

DNAMAN

Sequence Analysis Software

***Restriction  
Analysis and  
In-Silico Cloning***

**BIOINFORMATICS PLATFORM**

# DNAMAN Features

- ▶ Restriction Site Analysis
- ▶ Restriction Enzyme Map
- ▶ Restriction Pattern – In Silico Gel
- ▶ In-Silico Cloning
- ▶ Restriction DNA Fragment

# Restriction Analysis

## General Information

1. Open a sequence file
2. Click Restriction Analysis tool in Quick Analysis tab

DNAMAN - AY599226.seq

Home Sequence Protein & Database View

Clipboard Editing Quick Analysis Information

Channel AY599226.seq

**1** AY599226.seq

**2** Restriction Analysis

**All Channels**

- Channel 1: AY599226 (DNA)
- Channel 2: Empty
- Channel 3: Empty
- Channel 4: Empty
- Channel 5: Empty
- Channel 6: Empty
- Channel 7: Empty
- Channel 8: Empty
- Channel 9: Empty
- Channel 10: Empty
- Channel 11: Empty
- Channel 12: Empty
- Channel 13: Empty
- Channel 14: Empty
- Channel 15: Empty
- Channel 16: Empty
- Channel 17: Empty
- Channel 18: Empty
- Channel 19: Empty
- Channel 20: Empty

SEQ AY599226 2934

Composition 746 A; 729 C; 717 G; 742 T; 0 OTHER

Percentage 25.4% A; 24.8% C; 24.4% G; 25.3% T; 0.0% OTHER

MW (kDa) 905.64 ssDNA 1808.79 dsDNA

KEYWORDS CIRCULAR

COLOURS

sequence = 1

features = 0

FEATURES

source 1..2934

/source="Cloning\_vector"

misc\_feature 582..671

/attributes="MCS; multiple\_c;"

gene complement(1874..2734)

/name="bla"

cds complement(1874..2734)

/name="beta-lactamase"

ORIGIN

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TAGCCAGCT AGAGGACCAG CCGCGTAACC TGGCAAAATC GGTTACGGTT GAGTAATAAA

361 TGGATGCCCT GCGTAAGCGG GTGTGGCGCG ACAATAAGT CTTAAACTGA ACAAAATAGA

421 TCTAAACTAT GACAATAAAG TCTTAAACTA GACAGAATAG TTGTAACCTG AAATCAGTCC

481 AGTTATGCTG TGAAAAAGCA TACTGGACTT TTGTTATGCC TAAAGCAAAC TCTTCATTTT

541 CTGAAGTGCA AATTGCCCGT CGTATTAAAG AGGGCGGTGG GGTGCGACAT ATCATGCATG

601 TCGCGACTG TCGAAACCGG CCGCTGCGCG AATGCGGCGA GCAGCTTCCG CCGGCTAGCT

Log-cmd Channel Database File

12:2 Channel 1:DNA AY599226 2934bp CAP|NUM|SCLR

# Restriction Analysis

## General Information

1. Select Show sites on sequence button
2. Click Next Button

Restriction Analysis

**1**

**Results**

Show summary

Show sites on sequence

Draw restriction map

Draw restriction pattern

Ignore enzymes with more than  sites

Ignore enzymes with less than  sites

List site order and non-cutting enzymes

bases per line

With double-stranded sequence

With enzyme position

Including annotations

**Target DNA**

Circular

dam methylation

All DNA in sequence channels

dcm methylation

**2**

< Back **Next >** Cancel

# Restriction Analysis

## General Information

1. Select Blunt to limit enzymes with blunt ends
2. Click Select All to use all restriction enzymes in the list
3. Press Finish button

Enzyme Selection

Enzyme File: RESTRICT.ENZ Save list

**2** Select All >>

<<Clear

AhIII  
Ball  
BstD102I  
DraI  
Ecl136II  
Eco47III  
Eco72I  
EcoICRI  
EcoRV  
EheI  
HpaI  
MscI  
MstI  
NaeI  
NruI  
PmaCI  
PmeI

List: 0 Selected: 28

Cutter:  All **1**  >=5  >=6  >=8

End:  All  Blunt  5' Overhang  3' Overhang

**3** < Back Finish Cancel

# Restriction Analysis

## General Information

1. Enzyme List with site positions
2. List of sites in position order
3. List of enzymes that do not cut
4. Enzyme positions shown on the DNA sequence

AY599226.seq | AY599226\_Restriction\_A...

```

SciI CTC/GAG 1: 638
SmaI CCC/GGG 1: 620
SpoI TCG/CGA 1: 662
SspI AAT/ATT 1: 2751
StuI AGG/CCT 1: 668
XmnI GAANN/NNTTC 1: 2546
  
```

List by Site Order

237	EheI	638	SciI	1813	DraI	2524	AhaIII
258	MstI	662	NruI	1832	AhaIII	2524	DraI
590	EcoRV	662	SpoI	1832	DraI	2546	XmnI
602	EcoICRI	668	StuI	2169	MstI	2751	SspI
602	Ecl136II	987	BstD102I	2427	ScaI	2788	BstD102I
620	SmaI	1813	AhaIII				

Non Cut Enzymes

BalI	Eco47III	Eco72I	HpaI	MscI	NaeI
PmaCI	PmeI	PvuII	SnaBI	SrfI	SwaI

Restriction sites on AY599226

```

1      TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
      AGCGCGCAAAGCCACTACTGCCACTTTTGGAGACTGTGTACGTCGAGGGCCTCTGCCAGT

61     CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTG
      GTCGAACAGACATTGCGCTACGGCCCTCGTCTGTTCCGGGCAGTCCCGCGCAGTCGCCAC

121    TTGGCGGGTGTGCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTAAGTACTGAGAGTGC
      AACCGCCACAGCCCCGACCGAATTGATACGCCGTAGTCTCGTCTAACATGACTCTCAGC

181    ACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGGCGCC
      TGGTATACGCCCACTTTATGGCGTGTCTACGCATTCTCTTTATGGCGTAGTCCGCGG
  
```

EheI

MstI

# Restriction Map

1. Starting Restriction Analysis with DNA sequence in current channel, select Draw Restriction Map
2. Press Next button

## Restriction Analysis

### Results

- Show summary
- Show sites on sequence
- Draw restriction map
- Draw restriction pattern
- Ignore enzymes with more than  sites
- Ignore enzymes with less than  sites
- List site order and non-cutting enzymes
- bases per line
- With double-stranded sequence
- With enzyme position
- Including annotations

### Target DNA

- Circular
- dam methylation
- All DNA in sequence channels
- dcm methylation

< Back

Next >

Cancel

# Restriction Map

## Select Enzymes

1. Select Blunt to limit enzymes with blunt ends
2. Click Select All to use all restriction enzymes in the list
3. Press Finish button

Enzyme Selection

Enzyme File:

List: 0 Selected: 28

Cutter:  All  >=5  >=6  >=8

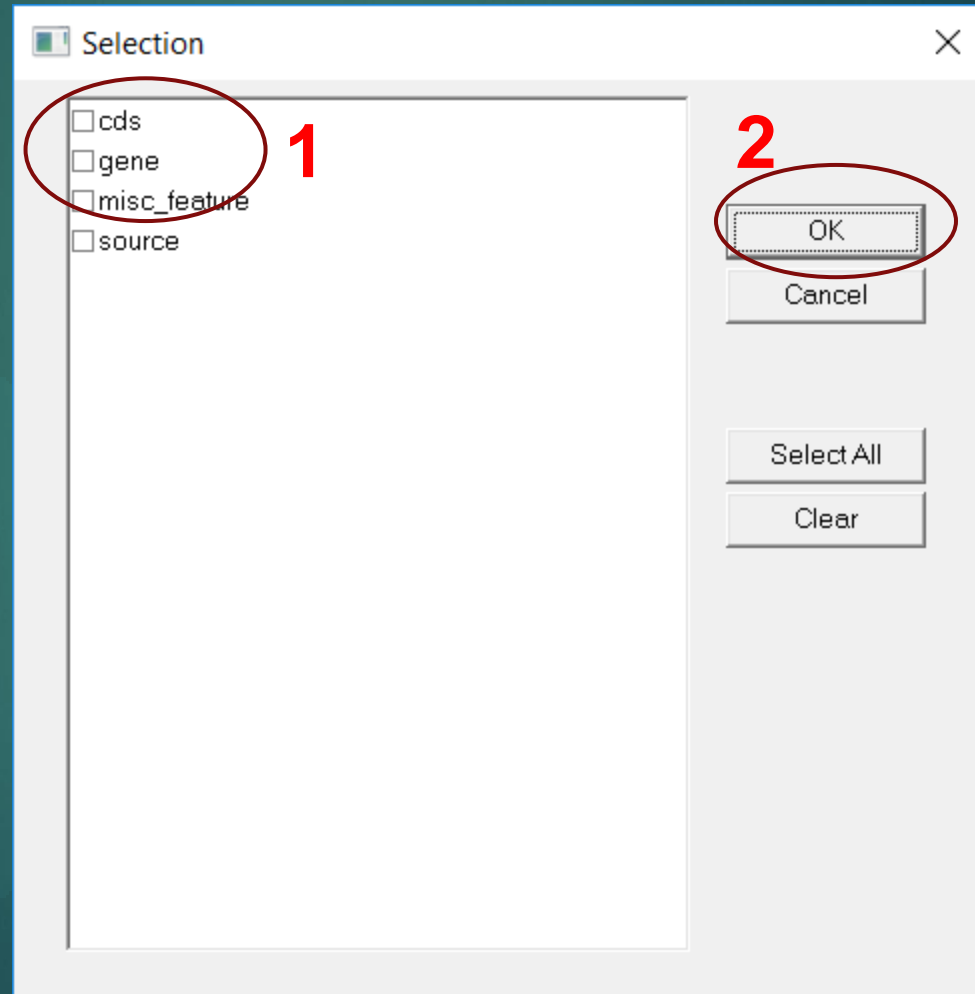
End:  All  Blunt  5' Overhang  3' Overhang



# Restriction Map

## Select Annotations

1. Select Annotations to show on the map
2. Press OK button



# Restriction Map

## Display Map: 3 panels

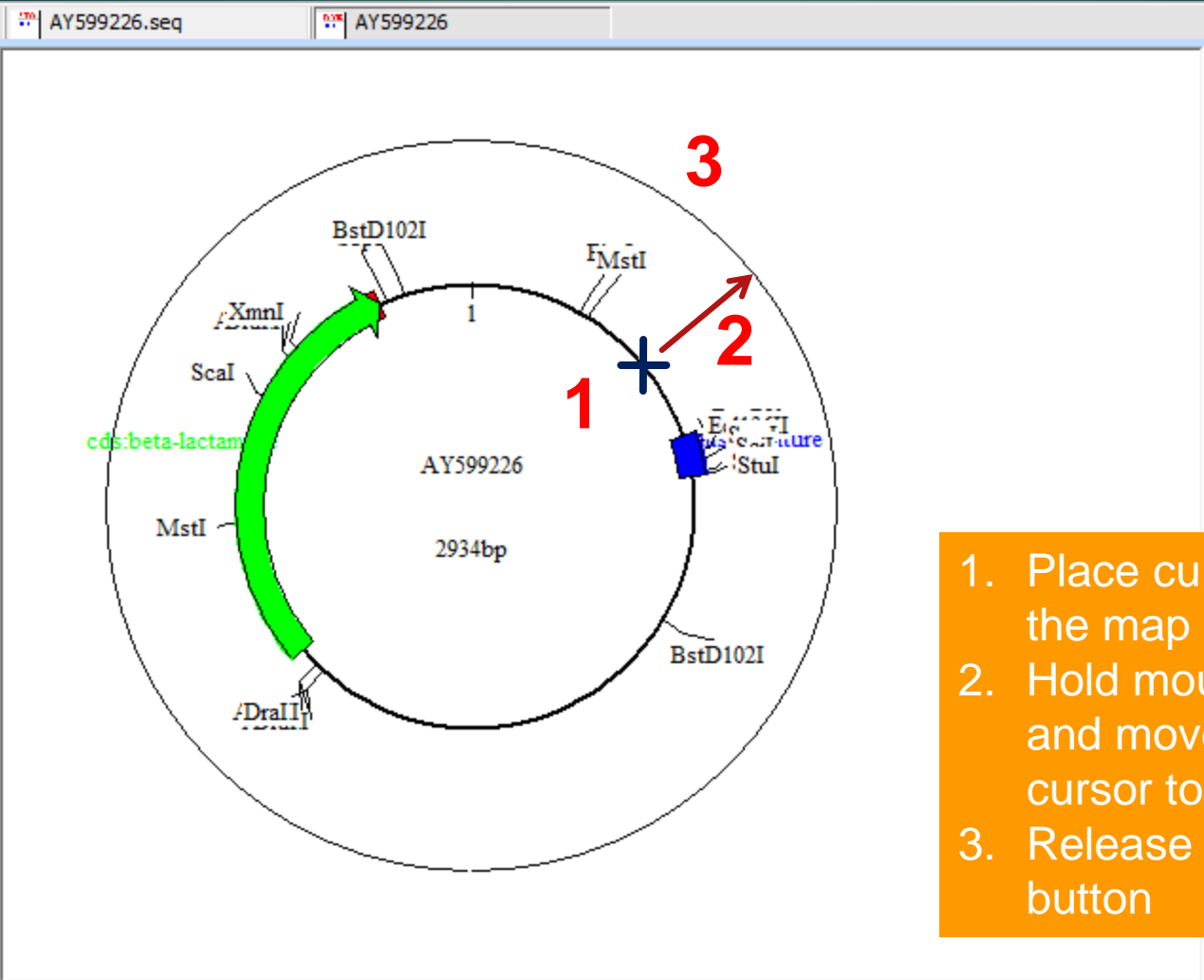
- 1. Map Panel
- 2. Sequence Panel
- 3. Map Info Panel

The screenshot displays a bioinformatics software interface for file AY599226. It is divided into three main panels:

- Map Panel (1):** A circular restriction map of a 2934bp plasmid. The map is labeled 'AY599226' and '2934bp'. A green arc on the left side is labeled 'beta-lactam'. Various restriction enzyme sites are marked around the circle: XmnI, ScaI, MstI, DraII, BstD102I, MstI, and StuI. A blue box on the right side is labeled 'EcoRI' and 'StuI'.
- Sequence Panel (2):** A linear DNA sequence view showing nucleotide positions from 10 to 240. The sequence is displayed in a monospaced font with line numbers every 10 positions. The sequence ends with 'EheI'.
- Map Info Panel (3):** A panel on the right side of the interface, currently showing the file name 'AY599226' in a blue box.

# Restriction Map

## Map Panel: Resizing Map



AY599226.seq | AY599226

AY599226

- General
- Elements
- Enzyme Sites
- Text Objects
- Graph Objects

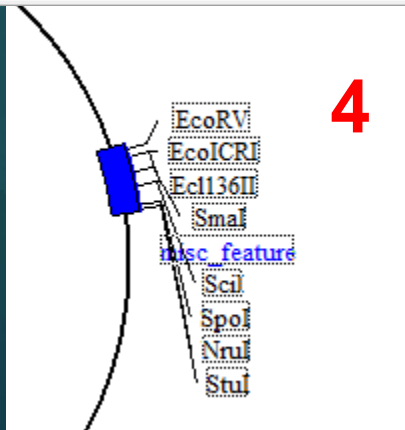
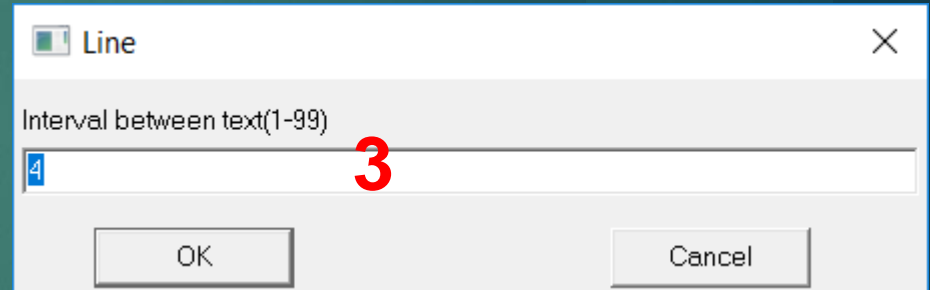
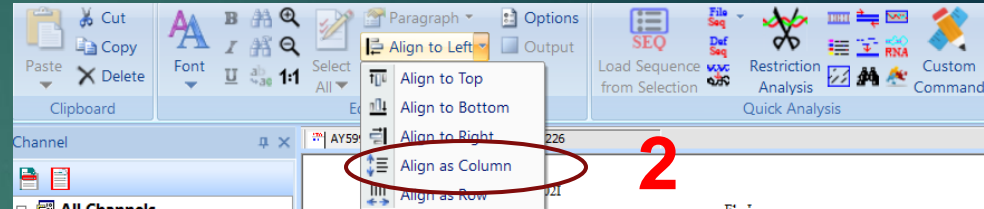
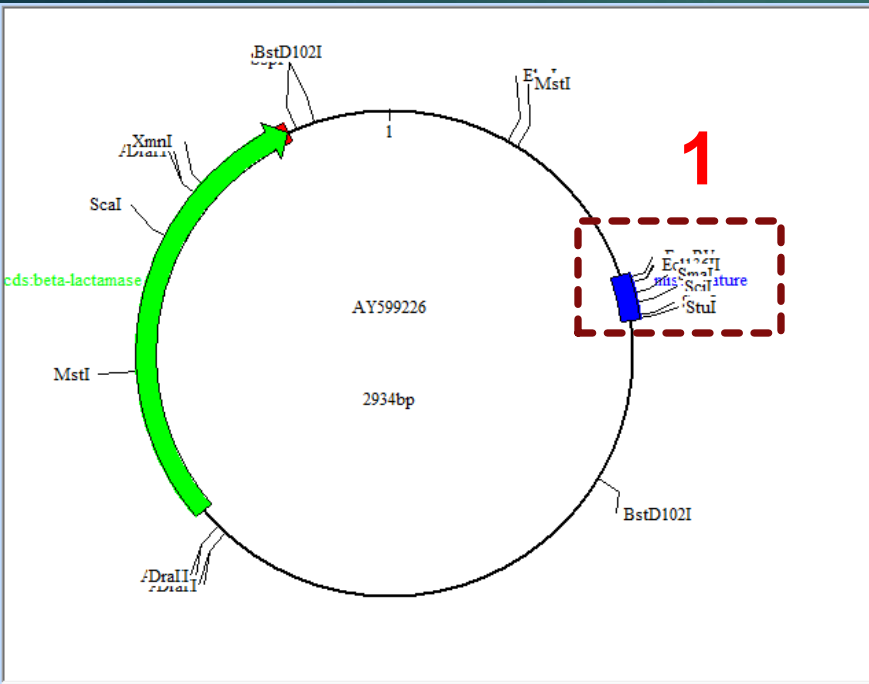
1. Place cursor on the map circle

2. Hold mouse button and move the cursor to resize

3. Release mouse button

# Restriction Map

## Map Panel: Overlapping Text Objects



1. Hold mouse button and select overlapping area
2. Select Align as Column menu from Edit tools
3. Enter space size between columns
4. Overlapping Text objects aligned as column

# Restriction Pattern In-Silico Gel

1. Starting Restriction Analysis with DNA sequence in current channel, select Draw Restriction Pattern
2. Press Next button

Restriction Analysis

Results

Show summary

Show sites on sequence

Draw restriction map

Draw restriction pattern

Ignore enzymes with more than  sites

Ignore enzymes with less than  sites

List site order and non-cutting enzymes

bases per line

With double-stranded sequence

With enzyme position

Including annotations

Target DNA

Circular

dam methylation

All DNA in sequence channels

dcm methylation

< Back **Next >** Cancel

# Restriction Pattern

## Select Enzymes

1. Select 5' Overhang to limit enzyme numbers (optional)
2. Double-Click BamHI and BglII to select
3. Press Finish button

Enzyme Selection

Enzyme File: **RESTRICT.ENZ** Save list

**BamHI**  
**BglII** 2

Acc65I  
AccIII  
AclI  
AflII  
AgeI  
Alw44I  
ApaLI  
AscI  
Asp718I  
AsuII  
AvrII  
BbvII  
BclI  
Bpu1102I  
Bsc91I  
BsiI  
Bsp1407I

Select All >>

<< Clear

List: 58 Selected: 2

Cutter:  All  >=5 **1**  >=6  >=8

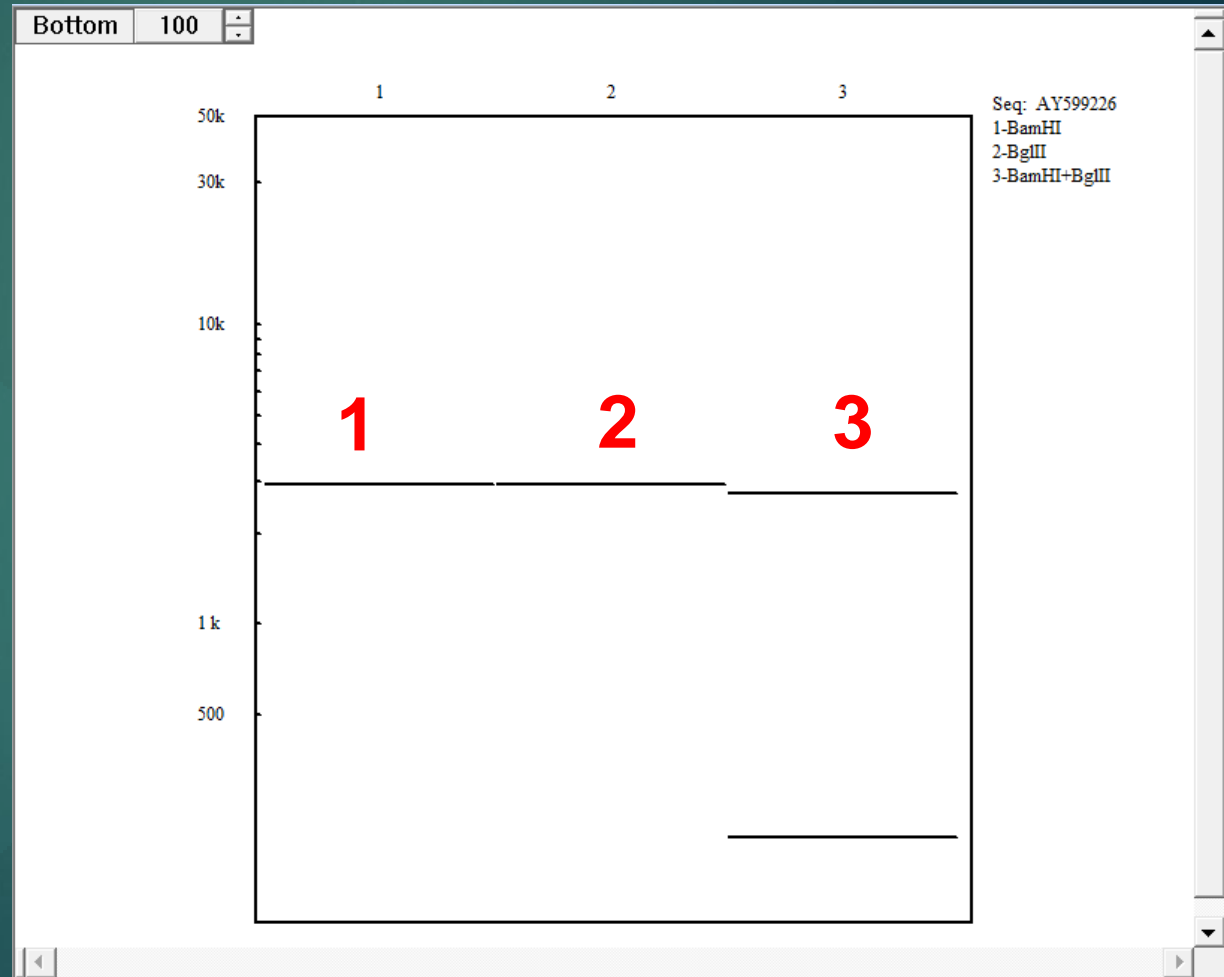
End:  All  Blunt  **3** 5' Overhang  3' Overhang

< Back **3** Finish Cancel

# Restriction Pattern

## In Silico Gel

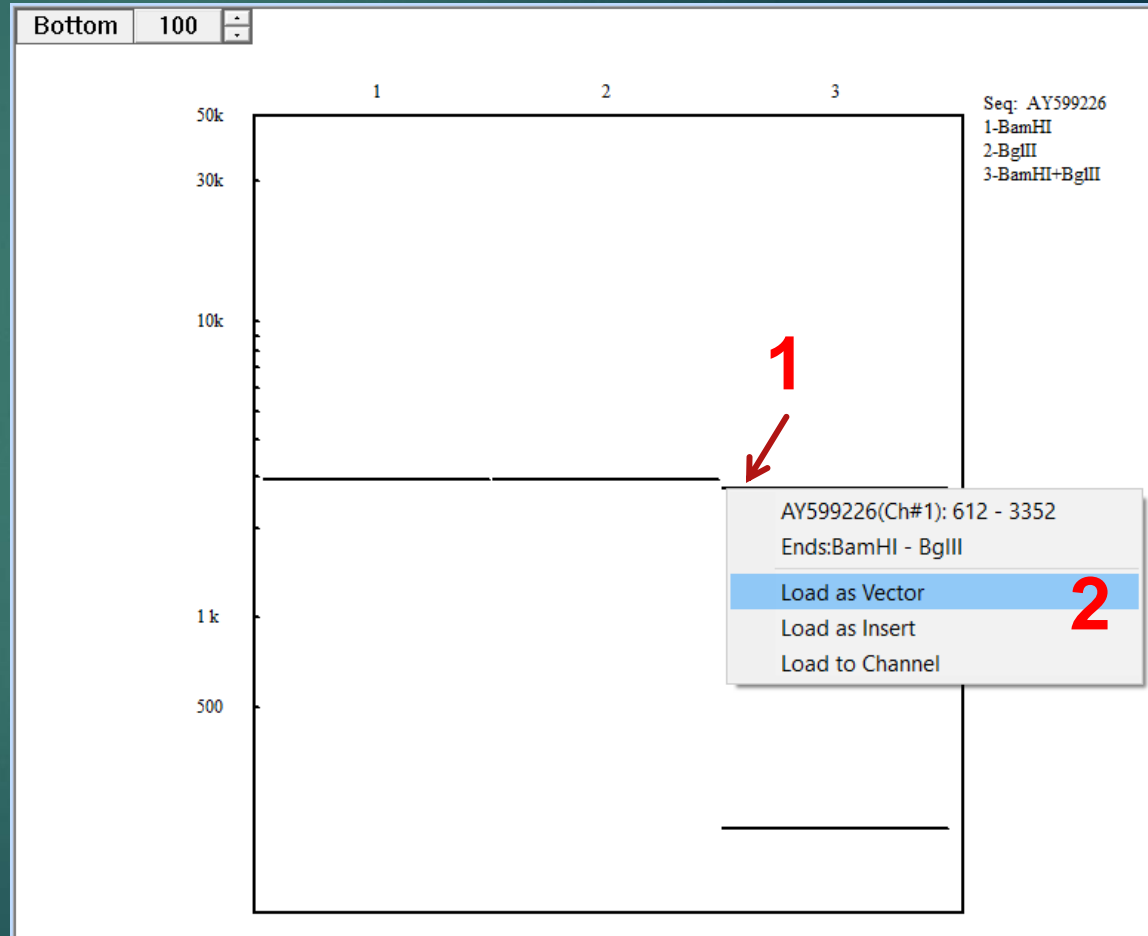
1. Single cut by BamH1
2. Single cut by BglII
3. Double cut by BamH1 and BglII



# Restriction Pattern

## In Silico Cloning

1. Click on the DNA band of interest
2. Choose Load as Vector menu

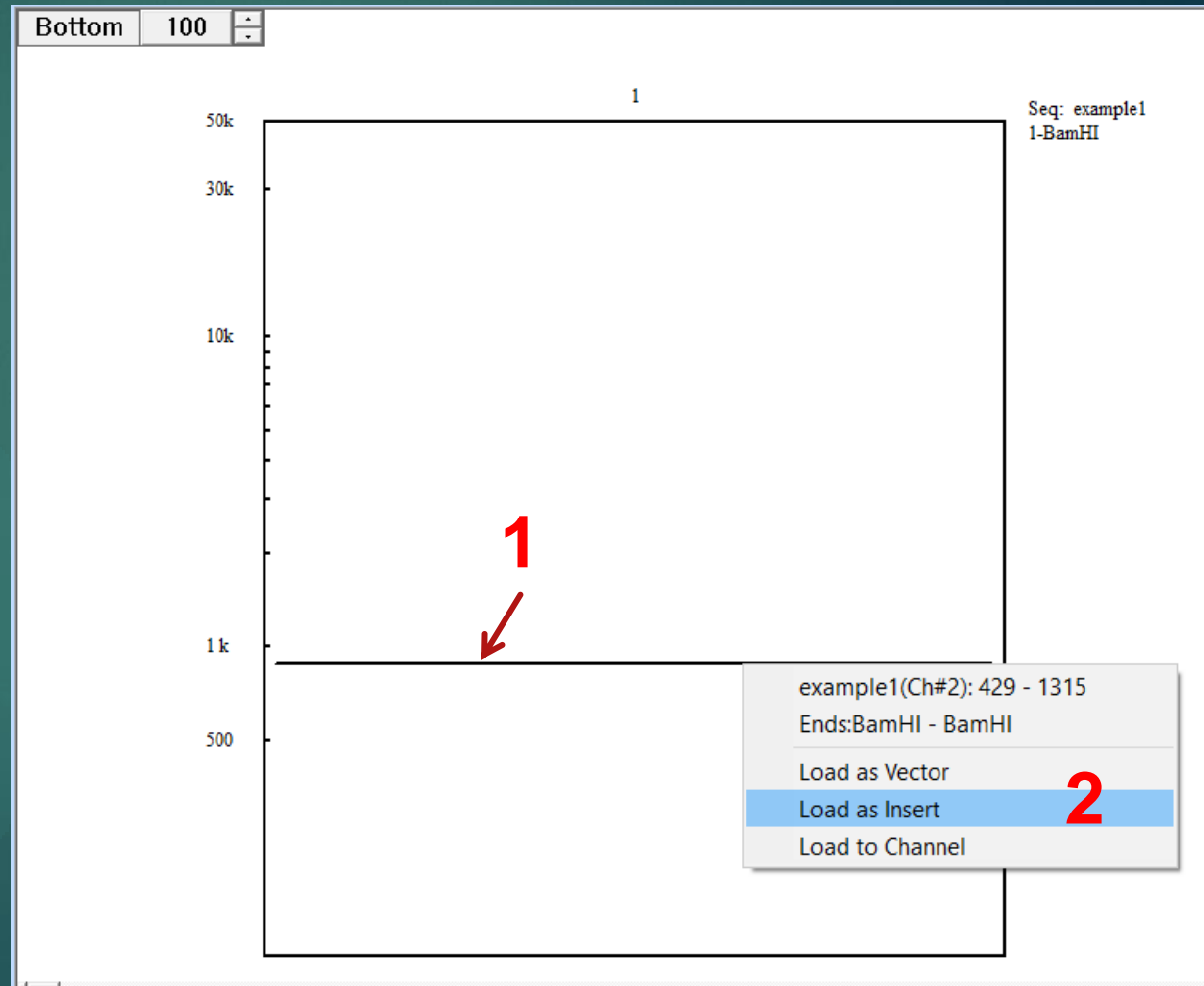




# Restriction Pattern In Silico Cloning

Repeat In Silico Gel  
as the previous  
slides with another  
DNA sample

1. Click on the DNA  
band of interest
2. Choose Load as  
Insert menu



# In Silico Cloning

## Compatible ends

1. Press Cloning tool in Restriction Analysis tab
2. Verify the end compatibilities. Modify ends if needed by pressing the button of each end
3. Click OK button

The screenshot shows the 'Cloning' dialog box with the following details:

- Vector:**
  - Channel: [ ] File: [ ] Database: [ ]
  - Name: AY599226
  - Size(bp): 2740
  - 5' End: +GATC [Keep]
  - 3' End: -GATC [Keep]
  - Vector recircularization
- Insert:**
  - Channel: [ ] File: [ ] Database: [ ]
  - Name: example1
  - Size(bp): 886
  - 5' End: +GATC [Keep]
  - 3' End: -GATC [Keep]
  - Reverse insert orientation
- Linkers:**
  - Vector-Insert: [ ]
  - Insert-Vector: [ ]
- Load new plasmid sequence to channel:** NO
- Buttons:** OK (circled in red), Cancel

# In Silico Cloning Results

1. New sequence constructed
2. Click Save button to save as new file

The screenshot shows the DNAMAN software interface for a 'New Construct'. The 'Save' button in the top-left toolbar is circled in red and labeled with a red '2'. The main window displays the following information:

**Plasmid Construction:**  
Vector from AY599226 2740bp  
Insert from example1 886bp;  
SEQ New 3626 bp  
Composition 939 A; 855 C; 869 G; 963 T; 0 OTHER  
Percentage 25.9% A; 23.6% C; 24.0% G; 26.6% T; 0.0% OTHER  
MW(kDa) 1119.66 ssDNA 2235.34 dsDNA  
KEYWORDS CIRCULAR

**COLOURS**  
sequence = 1  
features = 0

**FEATURES**  
gene complement(2566..3426) /name="bla"  
cds complement(2566..3426) /name="beta-lactamase"  
intron 906..1036 /name="Unknown"

**ORIGIN**

```
1 TCGCGGCTTT CGGTGATGAC GGTGAAAACC TGTGACACAT GCAGCTCCCG GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CGGCACAGAT CGGTAAGGAG AAAATACCGC ATCAGCGCGC
241 ATTCCGCATT CAGGCTGCGC AACTGTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
301 TACGCCAGCT AGAGGACCAG CGCGTAACC TGGCAAAATC GGTTACGGTT GAGTAATAAA
361 TGGATGCCCT CGGTAAGCGG GTGTGGGCGG ACAATAAAGT CTTAAACTGA ACAAAATAGA
421 TCCTGTTTGA AAACATGGGT GCCTATACTG TTGCAGCAGC TTCTACTTTC AACGGATTCC
481 AGAGGCCAAC AATACATTAT GCAGTGTCC TGTCGCTGTG AAAGTGGAAAT TGAGTATCCA
541 GCAACTTGTG CTTACGCTAG TATTAATGTA TAAATAATAA ATACTTAGAA ACTAACTGCA
601 AGTTTAGTCA TTGAATTTAG GGCATTTGGG GGACCAATAA CTTAATTCCT GCTAGATTTT
661 TTTAAGTGT TTTTAAAGG TTTAGGTTTG GCATAAACAC GACAAAAAAA TGACTAGGAG
721 ATGGGTACCA TTTATCTGTG TTCCTATGGA AACTATTTTG ATATTGTTT ATATGGATTT
781 TTATTCACIT TTCATGTATG CTAACATAAG ACTGACCCTC AGCTGTCAAA CAAGTATTTG
841 TAGCTTGTGT ATGGCAGAAAT GGGCTAAGCT TAGTGTTTGA CTGCCACTTC CTCGATGAAG
901 GTTTTACTGC CAAGGCATT CTGGACCAGA AAATTAATGA AGTTTCTTCT TCTGATGATA
```

The status bar at the bottom shows: 8:21 Channel 2:DNA example1 886bp CAP|NUM|SCRL|

# Restriction Fragment

Vector and Insert fragments can be loaded to channel from In Silico Gel and displayed in Text window.

1. Sequence original position
2. 5' and 3' end information

The fragment can be saved as file for future works.

DNAMAN - [AY599226]

Channel: AY599226.seq, EXAMPLE1.SEQ, New Construct, Pattern, AY599226

SEQ AY599226 2740 DNA  
Composition 683 A; 696 C; 674 G; 687 T; 0 OTHER  
Percentage 24.9% A; 25.4% C; 24.6% G; 25.1% T; 0.0% OTHER  
MW (kDa) 845.52 ssDNA 1689.21 dsDNA  
COLOURS  
sequence = 1  
features = 0

FEATURES

- gene complement (1262..2122) /name="bla"
- cds complement (1262..2122) /name="beta-lactamase"
- orit 2323..2323 /name="SeqOrigin"
- terminal 1..4 /name="5+" /source="GATC"
- terminal 1..4 /name="3-" /source="GATC"

ORIGIN

```
1      GATCCCCCGG GCTGCAGGAA TTCCTCGAGA AGCTTGGGCC CGGTACCTCG CGAAGGCCTT
61     GCAGGCCAAC CAGATAAGTG AAATCTAGTI CCAAACTATT TTGTCATTTT TAAITTTTCGT
121    ATTAGCTTAC GAGCGTACAC CCAAGTCCCA TCTATTTTGT CACTCTTCCC TAAATAATCC
181    TTAAAAACTC CATTTCCACC CCTCCAGTIT CCAAACTATT TTGTCGCGCC ACAGCGGGGGC
241    ATTTTTCCTC CTGTTATGTT TGGGCGCTGC ATTAATGAAT CGGCCAACGC GCGGGGAGAG
301    GCGGTTTGCG TATTGGGCGC TCTTCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTGG
361    TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGGCGT AATACGGTTA TCCACAGAAT
421    CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC AGGAACCGTA
481    AAAAGGCCGC GTTGCTGGCG TTTTCCATA GGCTCCGCCC CCTGACGAG CATCACAAAA
541    ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAGATAC CAGGCGTTTC
601    CCCCTGGAAG CTCCTCTGTG CGTCTCTCTG TTCCGACCCCT GCCCGTTACC GGATACCTGT
661    CCGCTTTTCT CCTTCGGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA
721    GTTCGGTGTA GGTCTGTTGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCC
781    ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCGGTAAGA CAGCAGTTAT
841    CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA GCGCGTGCTA
901    CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT
```

Log-c... Channel Databa... File

Ready Channel 3:DNA AY599226 2740bp CAP NUM SCRL