition sequence is close to a biotinylated 5´ end?

Generally, six bases on either side of the recognition sequence are required to ensure efficient cleavage (use at least eight bases for *Nde* I and *Not* I).

When working with biotinylated probes in particular, keep this general rule in mind to minimize steric hindrance. Another important aspect is the incubation time: in critical cases an extended incubation time of up to 20 hours can help to improve the cleavage rate.

12 The volume of the enzyme in the vial appears to be very low. Could short-filling or leakage during shipment be the cause?

Some enzymes are supplied in a very low volume; therefore, the vial appears to be empty. During shipment, the enzyme may be dispersed over the interior surface of the vial or gathered under the cap. To check whether the volume is correct, perform the following steps while keeping the enzyme cooled to 4°C:

- Carefully check the exterior of the enzyme vial, noting any glycerol leakage.
- Prepare a water blank containing the enzyme's expected volume to use as a counterbalance.
- Briefly spin the enzyme and blank in a microcentrifuge.
- With both vials on ice, estimate the volume of the enzyme by comparison to that of the blank.

13 How can I calculate the optimum number of enzyme units for my restriction digest?

Reference DNA is used in the specific unit assay for each restriction enzyme. This reference DNA is identified in the pack insert, in addition to the number of cleavage sites for the respective enzyme in this reference DNA.

As an example, for *Eco*R I, one unit of *Eco*R I is defined as the amount that completely cleaves 1 µg λDNA in 1 hour at 37°C. Since λDNA has a size of 48,502 bp and six cleavage sites for *Eco*R I, one unit of *Eco*R I, is, in general, sufficient to cleave DNA with an average cleavage-site frequency of 1/8084 bp.

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How can I calculate the optimum number of enzyme units for my restriction digest? *(continued)*

If you want to cleave 2.5 µg of a 4363-bp plasmid carrying two *Eco*R I sites, this corresponds to a site frequency of 1/2181 bp. Thus, for complete digestion of 1 µg of your plasmid in 1 hour, you would need approximately fourfold more enzyme units than for digesting λDNA. Multiplied by the DNA amount of 2.5 µg in your assay, this results in a total of 10 units you should apply in your assay.

Keep in mind also, that the nature of the substrate strongly influences the activity of restriction enzymes. For plasmid DNA, especially, you must consider the topology of DNA: supercoiled plasmids need more unit activity (up to fivefold; even 15-fold for *Sfi* I) for complete cleavage compared to linearized DNA.

14 What do you recommend for dilution of restriction enzymes, and how stable are diluted restriction enzymes?

In order to obtain the correct concentration, enzymes are often diluted prior to addition to the reaction mixture. The following dilution buffer is recommended: 20 mM potassium phosphate buffer (pH 7.4), 200 mM KCl, 1 mM EDTA, 7 mM β-mercaptoethanol, 10% glycerol and 0.2mg/ml BSA. After dilution with this buffer, the enzyme should be used within a day! Longtime storage is not recommended. Do not freeze the diluted enzyme.

Alternatively, restriction enzymes may also be diluted in the storage buffer that is indicated in the pack insert for each enzyme, and left for several weeks to months at -20°C. Do not dilute to a concentration of **less** than 1 U/µl. Note that for some enzymes, especially *Eco*R I, drastic loss of activity within two days after dilution in storage buffer (5 U/µl) has been reported. Therefore, stability of enzymes after dilution in storage buffer cannot be guaranteed.

Some laboratory manuals recommend dilution of enzymes with 1x reaction buffer before addition to the DNA. Such dilution might partially or completely inactivate the enzyme, especially if the reaction buffer has low ionic strength and contains no stabilizing agents such as BSA. Do not dilute restriction enzymes in reaction buffer.

Always add the restriction endonuclease last to the reaction mixture. Appropriate mixing of the enzyme is recommended before adding it to the reaction mixture; however, do not vortex dilutions or the final reaction mixture. Mix by gentle pipetting and avoid generation of air bubbles.

1 What are the conditions for a successful restriction enzyme digest?

Make sure your DNA preparation is pure and free of contaminants such as phenol, chloroform, ethanol, detergents, EDTA, and salts because these can partially or completely inhibit the activity of the restriction endonuclease. Such compounds should be completely removed by ethanol precipitation followed by drying, before the DNA is added to the restriction digest reaction.

- Optimal reaction conditions for each enzyme are indicated in the pack insert. They can also be found in the Roche Applied Science **Lab FAQs** guide or the **Laminated Buffer Chart**.
- Always use the recommended reaction buffer supplied with the enzyme. Low enzyme activity may sometimes be due to deterioration of the reaction buffer; in such cases, using fresh buffer will solve the problem.
- When working with a restriction enzyme, always keep it on ice and add it last to the digestion reaction mixture, using a fresh pipette tip.

2 How can the substrate influence the restriction digest?

In addition to the incubation conditions, the nature of the substrate strongly influences the activity of restriction enzymes. Important general parameters of the substrate are:

- Base distribution in natural DNA
- Site density per µg DNA
- Tertiary DNA structure
- Base composition of the flanking sequences
- Position of the cleavage sites with respect to each other
- Methylation status of the substrate

Flanking sequences: Restriction endonucleases differ in their ability to cleave at recognition sites close to the end of a DNA fragment. Cleavage close to the end of a fragment is important when two restriction sites are close together in the multiple cloning site of a plasmid or when cleaving near the ends of PCR products or oligonucleotides. Many restriction enzymes can cleave near a DNA end having one base pair remaining in addition to a 1 to 4 single-base overhang produced by an initial cleavage; others require at least 3 base pairs in addition to an overhang. When designing PCR primers containing restriction sites, adding eight random bases 5´ of the restriction site is recommended for complete digestion of the restriction sites. However, in these cases, higher quantities of enzyme and longer reaction times are also necessary. Normal reaction rates are achieved only with increasing length of the flanking sequences.The base composition within the flanking sequences is also important for the cleavage rates at the individual cleavage sites.

Plasmids: Supercoiled plasmids often require more restriction endonuclease to achieve complete digestion than linear DNA.

Double Digest

Digestion with multiple enzymes: would you recommend performing this simultaneously or sequentially?

Digesting DNA with two restriction enzymes is a common task, and often the two enzymes have different buffer requirements. Here are some recommendations for performing double digests:

- **Digest with both enzymes in the same buffer**
  In many cases, even if one given buffer is not optimal for an enzyme, cleavage rates are still quite good. Roche Applied Science provides a **double-digest table** (see below) which lists the best single buffer for performing specific double digests. If the reaction produces extra fragments, possibly caused by star activity, reduce the reaction time or the amount of enzyme. If the reaction is incomplete, test each enzyme individually to determine its ability to linearize the plasmid. A lack of cutting may indicate an inactive enzyme, absence of the expected site, or inhibitors in the template preparation. Test the enzyme on a second target as a control. If both enzymes are active and the restriction sites are within several bases of each other, there may be a problem cutting close to the end of the fragment.
- **Cut with one enzyme, then alter the buffer composition and cut with the second enzyme**
  Always perform the first digest with the enzyme requiring the lower salt buffer. Then adjust the buffer composition for the needs of the second enzyme and add the required amount of salt.
- **Change buffer between digestions with two enzymes**
  Perform one digestion, recover the DNA (by ethanol precipitation), then resuspend it in the buffer appropriate for the second enzyme.
- **Prepare a sequential digest** (with or without altering buffer composition) if using restriction enzymes requiring different reaction temperatures.

Double Digestion Table

Enzyme	100% Activity in Sure/ Cut Buffers	*Asp*718 I	*Bam*H I	*Bgl* II	*Bln* I	*Bst*1107 I	*Bst*X I [2]	*Cla* I	*Dpn* I	*Dra* II	*Eco*47 III	*Eco*R I	*Eco*R V	*Hind* III	*Kpn* I [4]	*Mlu* I	*Nae* I	*Nco* I	*Nde* I	*Nhe* I	*Not* I	*Nsp* I	*Pst* I	*Pvu* I	*Pvu* II	*Sac* I	*Sal* I	*Sfi* I [3]	*Sma* I [4]	*Spe* I	*Sph* I	*Stu* I	*Xba* I
		B	ABM	ABMH	H	H	BH	ABMH	A	AL	H	ABH	B	BM	L	H	AL	H	H	ALM	H	M	H	H	M	AL	H	M	A	MH	M	ABL	AH
*Bam*H I	A B M	B																														
Bgl II	A B M H	B	M																													
Bln I	H	H	B	H																												
*Bst*1107 I	H	H	B	H	H																											
Bst X I [2]	B H	B	B	H	H	H																										
Cla I	A B M H	B	B	H	H	H	H																									
Dpn I	A	A	A	A	H	H	H	A																								
Dra II	A L	A	A	A	B	B	B	A	A																							
*Eco*47 III	H	H	B	H	H	H	H	H	H	SD																						
*Eco*R I	A B H	B	A	H	H	H	H	H	A	A	H																					
*Eco*R V	B	B	B	B	H	H	B	B	B	B	H	B																				
Hind III	B M	B	B	B	H	H	B	B	B	A	H	B	B																			
Kpn I [4]	L	A	L	A	SD	SD	SD	A	A	L	SD	A	SD	A																		
Mlu I	H	H	H	H	H	H	H	H	H	SD	H	H	H	H	SD																	
Nae I	A L	A	A	A	A	A	SD	A	A	L	SD	A	SD	A	L	SD																
Nco I	H	H	B	H	H	H	H	H	H	L	H	H	H	H	L	H	L															
Nde I	H	B	B	H	H	H	H	H	H	B	H	H	H	B	SD	H	A	H														
Nhe I	A L M	A	M	A	A	M	B	M	A	A	SD	A	M	M	L	SD	L	L	M													
Not I	H	H	B	H	H	H	H	H	H	SD	H	H	H	H	SD	H	SD	H	H	SD												
Nsp I	M	B	M	M	B	B	B	M	M	L	B	M	B	M	L	SD	L	M	M	M	B											
Pst I	H	H	H	H	H	H	H	H	H	A	H	H	H	H	SD	H	A	H	H	M	H	M										
Pvu I	H	B	B	H	H	H	H	H	H	A	H	H	B	B	L	H	A	H	H	A	H	M	H									
Pvu II	M	M	M	M	H	H	H	M	M	M	SD	M	M	M	M	H	A	M	M	M	H	M	M	M								
Sac I	A L	A	A	A	A	A	SD	A	A	A	SD	A	A	A	L	SD	L	A	M	L	SD	L	A	A	M							
Sal I	H	H	H	H	H	H	H	H	H	SD	H	H	H	H	SD	H	SD	H	H	SD	H	SD	H	H	H	SD						
Sfi I [3]	M	M	M	M	M	H	B	M	M	L	SD	M	M	M	L	H	L	M	M	M	M	M	M	M	M	L	H					
Sma I [4]	A	A	A	A	A	A	SD	A	A	A	SD	A	A	A	A	SD	A	A	A	A	SD	A	A	A	A	A	SD	A				
Spe I	M H	B	M	H	H	H	H	H	H	L	H	H	H	M	L	H	A	H	H	M	H	M	H	H	M	A	H	M	A			
Sph I	M	B	M	M	H	H	H	M	M	A	H	M	B	M	A	H	A	H	H	M	H	M	H	H	M	M	H	M	A	M		
Stu I	A B L	B	A	A	H	H	B	B	A	L	H	A	B	B	L	H	L	H	B	L	H	M	H	B	M	L	H	M	A	L	M	
Xba I	A H	B	A	H	H	H	H	H	A	A	H	H	B	B	A	H	A	H	H	A	H	M	H	H	M	A	H	M	A	H	M	A
Xho I	H	B	B	H	H	H	H	H	H	B	H	H	B	M	L	H	A	H	H	M	H	M	H	H	H	A	H	M	A	H	H	B	H

Please remember that star activity may be activated by high glycerol concentration, significant overdigestion, and long incubation times (>16 h).

Legend:

SD Sequential digest is recommended.
One enzyme is less than 50% active in specified buffer.

[1] Enzyme requires addition of BSA.
[2] Enzyme requires incubation at 45°C.

[3] Enzyme requires incubation at 50°C.
[4] Enzyme requires incubation at 25°C.

1 After restriction enzyme digestion of genomic DNA to be subsequently used in a Southern blot, how can you check if digestion is complete?

A complete digestion is characterized by a stable pattern, to be distinguished from an incomplete or degraded pattern. The digestion is complete when a similar pattern of DNA fragmentation appears in consecutive samples of decreasing enzyme concentration within the serial digest. If samples with high enzyme concentration show smears that contain fragments smaller than those seen in samples containing less enzyme, then most probably degradation is occurring. If the sample containing the most enzyme is the only sample demonstrating a complete digest, then the subsequent samples (containing less enzyme) will demonstrate progressively larger fragments. A uniformly banded pattern will not occur in serial samples unless the samples are all completely cut or completely uncut.

For a detailed protocol how to perform a Southern blot in combination with DIG labeled probes please refer to our website:
http://www.roche-applied-science.com/DIG
or the DIG Application Manual for Filter Hybridization.

1 What is meant by DNA methylation, and how can it affect a restriction enzyme digest?

① DNA methylation is the covalent modification of DNA by the transfer of a methyl group from S-adenosylmethionine to one of a few possible sites on cytosine or adenine.

DNA methylation is involved in several biological processes, including restriction and modification, mismatch error correction (a DNA repair process), and the control of eukaryotic gene expression.

In prokaryotes, both adenine and cytosine methylation occurs, whereby N^6-methyladenine is the major methylated base and to a lesser extent N^4-methylcytosine. Methylation in bacteria serves two functions: either to protect special sites against cleavage by restriction endonucleases or to correct mismatch error (as methylation of A residues in the sequence 5´-GATC-3´ in *E. coli*). Furthermore, it plays a role in controlling initiation of DNA replication.

In eukaryotes, methylation occurs at the 5´ position of the pyrimidin ring of cytosines.

In animals, methylation is found primarily in C residues that are immediately 5´ to G residues (that is, in the sequence CpG). When such a C is methylated, so is the corresponding C in the complementary strand. In plant DNA, in addition to CpG, the methylated sequence can also be CpNpGp with N being any base. In comparison to animals, plants have a much higher proportion of methylated cytosine (30% versus 2 - 5%).

② Depending on the particular strain, *E. coli* expresses four different methylases that are able to methylate adenines or cytosines which – when located in a cleavage site – may inhibit cleavage by a restriction enzyme:

- The most widely distributed methylase systems are the **M·Eco dam I methylase** (encoded by the *dam* gene) and the **M·Eco dcm I methylase** (encoded by the *dcm* gene). The dam methylase recognizes the sequence GATC and methylates the N^6 position of adenine. The *dcm* methylase recognizes the sequence CC(A/T)GG and methylates the internal cytosine at the C^5 position. *E. coli* may harbor both methylases at the same time.

- The EcoB and EcoK methylases are encoded by the *eco*B/*eco*K gene located on the same locus. Therefore, *E. coli* can only contain EcoB or EcoK, not both at the same time. Both methylases have rather long and therefore rare methylation sites (A^{m6}ACGTGC and GC^{m6}ACGTT), and do not play a major role in affecting restriction enzyme cleavage.

continued on next page

What is meant by DNA methylation, and how can it affect a restriction enzyme digest? *(continued)*

Thus, plasmid DNA from normal strains may be cleaved partially or not at all by restriction endonucleases that are sensitive to methylation. This can be avoided by preparing plasmid DNA from strains that lack these methylases.

Methylation problems can also arise when working with mammalian or plant DNA.

Methylation patterns in eukaryotic genomic DNA can be investigated by using the different methylation sensitivities of isoschizomers (for example, *Mbo* I and *Sau*3A I are isoschizomers that recognize and cleave the sequence GATC, identical to the recognition sequence of *dam* methylase; however, while digestion of G^{m}ATC by *Mbo* I is completely inhibited, digestion by *Sau*3A I is unaffected by methylation).

Note:

- A list of the most important cloning strains and information about methylation sensitivity is always provided in the pack insert of the respective enzyme.
- A sub-group of Class II restriction enzymes (Type IIM) requires a methylated recognition site for cleavage (an example is *Dpn* I).

2 I would like to know whether the restriction enzyme I want to use is sensitive to the methylation pattern of my DNA. Can you explain how to use the symbols of your "Recognition Sequences of Restriction Enzymes" poster in this respect?

Methylation will interfere with your restriction digestion when cutting DNA cloned in *dam*$^{+}$ or *dcm*$^{+}$ *E. coli* strains, or when cutting genomic DNA from certain eukaryotic organisms.

The $^{+}$ **symbol** above an A or C in the recognition sequence indicates that cleavage by the corresponding enzyme is **inhibited** by N^{6}-methyladenine or 5-methylcytosine. The methylation of either A or C in the recognition sequence may occur in *E. coli* strains featuring *dam* or *dcm* methylation. Note that the recognition sequence of the restriction enzyme doesn't have to include the entire methylation site to be blocked. Overlapping of the methylation site with the recognition sequence will also cause problems. *Xba* I, for example, has the recognition sequence 5´TCTAGA3´ which lacks the GATC *dam* methylase target site. Nevertheless, if the preceding 5´ two bases are GA, giving **GATC**-TAGA, or the following 3´ bases are TC, giving TCTA**GATC**, then the *dam* methylase will block *Xba* I from cutting.

The ^{m} **symbol** above the A or C in a recognition sequence indicates that an N^{6}-methyladenine or 5-methylcytosine is **required** for cleavage. In this case, the enzyme you are intending to use requires a methylated A or C in its sequence to cut efficiently.

continued on next page

I would like to know whether the restriction enzyme I want to use is sensitive to the methylation pattern of my DNA. Can you explain how to use the symbols of your "Recognition Sequences of Restriction Enzymes" poster in this respect? *(continued)*

The ° **symbol** above an A or C indicates that cleavage is **not influenced** by N^6-methyl-adenine or 5-methylcytosine. If A or C are not marked by a symbol, the influence of methylation on restriction enzyme activity is still unknown or ambiguous.

A **dotted line under** a sequence (*e.g.*, *Bcl* I TGATCA) indicates the complete or partial recognition sequence of the *E. coli dam*-gene encoded methylase M-*Eco dam* I.

A **dotted line above a** sequence (*e.g.*, Sex AI ACC(A/T)GGT) indicates the complete or partial recognition sequence of the *E. coli dcm*-gene encoded M-*Eco dcm* I methylase.

If the M-*Eco dam* or M-*Eco dcm* methylation sequence is only partial, the flanking nucleotides must complete the GATC or CC(A)GG complement in order for cleavage inhibition to occur.

Note that the presence of a methylase site within a cleavage site does not mean that the corresponding enzyme is in any case inhibited by methylation: while *Bam*H I is not inhibited by GGA^{m6}TCC, *Bcl* I is inhibited by TGA^{m6}TCA.

1 After analyzing the restriction enzyme digest by gel electrophoresis, I observe no cleavage at all. What could be the cause for this?

- **Check the amount and nature** of your DNA. See the FAQs "How can I calculate the optimum number of enzyme units for my restriction digest?" and "How can the substrate influence the restriction digest?" for details.

- **Check the purity of your template.** Residual inhibiting compounds like EDTA, phenol, chloroform, ethanol, CsCl, NaCl, or metal ions in the substrate DNA solution could interfere with the reaction (see the FAQ concerning star activity).

  ▸ To purify your substrate, use
  - the High Pure PCR Template Preparation Kit†, the DNA Isolation Kit for Mammalian Blood†, or the DNA Isolation Kit for Cells and Tissues† when using genomic DNA
  - the Genopure Plasmid Midi or Maxi Isolation Kit when using plasmid DNA.

- **Is your DNA methylated and your enzyme sensitive to methylation?** Digest your DNA with an isoschizomer that is insensitive to methylation. If you are working with plasmid DNA, use a host strain which lacks *dam* and *dcm* methylases.

- **Check the temperature chosen for the digest.** Some restriction enzymes require special incubation temperatures (*e.g., Sfi* I (50°C) or *Sma* I (25°C). The Laminated Buffer Chart lists all enzymes with incubation temperatures other than 37°C.

- **If the enzyme cleaves a particular substrate poorly,** the enzyme's activity can be checked by using λDNA along with the test DNA mixed with λDNA. If the enzyme's activity on λDNA alone corresponds to that indicated in the pack insert, and the λDNA mixed with the DNA of interest is digested poorly, the DNA should be re-purified.

- **Check if the enzyme's expiration date** has been exceeded.

- **Check if the enzyme's storage conditions** were optimal. Always keep restriction enzymes on ice, even during setup of the reaction.

- **Check the dilution and addition of enzyme.** Some restriction enzymes are very sensitive to the concentration of glycerol in the reaction mixture. Since our restriction enzymes are supplied in 50% glycerol, the enzyme should comprise not more than 1/10 of the final reaction volume (*i.e.,* no more than 5% glycerol). Always add the restriction enzyme last to the reaction mixture. Mix gently. Ensure thorough mixing of the reaction, but do not vortex.

2 What could be the reason for partial cleavage?

In this case, check the following:

- Is the substrate DNA pure enough? The efficiency of the restriction endonuclease reaction is very dependent upon the purity of the substrate DNA. Contaminants found in some DNA preparations (*e.g.*, protein, phenol, chloroform, ethanol, EDTA, SDS, CsCl, high salt concentration) may inhibit restriction endonuclease activity.
- After addition of restriction enzyme to the reaction mixture, did you thoroughly mix to ensure even distribution?
- Is the concentration of substrate DNA too high for the applied number of enzyme units?
- Is the substrate DNA supercoiled? In this case you could add more enzyme (up to 20 fold) to the reaction mix, as supercoiled DNA requires more units of enzyme for complete digestion. This also applies to some viral DNAs.
- Some special enzymes show site preferences on different substrates or are influenced by sequences flanking the cleavage site. Several cleavage sites on these DNAs are cleaved at extremely slow rates and complete digestion is obtained only with excess of enzyme units (examples are *Nae* I, *Nar* I, *Sac* II, *Xma* III) For more information, please refer to the respective pack insert.

3 It seems that the enzyme I am using has a lower enzyme activity than stated.

- Make sure that you followed exactly the instructions in the pack insert (*e.g.*, reaction temperature, buffer system, and correct storage and handling of enzyme).
- Is the substrate DNA supercoiled? In this case you could add more enzyme to the reaction mix, as supercoiled DNA requires more units of enzyme for complete digestion.
- If the enzyme was diluted prior to use, check the dilution.
  Note: Diluted enzymes which are not diluted in storage buffer should be used within one day.

4 Additional DNA bands not typical for the expected fragment pattern appeared on my gel. What can I do?

- Star activity could be the reason for your problem. A restriction enzyme shows star activity under non-optimal conditions such as low ionic strength, high pH of the reaction buffer, excess concentration of enzyme, excess concentration of glycerol, manganese, or divalent cations other than magnesium. Under these conditions, restriction enzymes begin to cleave the substrate in other sites in addition to the normally recognized sequences. You should observe additional bands lower than the expected bands and no additional bands higher than the largest fragment. Addition of more enzyme and a prolonged incubation time will lead to an increase in additional bands and decrease of the typical banding pattern, if star activity is the cause of your problem.
  Note: To avoid star activity, add less enzyme, perform the reaction in the recommended buffer, and avoid a too-high concentration (>5%) of glycerol in the reaction mix. See the FAQ on star activity for details.

- If low-intensity bands are present above the expected bands on the gel and there are no bands below the smallest band, then your RE digest is incomplete. Increase the incubation time and the amount of enzyme, and the bands will disappear.

- If following these suggestions do not help you to achieve the correct banding pattern, it could be that
  - your enzyme is contaminated with another enzyme, which can occur due to improper handling,
  - or the substrate DNA is contaminated with DNases, which is often the case for “miniprep” plasmid preparations.

5 After digestion there is only a smear on my gel - what went wrong?

This indicates a probable nuclease contamination from the bacterial host or the reagents used. Work under sterile conditions, wear gloves, and do not re-use tips.

6 How can I set up a control reaction?

Control reactions are an essential part of good laboratory practice.

For your restriction enzyme digest, prepare one vial containing sample DNA reaction buffer and no restriction enzyme. DNA degradation in your control vial indicates that you might have a nuclease contamination in your reaction buffer or the DNA preparation.

7 The ligation efficiency is poor. What could be the reason?

There could be several reasons for this, such as:

- Degradation of ligation buffer components such as ATP and DTT. Repeat the ligation with fresh buffer.
- The restriction enzyme is still active in the ligation mixture. Heat inactivate the RE reaction mixture after digestion at 65°C; treat with phenol/chloroform, and/or precipitate with ethanol before ligation, depending on the possibility of inactivating the restriction enzyme by heating.
- Check if the DNA preparation, the ligation reaction, or the restriction enzyme reaction is contaminated with nuclease.
- Check the ligase concentration. Ligation of blunt-ended fragments need more ligase activity (0.1-0.5 units/µl).
- Has the vector ligated to itself? If the vector has compatible ends: Were the 5´ phosphate groups removed by dephosphorylation and was the success of dephosphorylation checked?
- Another reason for a failed ligation could be that the molar ratio of vector and insert is not sufficient. For suggestions, see the pack insert of our Rapid DNA Ligation Kit.
- Check if the competent cells used are really functional. Perform a control transformation.

References

Williams, R.J.
Mol. Biotechnol. 23: 225-243 (2003).
Restriction endonucleases: Classification, properties, and applications.

Jeltsch, A.
Chembiochem 3: 274-293 (2002).
Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases.

Perona, J.J.
Methods 28: 353-364 (2002).
Type II restriction endonucleases.

Pingoud, A., Jeltsch, A.
Nucleic Acids Res. 29: 3705-3727 (2001).
Structure and function of type II restriction endonucleases.

Raleigh, E.A., Brooks, J.E.
(1998) In Bacterial Genomes (De Bruijn, F.J., Lupski, J.R., Weinstock, G.M. Ed.). pp 78-92. Chapman & Hall, New York.
Restriction modification systems: where they are and what they do.

Smith, D.R.
(1996) In Methods Mol. Biol. Vol 58.
(A. Harwood Ed.). pp 11-15. Humana Press, Inc., Totowa, NJ.
Restriction endonuclease digestion of DNA.

Palmer, B.R., Marinus, M.G.
Gene 143: 1-12 (1994).
The dam and dcm strains of Escherichia coli - a review.

Bhagwat, A.S.
Methods Enzymol. 216: 199-224 (1992).
Restriction enzymes: Properties and Use.

Bird, A.
Cell 70: 5-8 (1992).
The essentials of DNA methylation.

Roberts, R.J., Macelis, D.
Nucleic Acids Res. 19: 2077-2109 (1991).
Restriction enzymes and their isoschizomers.

Kessler, C., Manta, V.
Gene 92: 1-248 (1990).
Specificity of restriction endonucleases and DNA modification methyltransferases - a review (Edition 3).

Gerstein, AS.
Molecular Biology Problem Solver: A Laboratory Guide. Chapter 9: Restriction Endonucleases (2001) by Wiley-Liss, Inc.
ISBNs: 0-471-37972-7 (Paper); 0-471-22390-5 (Electronic).

Ausubel, FM., Brent, R., Kingston, RE., Moore DD, J. G. Seidmann JG, Smith JA., Struhl K. (Eds)
Current Protocols in Molecular Biology (1998)
by Whiley & Sons; Four Volumes 0-471-50338-X-Looseleaf or 0-471-30661-4-CD-Rom.

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
Aat I	→ *see isoschizomer* ***Stu* I**			
***Aat* II**	GACGT↓C	1–5	10 775 207 001	250
***Acc* I**	GT↓(A,C)(T,G)AC	5	10 728 420 001	100
		5	10 728 438 001	500
Acc III	→ *see isoschizomer* ***Mro* I**			
***Acs* I**	(A,G)↓AATT(T,C)	10	11 526 456 001	200
***Acy* I (*Aha* II)**	G(A,G)↓CG(C,T)C	5	11 081 314 001	200
Afl I	→ *see isoschizomer* ***Ava* II**			
Afl II	→ *see isoschizomer* ***Bfr* I**			
***Afl* III**	A↓C(A,G)(T,C)GT	5	11 209 183 001	100
Age I	→ *see isoschizomer* ***Pin*A I**			
Aha II	→ *see isoschizomer* ***Acy* I**			
Aha III	→ *see isoschizomer* ***Dra* I**			
***Alu* I**	AG↓CT	10	10 239 275 001	500
		10	10 656 267 001	2000
***Alw44* I (*Sno* I)**	G↓TGCAC	10	11 450 506 001	1000
Aos I	→ *see isoschizomer* ***Avi* II**			
***Apa* I**	GGGCC↓C	10	10 899 208 001	5000
		40	10 703 745 001	5000
		40	10 703 753 001	20000
*Apa*L I	→ *see isoschizomer* ***Alw44* I**			
Apo I	→ *see isoschizomer* ***Acs* I**			
Apy I	→ *see isoschizomer* ***Eco*R II, *Mva* I**			
***Asp* I (*Tth111* I)**	GACN↓NNGTC	10	11 131 354 001	400
***Asp*700 (*Xmn* I)**	GAANN↓NNTTC	10	10 835 277 001	500
		10	10 835 285 001	2500
***Asp*718**	G↓GTACC	10	10 814 245 001	1000
		10	10 814 253 001	5000
		40	11 175 050 001	5000
***Asp*E I**	GACNNN↓NNGTC	10	11 428 179 001	200
***Asu* II**	→ *see isoschizomer* ***Sfu* I**			
***Ava* I**	C↓(T,C)CG(A,G)G	5	10 740 721 001	200
		5	10 740 730 001	1000
***Ava* II**	G↓G(A,T)CC	5	10 740 756 001	500
***Avi* II (*Aos* I)**	TGC↓GCA	10	11 481 436 001	200
Avr II	→ *see isoschizomer* ***Bln* I**			
Bal I	→ *see isoschizomer* ***Mlu*N I**			

Ordering Information

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Bam*H I**	G↓GATCC	10	10 220 612 001	1000
		10	10 567 604 001	2500
		10	10 656 275 001	10000
		40	10 798 975 001	10000
		40	11 274 031 001	50000
***Ban* II**	G(A,G)GC(T,C)↓C	10	10 775 240 001	500
***Bbr*P I (*Pma*C I)**	CAC↓GTG	10	11 168 860 001	500
Bbs I	→ *see isoschizomer* ***Bpu*A I**			
***Bcl* I**	T↓GATCA	10	10 693 952 001	500
		10	10 693 979 001	2500
		40	11 097 059 001	2500
***Bfr* I (*Afl* II)**	C↓TTAAG	10	11 198 939 001	500
***Bgl* I**	GCC(N)4↓NGGC	10	10 404 101 001	1000
		10	10 621 641 001	5000
		40	11 047 604 001	5000
***Bgl* II**	A↓GATCT	10	10 348 767 001	500
		10	10 567 639 001	2000
		40	10 899 224 001	2000
		40	11 175 068 001	10000
***Bln* I (*Avr* II)**	C↓CTAGG	10	11 558 161 001	200
		10	11 558 170 001	1000
***Bpu*A I**	GAAGAC(N)2/6	10	11 497 944 001	200
***Bse*A I**	T↓CCGGA	10	11 417 169 001	200
*Bse*P I	→ *see isoschizomer* ***Bss*H II**			
***Bsi*W I**	C↓GTACG	10	11 388 959 001	300
***Bsi*Y I**	CCNNNNN↓NNGG	10	11 388 916 001	200
***Bsm* I**	GAATGCN↓N	10	11 292 307 001	200
*Bsp*1407 I	→ *see isoschizomer* ***Ssp*B I**			
*Bsp*H I	→ *see isoschizomer* ***Rca* I**			
***Bsp*LU11 I**	A↓CATGT	10	11 693 743 001	200
***Bss*H II**	G↓CGCGC	10	11 168 851 001	200
*Bss*G I	→ *see isoschizomer* ***Bst*X I**			
***Bst*1107 I**	GTA↓TAC	10	11 378 953 001	200
*Bst*B I	→ *see isoschizomer* ***Sfu* I**			
***Bst*E II**	G↓GTNACC	10	10 404 233 001	500
*Bst*N I	→ *see isoschizomer* ***Mva* I, *Eco*R II**			
***Bst*X I**	CCA(N)5↓NTGG	10	11 117 777 001	250
		10	11 117 785 001	1250
***Cel* II (*Esp* I)**	GC↓TNAGC	10	11 449 397 001	200
***Cfo* I (*Hha* I)**	GCG↓C	10	10 688 541 001	1000
		10	10 688 550 001	5000
Cfr I	→ *see isoschizomer* ***Eae* I**			

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Cla* I**	AT↓CGAT	10	10 404 217 001	500
		10	10 656 291 001	2500
		40	11 092 758 001	2500
***Dde* I**	C↓TNAG	10	10 835 293 001	200
		10	10 835 307 001	1000
***Dpn* I**	GA↓TC	10	10 742 970 001	200
		10	10 742 988 001	1000
***Dra* I**	TTT↓AAA	10	10 779 695 001	1000
		10	10 827 754 001	5000
		40	11 175 076 001	10000
***Dra* II**	(A,G)G↓GNCC(T,C)	1–3	10 843 512 001	250
***Dra* III**	CACNNN↓GTG	1–5	10 843 539 001	100
		1–5	10 843 547 001	500
***Eae* I (*Cfr* I)**	(T,C)↓GGCC(A,G)	10	11 062 557 001	200
Eag I	→ *see isoschizomer* ***EclX* I**			
*Eam*1105 I	→ *see isoschizomer* ***Asp*E I**			
***EclX* I (*Xma* III)**	C↓GGCCG	10	11 131 389 001	200
		10	11 131 397 001	1000
***Eco*47 III**	AGC↓GCT	5	11 167 103 001	100
***Eco*R I**	G↓AATTC	10	10 703 737 001	5000
		10	11 175 084 001	10000
		40	10 200 310 001	10000
		40	10 606 189 001	50000
***Eco*R II**	↓CC(A,T)GG	10	11 427 881 001	200
***Eco*R V**	GAT↓ATC	10	10 667 145 001	2000
		10	10 667 153 001	10000
		40	11 040 197 001	10000
Esp I	→ *see isoschizomer* ***Cel* II**			
*Fnu*D II	→ *see isoschizomer* ***Mvn* I**			
Fnu 4H I	→ *see isoschizomer* ***Ita* I**			
***Fok* I**	GGATG(N)9 ↓ CCTAC(N)13 ↑	1–5	11 004 816 001	100
Fsp I	→ *see isoschizomer* ***Avi* II**			
***Hae* II**	(A,G)GCGC↓(T,C)	5	10 693 910 001	100
***Hae* III**	GG↓CC	10	10 693 936 001	1000
		10	10 693 944 001	5000
Hha I	→ *see isoschizomer* ***Cfo* I**			
Hinc II	→ *see isoschizomer* ***Hind* II**			

Ordering Information

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Hin*d II**	GT(T,C)↓(A,G)AC	3–10	10 567 655 001	500
		3–10	10 656 305 001	2500
***Hin*d III**	A↓AGCTT	10	10 656 313 001	5000
		10	10 656 321 001	10000
		40	10 798 983 001	10000
		40	11 274 040 001	50000
***Hin*f I**	G↓ANTC	10	10 779 652 001	1000
		10	10 779 679 001	5000
		40	11 097 067 001	5000
		40	11 274 082 001	20000
***Hpa* I**	GTT↓AAC	3–10	10 380 385 001	100
		3–10	10 567 647 001	500
***Hpa* II**	C↓CGG	10	10 239 291 001	1000
		10	10 656 330 001	5000
		40	11 207 598 001	5000
***Ita* I**	GC↓NGC	10	11 497 979 001	200
***Kpn* I**	GGTAC↓C	10	10 899 186 001	5000
		40	10 742 945 001	2000
		40	10 742 953 001	10000
***Ksp* I (*Sac* II)**	CCGC↓GG	10	11 117 807 001	1000
***Ksp*632 I**	CTCTTC(N)1↓ GAGAAG(N)4↑	10	11 081 276 001	200
***Mae* I**	C↓TAG	1–5	10 822 213 001	50
		1–5	10 822 221 001	250
***Mae* II**	A↓CGT	1–5	10 862 495 001	50
***Mae* III**	↓GTNAC	1–5	10 822 230 001	50
		1–5	10 822 248 001	250
***Mam* I**	GATNN↓NNATC	10	11 131 281 001	200
Mbo I	→ *see isoschizomer* ***Nde* II**			
Meganuclease I-*Sce* I		8–12	11 362 399 001	1000
Mfe I	→ *see isoschizomer* ***Mun* I**			
***Mlu* I**	A↓CGCGT	10	10 909 700 001	500
		10	10 909 718 001	2500
		40	11 207 601 001	2500
***Mlu*NI (*Bal* I)**	TGG↓CCA	10	11 526 430 001	200
***Mro* I (*Acc* III)**	T↓CCGGA	1–5	11 102 982 001	100
Msc I	→ *see isoschizomer* ***Mlu*N I**			
Mse I	→ *see isoschizomer* ***Tru*9 I**			

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Msp* I**	C↓CGG	10	10 633 518 001	1000
		10	10 633 526 001	5000
		40	11 047 647 001	5000
Mst I	➔ *see isoschizomer* ***Avi* II**			
***Mun* I (*Mfe* I)**	C↓AATTG	10	11 441 337 001	200
***Mva* I (*Bst*N I)**	CC↓(A,T)GG	10	11 288 067 001	1000
		10	11 288 075 001	5000
***Mvn* I (*Fnu*D II)**	CG↓CG	10	11 062 573 001	200
***Nae* I**	GCC↓GGC	10	10 786 314 001	250
		10	10 786 322 001	1000
***Nar* I**	GG↓CGCC	10	11 103 016 001	200
		10	11 103 024 001	1000
***Nco* I**	C↓CATGG	10	10 835 315 001	200
		10	10 835 323 001	1000
		40	11 047 698 001	1000
***Nde* I**	CA↓TATG	10	11 040 219 001	200
		10	11 040 227 001	1000
***Nde* II (*Mbo* I)**	↓GATC	5	11 040 235 001	200
		5	11 040 243 001	1000
***Nhe* I**	G↓CTAGC	10	10 885 843 001	200
		10	10 885 851 001	1000
		40	10 885 860 001	1500
***Not* I**	GC↓GGCCGC	10	11 014 706 001	200
		10	11 014 714 001	1000
		40	11 037 668 001	1000
***Nru* I**	TCG↓CGA	10	10 776 769 001	200
		10	10 776 777 001	1000
***Nsi* I**	ATGCA↓T	10	10 909 831 001	200
		10	10 909 840 001	1000
		40	11 207 628 001	1000
***Nsp* I**	(A,G)CATG↓(T,C)	10	11 131 419 001	200
Nsp II	➔ *see isoschizomer* ***Bmy* I**			
Nsp V	➔ *see isoschizomer* ***Sfu* I**			
***Pin*A I (*Age* I)**	A↓CCGGT	10	11 464 841 001	200
		10	11 464 850 001	1000
*Pma*C I	➔ *see isoschizomer* ***Bbr*P I**			
Pml I	➔ *see isoschizomer* ***Bbr*P I**			
***Psp*1406 I**	AA↓CGTT	5	11 533 860 001	200

Ordering Information

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Pst* I**	CTGCA↓G	10	10 621 625 001	3000
		10	10 621 633 001	10000
		40	10 798 991 001	10000
		40	11 274 066 001	50000
***Pvu* I**	CGAT↓CG	5	10 650 137 001	100
		5	10 650 129 001	500
***Pvu* II**	CAG↓CTG	10	10 642 690 001	1000
		10	10 642 703 001	5000
		40	10 899 216 001	5000
***Rca* I (*Bsp*H I)**	T↓CATGA	5	11 467 123 001	200
***Rsa* I**	GT↓AC	10	11 729 124 001	1000
		10	11 729 132 001	5000
		40	11 047 671 001	5000
***Rsr* II**	CG↓G(A,T)CCG	10	11 292 587 001	200
		10	11 292 595 001	1000
***Sac* I (*Sst* I)**	GAGCT↓C	10	10 669 792 001	1000
		10	10 669 806 001	5000
		40	11 047 655 001	5000
Sac II	→ *see isoschizomer* ***Ksp* I**			
***Sal* I**	G↓TCGAC	10	10 348 783 001	500
		10	10 567 663 001	2500
		40	11 047 612 001	2500
***Sau*3A I**	↓GATC	1–5	10 709 743 001	100
		1–5	10 709 751 001	500
***Sau*96 I**	G↓GNCC	10	10 651 303 001	300
***Sca* I**	AGT↓ACT	10	10 775 258 001	500
		10	10 775 266 001	2500
		40	11 207 636 001	5000
***Scr*F I (*Dsa* V)**	CC↓NGG	10	11 081 292 001	500
***Sex*A I**	A↓CC(A,T)GGT	10	11 497 995 001	200
***Sfi* I**	GGCC(N)4↓NGGCC	10	11 288 016 001	250
		10	11 288 024 001	1250
		40	11 288 032 001	1250
		40	11 288 059 001	5000
***Sfu* I (*Asu* II)**	TT↓CGAA	10	11 243 497 001	2000
***Sgr*A I**	C(A,G)↓CCGG(T,C)G	10	11 277 014 001	200
***Sma* I**	CCC↓GGG	10	10 220 566 001	1000
		10	10 656 348 001	5000
		40	11 047 639 001	5000
***Sna*B I**	TAC↓GTA	10	10 997 480 001	200
Sno I	→ *see isoschizomer* ***Alw*44 I**			

Product	Sequence	Volume Activity (units/µl)	Cat. No.	Pack Size (units)
***Spe* I**	A↓CTAGT	10	11 008 943 001	200
		10	11 008 951 001	1000
		40	11 207 644 001	1000
***Sph* I**	GCATG↓C	10	11 026 950 001	200
		10	10 606 120 001	500
		10	11 026 534 001	2500
		40	11 026 542 001	2500
***Ssp* I**	AAT↓ATT	10	10 972 967 001	200
		10	10 972 975 001	1000
		40	11 207 652 001	1000
***Ssp*B I**	T↓GTACA	10	11 497 901 001	200
Sst I	→ *see isoschizomer* ***Sac* I**			
Sst II	→ *see isoschizomer* ***Ksp* I**			
***Stu* I**	AGG↓CCT	10	10 753 351 001	500
		10	10 753 360 001	2500
		40	11 047 680 001	2500
***Sty* I**	C↓C(A,T)(A,T)GG	10	11 047 744 001	2000
***Swa* I**	ATTT↓AAAT	10	11 371 517 001	200
		10	11 371 525 001	1000
***Taq* I**	T↓CGA	10	10 404 128 001	500
		10	10 567 671 001	2500
		10	11 175 114 001	10000
Tha I	→ *see isoschizomer* ***Mvn* I**			
***Tru*9 I**	T↓TAA	10	11 464 817 001	200
		10	11 464 825 001	1000
*Tth*111 I	→ *see isoschizomer* ***Asp* I**			
***Van*91 I (*Pfl*M I)**	CCA(N)4↓NTGG	5	11 379 275 001	200
***Xba* I**	T↓CTAGA	10	10 674 257 001	1000
		10	10 674 265 001	5000
		10	10 674 273 001	20000
		40	11 047 663 001	20000
***Xho* I**	C↓TCGAG	10	10 899 194 001	5000
		40	10 703 770 001	2500
		40	10 703 788 001	12500
***Xho* II**	(A,G)↓GATC(T,C)	1–5	10 742 929 001	50
Xma III	→ *see isoschizomer* ***EclX* I**			
***Xma*C I**	C ↓CCGGG	10	11 743 392 001	200
Xmn I	→ *see isoschizomer* ***Asp* 700**			

Ordering Information

Product	Application	Cat. No.	Pack Size
Rapid DNA Ligation Kit	Ligation of sticky-end or blunt-end DNA fragments in just 5 min at 15 - 25 °C.	11 635 379 001	1 Kit (40 DNA ligations)
T4 DNA Ligase	Ligation of sticky- and blunt ended DNA fragments.	10 481 220 001 10 716 359 001	100 units 500 units
Alkaline Phosphatase, shrimp	Dephosphorylation of 5'-phosphate residues from nucleic acids. Heat inactivation: 15 min at 65 °C.	11 758 250 001	1000 units
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5'-phosphate residues from nucleic acids.	11 097 075 001	1000 units
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids.	11 388 983 001 11 388 991 001	100 g 500 g
High Pure PCR Product Purification Kit†	For purification of PCR reaction products.	11 732 668 001 11 732 676 001	1 Kit (50 purifications) 1 Kit (250 purifications)
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes.	11 082 035 001	1 ml each (10 x conc. solutions)
SuRE/Cut Buffer A	Restriction enzyme incubation.	11 417 959 001	5 x 1 ml (10 x conc. solution)
SuRE/Cut Buffer B	Restriction enzyme incubation.	11 417 967 001	5 x 1 ml (10 x conc. solution)
SuRE/Cut Buffer H	Restriction enzyme incubation.	11 417 991 001	5 x 1 ml (10 x conc. solution)
SuRE/Cut Buffer L	Restriction enzyme incubation.	11 417 975 001	5 x 1 ml (10 x conc. solution)
SuRE/Cut Buffer M	Restriction enzyme incubation.	11 417 983 001	5 x 1 ml (10 x conc. solution)
BSA, special quality for molecular biology	Maintaining enzyme stability	10 711 454 001	20 mg (1 ml)
Glycogen	Carrier for the precipitation of nucleic acids (DNA or RNA)	10 901 393 001	20 mg (1 ml)
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved water.	03 315 932 001	25 ml (25 vials of 1 ml)

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Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany