

ApE Window

The screenshot displays the ApE (ApE Window) software interface. The title bar indicates the file is 'pMLS280.apc'. The main window shows a DNA sequence of 2484 base pairs, with a 'circular' checkbox checked. The sequence is displayed in a monospaced font, with various features highlighted in different colors (yellow, green, red, blue, purple). A table of features is visible on the right side of the window.

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	535..553
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

The sequence is displayed in a monospaced font, with various features highlighted in different colors (yellow, green, red, blue, purple). The sequence is shown in a window with a title bar and a menu bar. The sequence is displayed in a monospaced font, with various features highlighted in different colors (yellow, green, red, blue, purple). The sequence is shown in a window with a title bar and a menu bar.

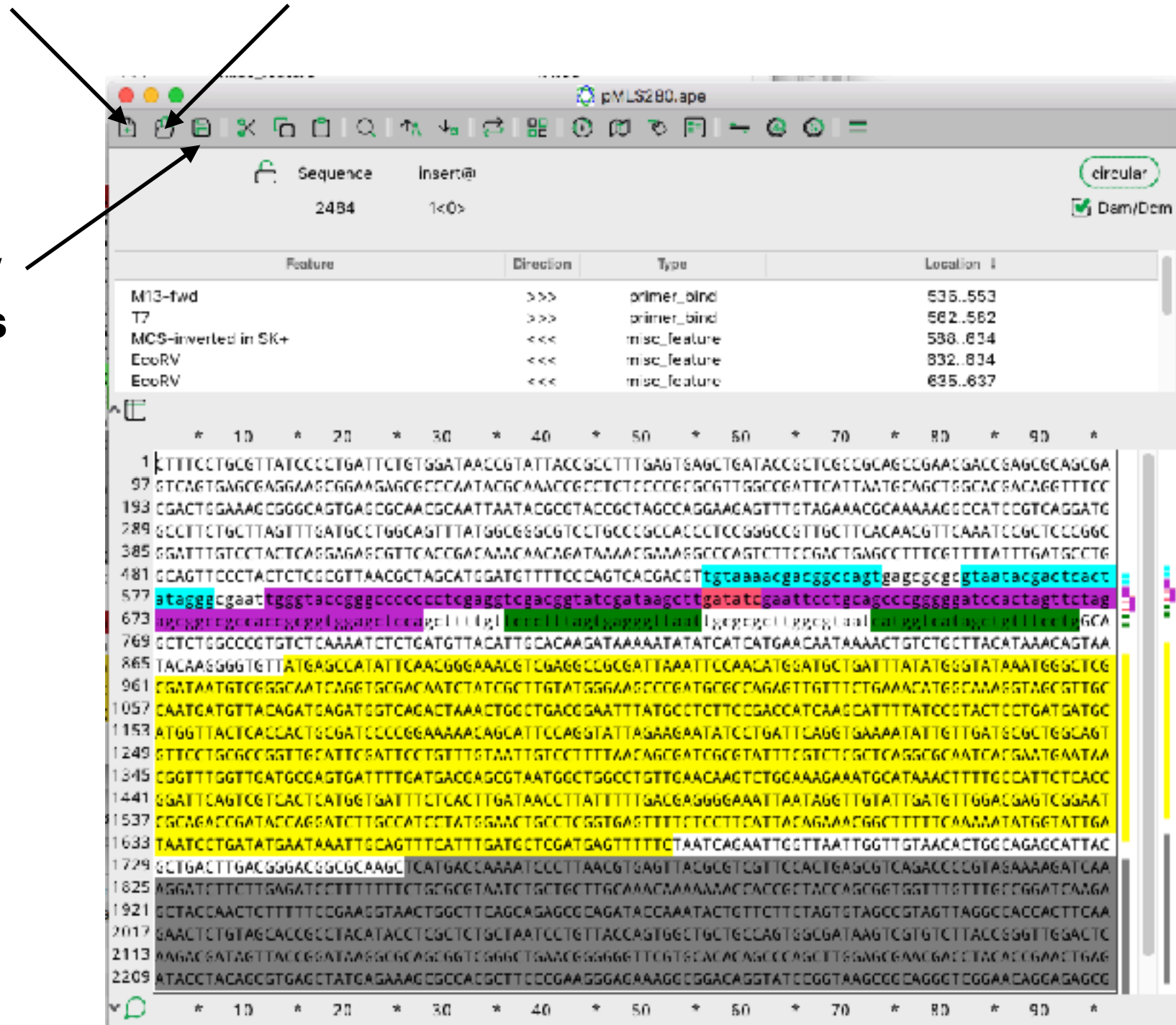
ApE Window

New window

Shift: Duplicate selection

Open File

Save window
Shift: Save as



Shortcuts

Command	Function
O	Open
N	New window
D	Duplicate selected region
Shift-D	Duplicate whole sequence
S	Save sequence
Shift-S	Save sequence as...

ApE Window

Circular/ Linear

Cursor location

Sequence length

Edit lock

[illegible]

Dam/Dcm status

ApE Window

Selection start Selection length Selection end
double click to set double click to set

The screenshot displays the ApE (ApE Window) software interface. The top toolbar contains various icons for file operations, editing, and viewing. Below the toolbar, a table lists sequence features with columns for Feature, Direction, Type, and Location. The main window shows a DNA sequence with a selection box highlighting a segment. The sequence is displayed in a grid format with row and column indices.

Feature	Direction	Type	Location
M18-fwd	>>>	primer_bind	536..552
T7	>>>	primer_bind	567..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

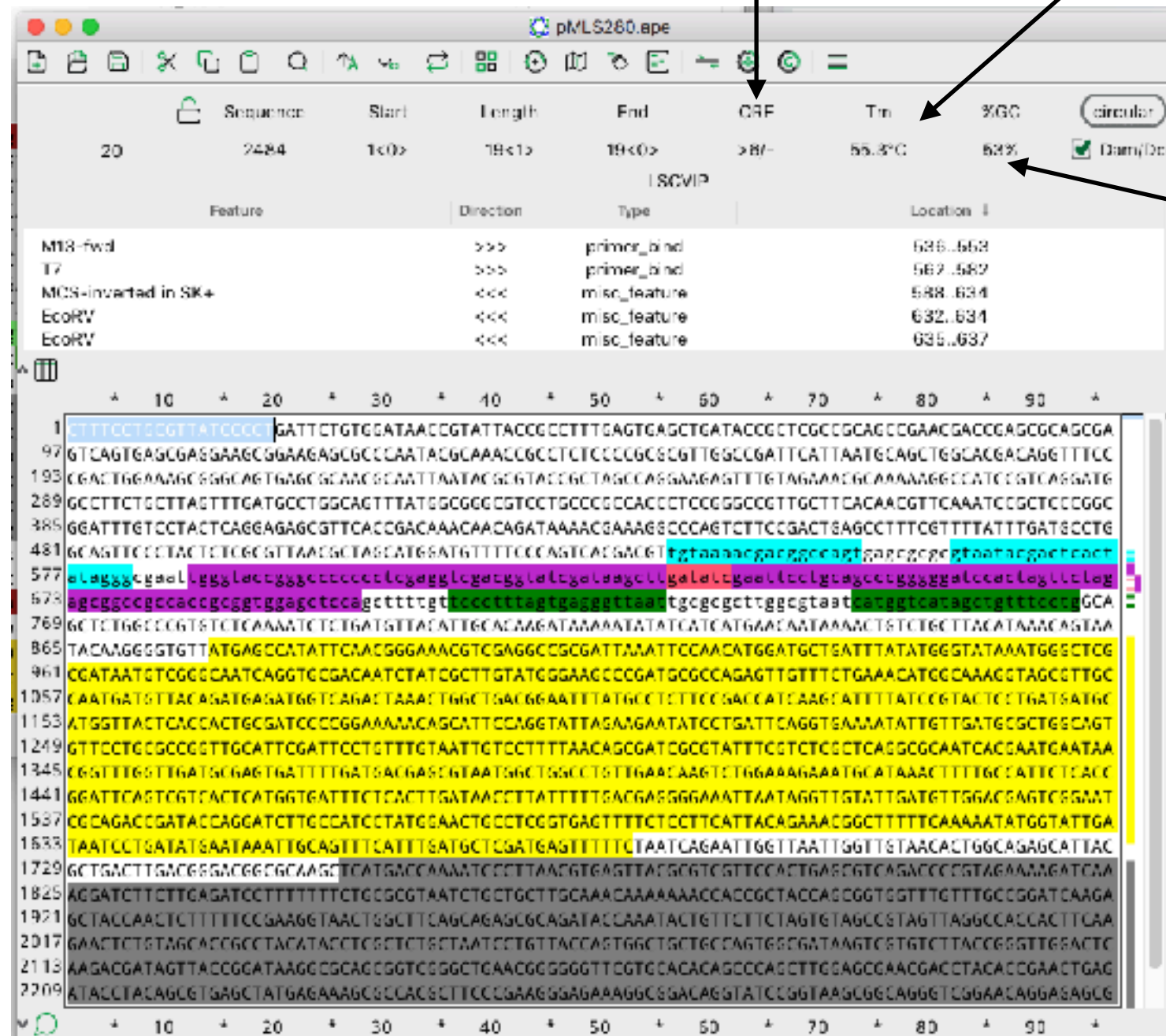
The DNA sequence is displayed in a grid format with row and column indices. The sequence is as follows:

```
1  TTTTCCTCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCCAGCGCAGCGA
97  GTCAAGTGAGCGAGGAAAGCGGAAAGAGCGGCCAATACGCAACCGCCTCTCCCGCGCGTTGCGCGATTTCATTAAATGAGCTGCGACGACAGGTTTCC
193  CGACTGGAAAGCGGCGAGTGAGCGCAACGCAATTAAATACGGTACCGCTAGCCAGGAAGAGTTTGTAGAAAGCGCAAAAAGGCGATCGTCAGGATG
289  GCCTTCTGCTTAGTTTTCATGCGCTGSCAGTTTATGCGCGCGCTGCTGCGCGCCACCTCCCGGGCGGTTGCTTCACAACTGTCAAATCCGCTCCCGGC
385  GGATTTGTCTACTCAGGAGAGCGTTTACCGGACAAACACAGATAAAACGAAAGSCCCAGTCTTCCGACTGAGCGCTTTCGTTTATTTGATSCCTG
481  GCAGTTCTCTACTCTCGGCTTAACGCTAGCATGATGTTTTCGAGTCAGGAGCTTgtaaaacgagggcagtgagggcggttaataaggaactact
577  ataggcggaalggglaacggggcccccctcagggcggagggatcagagagcctgagatcgaatcccgtagcctggggagatccatagctcag
673  agcgggccgcccacggcggtggagctccagcttttcttcccttttagtgagggatgaattgcgcgcttgggcgtaatacaggtcagagcgttttccg
769  GCTCTGGCCCGTSTCTCAAAATCTCTGATGTTACATTCACAAAGATATAAATATATCATCATGAACATAAAACTSTCTGCTTACATAAACAGTAA
865  TACAAGGCGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCGCGGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCG
961  CCATAATCTCGGSCAATCAGGTGCGACAATCTATCGCTTGATCGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGCGCAAGCTAGCCTTGC
1057  CAATGATGTTACAGATGAGATGGTCAAGCTAAATGAGCTGACGGAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTACTCTGATGATG
1153  ATGGTTACTCACCCTGCGATCCCGGAAACACAGCATTCCAGGTATTAGAGAATATCTGATTCAGGTGAAATATTTGATGCGCTGCGAGT
1249  GTTCTCGCGCGCTTGGATTGATTCTCTTTGTAATGTCCTTTTAAACAGCGATCGCGTATTTCTCTCCTCAGCGCGCAATCAGCAATCAATAA
1345  CGGTTTGGTTGATGAGAGTGATTTTGATGAGAGCGTAATGAGTGGCTGAGCTGTTGAAACAAAGTGTGAAAGAAATGCAAACTTTTGGCTATTC
1441  GGATTCAGTGGTCACTCATGGTGAATTTCTACTTGATAACCTTATTTTGAAGAGGGGAAATTAATAGGTTGATTTGATGTTGGACGAGTCSGAT
1537  CCGAGACGATACCGAGATCTTCCATCCTATGGAACGCTCGGTGAGCTTTCTCCTTCATTACAGAAACGGCTTTTCAAAAATATGGTATTGA
1633  TAATCTGATATGAATAAATTGCAATTTTCAATTTGATGCTGATGATTTTCTAATCAGAAATGTTAATTGGTTSTAACACTGGCAGAGCATTAC
1729  GCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCTCTTAAGGTGAGTTATGCTGCTTCCACTGAGCGTCAGACCCCTAGAAAAGATCAA
1825  AGGATCTTCTGAGATCCTTTTCTCGCGGTAATCTGCTGCTGCTGCAAAACAAACACCGCTACCGCGGTGTTTGTGTTTGGCGGATCAAGA
1921  GCTACCAACTCTTTTTCCGAAAGTAACCTGCTTCAAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCSTAGTTAGGCCACCACTCAA
2017  GAATCTCTAGCAGCGGCTACATACCTCTCTCTCTAATCTGTTACCAAGTGGCTGCTGCGAGTGCGGATAAGTCTGTCTTACCGGTTGAGTCT
2113  AAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACCGGGGTTGCTGCAACAGGCCAGCTTGGAGCGAACGACCTACACCGAACTGAG
2209  ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAAGGAGAAAGGCGGACAGGTATCGGTAAGCGGCGAGGTCGGAAACAGGAGAGCG
```

ApE Window

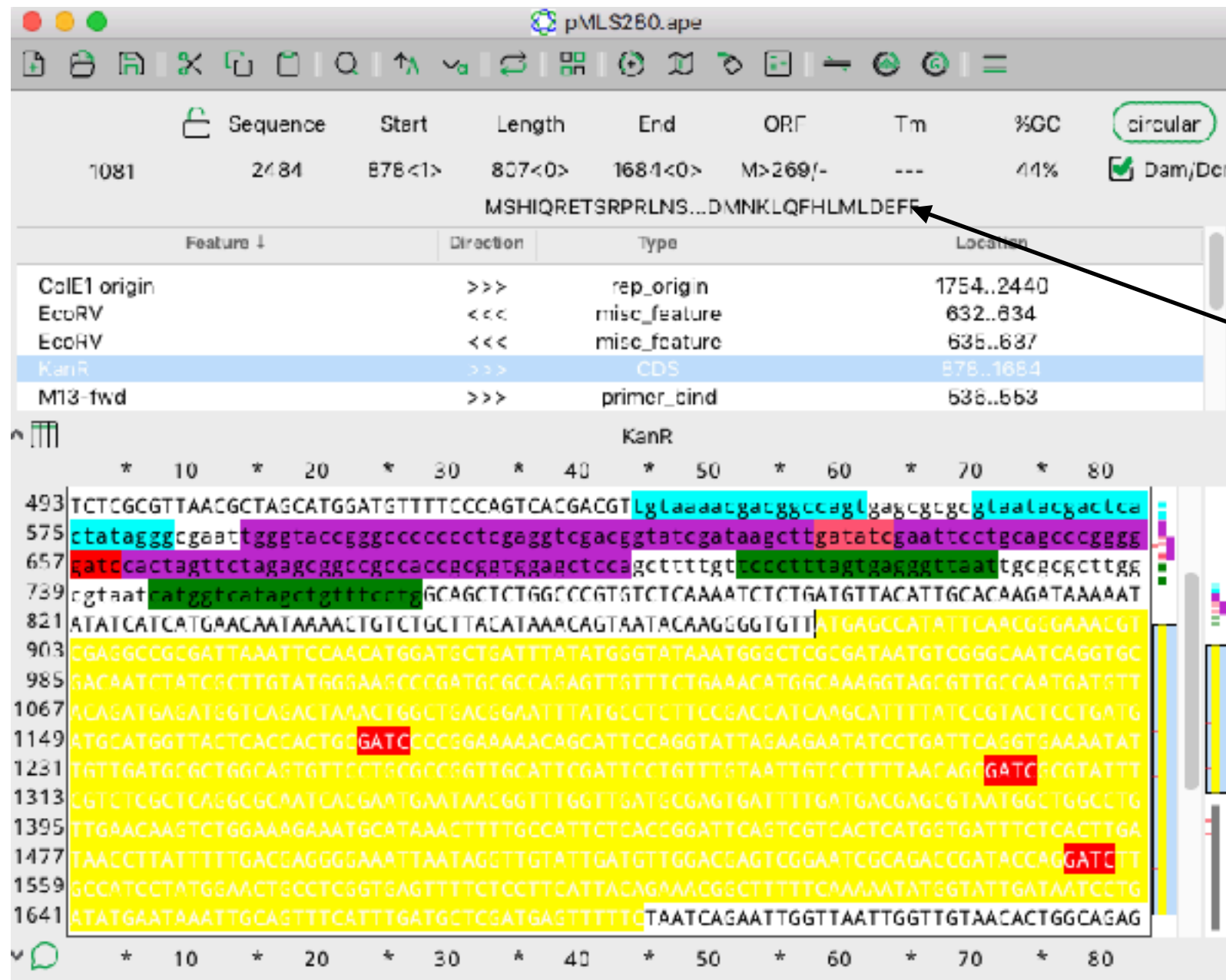
ORF state

Selection melting temperature



%GC

Selection translation



The translation of the selected region is shown here

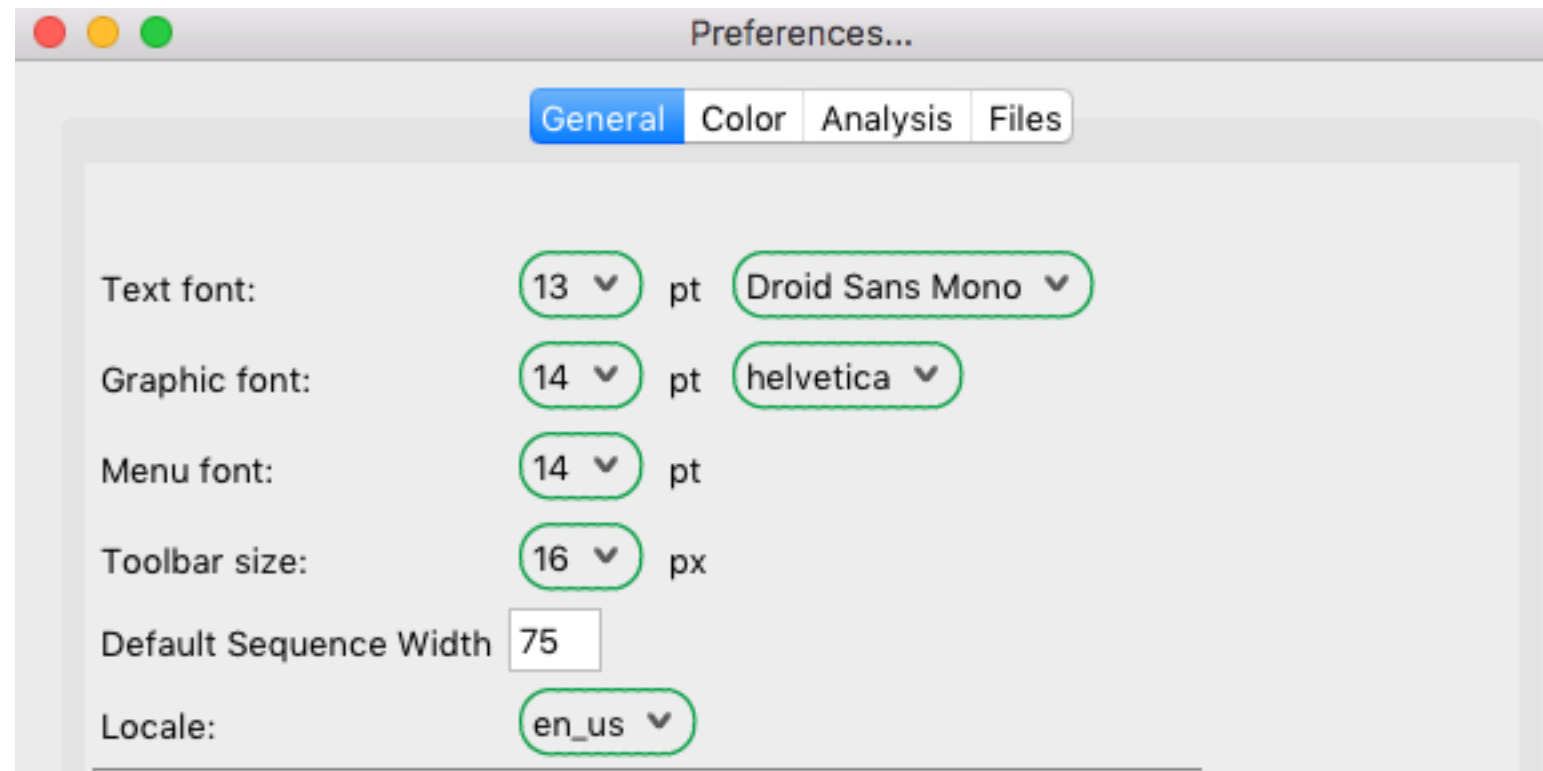
Selection translation

The screenshot shows a bioinformatics software window titled "pMLS280.apc". The top toolbar contains various icons for file operations and analysis. Below the toolbar, a table displays sequence statistics: Sequence (2484), Start (878<1>), Length (807<0>), End (1684<0>), ORF (M>269/-), Tm (---), %GC (44%), and a "circular" checkbox. A dropdown menu is open, showing options: "Forward" (checked), "Reverse", and "Uppercase ONLY". An arrow points from the text "Right click here to set the translation direction" to the "Forward" option. Below the menu, a table lists features: Feature, Direction, Type, and Location. The features include ColE1 origin, EcoRV, KanR, and M13-fwd. The KanR feature is highlighted in blue. Below the table, a sequence alignment is shown with positions 493 to 1641. The sequence is color-coded by codon: TCTCGGTTAACGCTAGCATGGATGTTTCCAGTCACGACGT (blue), tgtaaaacgargggcagtgagcgcgcgtaatacgaactca (red), ctatagggcgaattgggtaccgggccccccctcgaggtcgacggatcgataagcttgatatcgaattcctgcagccccgggg (green), gatccactagttctagagcggccgccaccgcgggtggagctccagcttttcttcccttttagtgagggttaattgcgcgccttgg (blue), cgtaatcaggtcatagctgtttcctgGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAAT (red), ATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGT (blue), CGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCCCGATAATGTCCGGCAATCAGGTGC (green), GACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTT (red), ACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATG (blue), ATGCATGGTTACTCACCCTGCTGATCCTCCGGAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAATAT (green), TGTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTGTAATTGTCCTTTTAAACAGCGATCGGTATTT (red), CGTCTCGCTCAGGCGCAATCAGCAATGAATAACGGTTTGGTTGATGCGAGTGATTTGATGACGAGCGTAATGGCTGCCCTG (blue), TTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCATTCTCACCAGATTCAAGTCGTCATGGTGATTTCTCACTTGA (green), TAACCTTATTTTGGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATAACAGGATCTT (red), GCCATCTATGGAACCTGCTCGGTGAGTTTCTCCTTCATTACAGAAACGGCTTTTCAAAAATATGGTATTGATAATCCTG (blue), ATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTCTTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAG (green).

Feature ↓	Direction	Type	Location
ColE1 origin	>>>	rep_or	1754..2440
EcoRV	<<<	misc_feature	532..634
EcoRV	<<<	misc_feature	635..637
KanR	>>>	CDS	878..1684
M13-fwd	>>>	primer_bind	536..553

Right click here to set the translation direction

Preferences



ApE Window

Cut

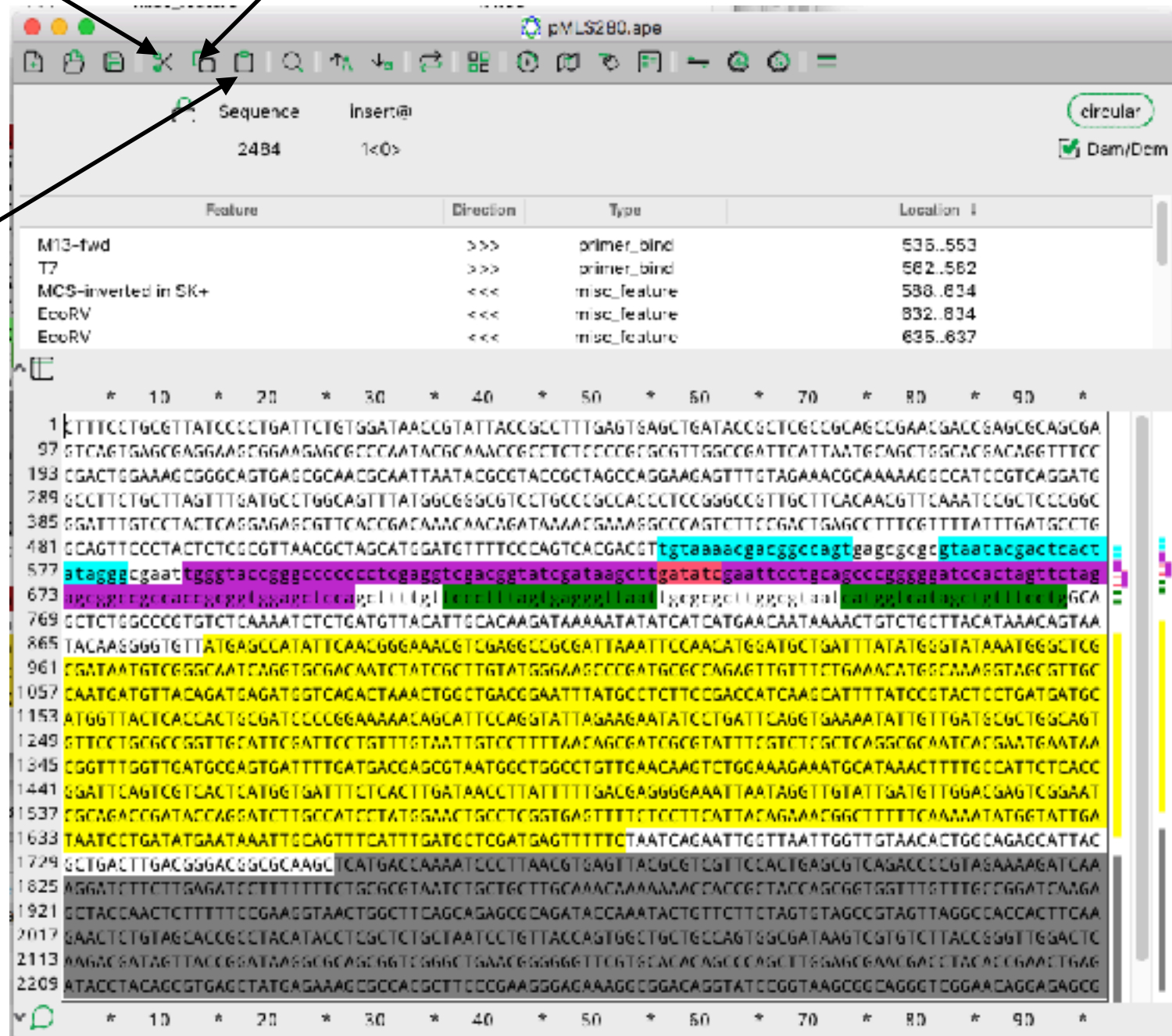
Shift: Cut rev-com

Copy

Shift: Copy rev-com

Paste

Shift: Paste rev com



Shortcuts

Command	Function
X	Cut
Shift-X	Cut rev-com
C	Copy
Shift-C	Copy rev-com
V	Paste
Shift-V	Paste rev-com

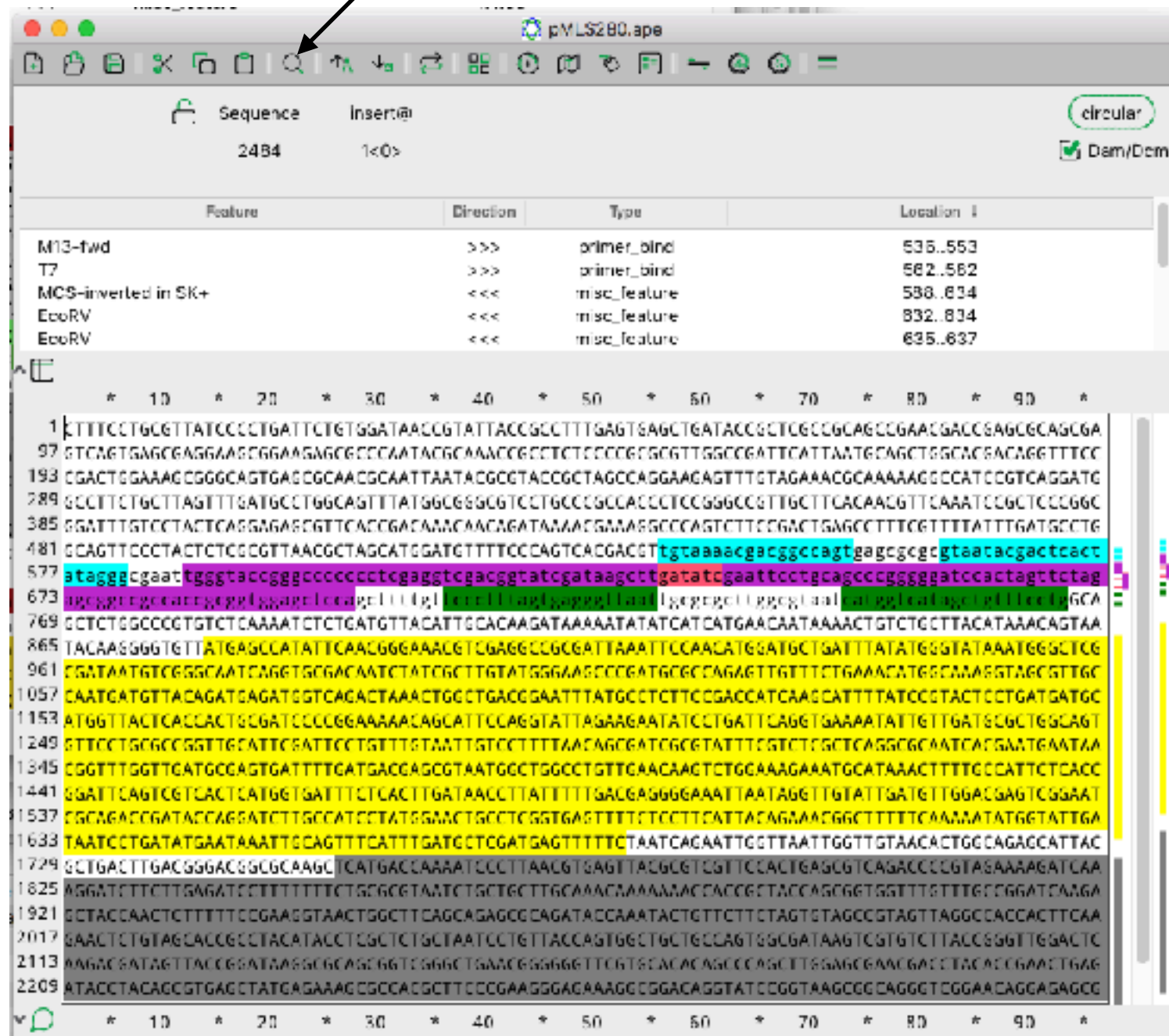
Copy Special

Copy Special		
Select All	⌘A	Copy All as Genbank
Select From-To...	⇧⌘A	Copy Uppercase
Jump To...	⌘J	Copy Uppercase Rev-Com
Find...	⌘F	Copy Translated
Find Again	⌘G	Copy Uppercase Translated
Clear Find Highlighting	⇧⌘F	Copy Translated Rev-Com
New Features From Highlighting	⇧⌘G	Copy Uppercase Translated Rev-Com
Convert to UPPERCASE	⌘+	Copy as FASTA
Convert to lowercase	⌘-	Copy Reverse (NOT A GOOD IDEA)
UPPER<->lower	⌘=	Copy Complement (NOT A GOOD IDEA)
Reverse-Complement	⌘/	Copy Features as NCBI Bankit table

ApE Window

Find

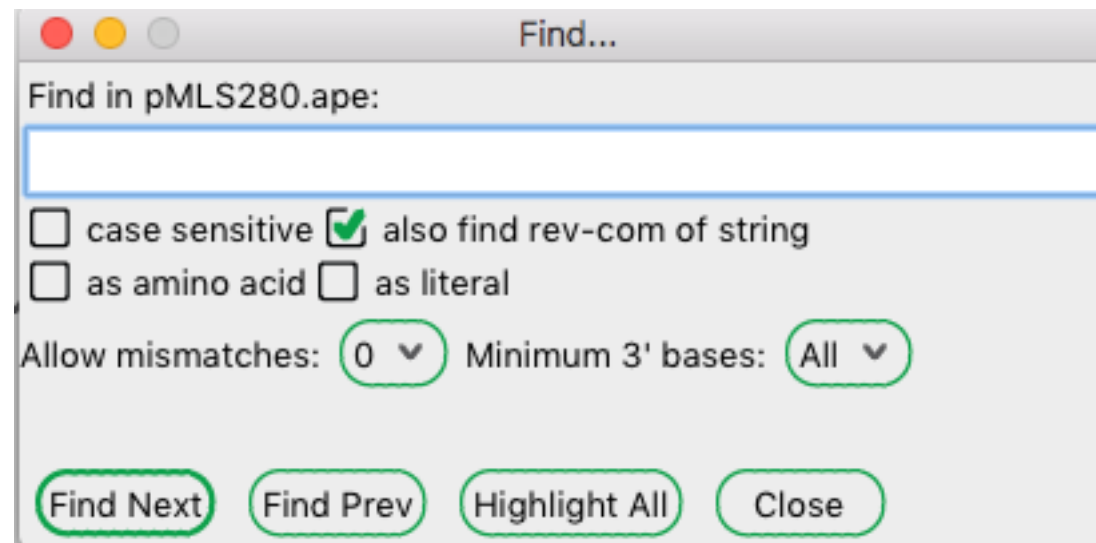
Shift: Clear find highlight



Shortcuts

Command	Function
F	Find
Shift-F	Clear find highlight
G	Do find again
Shift-G	New feature from find highlight

Find dialog



Find...
Find in pMLS280.ape:

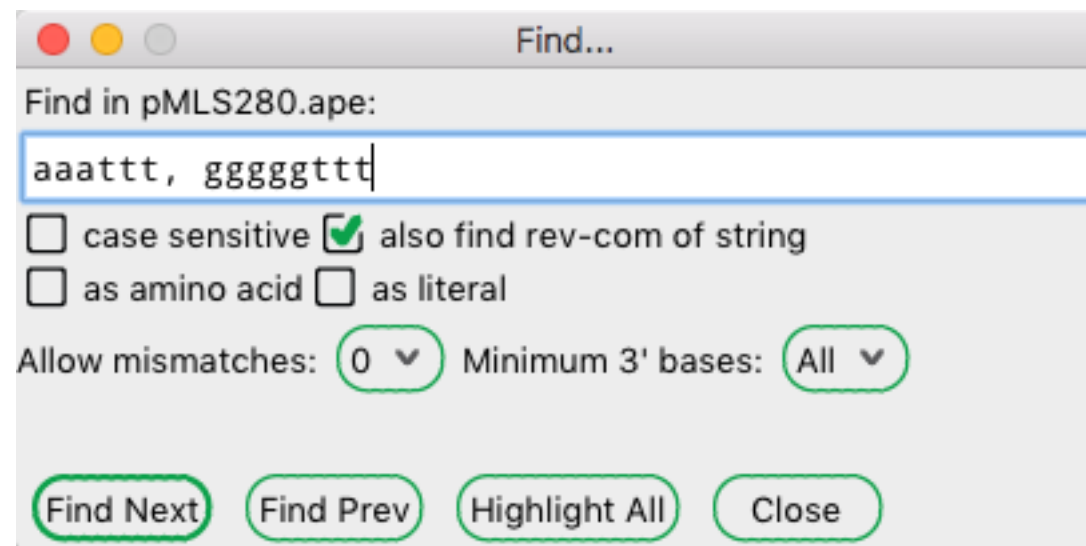
☐ case sensitive ☒ also find rev-com of string
☐ as amino acid ☐ as literal

Allow mismatches: 0 Minimum 3' bases: All

Find Next Find Prev Highlight All Close

Find dialog

You can search for multiple patterns with a comma or bar



ApE Window

Sequence: 2484 Start: 1<0> Length: 19<1> End: 19<0> ORF: >8/- Tm: 55.3°C %GC: 63% circular

ISCVP

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	526..552
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

1 TTTTCCTCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCCAGCGCAGCGA
97 GTCAGTGAGCGAGGAAAGCGAAGAGCGCCCAATACGCAACCGCCTCTCCCGCGCGTTGCGCGATTTCATTAAATGCAAGCTGGCAGACAGGTTTCC
193 CGACTGGAAAGCGGGCAGTGAGCGCAACSCAATTAAATACGGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCGTCAGGATG
289 GCCTTCTGCTTAGTTTTCATGCGCTGGCAGTTTATGGCGGGCGTCTGCCCGCCACCTCCCGGGCGGTTGCTTCAACAAGTTCAAATCCGCTCCCGGC
385 GGATTTGTCTACTCAGGAGAGCGTTACCGGACAAACAAGATAAACGAAAGSCCCAGTCTTCCGACTGAGCCCTTCGTTTATTTGATSCCTG
481 GCAGTTCCTACTCTCGGTTAAAGCTAGCATGATGTTTTCGAGTCAGGAGCTTgaataaacgagggcagtgagggcgaggaataggactcaact
577 ataggggcgaalggglaacggggcccccctcagggcaggggaltgalaagcttgalaatggalttcgtagcctgggggaltccatagttcag
673 agcgggccgcccacggcggtggagctccagcttttctcccttttagtgagggatgaatggcgcgcttggcgtaatcagggcagagcgttttcccgca
769 GCTCTGGCCCGTSTCCCAAAATCTCTGATGTTACATTGCAAGATATAAATATATCATCATGAACATAAAACTSTCTGCTTACATAAACAGTAA
865 TACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCGCGGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCG
961 CCATAATSTCGGCCAATCAGGTGCGACAATCTATCGCTTGATGCGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGCGCAAGGTAGCCTTG
1057 CAATGATGTTACAGATGAGATGGTCAAGCTAAATGAGCTGACGGAATTTATGCTCTTCCGACCATCAAGCATTTTATCGGTACTCTGATGATG
1153 ATGGTTACTCACCCTGCGATCCCGGAAACAGCATTCAGGTATTAGAGAATATCTGATTCAGGTGAAATATTTGATGCGCTGCGAGT
1249 GTTCTCTCGCGCGTTGCAATTCGATTCCTGTTTGAATGTCCTTTTAAACAGCGATCGCGTATTTCCTCTCGCTCAGCGCGAATCAGCAATCAATAA
1345 CGGTTTGGTTGATGAGAGTGATTTTGATGAGAGCGTAATGGCTGGGCTGTTGAACAAAGTGTGGAAGAGAAATGCATAAACTTTTGGCTATTCAC
1441 GGATTCAGTGGTCACTCATGGTATTTCTCACTTGATAACCTTATTTTGAAGAGGGGAAATTAATAGGTTGATTTGATGTTGGACGAGTCSGAT
1537 CCGAGACGATACCGAGATCTTCCATCCTATGCAACTGCGCTCGGTGAGTTCCTCTCATTACAGAAACGGCTTTTCAAAAATATGGTATTGA
1633 TAATCCTGATATGAATAAATGCAATTTGATTTGATGCTGATGATGATTTTCTAATCAGAAATGTTAATTGGTTSTAACACTGGCAGAGCATTAC
1729 GCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAAGGTGAGTTACGGCTCTCCACTGAGGCGTCAGACCCCTAGAAAAGATCAA
1825 AGGATCTTCTGAGATCCTTTTCTCGCGGTAATCTGCTGCTGCTGCAAAACAAAAAACCCGCTACCGCGGTGTTTGTGTTGCGGATCAAGA
1921 GCTACCACTCTTTTTCCGAAAGGTAAGTGGCTTCAAGCAGAGCGCAGATACCAATACTGTTCTTCTAGGTAGCCSTAGTTAGGCCACCACTTCAA
2017 GAATCTGTAGCAGCGCTACATACCTCTCTCTCTAATCTGTATACCAAGTGGCTGCTGCGAGTGGGATAAGTCTGTCTTACCGGTTGAGCTC
2113 AAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACCGGGGTTGCTGCAACAGGCCAGCTTGGAGCGAACGACCTACACCGAACTGAG
2209 ATACCTACAGCGTGAAGCTATGAGAAAGCGCCACGCTTCCGAAAGGAGAAAGGCGGACAGGTATCGGTAAGCGGCAAGGTGGGACAGGAGAGCG

Open this to
show the file
comment

Shortcuts

Command	Function
.	New Feature
K	Add features using feature library
Shift-K	Clear all features

Enzymes

Select Enzymes

The screenshot shows a bioinformatics software interface with a DNA sequence and a table of features. An arrow points to the 'Enzyme Selection Dialog' button in the toolbar.

Enzyme Selection Dialog
Shift+Toggle Selected Only

Sequence
2484

Insert@
1<0>

Features Table:

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	636..653
T7	>>>	primer_bind	662..682
MCS-Inverted in 5K+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

DNA Sequence:

```
1 CTTTCCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGACGCCAACGACCGAGCGCAGCGA
97 GTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCTCTCCCGCGCGTTGGCCGATTCAATGACGCTGGCAGGACAGGTTTCC
193 CGACTGGAAAGCGGSCAGTGAGCGCAACGCAATTAAACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGCCATCCGTCAGGATG
289 CCCTTCTGCTTAGTTTGATGCTGGCAGTTTATGCGCGCGCTCTGCGCGCCACCTCCGCGCCGTTGCTTCACAACTTCAAATCCGCTCCCGGC
385 GGATTTGTCTACTCAGGAGAGCGTTCACCGACAAACAGATAAACGAAAGGCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCTG
481 GCAGTTCCTACTCTCGCGTTAACGCTAGCATGATGTTTTCCAGTACAGACGTtgtaaaacgacggccagtgagcgcgctaatatcgactcact
577 atagggcgaaatgggtaacgggcccccccgagggtgagggtatcgataggttgatcgaatctcagagcggggagatccactagtttag
578 agcggcgccacggcggtggagctccagcttttggctccctttagtgagggttaattgcgcgcttgggcgtaattcatgggtcatagctgtttccctgca
769 CCTCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCAAGATAAAATATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAA
865 TACAAGGGGTGTTATGAGCCATATTCAACGGGAACGTCGAGGCCGCGATTAAATTCACATGGATGCTGATTTATATGGGTATAAATGGGCTCG
961 CGATAATGTCGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGGCCGATGCGCCAGAGTTGTTTCTCAAACTGCGAAAGGTAGCGTTGC
1057 CAATGATGTTACAGATGAGATGCTCAGACTAATCTGCTGACGGAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGC
1153 ATGGTTACTCACCCTGCGATCCCGGAAAAACAGCATTCAGGTATTAGAAATATCTCTGATTCAGGTCAAAATATTGTTGATGCGCTGCGCAST
1249 GTTCCTGCGCGGTTGCAATTCGATTCCTGTTTGTAAATGCTCTTTTAAACAGCGATCGGCTATTTGCTCTGCTCAGGCGCAATCAGGAATGAATAA
1345 CGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGCTGGCTGTTGAAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACC
1441 GGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTGGACGAGGCGAAATTAATAGGTTGATTCATGTTGGACGAGTGGGAAT
1537 CGCAGACCGATACCAAGATCTTGCCATCCTATGSAACTGCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGA
1633 TAATCCTGATATGAATAAATTCAGTTTCATTTGATGCTCGATGAGTTTTCTAATCAGAATTGGTTAATTGTTGTAACACTGGCAGAGCATTAC
1729 GCTGACTTGACGGGACGCGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACCGCTCGTTCCACTGAGCGTCAGACCCCGTAGAAAGATCA
1825 AGGATCTTCTGACATCTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACACCGCTACCAAGCGTGGTTTCTTTGCGGATCAAGA
1921 GCTACCAACTCTTTTTCGAAGGTAACCTGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCGTAGTTAGGCCACCACTTCAM
2017 GAACTCTGTAGACCCGCTACATACCTCGCTCTGCTAATCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGTTGGACTC
2113 AAGACGATAGTTACCGGATAAGGCGCAGCGCTCGGCTGAAACGGGGGTTGCTGCACACAGCCAGCTTGAAGCGAACGACCTACACCGAAGTGA
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGACAGGTCGGAACAGGAGAGCG
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Select an enzyme to highlight

Enzyme Selection...

Window pMLS280.ape ☐ Selection: 1 - 2484 ☒ Dam/Dcm

AatII (0)	BanII (3)	BspLU11I (1)	Eco47III (0)	KpnI (1)	NlaIV (5)	Sall (1)	Swal (0)
AbSI (1)	BbeI (0)	BsrGI (0)	EcoNI (1)	MaeI (5)	NotI (1)	SanDI (0)	TaqI (8)
Acc65I (1)	BceAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	NruI (1)	SapI (1)	TatI (0)
AccB1I (1)	BclI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	NsiI (2)	SbfI (0)	TspEI (11)
AccI (1)	BfmI (4)	BstBI (0)	EcoRV (1)	MboI (0)	Nspl (1)	Scal (0)	TspGWI (3)
AccII (14)	BglI (0)	BstEII (0)	FseI (0)	MfeI (0)	OliI (1)	Sdul (4)	Tth111I (0)
AccIII (0)	BglII (0)	BstXI (1)	FspAI (0)	MluI (2)	PacI (0)	SexAI (0)	Vspl (3)
AcII (1)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (7)	PfIMI (1)	SfiI (0)	XbaI (1)
Acyl (1)	BsaAI (0)	Bsu36I (0)	HaeII (2)	MreI (0)	PfoI (0)	SfoI (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaeIII (15)	MscI (0)	PmeI (0)	Sgfl (1)	XhoI (1)
AflIII (3)	Bsal (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	BsaWI (3)	Cfr10I (1)	HincII (2)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (1)
AluI (12)	BseRI (0)	CfrI (3)	HindIII (1)	MspA1I (4)	PshAI (0)	SmaI (1)	Xmnl (0)
AlwNI (1)	BseSI (2)	Clal (1)	HpaI (1)	MwoI (11)	PsiI (0)	SmlI (4)	ZraI (0)
Apal (1)	BsiEI (4)	DpnI (8)	HpaII (11)	NaeI (0)	PstI (1)	SnaBI (0)	
ApalI (1)	BsiHKA1 (2)	DraI (0)	Hpy188III (17)	NarI (0)	PvuI (1)	SpeI (1)	
ApoI (3)	BsiWI (0)	DraII (1)	Hpy8I (4)	NcoI (0)	PvuII (1)	SphI (0)	
AscI (0)	BsiYI (10)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	BsmBI (1)	DrdI (1)	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	Sspl (1)	
AvrII (0)	Bsp120I (1)	EagI (1)	HpyCH4V (9)	NheI (2)	SacI (1)	StuI (0)	

Select Enzymes unique (1) All Select De-select AND clear all Sel to Mem

Perform Action Graphic Map Graphic Map +U Digest Digest with All Highlight Text Close

☐ Keep Selector Dialog Open

Enzyme highlights

Enzyme name and sequence shows up here when you put the mouse over the sequence

The screenshot shows a bioinformatics software window titled 'pML5280.apc'. It displays a DNA sequence with various features highlighted. A table at the top lists features with their start, end, and type. The sequence below is color-coded to show these features.

Feature	Start	End	Type
M13-fwd	536	553	primer_bind
EcoRV	552	562	misc_feature
EcoRI	563	573	misc_feature
EcoRV	574	584	misc_feature
EcoRI	585	595	misc_feature

The sequence is displayed in a window with a toolbar and a status bar. The sequence is color-coded to show features. A mouse cursor is hovering over the sequence, and a tooltip shows the enzyme name and sequence.

Enzymes can be highlighted

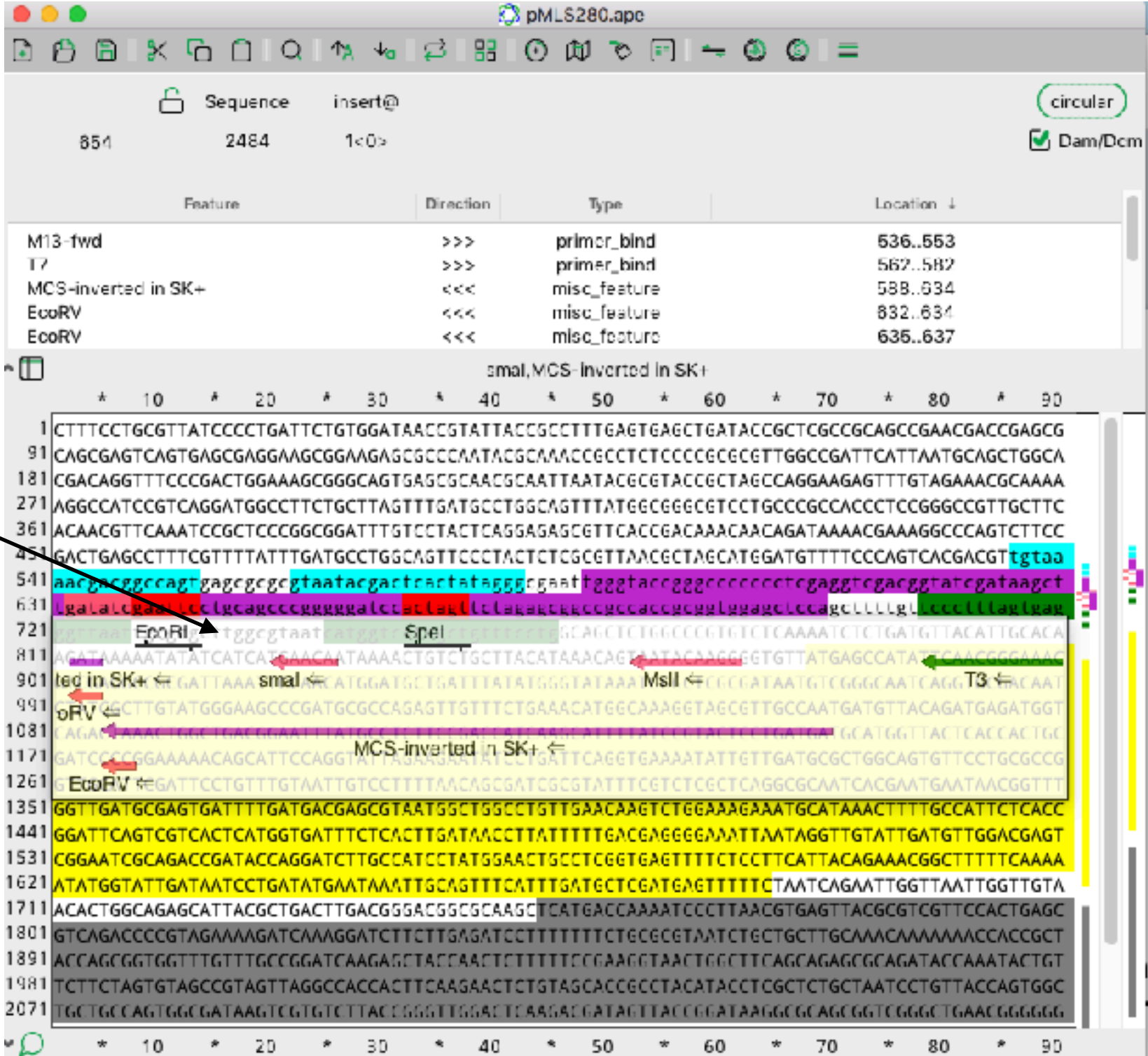
X-ray window

[illegible]

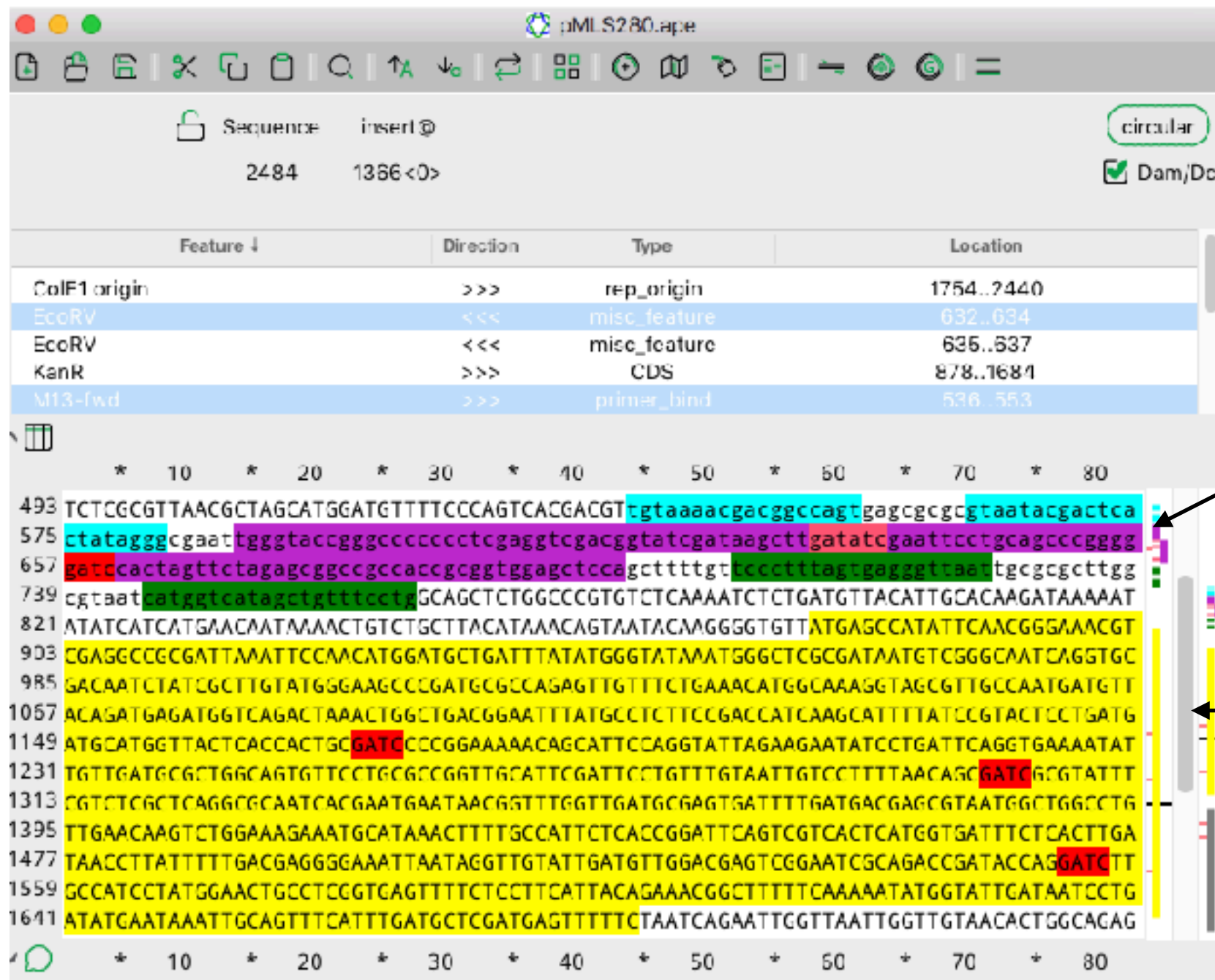
spacebar turns on the X-ray window

X-ray window

The X-ray window shows all the features and enzyme sites in a row



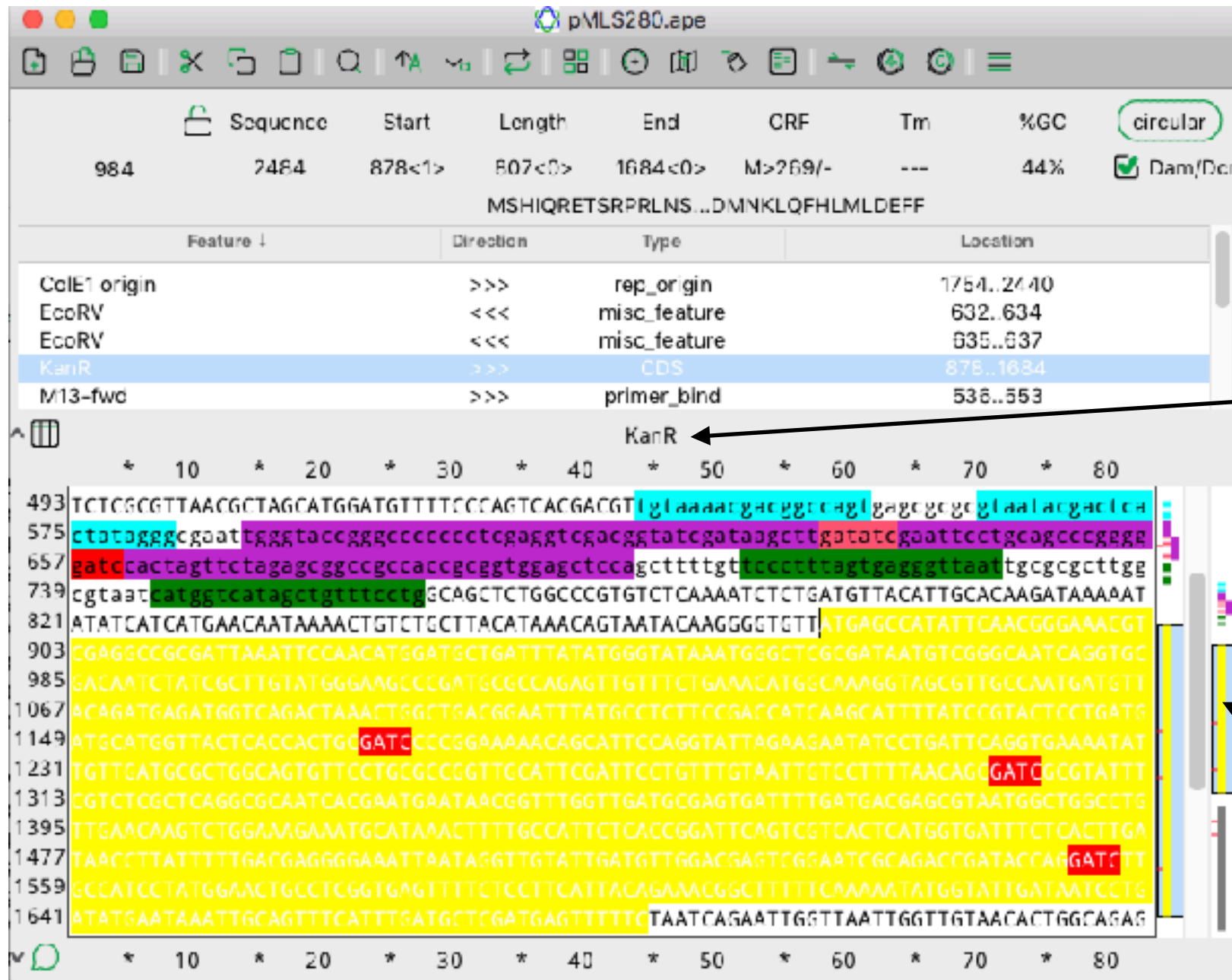
Side maps



First map shows the features in the current lines

Second map shows the features in the whole sequence

Side maps



Side map features under the pointer are listed here

Click on any of these to select a feature in the feature table and set the selection to that feature

Virtual Gel

Select Enzymes

The screenshot shows the pML S280.lape software interface. At the top, there is a menu bar with various icons. Below the menu bar, there is a toolbar with icons for file operations, editing, and visualization. A tooltip for the 'Enzyme Selection Dialog' icon reads 'Enzyme Selection Dialog Shift+Toggle Selected Only'. The main window displays a sequence editor with a table of features and a DNA sequence view.

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	636..653
T7	>>>	primer_bind	662..682
MCS-Inverted in 5K+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	636..637

The DNA sequence view shows a sequence of 2484 bases. The sequence is displayed in a monospaced font, with line numbers on the left and base positions at the top. The sequence is color-coded to highlight specific features, such as restriction enzyme sites and primers.

Virtual Gel

Enzyme Selection...

Window pMLS280.apex ☐ Selection: 1 - 2484 ☒ Dam/Dcm

AatII (0)	BanII (3)	BspLU11I (1)	Eco47III (0)	KpnI (1)	NlaIV (5)	Sall (1)	Swal (0)
<u>AbsI (1)</u>	BbeI (0)	BsrGI (0)	<u>EcoNI (1)</u>	MaeI (5)	<u>NotI (1)</u>	SanDI (0)	TaqI (8)
<u>Acc65I (1)</u>	BceAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	<u>NruI (1)</u>	<u>SapI (1)</u>	TatI (0)
<u>AccB1I (1)</u>	BclI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	Nsil (2)	Sbfl (0)	TspEI (11)
<u>AccI (1)</u>	Bfml (4)	BstBI (0)	<u>EcoRV (1)</u>	Mbol (0)	<u>Nspl (1)</u>	Scal (0)	TspGWI (3)
AccII (14)	BglI (0)	BstEII (0)	FseI (0)	Mfel (0)	<u>Olil (1)</u>	Sdul (4)	Tth111I (0)
AccIII (0)	BglII (0)	<u>BstXI (1)</u>	FspAI (0)	Mlul (2)	PacI (0)	SexAI (0)	Vspl (3)
<u>AcII (1)</u>	BlpI (0)	BstZ17I (0)	Fspl (0)	Mmel (7)	<u>PfIMI (1)</u>	Sfil (0)	<u>XbaI (1)</u>
<u>Acyl (1)</u>	BsaAI (0)	Bsu36I (0)	Haell (2)	Mrel (0)	Pfol (0)	Sfol (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaellI (15)	MscI (0)	Pmel (0)	<u>Sgfl (1)</u>	<u>XhoI (1)</u>
AflIII (3)	Bsal (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	BsaWI (3)	<u>Cfr10I (1)</u>	HincII (2)	<u>MslI (1)</u>	PpuMI (0)	SgrDI (0)	<u>XmaI (1)</u>
AluI (12)	BseRI (0)	CfrI (3)	<u>HindIII (1)</u>	MspAII (4)	PshAI (0)	<u>Smal (1)</u>	XmnI (0)
<u>AlwNI (1)</u>	BseSI (2)	<u>Clal (1)</u>	<u>HpaI (1)</u>	Mwol (11)	Psil (0)	SmlI (4)	ZraI (0)
<u>Apal (1)</u>	BsiEI (4)	DpnI (8)	HpaII (11)	NaeI (0)	<u>PstI (1)</u>	SnaBI (0)	
<u>ApaLI (1)</u>	BsiHKAII (2)	DraI (0)	Hpy188III (17)	NarI (0)	<u>PvuI (1)</u>	SpeI (1)	
ApoI (3)	BsiWI (0)	<u>Drall (1)</u>	Hpy8I (4)	NcoI (0)	<u>PvuII (1)</u>	SphI (0)	
AscI (0)	BsiYI (10)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	<u>BsmBI (1)</u>	<u>DrdI (1)</u>	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	<u>Sspl (1)</u>	
AvrII (0)	<u>Bsp120I (1)</u>	<u>EagI (1)</u>	HpyCH4V (9)	NheI (2)	<u>SacI (1)</u>	StuI (0)	

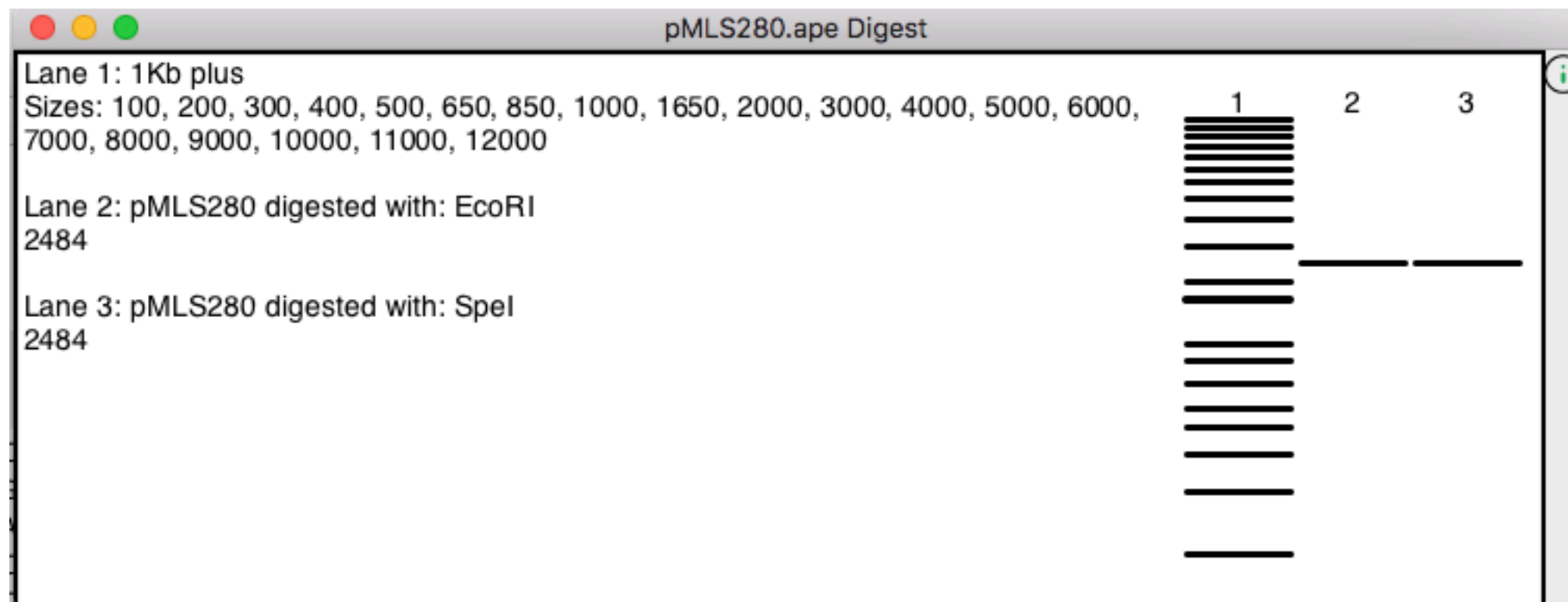
Select Enzymes

Perform Action

☐ Keep Selector Dialog Open

Click here to digest the sequence
with each enzyme individually

Virtual Gel



Virtual Gel

Enzyme Selection...

Window: pMLS280.apex

Selection: 1 - 2484 ☒ Dam/Dcm

AatII (0)	BanII (3)	BspLU11I (1)	Eco47III (0)	KpnI (1)	NlaIV (5)	Sall (1)	Swal (0)
AbsI (1)	BbeI (0)	BsrGI (0)	EcoNI (1)	MaeI (5)	NotI (1)	SanDI (0)	TaqI (8)
Acc65I (1)	BceAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	NruI (1)	SapI (1)	TatI (0)
AccB1I (1)	BclI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	Nsil (2)	Sbfl (0)	TspEI (11)
AccI (1)	BfmI (4)	BstBI (0)	EcoRV (1)	Mbol (0)	Nspl (1)	Scal (0)	TspGWI (3)
AccII (14)	BglI (0)	BstEII (0)	FseI (0)	Mfel (0)	OliI (1)	Sdul (4)	Tth111I (0)
AccIII (0)	BglII (0)	BstXI (1)	FspAI (0)	MluI (2)	PacI (0)	SexAI (0)	Vspl (3)
AcII (1)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (7)	PfIMI (1)	Sfil (0)	XbaI (1)
Acyl (1)	BsaAI (0)	Bsu36I (0)	HaeII (2)	MreI (0)	PfoI (0)	Sfol (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaeIII (15)	MscI (0)	PmeI (0)	Sgfl (1)	XhoI (1)
AflIII (3)	Bsal (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	BsaWI (3)	Cfr10I (1)	HincII (2)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (1)
AluI (12)	BseRI (0)	CfrI (3)	HindIII (1)	MspAII (4)	PshAI (0)	SmaI (1)	XmnI (0)
AlwNI (1)	BseSI (2)	Clal (1)	HpaI (1)	MwoI (11)	Psil (0)	SmlI (4)	ZraI (0)
Apal (1)	BsiEI (4)	DpnI (8)	HpaII (11)	NaeI (0)	PstI (1)	SnaBI (0)	
ApaLI (1)	BsiHKAII (2)	DraI (0)	Hpy188III (17)	NarI (0)	PvuI (1)	SpeI (1)	
ApoI (3)	BsiWI (0)	Drall (1)	Hpy8I (4)	NcoI (0)	PvuII (1)	SphI (0)	
AscI (0)	BsiYI (10)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	BsmBI (1)	DrdI (1)	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	Sspl (1)	
AvrII (0)	Bsp120I (1)	EagI (1)	HpyCH4V (9)	NheI (2)	SacI (1)	StuI (0)	

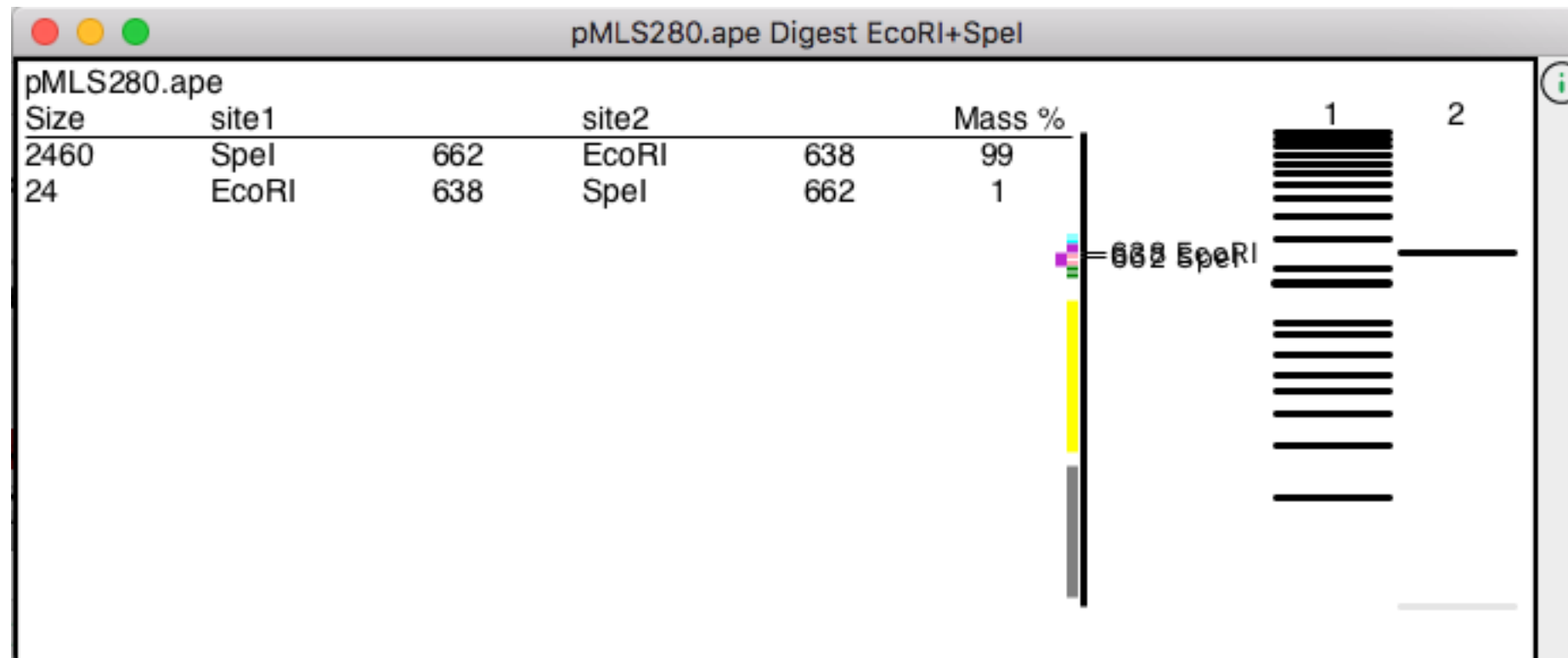
Select Enzymes: unique (1) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map +U **Digest** Digest with All Highlight Text Close

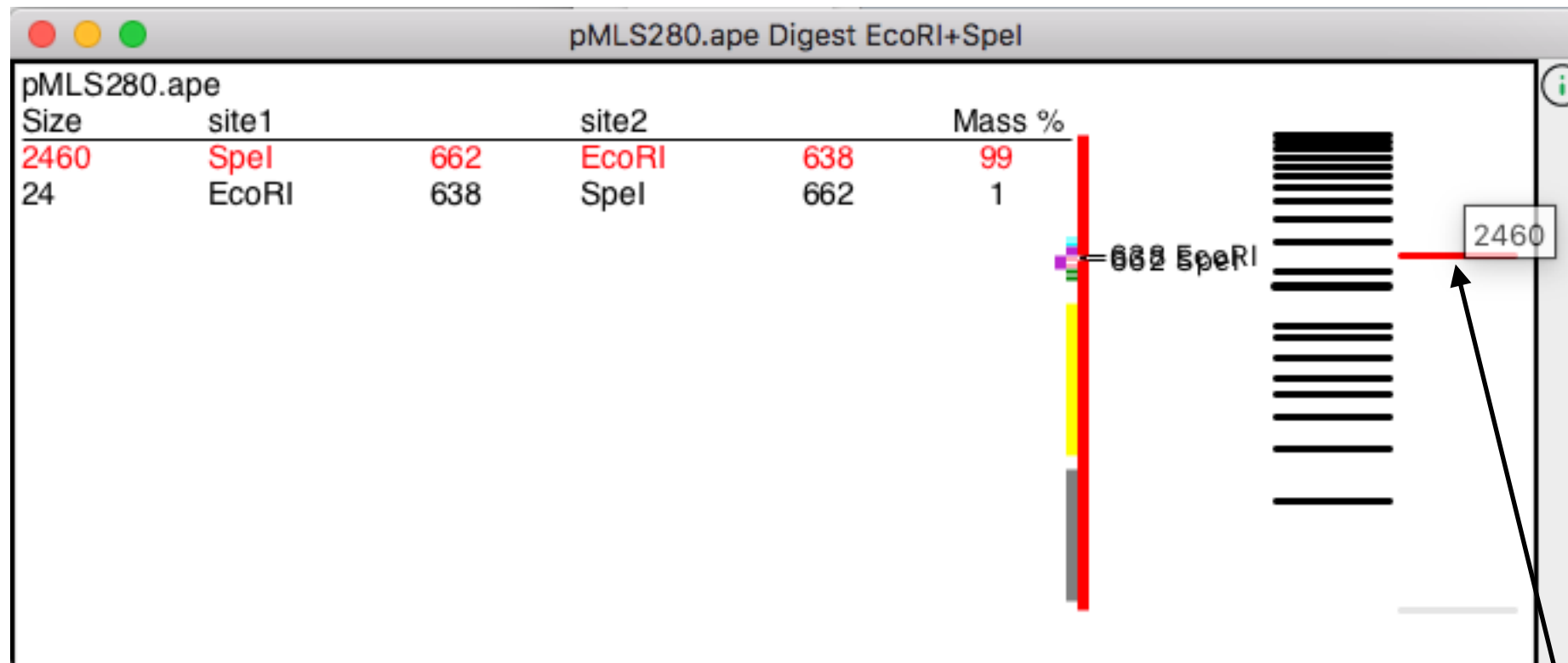
☐ Keep Selector Dialog Open

Click here to digest the sequence with each enzyme at one time

Virtual Gel

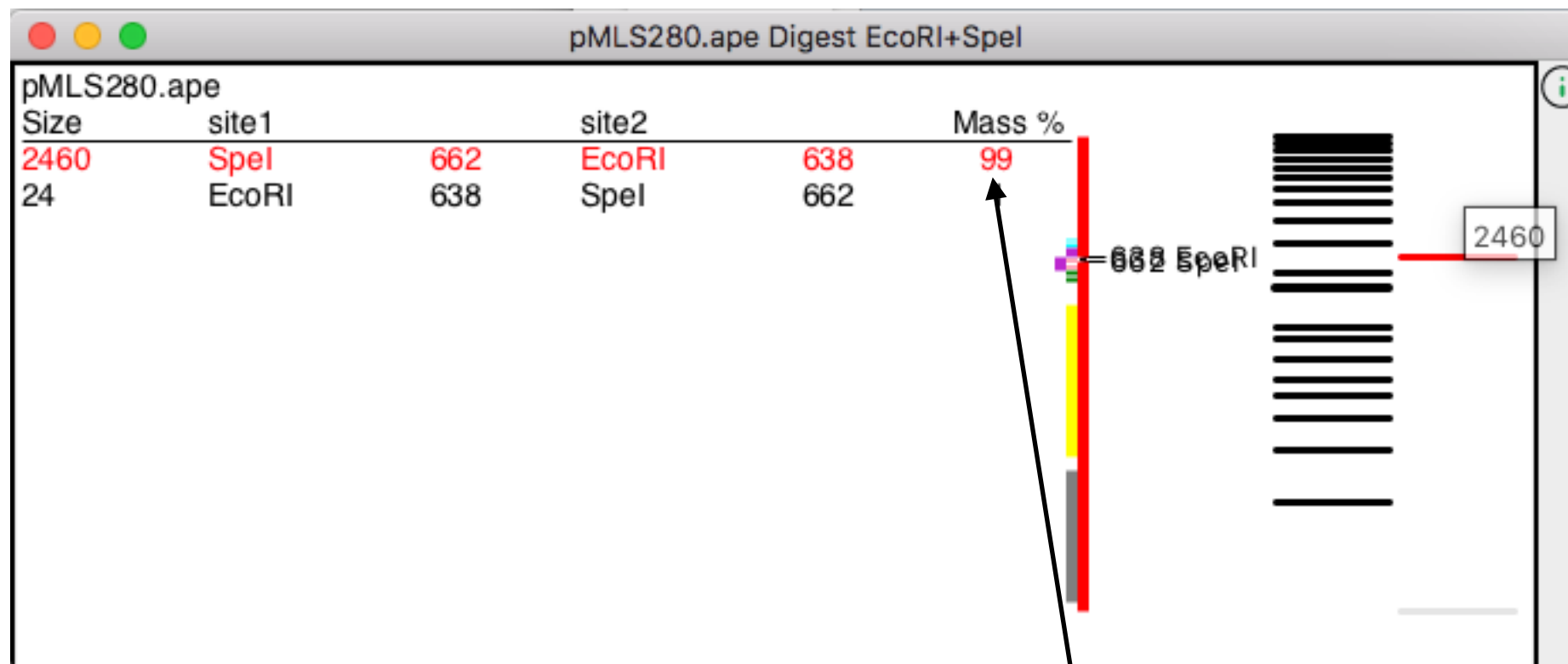


Virtual Gel

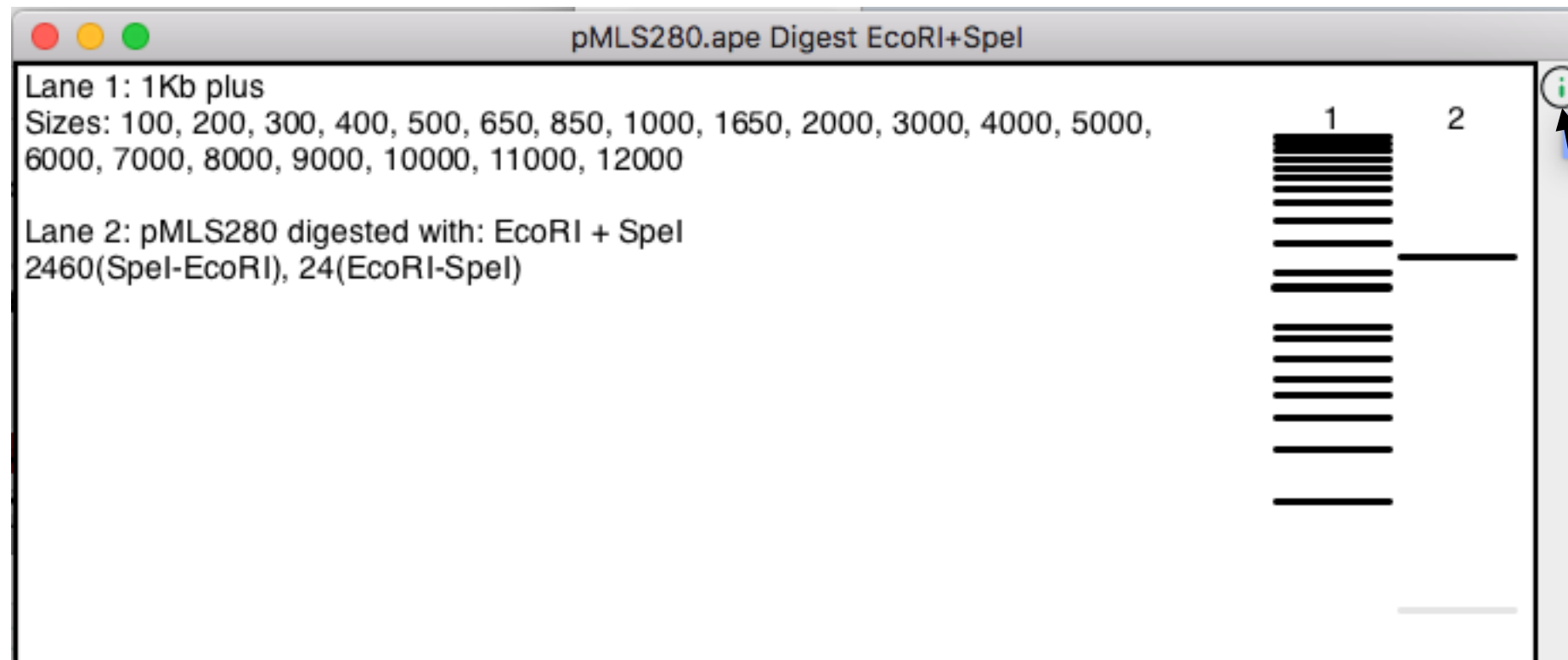


Mouse here to highlight the band
Double-click to select that region
in the sequence

Virtual Gel

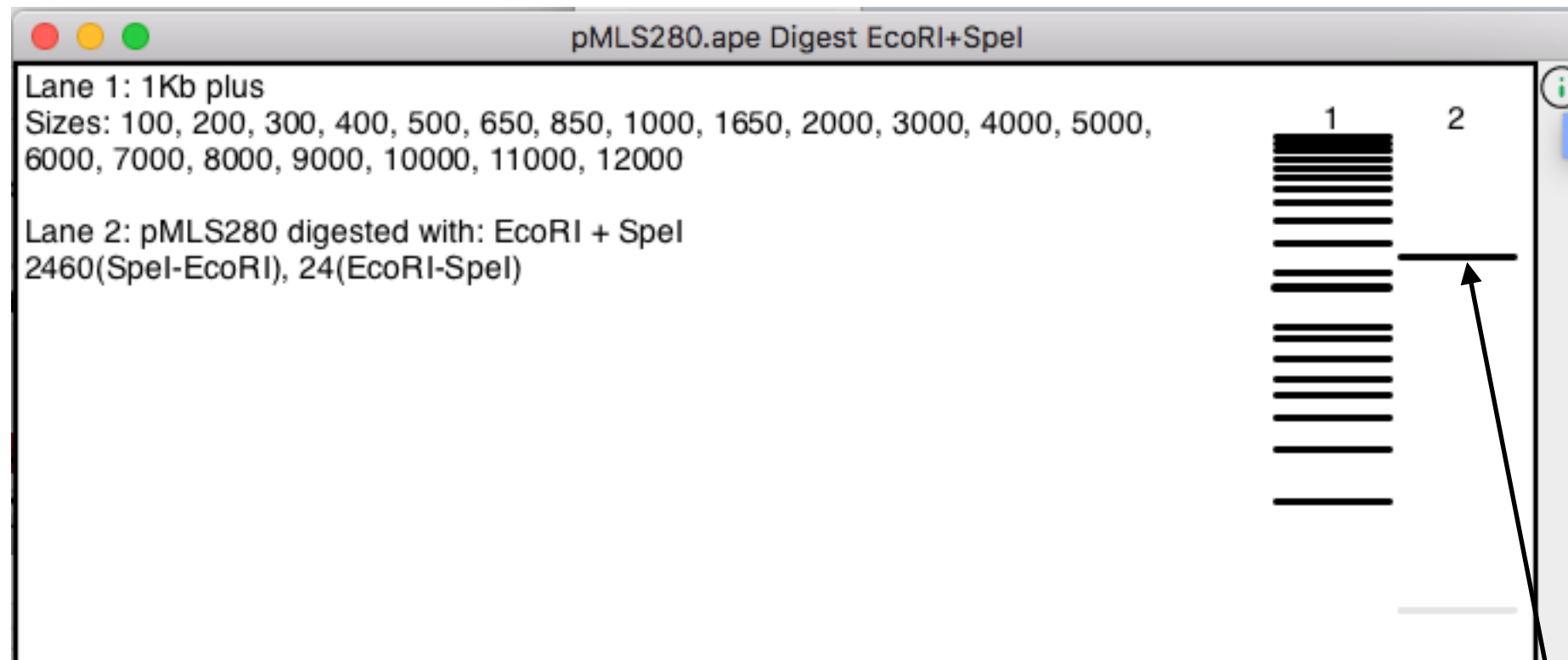


Virtual Gel



Click here to see a list of digest lanes

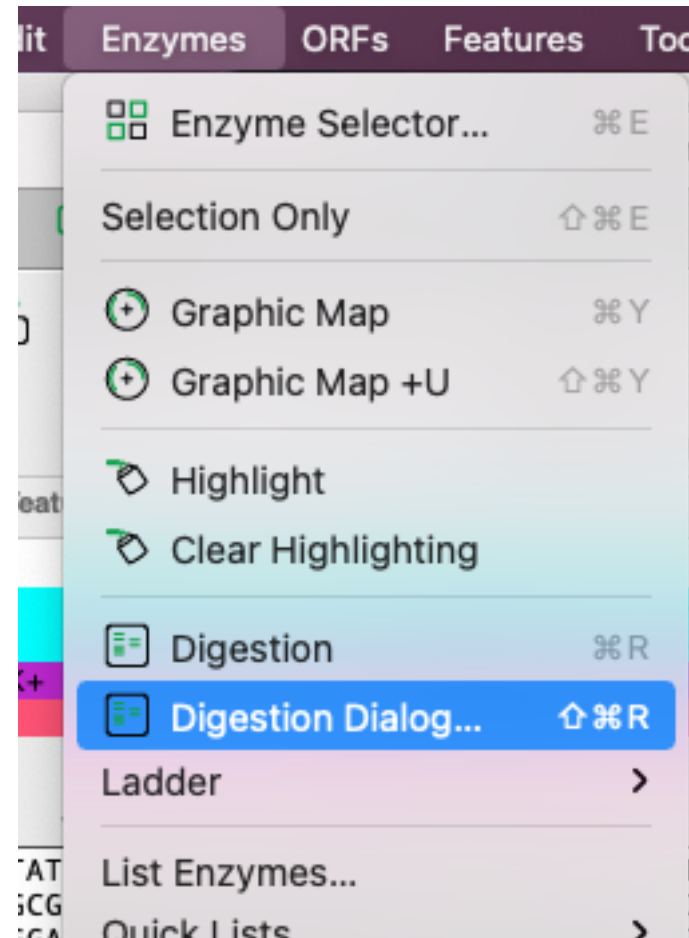
Virtual Gel



Shortcuts

Command	Function
R	Digest with currently selected enzymes
Shift-R	Digest Dialog
E	Enzyme selection dialog
Shift-E	Set all functions to apply to selection

Digestion dialog

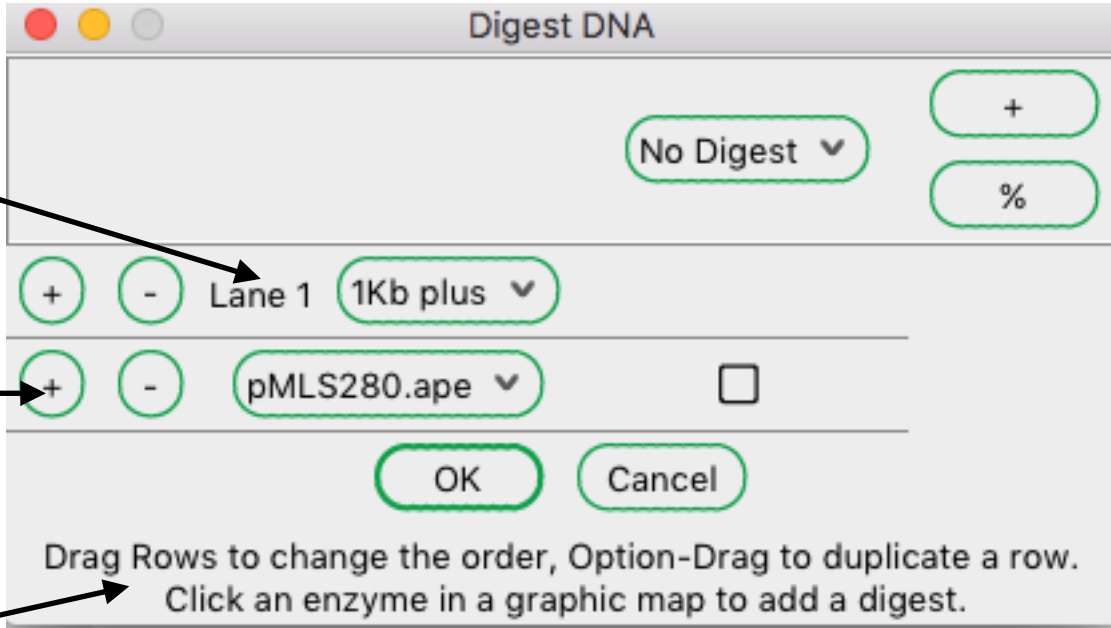


Digestion dialog

Each gel lane is a table row

Add and remove rows with these buttons

Rows can be dragged and duplicated



The screenshot shows a 'Digest DNA' dialog box with a table of gel lanes. The table has two rows. The first row is labeled 'Lane 1' and has a '1Kb plus' dropdown menu. The second row has a 'pMLS280.apc' dropdown menu and a checkbox. Above the table are buttons for '+', '-', and '%'. Below the table are 'OK' and 'Cancel' buttons. A text box at the bottom explains that rows can be dragged to change order or duplicated with Option-Click, and that clicking an enzyme in a graphic map adds a digest.

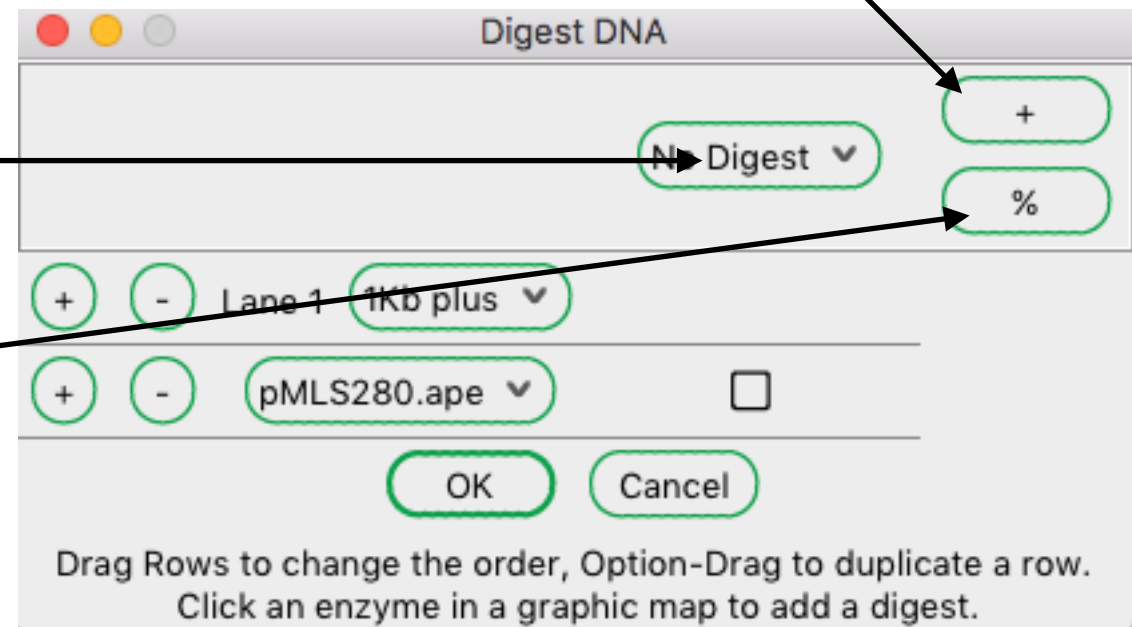
Digest DNA			
No Digest ▾			
+			
%			
+	-	Lane 1	1Kb plus ▾
+	-	pMLS280.apc ▾	<input type="checkbox"/>
OK Cancel			
Drag Rows to change the order, Option-Click to duplicate a row. Click an enzyme in a graphic map to add a digest.			

Digestion dialog

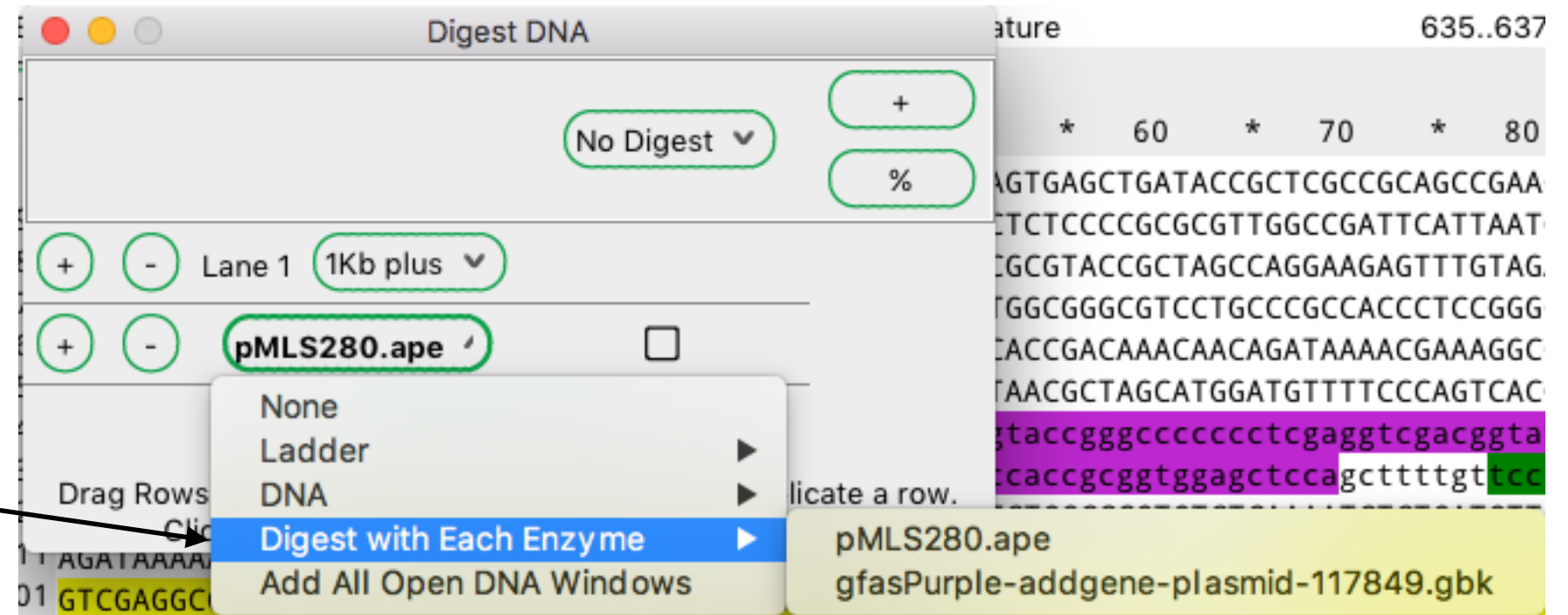
Add enzyme columns
with this button

Enzymes for digestions
are added here

Show partial digest
options with this
button

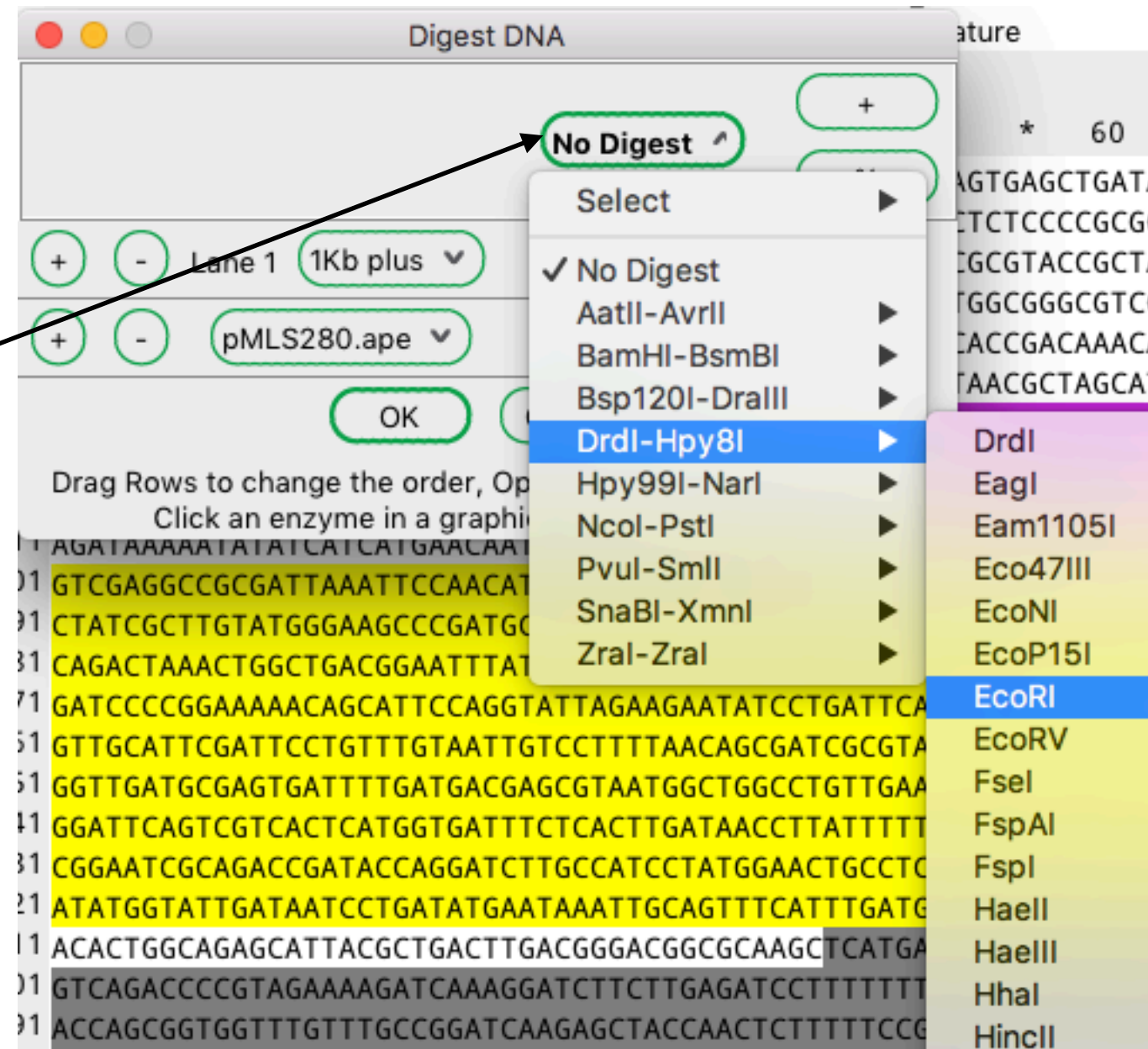


Digestion dialog

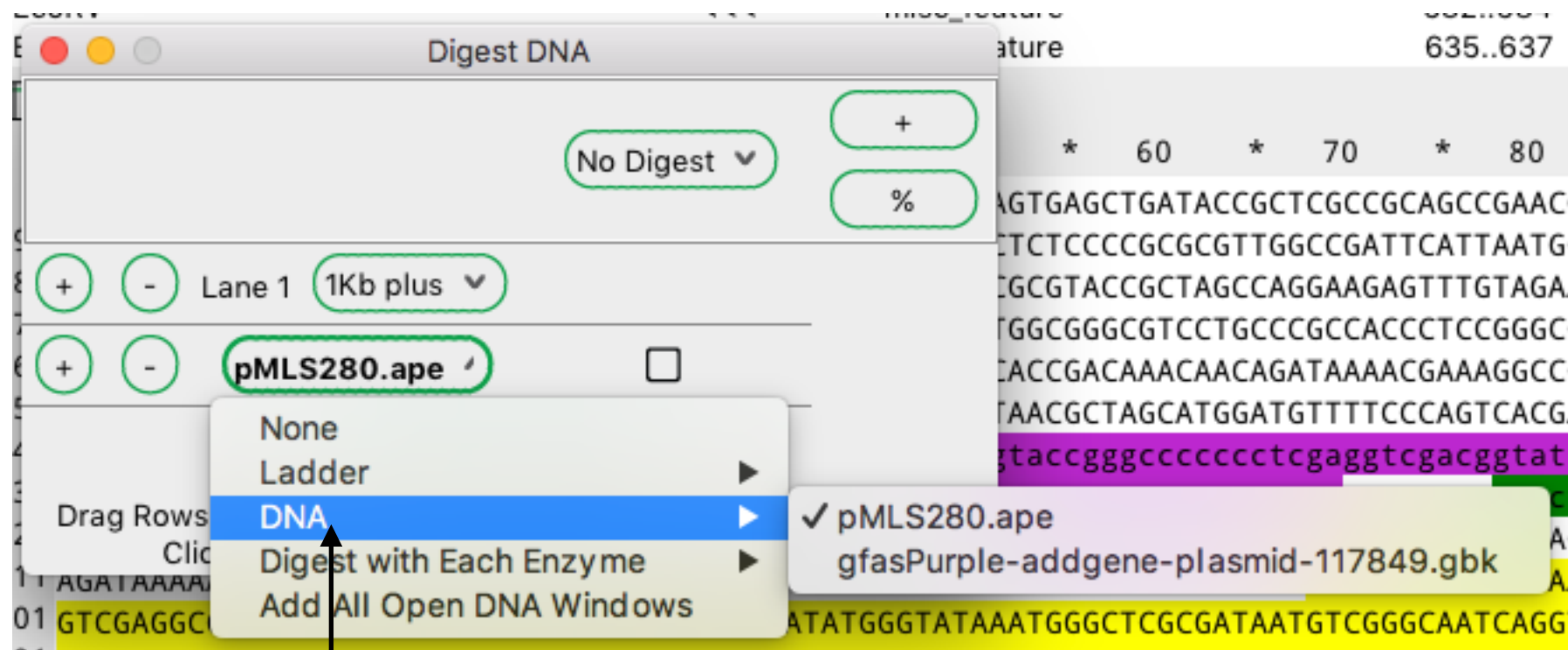


Digestion dialog

Change the enzyme
with this menu



Digestion Dialog

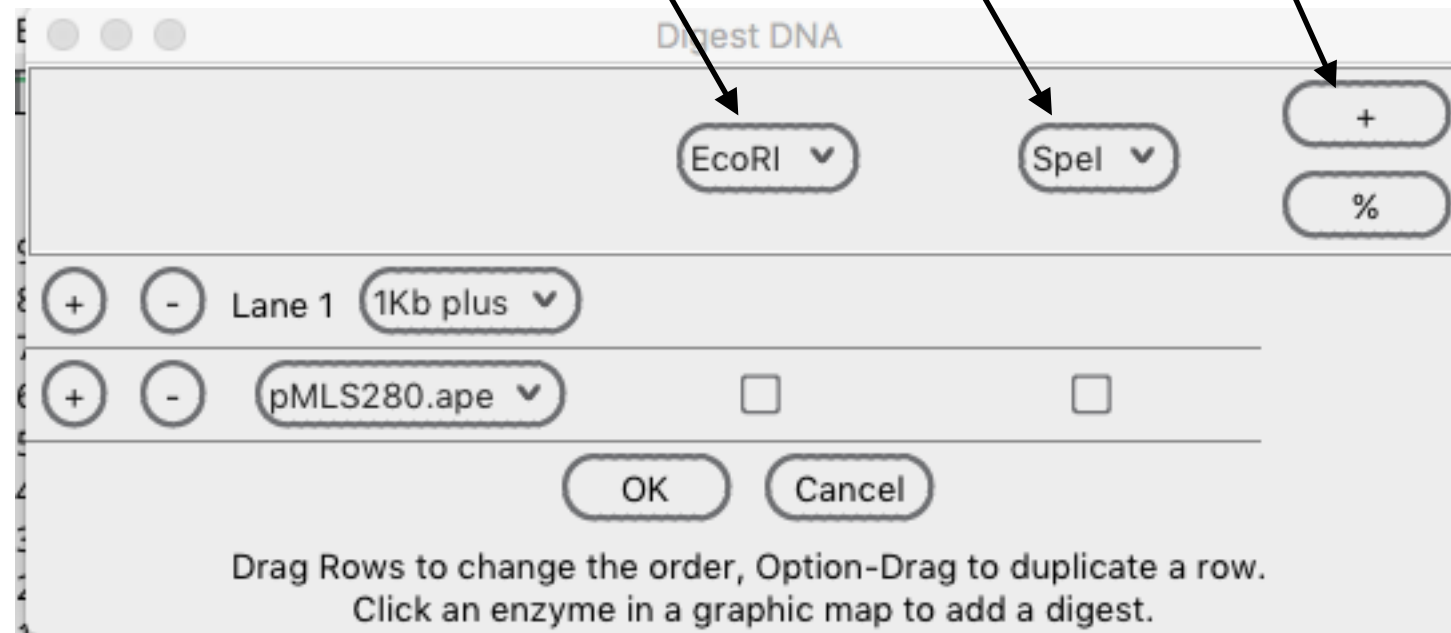


Select the DNA or ladder for each lane

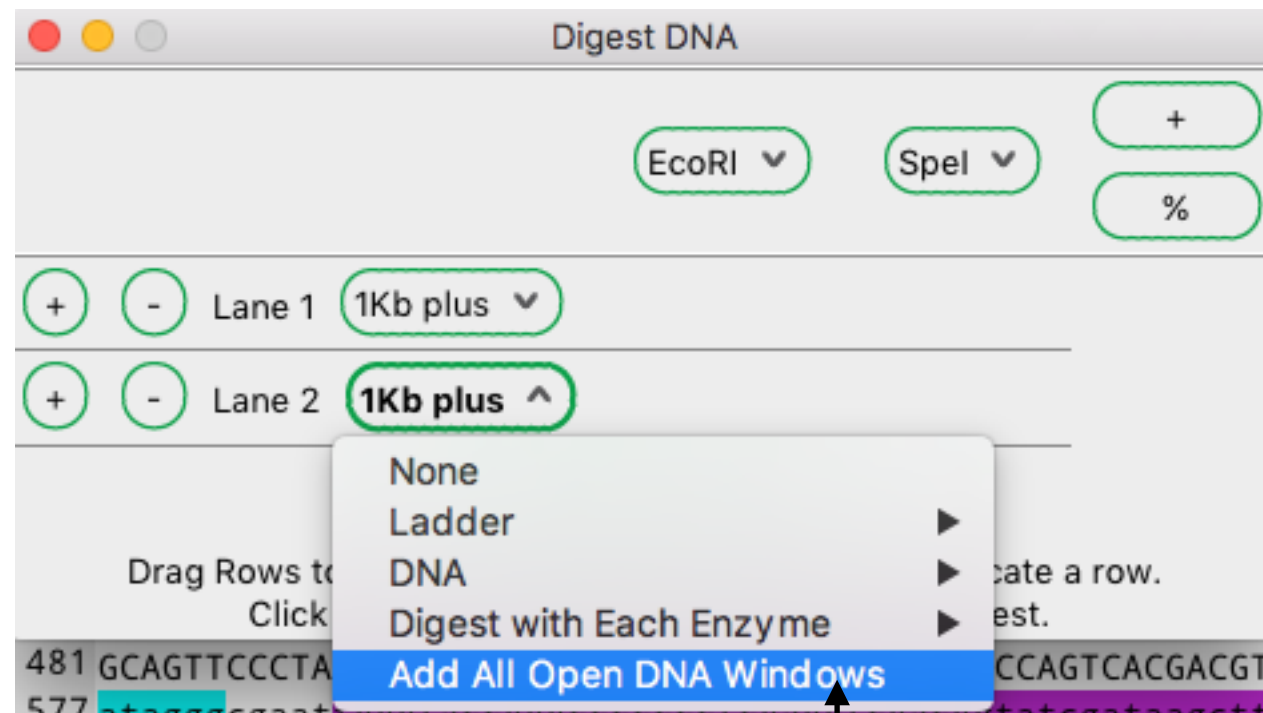
Digestion Dialog

Add an Enzyme Column

Select EcoRI and SpeI

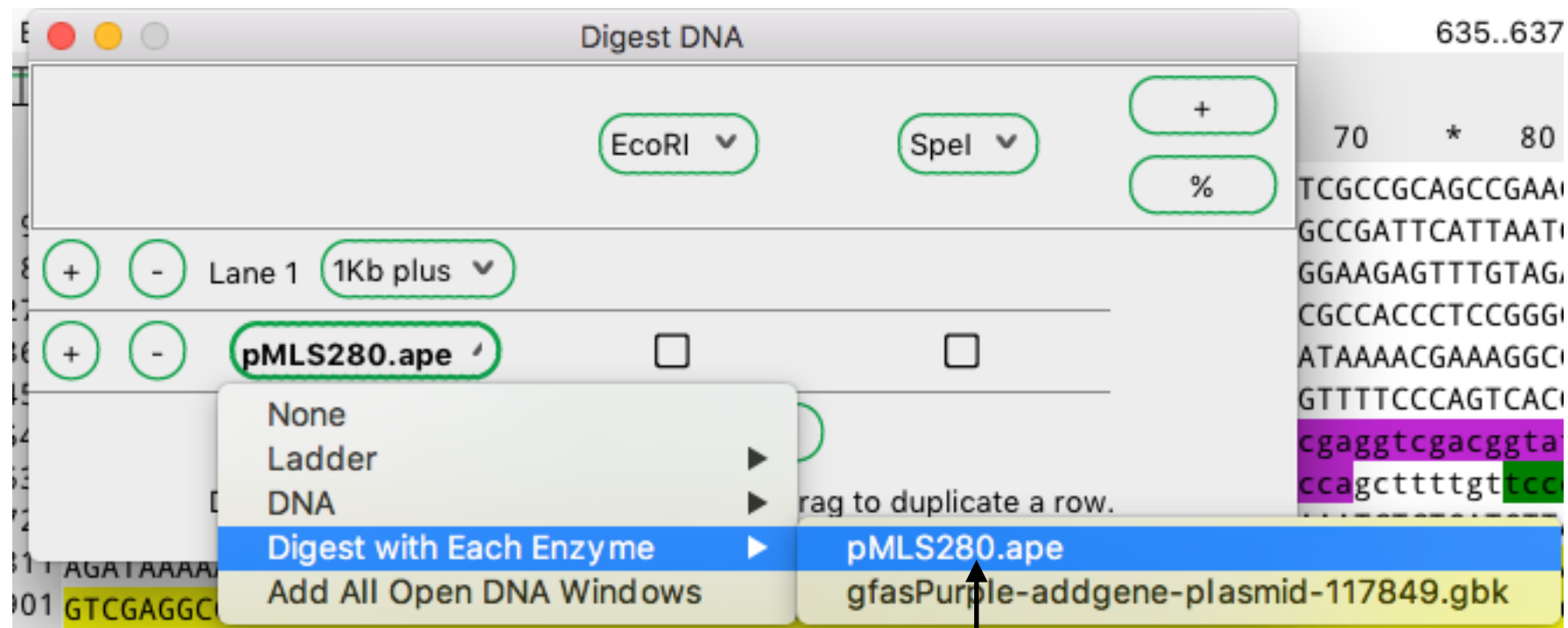


Digestion Dialog



You can also just create a set of new lanes with each open DNA

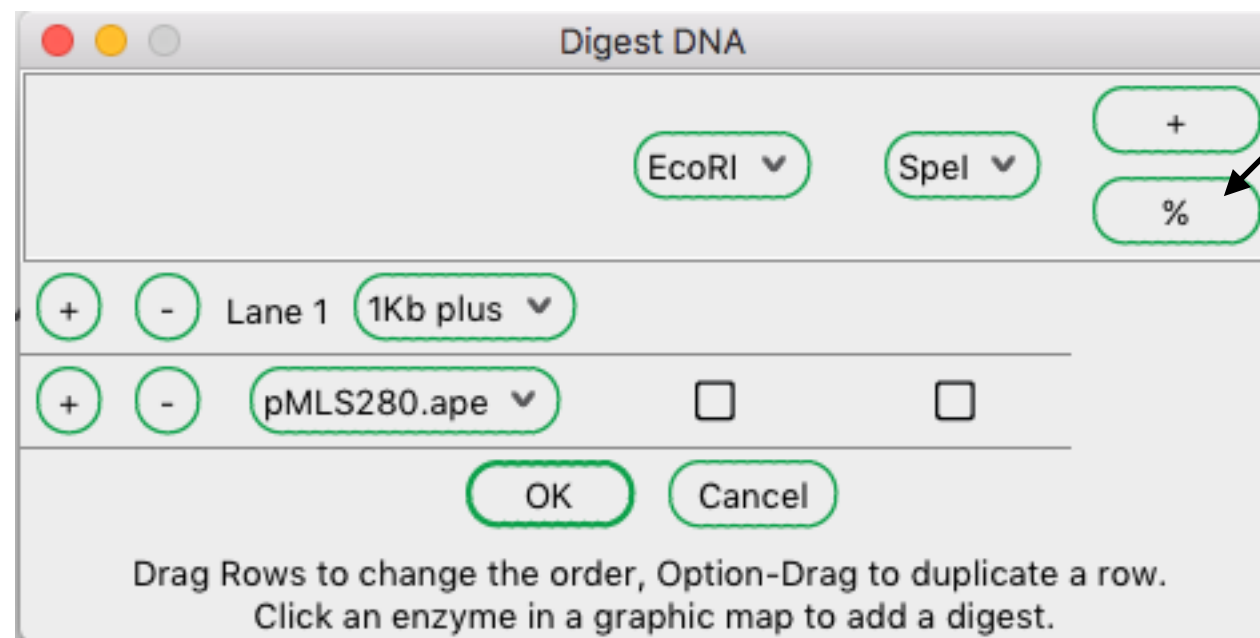
Digestion Dialog



For convenience, you can digest a single DNA with all current enzymes

Digestion Dialog

Press this to do partial digests



The screenshot shows a 'Digest DNA' dialog box. At the top, there are two enzyme selection buttons: 'EcoRI' and 'SpeI', each with a dropdown arrow. To their right are two buttons: a '+' button and a '%' button. An arrow points from the text 'Press this to do partial digests' to the '%' button. Below these are two rows for lane configuration. The first row is labeled 'Lane 1' and has a '1Kb plus' dropdown. The second row has a 'pMLS280.apc' dropdown and two checkboxes. At the bottom are 'OK' and 'Cancel' buttons. A note at the very bottom reads: 'Drag Rows to change the order, Option-Drag to duplicate a row. Click an enzyme in a graphic map to add a digest.'

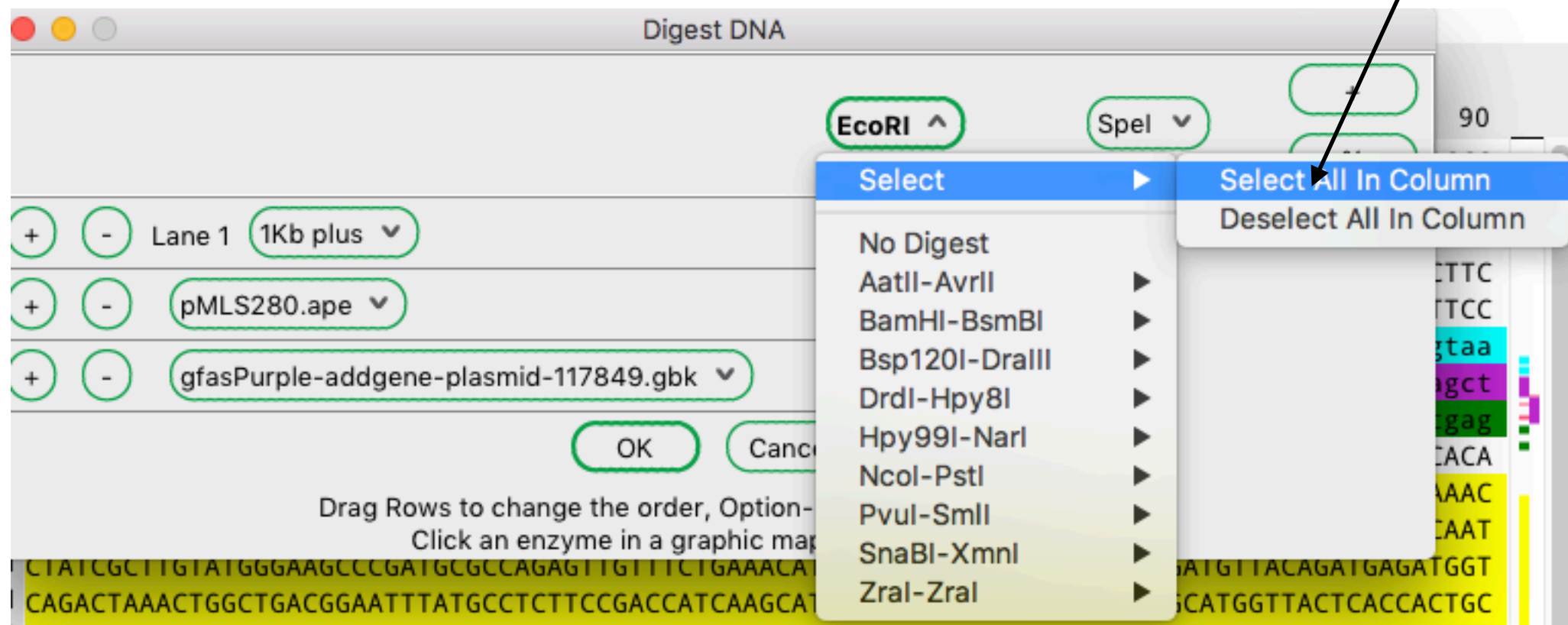
		EcoRI ▾	SpeI ▾	+	%
+	-	Lane 1 1Kb plus ▾			
+	-	pMLS280.apc ▾ <input type="checkbox"/> <input type="checkbox"/>			

OK Cancel

Drag Rows to change the order, Option-Drag to duplicate a row.
Click an enzyme in a graphic map to add a digest.

Digestion Dialog

Press this to select EcoRI in all columns



Digestion Dialog

Digest DNA

EcoRI ▾ SpeI ▾ + %

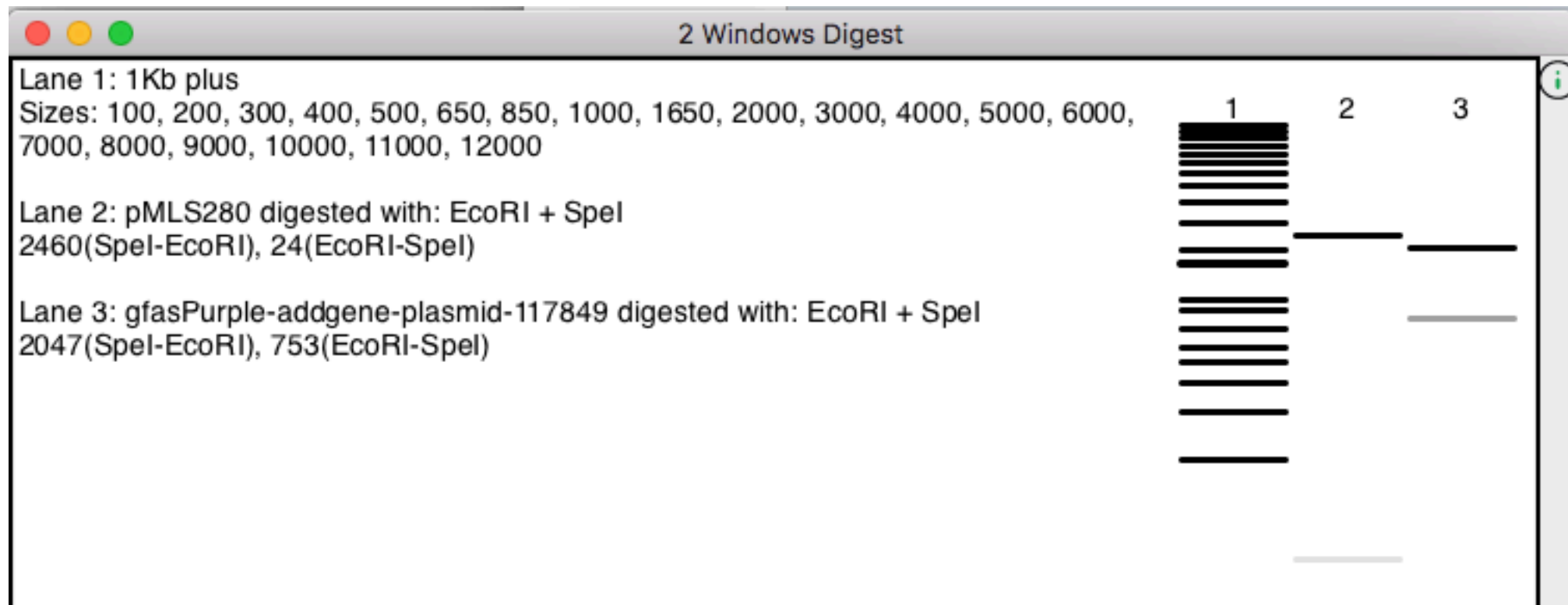
+ -	Lane 1	1Kb plus ▾		
+ -	Lane 2	pMLS280.apc ▾	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
+ -	Lane 3	gfasPurple-addgene-plasmid-117849.gbk ▾	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

OK Cancel

Drag Rows to change the order, Option-Drag to duplicate a row.
Click an enzyme in a graphic map to add a digest.

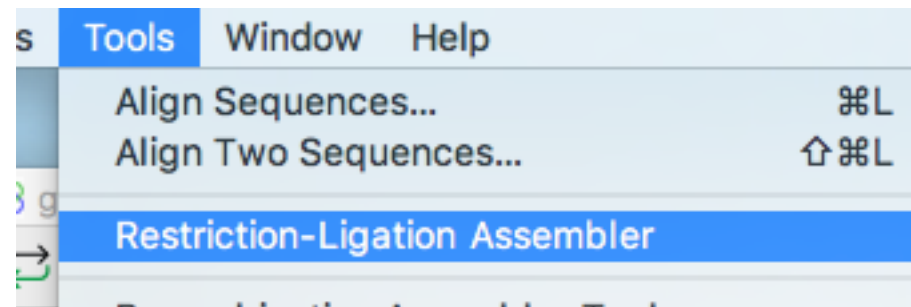
**This will digest pMLS280 and
gfasPurple with EcoRI and SpeI**

Digestion Dialog

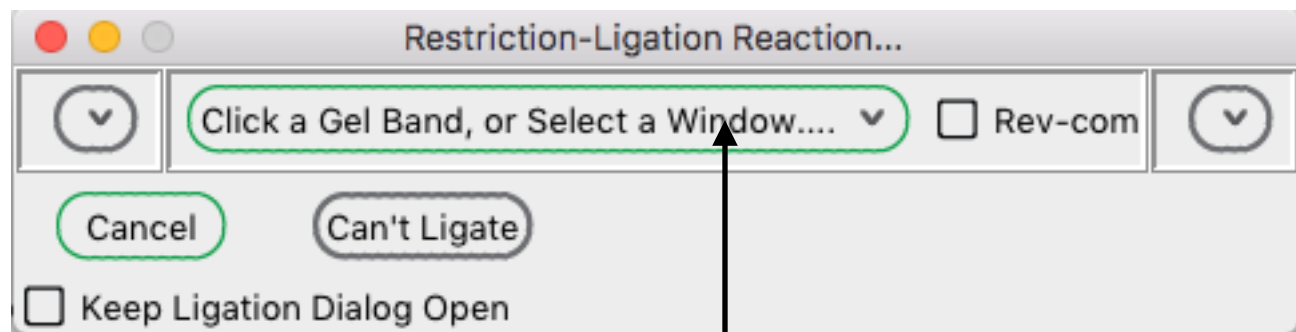
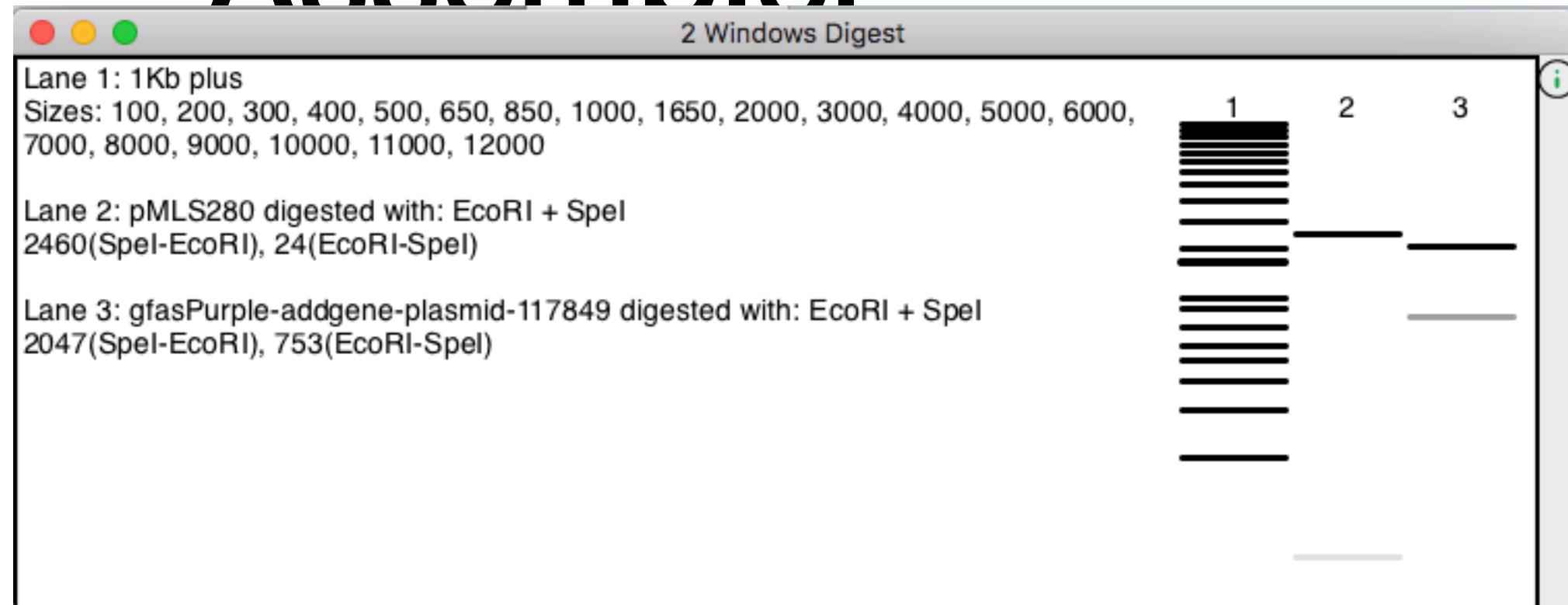


**This will digest pMLS280 and
gfasPurple with EcoRI and SpeI**

Restriction-Ligation Assembler

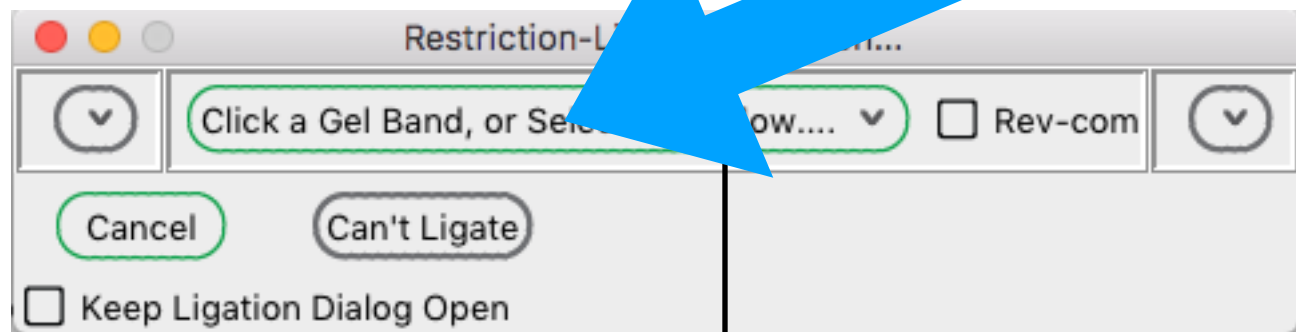
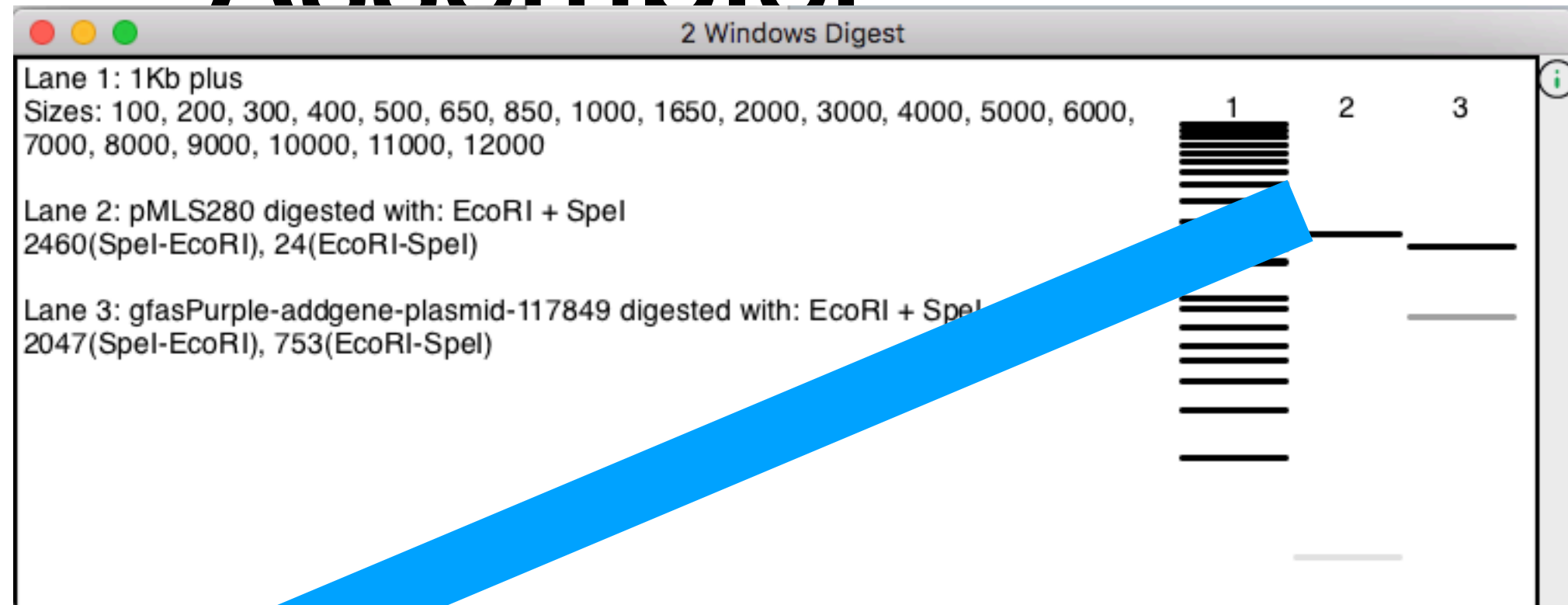


Restriction-Ligation Assembler



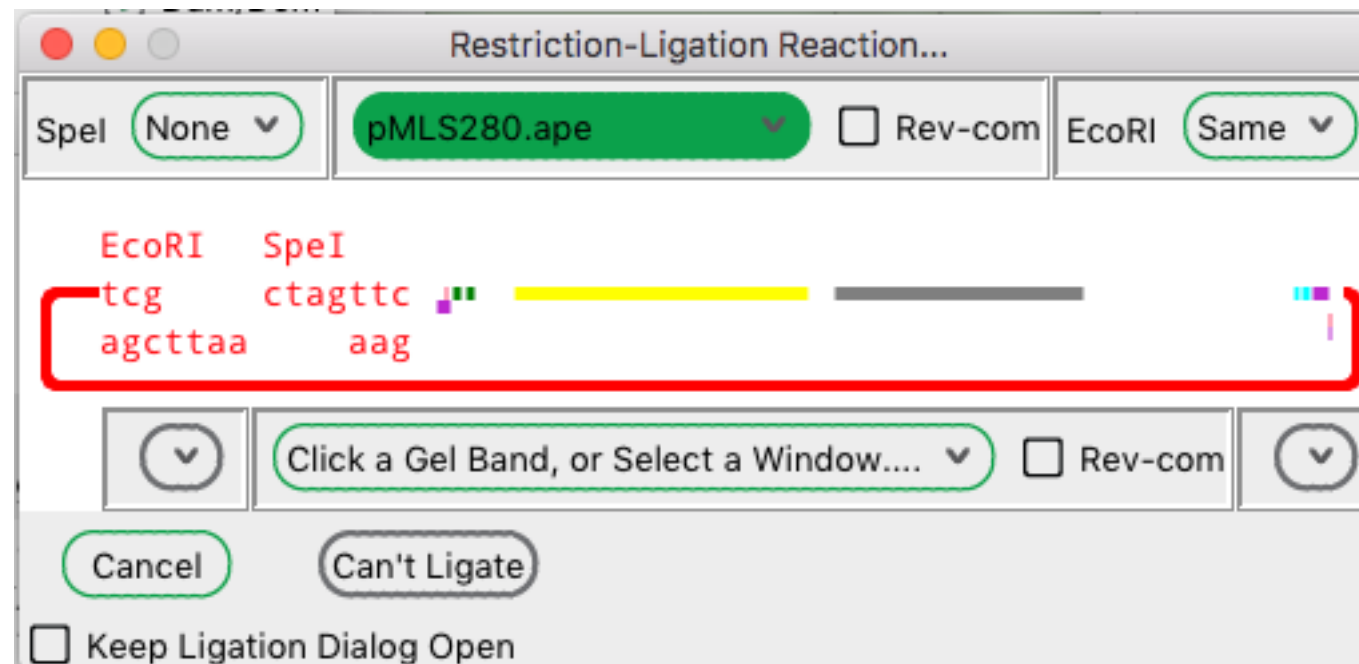
Click and drag a gel lane here

Restriction-Ligation Assembler

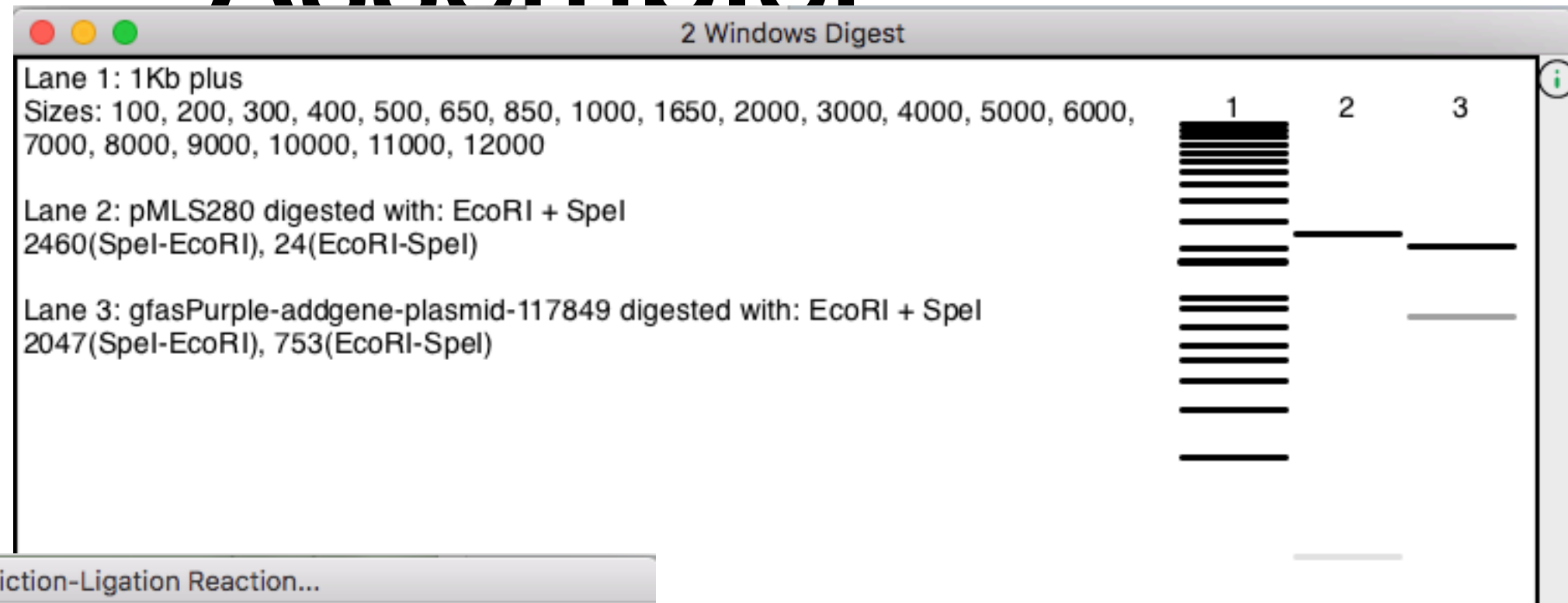


Click and drag a gel lane here

Restriction-Ligation Assembler



Restriction-Ligation Assembler



Restriction-Ligation Reaction...

SpeI None ▾ pMLS280.ape ▾ ☐ Rev-com EcoRI Same ▾

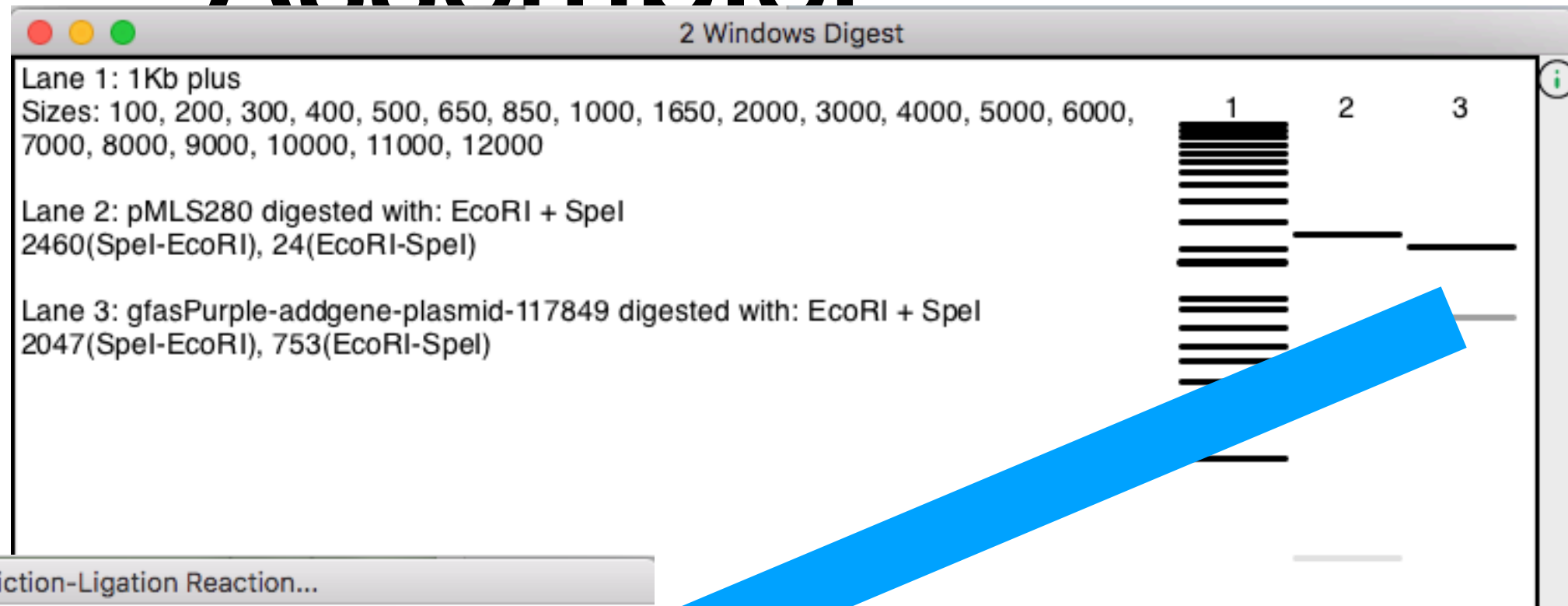
EcoRI SpeI
tcg ctagttc
agcttaa aag

Click a Gel Band, or Select a Window... ▾ ☐ Rev-com

Cancel Can't Ligate

☐ Keep Ligation Dialog Open

Restriction-Ligation Assembler



Restriction-Ligation Reaction...

SpeI None ▾ pMLS280.apc ▾ ☐ Rev-com EcoRI Same ▾

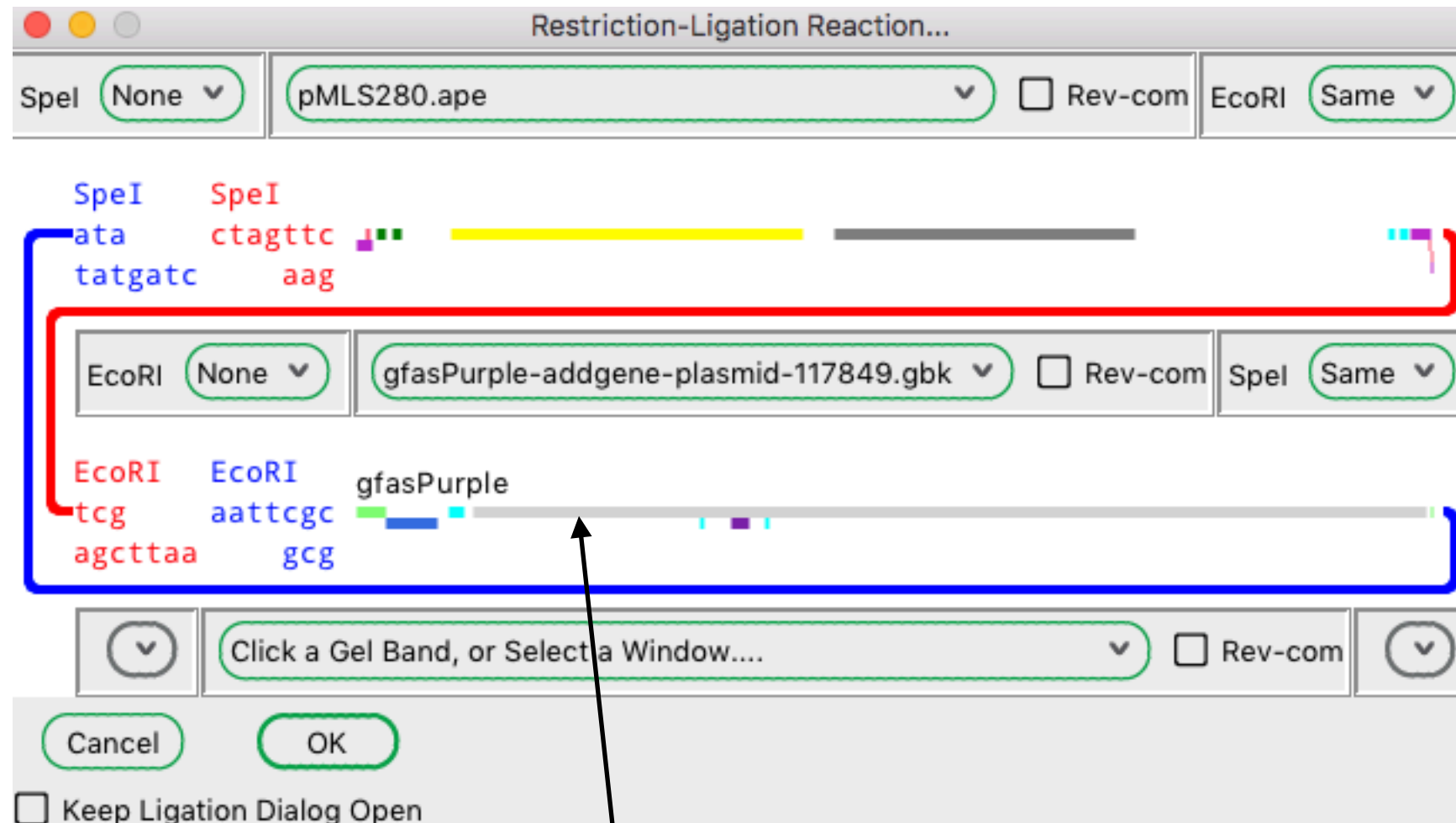
EcoRI SpeI
tcg ctagttc
agcttaa aag

Click a Gel Band, or Select a Window... ▾ ☐ Rev-com

Cancel Can't Ligate

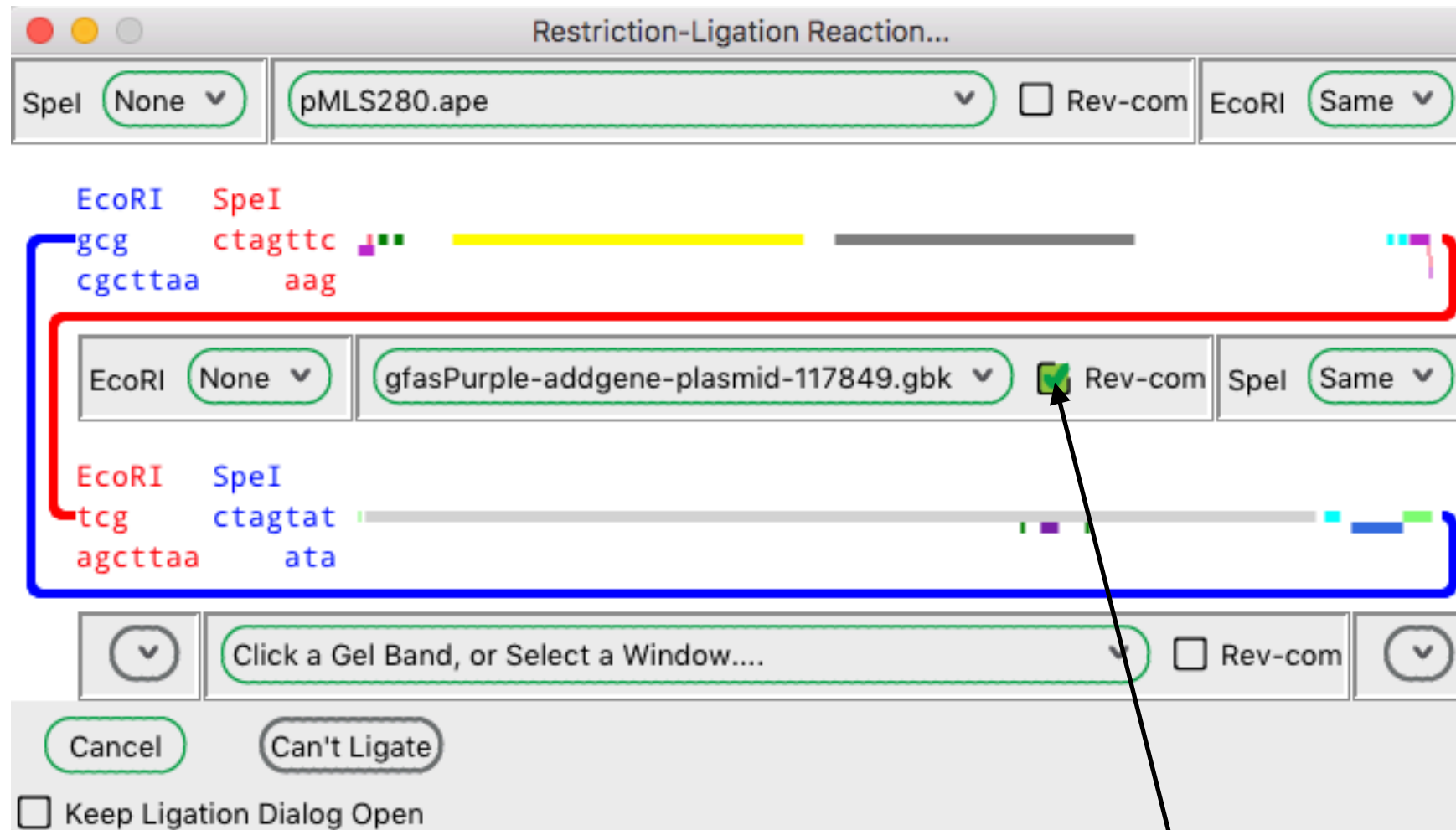
☐ Keep Ligation Dialog Open

Restriction-Ligation Assembler



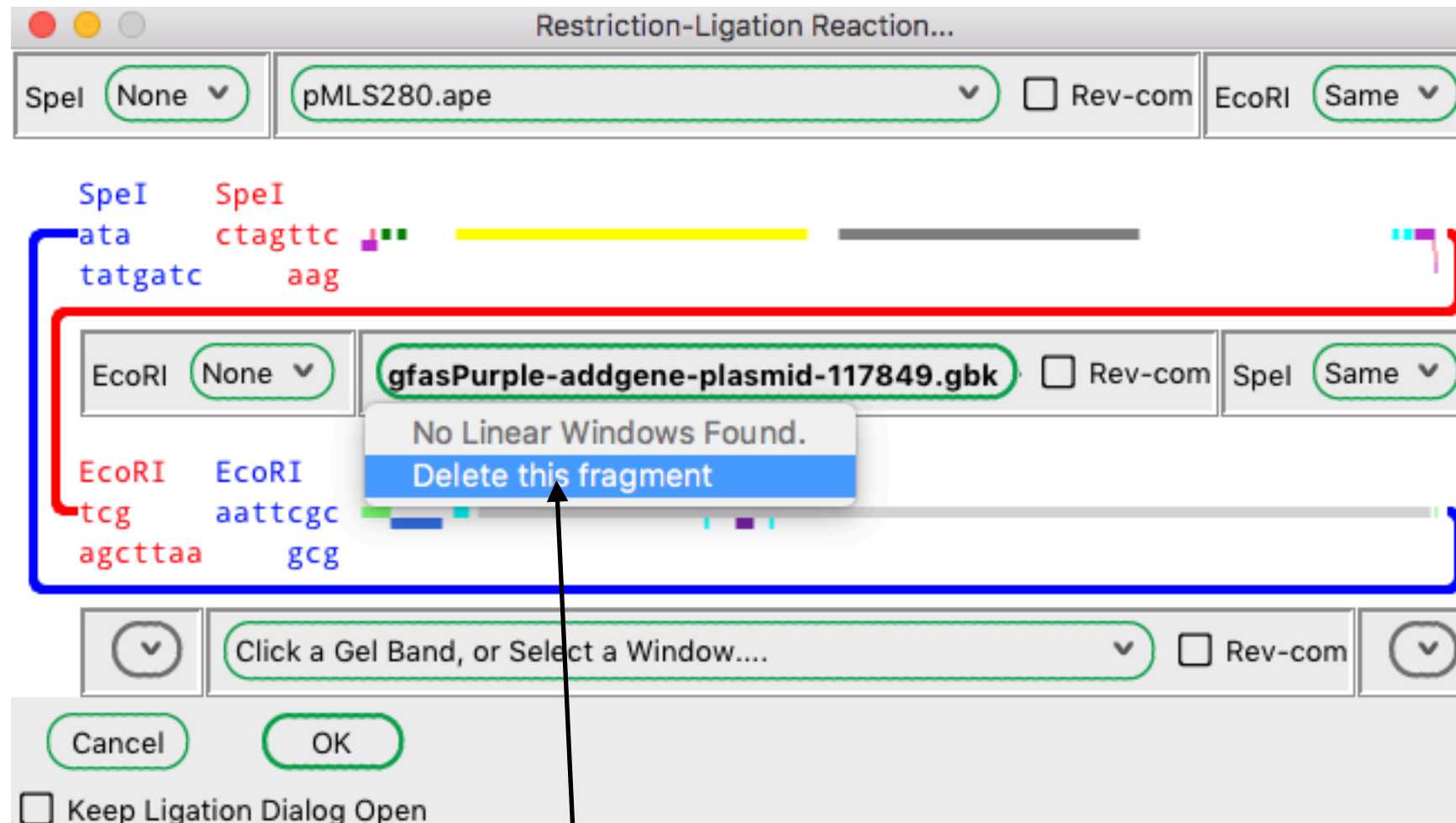
mouse here to see the feature
names

Restriction-Ligation Assembler



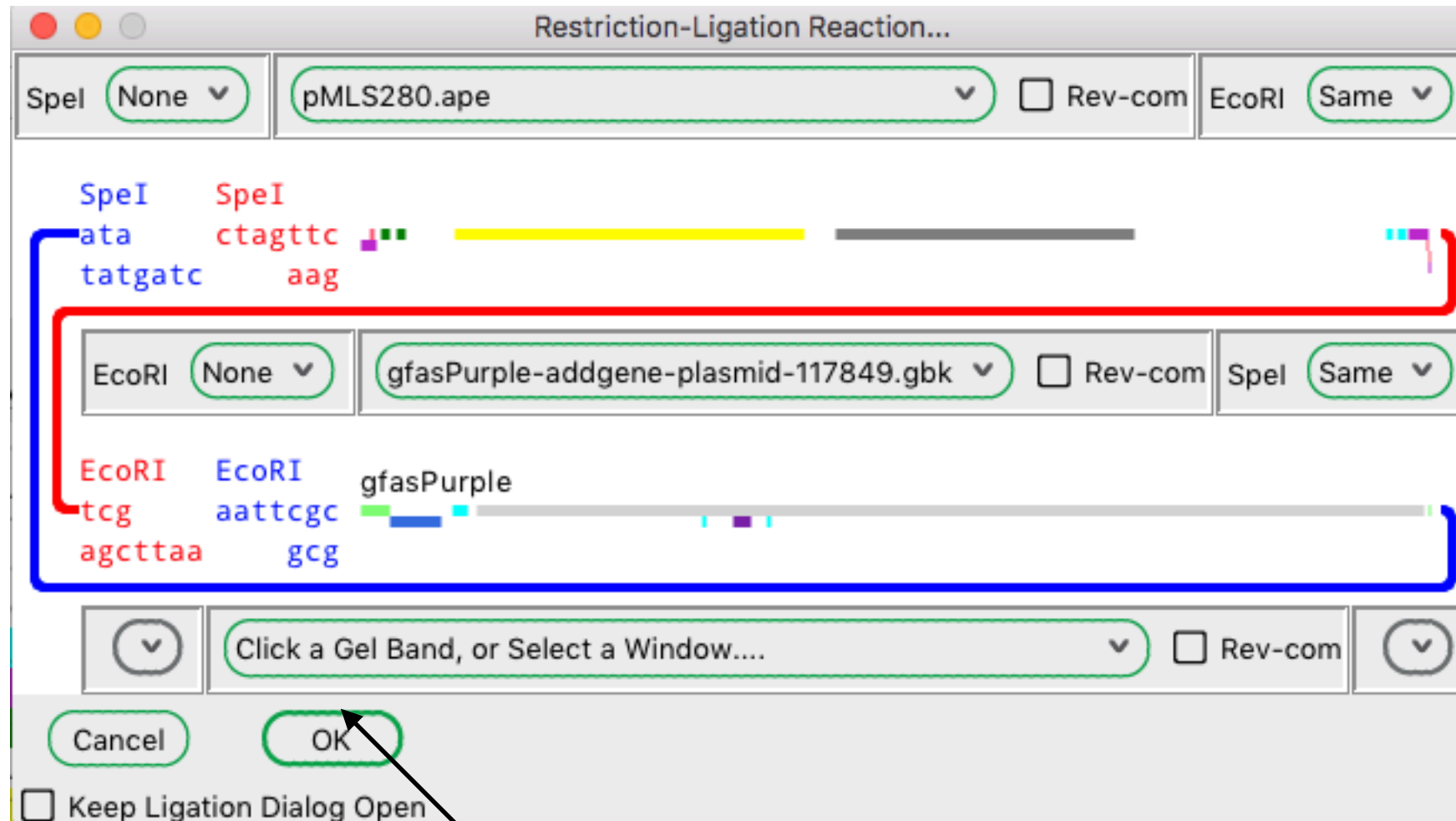
click here to flip a fragment

Restriction-Ligation Assembler



click here to delete a fragment

Restriction-Ligation Assembler

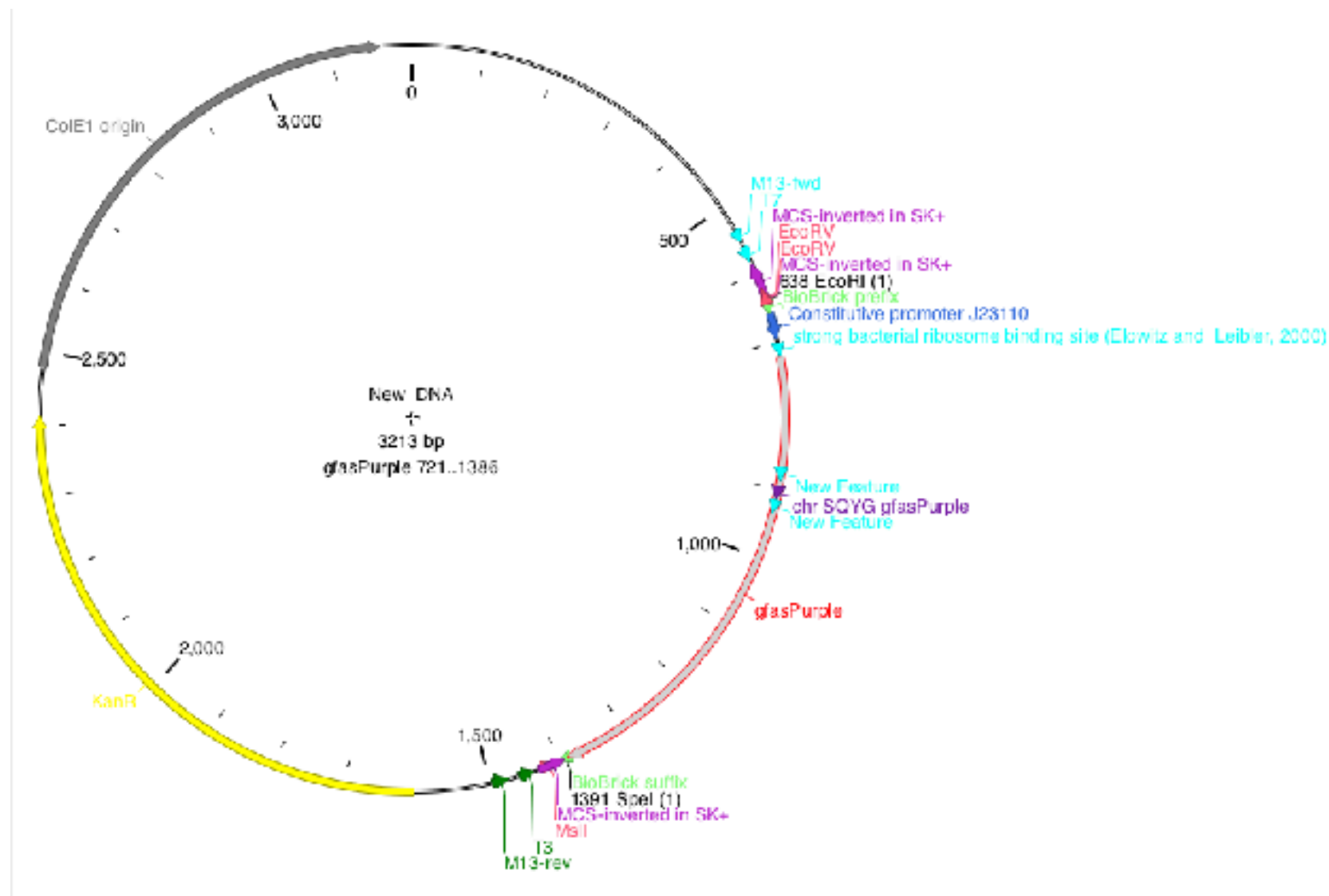


When everything works, you can
click OK

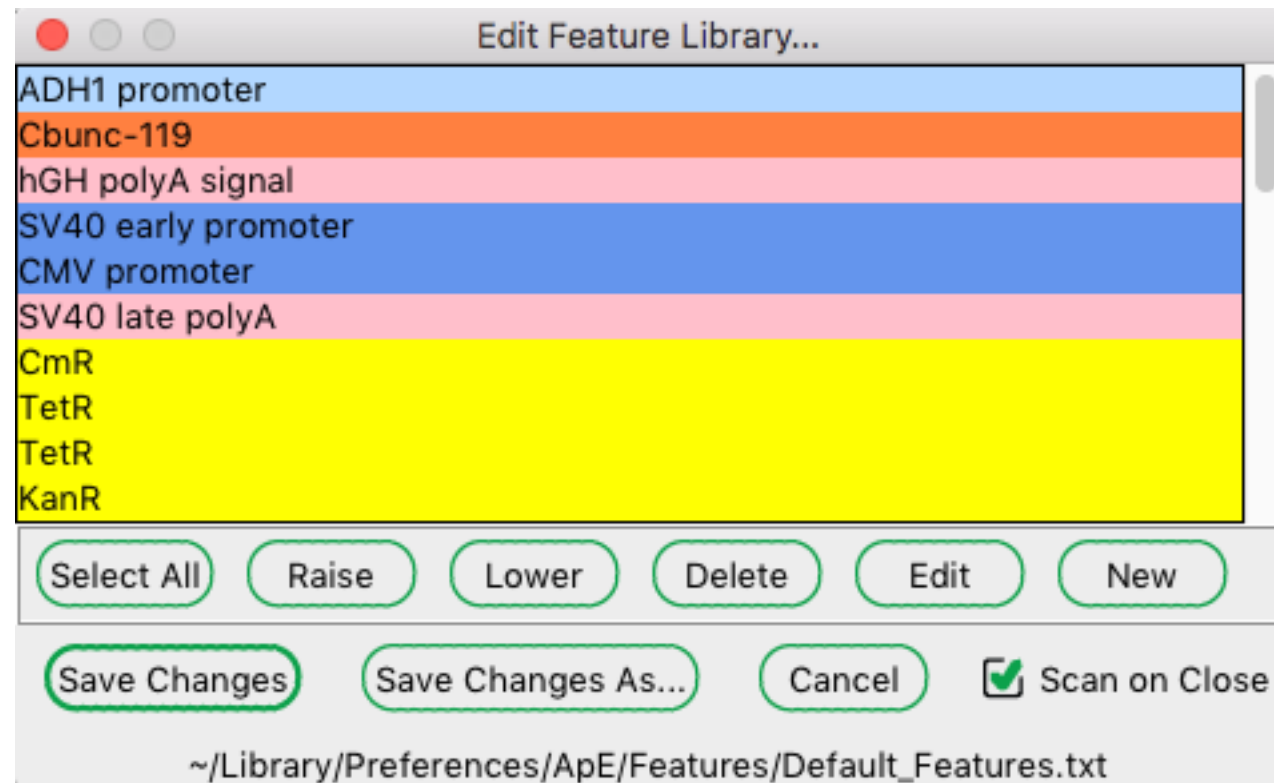
Restriction-Ligation Assembler

Geneious software interface showing a DNA sequence with various annotations. The top bar includes icons for file operations and a "New_DNA" label. The main window displays a sequence with features like "M13-fwd", "T7", "MCS-inverted in SK+", and "EcoRV" highlighted. A "gfasPurple" annotation is visible above the sequence. The sequence itself is color-coded with yellow, green, and red highlights, and a "circular" button is in the top right corner.

Restriction-Ligation Assembler



Feature library



New Feature

1..19 ☐ Rev-Com

Uppercase Only Feature Plus Selection Feature Minus Selection

Add Qualifier

Feature type: misc_feature

Forward color: ■ Reverse color: ■ Same Favorites

Place Directly Above Feature: EcoRV

OK ☐ Make format default for feature type

Features

Current features
under the mouse
pointer are listed
here

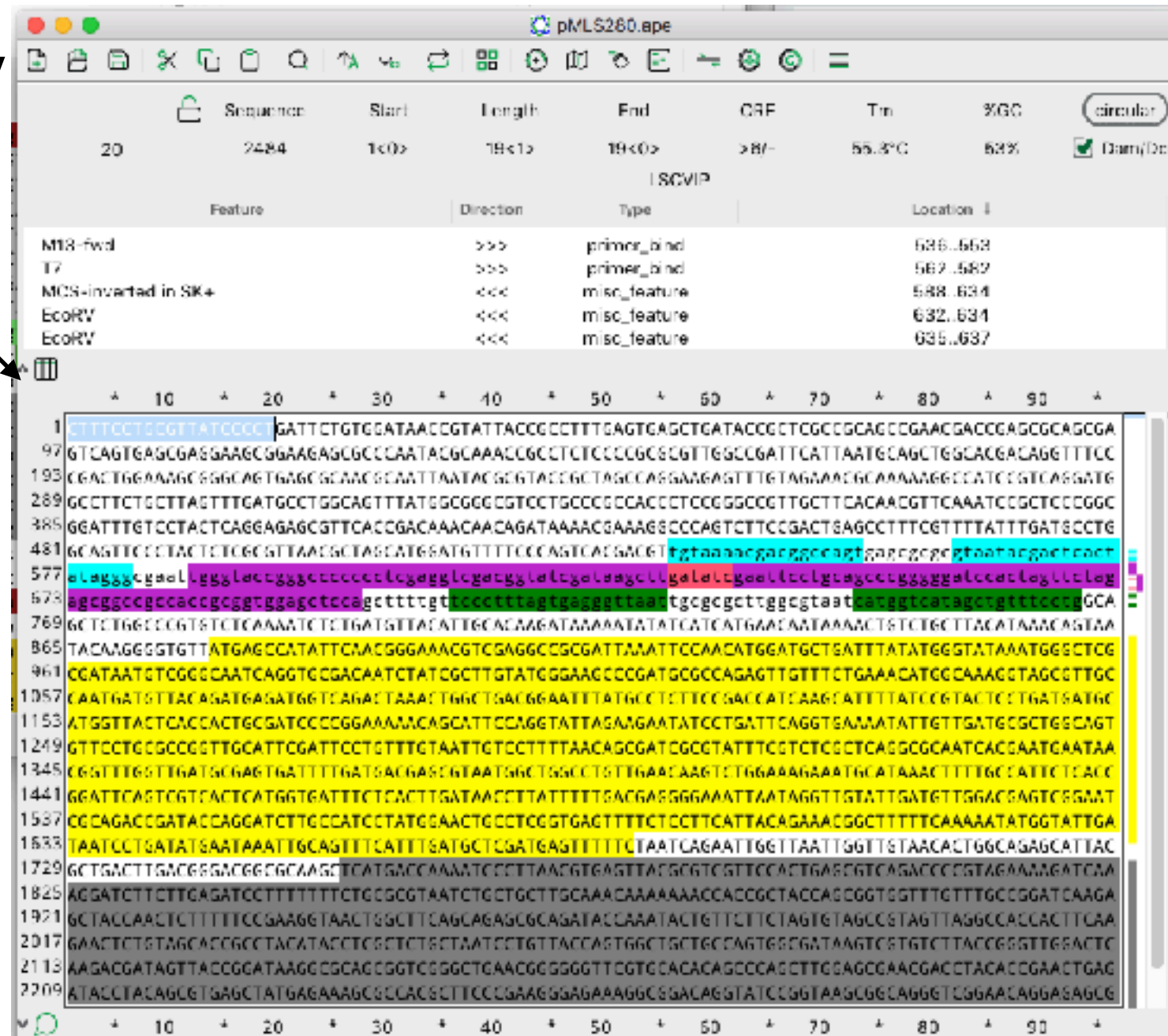
The screenshot shows a bioinformatics software interface with a sequence alignment view and a table of features. The sequence alignment is displayed in a window titled "pML S280.apc". The sequence is shown in a grid with columns numbered 10, 20, 30, 40, 50, 60, 70, 80, 90. The sequence is color-coded by codon, with each codon having a unique color. The features table is located at the top of the window and lists the following features:

Feature	Direction	Type	Location
ColE1 origin	>>>	rep_origin	1754..2440
EcoRV	<<<	misc_feature	532..634
EcoRV	<<<	misc_feature	635..637
KanR	>>>	CDS	878..1684
M13-fwd	>>>	primer_bind	536..553

An arrow points from the text "Current features under the mouse pointer are listed here" to the "KanR" feature in the table.

Features

Click this to hide/
show the feature
table



Features

Right-click here
to edit a feature

The screenshot shows a bioinformatics software window titled 'pML S280.spe'. The main display is a DNA sequence with various features highlighted. A right-click context menu is open over the 'M13-fwd' feature, showing options: 'Edit Feature Name', 'Change Color', 'Edit Feature (Dialog)', 'Arrange', 'Hide Feature', 'Hide All Features', and 'Delete Feature'. The menu is also open over the 'M13-fwd' feature in the list.

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	535..553
M13-rev	<<<	primer_bind	562..582
MCS-In	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

The DNA sequence is displayed below the table, with features highlighted in different colors. The sequence is: 1 CTT... 97 GTC... 193 CCA... 289 GCT... 385 GGA... 481 GCA... 577 GTC... 673 GTC... 769 GCT... 865 TAC... 961 CGA... 1057 CAAT... 1153 ATG... 1249 GTT... 1345 CGG... 1441 GGA... 1537 CCA... 1633 TAA... 1729 GCT... 1825 AGG... 1921 GCT... 2017 GAA... 2113 AAG... 2209 ATC...

Features

**Click this to sort
the feature table**

Sequence Insert@

2484 1366<0>

circular

Dam/Dcm

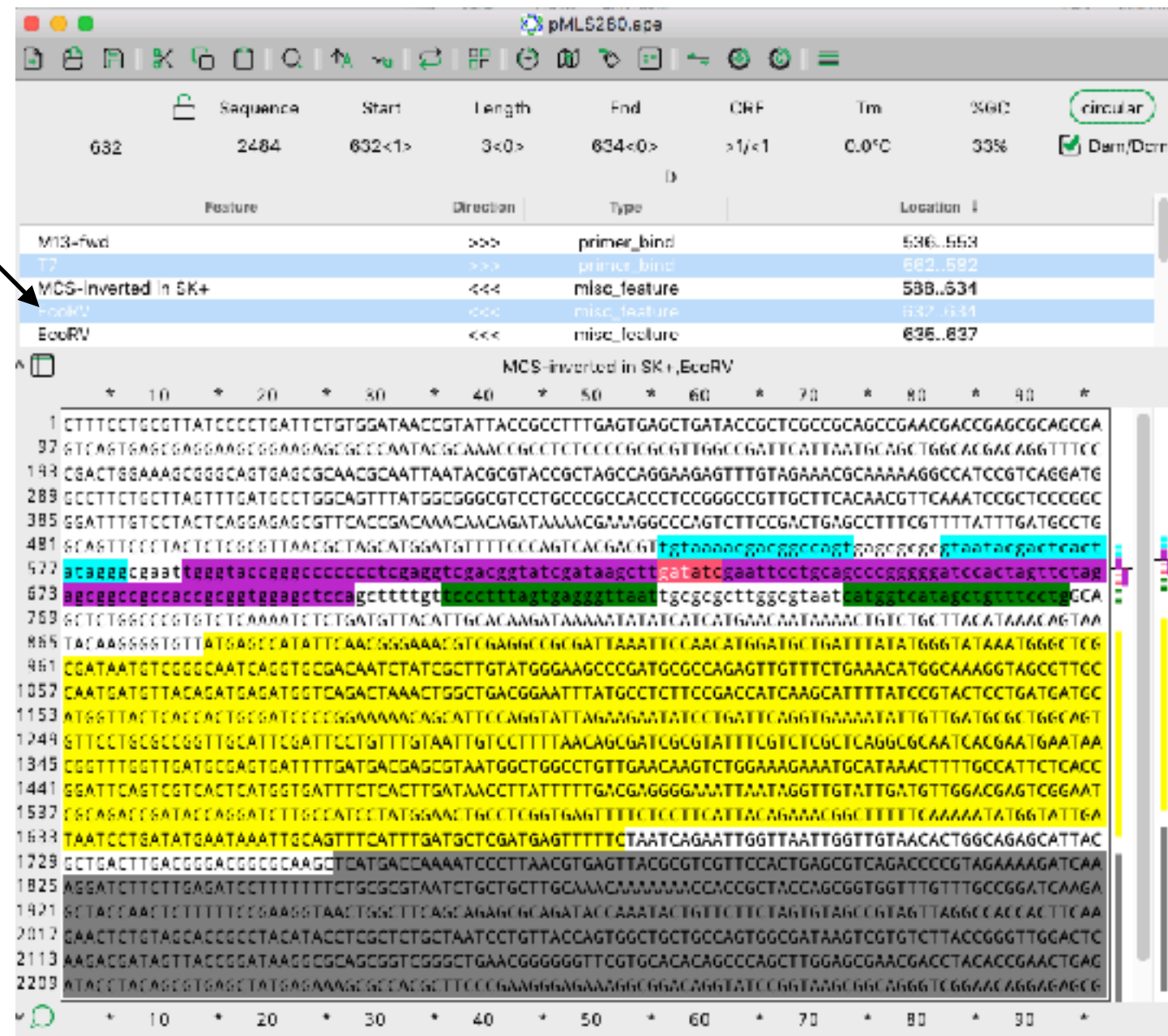
Feature ↓	Direction	Type	Location
ColE1 origin	>>>	rep_origin	1754..2440
EcoRV	<<<	misc_feature	632..634
FcoRV	<<<	misc_feature	635..637
KanR	>>>	CDS	878..1684
M13-fwd	>>>	primer_bind	538..553

1 CTTTCCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCTTTGAGTGAGCTGATACCGCTCECCGAGCCGAACGACCGAGCGCAGCGA
97 GTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAACCGCCTCTCCCGCGCGTTGGCCGATTCTTAATGCAGCTGGCAGCAGAGGTTTCC
193 CGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTGAGGATG
289 GCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGCGGGCGTCTGCGCCGACCCCTCCGGGCCGTTGCTTCACAACGTTCAAATCCGCTCCCGGC
385 GGATTTGTCTACTCAGGAGAGGTTTACCGACAAACAACAGATAAAACGAAAGGCCAGTCTTCCGACTGAGCCTTTCTGTTTTATTTGATGCTG
481 GCAGTTCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCCAGTCACGACGTtgtaaaaacgacggccagtgagcgcgcgtaatacagactcact
577 atagggcggaattgggtaccggggccccccctcgagggtcgacgggtatcgataagcttgatatagaattcctgcagcccgggggggtccactagttctag
673 agcgggcgccaccgcgggtggagctccagcttttgcctttagtgaggggttaattgcgcgcgttgcgtaaatcatggtcatagctgtttcctcgc
769 GCTCTGGCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAA
865 TACAAGGGGTGTTATGAGCCATATTCAACGGGAACGTCGAGGCCGCGATTAAATTCACATGATGCTGATTTATATGGGTATAAATGGGCTCG
961 CGATAATGTGGGCAATCAGGTGCGACAATCTATCGCTTGATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAGGTAGCGTTGC
1057 CAATGATGTTACAGATGAGATGCTCAGACTAACTGCTGACGGAATTTATGCTCTCTCCGACCATCAAGCATTTTATCCGTACTCTCTGATGATGC
1153 ATGGTTACTCACCCTGCGATCCCCGAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATCGCTGGCAGT
1249 GTTCCTGCGCCGTTGCATTGATTCCTGTTTGTAAATTGTCCTTTTAAACAGCGATCGGTATTTCTGCTCGCTCAGGCGCAATCAGCAATGAATAA
1345 CGTTTGGTTGATGCGAGTGATTTTGTATGACGAGCGTAATGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACC
1441 GGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTGGACGAGGGGAAATTAATAGTGTGATTGATGTTGGACGAGTCGGAAT
1537 CGCAGACCGATACCAGGATTTGCCATCCTATGGAACCTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGA
1633 TAATCCTGATATGAATAAATTTGCAGTTTCATTTGATGCTCGATGAGTTTTCTTAATCAGAATTGGTTAATTGTTGTAACACTGGCAGAGCATTAC
1729 GCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCTCGTTCCACTGAGGTCAGACCCCGTAGAAAAAGATCAA
1825 AGGATCTTCTTGAATCTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAAACACCGCTACCAGCGGTGGTTTGGTTTGGCGGATCAAGA
1921 GCTACCAACTCTTTTCCGAAAGTAATGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTTCAA
2017 GAACTCTGTAGCACCBCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTC
2113 AAGACGATAGTTACCGGATAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAAGCAGCTACACCGAAGTGA
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAAACAGGAGAGCG

Features

Command-click
here to select
multiple features

Shift-click here
to select a range
of features



The screenshot shows a bioinformatics software interface with a sequence alignment and a table of features. The sequence alignment is displayed in a window titled "pML5280.sce". The sequence is 2484 bp long, with a GC content of 33%. The alignment is shown in a window titled "MCS-inverted in SK+ EcoRV". The sequence is displayed in a window titled "MCS-inverted in SK+ EcoRV". The sequence is displayed in a window titled "MCS-inverted in SK+ EcoRV".

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	536..553
T7	>>>	primer_bind	582..592
MCS-inverted in SK+	<<<	misc_feature	538..631
EcoRV	<<<	misc_feature	632..637

The sequence alignment is displayed in a window titled "MCS-inverted in SK+ EcoRV". The sequence is displayed in a window titled "MCS-inverted in SK+ EcoRV". The sequence is displayed in a window titled "MCS-inverted in SK+ EcoRV".

Features

**Right-click here
to edit a range of
features**

The screenshot displays the SnapGene software interface for editing a plasmid map. The top toolbar includes standard file and editing icons. Below the toolbar, the plasmid map is shown with a circular layout. The map contains several features, including 'MCS-inverted in SK+', which is currently selected. A context menu is open over this feature, providing various editing options. The sequence view at the bottom shows the DNA sequence with various features highlighted in different colors.

Feature	Direction	Type	Location
MCS-inverted in SK+	>>>	primer_bind	536..553
7	>>>	primer_bind	562..562
MCS-inverted in SK+	<<<	misc_feature	588..634
7	<<<	misc_feature	632..634
7	<<<	misc_feature	635..637

The context menu options are:

- Edit Feature Name
- Change Color
- Edit Feature (Dialog)
- Arrange
- Hide Feature
- Hide All Features
- Delete Feature
- Delete 4 Selected Features
- Raise 4 Selected Features to Top
- Lower 4 Selected Features to Bottom
- Hide 4 Selected Features
- Show 4 Selected Features

The sequence view at the bottom shows the DNA sequence with various features highlighted in different colors.

Features

Right-click here
to edit a feature's
direction

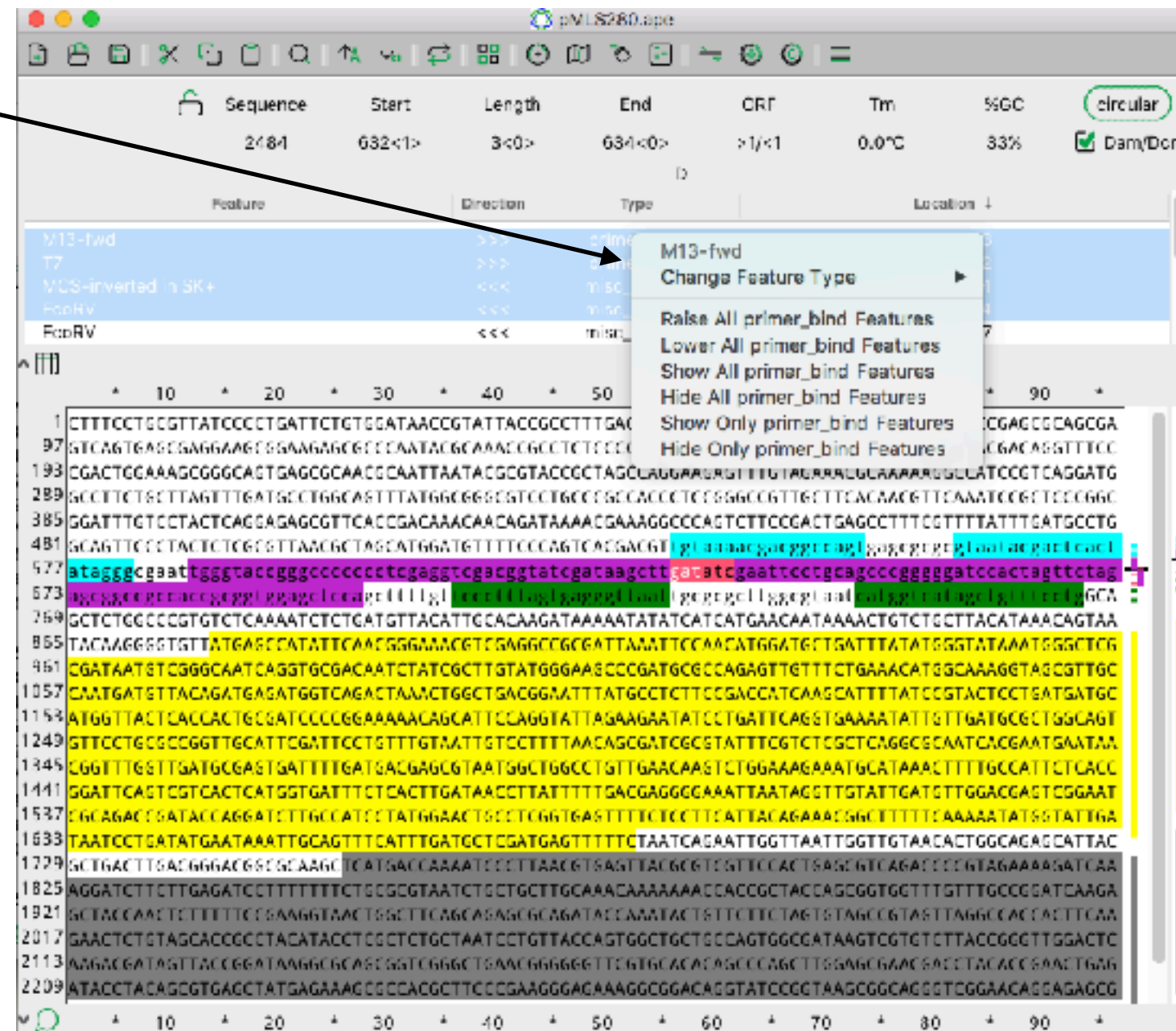
The screenshot shows a bioinformatics software interface with a sequence viewer. The top toolbar includes icons for file operations, editing, and viewing. Below the toolbar is a table with columns: Sequence, Start, Length, End, ORF, Tm, %GC, and a 'circular' checkbox. The sequence is 632 bases long, starting at 7484 and ending at 634. The ORF is >1/<1, Tm is 0.0°C, and %GC is 33%. Below this is a table of features:

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	536..553
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	586..634
EcoRV	<<<	misc_feature	632..637

A right-click context menu is open over the 'T7' feature, showing options: 'T7' and 'Reverse Feature'. The sequence viewer below shows the DNA sequence with various features highlighted in different colors. The sequence is: 1 CTTTCCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCGGAGCCGAACGACCGAGCGGAGCGA 97 CTCAGTCAGCGAGGAAGCGGAGAGCGCCCAATACGCAACCGCCTCTCCCGCGCGTTGGCGGATTCAATATGCAGCTGGCAGGACAGGTTTCC 193 CGACTGGAAAGCGGSCASTGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAGGCCATCCGTCAGGATG 289 GCCTTCTGCTTAGTTTGTATGCTGGCAGTTTATGGCGGCGCTCTGCGGCCACCTCCGGGCGGTTGCTTACACACGTTCAATCCGCTCCCGGC 385 GGATTTGCTCTACTCAGGAGAGCGTTACCGGACAAACACAGATAAAGCAAAAGGCCAGTCTTCCGACTGAGCCTTTGCTTTTATTTGATGCTG 481 GCAGTTCCCTACTCTCGGTTAAGCTAGCATGGATGTTTTCCAGTCACGACGTGtaaaacgagggcagtgagcgggtaatacagactcact 577 ataggcggaattgggtaccgggccccccctcgaggctcgacgggtatcgataagcttatatcgaaattcctgcagcccgsgggatccactagttctag 673 agcggcgccaccgcggtggagctccagctttgttcccttttagtgagggttaattgcgcgcttgccgtaattcatggtcagctgtttccctgSCA 769 GCTCTGCCCGTGTCTCMAATCTCTGATGTTACATTGCACAGATAMAAATATATCATCATGAACMAAAMACTGTCTGCTTACATAAACAGTAA 865 TACAAGGGTGTATCAGCCATATTCAACGGGAACCGTCGAGGCGCGGATTAAATTCACACATGATGCTGATTTATATGGGTATAAATGGGCTCG 961 CGATAATGTCGGCAATCAGGTGCGAATCTATCGCTTGTATGGAAGCCCGATCGCCACAGTTGTTTCTSAACATGGCAAGGTAGCGTTGC 1057 CAATGATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTATCCGTAATCCTGATGATGC 1153 ATGETTACTCACCCTGCGATCCCGGAAAAACAGCATTCCAGGTATTAGAAAGATATCCTGATTCAAGTGAAATATTGTTGATGCGCTGGCAGT 1249 GTTCCTGCGCGGTTGCAATCGATTCTGTTTGTAAATTTGCTTTTAAACAGCATCGCGTATTTGCTCTGCTCAGGCGCAATCAGCAATGAATA 1345 CGGTTTGGTTGATGCGAGTGATTTTGTATGACGAGCGTAATGGCTGGCTGTTCAACAAGCTCTGGAAGAAATGCATAAACTTTTSCCATTCTCACC 1441 GGATTCAGTCGTCACTCATGCTGATTTCTCACTTGATAACCTTATTTTGAAGAGGGGAAATTAATAGGTTGATTGATGTTGACGAGTCGGAAT 1537 CGCAGACCGATACCAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTCTCCTTCATTACAGAAACGGCTTTTCAAAATATGATATTGA 1633 TAATCCTGATATGAATAAATTGCAGTTTCATTGTATGCTCGATGAGTTTCTTAATCAGAATTGTTAATTGTTGTAACACTGCGAGAGCATTAC 1729 GCTCACTTGACGCGGACGGCGCAAGCTCATSACCAAAATCCCTTAACGTGASTTACCGGTGCTTCCACTGAGGCTCAGACCCCGTAGAAAAGATCAA 1825 AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCACCAAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGATCAAGA 1921 GCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAA 2017 GAACCTCTGTAGCACCAGCTACATACCTCGCTCTGCTAATCTGTTACCAAGTGGCTGCTGCCAGTGCGGATAAGTGTGCTTACCGGTTGGACTC 2113 AAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTGCTGCACACAGCCAGCTTGGAGCGAACGACCTACACGAACTGAG 2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCGAGGTCGGAACAGGAGAGCG

Features

**Right-click here
to edit a feature's
type, or to raise,
lower or hide all
of a type of
feature**



Features

The screenshot shows the pML5280.spe software interface. At the top, there's a toolbar with various icons. Below it, a summary bar displays sequence statistics: Sequence (2484), Start (832<1>), Length (8<0>), End (834<0>), ORF (>1/<1), Tm (0.0°C), %GC (38%), and a 'circular' checkbox. Below this is a table of features:

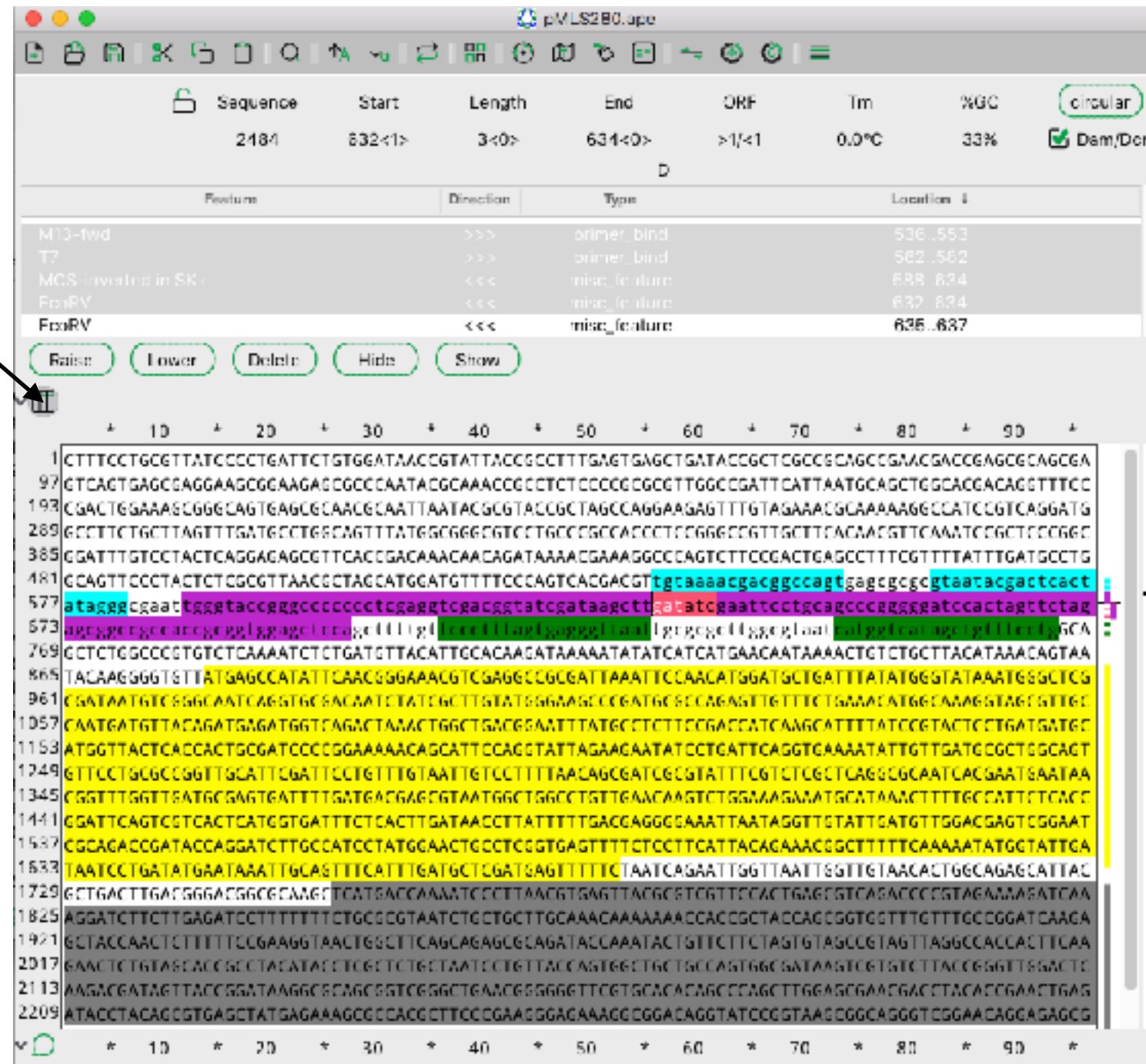
Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	832-834
T7	>>>	primer_bind	832-834
MCS: inverted in SK+	<<<	misc_feature	832-834
FcoRV	<<<	misc_feature	832-834
FcoRV	<<<	misc_feature	832-834

Below the table is a sequence viewer showing a DNA sequence with various features highlighted in different colors. A right-click context menu is open over the 'T7' feature, showing options: 'Uppercase Only', 'Feature Plus Selection', and 'Feature Minus Selection'. An arrow points from the text 'Right-click here to edit a feature's range' to the context menu.

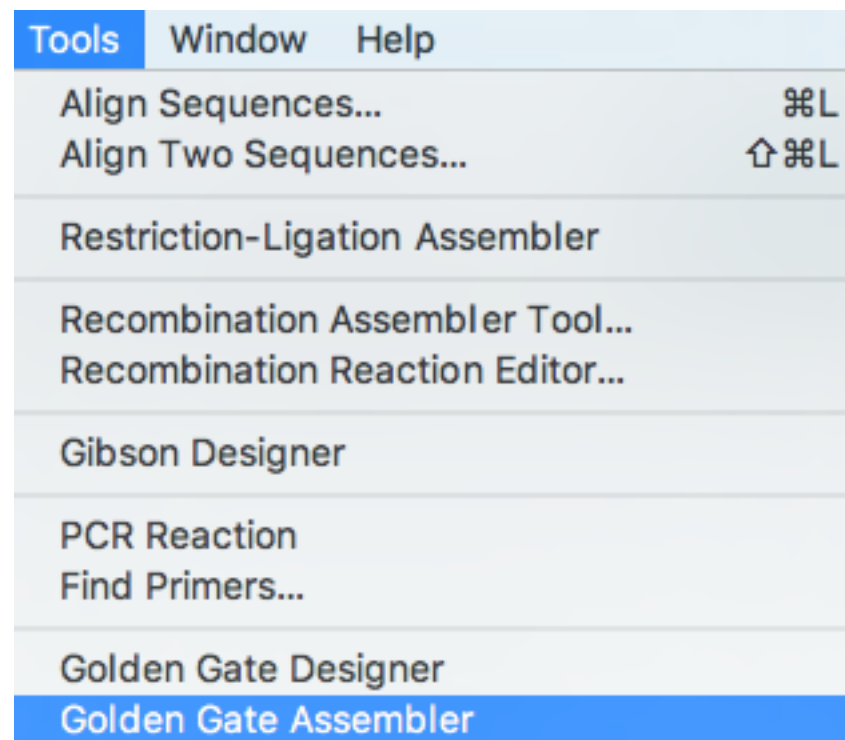
Right-click here
to edit a feature's
range

Features

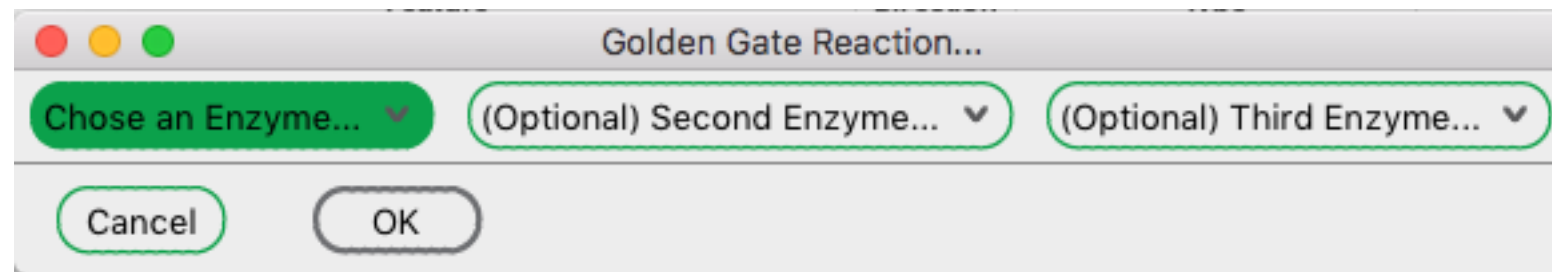
**Shift-click this to
hide/show the
feature table
buttons**



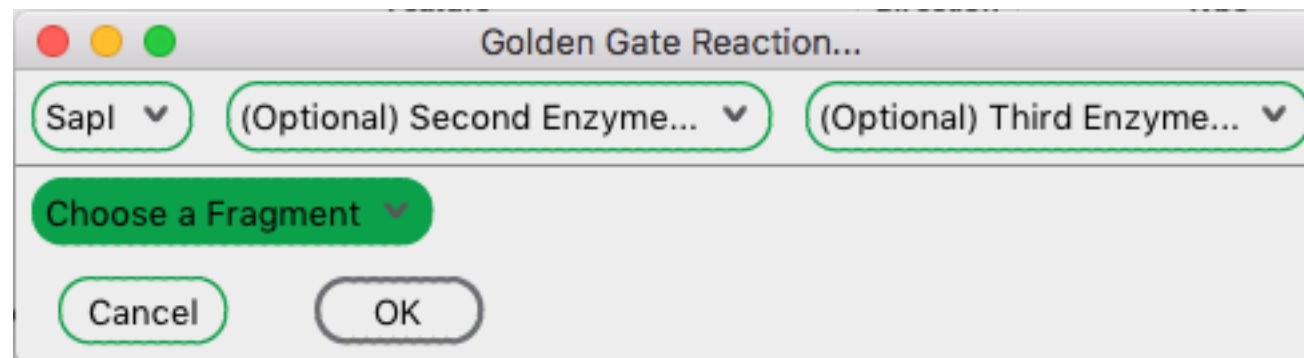
Golden Gate Assembler



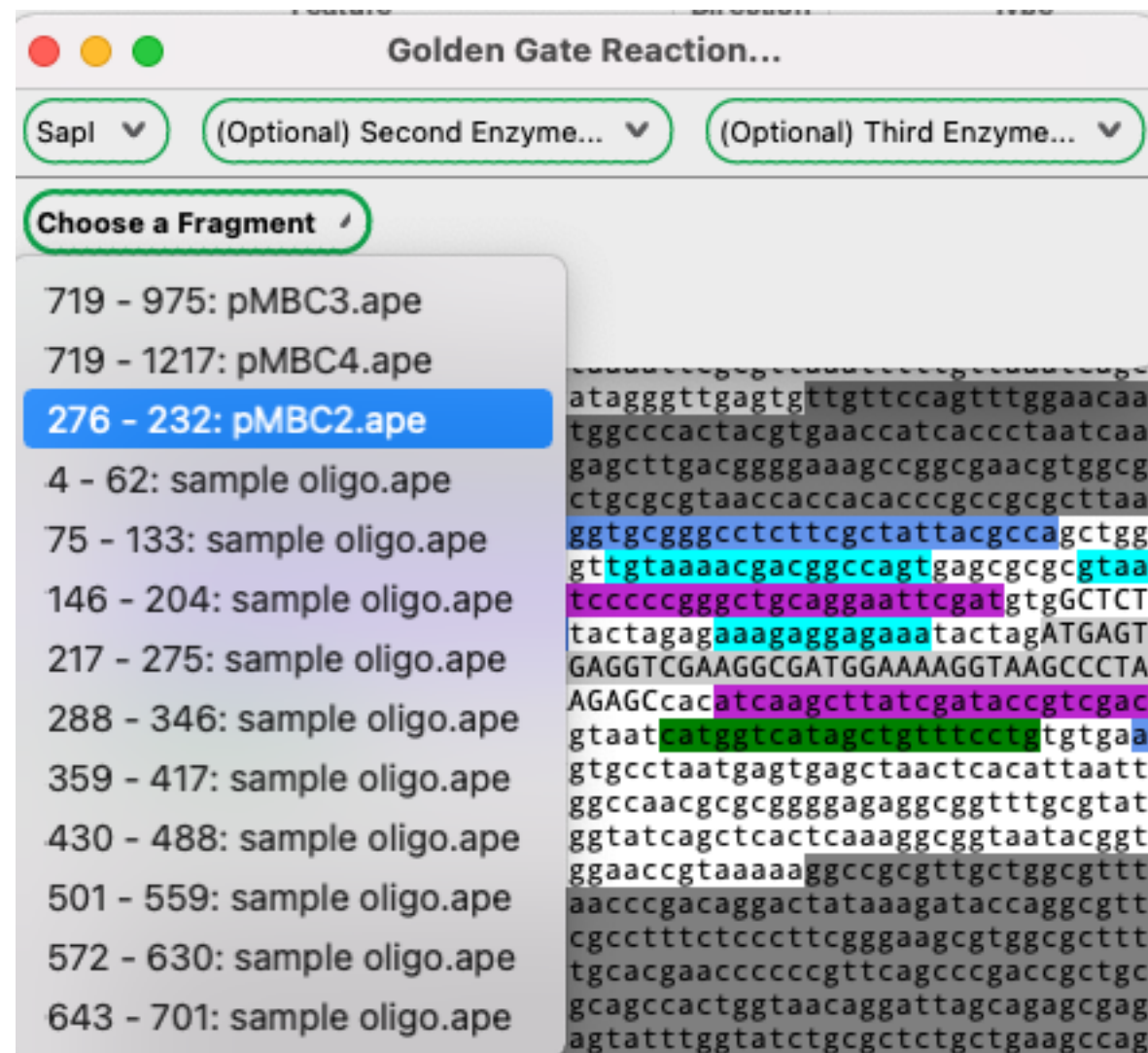
Golden Gate Assembler



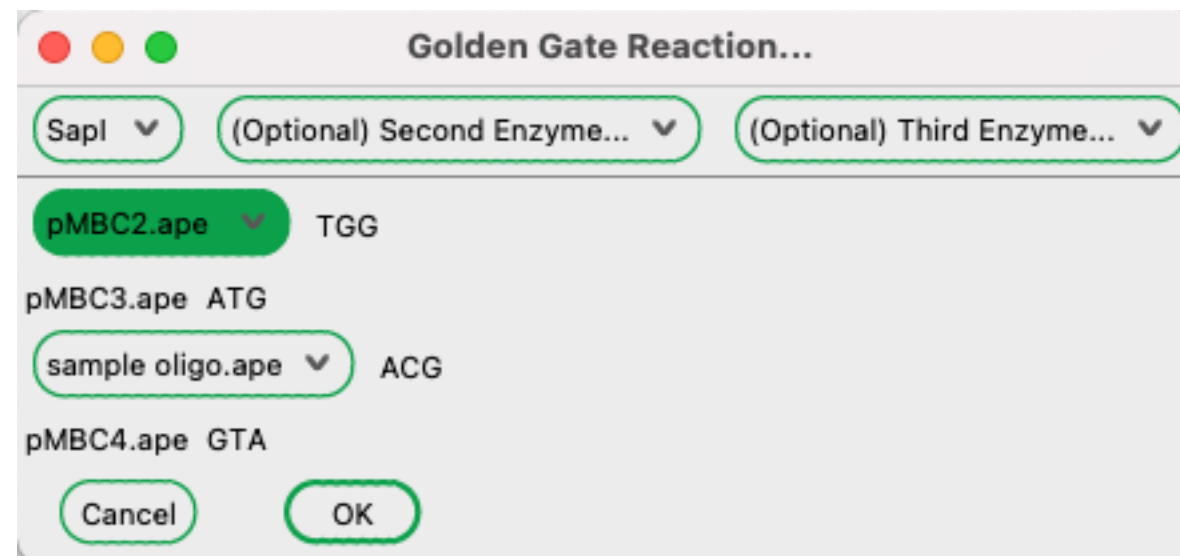
Golden Gate Assembler



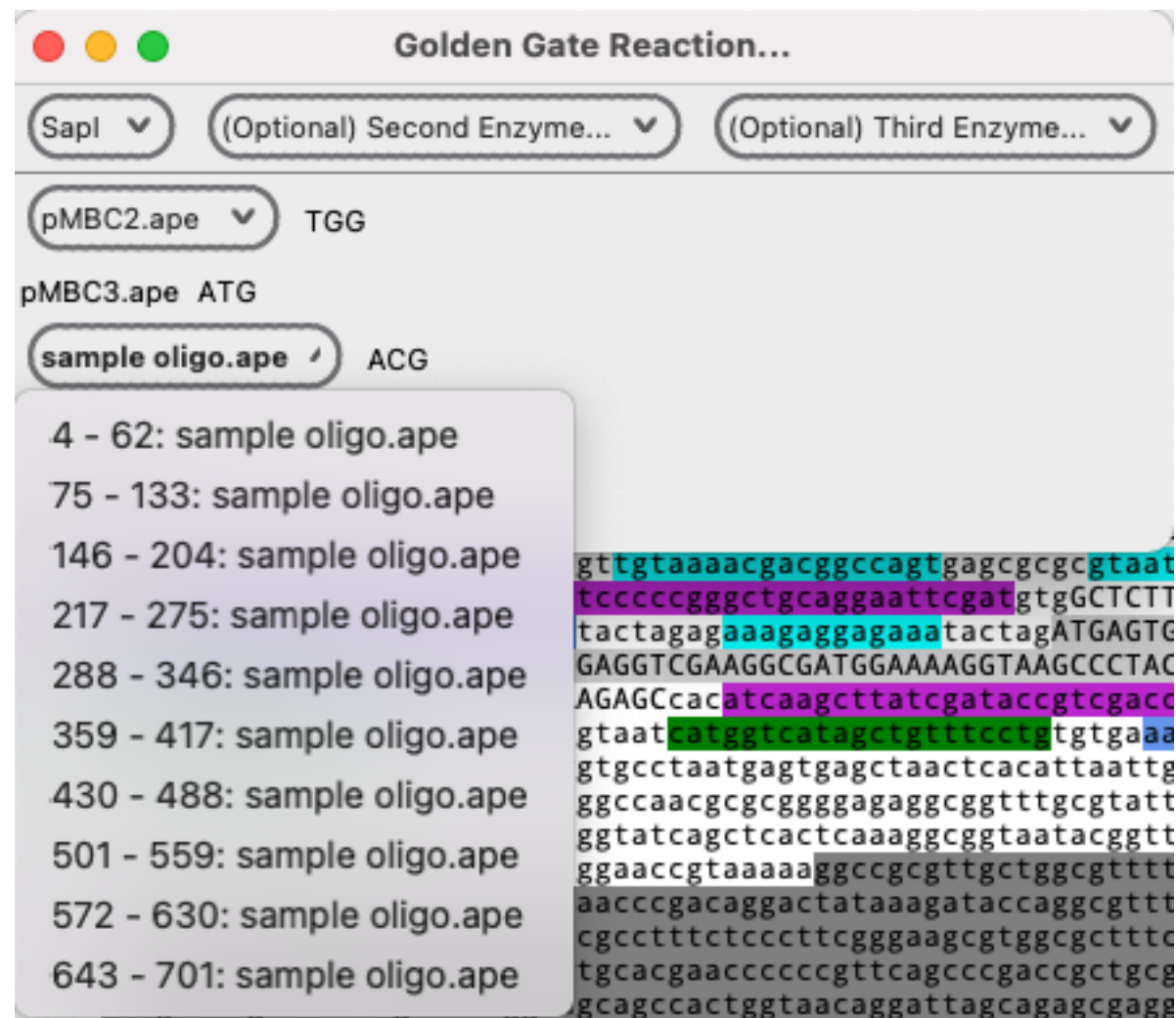
Golden Gate Assembler



Golden Gate Assembler



Golden Gate Assembler



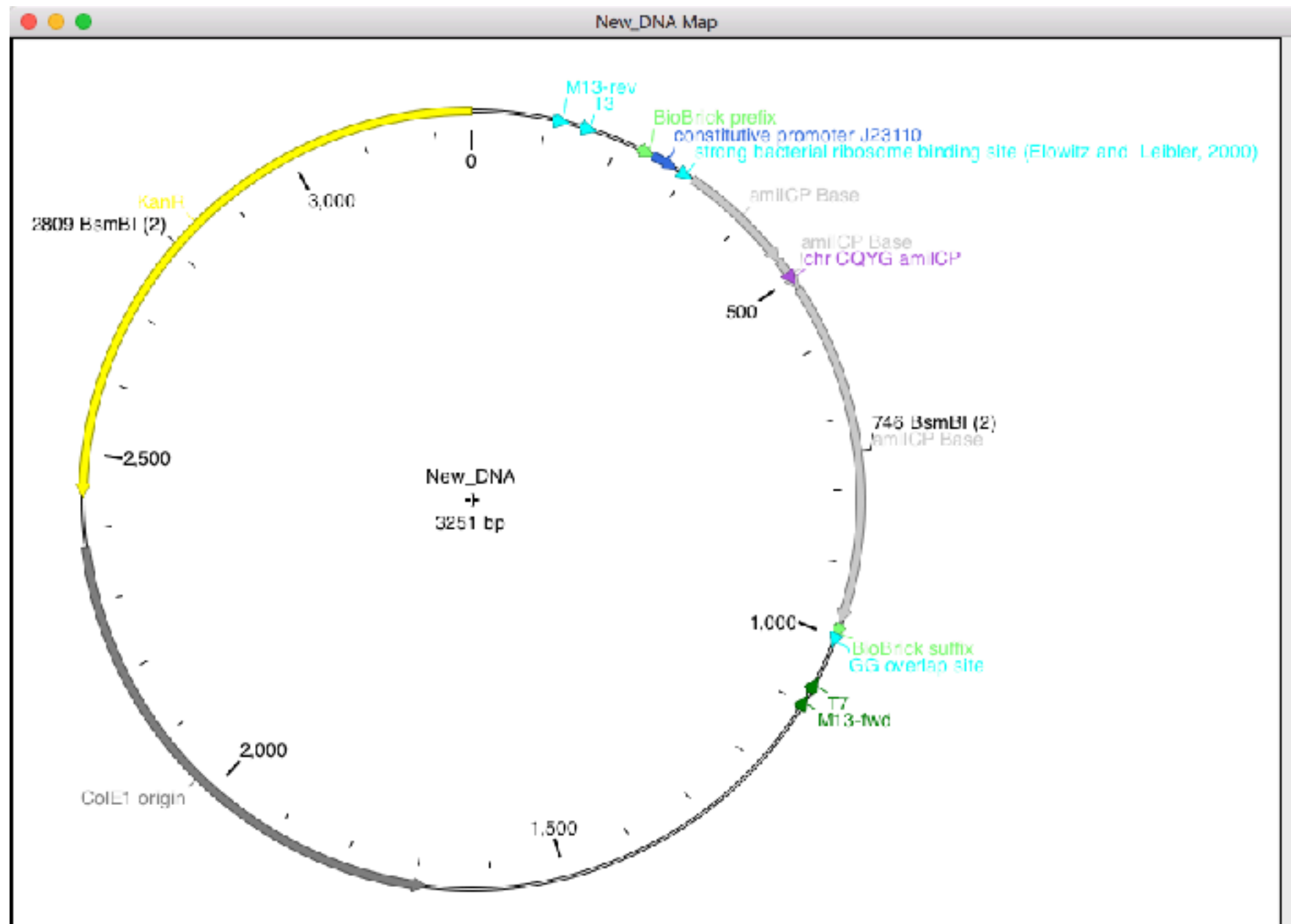
Golden Gate Assembler

The screenshot displays the Golden Gate Assembler (GGA) software interface. The window title is "New_DNA". The top toolbar contains various icons for file operations, editing, and visualization. Below the toolbar, there are fields for "Sequence" (1), "insert@" (3251), and "1<0>". A "circular" checkbox is checked, and a "Dam/Dcm" checkbox is also checked. The main area shows a table of features:

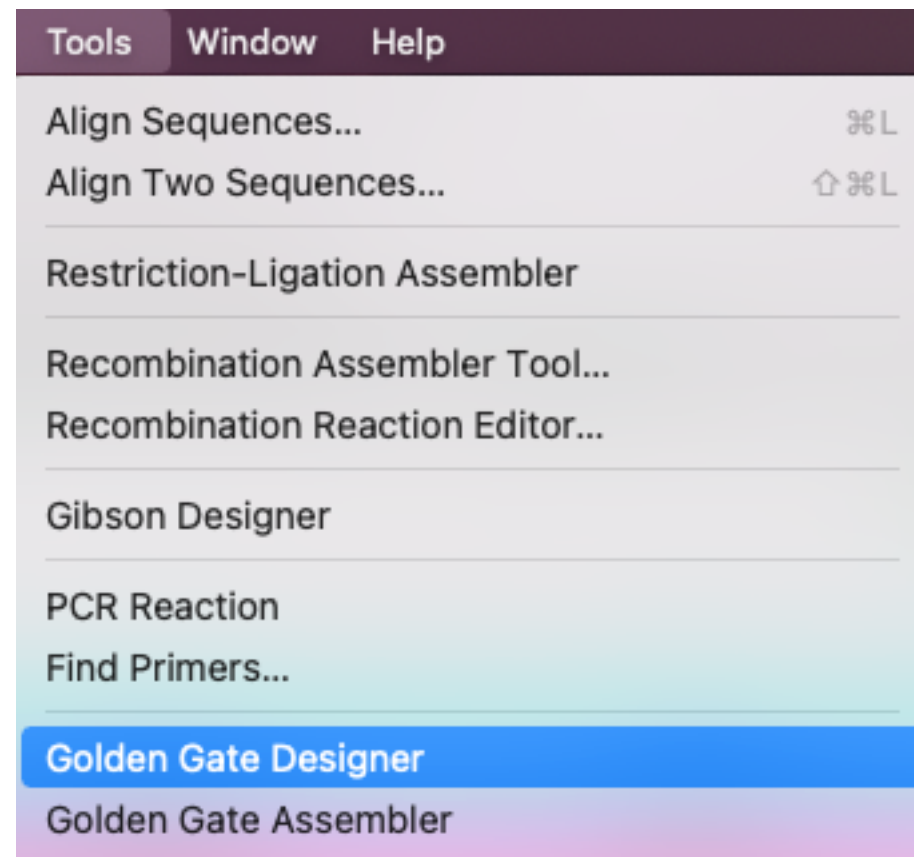
Feature	Direction	Type	Location
> Hidden			
M13-rev	>>>	primer_bind	111..131
T3	>>>	primer_bind	149..168
> BioBrick prefix	>>>	misc_feature	231..252
constitutive promoter J23110	>>>	promoter	263..287

Below the table, a DNA sequence is displayed with line numbers on the left (1, 94, 187, 280, 373, 466, 559, 652, 745, 838, 931, 1024, 1117, 1210, 1303, 1396, 1489, 1582, 1675, 1768, 1861, 1954, 2047, 2140, ~~~~). The sequence is color-coded: blue for primer binding sites, green for the BioBrick prefix, and red for the constitutive promoter. The sequence is: 1 CACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAG 94 ACACGGGCCAGAGC16C caggaaacagctatgaccatgattacgccaaagcgcgca attaaacctcactaaagggaacaaaagctggagctcca 187 ccgcgggtggggccgctctagaagggctgcaggaattcgatTGG gaattcgcggcgccgttctagagtttacggctagctcagtccttaggtaca 280 atgctagctactagagaaagaggagaaa tactagATGAGTGTGA1CGC1AAACAAATGAC1ACAAGG11TATA1GT CAGGCACGGTCAA1GG 373 ACCTACTTTTCAGCTCGAAGCCGATGGAAAGGTAAGCCCTACCAGCGGGAGCAGACCGGTAAACCTCACTGT CACCAAGGGCGCACCTCTGCC 466 ATTTGCATGGGATATTTTATCACCACAGTGTCTAGTACGGAAGCATACCATTCACgaagtacccctgaagacatccctgactatgtaaagcagtc 559 ATTCCCGGAGCGCTATACATCGGAGAGGATCATCAACTTTGAACATCGTGCAGTGTCTACTGTCAGCAATGATTCCAGCATCCAAGCCAACTG 652 TTTCATCTACCATGTCAAGTTCTCTGTTTGAACCTTTCTCCCAATGGACCTGTCTATGCAGAGAAGACACAGGGCTGGGAACCCAACTGA 745 GCGTCTCT1TGCACGAGATGGAATGCTGCTAGGAAACAAT1TA1GGCTCTGAAG1TAGAAGGAGGCGGTCAC1AT1TGTGTGAAT1TAAAC 838 TACTTACAAGCCAAAGAAGCCTGTGAAGATGCCAGGGTATCACTATGTTGACCCCAAACTGGATGTAACCAATCACAACAAGGATTACACTTC 931 GGTGAGCAGTGTGAAATTTCCATTGCACGCAAACTGTGGTCCGCTAATcaatcactagtagcggcgctgcagGTAatcaagcttatcgatac 1024 cgtcgacctcgagggggggcccggtacccaattcg cctatagtagagtcgtattacgcgcgtc actggcggcggtttacaACGTCTGTGACT 1117 GGGAAAACATCCATGCTAGCTTAACGCGAGAGTAGGGAAGTGGCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGCTTTCTGT 1210 TTTATCTGTTGTTTGTGCGGTGAACGGCTCTCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGTGAAGCAACGGCCCGGAGGGTGG 1303 CGGGCAGGAGCCCGCCATAACTGCCAGGCATCAAATAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTCGCTTTCTACAACTCTTCCT 1396 GGTAGCGG1ACGG1AT1AA1TGC1T1GCGT1CAC1GCGCGCT1TCCAGT1CGGGAAC1G1CG1GCCAGCTGCA1TAA1GAA1CGGCAAC 1489 GCGCGGGGAGAGCGGTTTGCCTATTGGGCGCTcatatgCGCTTCTCTGCTCACTGACTCGCTCGCTCGGTCTTCGCTGCTGCGGAGCGGT 1582 ATCAGCTCACTCAAGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGCAAGGAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAG 1675 CAACCGTAAAAAGCCCGCGTTGCTGGCGTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCG 1768 AAACCTGACAGGACTATAAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTGTGCGCTCTCTGTTCCGACCTGCGCTTACCGGATACCT 1861 GTCCGCTT1TCTCCCT1CGGGAAGCGTGGCGCT1TCTCATAGCTCACGCTGTAGG1ATCTCAGT1CGG1GTAGG1CGT1CGCTCCAGCTGGG 1954 CTGTGTGACGAACCCCCGTTCCAGCCCCGACCGCTGCGCCTTATCCGGTAACTATCCTCTTGAGTCCAACCCGTTAAGACACGACTTATCGCC 2047 ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC 2140 TAGAAGAACACTATTTGGTATCTCCGCTCTCCTCAAGCCAGTTACCTTCGGAAAAACAGTTGGTAGCTCTTGATCCCGCAAAACAAACCACCGC ~~~~

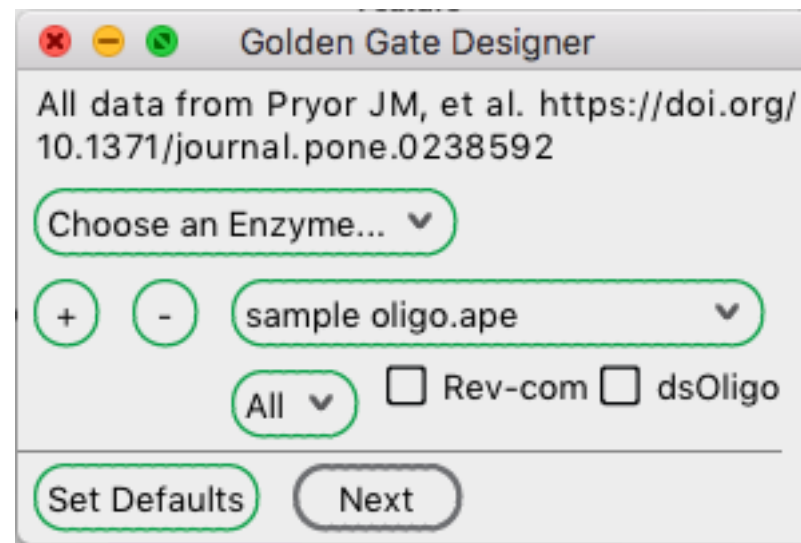
Golden Gate Assembler



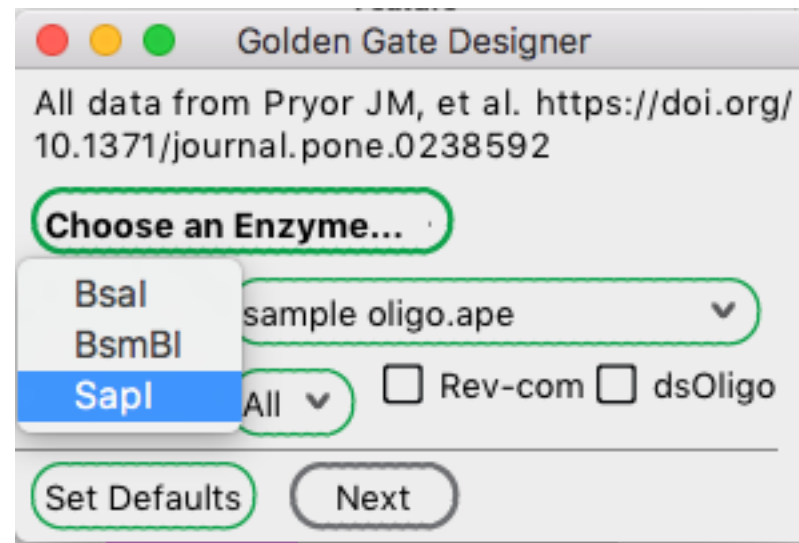
Golden Gate Designer



Golden Gate Designer

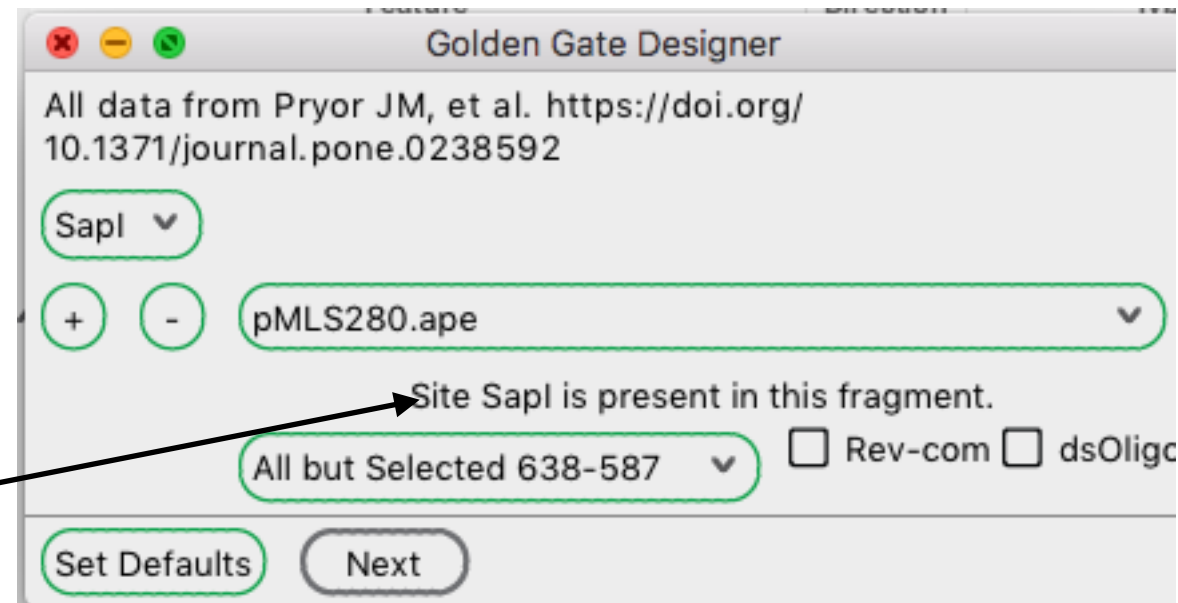


Golden Gate Designer

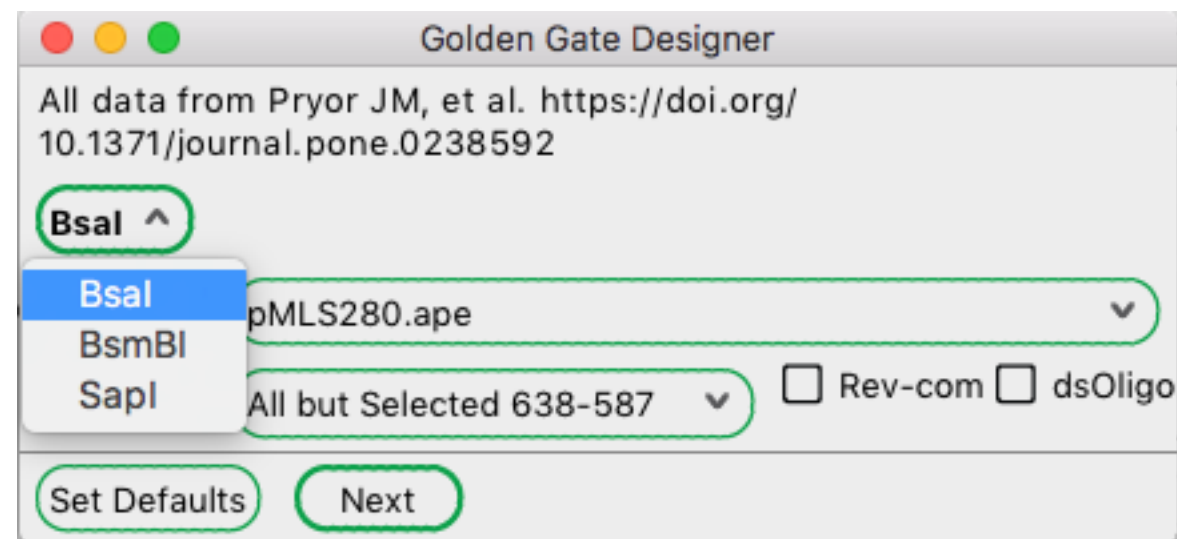


Golden Gate Designer

You can't use a fragment that has a site already in it



Golden Gate Designer



Golden Gate Designer

Add fragments using
this button

Golden Gate Designer

All data from Pryor JM, et al. <https://doi.org/10.1371/journal.pone.0238592>

Bsal ▼

+ - pMLS280.apc ▼

All but Selected 638-587 ▼ ☐ Rev-com ☐ dsOligo

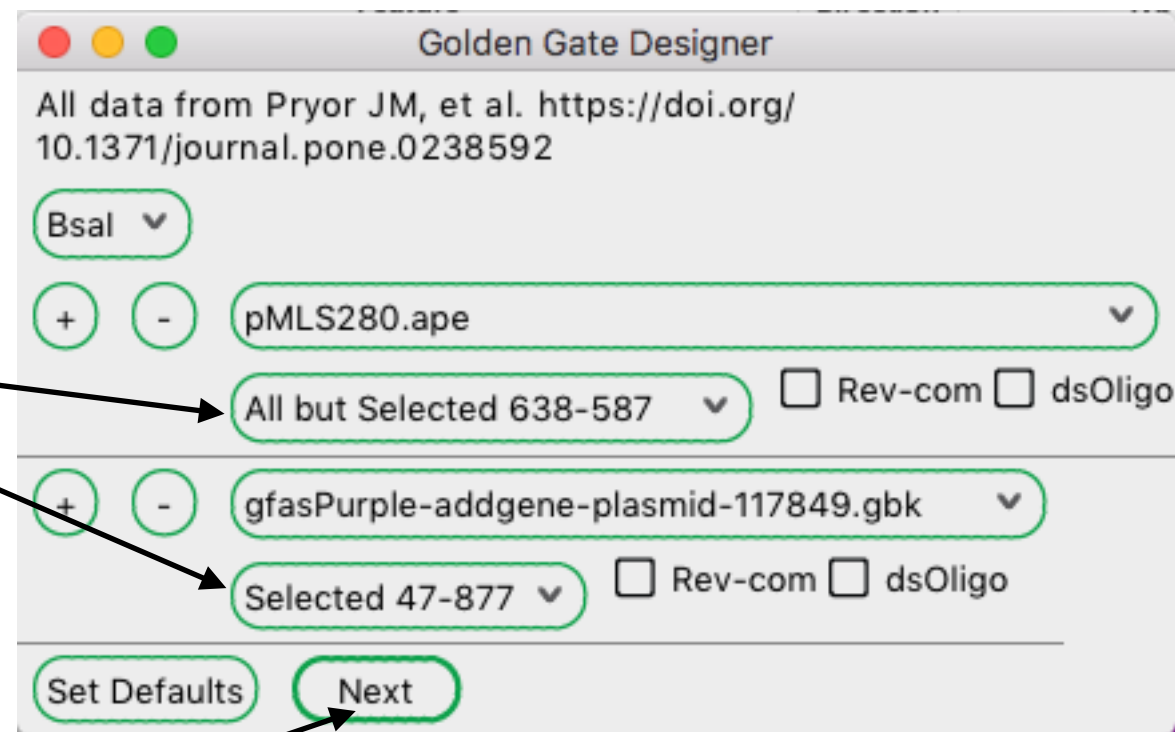
+ - gfasPurple-addgene-plasmid-117849.gbk ▼

Selected 47-877 ▼ ☐ Rev-com ☐ dsOligo

Set Defaults Next

Golden Gate Designer

Select these ranges



The screenshot shows the 'Golden Gate Designer' window. At the top, it says 'All data from Pryor JM, et al. <https://doi.org/10.1371/journal.pone.0238592>'. Below this, there are two main sections for selecting DNA ranges. The first section has a 'Bsal' restriction enzyme dropdown, followed by '+' and '-' buttons, a dropdown menu showing 'pMLS280.ape', and another dropdown menu showing 'All but Selected 638-587'. To the right of this are checkboxes for 'Rev-com' and 'dsOligo'. The second section has '+' and '-' buttons, a dropdown menu showing 'gfasPurple-addgene-plasmid-117849.gbk', and a dropdown menu showing 'Selected 47-877'. To the right of this are also checkboxes for 'Rev-com' and 'dsOligo'. At the bottom, there are two buttons: 'Set Defaults' and 'Next'. Arrows from the text 'Select these ranges' point to the 'All but Selected 638-587' and 'Selected 47-877' dropdown menus. An arrow from the text 'Then click "Next"' points to the 'Next' button.

Golden Gate Designer

All data from Pryor JM, et al. <https://doi.org/10.1371/journal.pone.0238592>

Bsal ▼

+ - pMLS280.ape ▼

All but Selected 638-587 ▼ ☐ Rev-com ☐ dsOligo

+ - gfasPurple-addgene-plasmid-117849.gbk ▼

Selected 47-877 ▼ ☐ Rev-com ☐ dsOligo

Set Defaults Next

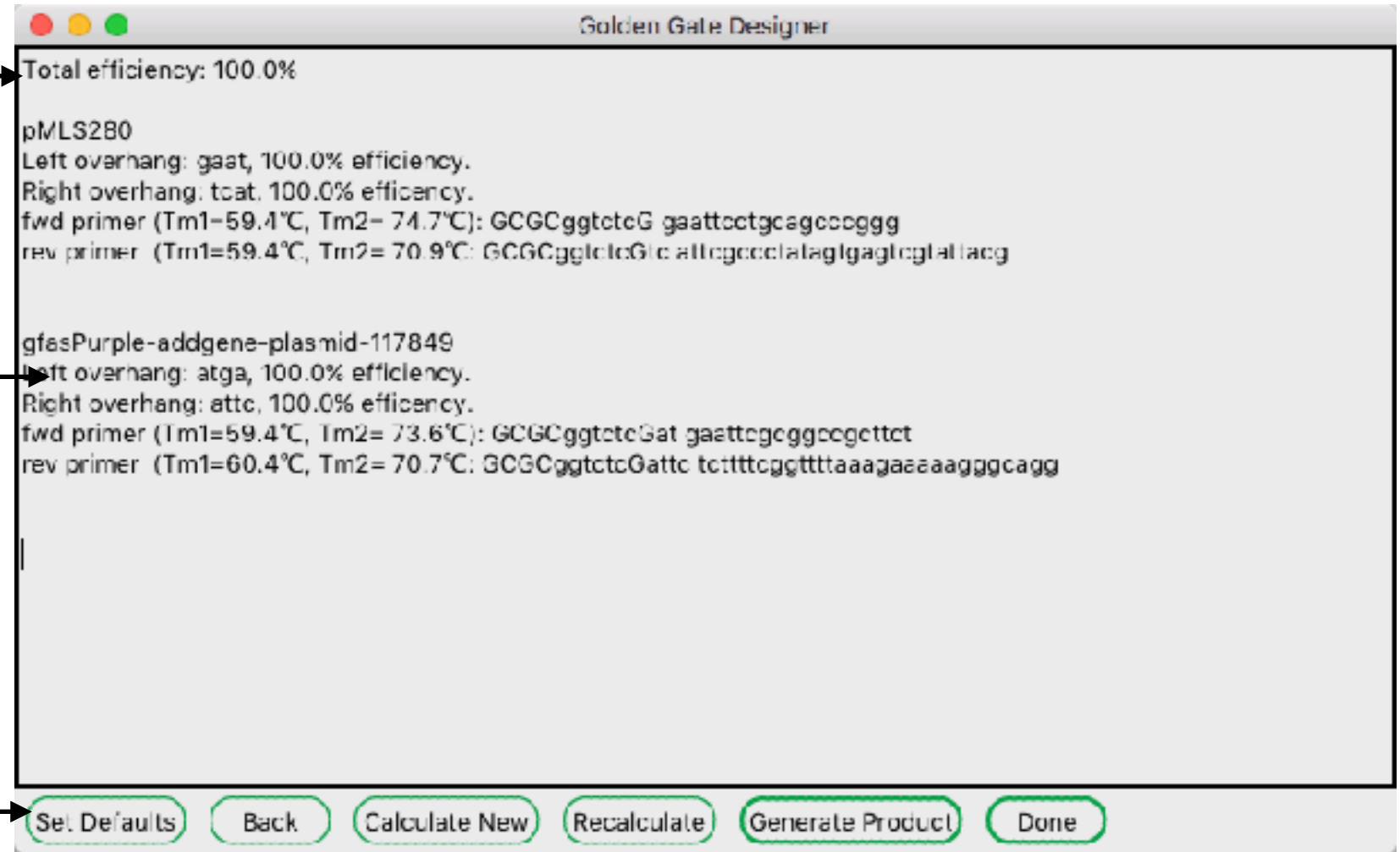
Then click "Next"

Golden Gate Designer

Calculates the total efficiency (on-target to off target)

Each fragment and the primers needed

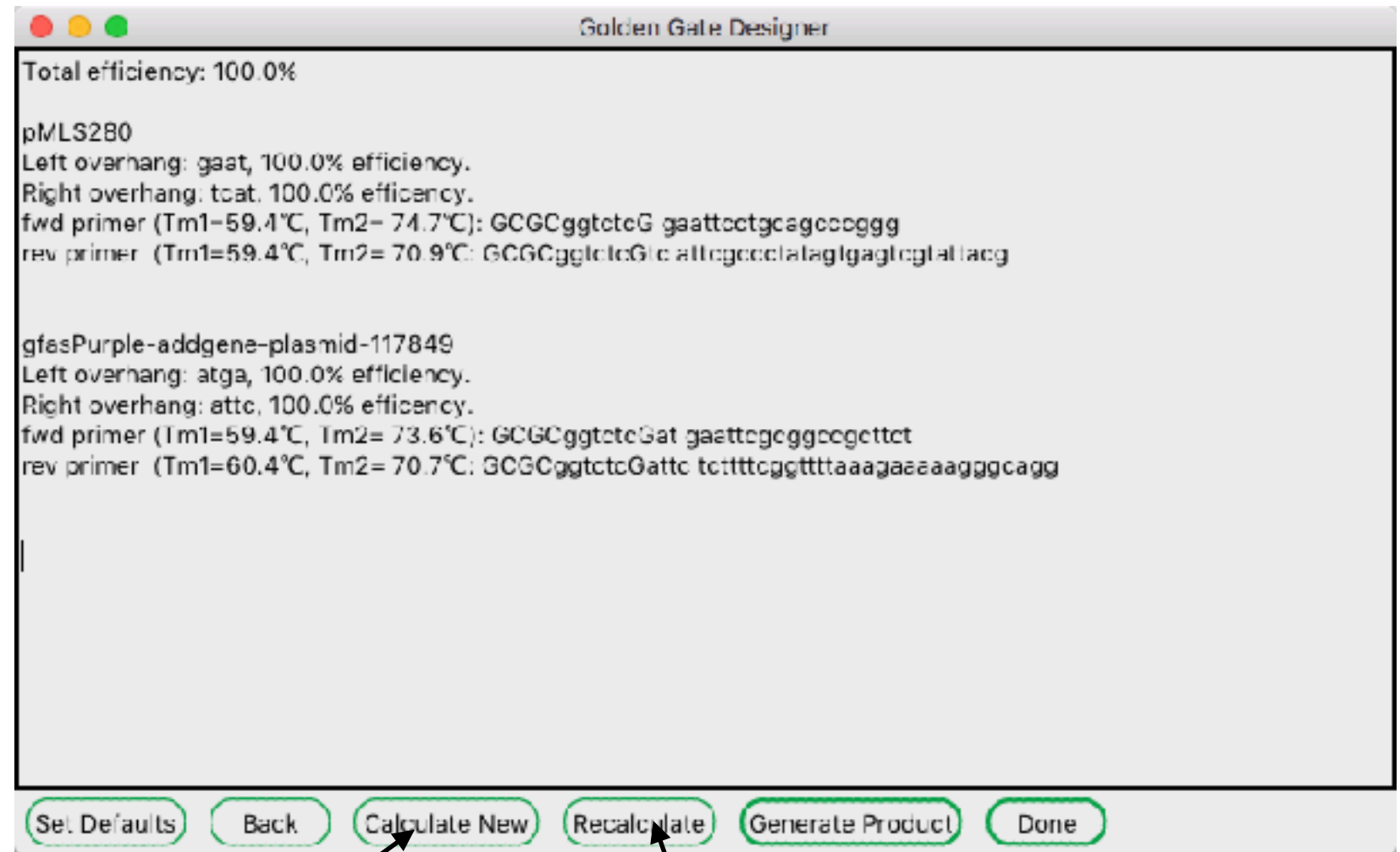
You can set default values here bases



Golden Gate Designer



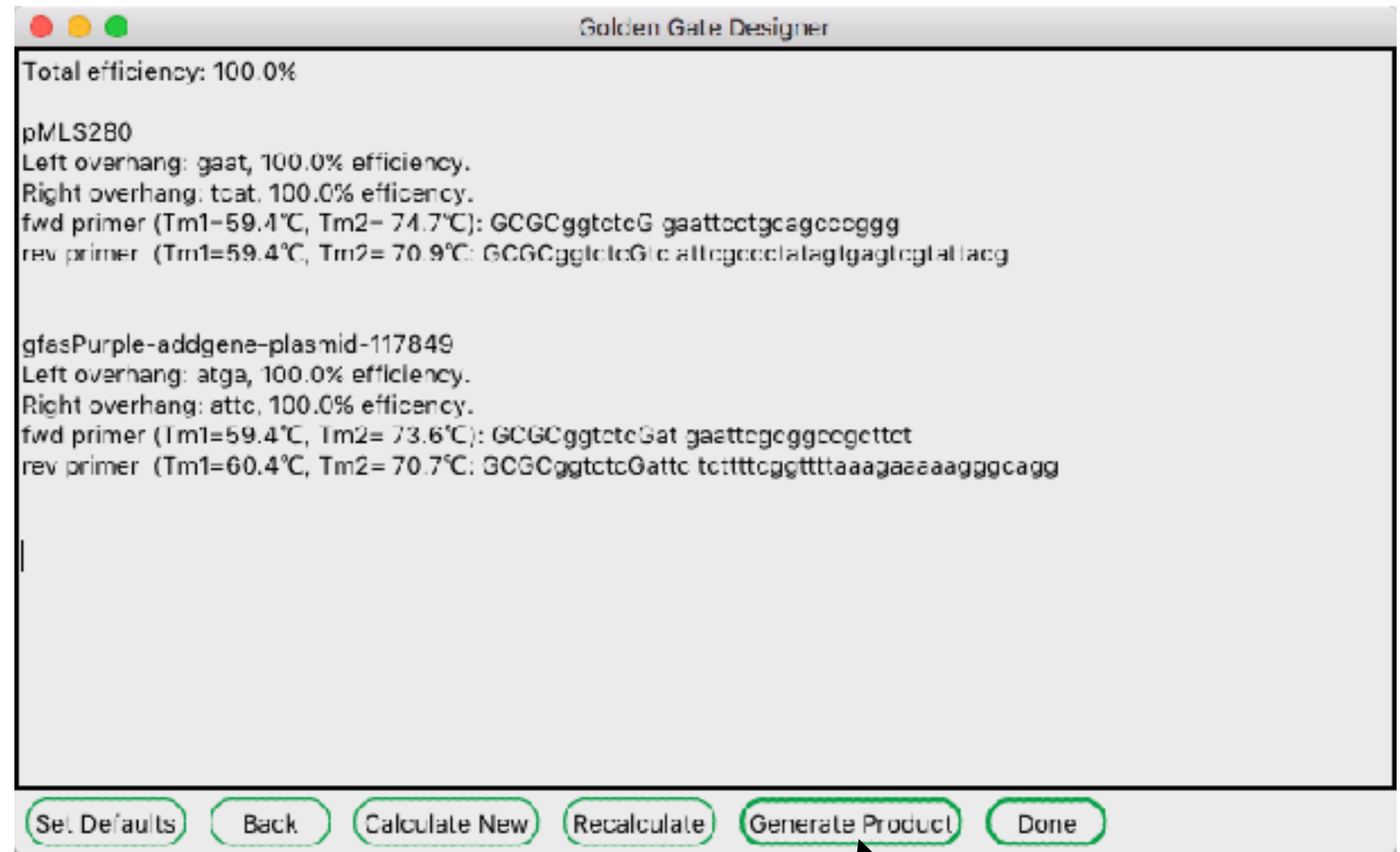
Golden Gate Designer



**Re-start the
calculation if it's stuck
in an unfavorable
search**

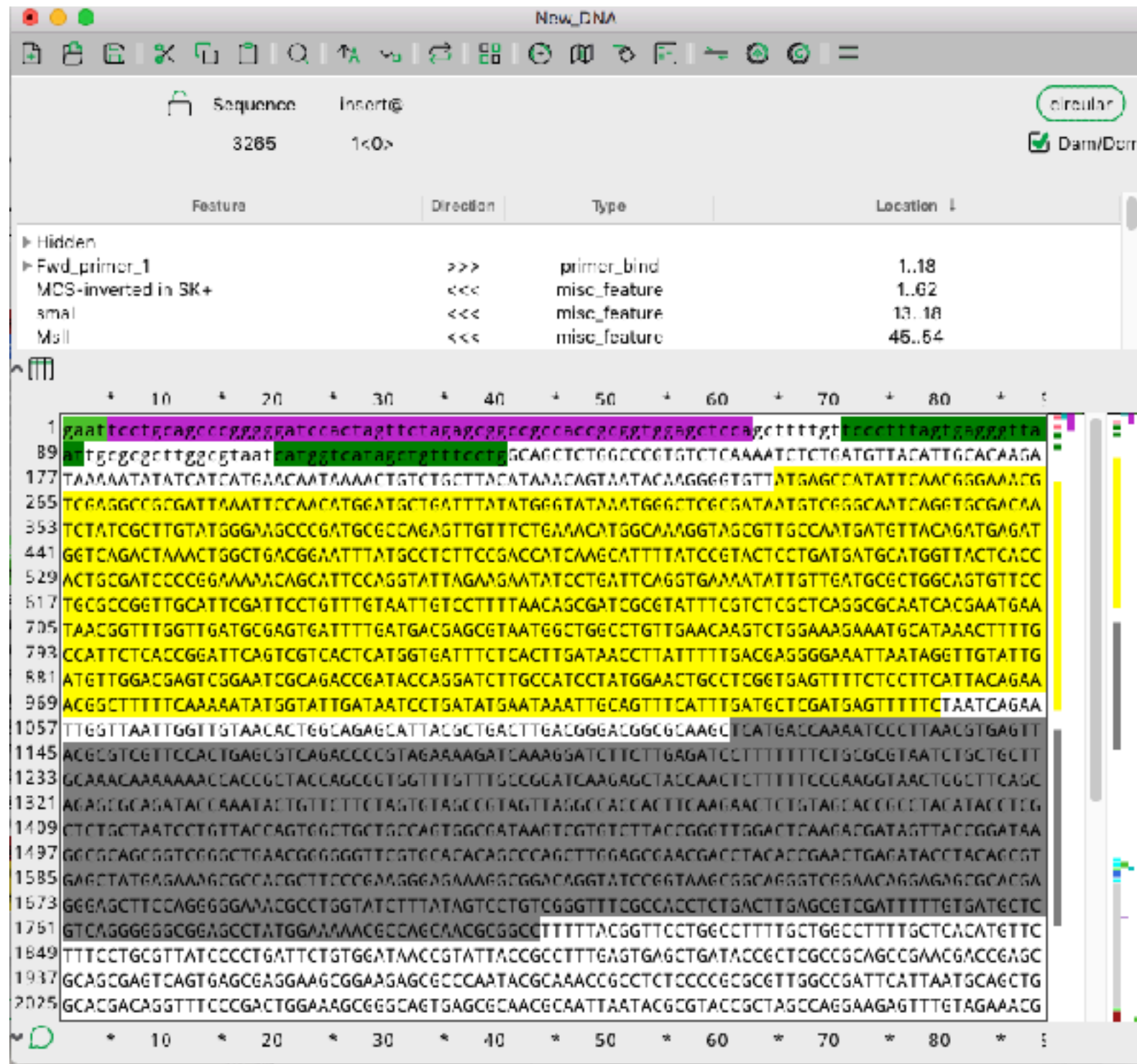
**Do a longer calculation
from the current
search position**

Golden Gate Designer



**Generate the new
product**

Golden Gate Designer



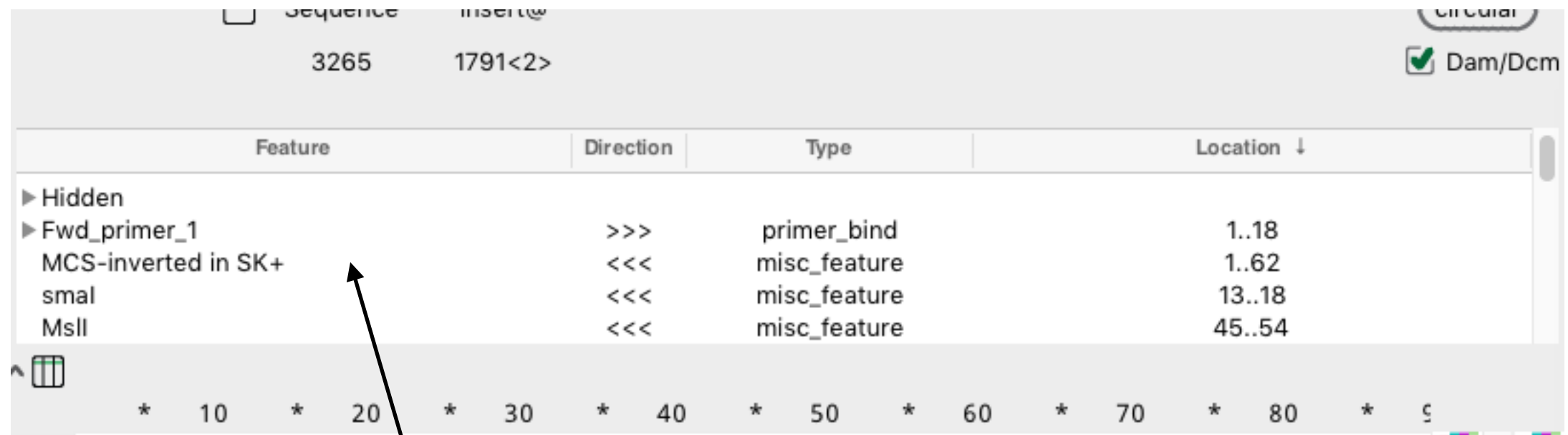
Golden Gate Designer

The screenshot displays the Golden Gate Designer interface. At the top, a DNA sequence is shown with line numbers 1497, 1585, 1673, 1761, 1849, 1937, and 2025. The sequence is: 1497 GGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGT 1585 GAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGGAACAGGAGAGCGCACGA 1673 GGGAGCTTCCAGGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTC 1761 GTCAGGGGGGCGGAGCCTATGGAAAAACGC|CAGCAACGCGGCC|TTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTT 1849 TTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGC 1937 GCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTG 2025 GCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACG. A vertical bar is present between positions 1761 and 1762. Below the sequence is a progress bar with markers at 10, 20, 30, 40, 50, 60, 70, 80, and 90. At the bottom, a text box contains the following information: Golden Gate reaction: Bsal, PCR: pMLS280, Fwd_primer_1 GCGCggtctcGgaattcctgcagcccggg 59.4, 74.7, Rev_primer_1 GCGCggtctcGtcattcgccctatagtgagtcgtattacg 59.4, 70.9, and Product length : 2458. An arrow points from the text 'The primers and PCR conditions are in the comments' to the primer sequences in the text box.

Golden Gate reaction: Bsal
PCR: pMLS280
Fwd_primer_1 GCGCggtctcGgaattcctgcagcccggg 59.4, 74.7
Rev_primer_1 GCGCggtctcGtcattcgccctatagtgagtcgtattacg 59.4, 70.9
Product length : 2458

**The primers and PCR
conditions are in the
comments**

Golden Gate Designer

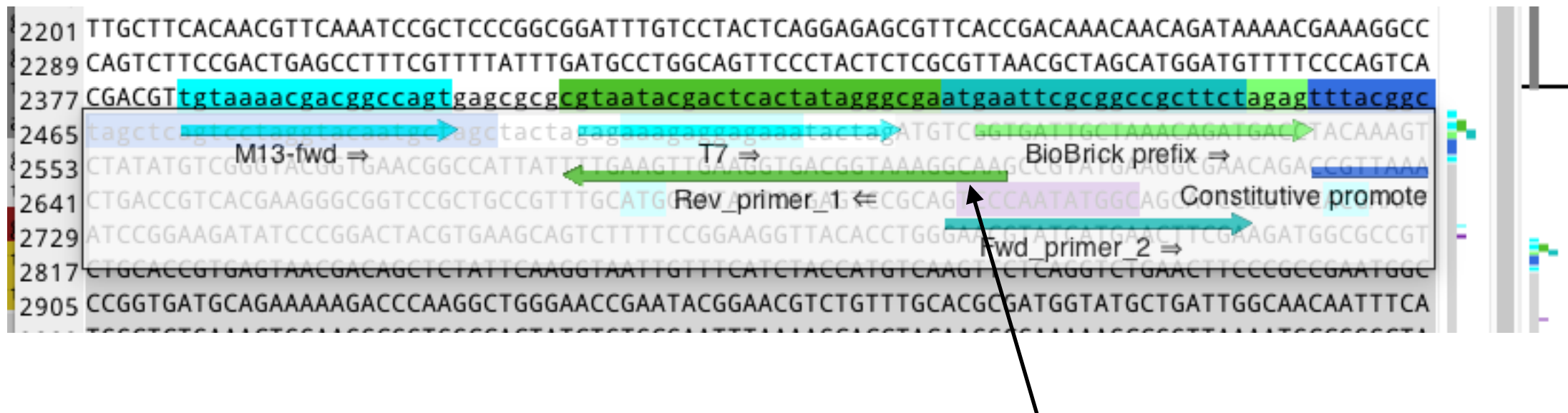


The screenshot shows the Golden Gate Designer interface. At the top, there are fields for 'Sequence' (3265) and 'Insert' (1791<2>). A 'Circular' button is on the right. Below these is a table with columns: Feature, Direction, Type, and Location ↓. The table lists several features, including 'Fwd_primer_1' and 'MCS-inverted in SK+'. Below the table is a sequence map with positions 10, 20, 30, 40, 50, 60, 70, 80, and 90. An arrow points from the text 'The primers are new features in the feature table' to the 'Fwd_primer_1' entry in the table.

Feature	Direction	Type	Location ↓
▶ Hidden			
▶ Fwd_primer_1	>>>	primer_bind	1..18
MCS-inverted in SK+	<<<	misc_feature	1..62
smal	<<<	misc_feature	13..18
MslI	<<<	misc_feature	45..54

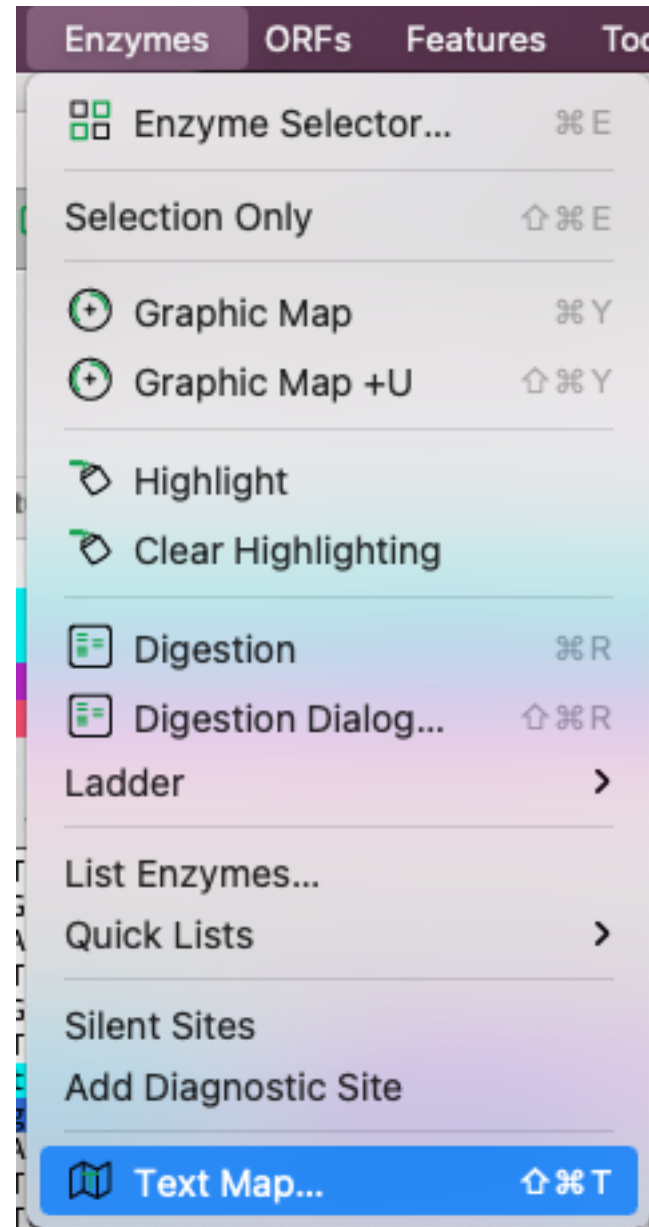
The primers are new
features in the feature
table

Golden Gate Designer



The primers are new features in the sequence

Text map dialog



Text map dialog

Each box is an analysis line

Turn off the line by unchecking this box

Drag the frames to change the order

The 'Text Map...' dialog box contains several sections for configuring sequence analysis display:

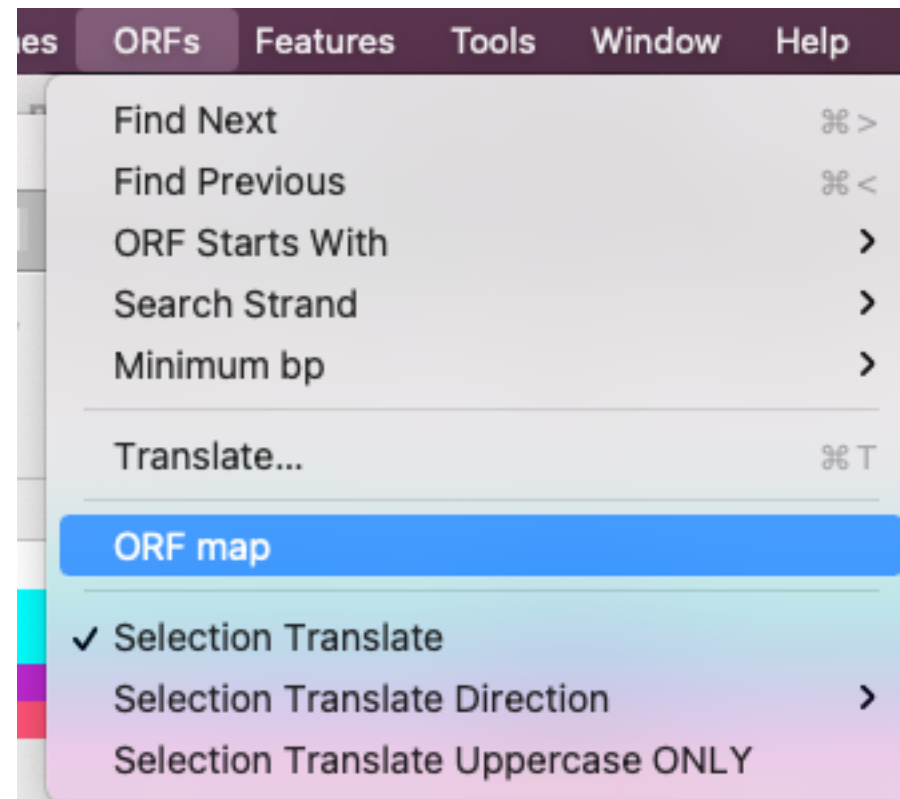
- Enzymes:** Includes a 'Show' checkbox (checked) and radio buttons for 'All' and 'Selected Enzymes Only'.
- Index Line:** Includes a 'Show' checkbox (checked), two dropdown menus for 'character every' (set to 10 and 5), and a 'Numbers' checkbox (checked).
- DNA:** Includes radio buttons for 'All' (selected) and 'Selected DNA only', a 'Copy Highlighting' checkbox (checked), a 'Characters/line' field (100), and a 'Line Numbers' dropdown (Both).
- Translation:** Includes a 'Show' checkbox (unchecked) and a grid of radio buttons for different frame and letter combinations. The '3 frame 1 letter' option is selected.
- 2nd Strand:** Includes a 'Show' checkbox (unchecked).
- Features:** Includes a 'Show' checkbox (checked), a 'Crop Labels to Sequence Width' checkbox (checked), and a 'Show Hidden Features' checkbox (unchecked).
- Uppercase as Genes:** Includes a 'Show' checkbox (unchecked).
- Footer:** Includes the text 'Drag frames to set the display order' and 'Cancel' and 'OK' buttons.

Annotations with arrows point to the 'Show' checkbox in the Enzymes section, the 'Show' checkbox in the Translation section, and the 'Drag frames to set the display order' text.

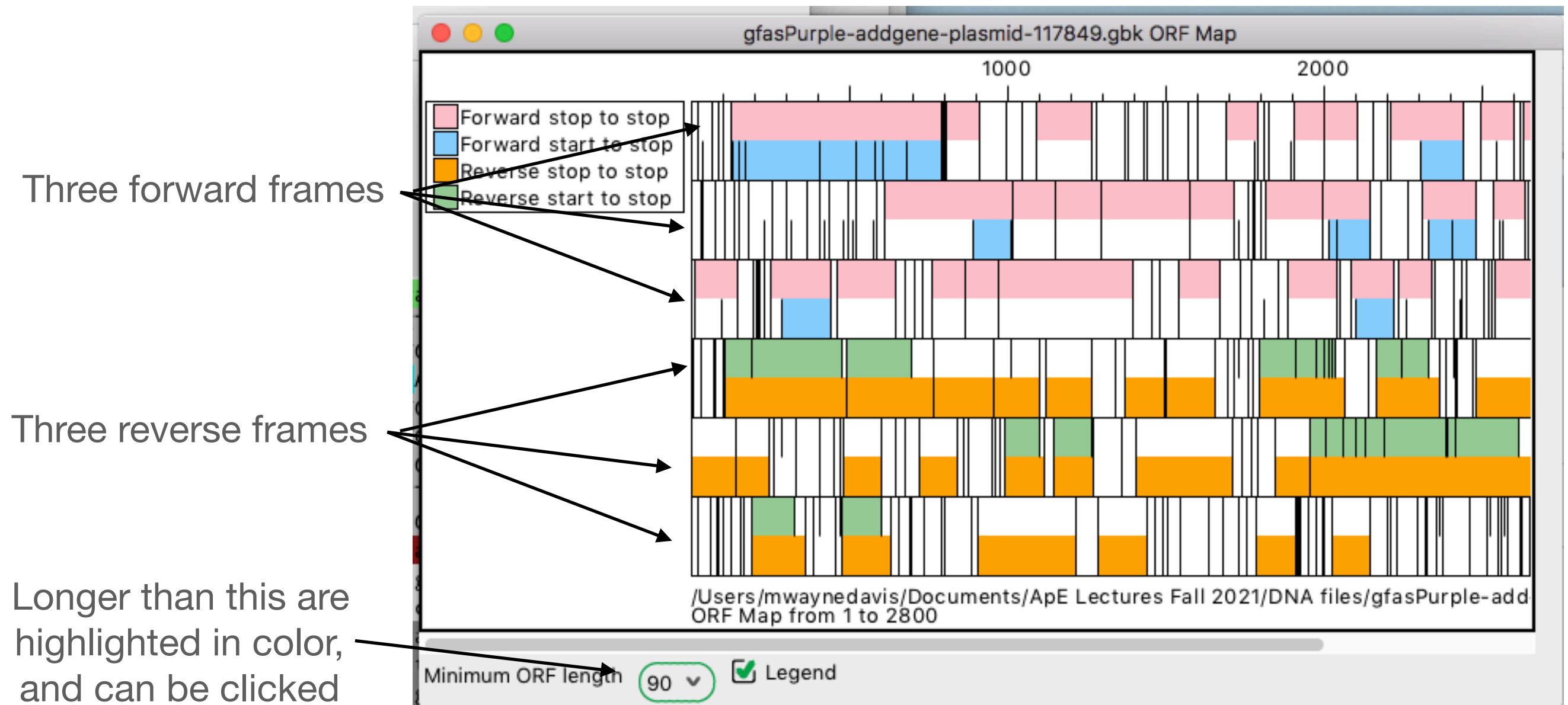
Text map dialog

[illegible]

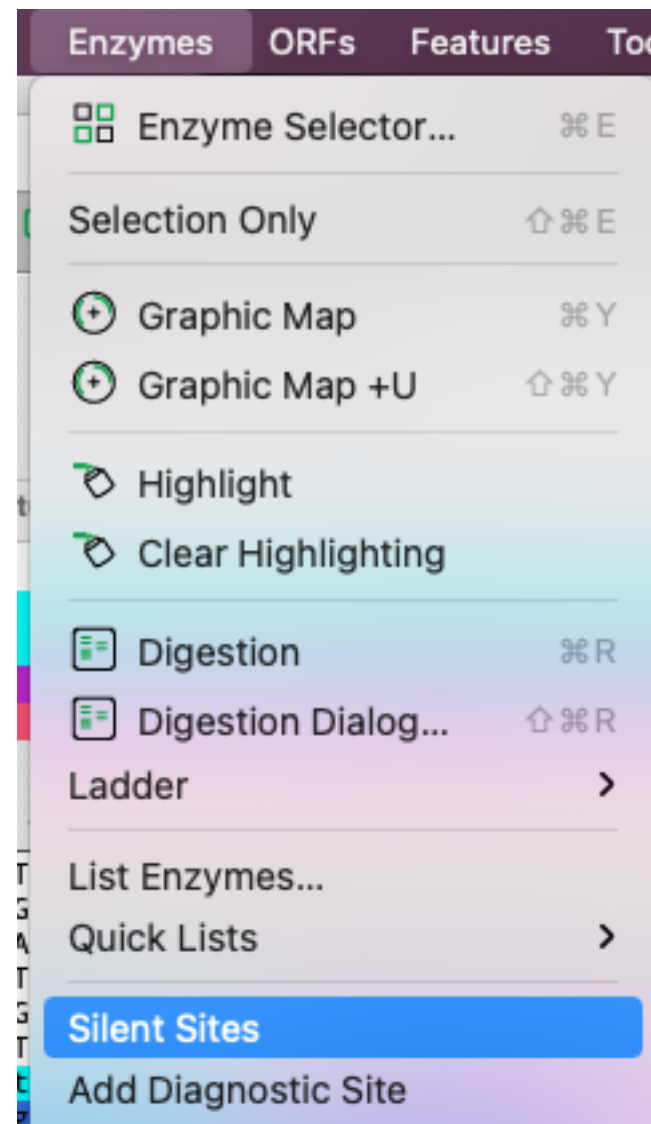
ORF map dialog



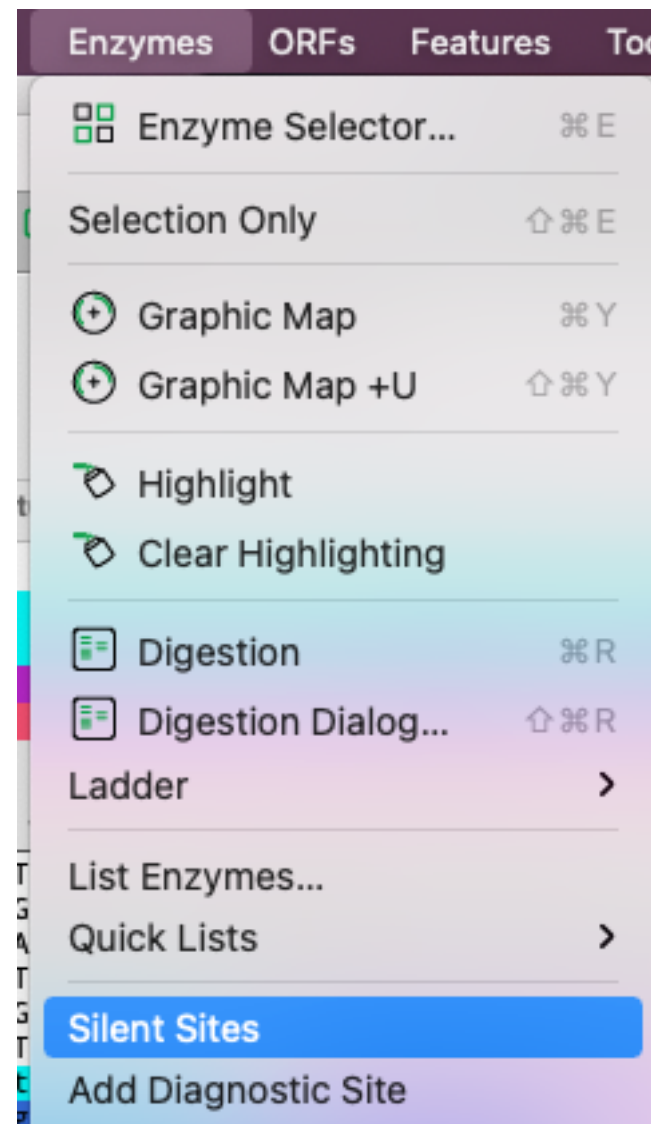
ORF map dialog



Silent/ diagnostic sites dialog



Silent/ diagnostic sites dialog



***Select a forward ORF and a set of restriction enzymes before selecting the menu item

Silent/ diagnostic sites dialog

```
gfasPurple-addgene-plasmid-117849.gb...
Wed Oct 06, 2021 10:20 MDT
gfasPurple-addgene-plasmid-117849.gbk
From 130 to 795.
Silent Sites

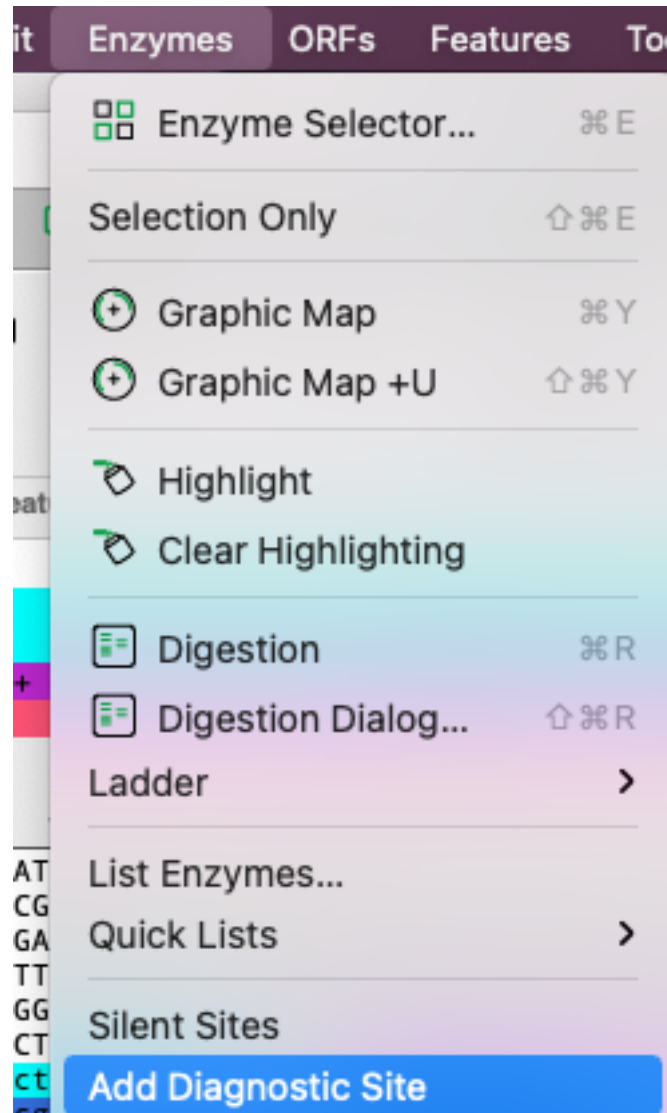
136 GTG ATT
    GTG ATC
MboI  G ATC
      V  I

139 ATT GCT AAA
    ATC GCG AAA
NruI  TC GCG A
      I  A  K

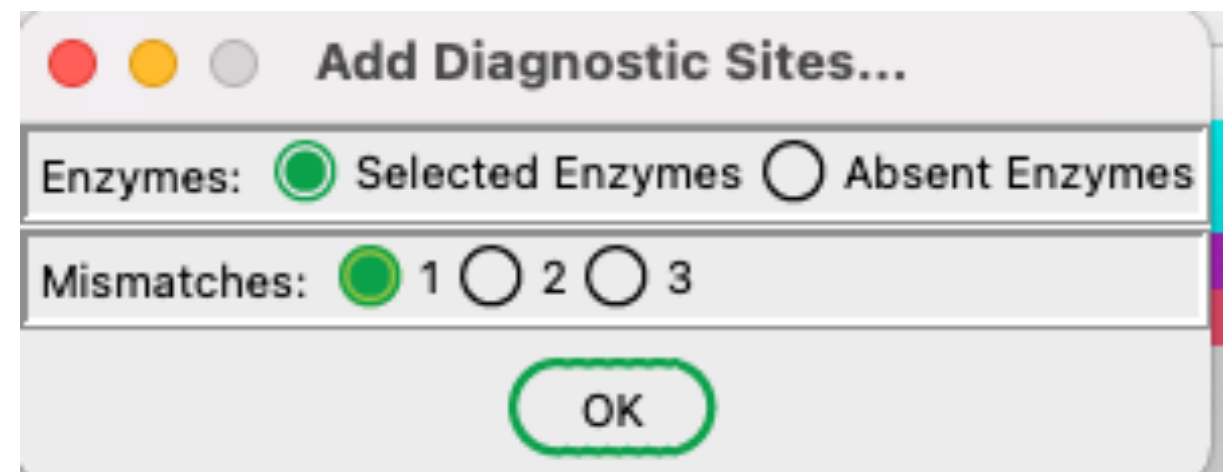
142 GCT AAA CAG
    GCT AAG CAG
BlpI  GCT NAG C
      A  K  Q

154 ACC TAC AAA
    ACT TAT AAA
PsiI  T TAT AA
      T  Y  K
```

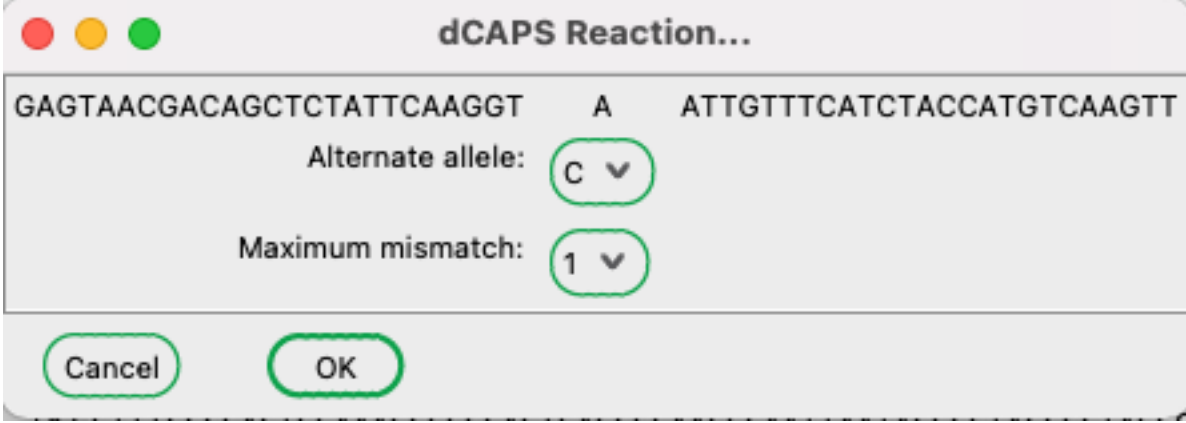
Silent/ diagnostic sites dialog



Select diagnostic site if
you are adding a site
that is NOT in an ORF



dCAPS dialog



A screenshot of a macOS-style dialog box titled "dCAPS Reaction...". The dialog has a light gray background and a title bar with three colored window control buttons (red, yellow, green) on the left. The main content area is divided into two sections. The top section displays a DNA sequence "GAGTAACGACAGCTCTATTCAAGGT" on the left, a central letter "A", and another sequence "ATTGTTTCATCTACCATGTCAAGTT" on the right. Below this, the label "Alternate allele:" is followed by a dropdown menu showing "C" with a downward arrow. Further down, the label "Maximum mismatch:" is followed by a dropdown menu showing "1" with a downward arrow. At the bottom of the dialog, there are two buttons: "Cancel" on the left and "OK" on the right, both with rounded rectangular shapes and green borders.

dCAPS Reaction...		
GAGTAACGACAGCTCTATTCAAGGT	A	ATTGTTTCATCTACCATGTCAAGTT
Alternate allele: C ▼		
Maximum mismatch: 1 ▼		
Cancel	OK	

dCAPS dialog

Two orientations

WT or mutant cut

Red is the variant base

Green is a mutant primer base

```
1. Ligation Product.apc dCAPS
Tue Jul 27, 2021 21:24 NDI
1. Ligation Product.apc
GAGTAACGACAGCTCTATTCAAGGT(A/C)ATTGTTTCATCTACCATGTCAAGTT

Forward primer, wild-type cut
TspEI      GAGTAACGACAGCTCTATTCAAGGTAAAT
           GAGTAACGACAGCTCTATTCAAGGTAAAT

MfeI       GAGTAACGACAGCTCTATTCAAGGAA
           GAGTAACGACAGCTCTATTCAAGGAA

MfeI       GAGTAACGACAGCTCTATTCAAGGCAATTC
           GAGTAACGACAGCTCTATTCAAGGCAATTC

XmnI       GAGTAACGACAGCTCTATTCAAGGCAATTC
           GAGTAACGACAGCTCTATTCAAGGCAATTC

Reverse primer, wild-type cut
TspEI      AACTTGACATGGTAGATGAAACAATT
           AACTTGACATGGTAGATGAAACAATT

RsaI       AACTTGACATGGTAGATGAAACAATTAC
           AACTTGACATGGTAGATGAAACAATTAC

Forward primer, alternate allele cut
MboI       GAGTAACGACAGCTCTATTCAAGGTC
           GAGTAACGACAGCTCTATTCAAGGTC
```


dCAPS dialog

Sequence of the DNA

Enzyme recognition
sequence

Primer sequence

```
1. Ligation Product.apc dCAPS
Tue Jul 27, 2021 21:24 NDI
1. Ligation Product.apc
GAGTAACGACAGCTCTATTCAAGGT(A/C)ATTGTTTCATCTACCATGTCAAGTT

Forward primer, wild-type cut
TspEI  GAGTAACGACAGCTCTATTCAAGGTAAATT
      GAGTAACGACAGCTCTATTCAAGGTAAATT

MseI   GAGTAACGACAGCTCTATTCAAGGTTAA
      GAGTAACGACAGCTCTATTCAAGGTTAA

MfeI   GAGTAACGACAGCTCTATTCAAGGTTAA
      GAGTAACGACAGCTCTATTCAAGGTTAA

XmnI   GAGTAACGACAGCTCTATTCAAGGTTAA
      GAGTAACGACAGCTCTATTCAAGGTTAA

Reverse primer, wild-type cut
TspEI  AACTTGACATGGTAGATGAAACAATT
      AACTTGACATGGTAGATGAAACAATT

RsaI   AACTTGACATGGTAGATGAAACAATT
      AACTTGACATGGTAGATGAAACAATT

Forward primer, alternate allele cut
MboI   GAGTAACGACAGCTCTATTCAAGGTC
      GAGTAACGACAGCTCTATTCAAGGTC
```


Primers- hand designed

**Drag the selection
where you want the
primer**

Sequence Start Length End ORF Tm %GC Circular Dam/Dom

617 2484 586<1> 21<0> 516<0> >7/<7 59.2°C 81%

GPPI FVD

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	535..553
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

MCS-Inverted in SK+

* 10 * 20 * 30 * 40 * 50 * 60 * 70 * 80 * 90 *

1 CTTTCCTGCGTTATCCCTGATTCTGTGATAACCGTATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGACGCGAACGACCGAGCGCAGCGA
97 GTCACTGAGCGAGGAAGCGGAAGSAGCGCCCAATACGCAACCGCCTCTCCCCGCGCGTTGGCCGATTCTTAATGACAGCTGGCACGACAGSTTTCC
193 CGACTGGAAGCGGCGAGTGAGGCGACGCAATTAATACGCTACCGCTAGCGAGGAAGAGTTTGTAGAAGCGCAAAAGGCCATCCGTCAGGATG
289 GCCTTCTGCTTAGTTTGTATGCTGCGAGTTTATGCGGGGCTCTCTCGCCGCAACCTCCGGGCGGTTGCTTCACAAAGCTTCAATCCGCTCCCGCC
385 GCATTGTCTACTCAGGAGAGCGTTACCGACAAACAGATAAAACGAAAGGCCAGTCTTCCGACTGAGCGCTTTCGTTTTATTTGATGCTG
481 GCAGTTCCCTACTCTCCGCTTAAGCTAGCATGATGTTTTCCAGTCAAGAGCTTGTAAAACGACGCGCAGTGTAGCGCGCTTAATACGACTCACT
577 ATAGGGCGAATTGGGTACCGGGCCCCCTCGAGGTGCGAGGTATCGATAAGCTTGATATCGAATTCCTGCAGCGCGGGGGATCCACTAGTCTAG
673 AGTGGCGGACGTGGGTTGGAGTTTGGCTTTTGTCTTGTATGAGTGGGTTTGTGAGGCTTGGGTTAATAGGTTTGTATGAGTGGGTTTGTGCA
769 GCTCTGCCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAGATAAAAATATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAA
865 TACAAGGSETGTTATGAGCCATATTCAACGCGAACGTCGAGGCCGCGATTAAATTECAACATGATGCTGATTTATATGGGTATAAATGSGCTCG
961 CGATAATGTCCGGCAATCAGGTGCGACAACTCTATCGCTTGTATGCGAAGCCCGATCGCCAGAGTGTGTTCTCAAAACATGCGAAAGGTACGGTTCC
1057 CAATGATGTTACAGATGAGATGTCAGACTAACTGGCTGACGGAAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGC
1153 ATGGTTACTCACCACCTGCGATCCCGGAAAAACAGCATTCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGT
1249 GTTCCTGCGCGGTTGCATTGATTCCTGTTTGTAAATTGCTCTTTAACAGCGATCGCGTATTTGCTCTCGCTCAGGCGCAATCAGGAATGAATAA
1345 CCGTTTGGTTGATGCGAGTGATTTTGTATGACGAGCGTAATGSGCTGCGCTGTTGAACAAGTCTGGAAGAAGTGCATAACTTTTGCCATTCTCACC
1441 GCATTCACTCGTCACTCATGGTGAATTTCTCACTTGATAACCTTATTTTGAACGAGCGGAAATTAATAGSTTGTATTGATGTTGACGAGTCCGAAT
1537 CGCAGACCGATACCAGGATCTTSCCATCTATGGAAGTGCCTCGSTGAGTTTTCTCCTTCATTACAGAAACGCTTTTTCAAAAATATGATTTGA
1633 TAATCCTGATATGAATAAATTGCATTTTCAATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGCCAGACATTAC
1729 GCTGACTTGACGCGACGCGSCAAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
1825 AGGATCTCTTGAGATCTTTTTTCTGCGCGTAATCTGCTGCTTGCACAAACAAAACCCAGCGCTACCGCGGTGGTTTGTGTCGGGATCAAGA
1921 GCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTATAGGCCACCACTTCAA
2017 GAACCTCTGAGACCGCTACATACCTCGCTCTGCTAATCTGTTACCACTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGAGCTC
2113 AAGACGATAGTTACCGGATAAGGCGCAGCGCTCGGCGCTGAACGGGCGGTTCTGTGACACAGCCAGCTTGGAGCGAAGGACCTACACCGAAGTGA
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGAGAGAAAGCGGACAGGTATCCGTAAGCGGACAGGCTCGGAACAGGAGAGCG

* 10 * 20 * 30 * 40 * 50 * 60 * 70 * 80 * 90 *

Primers- hand designed

**Drag the selection
until the Tm is what
you want**

Sequence Start Length End ORF Tm %GC

617 2484 586<1> 21<0> 516<0> >7/<7> 59.2°C 81%

GPPI FVD

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	535..553
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

MCS-Inverted in SK+

10 20 30 40 50 60 70 80 90

1 CTTTCCTGCGTTATCCCTGATTCTGTGATAACCGTATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGACGCGAACGACCGAGCGCAGCGA
97 GTCACTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGAGCTGGCAGCGACAGSTTTCC
193 CGACTGGAAGCGCGGCGAGTGAGCGCAGCAATTAATACGCTACCGCTAGCGAGGAAGAGTTTGTAGAAGCGCAAAAGGCCATCCGTCAGGATG
289 GCCTTCTGCTTAGTTTGTATGCTGCGAGTTTATGCGGGGCTCTCTGCGGCGACCGCTCCGGGCGGTTGCTTCACAAAGCTTCAATCCGCTCCCGG
385 GGATTTGTCTACTCAGGAGAGCGTTCCACCGACPAACACAGATAAAACGPAAGGCCAGTCTTCCGACTGAGCGCTTTCGTTTTATTTGATGCTG
481 GCAGTTCCCTACTCTCGGTTAACGCTAGCATGATGTTTTCACAGTCAGGAGCTTGTAAACGACGCGCAGTGTAGCGCGCGTAATACGACTCACT
577 ATAGGGCGAATTGGGTACCGGGCCCCCTCGAGGTGCGAGGTATCGATAAGCTTGATATCGAATTCCTGCAGCGCGGGGGATCCACTAGTCTAG
673 AGTGGTGGTACGCGGGTGGAGTTCAGGCTTTGTCCTTATGTTGGGTTAAATGGGCGCTTGGGTTAAATAGGTTATAGGTTTTCGCA
769 GCTCTGCGCGCTGTCTCAAAATCTCTGATGTTACATTGCACAGATAAAAATATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAA
865 TACAAGGSETGTTATGAGCCATATTCAACGCGAACGTCGAGGCCGCGATTAAATTECAACATGGATGCTGATTTATATGGGTATAAATGSGCTCG
961 CGATAATGTCCGSCAATCAGGTGCGACAATCTATCGCTTGTATGCGAAGCCCGATCGCCCGAGATTTGTTCTCAAAACATGCGAAAGGTACGGTTCC
1057 CAATGATGTTACAGATGAGATGTCAGACTAACTGGCTGACGGAAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGC
1153 ATGGTTACTCACCAGTCCGATCCCGGAAAAACAGCATTCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGT
1249 GTTCCTGCGCGGTTGCATTGATTCCTGTTTGTAAATTGCTCTTTAACAGCGATCGCGTATTTGCTCTCGCTCAGGCGCAATCAGGAATGAATAA
1345 CCGTTTGGTTGATGCGAGTGATTTTGTATGACGAGCGTAATGSGCTGCGCTGTTGAACAAGTCTGGAAGAATGCAAACTTTTGCCATTCTCACC
1441 GCATTCACTCGTCACTCATGGTGAATTTCTCACTTGATAACCTTATTTTGAACGAGCGCAAAATTAATAGSTTGTATTGATGTTGAGCGAGTCCGAAT
1537 CGCAGACCGATACCAGGATCTTSCCATCTATGGAAGTGCCTCGSTGAGTTTTCTCCTTCATTACAGAAACGCTTTTTCAAAAATATGATTTGA
1633 TAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGCCAGACCATTA
1729 GCTGACTTGACGCGGACGCGSCAAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
1825 AGGATCTCTTGAGATCTTTTTTCTGCGCGTAATCTGCTGCTTGCACAAACAAAACCCAGCGCTACCGCGGTGGTTTGTGTCGCGATCAAGA
1921 GCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTATAGGCCACCACTTCAA
2017 GAACCTCTGAGACCGGCTACATACCTCGCTCTGCTAATCTGTTACCACTGGCTGCTGCCAGTGCGGATAAGTCTGCTTACCGGGTTGAGCTC
2113 AAGACGATAGTTACCGGATAAGGCGCAGCGCTCGGCGCTGAACGGGCGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAAGTGA
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGAGAGAAAGCGGACAGGTATCCGGAAGCGCAGGCTCGGAACAGGAGAGCG

Primers- hand designed

Try to keep the %GC near 50%, if possible

pML S280.apc

Sequence Start Length End ORF Tm %GC circular Dam/Dcm

617 2484 596<1> 21<0> 516<0> >7/<7 59.2°C 81%

GPPI FVD

Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	535..559
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

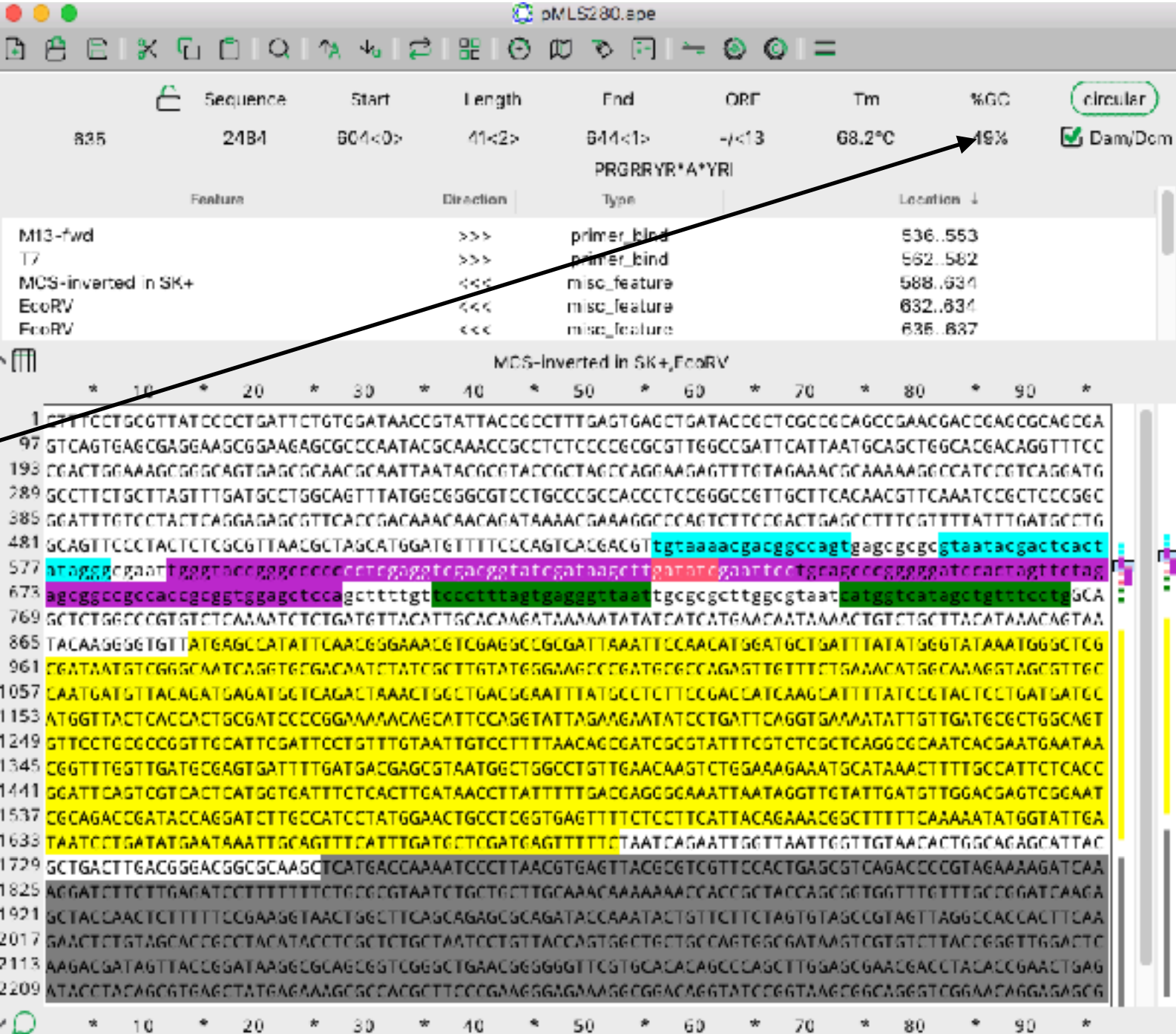
MCS-Inverted In SK+

* 10 * 20 * 30 * 40 * 50 * 60 * 70 * 80 * 90 *

1 CTCTCCTGCGTTATCCCTGATTCTGTGATAACCGTATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGCAAGCGAAGCGAGCGCAGCGA
97 GTCACTGAGCGAGGAAGCGGAAGSAGCGCCAATACGCCAACCGCCTCTCCCCGGCGSTTGCCGATTTCATTAATGAGCTGGCACGACAGSTTTCC
193 CGACTGGAAGCGCGGCGAGTGAGCGCAGCAATTAATACGCTACCGCTAGCGAGGAAGAGTTTGTAGAAGCGCAAAAGGCCATCCGTCAGGATG
289 GCCTTCTGCTTAGTTTGTATGCTGCGAGTTTATGCGGGGCGTCTGCGCGCCACCCTCCGGGCGGTGCTTCACAACGTTCAAATCCGCTCCCGCC
385 GGATTTGTCTACTCAGGAGAGCGTTCCACCGACPAACAACAGATAAAACGPAAGGCCAGTCTTCCGACTGAGCCTTTGTTTTATTTGATGCTG
481 GCAGTTCCCTACTCTCGGTTAACGCTAGCATGGATGTTTTCCAGCTCAGGACGTtgraaaacgagcgccagttagcgccgcgtaatacgaactcaat
577 ataggsggaattgggtaccgggcccccccctcgagggtcgadgggatcgataagcttgatatcgaaattcctgcagcccggggatccactagttctag
673 agtgggtggcagcgggggggaggttcaggttttgttccctttagtgagggttaaallggcgagcttggggtaaatatgggtalagctgttgcgca
769 GCTCTGCGCCGTTGCTCAAAATCTCTGATGTTACATTGCACAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAA
865 TACAAGSESETGTTATGAGCCATATTCAACGEGAMACGTCGASGCCCGGATTAANTTECAACATGGATGCTGATTTATATGGGTATAAATGSGCTCG
961 CCATAATGTCCGSCAATCAGGTGCGACAATCTATCCCTTGTATGCGAAGCCCAGTCGCCAGAGATTGTTTCTCAAAACATCGCAAASGTACCGTTCC
1057 CAATGATGTTACAGATGAGATGTCAGACTAACTGGCTGACGGAAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTAATCTCTGATGATGC
1153 ATGGTTACTCACCFACTGCGATCCCGGAAAAACAGCATTCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGT
1249 GTTCCTGCGCGGTTGCATTGCAATTCCTGTTTGTAAATGTCTTTTAACAGCGATCGGATTTTCGTCTCGCTCAGGCGCAATCAGGAATGAATPA
1345 CCGTTTGGTTGATGCGAGTGATTTTGTATGACGAGCGTAATGCTGCGCTGTTGAACAAGTCTGSAANAGAANTGCATAACTTTTGCCATTCTCACC
1441 GCATTCAGTCTGCTCACTCATGGTGAATTTCTCACTTGATAACCTTATTTTGAACGAGCGCAAAATTAATAGCTTGTATTGATGTTGACGAGTCCGAAT
1537 CGCAGACCGATACCAGGATCTTGCCATCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTCAAAAATATGCTATTGA
1633 TAATCCTGATATGAATAAATTGCASTTTCATTTGATGCTCGATGACTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGCCAGACATTAC
1729 GCTGACTTGACGSGACGSGSCAAAGTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
1825 AGSATCTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACCGACGCTGGTTTGTGTCGGGATCAAGA
1921 GCTACCAACTCTTTTTCCGAGGTAACGTGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAA
2017 GAACCTCTGAGACCGGCTACATACTCTGCTCTGTAATCTGTTACCACTGGCTGCTGCCAGTGCGGATAAGTCTGTCTTACCGGGTTGAGCTC
2113 AAGACGATAGTTACCGGATAAGGCGCASCCTCGGCTGAAACGGGCGGTTCTGTGCACACAGCCAGCTTGGAGCGAAGCAGCTACACCGAAGTGA
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGAGAAAGGCGGACAGGTATCCGGAAGCGGAGGCTCGGAACAGGAGAGCG

+ 10 + 20 + 30 + 40 + 50 + 60 + 70 + 80 + 90 +

Primers- hand designed



Sequence Start Length End ORF Tm %GC

835 2484 604<0> 644<1> -/13 68.2°C 49%

PRRRYR*A*YRI

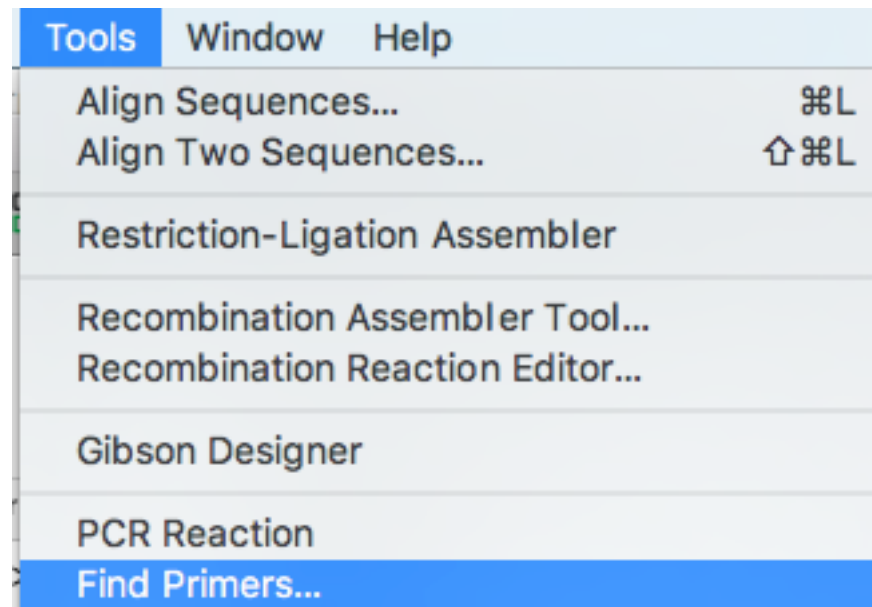
Feature	Direction	Type	Location
M13-fwd	>>>	primer_bind	536..553
T7	>>>	primer_bind	562..582
MCS-inverted in SK+	<<<	misc_feature	588..634
EcoRV	<<<	misc_feature	632..634
EcoRV	<<<	misc_feature	635..637

MCS-Inverted in SK+, EcoRV

1 GTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGACCGCAGCGA
97 STCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCTCTCCCCGCGCTTGCCGATTATTATGCACTGGCAGCAGAGGTTTCC
193 CCACTGGAAAGCGGCGAGTGAGCGCAACGCAATTAATACCGCTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAGGCCATCCGTCAGGATG
289 GCCTTCTGCTTAGTTTATGCTGCGAGTTTATGCGCGGCTCTGCCCCGACCCCTCCGCGCGCTTGCTTCACAACGTTCAAATCCGCTCCCGGC
385 GGATTTGCTCTACTCAGGAGAGCGTTACCGGACAAACACAGATAAAACGAAAGGCCAGCTCTCCGACTGAGCCTTTCTTTTATTTGATGCTG
481 GCAGTTCCTACTCTCGGTTAACGCTAGCATGGATGTTTTCCAGTCACGACGTTgttaaacgacggccagtgagcgcgcgtaatacagactcact
577 ataggcggaatgggtacggggccccctggaggtgagagatgataagcttgaratcgaaatctctgagccggggagatcactagttctag
673 agcgscggccaccggggtggagctccagctttgttcccttttagtgaggggttaatgcgcgcttgccgtaatcatggatcagctgttccctgca
769 GCTCTGCGCGTGTCTCAAAATCTCTGATGTTACATTGACAGAGATAAAATATATCATCATGAACAATAAACTGTCTGCTTACATAACAGTAA
865 TACAAGGGGTGTTATGAGCCATATTCAACGGGAACGTCGAGGCGCGGATTAAATTCACATGATGCTGATTATATGGGTATAAATGGGCTCG
961 CGATAATGTGCGGCAATCAGGTGCGCAATCTATCGCTTGTATGGGAAGCCGATGCGCCAGAGTTGTTTCTGAACATGGCAAGGTAGCGTTGC
1057 CAATGATGTTACAGATGAGATGCTCAGACTAACTGCTGACGCAATTTATGCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGC
1153 ATGGTTACTCACCCTGCGATCCCCGGMAAACAGCATTCAGGTATTAGAGAATATCCTGATTGAGGTGMAAATATTGTTGATGCGCTGCGAGT
1249 GTTCTGCGCGGTTGCAATCGATTCCTGTTTGTAAATGTCCTTTTAACAGCGATCGGATTTCTGCTCCTCAGGCGCAATCACGAATGAATA
1345 CGGTTTGTTGATGCGAGTGATTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAGAGAAATSCATAAATTTTSCCATTTCTCACC
1441 GCATTCAGTCGTCATGCTGATTTCTCACTTCATAACCTTATTTTGAAGAGCGCAATTAATAGGTTGATTTGATGTTGGACGAGTCGCAAT
1537 CGCAGACCGATACCAGGATCTTGCCATCCTATGGAACGCTCGGTGAGTTTCTCCTTCATTACAGAAACGCTTTTCAAAAATATGGTATTGA
1633 TAATCCTGATATGAATAAATTCAGTTTCATTTGATGCTCGATGAGTTTCTAATCAGAATTGGTTAATTTGGTTGTAACACTGGCAGAGCATTAC
1729 GCTGACTTGACGGGACGGGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
1825 AGGATCTTCTTGAGATCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCCGCTACCGAGCGGTGGTTTGTGTTGCGGATCAAGA
1921 GCTACCAACTCTTTTCCGAAGGTAACCTGCTTCAGCAGAGCGCAGATACCAAACTACTGTTCTTCTAGTGTAAGCGTAGTTAGGCCACCACTTCAA
2017 GAACCTCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGCTTGAAGTCTC
2113 AAGACGATAGTTACCGGATAGGCGCAGCGCTCGGCTGAACGGGGGTTCTGTGACACAGCCAGCTTGAGCGAACGACCTACACCGAAGTGAAG
2209 ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGATATCCGGTAAGCGGCAAGGTCGGAACAGGAGAGCG

Try to keep the %GC near 50%, if possible

Primers- ApE designed



Primers- ApE designed

[illegible]

Primers- ApE designed

Find Primer

	Minimum	Maximum
Length:	20	25
Tm:	55	60
%GC:	45	60
GC clamp:	1	2
Consecutive bases:		3
Salt (mM):	50	
Primer (nM):	250	

	total	adjacent	3'
<input checked="" type="checkbox"/> Self complement:	10	5	3
Heterodimer:	10	5	3

Orientation: 3'<--5' ▼

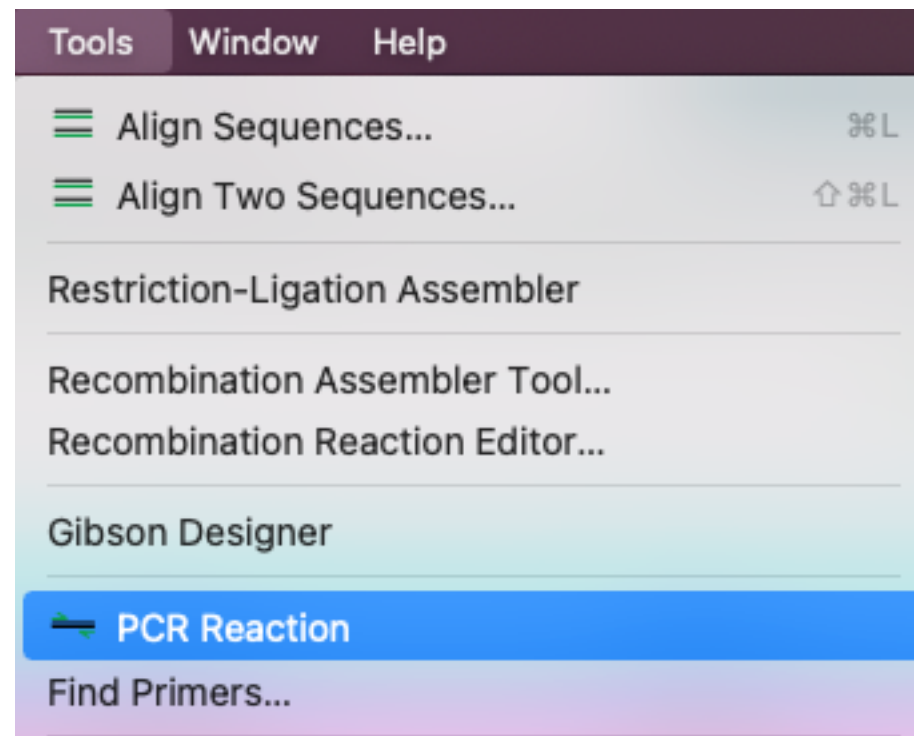
Sort by: 5' ▼

OK

Primers- ApE designed

Primer (5'-->3')					length	%GC	Tm(°C)	self/other(max adj 3')
Sun Jul 25, 2021 16:33 NDT								
pMLS280.apc								
From 406 to 998 (5'-->3')								
Find Primers								
Primer (5'-->3')					length	%GC	Tm(°C)	self/other(max adj 3')
443	AGTCTTCGACTGAGCCTTTC	483			21	52	58	8 4 2 /-----
444	GTCTTCGACTGAGCCTTTC	463			20	55	56	6 3 2 /-----
467	TTATTTSATGCTGGCACTTC	488			22	45	57	8 4 2 /-----
468	TATTTGATGCTGGCACTTC	488			21	47	57	8 4 2 /-----
469	ATTTSATGCTGGCACTTC	488			20	50	57	8 4 2 /-----
472	TGATGCTGGCACTTCCTAC	492			21	57	60	8 4 2 /-----
473	GATGCTGGCACTTCCTAC	492			20	60	59	8 4 2 /-----
474	ATGCTGGCACTTCCTACTC	494			21	57	60	8 4 1 /-----
475	TGCTGGCACTTCCTACTC	494			20	60	60	8 4 1 /-----
477	GCTGGCACTTCCTACTCTC	496			20	60	57	8 3 1 /-----
478	CTGGCACTTCCTACTCTCG	497			20	60	58	8 3 1 /-----
709	CCCTTAGTGAGGGTTAATTGC	730			22	45	55	8 4 1 /-----
730	CGCGCTTGCGTAATCATG	749			20	60	60	8 4 1 /-----
731	GCCTTGCGTAATCATGTC	751			21	57	60	8 4 1 /-----
732	CGCTTGCGTAATCATGTC	751			20	55	57	6 4 1 /-----
733	GCTTGCGTAATCATGTCATG	755			23	47	57	8 4 2 /-----
734	CTTGCGTAATCATGTCATG	755			22	45	55	8 4 2 /-----
738	GCCTAATCATGTCATGTCG	758			21	47	55	8 4 2 /-----
756	CTTTTCTGCGAGCTCTG	775			20	60	59	8 4 2 /-----
878	ATGACCATATTCAACGGGAACG	901			24	45	59	8 4 2 /-----
879	TGAGCCATATTCAACGGGAACG	901			23	47	59	8 4 2 /-----
879	TGAGCCATATTCAACGGGAAC	900			22	45	57	8 4 1 /-----
880	GAGCCATATTCAACGGGAACG	901			22	50	58	8 4 2 /-----
880	GAGCCATATTCAACGGGAAC	900			21	47	55	8 4 1 /-----
881	AGCCATATTCAACGGGAACG	901			21	47	57	8 4 2 /-----
882	GCCATATTCAACGGGAACG	901			20	50	55	8 4 2 /-----
883	CCATATTCAACGGGAACGTC	903			21	47	55	8 4 2 /-----
884	CATATTCAACGGGAACGTCG	904			21	47	55	8 4 2 /-----
903	CGAGCCCGGATTAAATTCC	922			20	55	57	8 4 2 /-----
960	GCATAATGTCGGGCAATCAG	980			21	57	57	8 3 1 /-----
961	CGATAATGTCGGGCAATCAG	980			20	50	55	8 3 1 /-----
962	GATAATGTCGGGCAATCAGTG	983			22	50	57	8 3 2 /-----
963	ATAATGTCGGGCAATCAGTG	983			21	47	56	8 2 2 /-----
964	TAATGTCGGGCAATCAGTG	983			20	50	56	8 2 2 /-----
971	GGCAATCAGGTGGGCAATC	991			21	57	60	6 3 2 /-----
972	GGCAATCAGGTGGGCAATCTATC	995			24	50	60	8 3 2 /-----
972	GGCAATCAGGTGGGCAATC	991			20	55	58	6 3 2 /-----
973	GCAATCAGGTGGGCAATCTATC	995			23	47	58	8 3 2 /-----
974	CAATCAGGTGGGCAATCTATC	995			22	45	55	8 2 2 /-----

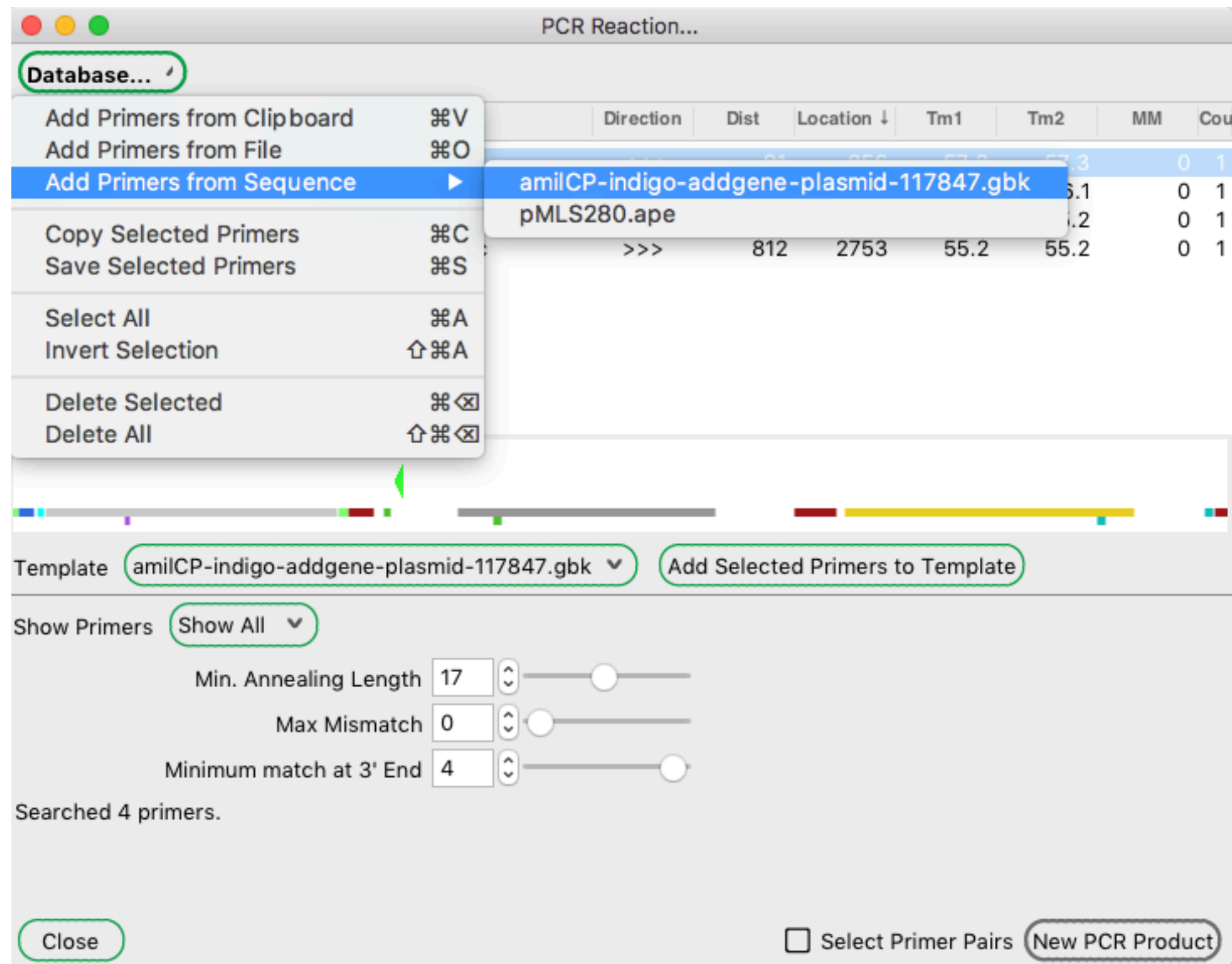
PCR reaction tool



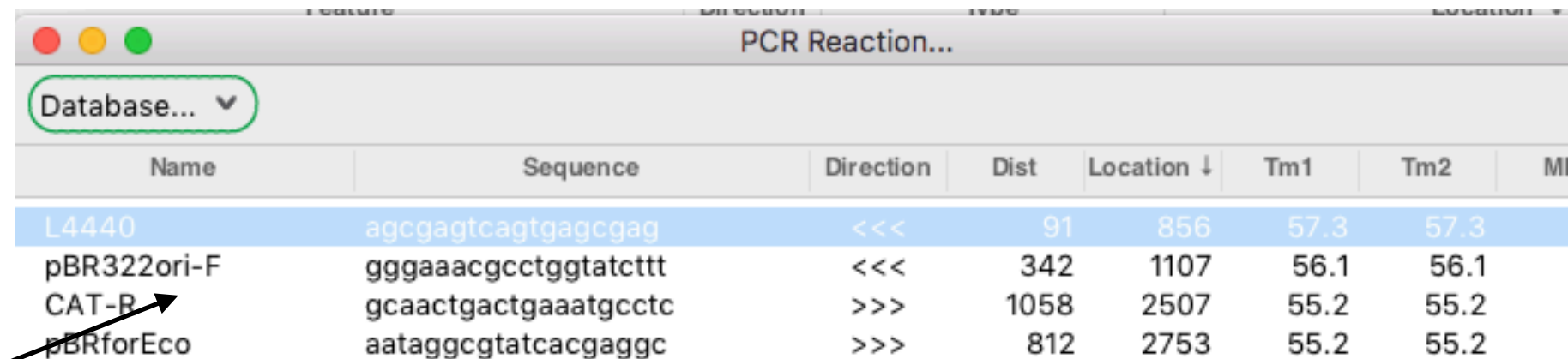
PCR reaction tool

[illegible]

PCR reaction tool

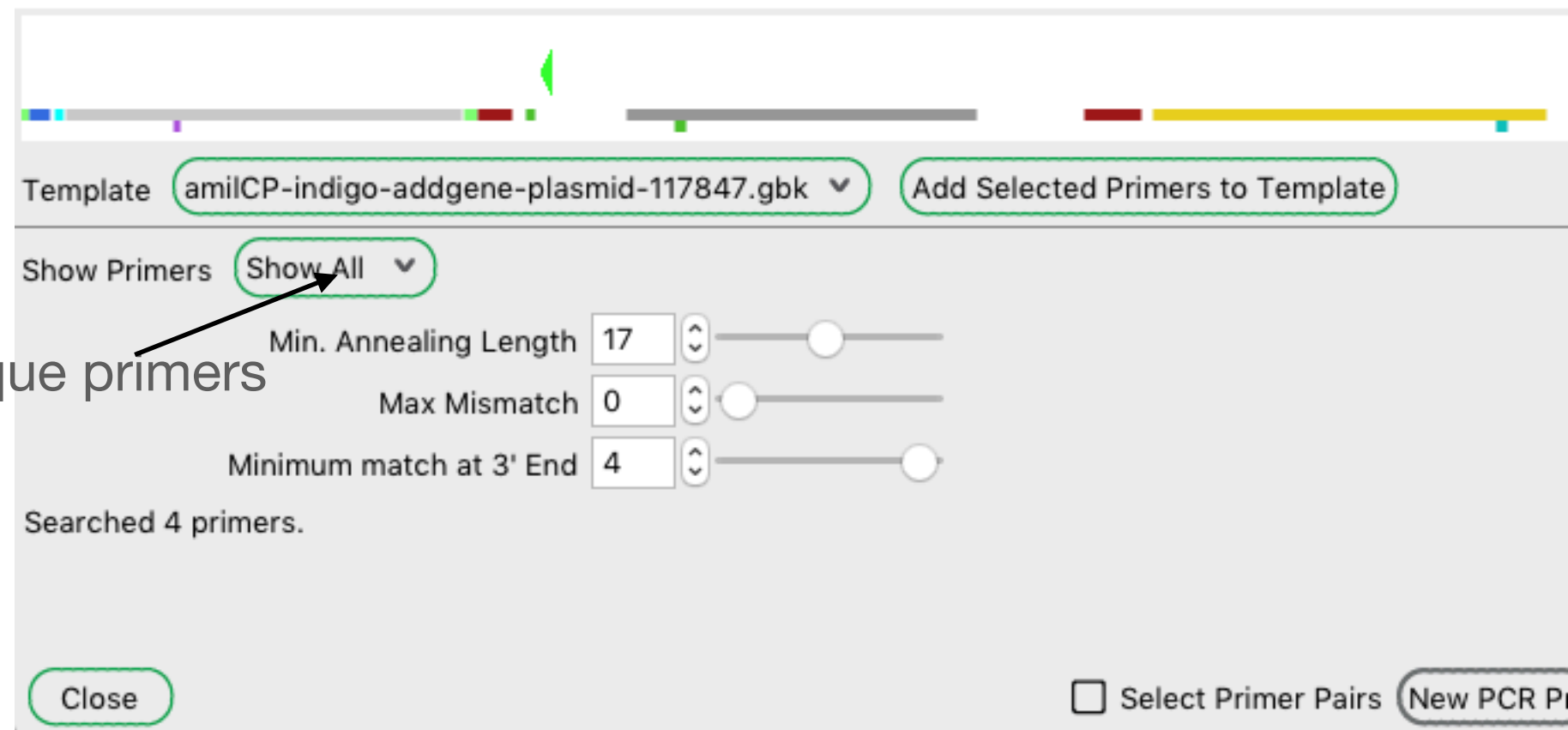


PCR reaction tool



Name	Sequence	Direction	Dist	Location ↓	Tm1	Tm2	M
L4440	agcgagtcagtgagcgag	<<<	91	856	57.3	57.3	
pBR322ori-F	gggaaacgcctgggatcttt	<<<	342	1107	56.1	56.1	
CAT-R	gcaactgactgaaatgcctc	>>>	1058	2507	55.2	55.2	
pBRforEco	aataggcgtatcacgaggc	>>>	812	2753	55.2	55.2	

Active primers show up here



Template: amilCP-indigo-addgene-plasmid-117847.gbk Add Selected Primers to Template

Show Primers: Show All

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Searched 4 primers.

Close ☐ Select Primer Pairs New PCR P

Select this to activate just unique primers

PCR reaction tool

Select primers to show
them in the sequence
map

Name	Sequence	Direction	Dist	Location ↓	Tm1	Tm2	MM	Cou
L4440	agcgagtcagtcgagcgag	<<<	91	856	57.3	57.3	0	1
pBR322ori-F	gggaaacgcctgggtatctt	<<<	342	1107	56.1	56.1	0	1
CAT-R	gcaactgactgaaatgctc	>>>	1058	2507	55.2	55.2	0	1
pBRforEco	actagcgctatcaacgaggc	>>>	812	2763	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk Add Selected Primers to Template

Show Primers: Unique in Template

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Searched 4 primers.

Close Select Primer Pairs New PCR Product

PCR reaction tool

Click here to add
selected primers as
new primer_bind
features

PCR Reaction...

Database...

Name	Sequence	Direction	Dist	Location ↓	Tm1	Tm2	MM	Cou
L4440	agcgagtcagtcgagcgag	<<<	91	856	57.3	57.3	0	1
pBR322ori-F	gggaaacgcctggatcttt	<<<	342	1107	56.1	56.1	0	1
CAT-R	gcaactgactgaaatgctc	>>>	1058	2507	55.2	55.2	0	1
pBRforEco	actagcgctatcaacgagc	>>>	812	2763	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk

Show Primers: Unique in Template

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Searched 4 primers.

Close

☐ Select Primer Pairs

New PCR Product

Add Selected Primers to Template

PCR reaction tool

Database...

Name	Sequence	Direction	Dist	Location ↓	Tm1	Tm2	MM	Cou
L4440	agcgagtcagtcgagcgag	<<<	91	856	57.3	57.3	0	1
pBR322ori-F	gggaaacgcctgggtatctt	<<<	342	1107	56.1	56.1	0	1
CAT-R	gcaactgactgaaatgctc	>>>	1058	2507	55.2	55.2	0	1
pBRforEco	actagcgctatcaacgaggc	>>>	812	2763	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk Add Selected Primers to Template

Show Primers: Unique in Template

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Searched 4 primers.

Close

☐ Select Primer Pairs New PCR Product

Modify the priming
search parameters
here

PCR reaction tool

Select all of the class primers and sequences then copy

	A	B	C	D
1	oMBC1	gaattcggcgccgcttctagag		
2	oMBC2	agcgagtcagtgagcgaggaagc		
3	oMBC3	gcttagcatttgtacctaaggactgagctag		
4	oMBC4	GCTtaactagtagogggcgctgcag		
5	oMBC5	tactagagaaagaggagaaatctatagAT		
6	oMBC6	ttattTAGGCGACCACAGGTTTGGG		
7	oMBC7	gtgGCTCTTCgTGGgaattcgogggcg		
8	oMBC8	gtgGCTCTTCgCAAGCAAATGCCAGAGG		
9	oMBC9	gtgGCTCTTCgACgAAGTACCCTGAAGA		
10	oMBC10	gtgGCTCTTCgTACctgcagcgggcgct		
11	oMBC11	gtgGCTCTTCgCGTGAATGGTATGCTTC		
12	oMBC12	gtgGCTCTTCgATGGGATATTTTATCAC		
13	oMBC13	gtgGCTCTTCgATGGGATATTTTATCAC		
14	oMBC14	gtgGCTCTTCgATGGGATATTTTATCAC		
15	oMBC15	gtgGCTCTTCgATGGGATATTTTATCAC		
16	oMBC16	gtgGCTCTTCgATGGGATATTTTATCAC		
17	oMBC17	gtgGCTCTTCgATGGGATATTTTATCAC		
18	oMBC18	gtgGCTCTTCgATGGGATATTTTATCAC		
19	oMBC19	gtgGCTCTTCgATGGGATATTTTATCAC		
20	oMBC20	gtgGCTCTTCgATGGGATATTTTATCAC		
21	oMBC21	gtgGCTCTTCgATGGGATATTTTATCAC		
22	oMBC22	/phos/CTGTGGTGATAAAATATCCCAA		
23	oMBC23	/phos/NNKXNKTACGGAAGCATAACCAT		
24	oMBC24	ggggacaagttttgtacaaaaaagcaggc		
25	oMBC25	ggggaccacgtttgtacaagaagaagctggg		
26	oMBC26	actggcgtcgttttaca		
27	oMBC27	catggtcatagctgtttctg		
28	oMBC28	tgtaaaacgacggccagtggaattcgccgcttctagag		
29	oMBC29	CAGGGTACTTGGTGAATGGTATGCT		
30	oMBC30	AGCATACCATTACCAAGTACCCTG		
31	oMBC31	caggaaacagctatgaccatgctgcagcgggcgctacta		
32	oMBC32	caggaaacagctatgaccatg		
33	oMBC33	tgtaaaacgacggccagt		
34	oMBC22	CTGTGGTGATAAAATATCCCAAAGCAAAT		
35	oMBC23	NNKXNKTACGGAAGCATAACCATTCACCA		
36				
37				

PCR reaction tool

Primers can be:

sequence

name (tab) sequence

name (tab) sequence (tab) note

feature library format

PCR reaction tool

PCR Reaction...

Database...

- Add Primers from Clipboard ⌘V
- Add Primers from File ⌘O
- Add Primers from Sequence ▶
- Copy Selected Primers ⌘C
- Save Selected Primers ⌘S
- Select All ⌘A
- Invert Selection ⇧⌘A
- Delete Selected ⌘⌫
- Delete All ⇧⌘⌫

	Direction	Dist	Location	Tm1	Tm2	MM	Cou
	>>>	1058	2507	55.2	55.2	0	1
	<<<	91	856	57.3	57.3	0	1
	<<<	342	1107	56.1	56.1	0	1
	>>>	812	2753	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk ▼ Add Selected Primers to Template

Show Primers: Unique in Template ▼

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: pBRforEco
Reverse primer: L4440

Close

☒ Select Primer Pairs New PCR Product

PCR reaction tool

PCR Reaction...

Database... ▾

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
CAT-R	gcaactgactgaaatgcctc	>>>	1058	2507	55.2	55.2	0	1
L4440	agcgagtcagtgagcgag	<<<	91	856	57.3	57.3	0	1
oMBC1	gaattcgcgccgcttctagag	>>>	741	24	61.4	61.4	0	1
oMBC2	agcgagtcagtgagcgaggaagc	<<<	86	851	64.4	64.4	0	1
oMBC3	gctagcattgtacctaggactgagctag	<<<	2066	31	61.4	61.4	0	1
oMBC4	GCTtactagtagcggccgctgcag	>>>	2790	775	62.4	65.0	0	1
oMBC5	tactagagaaagaggagaaatactag/	>>>	671	94	60.1	60.1	0	1
oMBC6	ttaTTAGGCGACCACAGGTTTC	<<<	2765	730	61.3	61.3	0	1
oMBC7	gtgGCTCTTCgTGGgaattcgcg	>>>	741	24	65.1	73.0	0	1
oMBC8	gtgGCTCTTCgCATGCAAATGG	<<<	2261	226	56.2	69.8	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk ▾ Add Selected Primers to Template

Show Primers: Show All ▾

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: pBRforEco
Reverse primer: L4440

Close

☒ Select Primer Pairs New PCR Product

PCR reaction tool

Select oMBC22 and
23 primers.
These are the around
the horn mutagenic
primers.

PCR Reaction...

Database... ▾

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
oMBC16	gtgGCTCTTCgATGGGATATTT							0
oMBC17	gtgGCTCTTCgATGGGATATTT							0
oMBC18	gtgGCTCTTCgATGGGATATTT							0
oMBC19	gtgGCTCTTCgATGGGATATTT							0
oMBC20	gtgGCTCTTCgATGGGATATTT							0
oMBC21	gtgGCTCTTCgATGGGATATTT							0
oMBC22	CTGTGGTGATAAAATATCCC	<<<	2270	235	60.3	60.3	0	1
oMBC23	NNKNNKTACGGAAGCATAC	>>>	463	302	64.9	65.2	0	1
oMBC24	ggggacaagtttgtacaaaaaagcag	>>>	741	24	65.2	75.3	0	1
oMBC25	ggggaccactttgtacaagaaagctgc	<<<	87	852	62.4	75.6	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk ▾ Add Selected Primers to Template

Show Primers: Show All ▾

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: oMBC23
Reverse primer: oMBC22

Close

☒ Select Primer Pairs New PCR Product

PCR reaction tool

PCR Reaction...

Database... ▾

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
oMBC16	gtgGCTCTTCgATGGGATATTT							0
oMBC17	gtgGCTCTTCgATGGGATATTT							0
oMBC18	gtgGCTCTTCgATGGGATATTT							0
oMBC19	gtgGCTCTTCgATGGGATATTT							0
oMBC20	gtgGCTCTTCgATGGGATATTT							0
oMBC21	gtgGCTCTTCgATGGGATATTT							0
oMBC22	CTGTGGTGATAAAATATCCC	<<<	2270	235	60.3	60.3	0	1
oMBC23	NNKNNKTACGGAAGCATAC	>>>	463	302	64.9	65.2	0	1
oMBC24	ggggacaagtttgtacaaaaagcag	>>>	741	24	65.2	75.3	0	1
oMBC25	ggggaccactttgtacaagaaagctgc	<<<	87	852	62.4	75.6	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk ▾ Add Selected Primers to Template

Show Primers: Show All ▾

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: oMBC23
Reverse primer: oMBC22

Close ☒ Select Primer Pairs New PCR Product

Select this to do a new
PCR reaction

PCR reaction tool

In primer pair mode, only one forward and one reverse primer can be selected. Selected primers are in bold.

The screenshot shows the 'PCR Reaction...' window. At the top is a 'Database...' dropdown. Below is a table of primers with columns: Name, Sequence, Direction, Dist, Location, Tm1, Tm2, MM, and Cou. Primers oMBC22 and oMBC23 are highlighted in blue, and oMBC22's sequence is bolded. Below the table is a genomic map with a green and blue double-headed arrow. The 'Template' section shows 'amilCP-indigo-addgene-plasmid-117847.gbk' and an 'Add Selected Primers to Template' button. The 'Show Primers' section has a 'Show All' dropdown and three sliders for 'Min. Annealing Length' (17), 'Max Mismatch' (0), and 'Minimum match at 3' End' (4). Below these are labels for 'Forward primer: oMBC23' and 'Reverse primer: oMBC22'. At the bottom are 'Close', 'Select Primer Pairs' (checked), and 'New PCR Product' buttons.

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
oMBC16	gtgGCTCTTCgATGGGATATTT							0
oMBC17	gtgGCTCTTCgATGGGATATTT							0
oMBC18	gtgGCTCTTCgATGGGATATTT							0
oMBC19	gtgGCTCTTCgATGGGATATTT							0
oMBC20	gtgGCTCTTCgATGGGATATTT							0
oMBC21	gtgGCTCTTCgATGGGATATTT							0
oMBC22	CTGTGGTGATAAAATATCCC	<<<	2270	235	60.3	60.3	0	1
oMBC23	NNKNNKTACGGAAGCATAC	>>>	463	302	64.9	65.2	0	1
oMBC24	ggggacaagttgtacaaaaagcag	>>>	741	24	65.2	75.3	0	1
oMBC25	ggggaccactttgtacaagaaagctgc	<<<	87	852	62.4	75.6	0	1

Template: amilCP-indigo-addgene-plasmid-117847.gbk

Show Primers: Show All

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

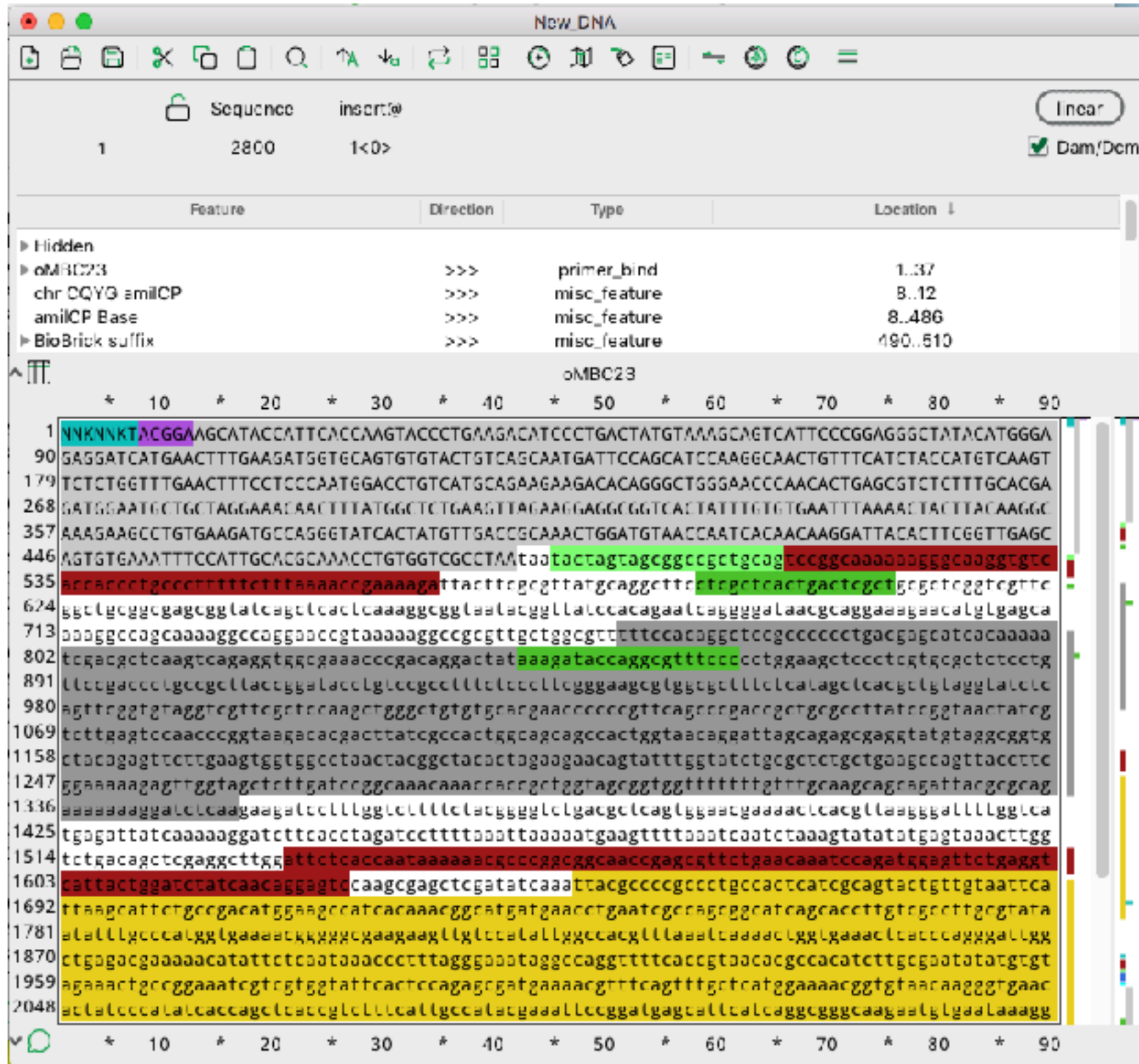
Forward primer: oMBC23

Reverse primer: oMBC22

Close Select Primer Pairs New PCR Product

New PCR will become active when a pair of primers is selected.

PCR reaction tool



Gibson Designer

Gibson Designer

Do oMBC26-oMBC27
PCR on pMLS280

PCR Reaction...

Database... ▾

Name	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
L4440	agcgagtcagtcagcgagg	>>>	2375	109	57.3	57.3	0	1
oMBC2	agcgagtcagtcagcgagggaagc	>>>	2370	114	64.4	64.4	0	1
oMBC25	ggggaccactttgtacaagaaagctggg	>>>	2371	113	62.4	75.6	0	1
oMBC26	actggccgctggtttaca	<<<	535	535	55.2	55.2	0	1
oMBC27	catggtcataagctgtttctg	>>>	1719	765	54.5	54.5	0	1
oMBC32	caggaaacagctatgaccatg	<<<	744	744	54.5	54.5	0	1
oMBC33	tgtaaacgacggccagt	>>>	1931	553	55.2	55.2	0	1
pBR322ori-F	gggaaacgocctggatcttt	>>>	142	2342	56.1	56.1	0	1

Template: pMLS280 ▾ Add Selected Primers to Template

Show Primers: Unique in Template ▾

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: oMBC27
Reverse primer: oMBC26

Close Select Primer Pairs New PCR Product

Gibson Designer

Do oMBC28-oMBC29
PCR on amilCP

The screenshot shows the 'PCR Reaction...' window in Gibson Designer. A table lists various primers and their properties. The primer pair oMBC28 and oMBC29 is highlighted in blue. Below the table, a template is selected as 'amilCP-indigo-addgene-plasmid-117847'. The 'Show Primers' dropdown is set to 'Unique in Template'. The 'Min. Annealing Length' is 17, 'Max Mismatch' is 0, and 'Minimum match at 3' End' is 4. The forward primer is oMBC28 and the reverse primer is oMBC29. The 'New PCR Product' button is highlighted.

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
oMBC22	CTGTGGTGATAAAATATCCCA	<<<	2270	235	60.3	60.3	0	1
oMBC23	NNKNNKTACGGAAGCATACCA	>>>	463	302	61.9	65.2	0	1
oMBC24	ggggacaaglllglacaaaaaagcaggr	>>>	741	74	65.7	75.3	0	1
oMBC25	ggggaccacftttgtacaagaaagctggc	<<<	87	852	62.4	75.6	0	1
oMBC28	tgtaaaacgacggccagtgaattgc	>>>	741	24	62.8	73.0	0	1
oMBC29	CAGGGTACTTGGTGAATGGT/	<<<	2312	277	60.4	60.4	0	1
oMBC30	AGCATACCATTACCAAGTACC	>>>	463	302	60.4	60.4	0	1
oMBC31	caggaaacagclalgacclgclgcagc	<<<	2789	754	64.7	73.7	0	1
p3R322ori-F	gggaaacgcctggtatcttt	<<<	342	1107	56.1	56.1	0	1
p3RforEco	aataggcgtatcagcgggc	>>>	812	2753	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847

Show Primers: Unique in Template

Min. Annealing Length: 17

Max Mismatch: 0

Minimum match at 3' End: 4

Forward primer: oMBC28
Reverse primer: oMBC29

Select Primer Pairs **New PCR Product**

Gibson Designer

Do oMBC30-oMBC31
PCR on amilCP

PCR Reaction...

Database... ▾

Name ↓	Sequence	Direction	Dist	Location	Tm1	Tm2	MM	Cou
oMBC22	CTGTGGTGATAAAATATCCCA	<<<	2270	235	60.3	60.3	0	1
oMBC23	NNKNNKTACGGAAGCATACCA	>>>	463	302	64.9	65.2	0	1
oMBC24	ggggacaagtttgtacaaaaaagcagg	>>>	741	24	65.2	75.3	0	1
oMBC25	ggggaccacttgtacaaagagctggc	<<<	87	852	62.4	75.6	0	1
oMBC28	tgtacaaagcaggccagtgattcgcc	>>>	741	24	62.8	73.0	0	1
oMBC29	CAGGGTACTTGGTGAATGGTA	<<<	2312	277	60.4	60.4	0	1
oMBC30	AGCATACCATTACCAAGTAC	>>>	463	302	60.4	60.4	0	1
oMBC31	caggaaacagctatgaccatgctgca	<<<	2789	754	64.7	73.2	0	1
pRR322ori-F	gggaaacggcgggaaacggcggg	<<<	342	1107	56.1	56.1	0	1
p3RforEco	aataggcgtatcagcaggc	>>>	812	2753	55.2	55.2	0	1

Template: amilCP-indigo-addgene-plasmid-117847 ▾ Add Selected Primers to Template

Show Primers: Unique In Template ▾

Min. Annealing Length: 17

Max Mismatch: 0

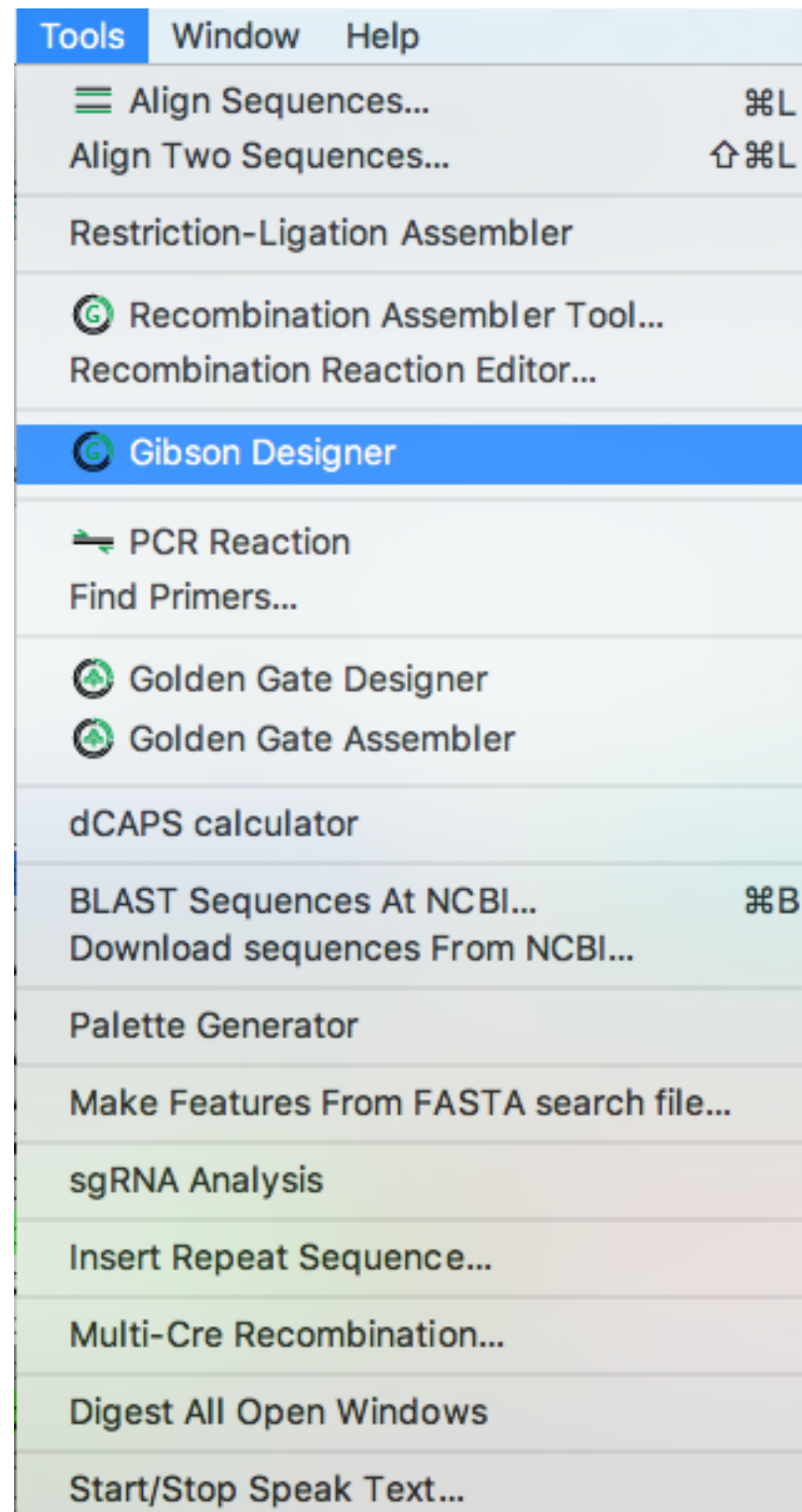
Minimum match at 3' End: 4

Forward primer: oMBC30
Reverse primer: oMBC31

Close

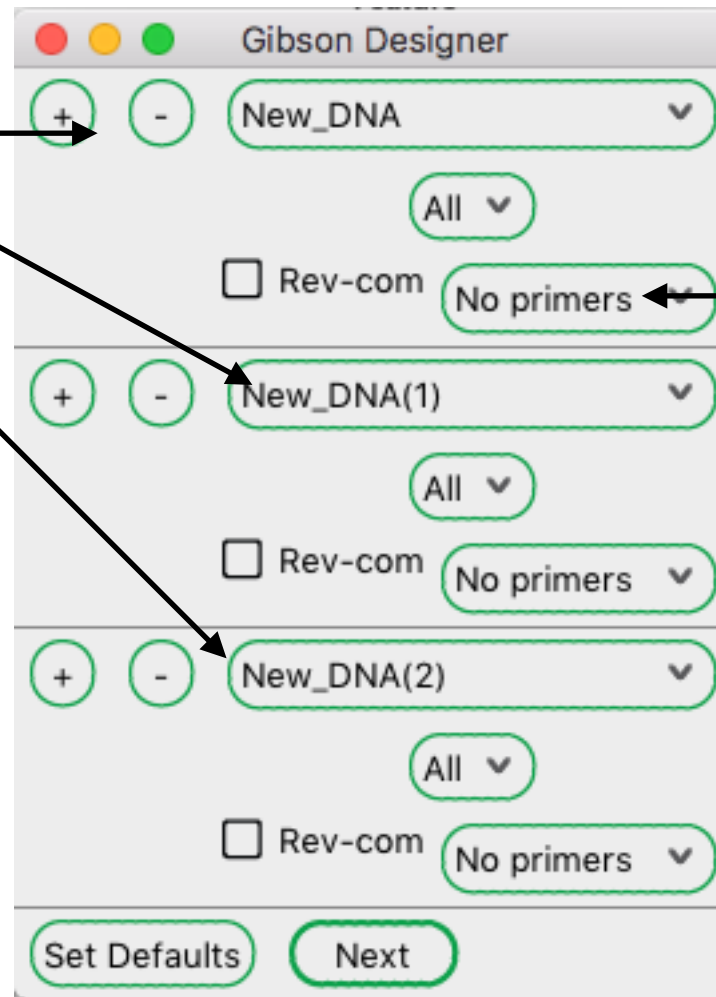
☒ Select Primer Pairs **New PCR Product**

Gibson Designer



Gibson Designer

add the three new
DNA sequences in
order



The screenshot shows the 'Gibson Designer' window with three DNA sequence entries. Each entry has a '+' and '-' button, a text field, a dropdown menu, a checkbox for 'Rev-com', and a 'No primers' dropdown. The entries are labeled 'New_DNA', 'New_DNA(1)', and 'New_DNA(2)'. At the bottom are 'Set Defaults' and 'Next' buttons.

Entry	Label	Rev-com	Primers
1	New_DNA	<input type="checkbox"/>	No primers
2	New_DNA(1)	<input type="checkbox"/>	No primers
3	New_DNA(2)	<input type="checkbox"/>	No primers

In this case, we don't
need new primers. We
are doing a Gibson
using someone else's
design

Gibson Designer

First fragment

The screenshot shows the Gibson Designer application window. The title bar reads 'Gibson Designer'. The main text area displays two DNA fragments: 'New_DNA 1..2293' with the sequence 'tgtaaaacgacggccagt' followed by 18 vertical bars representing the overlap, and 'New_DNA(1) 1..318' with the sequence 'tgtaaaacgacggccagt'. Below the sequences are input fields for 'Rev primer Tm', 'Fwd primer Tm', and 'Template Overlap' (set to 18). The 'Overlap Tm' is calculated as 55.2°C 18b. At the bottom, there are buttons for 'Set Defaults', 'Back', and 'Next' (highlighted in green). An arrow points from the 'Next' button to the text 'Press next to see each of the three junctions'.

New_DNA 1..2293

tgtaaaacgacggccagt

|||||

|||||

|||||

|||||

|||||

tgtaaaacgacggccagt

New_DNA(1) 1..318

Rev primer Tm:

Fwd primer Tm:

Template Overlap: 18

Overlap Tm: 55.2°C 18b

Both Primer Tail: 0

Set Defaults Back Next

Second fragment

Press next to see each of the three junctions

Gibson Designer

Second fragment

The screenshot shows the Gibson Designer application window. At the top, the title bar reads 'Gibson Designer'. The main area displays two DNA fragments. The first fragment is labeled 'New_DNA(1) 1..519' and contains the sequence 'catgggtcatagctgtttcctg' followed by a series of vertical bars representing a sequence. The second fragment is labeled 'New_DNA(2) 1..519' and contains the same sequence. Below the fragments, there are input fields for 'Rev primer Tm', 'Fwd primer Tm', and 'Template Overlap' (set to 21). The 'Overlap Tm' is set to 54.5°C 21b. At the bottom, there are buttons for 'Set Defaults', 'Back', and 'Finish'.

New_DNA(2) 1..519
catgggtcatagctgtttcctg
catgggtcatagctgtttcctg
New_DNA 1..2293

Rev primer Tm:
Fwd primer Tm:
Template Overlap: 21
Overlap Tm: 54.5°C 21b
Both Primer Tail: 0

Set Defaults Back Finish

Final fragment

Generate product

Gibson Designer

The first template sequence

The reverse primer sequence

The forward primer sequence

The second template sequence

The screenshot shows the Gibson Designer interface with the following elements:

- 1. Ligation Product 1429..587**
 - actatagggcgaat (with a right-pointing arrow below it)
 - primer1_rev (in a text box, with an arrow pointing to the sequence actatagggcgaattgggtaccggg)
 - primer2_fwd (in a text box, with an arrow pointing to the sequence tgggtaccggg)
 - tgggtaccggg (with a right-pointing arrow below it)
- 1. Ligation Product 588..694**
- Rev primer Tm: 59 (with a dropdown arrow), 59.4°C 81.0°C 25b
- Fwd primer Tm: 59 (with a dropdown arrow), 60.8°C 60.8°C 11b
- Template Overlap: None (in a dropdown menu)
- Overlap Tm: 55 (with a dropdown arrow), 60.8°C 11b
- Forward (in a dropdown menu), Primer Tail: 0 (with a dropdown arrow)
- Buttons: Set Defaults, Back, Next

Gibson Designer

Design a new Gibson reaction

Gibson Designer

Use the open plasmids
with these selection
ranges

The screenshot shows the Gibson Designer application window. It contains two plasmid entries, each with a selection range and a 'Rev-com' checkbox. The first entry is 'pMLS280.apc' with a selection range of 'All but Selected 638-587'. The second entry is 'amilCP-indigo-addgene-plasmid-117847.gbk' with a selection range of 'Selected 25-833'. Both entries have a 'PCR with tails' option selected. At the bottom, there are 'Set Defaults' and 'Next' buttons. Two arrows point from the text 'Use the open plasmids with these selection ranges' to the selection range dropdowns of the two plasmids.

Plasmid	Selection Range	Rev-com	PCR with tails
pMLS280.apc	All but Selected 638-587	<input type="checkbox"/>	<input checked="" type="checkbox"/>
amilCP-indigo-addgene-plasmid-117847.gbk	Selected 25-833	<input type="checkbox"/>	<input checked="" type="checkbox"/>

Gibson Designer

First fragment → pMLS280 638..587

Reverse primer → primer1_rev

Forward primer → primer2_fwd

Second fragment → amilCP-indigo-addgene-plasmid-117847 25..833

Rev primer Tm: 59 59.4°C 70.0°C 47b

Fwd primer Tm: 59 59.1°C 59.1°C 23b

Template Overlap: None

Overlap Tm: 56 56.5°C 20b

Forward Primer Tail: 0

Set Defaults Back Next

Gibson Designer

priming hybridization

Gibson hybridization

The screenshot shows the Gibson Designer software interface. At the top, the window title is "Gibson Designer". The main area displays two DNA sequences with their coordinates: "pMLS280 638..587" and "amilCP-indigo-addgene-plasmid-117847 25..833". The first sequence is "cgtaatacgaactcactatagggcgaat" with a right-pointing arrow above it. The second sequence is "cgtaatacgaactcactatagggcgaattttacggctagctcagtcct" with a left-pointing arrow above it. Below these sequences are two input fields: "primer1_rev" and "primer2_fwd". Arrows from the text "priming hybridization" and "Gibson hybridization" point to the "primer1_rev" and "primer2_fwd" fields respectively. The bottom section contains several controls: "Rev primer Tm: 59" (with a dropdown showing "59.4°C 70.0°C 47b"), "Fwd primer Tm: 59" (with a dropdown showing "59.1°C 59.1°C 23b"), "Template Overlap: None" (with a dropdown arrow), "Overlap Tm: 56" (with a dropdown showing "56.5°C 20b"), "Forward" (with a dropdown arrow), "Primer Tail: 0" (with a dropdown arrow), and three buttons: "Set Defaults", "Back", and "Next".

pMLS280 638..587

cgtaatacgaactcactatagggcgaat

cgtaatacgaactcactatagggcgaattttacggctagctcagtcct

primer1_rev

primer2_fwd

amilCP-indigo-addgene-plasmid-117847 25..833

Rev primer Tm: 59 59.4°C 70.0°C 47b

Fwd primer Tm: 59 59.1°C 59.1°C 23b

Template Overlap: None

Overlap Tm: 56 56.5°C 20b

Forward Primer Tail: 0

Set Defaults Back Next

Gibson Designer

Adjust the Tm

Adjust the Tm

If there is already overlap
between the templates, you
can use that

Adjust the overlap Tm

The screenshot shows the Gibson Designer interface with the following details:

- Window Title:** Gibson Designer
- Top Right:** pMLS280 638..587
- Sequence 1:** cgtaatac gactcactatagggcgaat (with a left-pointing arrow below it)
- primer1_rev:** cgtaatac gactcactatagggcgaattttacggctagctcagtcct
- primer2_fwd:** ttacggctagctcagtcctagg
- Sequence 2:** ttacggctagctcagtcctagg (with a right-pointing arrow below it)
- Bottom Right:** amilCP-indigo-addgene-plasmid-117847 25..833
- Rev primer Tm:** 59 (with a dropdown showing 59.4°C 70.0°C 47b)
- Fwd primer Tm:** 59 (with a dropdown showing 59.1°C 59.1°C 23b)
- Template Overlap:** None (dropdown menu)
- Overlap Tm:** 56 (with a dropdown showing 56.5°C 20b)
- Forward:** (dropdown menu)
- Primer Tail:** 0 (with a dropdown)
- Buttons:** Set Defaults, Back, Next

Gibson Designer

You can also add non-templated sequence here

Set which fragment gets the overlap

Add overlap to the other side

The screenshot shows the Gibson Designer application window. At the top, it displays the sequence `pMLS280 638..587` with a reverse primer `cgtaatacgaactcactatagggcgaat` and a forward primer `tttacggctagctcagtccttagg`. Below this, the sequence `amilCP-indigo-addgene-plasmid-117847 25..833` is shown. The interface includes input fields for `primer1_rev` and `primer2_fwd`. The `Rev primer Tm` is set to 59, with a calculated range of 59.4°C to 70.0°C over 47bp. The `Fwd primer Tm` is set to 59, with a calculated range of 59.1°C to 59.1°C over 23bp. The `Template Overlap` is set to `None`. The `Overlap Tm` is set to 56, with a calculated range of 56.5°C over 20bp. The `Forward` dropdown is set to `Forward`, and the `Primer Tail` is set to 0. At the bottom, there are buttons for `Set Defaults`, `Back`, and `Next`.

Gibson Designer

The screenshot shows the Gibson Designer window with the following details:

- Top Bar:** "Gibson Designer" title and window controls.
- Sequence Alignment:**
 - Top sequence: pMLS280 638..587, sequence `cgtaatacgaactcactatagggcgaat` with a left-pointing arrow below it.
 - Bottom sequence: amilCP-indigo-addgene-plasmid-117847 25..833, sequence `tttacggctagctcagtcctagg` with a right-pointing arrow above it.
 - Overlap region: `cgtaatacgaactcactatagggcgaattttacggctagctcagtcct` (top) and `tttacggctagctcagtcctagg` (bottom).
- Primer Fields:**
 - `primer1_rev` (corresponds to the top sequence)
 - `primer2_fwd` (corresponds to the bottom sequence)
- Parameters:**
 - Rev primer Tm: 59 (59.4°C 70.0°C 47b)
 - Fwd primer Tm: 59 (59.1°C 59.1°C 23b)
 - Template Overlap: None (dropdown)
 - Overlap Tm: 56 (56.5°C 20b)
 - Forward (dropdown) Primer Tail: 0
- Buttons:** Set Defaults, Back, Next.

You have to examine each junction

Gibson Designer

The screenshot displays the Gibson Designer software interface. At the top, the title bar reads "New_DNA". Below it is a toolbar with various icons for file operations and editing. The main window is divided into several sections:

- Sequence:** Shows a sequence of 2500 bases, with a current position of 3243. The sequence is displayed as "2500 3243 2500<0>".
- Features:** A table listing features with columns for Feature, Direction, Type, and Location.
- Sequence View:** A detailed view of the DNA sequence, with positions 10, 20, 30, 40, 50, 60, 70, 80, and 90 marked. The sequence is displayed in a monospaced font, with some regions highlighted in different colors (blue, green, red, yellow).

Feature	Direction	Type	Location
Hidden			
primer1_fwd	>>>	primer_bind	1..18
MCS-Inverted in SK+	<<<	misc_feature	1..62
attR1	<<<	misc_feature	13..18
MsiI	<<<	misc_feature	45..54

The sequence view shows a DNA sequence starting with "ACCGTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTT". The sequence is displayed in a monospaced font, with some regions highlighted in different colors (blue, green, red, yellow).

Gibson Designer

The screenshot displays the Gibson Designer interface. At the top, a DNA sequence is shown with line numbers 2729, 2817, 2905, 2993, 3081, and 3169. The sequence is: TGTAAGCAGTCATTCCCGGAGGGCTATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACCATGTCAAGTTCTCTGGTTTGAACCTTCCTCCCAATGGACCTGTCATGCAGAAGAAGACACAGGGCTGGGAACCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGCTAGGAAACAACCTTTATGGCTCTGAAGTTAGAAGGAGGCGGTCACTATTTGTGTGAATTTAAACTACTTACAAGGCAAAGAAGCCTGTGAAGATGCCAGGGTATCACTATGTTGACCGCAAACCTGGATGTAACCAATCACAACAAGGATTACACTTCGGTTGAGCAGTGTGAAATTTCCATTGCACGCAAACCTGTGGTCGCCTAAtaatactagtagcggccgctgcagtccggcaaaaaagggaagggtgtcaccacacctgccctttttcttttaaaaccgaaaaga. The sequence is color-coded: green for the first part, red for the second part, and green for the third part. Below the sequence is a scale bar with markers at 10, 20, 30, 40, 50, 60, 70, and 80. In the bottom left corner, a box contains the following text: Gibson reaction: PCR: pMLS280 primer1_fwd gaattcctgcagcccgagg 59.4, 59.4 primer1_rev aggactgagctagccgtaaaattcgccctatagtgagtcgtattacg 59.4, 70.0 Product length : 2454. An arrow points from the text 'Gibson PCR products, primers etc. are in the file comment' to the primer1_rev sequence.

Gibson reaction:
PCR: pMLS280
primer1_fwd gaattcctgcagcccgagg 59.4, 59.4
primer1_rev aggactgagctagccgtaaaattcgccctatagtgagtcgtattacg 59.4, 70.0
Product length : 2454

**Gibson PCR products,
primers etc. are in the file
comment**

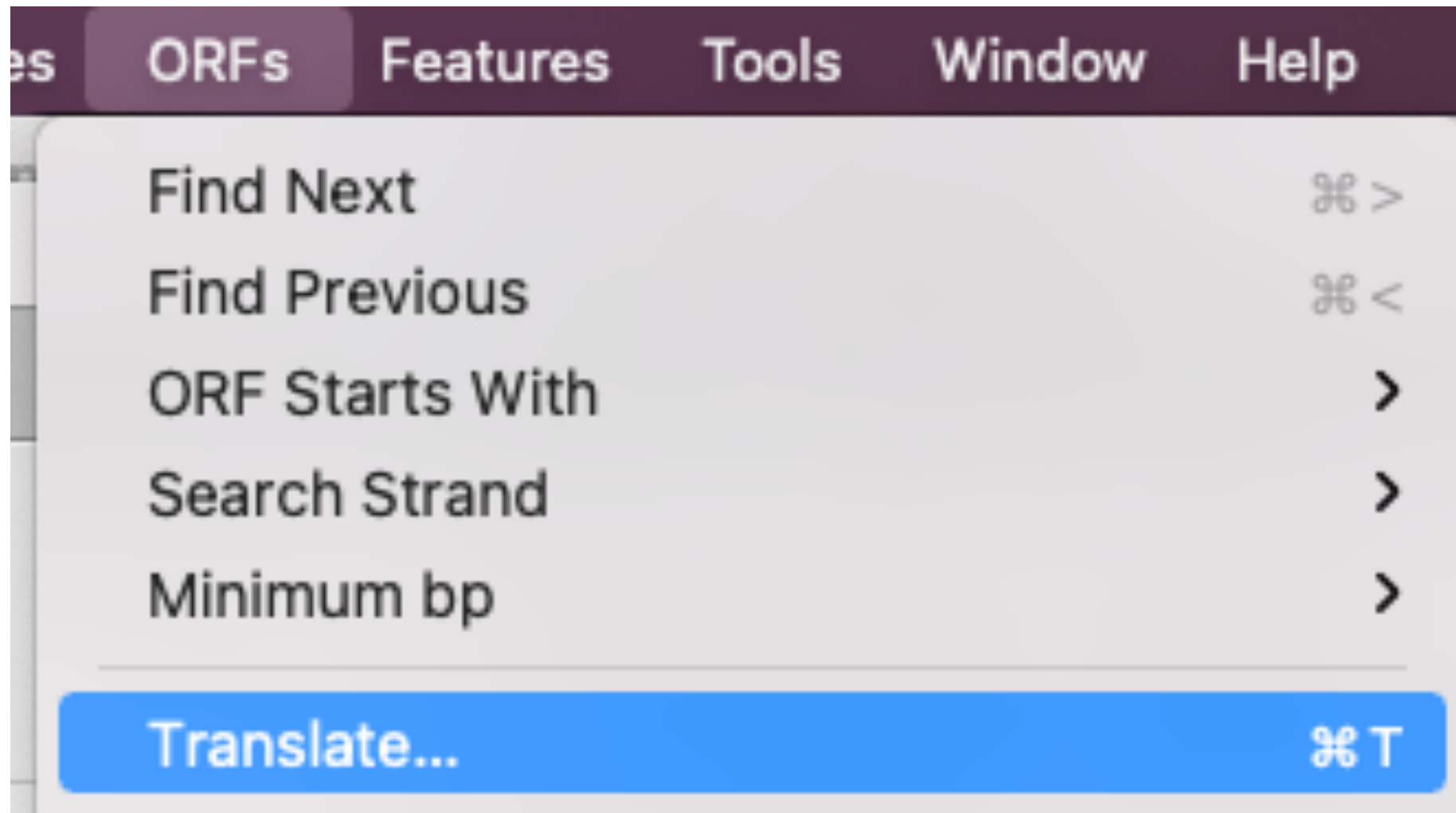
Gibson Designer

EFLQPG			
Feature	Direction	Type	Location ↓
▶ Hidden			
▼ primer1_fwd	>>>	primer_binc	1..18
		PCR_conditions	primer sequence:gaattcctgcagcccg
MCS-inverted in SK+	<<<	misc_feature	1..62
smal	<<<	misc_feature	13..18
^ MCS-inverted in SK+,primer1_fwd,primer2_rev			

2113 CAAAAGGCCATCCGTCAGGATGCGCTTCTGCTTAGTTTGATGCGCTGGCAGTTTATGGCGGGCGTCTGCGCGCCACCCCTCCGGGCGG
2201 TTGCTTCACAACGTTCAATCCGCTCCGCGCGGATTTGTCTACTCAGGAGAGCGTTACCGGACAAPCAACAGATAAACGAAAGGCC
2289 CAGTCTTCGACTGAGCCTTTGCTTTTATTTGATGCTGGCAGTTCCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCCAGTCA
2377 CGACGT **tgtaaaacgacggccagt** **gagcgcg** **cgtaatacgaactcactataggcggaattttacggctagctcagtccttaggtacaatg**
2465 ctagctctctagagagggaggaatctactagATCAGTGTGATCGCTAAACAAATGACCTACAGCTATATACAGGCTGGTCAAT
2553 **MS3-fwd** ⇒ **T7** ⇒ **primer2_fwd** ⇒
2641 CTCTGCCATTTGCTTGGGATATTTATCACCACAGTGTCACTACCGAA **primer1_rev** ⇒ **CCAGTACCTTCAACACATCCCTGACTA**
2729 TGTAAAGCACTCATTCCCGGAGGCTATACATGGGAGAGGATCATGAACCTTTGAAGATGG **constitutive promoter J23110** ⇒ CC
2817 TSCATCCAGGCAACTGTTTATTTACATGTCAGTTCTCTGGTTTGAACCTTCCCTCCCAATGGACCTGTCTGTCAGAGAGAGACAC
2905 AGCGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATCGAATGCTGCTAGGAACAACCTTTATGGCTCTGAAGTTAGAAGGAGG
2993 CGTCACTATTTGTGTGAATTTAAACTACTTACAAGGCAAAAGAGCCTGTGAAGATGCLAGGGTATCACTATTTGACCGCAACTG
3081 GATGTAAACCAATCACACAAAGGATTACACTTCGTTGACAGTGTGAATTTCCATTGCACGCAAACTGTGTCGCTAT **lacI**
3169 **amr** **aggcgccgctgcaattccgcgcaaaagggcgaagtctcaccacccctgccccttttcttttaaaacggaaa** **ga**

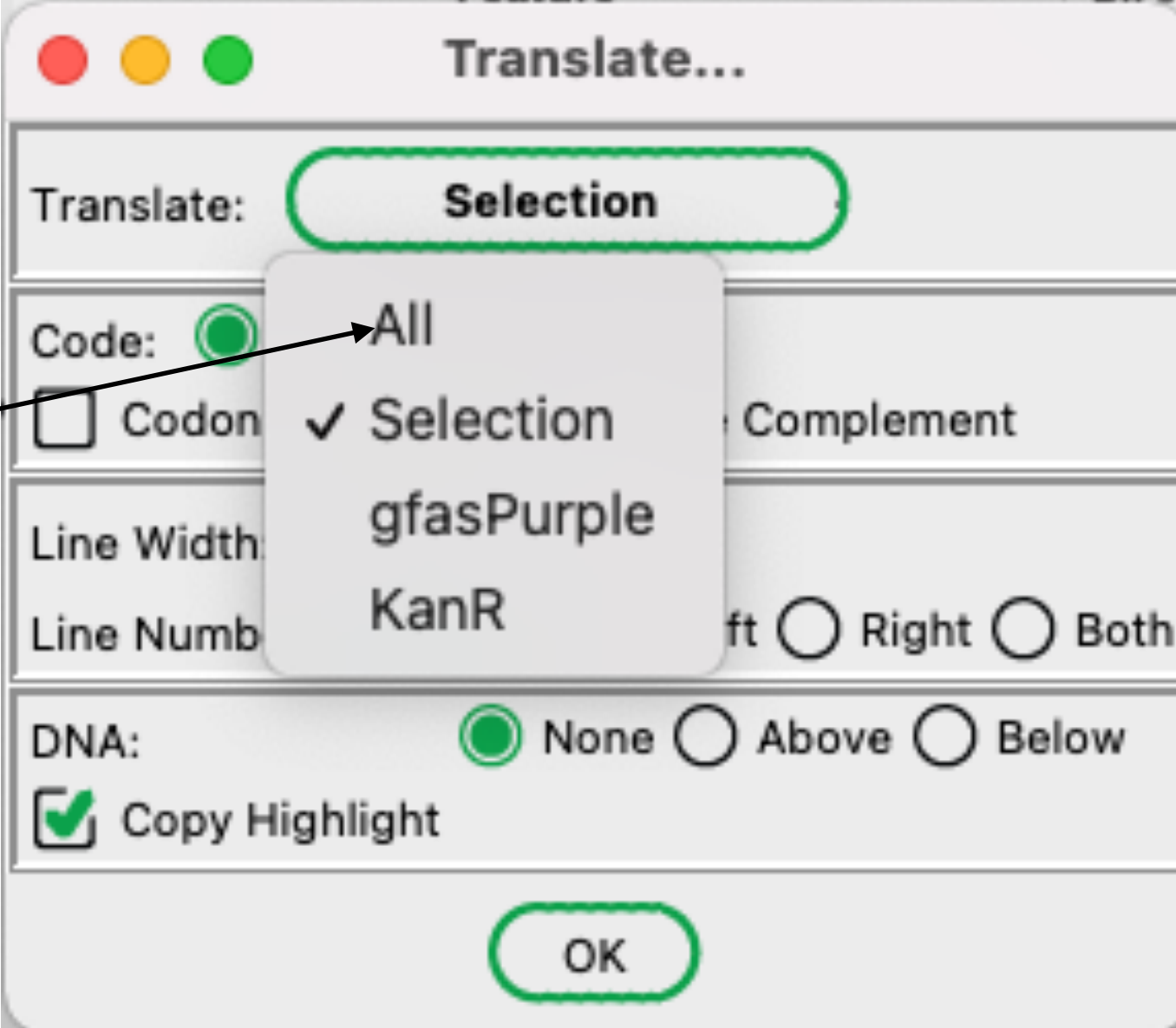
Gibson PCR primers are in the file features

Translation tool



Translation tool

Translate all of a
sequence, selected
text, or any “CDS”
type feature




The image shows a 'Translate...' dialog box with a dropdown menu open. The 'Translate:' field is set to 'Selection'. The dropdown menu lists 'All', 'Selection' (which is checked), 'gfasPurple', and 'KanR'. Other options in the dialog include 'Code' (radio button), 'Codon' (checkbox), 'Line Width', 'Line Numb', 'DNA' (radio buttons for None, Above, Below), 'Copy Highlight' (checkbox), and an 'OK' button. A green dashed line highlights the 'Selection' button and the 'OK' button. An arrow points from the text on the left to the 'Selection' option in the dropdown menu.

Field	Value / Option
Translate:	Selection
Code:	<input checked="" type="radio"/>
Codon	<input type="checkbox"/>
Line Width	
Line Numb	
DNA:	<input checked="" type="radio"/> None <input type="radio"/> Above <input type="radio"/> Below
Copy Highlight	<input checked="" type="checkbox"/>
OK	

Translation tool

Format the output



The image shows a 'Translate...' dialog box with the following settings:

- Translate:** gfasPurple
- Code:** ☒ 1 Letter ☐ 3 Letter
- ☐ Codon Spacing ☐ Reverse Complement
- Line Width:** 20
- Line Numbers:** ☒ None ☐ Left ☐ Right ☐ Both
- DNA:** ☒ None ☐ Above ☐ Below
- ☒ Copy Highlight
- OK**

Arrows from the text 'Format the output' point to the '1 Letter' radio button and the 'Line Width' field.

Translation tool

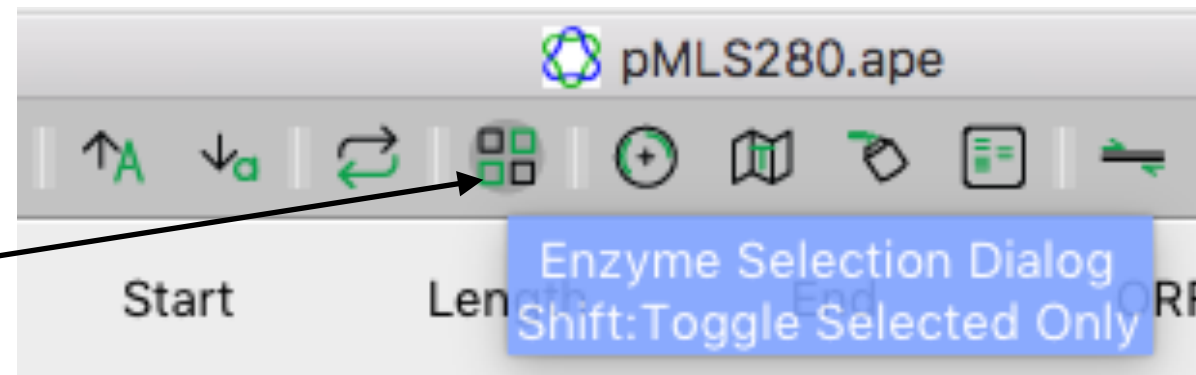


```
1. Ligation Product.ape Translation
Tue Jul 27, 2021 20:49 MDT
1. Ligation Product.ape
/Users/waynedavis/Downloads/1. Ligation Product.ape
gfasPurple
Translation 221 a.a. MW=24947.95

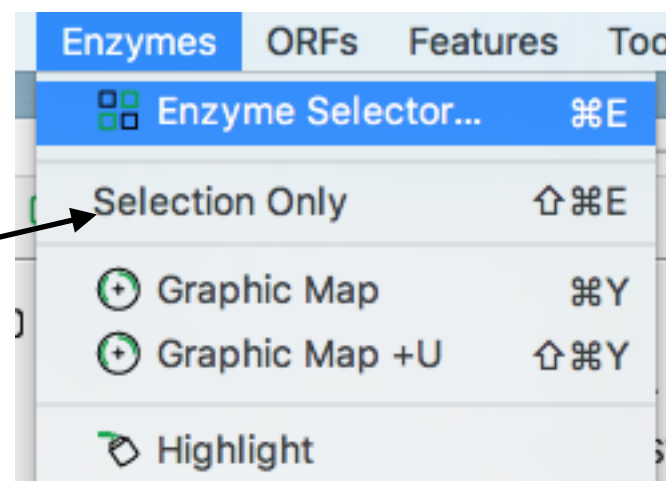
MSVIAKQMTYKVYMSGTVNG
HYFEVEGDGKGKPYEGEQTV
KLTVTKGGPLPFAWDILSPQ
SQYGSIPFTKYPEDIPDYVK
QSFPEGYTWERIMNFEDGAV
CTVSNDSSIQGNCFIYHVKF
SGLNFPPNGPVMQKKTQGWE
PENTERLFARDGMLIGNNFMA
LKLEGGGHYLCFEKSTYKAK
KPVKMPGYHYVDRKLDVTNH
NKDYTSVEQCEISIARKSVV
A*
```

Enzyme Selection

Shift-click to change
all tools to apply to
selection rather than
sequence

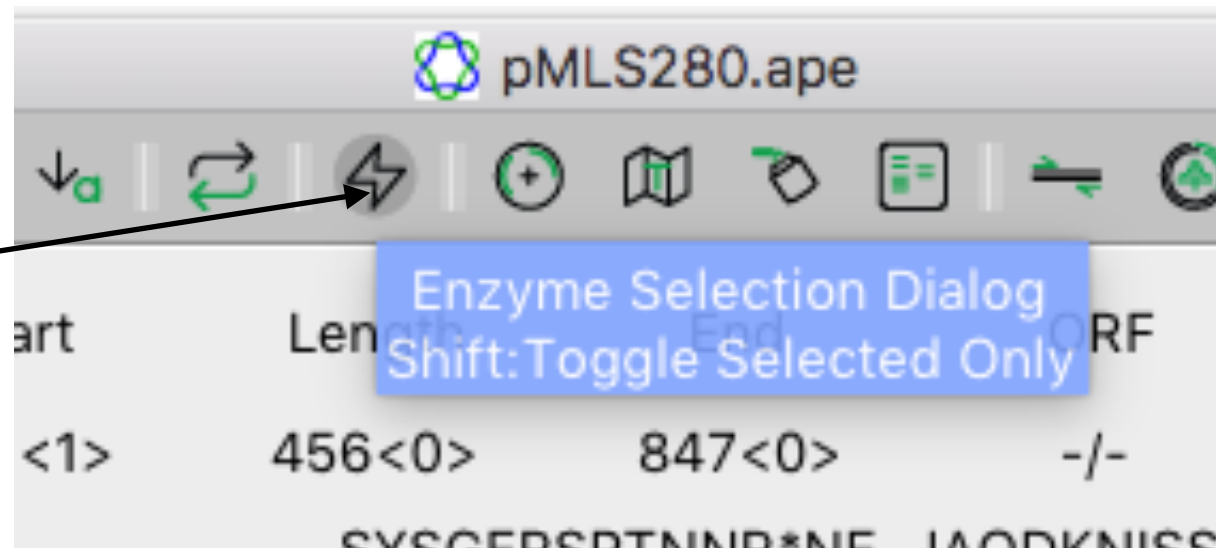


You can select this
option to change all
tools to apply to
selection rather than
sequence

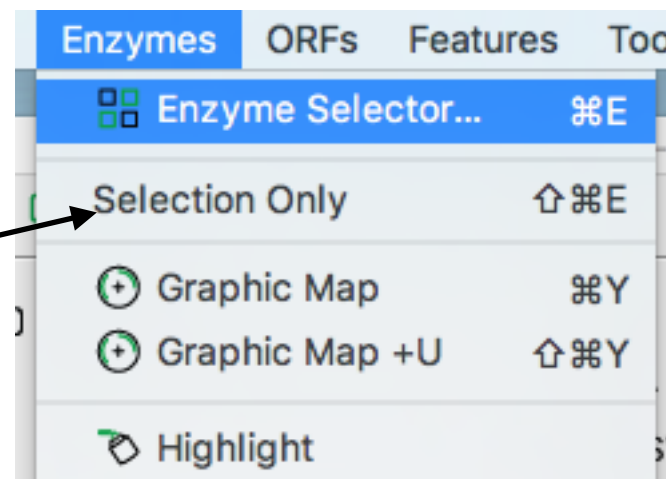


Enzyme Selection

This toolbar icon changes when you have “selection only” applied



You can select this option to change all tools to apply to selection rather than sequence



Enzyme Selection

The screenshot shows the 'Enzyme Selection...' dialog box for the file 'pML S280.ape'. The dialog displays a grid of enzymes and their counts. Annotations with arrows point to specific UI elements:

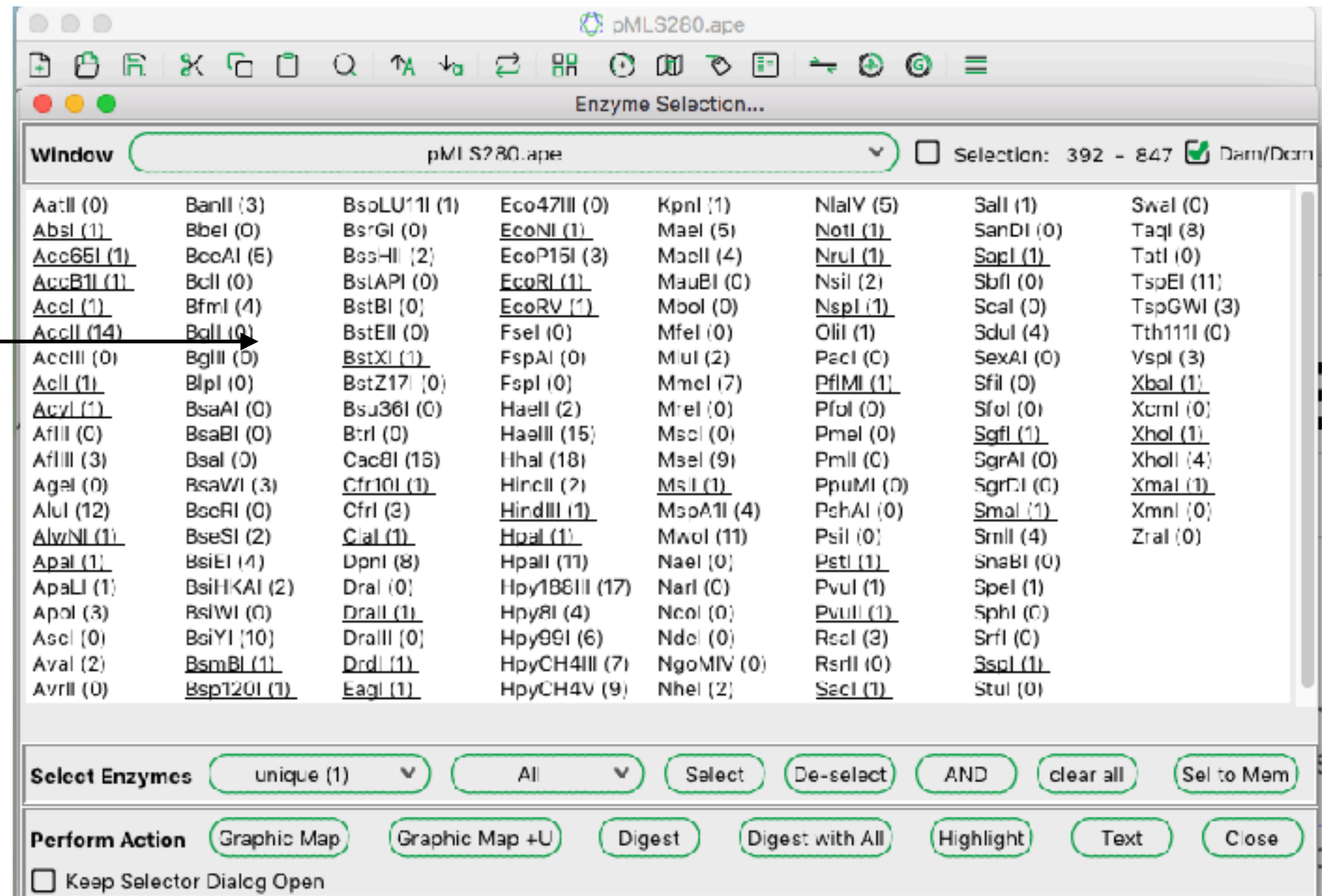
- Current window to search:** Points to the 'Window' dropdown menu at the top, which currently shows 'pML S280.ape'.
- Apply search to selection:** Points to the 'Selection: 392' status bar at the top right.
- Current selection in window (can be changed while the dialog is open):** Points to the 'unique (1)' dropdown in the 'Select Enzymes' section at the bottom.

The enzyme list is organized in columns. The first column lists enzymes like AatII, AbsI, Acc65I, etc. The second column lists enzymes like BanII, BbeI, BccAI, etc. The third column lists enzymes like BsoLU11I, BsrGI, BssHI, etc. The fourth column lists enzymes like Eco47III, EcoNI, EcoP15I, etc. The fifth column lists enzymes like KpnI, MaeI, MaeII, etc. The sixth column lists enzymes like NlaIV, NotI, NruI, etc. The seventh column lists enzymes like SalI, SanDI, SapI, etc. The eighth column lists enzymes like SmaI, TagI, TatI, etc. The ninth column lists enzymes like SbfI, Scal, SdaI, etc. The tenth column lists enzymes like SfiI, SfoI, SgrAI, etc. The eleventh column lists enzymes like SmaI, SmlI, SnaBI, etc. The twelfth column lists enzymes like SphI, SrfI, SspI, etc. The thirteenth column lists enzymes like StuI, Tth111I, VspI, etc. The fourteenth column lists enzymes like XbaI, XcmI, XhoI, etc. The fifteenth column lists enzymes like XmaI, XmnI, ZraI, etc.

At the bottom, the 'Select Enzymes' section includes a dropdown for 'unique (1)', buttons for 'All', 'Select', 'De-select', 'AND', 'clear all', and 'Sel to Mem'. The 'Perform Action' section includes buttons for 'Graphic Map', 'Graphic Map +U', 'Digest', 'Digest with All', 'Highlight', 'Text', and 'Close'. There is also a checkbox for 'Keep Selector Dialog Open'.

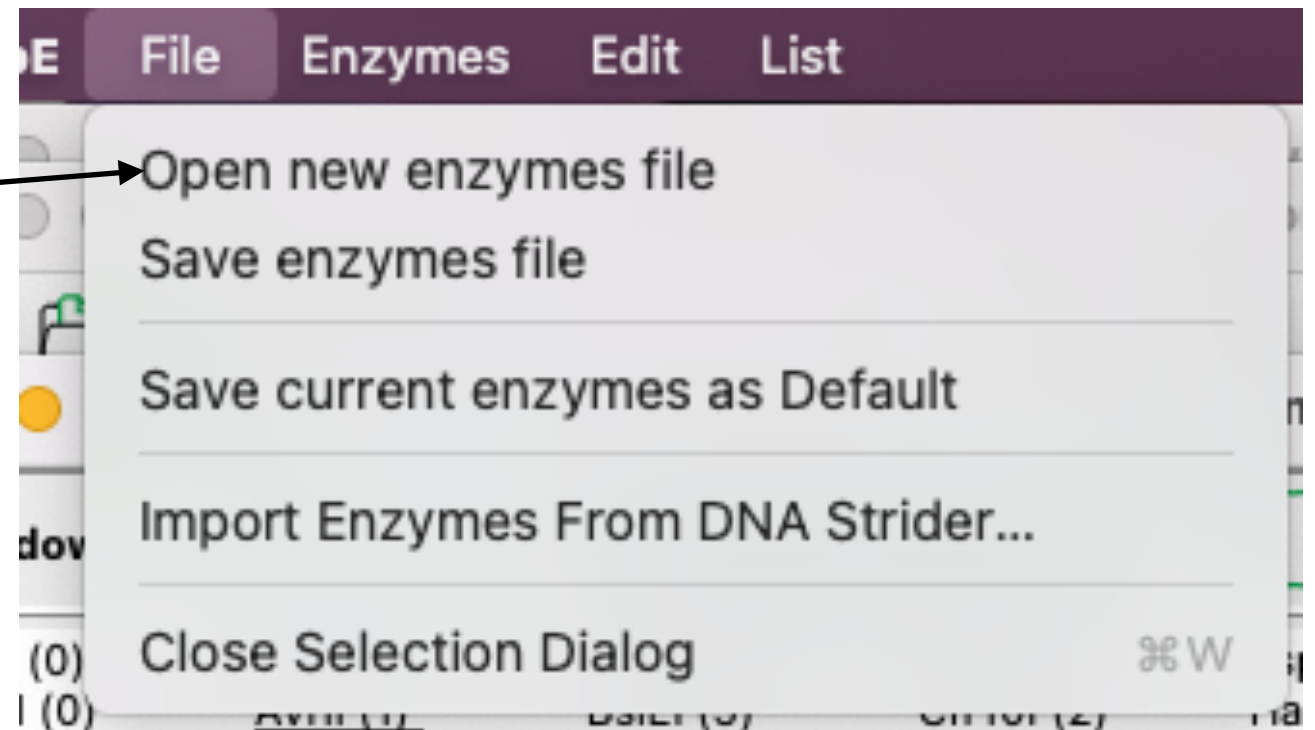
Enzyme Selection

All enzymes are shown
here

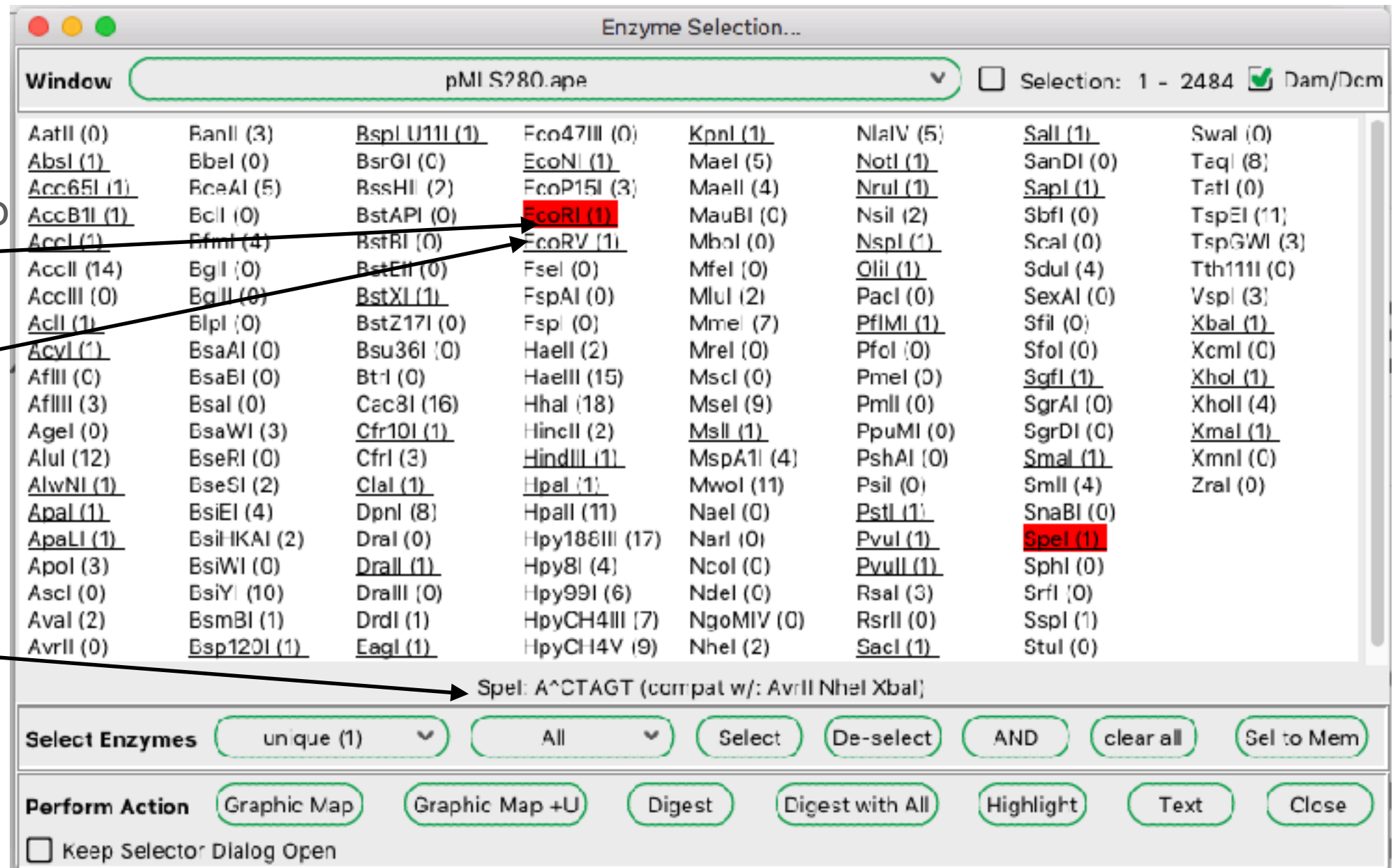


Enzyme Selection

The current enzymes
can be changed by
loading new ones
using the file menu
WHILE THE DIALOG IS
OPEN



Enzyme Selection



Enzyme Selection

Enzyme Selection...

Window: pMI S280.apa

Selection: 1 - 2484 ☒ Dam/Dcm

AatII (0)	BanII (3)	BspI U11I (1)	Eco47III (0)	KpnI (1)	NlaIV (5)	Sall (1)	Swal (0)
AbsI (1)	EbeI (0)	BsrGI (0)	EcoNI (1)	MaeI (5)	NotI (1)	SanDI (0)	TaqI (8)
Acc65I (1)	EcoAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	NruI (1)	SapI (1)	TatI (0)
AccB1I (1)	EcoRI (1)	BstAPI (0)	EcoRV (1)	MauBI (0)	NsiI (2)	SbfI (0)	TspEI (11)
AccI (1)	EcoRII (0)	BstBI (0)	FseI (0)	MboI (0)	NspI (1)	Scal (0)	TspGWI (3)
AccII (14)	EcoRII (0)	BstEII (0)	FspI (0)	MfeI (0)	OliI (1)	SduI (4)	Tth111I (0)
AccIII (0)	EcoRII (0)	BstXI (1)	FspAI (0)	MluI (2)	PacI (0)	SexAI (0)	VspI (3)
ActI (1)	EcoRII (0)	BstZ17I (0)	FspI (0)	MmeI (7)	PfIMI (1)	SfiI (0)	XbaI (1)
ActII (1)	EcoRII (0)	Bsu36I (0)	HaeII (2)	MreI (0)	PfoI (0)	SfoI (0)	XcmI (0)
AflII (0)	EcoRII (0)	BtrI (0)	HaeIII (15)	MscI (0)	PmeI (0)	SgfI (1)	XhoI (1)
AflIII (3)	EcoRII (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	EcoRII (0)	Cfr10I (1)	HincII (2)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (1)
AluI (12)	EcoRII (0)	CfrI (3)	HindIII (1)	MspA1I (4)	PshAI (0)	SmaI (1)	XmnI (0)
AlwNI (1)	EcoRII (0)	Clal (1)	HpaI (1)	MwoI (11)	PsiI (0)	SmlI (4)	ZraI (0)
ApaI (1)	EcoRII (0)	DpnI (8)	HpaII (11)	NaI (0)	PstI (1)	SnaBI (0)	
ApaLI (1)	EcoRII (0)	DraI (0)	Hpy188III (17)	NarI (0)	PvuI (1)	SpeI (1)	
ApoI (3)	EcoRII (0)	DraII (1)	Hpy8I (4)	NcoI (0)	PvuII (1)	SphI (0)	
AscI (0)	EcoRII (0)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	EcoRII (0)	DrdI (1)	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	Sspl (1)	
AvrII (0)	EcoRII (0)	EagI (1)	HpyCH4V (9)	NheI (2)	SacI (1)	StuI (0)	

SpeI: A^CTAGT (compat w/: AvrII NheI XbaI)

Select Enzymes:

Perform Action:

☐ Keep Selector Dialog Open

The selection criteria
are shown here

Enzyme Selection

Any enzyme matching both the count AND the group are underlined

This is the count

This is the group

The screenshot shows a window titled "Enzyme Selection...". At the top, there is a "Window" dropdown menu set to "pMI S280.apa" and a checkbox for "Selection: 1 - 2484" which is checked, with a "Dam/Dcm" checkbox also checked. The main area is a grid of enzymes, each followed by a count in parentheses. Some enzymes are underlined, indicating they match both the count and the group. The enzymes are arranged in 8 columns. At the bottom, there is a section for "Spel: A^CTAGT (compat w/: AvrII NheI XbaI)". Below this, there are two rows of buttons. The first row includes "Select Enzymes" (with a dropdown menu showing "unique (1)"), "All" (with a dropdown menu), "Select", "De-select", "AND", "clear all", and "Sel to Mem". The second row includes "Perform Action" (with a dropdown menu showing "Graphic Map"), "Graphic Map +U", "Digest", "Digest with All", "Highlight", "Text", and "Close". There is also a checkbox for "Keep Selector Dialog Open".

AatII (0)	BanII (3)	BspI U11I (1)	Eco47III (0)	KpnI (1)	NlaIV (5)	Sall (1)	Swal (0)
AbsI (1)	EbeI (0)	BsrGI (0)	EcoNI (1)	MaeI (5)	NotI (1)	SanDI (0)	TaqI (8)
Acc65I (1)	EcoAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	NruI (1)	SapI (1)	TatI (0)
AccB1I (1)	EcoI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	NsiI (2)	SbfI (0)	TspEI (11)
AccI (1)	EfmI (4)	BstBI (0)	EcoRV (1)	MboI (0)	NspI (1)	Scal (0)	TspGWI (3)
AccII (14)	EgII (0)	BstEII (0)	FseI (0)	MfeI (0)	OliI (1)	SduI (4)	Tth111I (0)
AccIII (0)	EgII (0)	BstXI (1)	FspAI (0)	MluI (2)	PacI (0)	SexAI (0)	VspI (3)
ActI (1)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (7)	PfIMI (1)	SfiI (0)	XbaI (1)
<u>Acyl (1)</u>	BsaAI (0)	Bsu36I (0)	HaeII (2)	MreI (0)	PfoI (0)	SfoI (0)	XcmI (0)
AfII (0)	BsaBI (0)	BtrI (0)	HaeIII (15)	MscI (0)	PmeI (0)	SgfI (1)	XhoI (1)
AfIII (3)	BsaI (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	BsaWI (3)	Cfr10I (1)	HincII (2)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (1)
AluI (12)	BseRI (0)	CfrI (3)	HindIII (1)	MspA1I (4)	PshAI (0)	SmaI (1)	XmnI (0)
<u>AlwNI (1)</u>	BseSI (2)	Clal (1)	HpaI (1)	MwoI (11)	Psil (0)	SmlI (4)	ZraI (0)
<u>ApaI (1)</u>	BsiEI (4)	DpnI (8)	HpaII (11)	NaI (0)	PstI (1)	SnaBI (0)	
<u>ApaLI (1)</u>	BsiHKAII (2)	DraI (0)	Hpy188III (17)	NarI (0)	PvuI (1)	SpeI (1)	
ApoI (3)	BsiWI (0)	<u>DraII (1)</u>	Hpy8I (4)	NcoI (0)	<u>PvuII (1)</u>	SphI (0)	
AscI (0)	BsiYI (10)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	BsmBI (1)	DrdI (1)	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	Sspl (1)	
AvrII (0)	<u>Bsp120I (1)</u>	<u>EagI (1)</u>	HpyCH4V (9)	NheI (2)	SacI (1)	StuI (0)	

Spel: A^CTAGT (compat w/: AvrII NheI XbaI)

Select Enzymes: unique (1) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map +U Digest Digest with All Highlight Text Close

☐ Keep Selector Dialog Open

Enzyme Selection

Enzyme Selection...

Window: pMI S280.apa

Selection: 1 - 2484 ☒ Dam/Dcm

AatII (0)	BanII (3)	<u>BspI</u> U11I (1)	Eco47III (0)	<u>KpnI</u> (1)	NlaIV (5)	<u>Sall</u> (1)	Swal (0)
<u>AbsI</u> (1)	Ebel (0)	BsrGI (0)	<u>EcoNI</u> (1)	MaeI (5)	<u>NotI</u> (1)	SanDI (0)	TaqI (8)
<u>Acc65I</u> (1)	EceAI (5)	BssHII (2)	EcoP15I (3)	Maell (4)	<u>NruI</u> (1)	<u>SapI</u> (1)	TatI (0)
<u>AccB1I</u> (1)	BclI (0)	BstAPI (0)	<u>EcoRI</u> (1)	MauBI (0)	Nsil (2)	SbfI (0)	TspEI (11)
<u>AccI</u> (1)	BfmI (4)	BstBI (0)	<u>EcoRV</u> (1)	Mbol (0)	<u>NspI</u> (1)	Scal (0)	TspGWI (3)
AccII (14)	BglI (0)	BstEII (0)	FseI (0)	Mfel (0)	<u>QliI</u> (1)	Sdul (4)	Tth111I (0)
AccIII (0)	BglII (0)	<u>BstXI</u> (1)	FspAI (0)	MLuI (2)	PacI (0)	SexAI (0)	VspI (3)
<u>AclI</u> (1)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (7)	<u>PfIMI</u> (1)	SfiI (0)	<u>XbaI</u> (1)
<u>AcyI</u> (1)	BsaAI (0)	Bsu36I (0)	HaeII (2)	MreI (0)	PfoI (0)	SfoI (0)	XcmI (0)
AfIII (0)	BsaBI (0)	BtrI (0)	HaeIII (15)	MscI (0)	PmeI (0)	<u>SgfI</u> (1)	<u>XhoI</u> (1)
AfIII (3)	Bsal (0)	Cac8I (16)	HhaI (18)	MseI (9)	PmlI (0)	SgrAI (0)	XhoII (4)
AgeI (0)	BsaWI (3)	<u>Cfr10I</u> (1)	HincII (2)	<u>MslI</u> (1)	PpuMI (0)	SgrDI (0)	<u>XmaI</u> (1)
AluI (12)	BseRI (0)	CfrI (3)	<u>HindIII</u> (1)	MspA1I (4)	PshAI (0)	<u>SmaI</u> (1)	XmnI (0)
<u>AlwNI</u> (1)	BseSI (2)	<u>Clal</u> (1)	<u>HpaI</u> (1)	MwoI (11)	Psil (0)	SmlI (4)	ZraI (0)
<u>ApaI</u> (1)	BsiEI (4)	DpnI (8)	HpaII (11)	Nael (0)	<u>PstI</u> (1)	SnaBI (0)	
<u>ApaLI</u> (1)	BsiHKAII (2)	DraI (0)	Hpy188III (17)	NarI (0)	<u>PvuI</u> (1)	<u>SpeI</u> (1)	
ApoI (3)	BsiWI (0)	<u>DraII</u> (1)	Hpy8I (4)	NcoI (0)	<u>PvuII</u> (1)	SphI (0)	
AscI (0)	BsiYI (10)	DraIII (0)	Hpy99I (6)	NdeI (0)	RsaI (3)	SrfI (0)	
AvaI (2)	BsmBI (1)	DrdI (1)	HpyCH4III (7)	NgoMIV (0)	RsrII (0)	Sspl (1)	
AvrII (0)	<u>Esp120I</u> (1)	<u>EagI</u> (1)	HpyCH4V (9)	NheI (2)	<u>SacI</u> (1)	StuI (0)	

SpeI: A^CTAGT (compat w/: AvrII NheI XbaI)

Select Enzymes: unique (1) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map HD Digest Digest with All Highlight Text Close

☐ Keep Selector Dialog Open

Click here to select all underlined enzymes

Click here to DE-select all underlined enzymes

Click here to de-select ALL enzymes

Enzyme Selection

Enzyme Selection...

Window: pMI S280.apc

Selection: 1 - 2484 ☒ Dam/Dcm

<u>AatII</u> (0)	<u>BanII</u> (3)	<u>BspI</u> <u>U11I</u> (1)	<u>Eco47III</u> (0)	<u>KpnI</u> (1)	<u>NlaIV</u> (5)	<u>Sall</u> (1)	<u>Swal</u> (0)
<u>AbsI</u> (1)	<u>EbeI</u> (0)	<u>BsrGI</u> (0)	<u>EcoNI</u> (1)	<u>MaeI</u> (5)	<u>NotI</u> (1)	<u>SanDI</u> (0)	<u>TaqI</u> (8)
<u>Acc65I</u> (1)	<u>EcoAI</u> (5)	<u>BssHII</u> (2)	<u>EcoP15I</u> (3)	<u>Maell</u> (4)	<u>NruI</u> (1)	<u>SapI</u> (1)	<u>TatI</u> (0)
<u>AccB1I</u> (1)	<u>EcoI</u> (0)	<u>BstAPI</u> (0)	<u>EcoRI</u> (1)	<u>MauBI</u> (0)	<u>NsiI</u> (2)	<u>SbfI</u> (0)	<u>TspEI</u> (11)
<u>AccI</u> (1)	<u>EfmI</u> (4)	<u>BstBI</u> (0)	<u>EcoRV</u> (1)	<u>MboI</u> (0)	<u>NspI</u> (1)	<u>Scal</u> (0)	<u>TspGWI</u> (3)
<u>AccII</u> (14)	<u>EgII</u> (0)	<u>BstEII</u> (0)	<u>FseI</u> (0)	<u>MfeI</u> (0)	<u>OliI</u> (1)	<u>SduI</u> (4)	<u>Tth111I</u> (0)
<u>AccIII</u> (0)	<u>EgII</u> (0)	<u>BstXI</u> (1)	<u>FspAI</u> (0)	<u>MluI</u> (2)	<u>PacI</u> (0)	<u>SexAI</u> (0)	<u>VspI</u> (3)
<u>ActI</u> (1)	<u>BlpI</u> (0)	<u>BstZ17I</u> (0)	<u>FspI</u> (0)	<u>MmeI</u> (7)	<u>PfIMI</u> (1)	<u>SfiI</u> (0)	<u>XbaI</u> (1)
<u>ActI</u> (1)	<u>BsaAI</u> (0)	<u>Bsu36I</u> (0)	<u>HaeII</u> (2)	<u>MreI</u> (0)	<u>PfoI</u> (0)	<u>SfoI</u> (0)	<u>XcmI</u> (0)
<u>AfIII</u> (0)	<u>BsaBI</u> (0)	<u>BtrI</u> (0)	<u>HaeIII</u> (15)	<u>MscI</u> (0)	<u>PmeI</u> (0)	<u>SgfI</u> (1)	<u>XhoI</u> (1)
<u>AfIII</u> (3)	<u>BsaI</u> (0)	<u>Cac8I</u> (16)	<u>HhaI</u> (18)	<u>MseI</u> (9)	<u>PmlI</u> (0)	<u>SgrAI</u> (0)	<u>XhoII</u> (4)
<u>AgeI</u> (0)	<u>BsaWI</u> (3)	<u>Cfr10I</u> (1)	<u>HincII</u> (2)	<u>MslI</u> (1)	<u>PpuMI</u> (0)	<u>SgrDI</u> (0)	<u>XmaI</u> (1)
<u>AluI</u> (12)	<u>BseRI</u> (0)	<u>CfrI</u> (3)	<u>HindIII</u> (1)	<u>MspA1I</u> (4)	<u>PshAI</u> (0)	<u>SmaI</u> (1)	<u>XmnI</u> (0)
<u>AlwNI</u> (1)	<u>BseSI</u> (2)	<u>Clal</u> (1)	<u>HpaI</u> (1)	<u>MwoI</u> (11)	<u>PsiI</u> (0)	<u>SmlI</u> (4)	<u>ZraI</u> (0)
<u>Apal</u> (1)	<u>BsiEI</u> (4)	<u>DpnI</u> (8)	<u>HpaII</u> (11)	<u>NaeI</u> (0)	<u>PstI</u> (1)	<u>SnaBI</u> (0)	
<u>ApalI</u> (1)	<u>BsiHKA1</u> (2)	<u>DraI</u> (0)	<u>Hpy188III</u> (17)	<u>NarI</u> (0)	<u>PvuI</u> (1)	<u>SpeI</u> (1)	
<u>ApoI</u> (3)	<u>BsiWI</u> (0)	<u>DraII</u> (1)	<u>Hpy8I</u> (4)	<u>NcoI</u> (0)	<u>PvuII</u> (1)	<u>SphI</u> (0)	
<u>AscI</u> (0)	<u>BsiYI</u> (10)	<u>DraIII</u> (0)	<u>Hpy99I</u> (6)	<u>NdeI</u> (0)	<u>RsaI</u> (3)	<u>SrfI</u> (0)	
<u>AvaI</u> (2)	<u>BsmBI</u> (1)	<u>DrdI</u> (1)	<u>HpyCH4III</u> (7)	<u>NgoMIV</u> (0)	<u>RsrII</u> (0)	<u>Sspl</u> (1)	
<u>AvrII</u> (0)	<u>Esp120I</u> (1)	<u>EagI</u> (1)	<u>HpyCH4V</u> (9)	<u>NheI</u> (2)	<u>SacI</u> (1)	<u>StuI</u> (0)	

SpeI: A^CTAGT (compat w/: AvrII NheI XbaI)

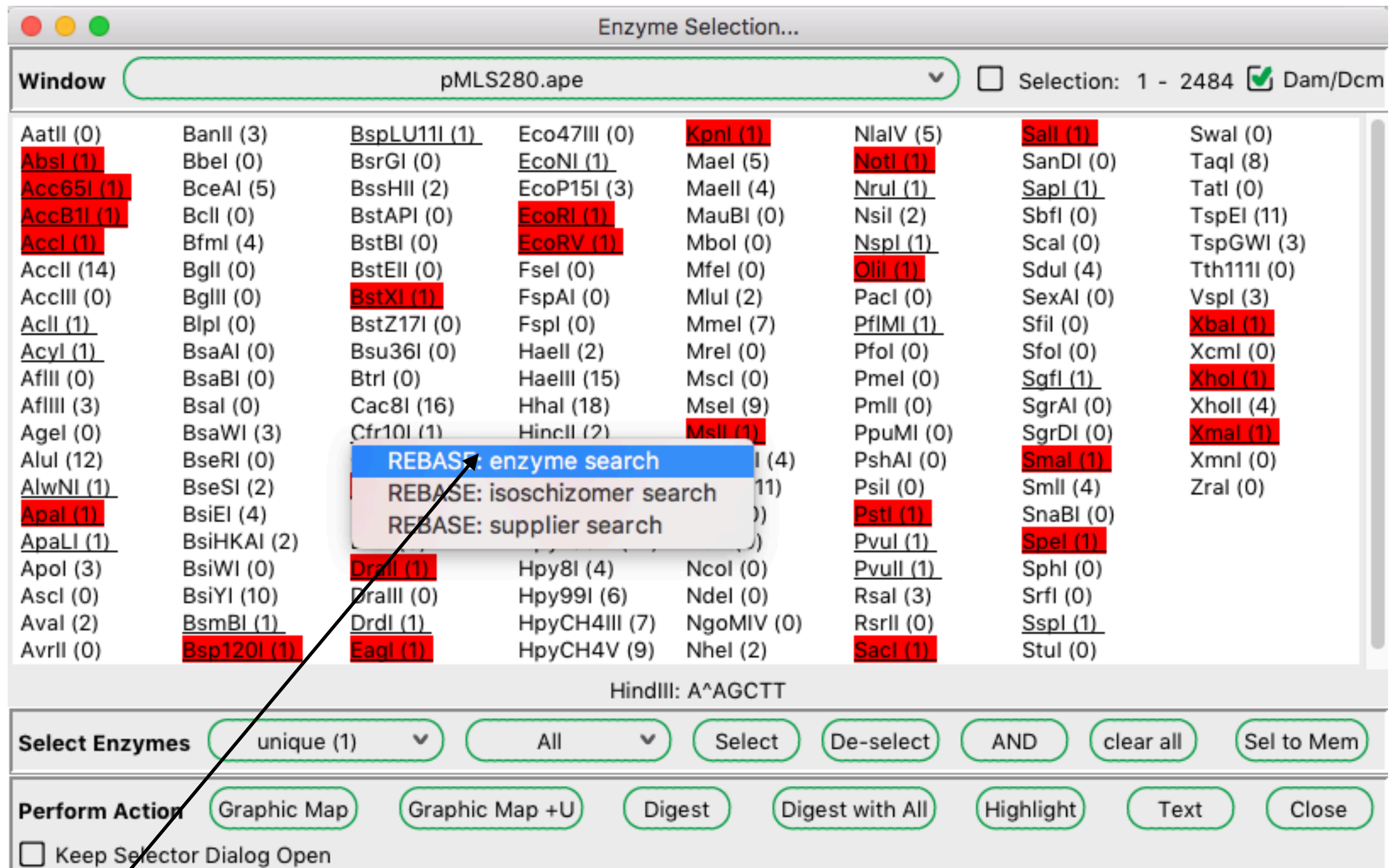
Select Enzymes: unique (1) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map +U Digest Digest with All Highlight Text Close

☐ Keep Selector Dialog Open

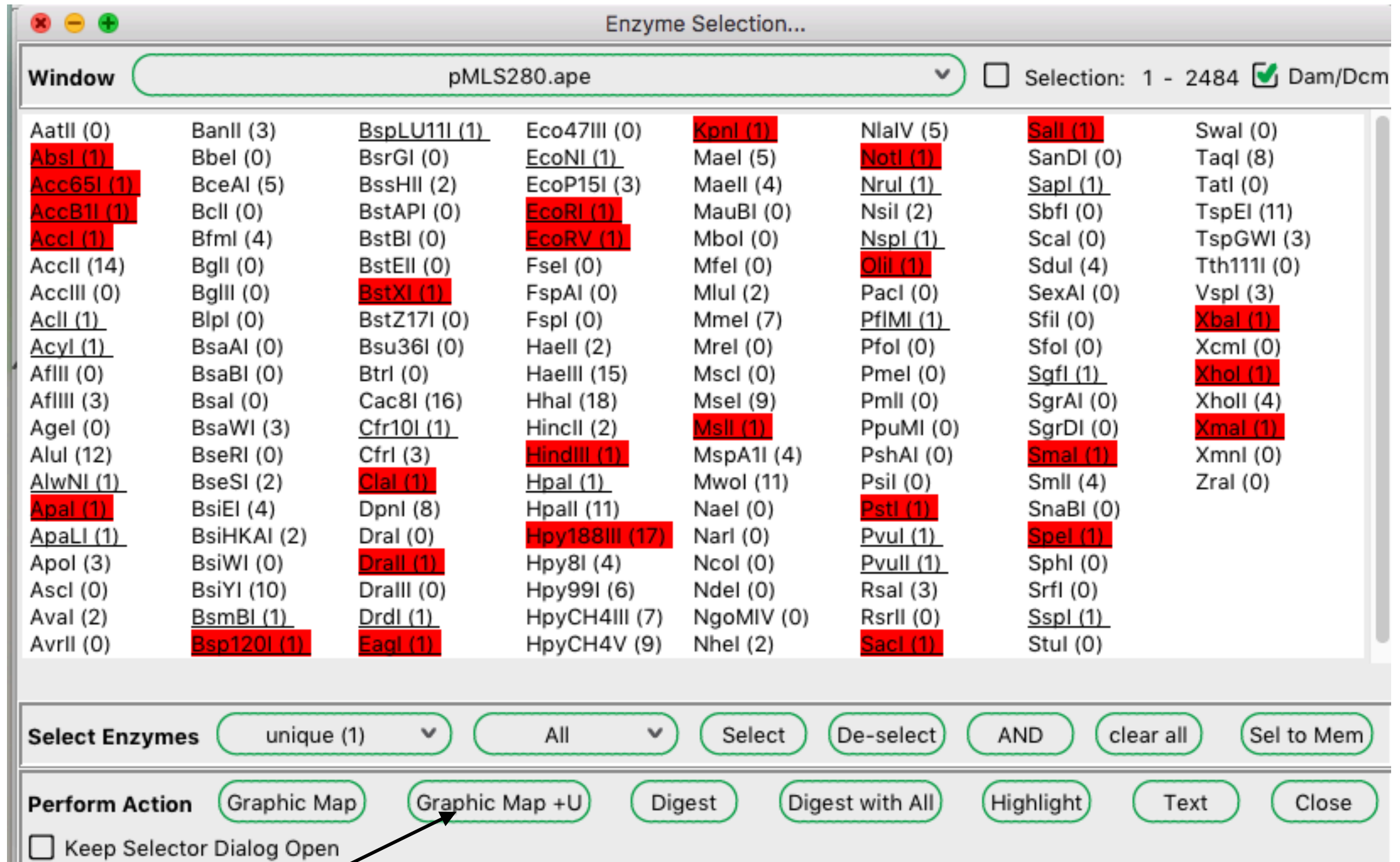
Click here to select
only enzymes that are
currently selected AND
underlined

Enzyme Selection



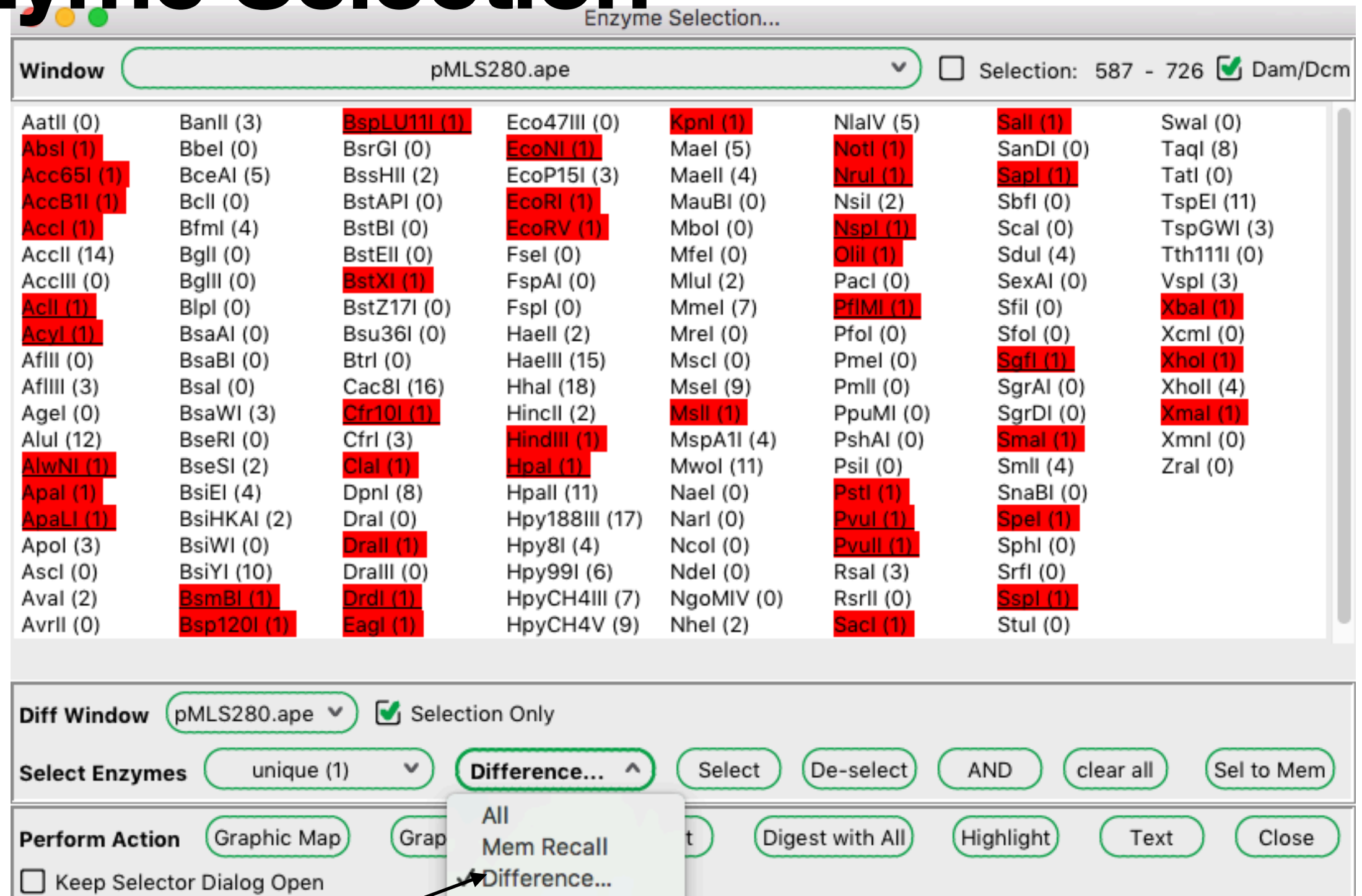
Right-click to search
for enzyme info

Enzyme Selection



Click here to do
analysis functions

Enzyme Selection

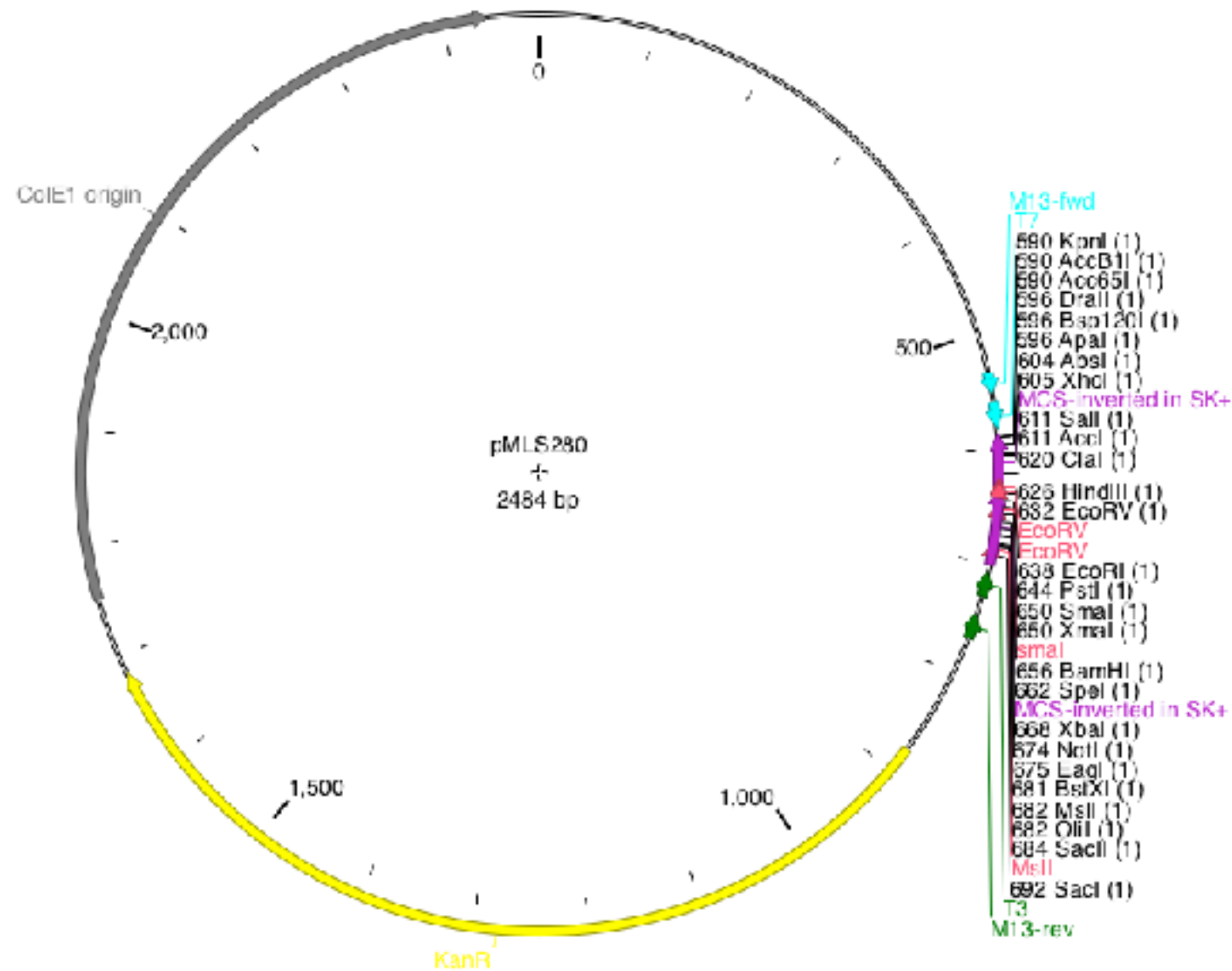


The difference group is special

Enzyme Selection

- Select the MCS in pMLS280
- Open the enzyme selector
- Select all unique enzymes in pMLS280
- Chose the difference group and pMLS280+selection within the difference selector
- Deselect the different enzymes
- Do a graphic map
- What do you see?

Enzyme Selection



Enzyme Selection

Find enzymes to clone amilCP
into pMLS280

Enzyme Selection

- Select from 25 to 751 in amilCP

Sequence analysis results for a 2800 bp sequence (positions 25 to 751).

Feature	Direction	Type	Location
Hidden			
BioBrick prefix	>>>	misc_feature	3..24
constitutive promoter J23110	>>>	promoter	25..59
strong bacterial ribosome binding site (Elowitz)	>>>	RBS	68..79
amilCP Base	>>>	misc_feature	86..751

Sequence (positions 1 to 90):

```
1 tgggaattcgcgccgcttctagagtttacggctagctcagtcctaggtacaatgctagctactagagaaagaggagaaatactagATGAG
91 TGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGTAA
181 GCCCTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGCGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACAGTGTCAT
271 GTACGGAAGCATACCATTACCAAGTACCCTGAAGACATCCCTGACTATGTAAAGCAGTCATTCCCGGAGGGCTATACATGGGAGAGGAT
361 CATGAACCTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACCATGTCAAGTTCTCTGG
451 TTTGAACCTTTCCTCCAATGGACCTGTCATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAAT
541 GCTGCTAGGAAACAACCTTTATGGCTCTGAAGTTAGAAGGAGGCGGTCCTATTGTTGTGAATTTAAACTACTTACAAGGCAAAGAAGCC
631 TGTGAAGATGCCAGGGTATCACTATGTTGACCGCAAACCTGGATGTAACCAATCACAACAAGGATTACACTTCGGTTGAGCAGTGTGAAAT
721 TTCCATTGCACGCAAACCTGTGGTCGCCTAA|taatactagtagcgccgctgcagtcaggcaaaaaagggaaggtgtcaccaccctgcc
811 ctttttctttaaaaccgaaaagattacttcgcgttatgcaggcttcctcgctcactgactcgctgcgctcggtcggttcggctgcggcgag
901 cggtatcagctcactcaaaggcggttaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaa
991
```

Enzyme Selection

- select all enzymes that are absent from the selection in amilCP

Enzyme Selection...

Window: amilCP-indigo-addgene-plasmid-117847.gbk

Selection: 25 - 751 Dam/Dcm

AatII (0)	BanII (0)	BspLU11I (0)	Eco47III (0)	KpnI (0)	NlaIV (1)	Sall (0)	Swal (0)
AbsI (0)	BbeI (0)	BsrGI (0)	EcoNI (0)	MaeI (6)	NotI (0)	SanDI (0)	TaqI (1)
Acc65I (0)	BceAI (1)	BssHII (0)	EcoP15I (0)	Maell (0)	NruI (0)	SapI (0)	TatI (1)
AccB1I (0)	BclI (0)	BstAPI (0)	EcoRI (0)	MauBI (0)	NsiI (0)	SbfI (0)	TspEI (2)
AccI (0)	Bfml (0)	BstBI (0)	EcoRV (0)	Mbol (0)	Nspl (0)	Scal (0)	TspGWI (1)
AccII (0)	BglI (0)	BstEII (0)	FseI (0)	MfeI (0)	OliI (1)	SduI (0)	Tth111I (0)
AccIII (0)	BglII (0)	BstXI (1)	FspAI (0)	MluI (0)	PacI (0)	SexAI (0)	Vspl (0)
AcII (0)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (0)	PfIMI (0)	SfiI (0)	XbaI (0)
Acyl (0)	BsaAI (0)	Bsu36I (0)	HaeII (0)	MreI (0)	PfoI (1)	SfoI (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaeIII (0)	MscI (0)	PmeI (0)	SgfI (0)	XhoI (0)
AflIII (0)	Bsal (0)	Cac8I (3)	HhaI (0)	MseI (1)	PmlI (0)	SgrAI (0)	XhoII (0)
AgeI (0)	BsaWI (0)	Cfr10I (0)	HincII (1)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (0)
AluI (3)	BseRI (1)	CfrI (0)	HindIII (0)	MspAII (0)	PshAI (0)	SmaI (0)	Xmnl (0)
AlwNI (0)	BseSI (0)	Clal (0)	HpaI (0)	MwoI (1)	PsiI (0)	SmlI (0)	ZraI (0)
Apal (0)	BsiEI (0)	Dpnl (2)	HpaII (1)	NaeI (0)	PstI (0)	SnaBI (0)	
ApaLI (0)	BsiHKAII (0)	DraI (1)	Hpy188III (0)	NarI (0)	PvuI (0)	SpeI (0)	
Apol (2)	BsiWI (0)	DraII (0)	Hpy8I (2)	NcoI (0)	PvuII (0)	SphI (0)	
Ascl (0)	BsiYI (3)	DraIII (0)	Hpy99I (0)	NdeI (0)	RsaI (4)	SrfI (0)	
Aval (0)	BsmBI (1)	DrdI (0)	HpyCH4III (6)	NgoMIV (0)	RsrII (0)	Sspl (0)	
AvrII (1)	Bsp120I (0)	EagI (0)	HpyCH4V (4)	NheI (2)	SacI (0)	StuI (0)	

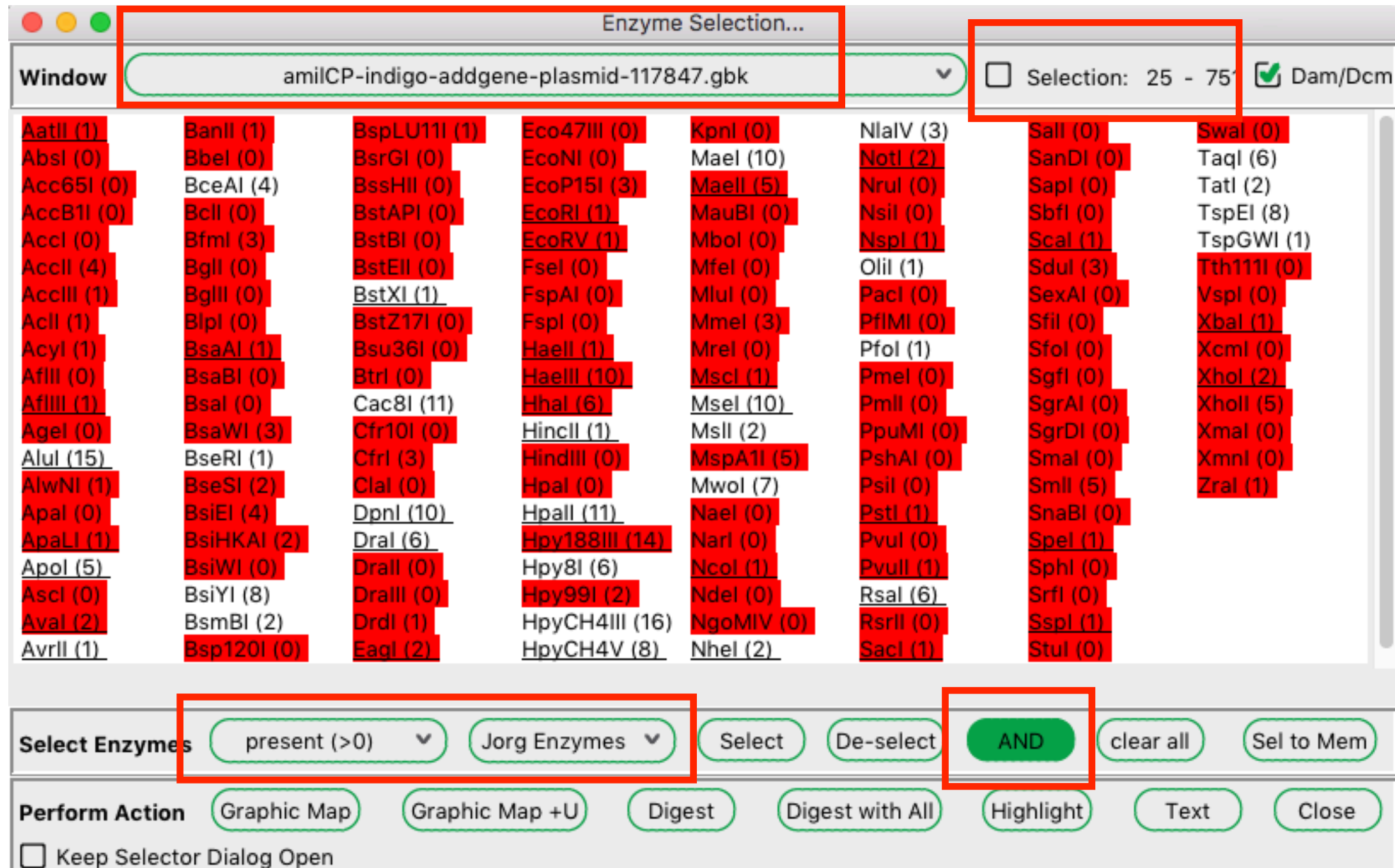
Select Enzymes: absent (0) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map +U Digest Digest with All Highlight Text Close

☐ Keep Selector Dialog Open

Enzyme Selection

- select all enzymes that are ALSO present somewhere in the plasmid, and in the Jorg collection



Enzyme Selection

- do a graphic map

Enzyme Selection...

Window amilCP-indigo-addgene-plasmid-117847.gbk ☐ Selection: 25 - 751 ☒ Dam/Dcm

AatII (1)	BanII (1)	BspLU11I (1)	Eco47III (0)	KpnI (0)	NlaIV (3)	Sall (0)	Swal (0)
AbsI (0)	BbeI (0)	BsrGI (0)	EcoNI (0)	MaeI (10)	NotI (2)	SanDI (0)	TaqI (6)
Acc65I (0)	BceAI (4)	BssHII (0)	EcoP15I (3)	Maell (5)	NruI (0)	SapI (0)	TatI (2)
AccB1I (0)	BclI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	NsiI (0)	SbfI (0)	TspEI (8)
AccI (0)	BfmI (3)	BstBI (0)	EcoRV (1)	MboI (0)	NspI (1)	Scal (1)	TspGWI (1)
AccII (4)	BglI (0)	BstEII (0)	FseI (0)	MfeI (0)	OliI (1)	SduI (3)	Tth111I (0)
AccIII (1)	BglII (0)	<u>BstXI (1)</u>	FspAI (0)	MluI (0)	PacI (0)	SexAI (0)	VspI (0)
ActI (1)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (3)	PfIMI (0)	SfiI (0)	XbaI (1)
AcylI (1)	BsaAI (1)	Bsu36I (0)	HaeII (1)	MreI (0)	PfoI (1)	SfoI (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaeIII (10)	MscI (1)	PmeI (0)	SgfI (0)	XhoI (2)
AflIII (1)	BsaI (0)	Cac8I (11)	HhaI (6)	<u>MseI (10)</u>	PmlI (0)	SgrAI (0)	XhoII (5)
AgeI (0)	BsaWI (3)	Cfr10I (0)	<u>HincII (1)</u>	MslI (2)	PpuMI (0)	SgrDI (0)	XmaI (0)
<u>AluI (15)</u>	BseRI (1)	CfrI (3)	HindIII (0)	MspA1I (5)	PshAI (0)	SmaI (0)	XmnI (0)
AlwNI (1)	BseSI (2)	Clal (0)	HpaI (0)	MwoI (7)	PsiI (0)	SmlI (5)	ZraI (1)
Apal (0)	BsiEI (4)	<u>DpnI (10)</u>	<u>HpaII (11)</u>	NaeI (0)	PstI (1)	SnaBI (0)	
ApaLI (1)	BsiHKA1 (2)	<u>DraI (6)</u>	Hpy188III (14)	NarI (0)	PvuI (0)	SpeI (1)	
<u>ApoI (5)</u>	BsiWI (0)	Drall (0)	Hpy8I (6)	NcoI (1)	PvuII (1)	SphI (0)	
AscI (0)	BsiYI (8)	Drall (0)	Hpy99I (2)	NdeI (0)	<u>RsaI (6)</u>	SrfI (0)	
AvaI (2)	BsmBI (2)	DrdI (1)	HpyCH4III (16)	NgoMIV (0)	RsrII (0)	SspI (1)	
<u>AvrII (1)</u>	Bsp120I (0)	EagI (2)	<u>HpyCH4V (8)</u>	<u>NheI (2)</u>	SacI (1)	StuI (0)	

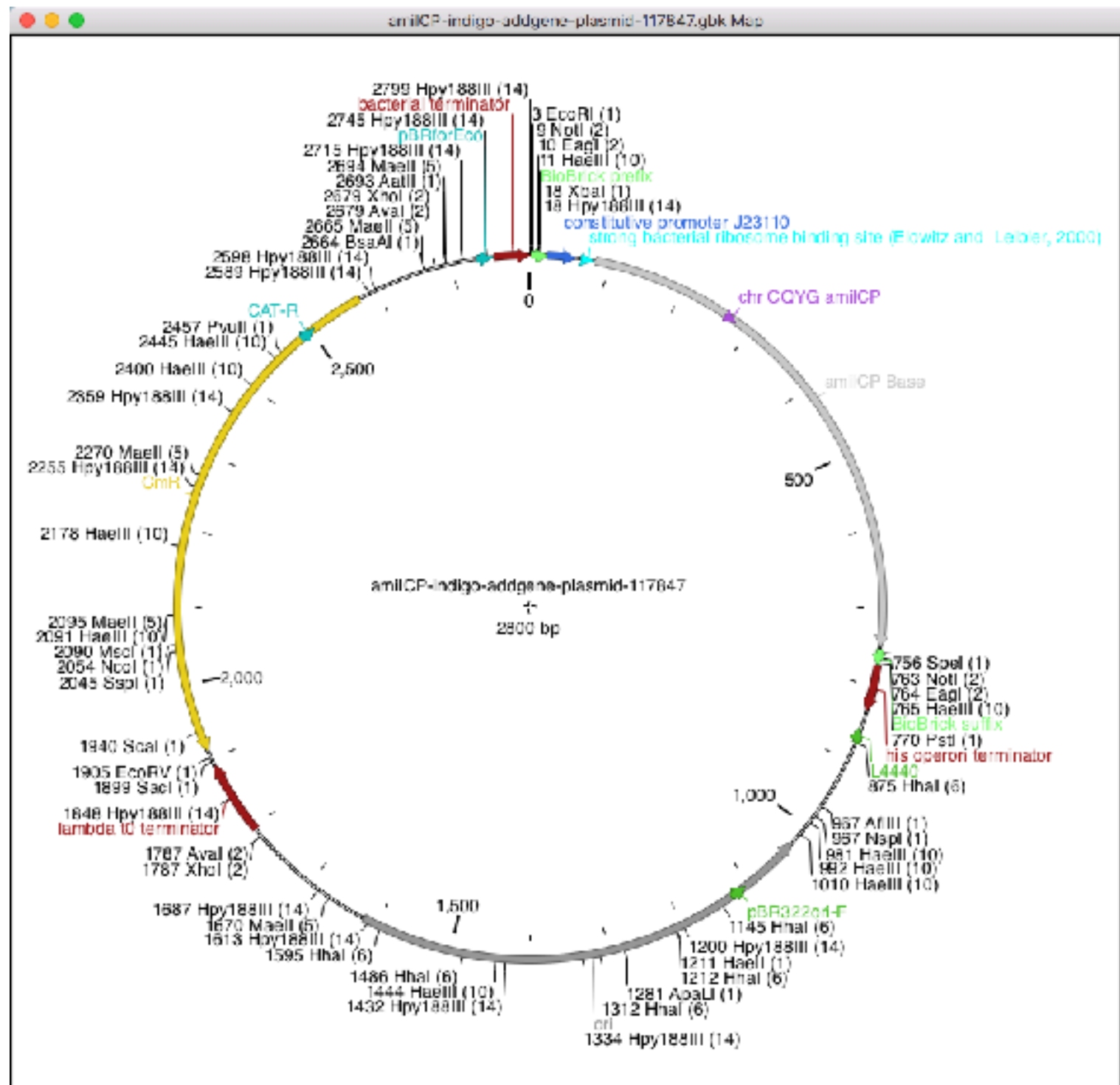
Select Enzymes present (>0)

Perform Action

☐ Keep Selector Dialog Open

Enzyme Selection

- do a graphic map



Enzyme Selection

- select all enzymes that are ALSO unique in the pMLS MCS

Enzyme Selection...

Window: pMLS280.ape

Selection: 587 - 699 Dam/Dcm

AatII (0)	BanII (2)	BspLU11I (0)	Eco47III (0)	KpnI (1)	NlaIV (4)	Sall (1)	Swal (0)
AbsI (1)	BbeI (0)	BsrGI (0)	EcoNI (0)	MaeI (2)	NotI (1)	SanDI (0)	TaqI (4)
Acc65I (1)	BceAI (0)	BssHII (0)	EcoP15I (0)	Maell (0)	NruI (0)	SapI (0)	TatI (0)
AccB1I (1)	BclI (0)	BstAPI (0)	EcoRI (1)	MauBI (0)	NsiI (0)	SbfI (0)	TspEI (1)
AccI (1)	BfmI (1)	BstBI (0)	EcoRV (1)	Mbol (0)	Nspl (0)	Scal (0)	TspGWI (0)
AccII (1)	BglI (0)	BstEII (0)	FseI (0)	Mfel (0)	OliI (1)	SduI (2)	Tth111I (0)
AccIII (0)	BglII (0)	BstXI (1)	FspAI (0)	MluI (0)	PacI (0)	SexAI (0)	VspI (0)
AcII (0)	BlpI (0)	BstZ17I (0)	FspI (0)	MmeI (0)	PfIMI (0)	SfiI (0)	XbaI (1)
Acyl (0)	BsaAI (0)	Bsu36I (0)	HaeII (0)	MreI (0)	PfoI (0)	SfoI (0)	XcmI (0)
AflII (0)	BsaBI (0)	BtrI (0)	HaeIII (2)	MscI (0)	PmeI (0)	Sgfl (0)	XhoI (1)
AflIII (0)	Bsal (0)	Cac8I (0)	HhaI (0)	MseI (0)	PmlI (0)	SgrAI (0)	XhoII (1)
AgeI (0)	BsaWI (0)	Cfr10I (0)	HincII (1)	MslI (1)	PpuMI (0)	SgrDI (0)	XmaI (1)
AluI (3)	BseRI (0)	CfrI (1)	HindIII (1)	MspA1I (1)	PshAI (0)	SmaI (1)	XmnI (0)
AlwNI (0)	BseSI (1)	Clal (1)	HpaI (0)	MwoI (1)	PsiI (0)	SmlI (1)	ZraI (0)
Apal (1)	BsiEI (1)	DpnI (1)	HpaII (2)	NaeI (0)	PstI (1)	SnaBI (0)	
ApaLI (0)	BsiHKA1 (1)	DraI (0)	Hpy188III (1)	NarI (0)	PvuI (0)	SpeI (1)	
ApoI (1)	BsiWI (0)	DraII (1)	Hpy8I (1)	NcoI (0)	PvuII (0)	SphI (0)	
AscI (0)	BsiYI (1)	DraIII (0)	Hpy99I (1)	NdeI (0)	RsaI (1)	SrfI (0)	
AvaI (2)	BsmBI (0)	DrdI (0)	HpyCH4III (1)	NgoMIV (0)	RsrII (0)	Sspl (0)	
AvrII (0)	Bsp120I (1)	EagI (1)	HpyCH4V (1)	NheI (0)	SacI (1)	StuI (0)	

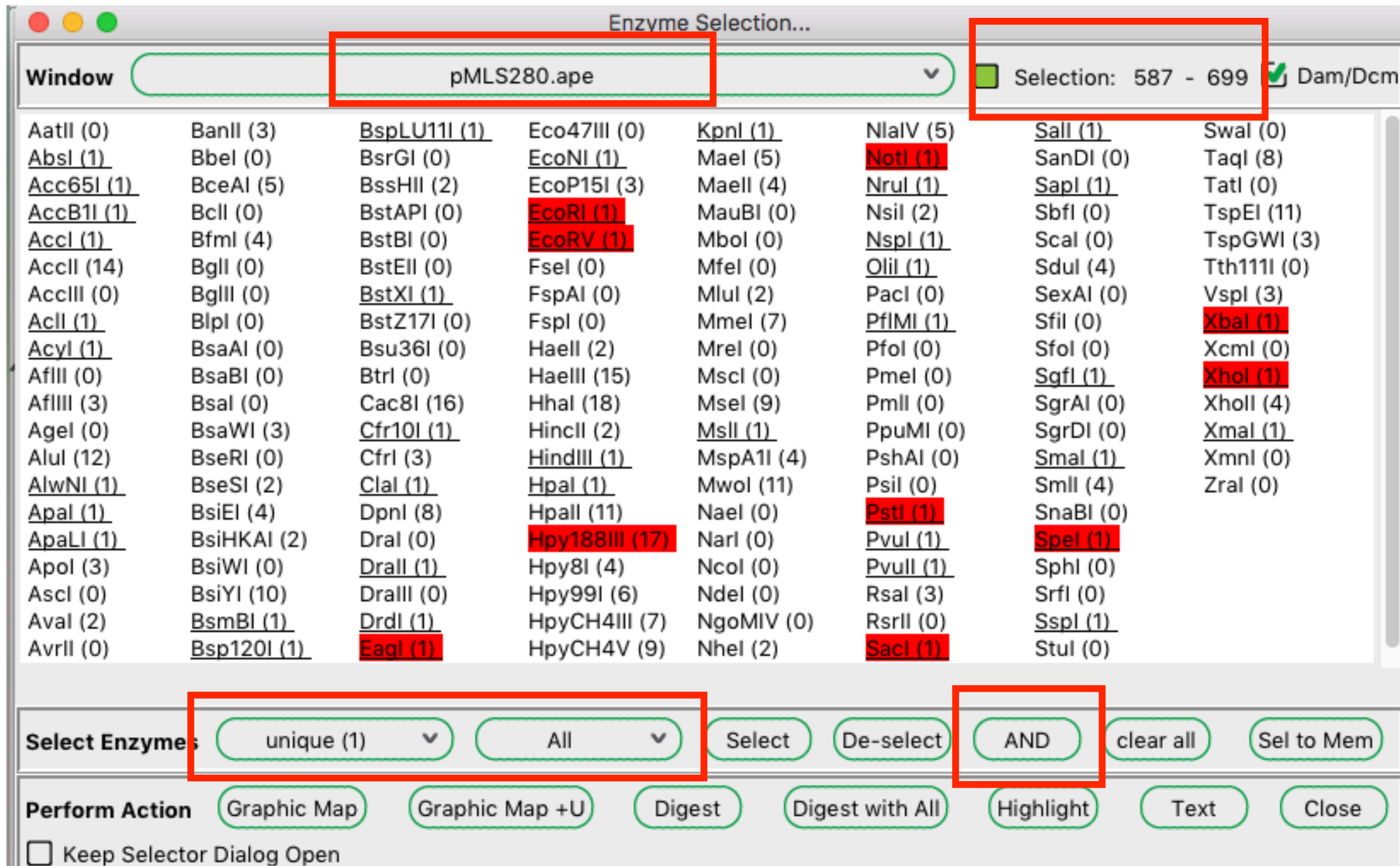
Select Enzymes: unique (1) All Select De-select AND clear all Sel to Mem

Perform Action: Graphic Map Graphic Map +U Digest Digest with All Highlight Text Close

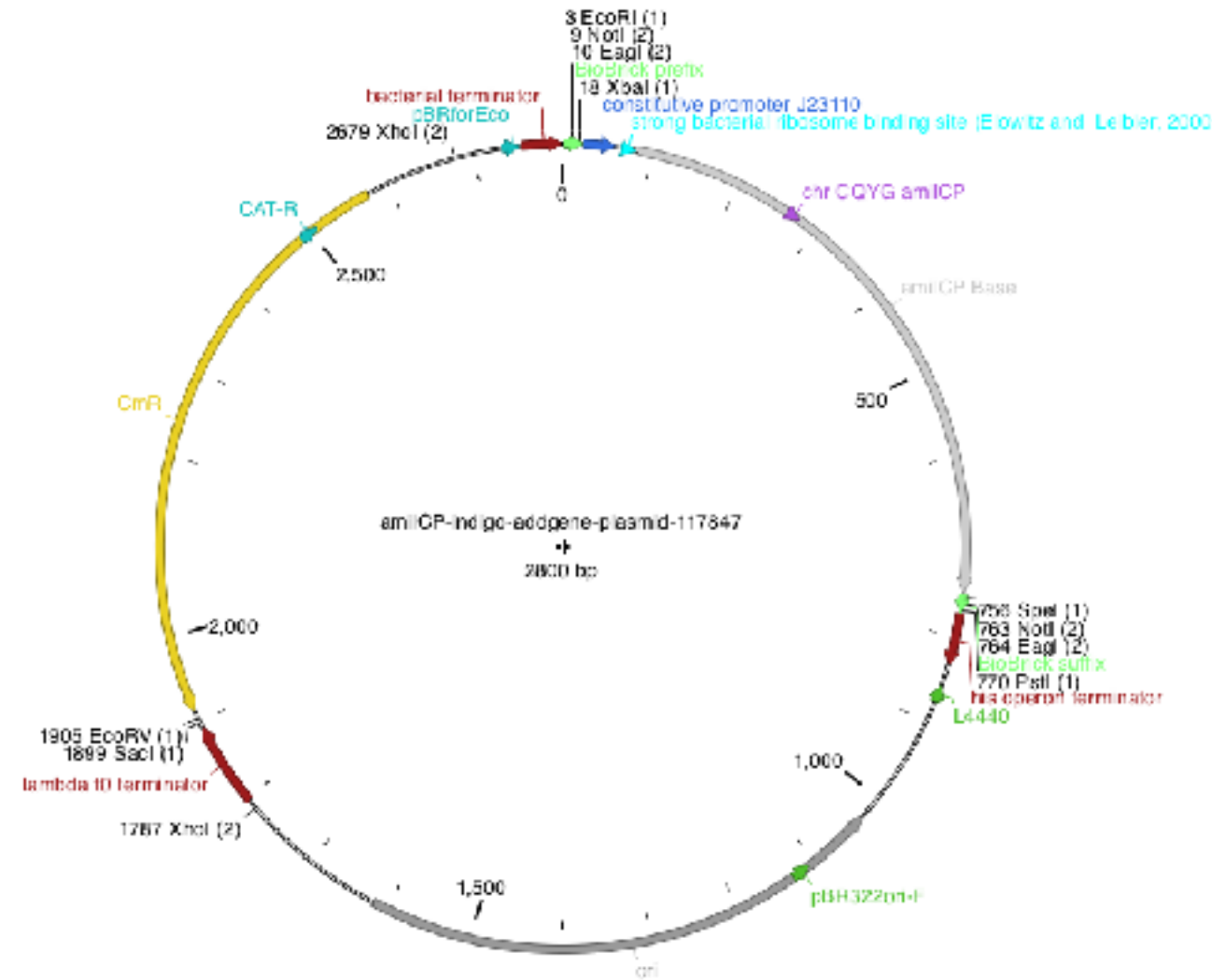
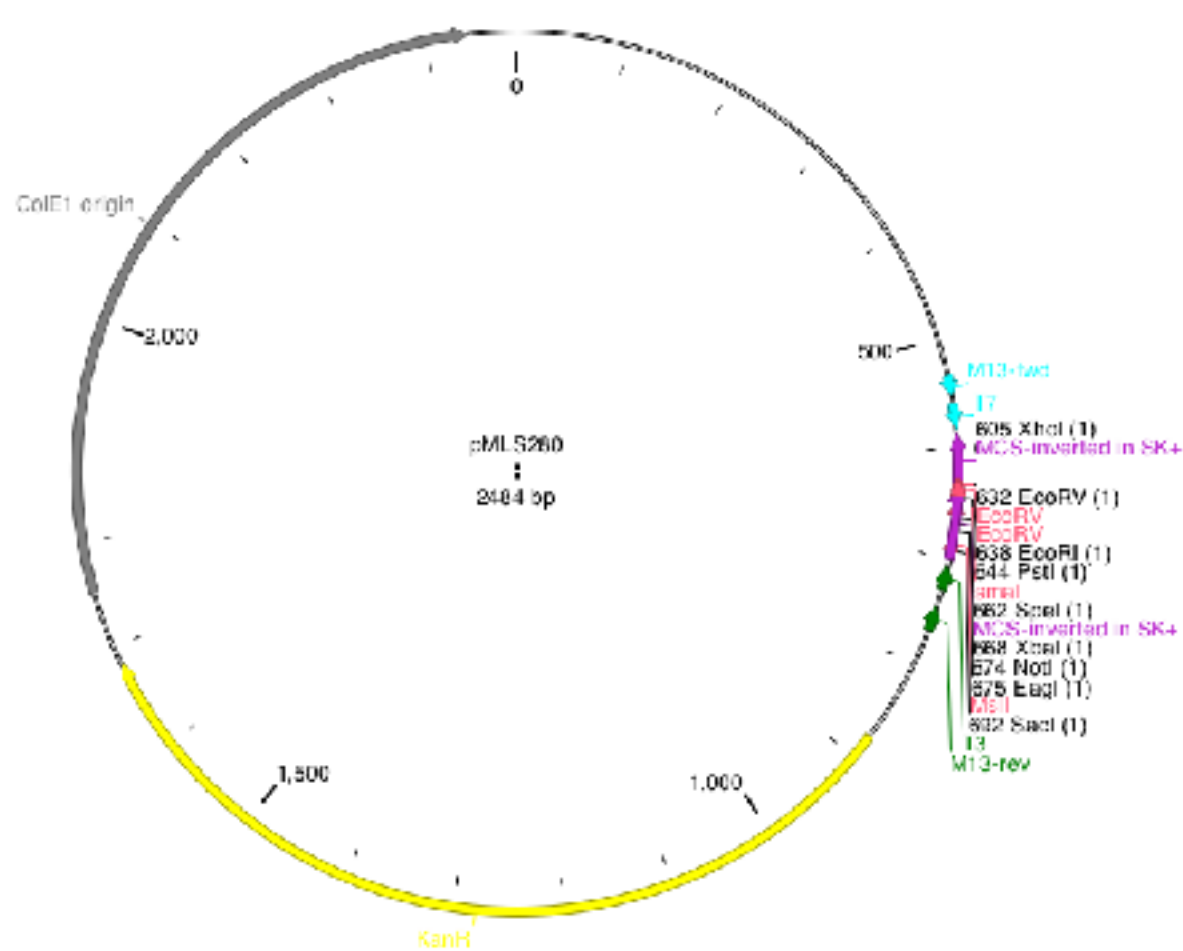
☐ Keep Selector Dialog Open

Enzyme Selection

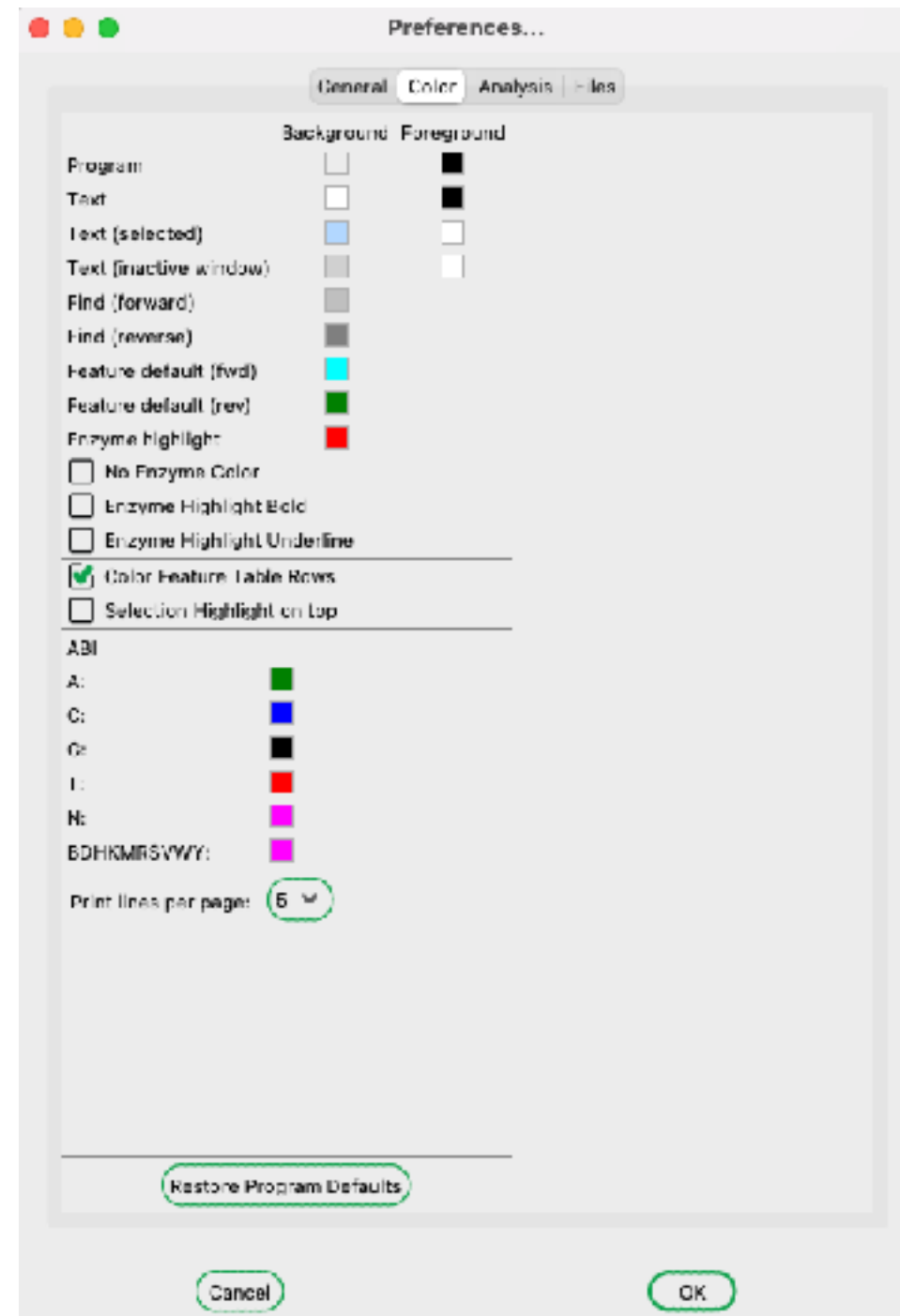
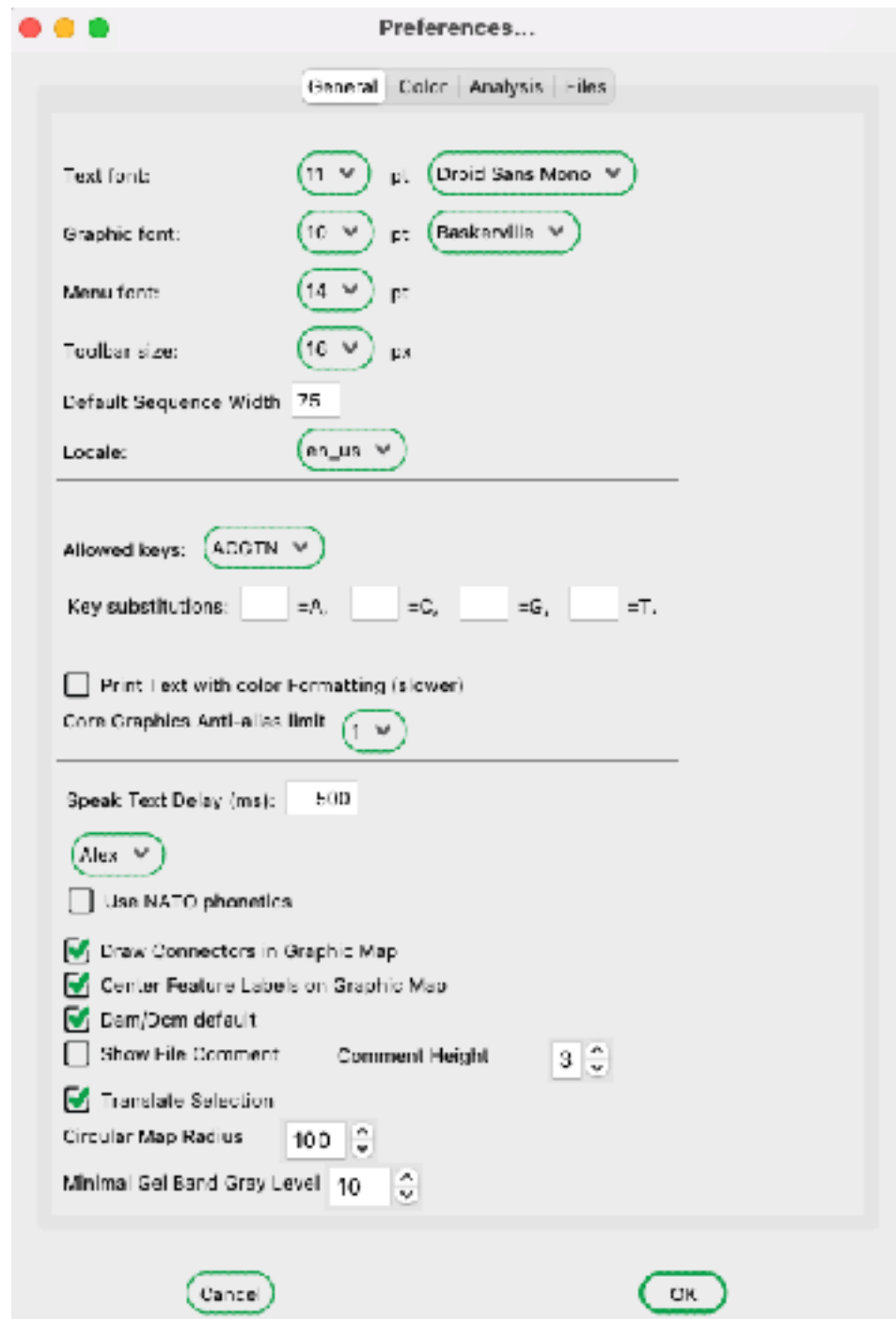
- select all enzymes that are ALSO unique in pMLS



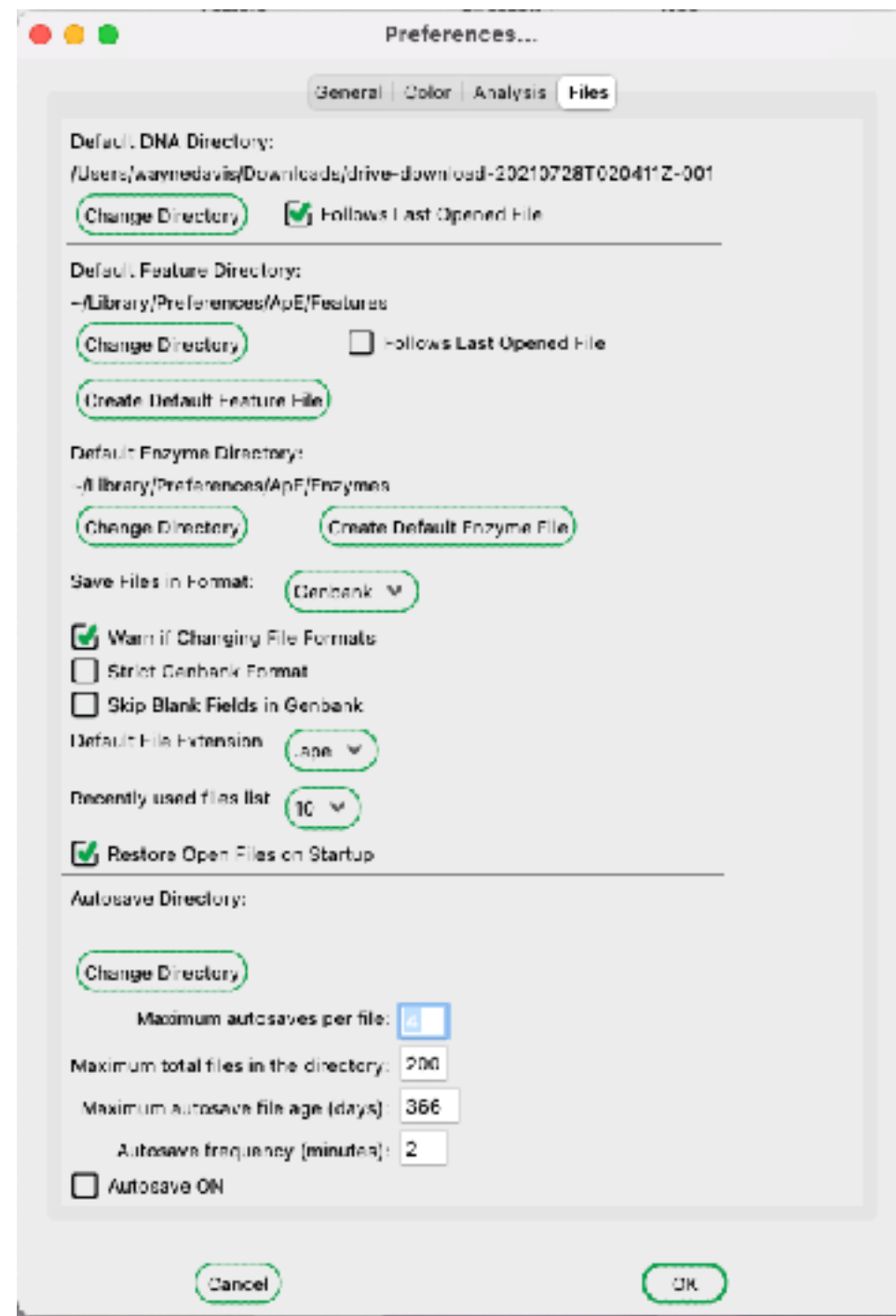
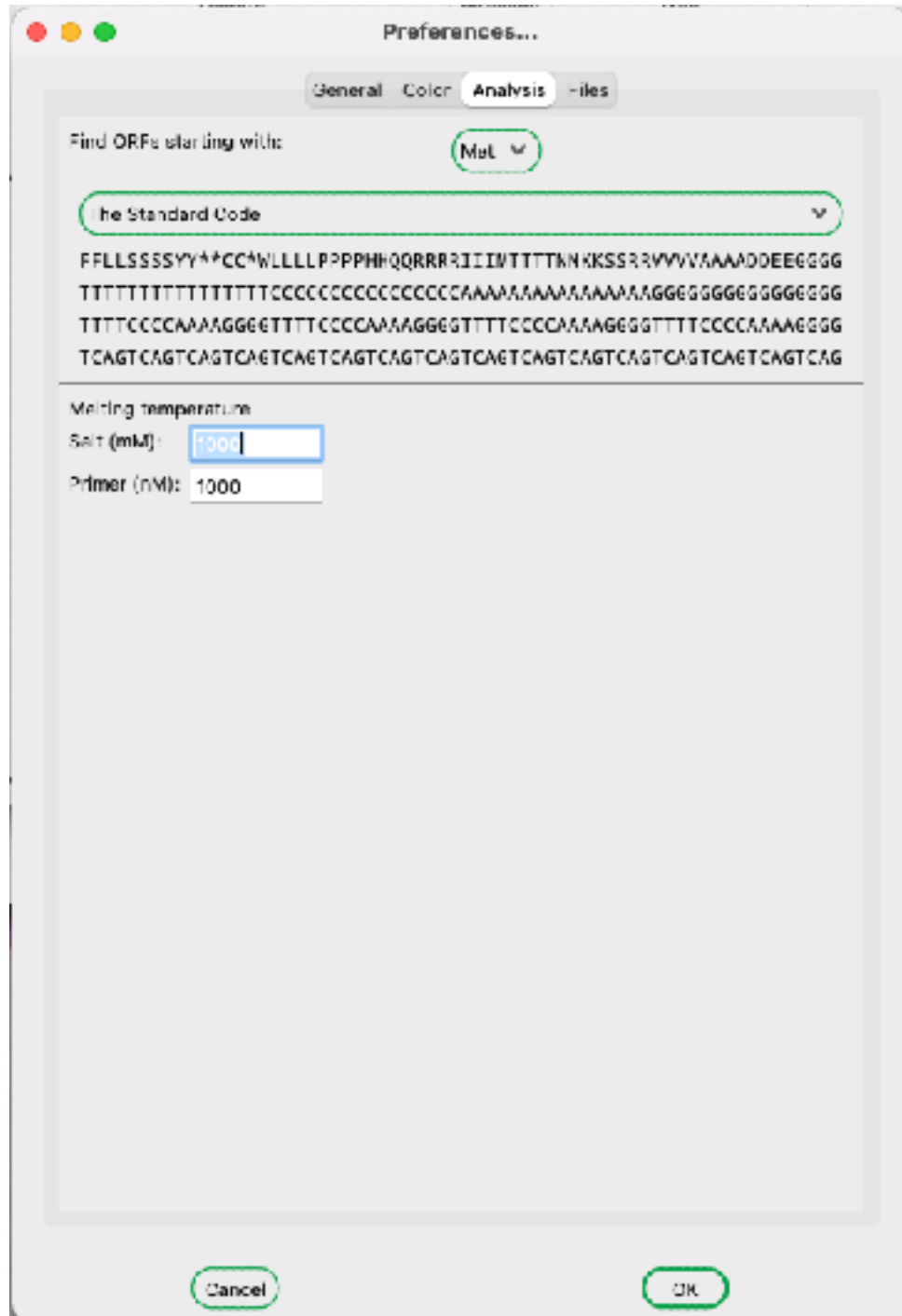
Enzyme Selection



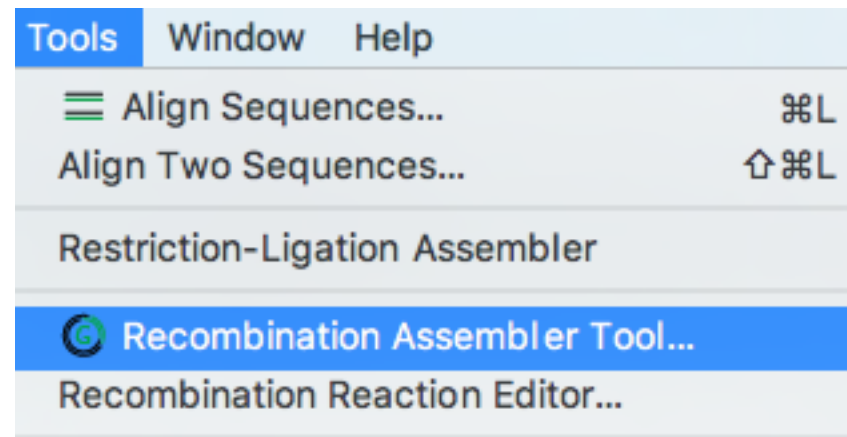
Preferences dialog



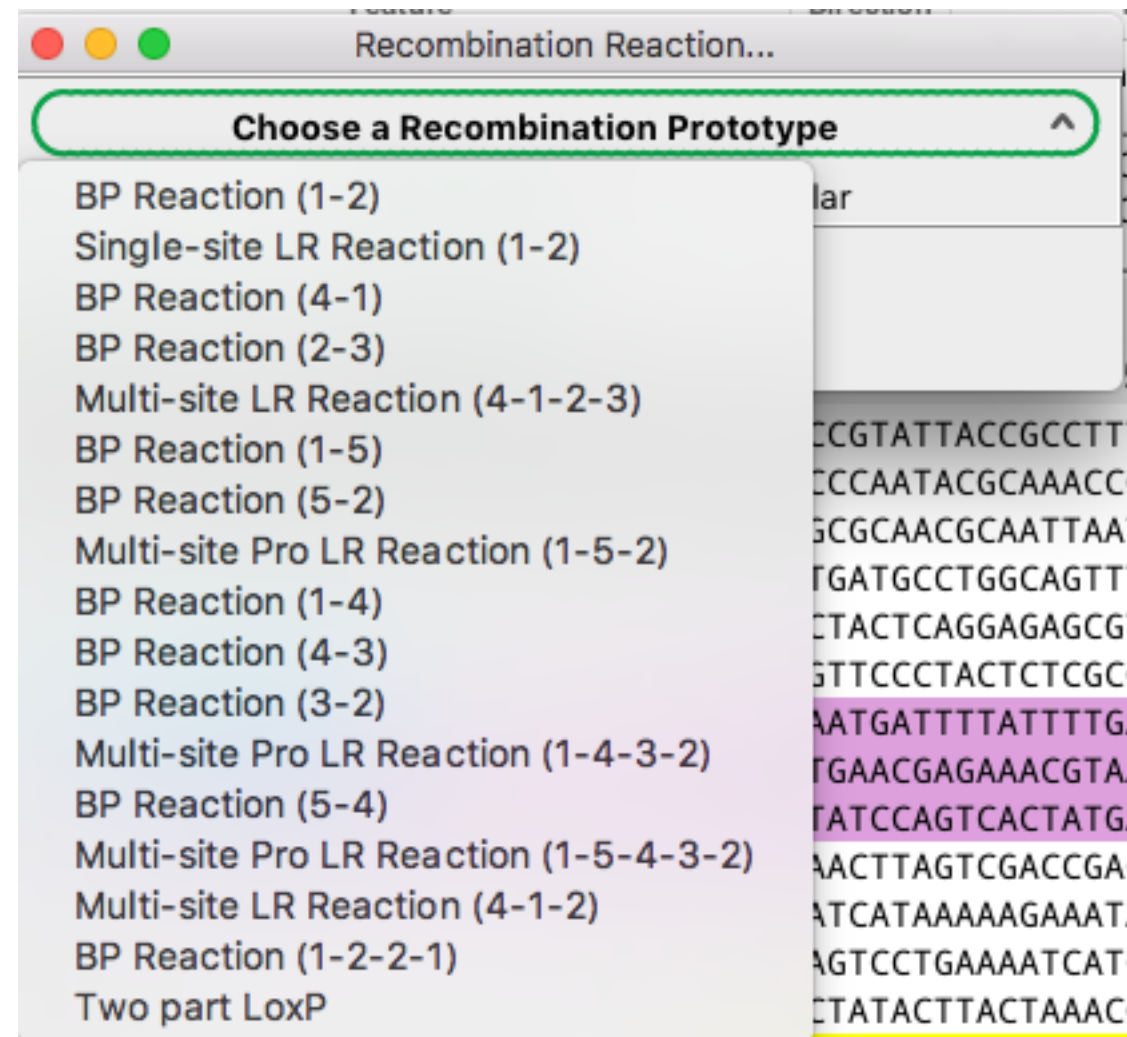
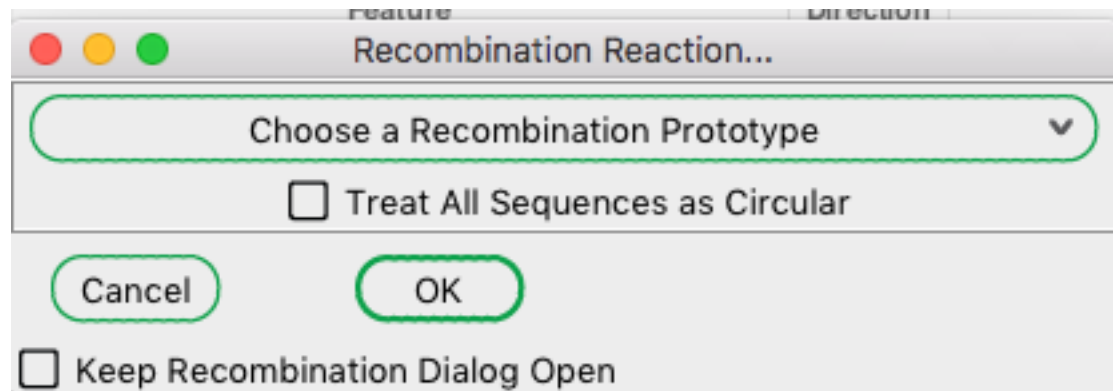
Preferences dialog



Recombination tool



Recombination tool



Recombination tool

Recombination Reaction...

BP Reaction (1-2) ^

☐ Treat All Sequences as Circular

Recombination Reaction Product

Insert pDONR 221.ape v

Backbone pDONR 221.ape v

Cancel OK

☐ Keep Recombination Dialog Open

Recombination Reaction...

BP Reaction (1-2) v

☐ Treat All Sequences as Circular

Recombination Reaction Product

Insert amilCP [oMBC24 oMBC25].ape v

Backbone pDONR 221.ape v

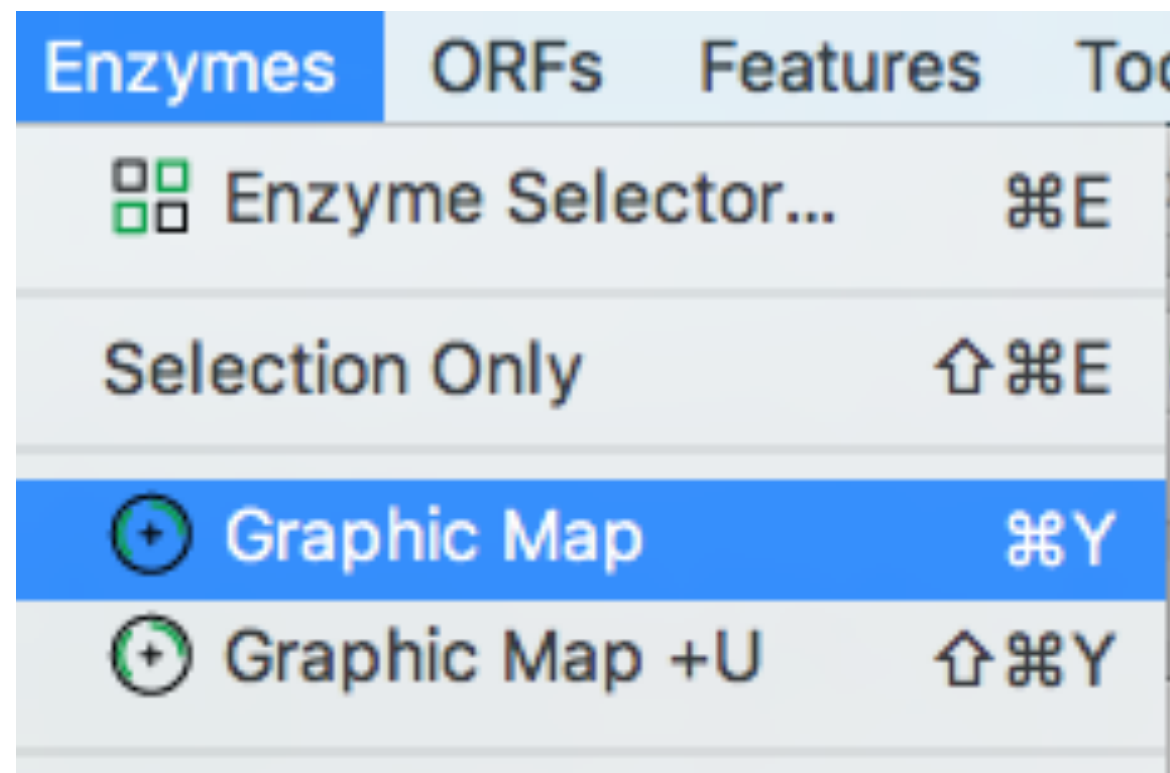
Cancel OK

☐ Keep Recombination Dialog Open

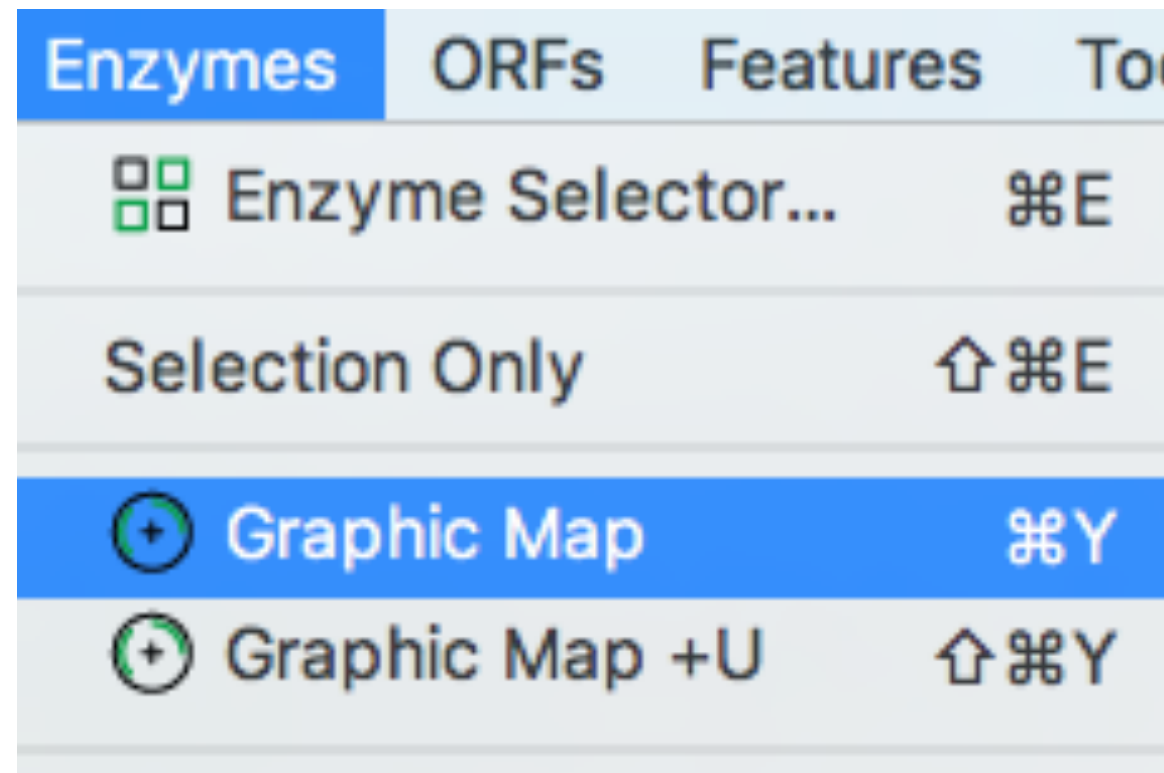
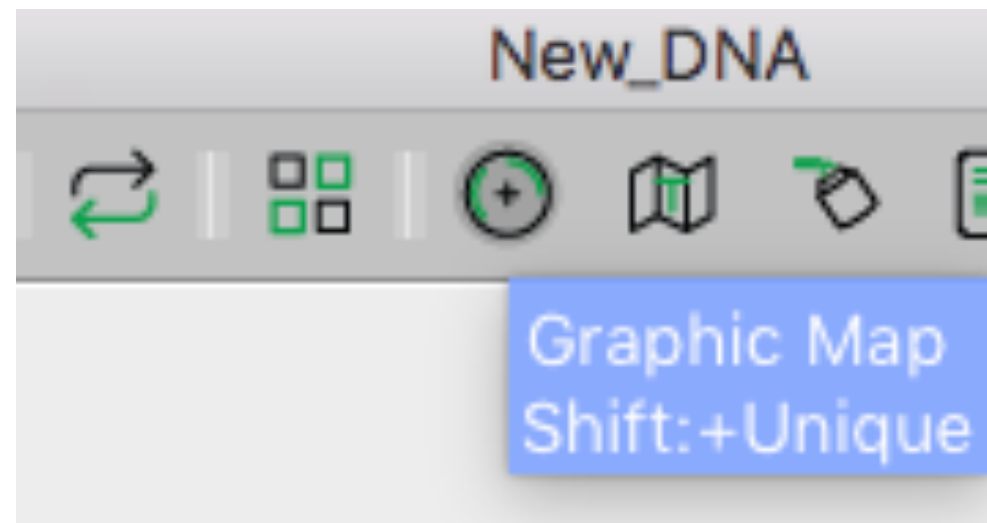
Recombination tool

The screenshot shows a DNA sequence editor window titled "New_DNA". The window displays a DNA sequence with various features highlighted. The sequence is shown in a text area with line numbers on the left. The sequence is: 1 CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCGAGCG 91 CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCA 181 CGACAGGTTTCCCGACTGGAAAAGCGGGCAGTGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAACGCAAAA 271 AGGCCATCCGTCAAGATGGCCTTCTGCTTAGTTTGTATGCCTGGCAGTTTATGGCGGGCGTCCTGCCCCGCCACCCTCCGGGCCGTTGCTTC 361 ACAACGTTCAAATCCGCTCCCGGGCGGATTTGTCTACTCAGGAGAGCGTTTACCCGACAAACAACAGATAAAACGAAAGGCCAGTCTTCC 451 GACTGAGCCTTTCTGTTTTATTTGATGCCTGGCAGTTCCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCCAGTCACGACGTTGTAA 541 AACGACGCGCCAGTCTTAAGCTCGGGCCCCAAATAATGATTTTATTTTACTGATAGTGACCTGTTTCTGTTGCAACAATGATGAGCAATG 631 CTTTTTTATAATGCCAACTTTgtacaaaaaagcaggctcgggaattcgcggccgcttctagagtttacggctagctcagtcctaggtacaa 721 tgctagctactagagaaagaggagaaatactagATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAAT 811 GGACACTACTTTGAGGTGGAAGGCGATGGAAAAGSTAAGCCCTACGACGGGAGCAGACGGTAAAGCTCACTGTCAACCAAGGGCGGACCT 901 CTGCCATTTGCTTGGGATATTTTATCACCACAGTGTCTCAGTACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGACTATGTA 991 AAGCAGTCATTCCCGGAGGGCTATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTGACCAATGATTCCAGCATC 1081 CAAGGCAACTGTTTCATCTACCATGTCAAGTTCTCTGGTTTGAACCTTCTCCCAATGGACCTGTCATGCAGAAGAAGACACAGGGCTCG 1171 GAACCCAACTGAGCGTCTCTTTGCACGAGATGSAATGCTGCTAGGAAACAACCTTATGGCTCTGAAGTTAGAAGGAGGCGGTCACTAT 1261 TTGTGTGAATTTAAACTACTTTACAAGGCAAGAAGGCTGTGAAGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAAT 1351 CACAAAGGATTACACTTCGTTGAGCAGTGTGAATTTCCATTGCACGCAAACTGTGGTCCCTAAtaatactagtagcggccgctg 1441 cagtccggcaaaaaagggaagggtgcaccaccctgccctttttctttaaaaccgaaaagaattacttcggttatgcaggcttcctcgcct 1531 factgactcgtcaccagctttCTTGTAACAAGTTGGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCCTATC 1621 AGTCAAAATAAAATCATTATTTGCCATCCAGCTGATATCCCTATAGTGAGTCGTATTACATGGTCATAGCTGTTTCTGGCAGCTCTGG 1711 CCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAATAATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAAT 1801 ACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGG 1891 GCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAA 1981 GGTAGCCTTCCCAATGATGTTACAGATGAGATGGTCAGACTAAACTCCCTCACGGAATTTATGCCTCTTCCGACCATCAAGCATTATTC 2071 CGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAA

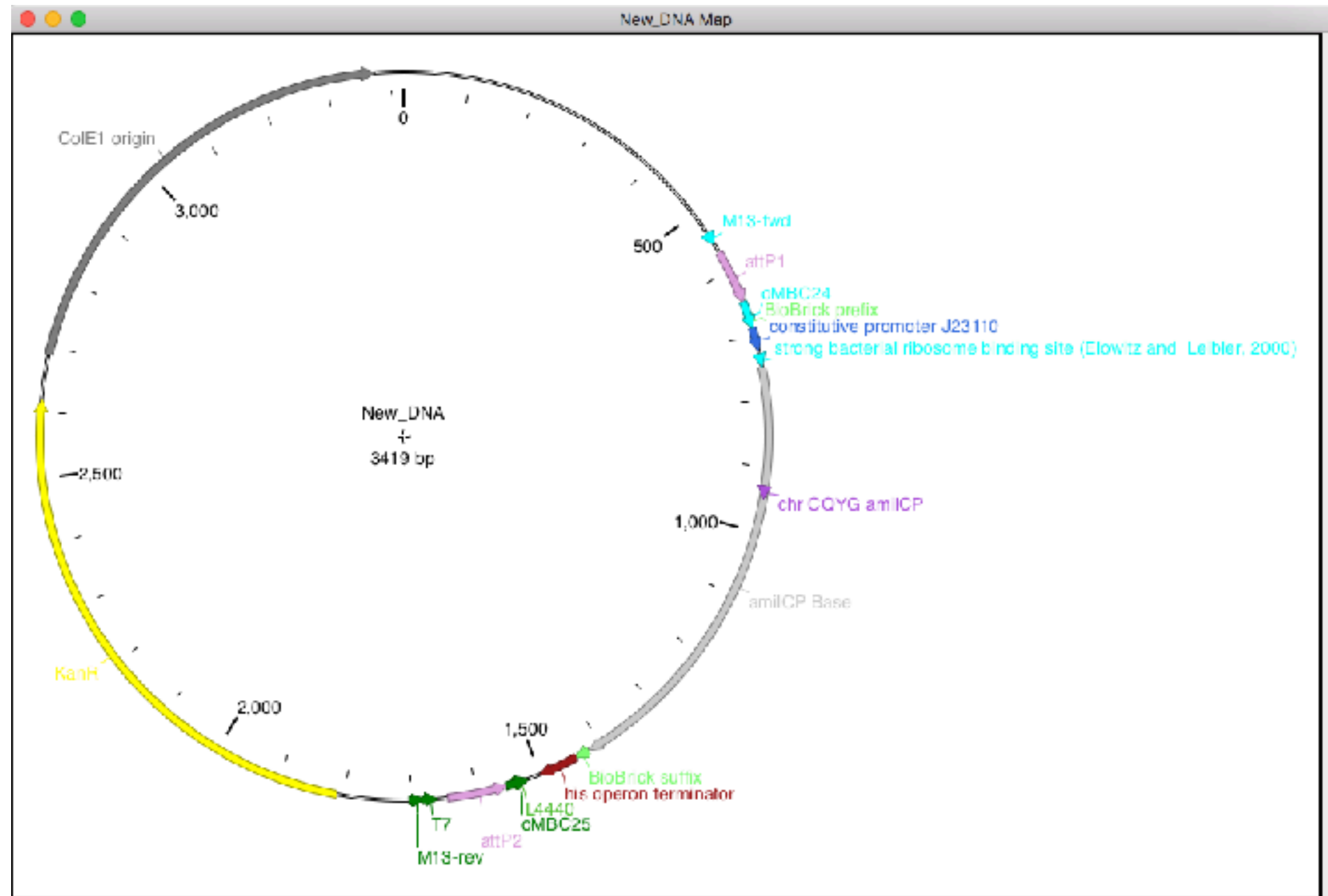
Graphic Map



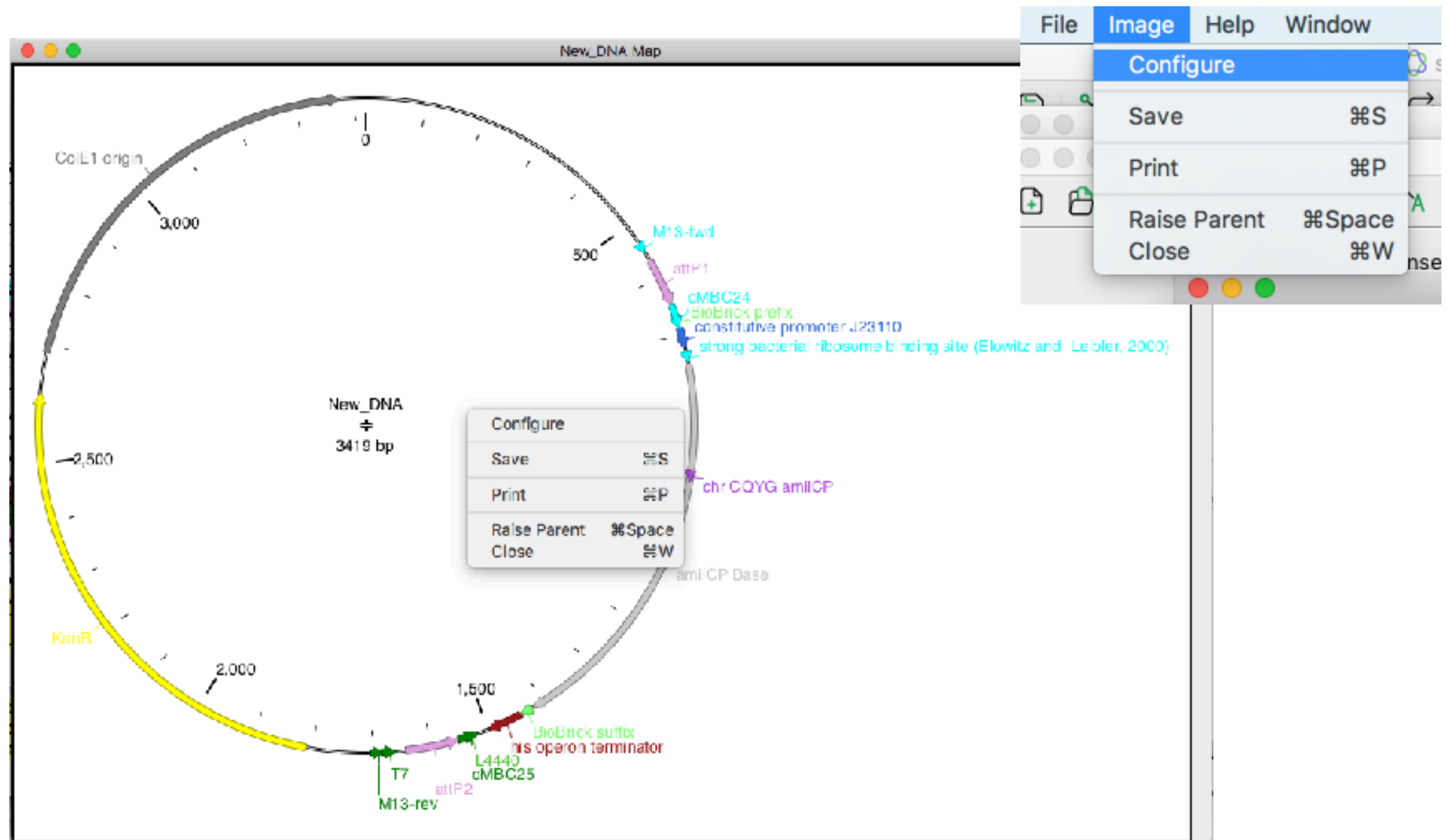
Graphic Map



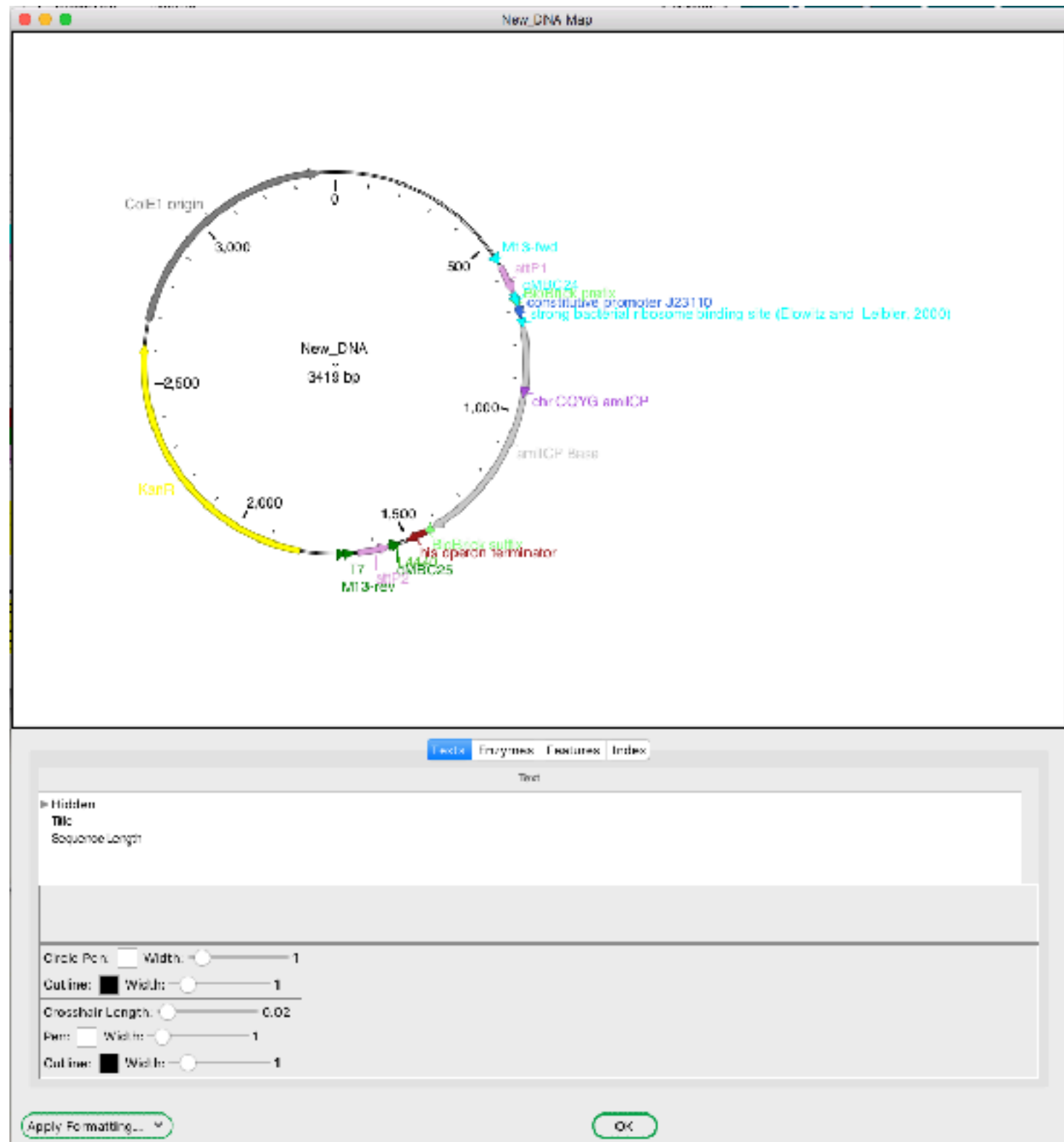
Graphic Map



Graphic Map



Graphic Map



Graphic Map

Texts Enzymes Features Index				
Feature	Direction	Type	Location ↓	Length
► Hidden				
M13-fwd	>>>	primer_bind	536	18
attP1	>>>	misc_recom	570	82
oMBC24	>>>	misc_featur	652	41
BioBrick prefix	>>>	misc_featur	671	22
constitutive promoter J23110	>>>	promoter	693	35
strong bacterial ribosome binding site (Elowitz and Leibler, 2	>>>	RBS	736	12

☒ Visible

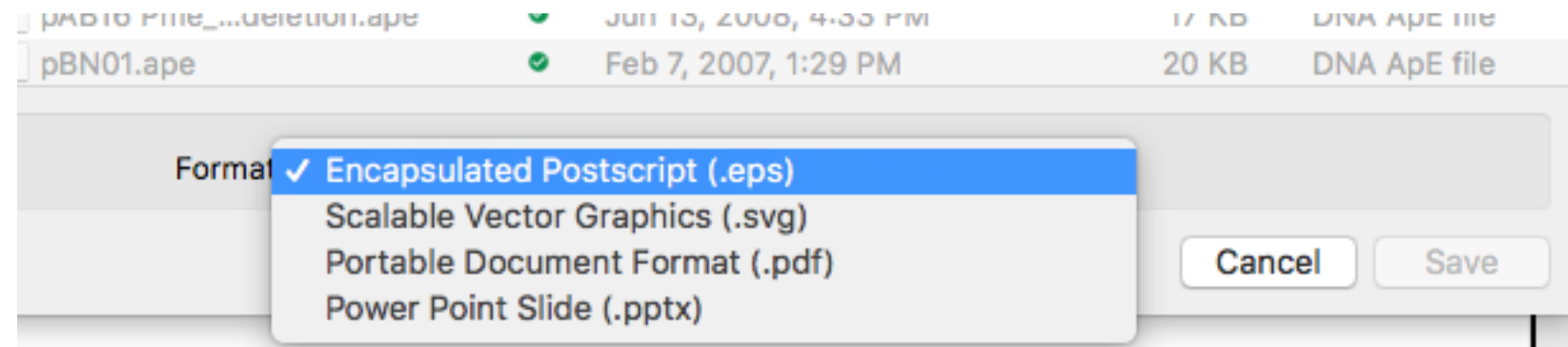
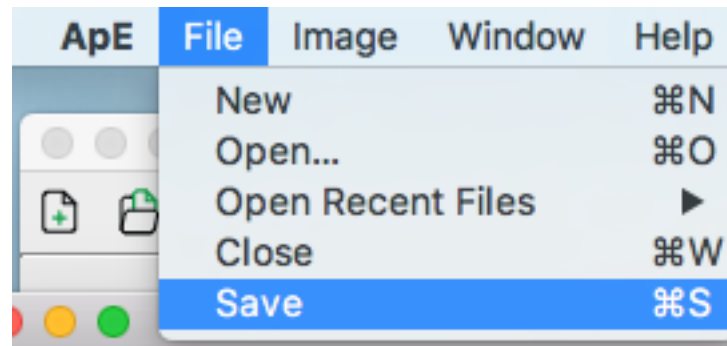
Label: % ▾ Helvetica ▾ 14 ▾ ☐ Use Arc Label

Fill: 5 Outline: % ▾ 1

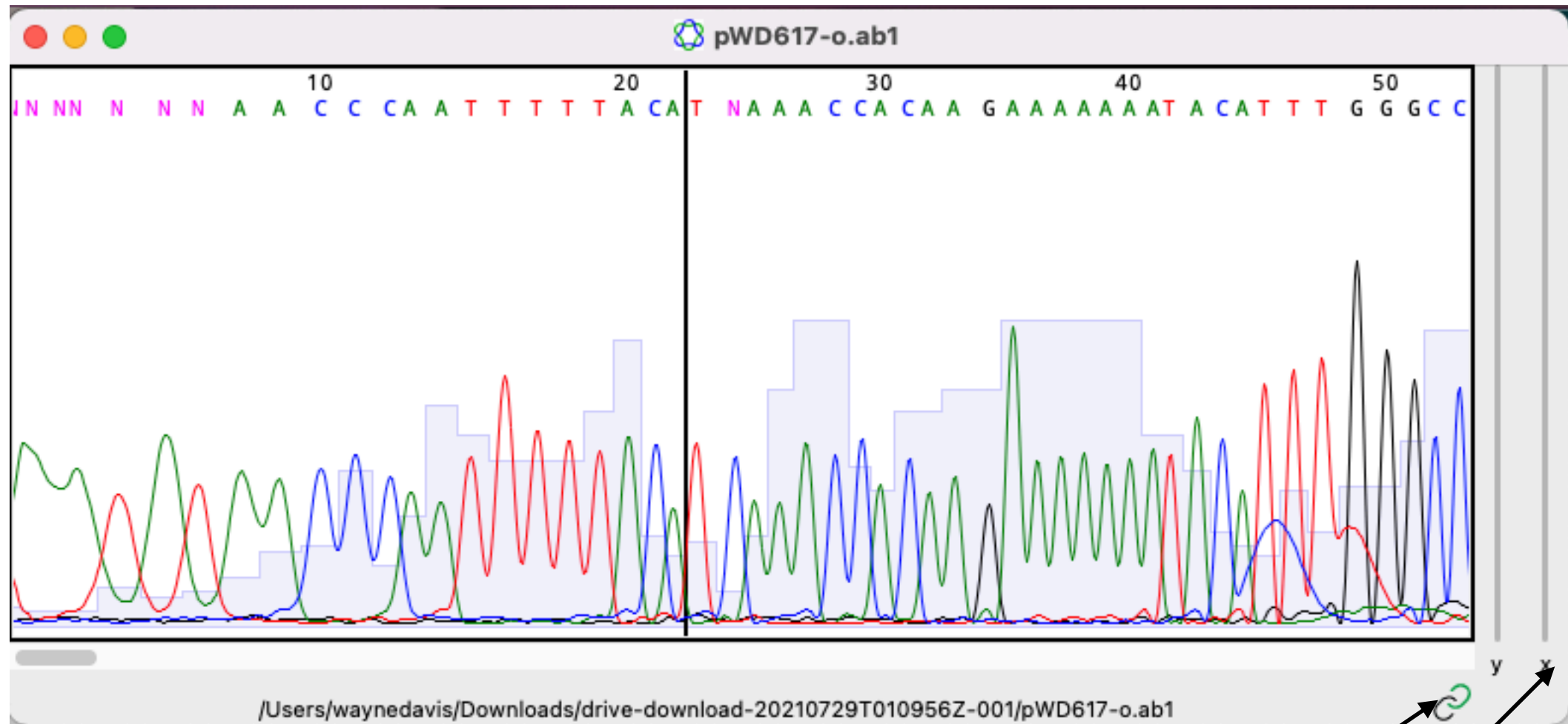
5' arrow: None ▾ 3' arrow: Barbed Arrow 1 ▾

Offset:

Graphic Map



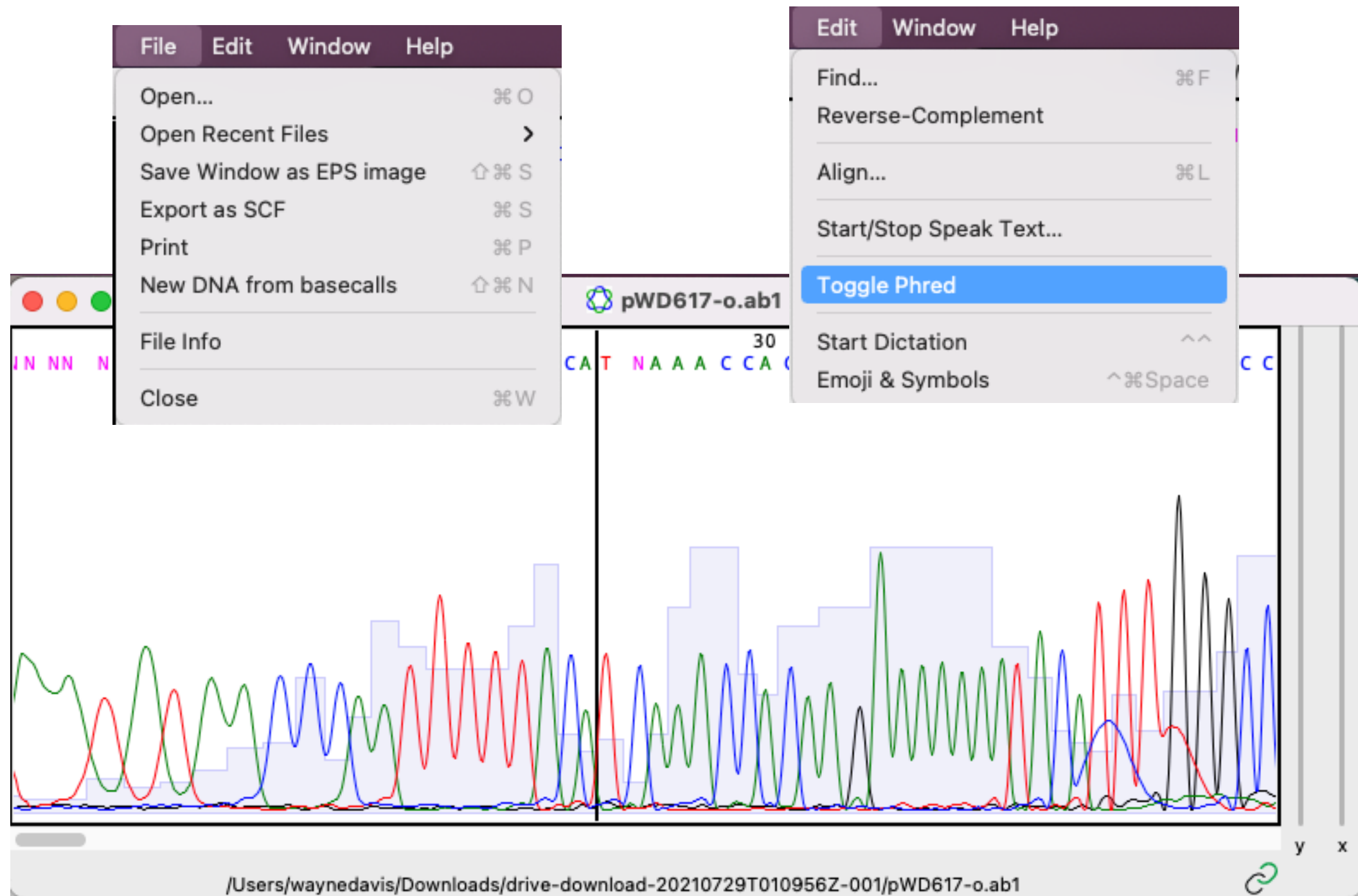
ABI files



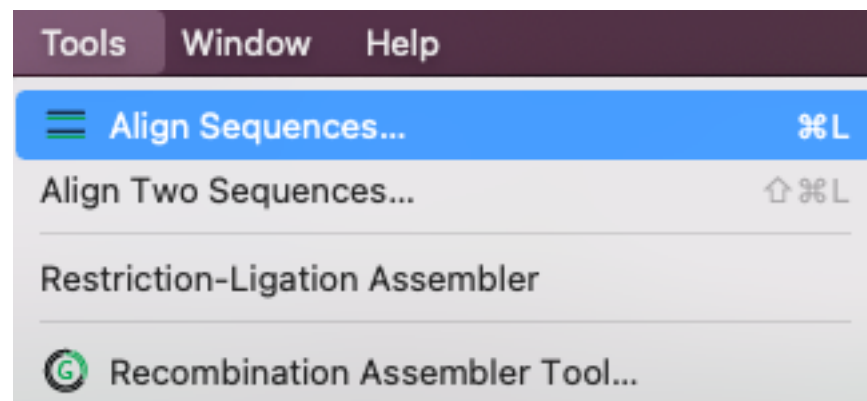
**Drag this to a sequence
window to add the abi file
to a sequence file**

Scale

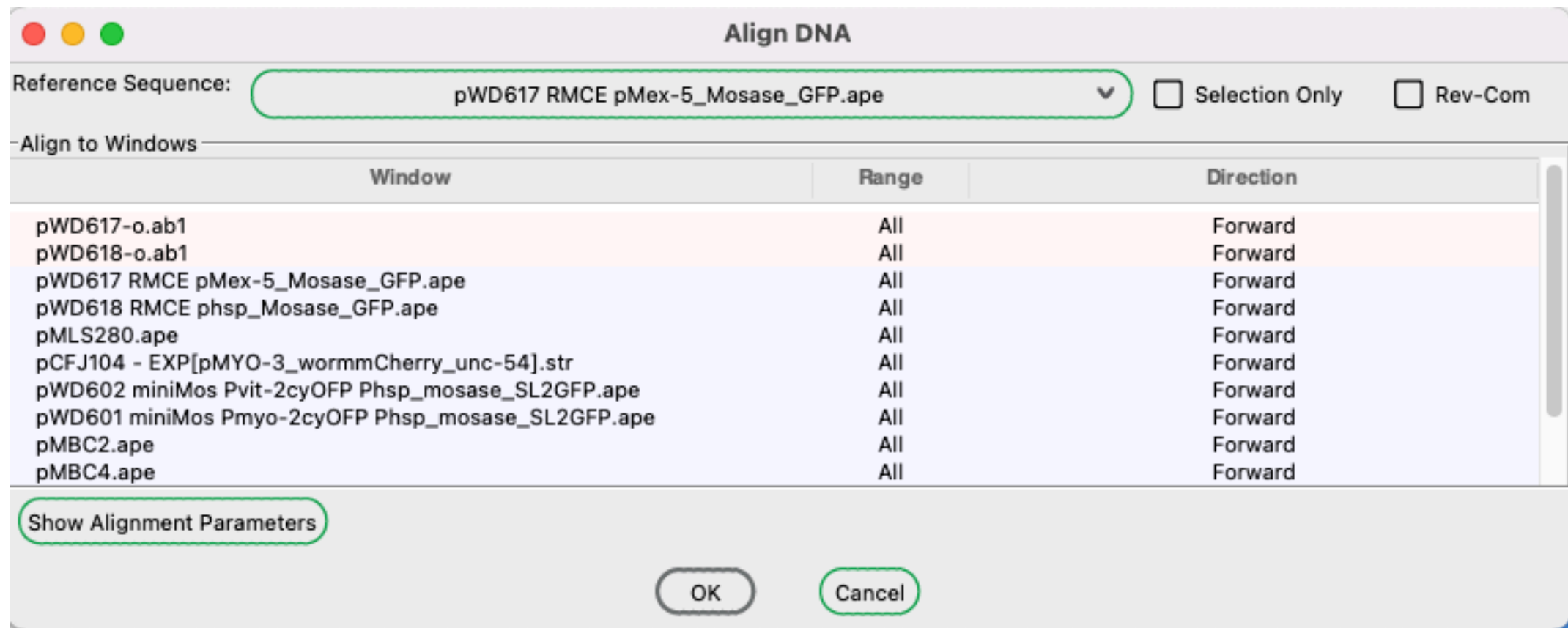
ABI files



Align



Align



The image shows a macOS-style dialog box titled "Align DNA". At the top, there are three window control buttons (red, yellow, green). Below the title bar, the "Reference Sequence:" is set to "pWD617 RMCE pMex-5_Mosase_GFP.ape" in a dropdown menu. To the right of this are two checkboxes: "Selection Only" and "Rev-Com", both of which are unchecked. Below this is a section labeled "Align to Windows" which contains a table. The table has three columns: "Window", "Range", and "Direction". It lists ten different plasmid and sequence files, all with a range of "All" and a direction of "Forward". The first two rows are highlighted in light orange, and the remaining eight rows are highlighted in light blue. At the bottom left of the dialog is a button labeled "Show Alignment Parameters". At the bottom center are two buttons: "OK" and "Cancel".

Align DNA

Reference Sequence: pWD617 RMCE pMex-5_Mosase_GFP.ape ☐ Selection Only ☐ Rev-Com

Align to Windows

Window	Range	Direction
pWD617-o.ab1	All	Forward
pWD618-o.ab1	All	Forward
pWD617 RMCE pMex-5_Mosase_GFP.ape	All	Forward
pWD618 RMCE phsp_Mosase_GFP.ape	All	Forward
pMLS280.ape	All	Forward
pCFJ104 - EXP[pMYO-3_wormmCherry_unc-54].str	All	Forward
pWD602 miniMos Pvit-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward
pWD601 miniMos Pmyo-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward
pMBC2.ape	All	Forward
pMBC4.ape	All	Forward

Show Alignment Parameters

OK Cancel

Align

pMLS280.ape	All	Forward
pCFJ104 - EXP[pMYO-3_wormmCherry_unc-54].str	All	Forward
pWD602 miniMos Pvit-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward
pWD601 miniMos Pmyo-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward
pMBC2.ape	All	Forward
pMBC4.ape	All	Forward

Alignment Parameters

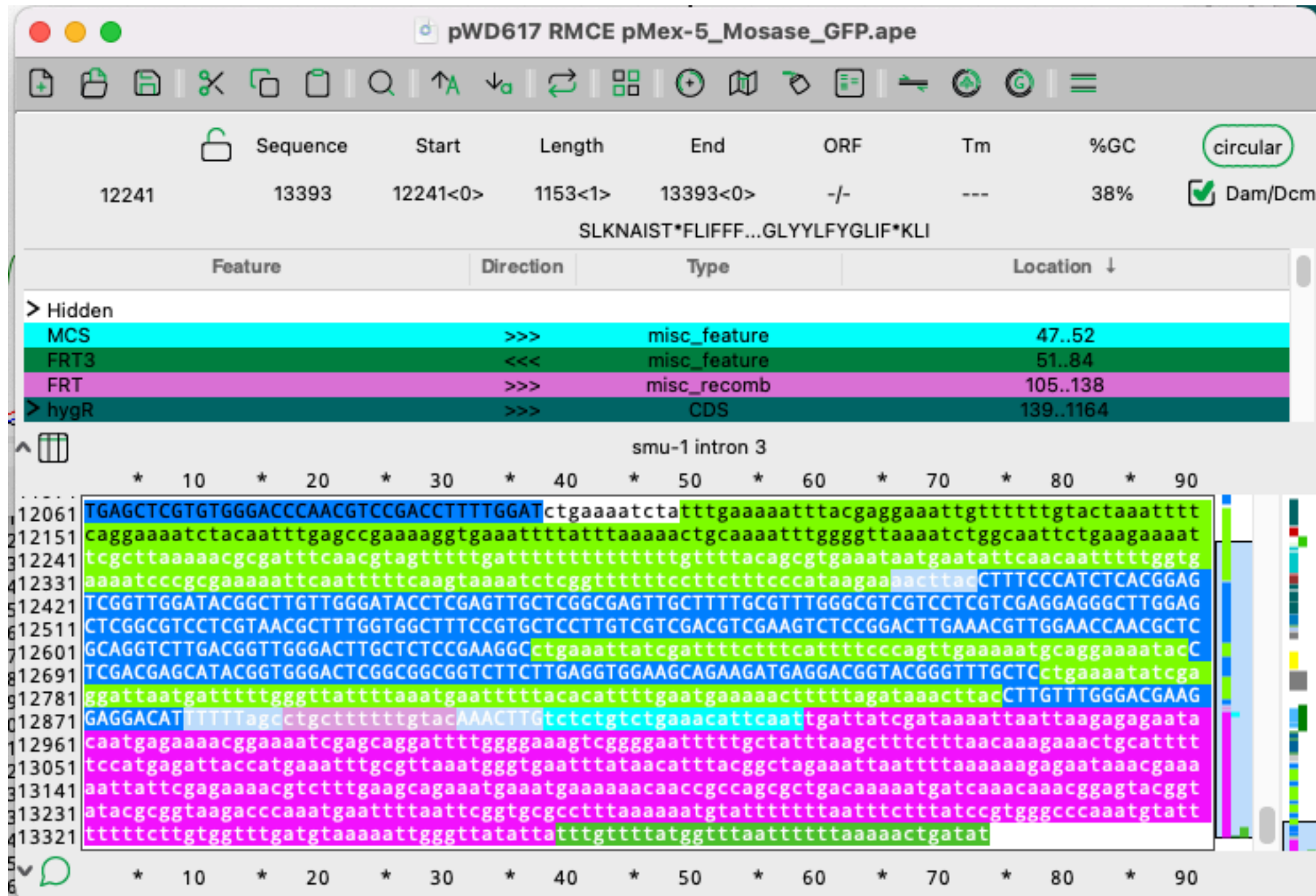
Blocks: N-W max:

Mismatch Penalty: Gap Penalty: Gap Ext. Penalty:

Line Width: ☒ Copy Highlighting from Sequence

Trim ends with Phred <: ☐ Show Only Aligned Regions

Align



Align

Align DNA

Reference Sequence:

pWD617 RMCE pMex-5_Mosase_GFP.ape

☒ Selection Only ☐ Rev-Com

Align to Windows

Window	Range	Direction
pWD617-o.ab1	All	Reverse
pWD618-o.ab1	All	Forward
pWD617 RMCE pMex-5_Mosase_GFP.ape	All	Forward
pWD618 RMCE phsp_Mosase_GFP.ape	All	Forward
pMLS280.ape	All	Forward
pCFJ104 - EXP[pMYO-3_wormmCherry_unc-54].str	All	Forward
pWD602 miniMos Pvit-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward
pWD601 miniMos Pmyo-2cyOFP Phsp_mosase_SL2GFP.ape	All	Forward

Align

```
pWD617 RMCE pMex-5_Mosase_GFP.ape Alignment to pWD617-o.ab1

Wed Jul 28, 2021 19:21 MDT
pWD617 RMCE pMex-5_Mosase_GFP.ape from 12241 to 13393
Alignment to
pWD617-o.ab1-- Matches:1071; Mismatches:16; Gaps:66; Unattempted:0

      *      *      *      *      *      *      *      *      *      *
12241>tcgcttaaaaaacgcgatttcaacgtagtttttgattttttttttttttgttttacagcgtgaaataatgaatattcaacaatttttggtgaaaatcccgc>12340
1087<-----NAGTTTGTGATTTT-----GTTTACAGCGTGAAATAATGANNNTCANCAA-----TTTGGTGAAAATCCCGC<1018

      *      *      *      *      *      *      *      *      *      *
                                     K G M E R L R N S V A Q Q
12341>gaaaaattcaatttttcaagtaaaatctcggttttttctttccataagaaacttacCTTCCCATCTCACGGAGTCGGTTGGATACGGCTTGT>12440
1017<GAAAAATCAA-----TTTCAAGTAAAATCTCGGTTTTTCTTTTCCATAAGNAACTTACCTTCCCATCTCACGNAGTCGGTTGGATACGGCTTGT<920

      *      *      *      *      *      *      *      *      *      *
      S V E L Q E A L Q K Q T Q A D D E D L L A Q L E A D E Y R K P P K
12441>GGGATACCTCGAGTTGCTCGGCGAGTTGCTTTGCGTTTGGGCGTCGTCCTCGTCGAGGAGGGCTTGGAGCTCGGCGTCCTCGTAACGCTTTGGTGGCT>12540
919<GGGATACCTCGAGTTGCTCGGCGAGTTGCTTTGCGTTTGGGCGTCGTCCTCGTCGAGGAGGGCTTGGAGCTCGGCGTCCTCGTAACGCTTTGGTGGCT<820

      *      *      *      *      *      *      *      *      *      *
      G H E K D D V D F D G S K F R Q F W R E C T K V T P V Q E G F A
12541>TCCGTGCTCCTTGTCTGTCGACGTCGAAGTCTCCGGACTTGAAACGTTGGAACCAACGCTCGCAGGTCTTGACGGTTGGGACTTGCTCTCCGAAGGCctga>12640
819<TCCGTGCTCCTTGTCTGTCGACGTCGAAGTCTCCGGACTTGAAACGTTGGAACCAACGCTCGCAGGTCTTGACGGTTGGGACTTGCTCTCCGAAGGCCTGA<720

      *      *      *      *      *      *      *      *      *      *
                                     E V L M R H S E A A T K K L H F C
12641>aattatcgatttttctttcattttccagttgaaaaatgcaggaaaatacCTCGACGAGCATACGGTGGGACTCGGCGGCGGTCTTCTTGAGGTGGAAGCA>12740
719<AATTATCGATTTCTTTCATTTTCCAGTTGAAAAATGCAGGAAAATACCTCGACGAGCATACGGTGGGACTCGGCGGCGGTCTTCTTGAGGTGGAAGCA<620
```

Align

GGCGAGTTGCTTTGCGTTTGGGCGTCGTCC

/ D F D G S K F R Q F
 ACgtcGAAGtcTCCggaCTTgaaACGttgGA/
 ACGTCGAAGTCTCCGGA^{CTT}GAAACGTTGGA/
 ACGTCGAAGTCTCCGGA^{CTT}GAAACGTTGGA/
 G C A
 A
 tttcccagttgaaaaatgcaggaaaatacC
 TTTTCCcAGTTGAAAAATGcAGGAAAATACCT