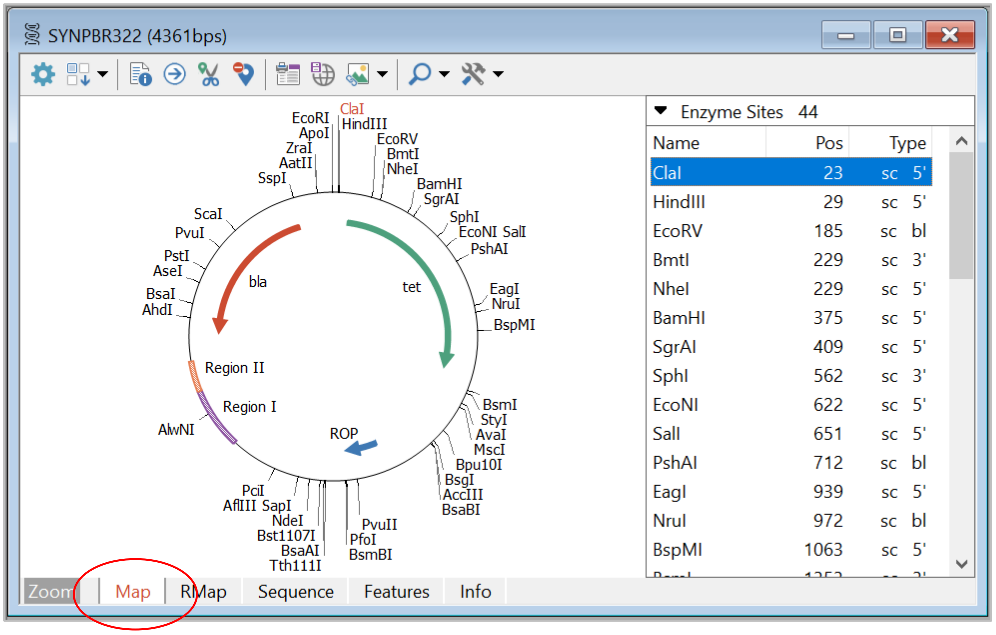
Molecule Viewer Window – Map View



Name	Pos	Type	
ClaI	23	sc	5'
HindIII	29	sc	5'
EcoRV	185	sc	bl
BmtI	229	sc	3'
NheI	229	sc	5'
BamHI	375	sc	5'
SgrAI	409	sc	5'
SphI	562	sc	3'
EcoNI	622	sc	5'
SalI	651	sc	5'
PshAI	712	sc	bl
EagI	939	sc	5'
NruI	972	sc	bl
BspMI	1063	sc	5'

Custom styles and colors applied to some genes in this map. Enzyme sites list shown at right.

Helpful toolbar buttons:

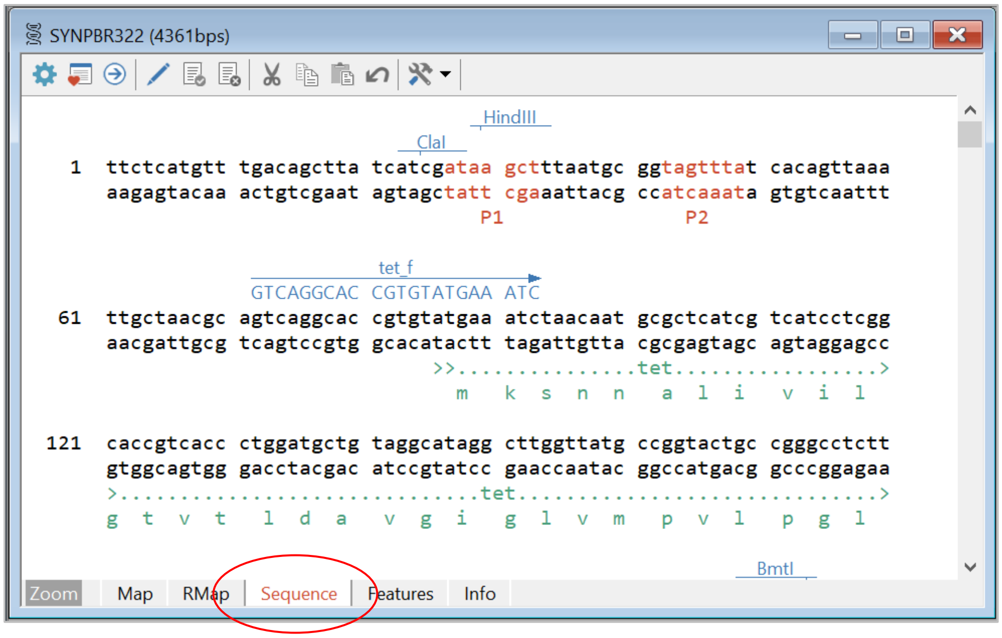
- Change Sites
- Site Properties
- Go To Sequence
- Save for Web
- Enhanced View

Sequence View

Formatted sequence shows enzyme sites, sequence labels, primers, translated genes.

Helpful toolbar buttons:

- My Style
- Go To BP Number
- Edit Sequence
- Tools > Find
- Tools > Enter as Feature



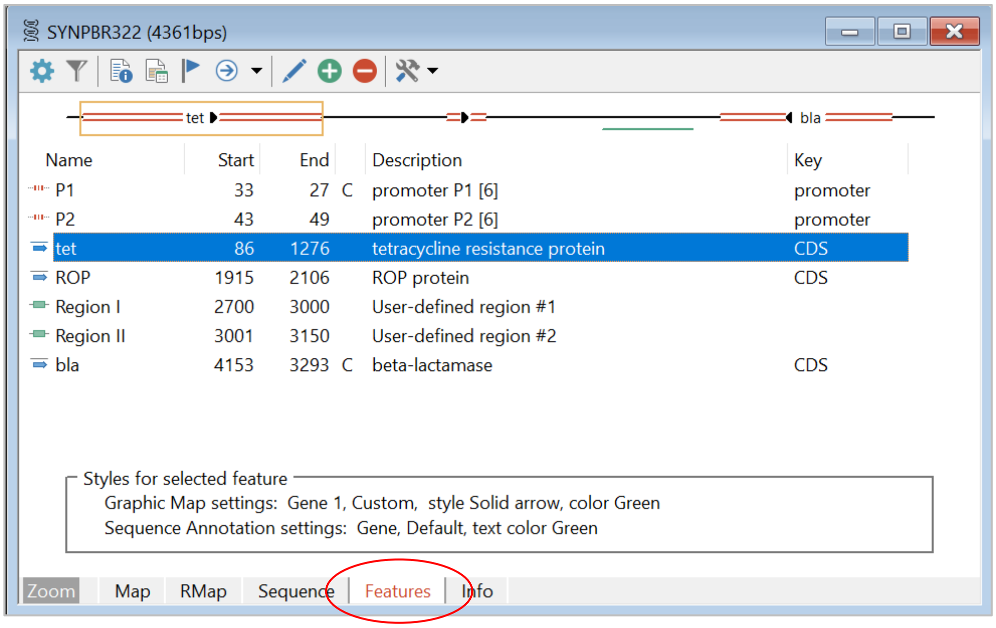
```
                                      HindIII
                                ClaI
   1  ttctcatgtt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa
      aagagtacaa actgtcgaat agtagctatt cgaaattacg ccatcaaata gtgtcaattt
                                      P1                P2

                             tet_f
                  GTCAGGCAC CGTGTATGAA ATC
  61  ttgctaacgc agtcaggcac cgtgtatgaa atctaacaat gcgctcatcg tcatcctcgg
      aacgattgcg tcagtccgtg gcacatactt tagattgtta cgcgagtagc agtaggagcc
                                  >>................tet...............>
                                    m   k  s  n  n   a  l  i   v  i  l

 121  caccgtcacc ctggatgctg taggcatagg cttggttatg ccggtactgc cgggcctctt
      gtggcagtgg gacctacgac atccgtatcc gaaccaatac ggccatgacg gcccggagaa
      >..............................tet..............................>
      g   t  v  t   l  d  a   v  g  i   g  l  v  m   p  v  l   p  g  l

                                                             BmtI
```

Features View



Name	Start	End		Description	Key
P1	33	27	C	promoter P1 [6]	promoter
P2	43	49		promoter P2 [6]	promoter
tet	86	1276		tetracycline resistance protein	CDS
ROP	1915	2106		ROP protein	CDS
Region I	2700	3000		User-defined region #1	
Region II	3001	3150		User-defined region #2	
bla	4153	3293	C	beta-lactamase	CDS

Styles for selected feature
Graphic Map settings: Gene 1, Custom, style Solid arrow, color Green
Sequence Annotation settings: Gene, Default, text color Green

Set to show simple features map above list and selected feature style in boxed area below list.

Helpful toolbar buttons:

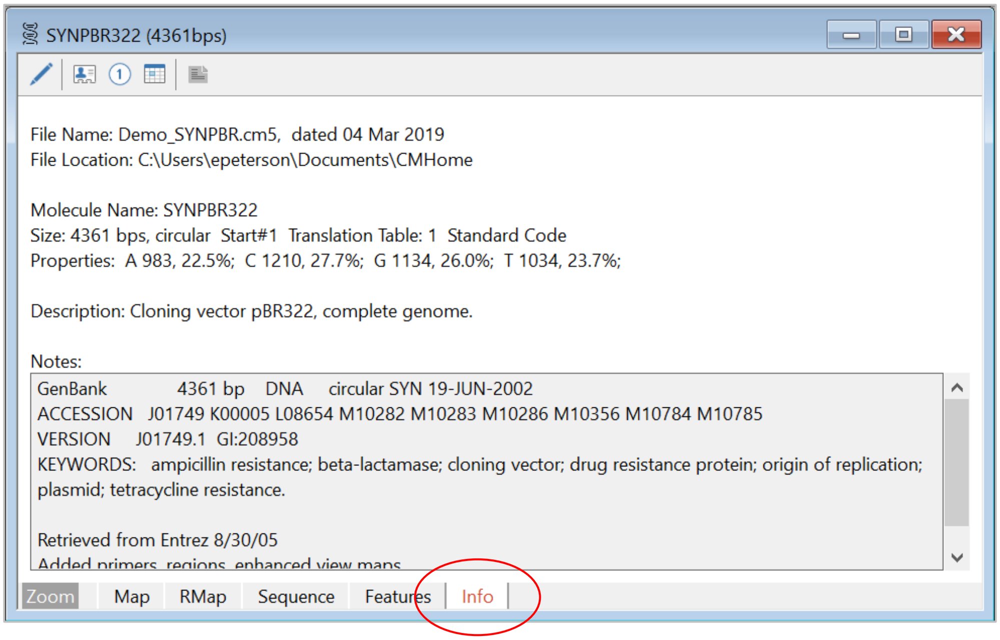
- Filter
- Feature Properties
- Customize Feature
- Go To Sequence
- Tools > Make Fragment

Info View

Modify molecule name and description. Add Notes to document molecule source or changes made.

Helpful toolbar buttons:

- Edit
- Author Stamp
- Base Number Start
- Translation Table
- GenBank Annotations



File Name: Demo_SYNPBR.cm5, dated 04 Mar 2019
File Location: C:\Users\epeterson\Documents\CMHome

Molecule Name: SYNPBR322
Size: 4361 bps, circular Start#1 Translation Table: 1 Standard Code
Properties: A 983, 22.5%; C 1210, 27.7%; G 1134, 26.0%; T 1034, 23.7%;

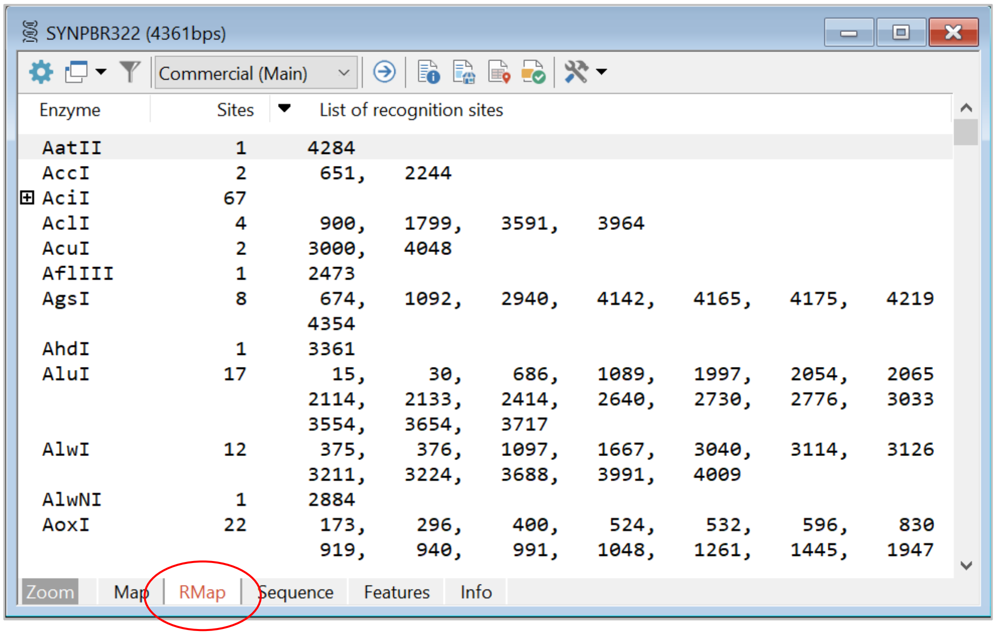
Description: Cloning vector pBR322, complete genome.

Notes:

```
GenBank          4361 bp    DNA     circular SYN 19-JUN-2002
ACCESSION   J01749 K00005 L08654 M10282 M10283 M10286 M10356 M10784 M10785
VERSION     J01749.1  GI:208958
KEYWORDS:   ampicillin resistance; beta-lactamase; cloning vector; drug resistance protein; origin of replication;
plasmid; tetracycline resistance.

Retrieved from Entrez 8/30/05
Added primers, regions, enhanced view maps
```

RMap View



```
Enzyme          Sites  ▼  List of recognition sites

AatII             1     4284
AccI              2      651,   2244
⊞ AciI           67
AclI              4      900,   1799,   3591,   3964
AcuI              2     3000,   4048
AflIII            1     2473
AgsI              8      674,   1092,   2940,   4142,   4165,   4175,   4219
                        4354
AhdI              1     3361
AluI             17       15,     30,    686,   1089,   1997,   2054,   2065
                        2114,   2133,   2414,   2640,   2730,   2776,   3033
                        3554,   3654,   3717
AlwI             12      375,    376,   1097,   1667,   3040,   3114,   3126
                        3211,   3224,   3688,   3991,   4009
AlwNI             1     2884
AoxI             22      173,    296,    400,    524,    532,    596,    830
                         919,    940,    991,   1048,   1261,   1445,   1947
```

Select to display List of Recognition Sites, Map of Recognition Sites, Fragment Sizes, or Gel View.

Helpful toolbar buttons:

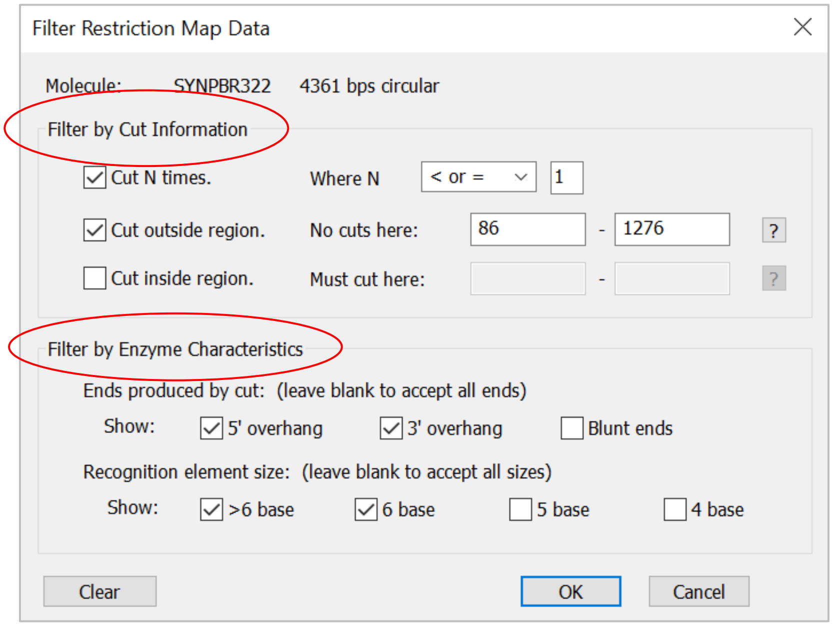
- Go To Enzyme
- Enzyme Properties
- Enzyme Suppliers
- Isoschizomers
- Compatible Ends

Filter RMap Display

Filter restriction map data by Cut Information and/or filter by Enzyme Characteristics.

Filtered restriction map data can be used to build a user enzyme list or enter all sites to your molecule map in one easy step.

Click the Tools button in the RMap display window to use these options on filtered data.



Molecule: SYNPBR322 4361 bps circular

Filter by Cut Information

☑ Cut N times. Where N < or = 1

☑ Cut outside region. No cuts here: 86 - 1276 ?

☐ Cut inside region. Must cut here: - ?

Filter by Enzyme Characteristics

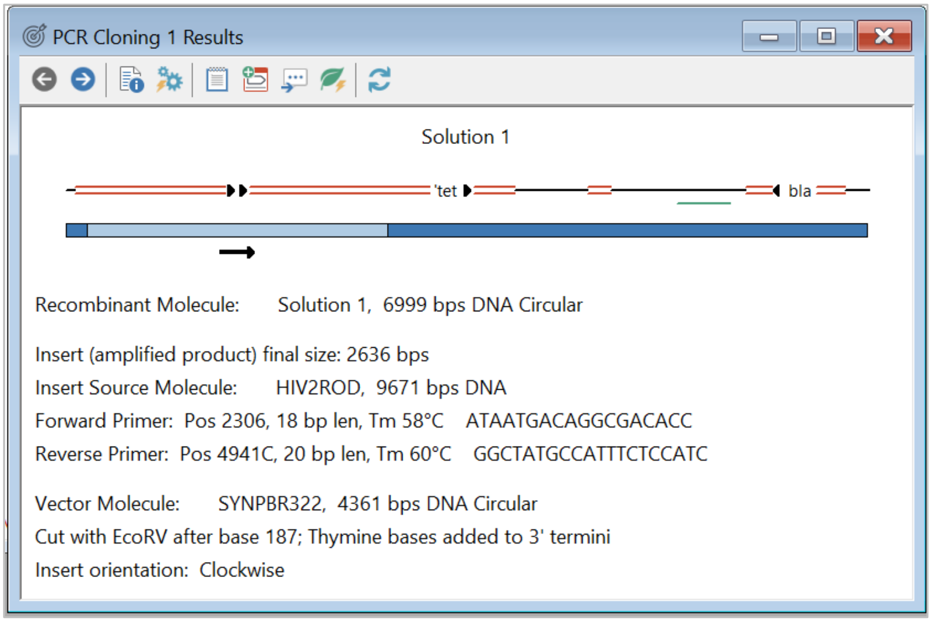
Ends produced by cut: (leave blank to accept all ends)

Show: ☑ 5' overhang ☑ 3' overhang ☐ Blunt ends

Recognition element size: (leave blank to accept all sizes)

Show: ☑ >6 base ☑ 6 base ☐ 5 base ☐ 4 base

Simulate Cloning – Use Cloning Wizards



Recombinant Molecule: Solution 1, 6999 bps DNA Circular

Insert (amplified product) final size: 2636 bps
Insert Source Molecule: HIV2ROD, 9671 bps DNA
Forward Primer: Pos 2306, 18 bp len, Tm 58°C ATAATGACAGGCGACACC
Reverse Primer: Pos 4941C, 20 bp len, Tm 60°C GGCTATGCCATTTCTCCATC

Vector Molecule: SYNPBR322, 4361 bps DNA Circular
Cut with EcoRV after base 187; Thymine bases added to 3' termini
Insert orientation: Clockwise

Cloning wizards will help you to select the appropriate components, show you the proposed result, and create the resulting recombinant molecule.

Helpful toolbar buttons:

- Solution Details
- Primer Pair Report
- Export Primer Sequences
- Create Molecule

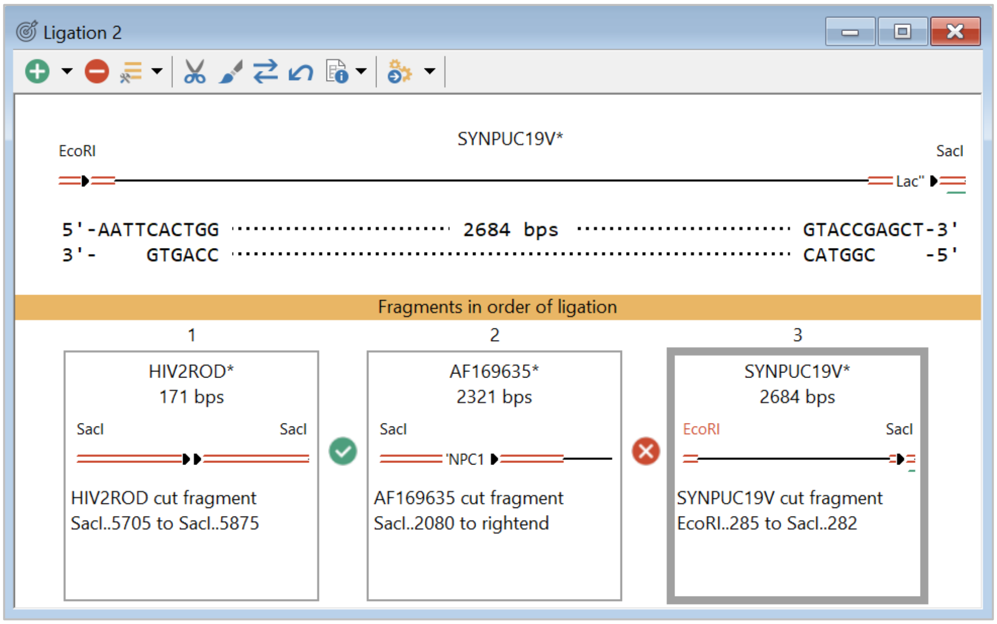
Ligate

Or use the Ligate module to do the cloning simulation.

Upper area: active molecule you can modify (cut, etc.) Lower area: fragments in correct order as they are being prepared for ligation.

Helpful toolbar buttons:

- Cut
- Modify Ends
- Invert Fragment
- Fragment Information



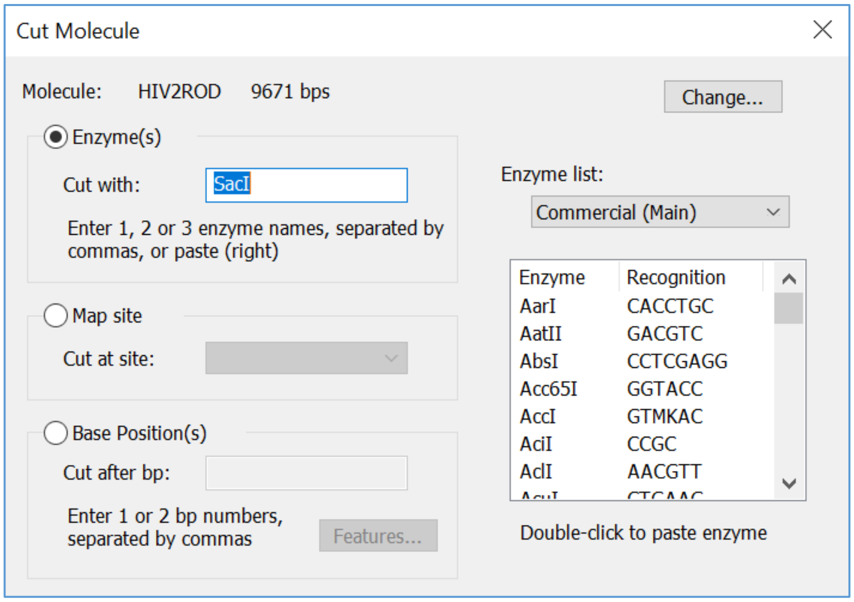
```
5'-AATTCACTGG ·························· 2684 bps ························· GTACCGAGCT-3'
3'-     GTGACC ················································································ CATGGC     -5'
```

HIV2ROD cut fragment
SacI..5705 to SacI..5875

AF169635 cut fragment
SacI..2080 to rightend

SYNPUC19V cut fragment
EcoRI..285 to SacI..282

Cut Molecule



Molecule: HIV2ROD 9671 bps

● Enzyme(s)

Cut with: SacI

Enter 1, 2 or 3 enzyme names, separated by commas, or paste (right)

○ Map site

Cut at site:

○ Base Position(s)

Cut after bp:

Enter 1 or 2 bp numbers, separated by commas

Enzyme list: Commercial (Main)

Enzyme	Recognition
AarI	CACCTGC
AatII	GACGTC
AbsI	CCTCGAGG
Acc65I	GGTACC
AccI	GTMKAC
AciI	CCGC
AclI	AACGTT

Double-click to paste enzyme

You can cut circular DNA to make it linear, cut out a region to be cloned, or cut with an enzyme to make a compatible end.

Cut at all enzyme cut sites for 1, 2 or 3 enzymes, or cut at one enzyme site on your map, or cut at user-specified basepair positions.

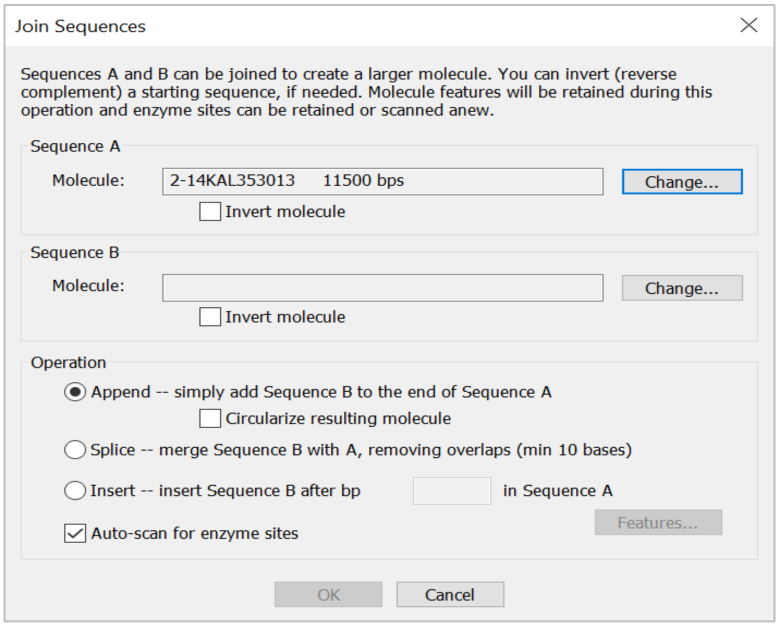
Use Modify Ends function, if needed.

Join Sequences

Simply join two sequences to create a larger molecule, selecting the join method.

Add one sequence to the end of the other (Append), or merge, removing overlaps (Splice), or insert within the other sequence (Insert).

Molecule features will be retained and basepair positions recalculated.



Sequences A and B can be joined to create a larger molecule. You can invert (reverse complement) a starting sequence, if needed. Molecule features will be retained during this operation and enzyme sites can be retained or scanned anew.

Sequence A

Molecule: 2-14KAL353013 11500 bps

☐ Invert molecule

Sequence B

Molecule:

☐ Invert molecule

Operation

● Append -- simply add Sequence B to the end of Sequence A

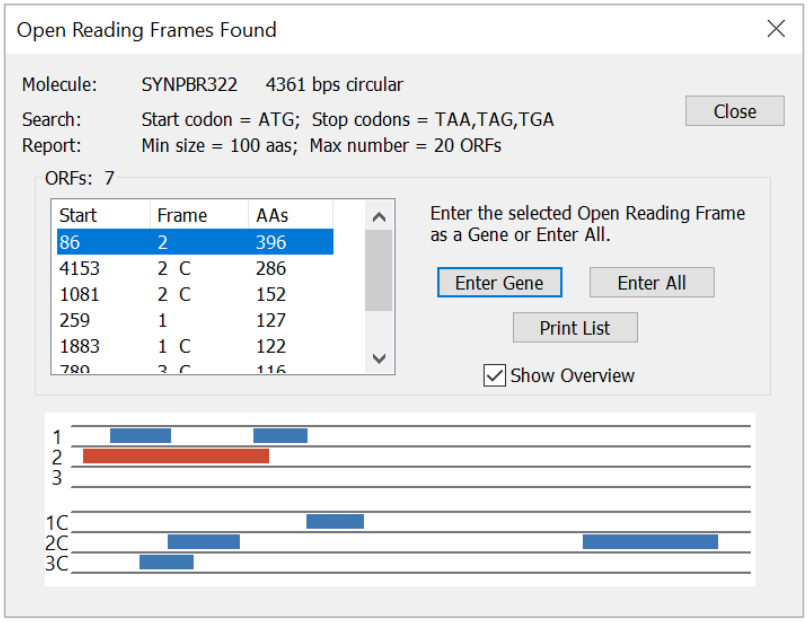
☐ Circularize resulting molecule

○ Splice -- merge Sequence B with A, removing overlaps (min 10 bases)

○ Insert -- insert Sequence B after bp in Sequence A

☑ Auto-scan for enzyme sites

Open Reading Frame Search



Open Reading Frames Found

Molecule: SYNPBR322 4361 bps circular

Search: Start codon = ATG; Stop codons = TAA,TAG,TGA

Report: Min size = 100 aas; Max number = 20 ORFs

ORFs: 7

Start	Frame	AAs
86	2	396
4153	2 C	286
1081	2 C	152
259	1	127
1883	1 C	122

Enter the selected Open Reading Frame as a Gene or Enter All.

☑ Show Overview

Use this option to find ORFs for the active molecule. Specify start and stop codons you require and minimum size cutoff.

View overview of results (shown here) or a list of each ORF found, with an arrow marking its location on a simple molecule map.

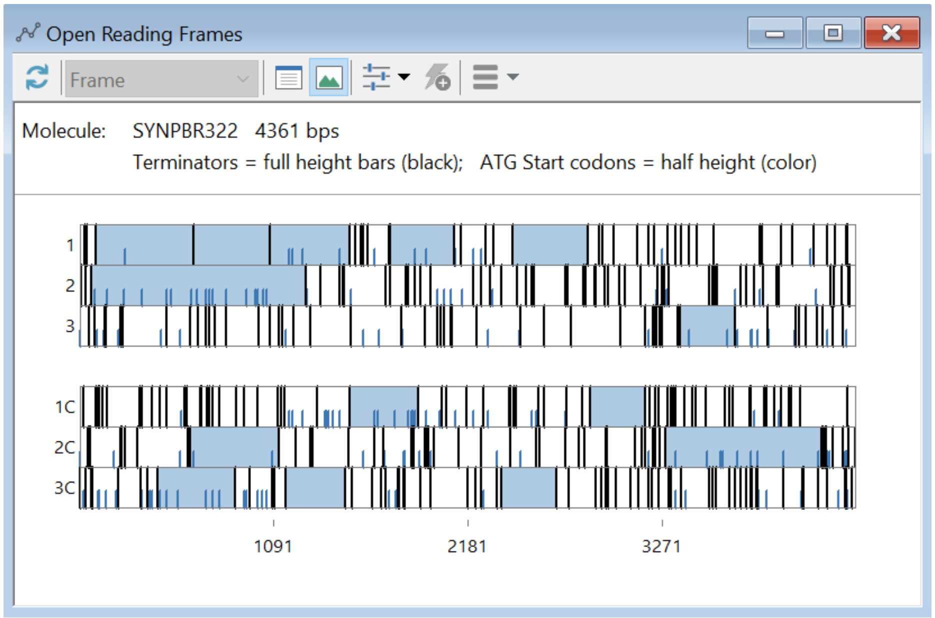
Click to enter an ORF as a gene in your molecule.

Analyze Open Reading Frames

Use the Analyze option to do an ORF analysis.

View a graphic results display showing an ORF map of all 6 reading frames. Full height bars mark terminators, half height bars mark selected start codons.

Or view a text display showing start and end bp positions, length in amino acids, frame, and Fickett's TESTCODE score values.

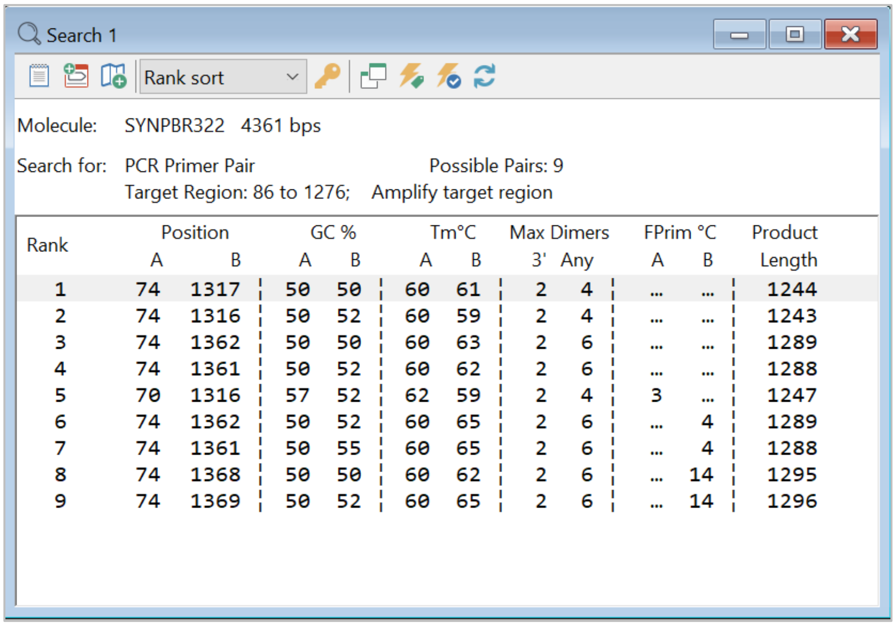


Open Reading Frames

Molecule: SYNPBR322 4361 bps

Terminators = full height bars (black); ATG Start codons = half height (color)

Design Primers, Search Results



Molecule: SYNPBR322 4361 bps

Search for: PCR Primer Pair Possible Pairs: 9
Target Region: 86 to 1276; Amplify target region

Rank	Position		GC %		Tm°C		Max Dimers		FPrim °C		Product
	A	B	A	B	A	B	3'	Any	A	B	Length
1	74	1317	50	50	60	61	2	4	…	…	1244
2	74	1316	50	52	60	59	2	4	…	…	1243
3	74	1362	50	50	60	63	2	6	…	…	1289
4	74	1361	50	52	60	62	2	6	…	…	1288
5	70	1316	57	52	62	59	2	4	3	…	1247
6	74	1362	50	52	60	65	2	6	…	4	1289
7	74	1361	50	55	60	65	2	6	…	4	1288
8	74	1368	50	50	60	62	2	6	…	14	1295
9	74	1369	50	52	60	65	2	6	…	14	1296

After a search for single primers or primer pairs, results will be listed in a table format showing basic information.

Helpful toolbar buttons:

- Primer Report
- Enter to Primer List
- Enter Primer Sites to Map
- Search Results / Stats
- Change sort method

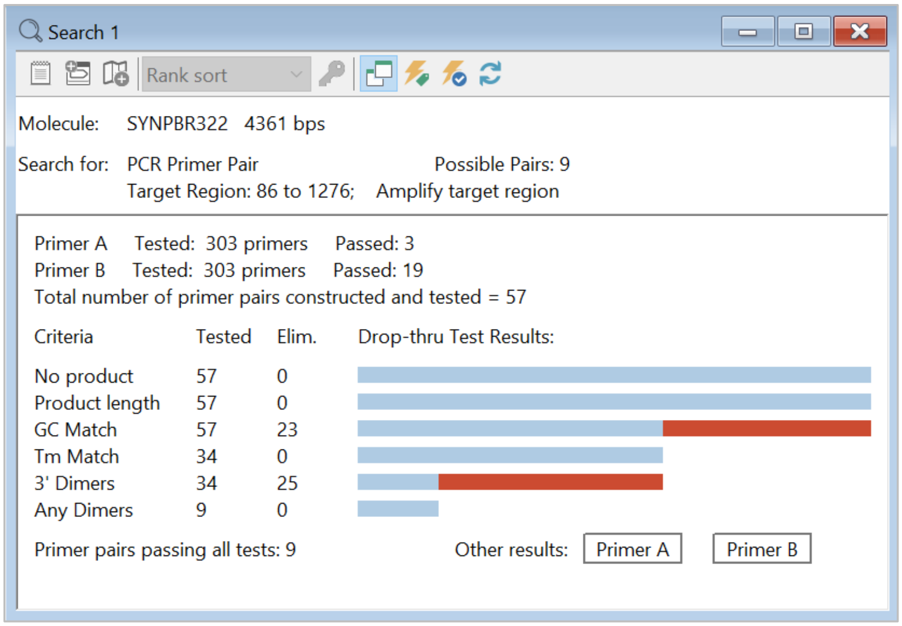
Primer Search Stats

Alternate search results view shows which criteria eliminated primers or pairs.

To find more primers, you can redefine the search region or modify primer length or evaluation criteria.

Helpful toolbar buttons:

- Auto Adjust Length
- Auto Adjust Criteria
- Redefine



Molecule: SYNPBR322 4361 bps

Search for: PCR Primer Pair Possible Pairs: 9
Target Region: 86 to 1276; Amplify target region

Primer A Tested: 303 primers Passed: 3
Primer B Tested: 303 primers Passed: 19
Total number of primer pairs constructed and tested = 57

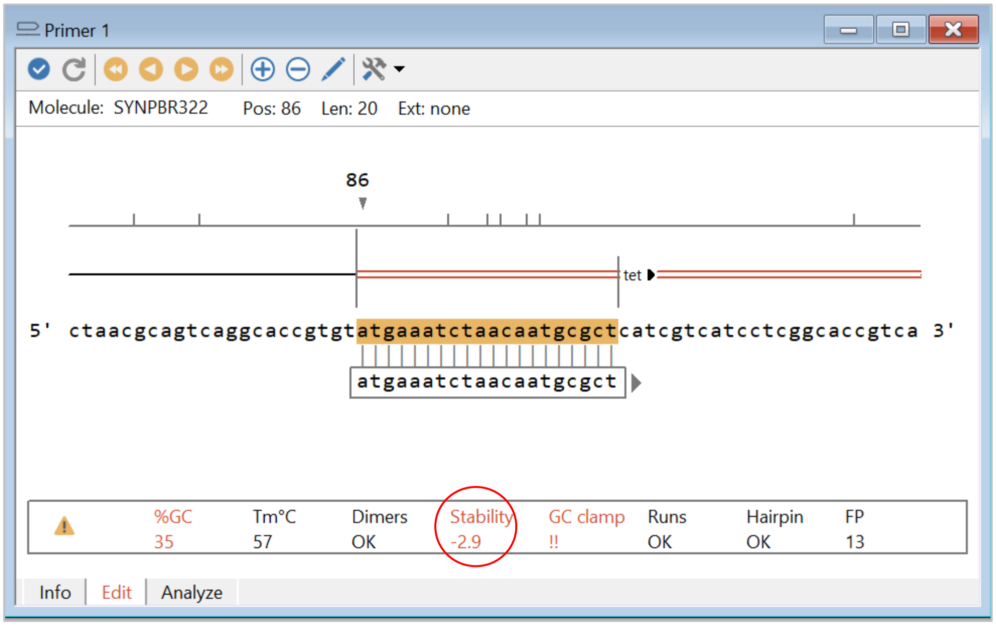
Criteria	Tested	Elim.
No product	57	0
Product length	57	0
GC Match	57	23
Tm Match	34	0
3' Dimers	34	25
Any Dimers	9	0

Drop-thru Test Results:

Primer pairs passing all tests: 9

Other results:

Create Primer from Molecule



```
Molecule:  SYNPBR322     Pos: 86    Len: 20    Ext: none

5'  ctaacgcagtcaggcaccgtgtatgaaatctaacaatgcgctcatcgtcatcctcggcaccgtca  3'
                            atgaaatctaacaatgcgct

%GC   Tm°C   Dimers   Stability   GC clamp   Runs   Hairpin   FP
35    57     OK       -2.9        !!         OK     OK        13
```

A primer of the length and at the position you specified is shown in the sequence, along with its evaluation. Change position or length to improve primer.

Click value to see analysis.

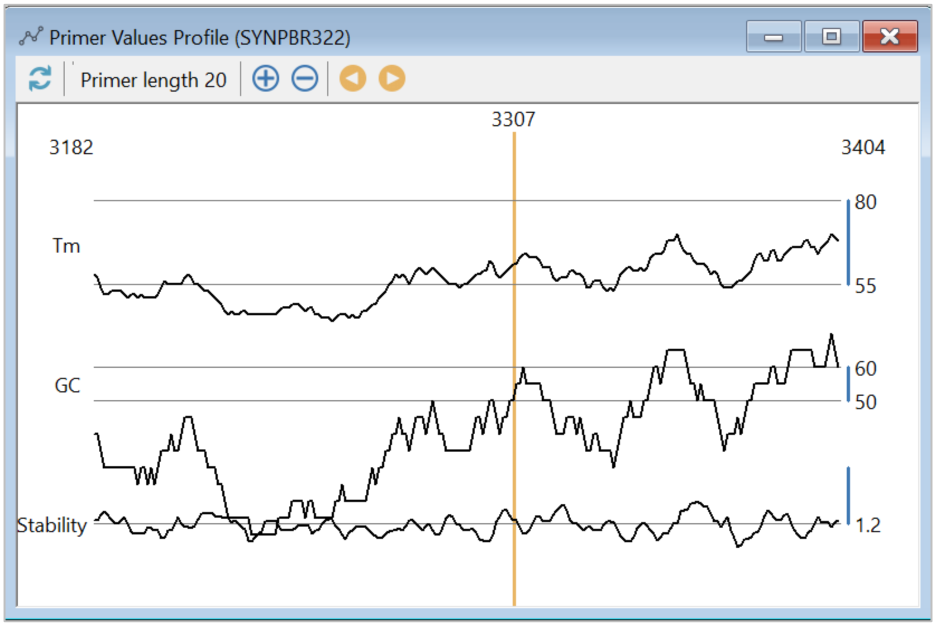
Helpful toolbar buttons:

- Move Left (or Right)
- Scan Left (or Right)
- Increase/decrease length

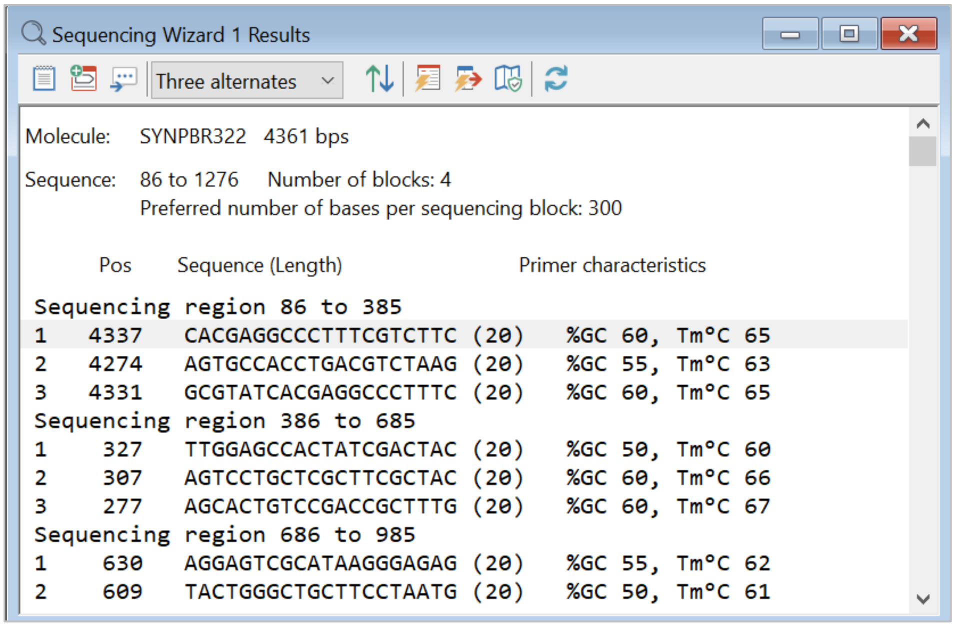
Primer Values Profile

Profile regions of your molecule and plot primer-related values to help you find regions for primers in difficult molecules.

Values that meet criteria will appear in the region of each graph marked with a green bar (at right). Click on an area where all three values are plotted in the green and read basepair position at top of ruler line.



Sequencing Wizard, Results View



```
Molecule:   SYNPBR322   4361 bps

Sequence:   86 to 1276    Number of blocks: 4
            Preferred number of bases per sequencing block: 300

        Pos     Sequence (Length)                Primer characteristics

Sequencing region 86 to 385
1   4337   CACGAGGCCCTTTCGTCTTC (20)   %GC 60, Tm°C 65
2   4274   AGTGCCACCTGACGTCTAAG (20)   %GC 55, Tm°C 63
3   4331   GCGTATCACGAGGCCCTTTC (20)   %GC 60, Tm°C 65
Sequencing region 386 to 685
1    327   TTGGAGCCACTATCGACTAC (20)   %GC 50, Tm°C 60
2    307   AGTCCTGCTCGCTTCGCTAC (20)   %GC 60, Tm°C 66
3    277   AGCACTGTCCGACCGCTTTG (20)   %GC 60, Tm°C 67
Sequencing region 686 to 985
1    630   AGGAGTCGCATAAGGGAGAG (20)   %GC 55, Tm°C 62
2    609   TACTGGGCTGCTTCCTAATG (20)   %GC 50, Tm°C 61
```

You can design a set of primers for sequencing a molecule region. This view shows three alternate primers for each sequencing block.

Helpful toolbar buttons:

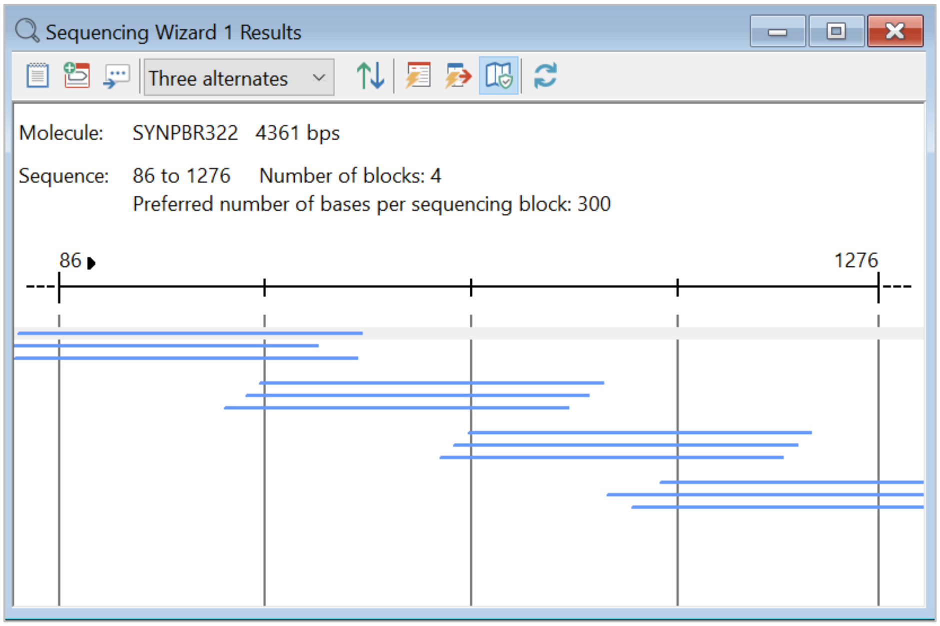
- View Primer Report for selected primer
- Export All Primer Sequences displayed
- Map Sequencing Coverage (graphic view)

Map Sequencing Coverage

Each of the expected sequencing blocks is shown by a colored bar on a separate line, in its approximate location.

Helpful toolbar buttons:

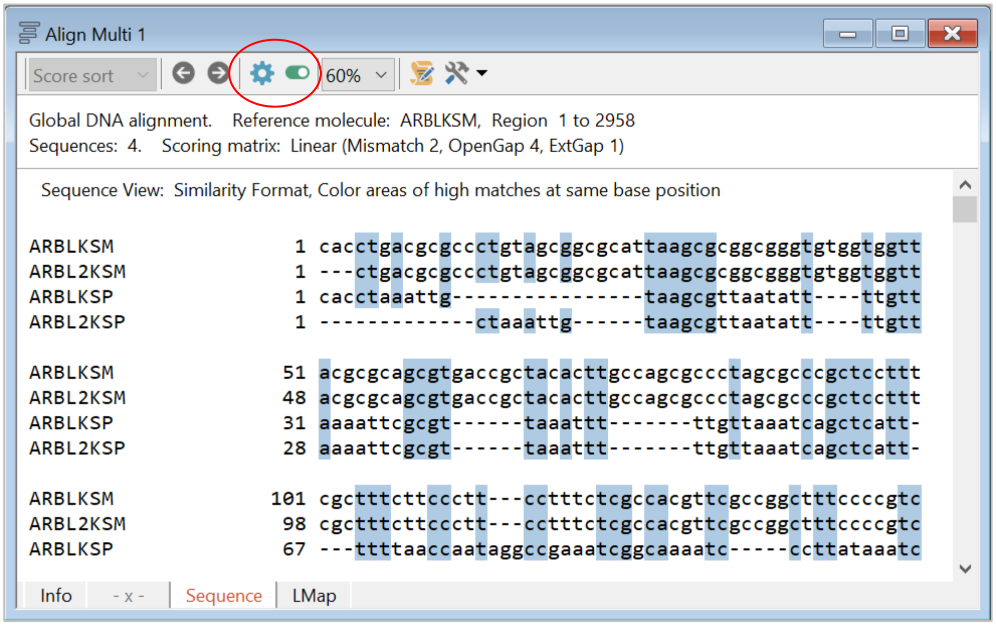
- Rotate Alternate Primers
- Show First Choice or Three Alternates
- Make New Primer Collection
- Redefine



Molecule: SYNPBR322 4361 bps

Sequence: 86 to 1276 Number of blocks: 4
Preferred number of bases per sequencing block: 300

Multiple Sequence Alignment – Global Ref



Global DNA alignment. Reference molecule: ARBLKSM, Region 1 to 2958
Sequences: 4. Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Sequence View: Similarity Format, Color areas of high matches at same base position

```
ARBLKSM                1 cacctgacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggtt
ARBL2KSM               1 ---ctgacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggtt
ARBLKSP                1 cacctaaattg----------------taagcgttaatatt----ttgtt
ARBL2KSP               1 -------------ctaaattg------taagcgttaatatt----ttgtt

ARBLKSM               51 acgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctccttt
ARBL2KSM              48 acgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctccttt
ARBLKSP               31 aaaattcgcgt------taaattt-------ttgttaaatcagctcatt-
ARBL2KSP              28 aaaattcgcgt------taaattt-------ttgttaaatcagctcatt-

ARBLKSM              101 cgctttcttccctt---cctttctcgccacgttcgccggctttccccgtc
ARBL2KSM              98 cgctttcttccctt---cctttctcgccacgttcgccggctttccccgtc
ARBLKSP               67 ---ttttaaccaataggccgaaatcggcaaaatc-----ccttataaatc
```

View aligned sequences in a variety of formats, styles, colors, emphasizing similarity or difference.

Click Settings button to access the full set of format options or set page style.

Click Show Similarity / Difference button to toggle quickly between default formats to get a quick impression.

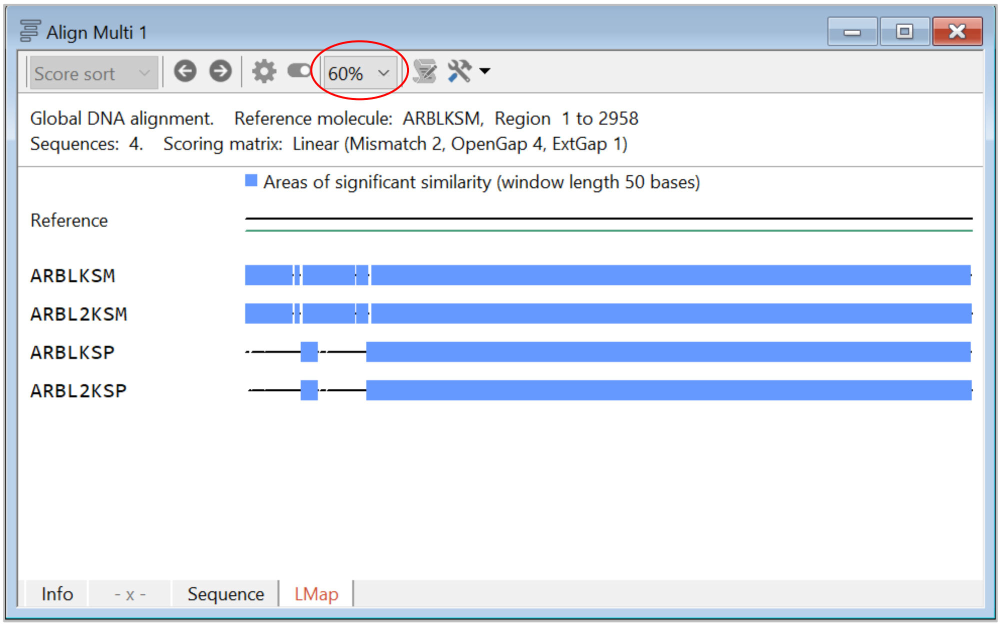
Location Map View

Summary of the extent and location of similarity of the sequences compared to the reference sequence.

Regions >= significance value are marked with colored bar.

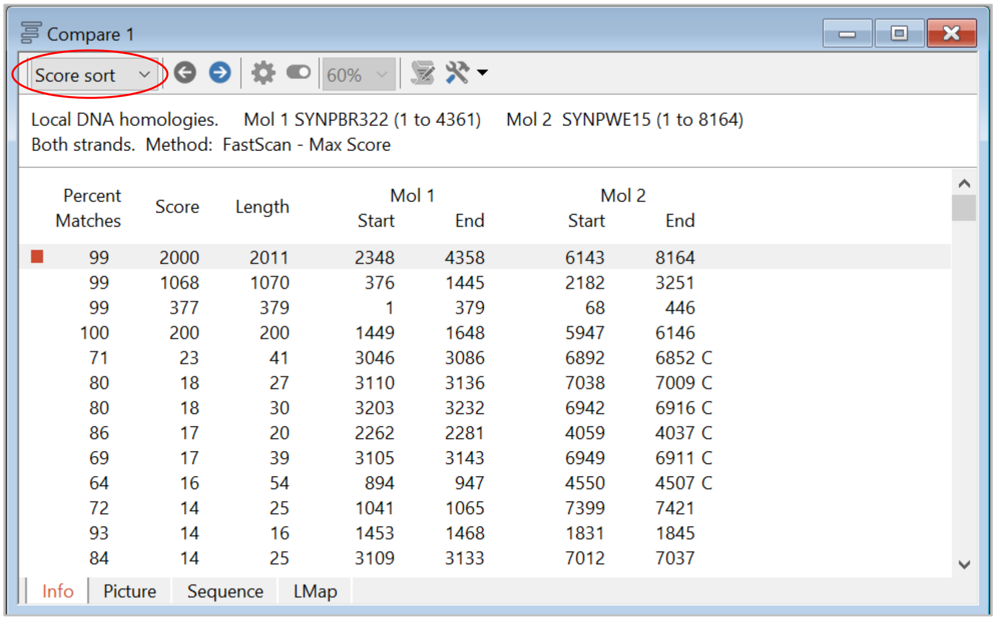
Helpful toolbar buttons:

- Tools > Compress View
- Tools > Overlay Composition Data
- Set Significance Value



Global DNA alignment. Reference molecule: ARBLKSM, Region 1 to 2958
Sequences: 4. Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Compare Two Sequences – Local Homology



Local DNA homologies. Mol 1 SYNPBR322 (1 to 4361) Mol 2 SYNPWE15 (1 to 8164)
Both strands. Method: FastScan - Max Score

Percent Matches	Score	Length	Mol 1 Start	Mol 1 End	Mol 2 Start	Mol 2 End
99	2000	2011	2348	4358	6143	8164
99	1068	1070	376	1445	2182	3251
99	377	379	1	379	68	446
100	200	200	1449	1648	5947	6146
71	23	41	3046	3086	6892	6852 C
80	18	27	3110	3136	7038	7009 C
80	18	30	3203	3232	6942	6916 C
86	17	20	2262	2281	4059	4037 C
69	17	39	3105	3143	6949	6911 C
64	16	54	894	947	4550	4507 C
72	14	25	1041	1065	7399	7421
93	14	16	1453	1468	1831	1845
84	14	25	3109	3133	7012	7037

Sort results by score, percent matches, length, or start position in either molecule.

Move highlight to select homology block. Then click the Sequence or LMap tab to view aligned sequence data or a detailed map for the selected homology block.

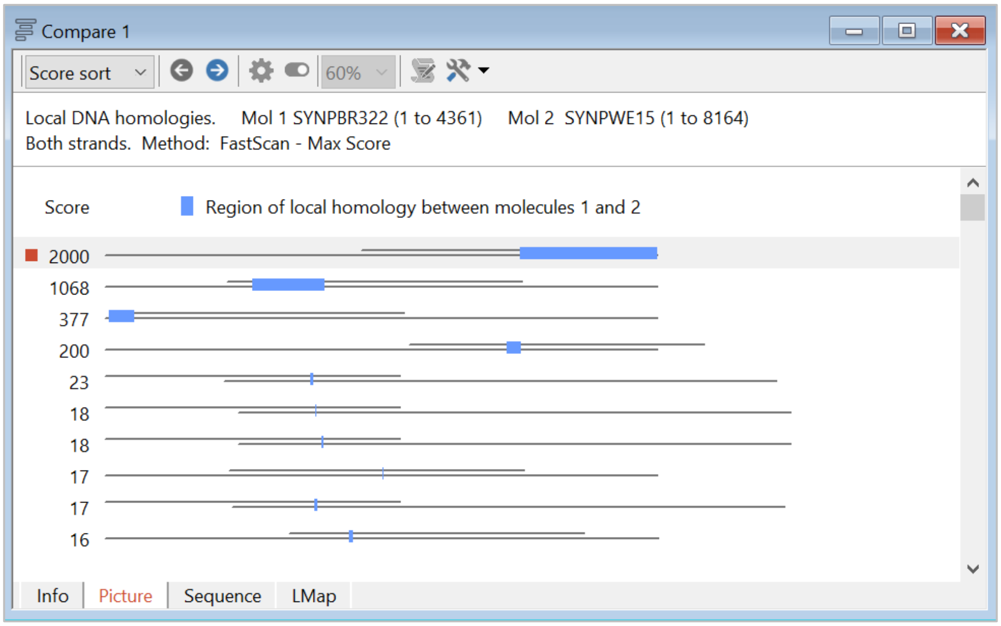
While viewing Sequence or LMap data, click Next Result button to move to data for the next homology block.

Picture View

Graphic view of the list of regions of homology found.

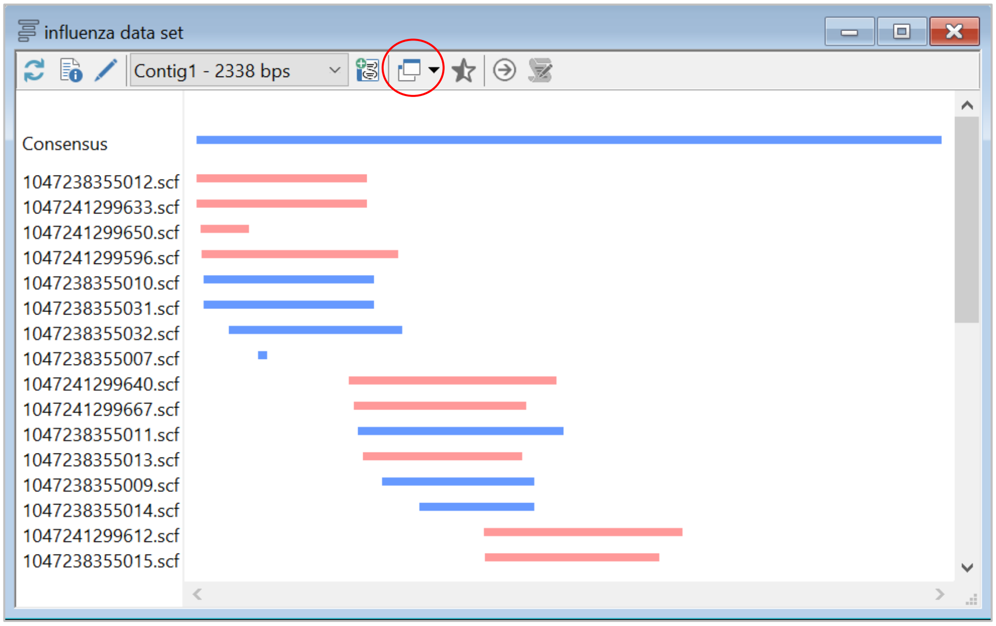
The region of local homology is shown by a colored bar, between lines representing the two molecules.

You can sort the results or select a region and then click the Sequence or LMap tab to view data for the selected homology block.



Local DNA homologies. Mol 1 SYNPBR322 (1 to 4361) Mol 2 SYNPWE15 (1 to 8164)
Both strands. Method: FastScan - Max Score

Sequence Assembly, Results View



```
Consensus
1047238355012.scf
1047241299633.scf
1047241299650.scf
1047241299596.scf
1047238355010.scf
1047238355031.scf
1047238355032.scf
1047238355007.scf
1047241299640.scf
1047241299667.scf
1047238355011.scf
1047238355013.scf
1047238355009.scf
1047238355014.scf
1047241299612.scf
1047238355015.scf
```

The Picture view shows alignment locations with respect to the consensus (map line in first position).

Bars show the approximate location of aligned regions. Sequence aligned 5’ to 3’ is blue; 3’ to 5’ is red.

Click Select View toolbar button to view Info, Sequence, Seq and Overview, Seq and Coverage or Seq and Trace (if trace data available).

Sequence and Overview

View the aligned sequences along with an overview of the sequence assembly.

Brackets above and below the overview show the position and extent of the aligned sequence shown.

Click on another area in the lower panel to move the brackets. Click Color Base or Background to highlight non-matching bases.

```
                                1420      1430      1440      1450      1460
                                   |         |         |         |         |
Consensus               1414  gagtggatagattctacaggatctgcaagttggtgggaatcaacatgagcaaaaag

1047241299612.scf       121C  gagtggatagattctacaggatctgcaagttggtgggaattaacttgagcaaaaag
1047238355015.scf       119C  gagtggatagattctacaggatctgcaagttggtgggaa
1047238355021.scf        501  gagtggatagattctacaggatctgcaagttggtgggaatcaacatgagcaaaaag
1047241299598.scf       252C  gagtggatagattctacaggatctgcaagttggtgggaatcaacatgagcaaaaag
1047238355024.scf        364  gagtggatagattctacaggatctgcaagttggtgggaatcaacatgagcaaaaag
1047238355016.scf        497  gagtggatagattctactggatctgcaagttggtgggaatcaacatgagcaaaaag
1047241299673.scf       575C  gagtggatagattctacaggatctgcaagttggtgggaatcaacatgagcaaaaag
1047238355023.scf       572C  gagtggatagattgtactggatctgcaagttggtgggaatcaacatgagcaaaaag
1047238355027.scf         52                                          caacatgagcaaaaag
```