Transitioning from:

- EditSeq to SeqBuilder Pro
- PrimerSelect to SeqBuilder Pro
- MegAlign to MegAlign Pro
- SeqMan Pro to SeqMan Ultra

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Why switch from the applications I currently use?

Why switch from the applications I currently use?

If you are a long-time user of DNASTAR software, you may be wondering why you need to make the switch to our newer applications, SeqBuilder Pro, MegAlign Pro and SeqMan Ultra.

Some of our 32-bit applications—EditSeq, PrimerSelect and MegAlign—were retired with the release of Lasergene 16.0 in mid-2019. Since then, all development efforts and new features related to sequence editing, primer design, and cloning are being focused exclusively on SeqBuilder Pro, while pairwise & multiple sequence alignment efforts are being focused on MegAlign Pro. With the release of Lasergene 17.0 in early, 2020, we replaced 32-bit SeqMan Pro with SeqMan Ultra, which features a modern, intuitive interface. For now, we are including both applications, but will be removing SeqMan Pro in a future release.

All three of the newer applications contain <u>numerous features not found in their classic counterparts</u>, and provide an improved user experience and many new capabilities compared to EditSeq, PrimerSelect, MegAlign and SeqMan Pro.

Try the new apps for yourself online. No download necessary!	Upgrade now to access powerful <u>new functionality</u>
Free Trial	Request a Quote

Why a special guide just for transitioning between applications?

During our preparations to retire three 1990's applications with the Lasergene 16.0 release in 2019, we realized that a substantial number of Lasergene users were still using legacy versions of our applications, despite having had <u>6 to 14 years</u> in which to switch to their replacements. Are you one of these loyal "classic app" fans? If you haven't yet made the switch, you are in for a treat! Lasergene's modern apps feature sleek, intuitive and customizable interfaces and a whole new world of time-saving functionality. Click the following links to learn how to transition from:

- EditSeq to SeqBuilder Pro
- <u>PrimerSelect to SeqBuilder Pro</u>
- MegAlign to MegAlign Pro
- SeqMan Pro to SeqMan Ultra

About this User Guide:

- For help INSTALLING Lasergene, see our separate Installation Guide.
- <u>Click here</u> for a list of all topics that include **VIDEOS**.
- To **PRINT** the current page of the User Guide, click the printer icon in the top right corner (
- To download a **PDF** of the entire User Guide, scroll to the bottom of the table of contents on the left, and press **Download as PDF**.

New functionality in SeqBuilder Pro, MegAlign Pro and SeqMan Ultra

Click the bullet-pointed links below to learn about added or improved functionality in the modern application compared to the "classic" versions. These links will take you away from this "Transitioning" guide to the relevant User Guide topic for SeqBuilder Pro or MegAlign Pro.

Note: For illustrated help showing how to perform your current EditSeq, PrimerSelect, MegAlign or SeqMan Pro tasks in the newer applications, see the following topics in this guide: <u>Transitioning from EditSeq to SeqBuilder Pro</u> / <u>PrimerSelect to SeqBuilder Pro</u> / <u>MegAlign to MegAlign Pro</u> or <u>SeqMan Pro to SeqMan Ultra</u>.

SeqBuilder Pro lets you:

- <u>Create a new sequence based on an existing sequence;</u> among others, by using the reverse complement, translation, or a feature, by sampling sequences* <u>Perform virtual cloning</u>
- <u>Auto-annotate sequences</u> with or without first <u>creating a custom feature database</u>
- Easily edit primers by using a mutation/codon change tool, by typing or by changing the primer length
- <u>Customize the appearance of features</u>
- Display the translation of a sequence below the original sequence
- Easily change the appearance of views and the layout of views and panels
- <u>Change the rendering style and font</u>
- Easily create, modify and join features, and show or hide features in the views
- Perform highly customizable agarose gel simulations
- Export a sequence in many different formats

MegAlign Pro lets you:

- Perform multiple alignments using the Clustal Omega, Clustal W, MAFFT or MUSCLE algorithms
- Perform genome-level multiple alignments with Mauve
- <u>Perform pairwise alignments</u> using the Smith-Waterman algorithm, or either of two variations of the Needleman-Wunsch algorithm; then easily perform subsequent pairwise alignments using different parameters or a different combination of sequences.
- Rename <u>a single sequence manually</u>, or <u>one or more sequences automatically</u>, using a customized naming convention
- Analyze results in several graphic-rich views, and customize the view layout
- <u>View details about any selection</u>: one or more sequences, a portion of one or more sequences, one or more tracks or features, or a portion of a pairwise alignment
- <u>Apply detail</u> for rulers, features, a translation, a consensus, the sequence logo, GC content or gap fraction histograms and many more.

- · Bookmark a sequence location or range of interest, so you can easily return to it later
- Export data, a phylogenetic tree, or an image of a view

SeqMan Ultra lets you:

- <u>Seamlessly access SeqMan NGen</u>, where you can create and build your assembly.
- Open finished assemblies for downstream analysis
- View information about the currently open assembly or about any selection.
- <u>Work with contigs</u> by organizing them into scaffolds, closing gaps between them and <u>seeing contig</u> <u>information in graphical or tabular views</u>.
- <u>Apply data tracks</u> that provide enhanced information about the contig, consensus and reads.
- <u>View features</u> in graphical, tabular or text format and filter features.
- View, filter and score variants.
- Search for sequences online in NCBI's BLAST and Entrez databases.
- · Customize the appearance and behavior of views, tables and the layout itself
- Export the consensus or data from a view.

Timeline for "classic" and modern applications

The following timeline shows the release dates of the "classic" Lasergene applications and their modern counterparts:

- **1988 EditSeq** was DNASTAR's original application for creating and editing sequences and their features.
- 1993 PrimerSelect was DNASTAR's original application for primer design and primer catalog creation, while MegAlign offered pairwise and multiple sequence alignment and the creation of phylogenetic trees.
- **2001** With increasing popularity of the Internet, **PrimerSelect**, **MegAlign** and **EditSeq** were upgraded to allow searching of NCBI's BLAST and Entrez databases.
- **2005 SeqBuilder** was added to the Lasergene software offering for virtual cloning, PCR primer design, plasmid map creation, and sequence editing.
- **2013 MegAlign Pro** was released with Lasergene 11.0 for multiple sequence alignments and visualization.
- 2017 SeqBuilder Pro was released with Lasergene 15.0 and SeqBuilder was retired. SeqBuilder
 Pro featured a beautiful new interface and supported additional cloning methods and agarose gel
 simulation. To smooth the transition from EditSeq and PrimerSelect, SeqBuilder Pro even featured
 a button that users could press to make the interface resemble their favorite classic applications.
- 2018 Pairwise alignment functionality was added to MegAlign Pro.
- **2019** With the release of Lasergene 16.0 in June, **EditSeq**, **PrimerSelect** and **MegAlign** were retired.
- **2020** With the release of Lasergene 17.0 in February, **SeqMan Ultra** was introduced as a replacement for SeqMan Pro. SeqMan Pro will remain in the package temporarily, but will be removed in a later release.

Transitioning from EditSeq to SeqBuilder Pro

As of Lasergene 16.0, SeqBuilder Pro completely replaced EditSeq. Compared to its co-predecessor, EditSeq, SeqBuilder Pro features a modern, colorful user interface and <u>greatly increased functionality</u>.

This short video is an overview of the SeqBuilder Pro application:

For an illustrated comparison showing how to perform an EditSeq task in SeqBuilder Pro, click any of the links below:

- Edit a sequence
- <u>Trim sequence ends</u>
- Translate a nucleotide sequence
 - Select and edit a genetic code
- Back translate a protein sequence
- Display a sequence and its translation simultaneously
- <u>View sequence statistics</u>
- Work with features
- Save or export a sequence

Make SeqBuilder Pro resemble EditSeq

To make the SeqBuilder Pro layout look similar to EditSeq, press the **Compact EditSeq Layout** it tool on the left of SeqBuilder Pro's document window. SeqBuilder Pro will display the Sequence view in the upper half of the Document window, without spacers or rulers, and the Features view in the lower half.

EditSeq application layout:

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SeqBuilder Pro application using **Compact EditSeq Layout** tool:

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Edit a sequence

To edit a sequence:

• EditSeq – Paste or type IUPAC characters into the sequence pane. To delete characters, use the **Backspace** or **Delete** keys.

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 SeqBuilder Pro – Paste or type IUPAC characters into the Sequence view. To delete characters, use the Backspace or Delete keys. For more information, see the SeqBuilder Pro User Guide topic <u>Use</u> <u>editing commands</u>.

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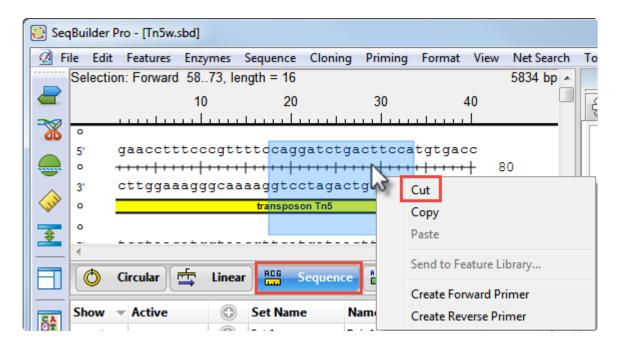
Trim sequence ends

To trim sequence ends:

• EditSeq – Use File > Open and press the Set Ends button, then specify cut points in that dialog.

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Look in: 🐌 Demo Data 👻	G 🎓 📂 🛄 🕶	
Name	Date modified	
LAMCG.seq	10/19/2004 1:36 PM	
pbr322.seq	10/19/2004 1:36 PM =	
pGEM-TEasy.seq	10/31/2004 5:25 PM	
🕅 puc18.seq	10/19/2004 1:28 PM	
tethis21.seq	10/19/2004 1:29 PM 👻	
<	Þ	
File name: pbr322.seq	Open	Set Ends
Files of type: Lasergene DNA Files (*.seq)	Cancel	pbr322.seq(1>4361)
pbr322.seq(1>4361) ttctcatgtttcttcaagaa	Help Set Ends	
Length: 4361 bp Range: 4361 bp		
		Length: 4361 bp Range: 4361 bp
Read Features	.4	OK Cancel

 SeqBuilder Pro – Select a portion of an already-open sequence in the the Circular, Linear or Sequence views and use Edit > Cut; or right-click on the selection and choose Cut. For more information, see the SeqBuilder Pro User Guide topic <u>Use editing commands</u>.



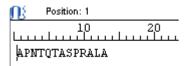
Translate a nucleotide sequence

To create a new document containing the translation of a selected portion of DNA/RNA sequence:

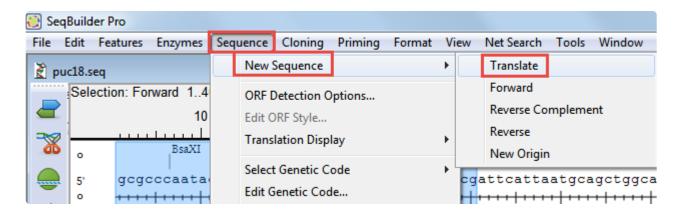
• EditSeq – Use Goodies > Translate DNA.

EditSeq - [puc18.seq : SEQUENCE]	
👫 File Edit Search Speech Features	Goodies Net Search Window Help
Selection: 1 -> 69 = 69	Reverse Complement
	Reverse Sequence
gcgcccaatacgcaaaccgcctctccccgcgcgti gctcgtatgttgtgtggaattgtgagcggataaca	Translate DNA
gaaaaccctggcgttacccaacttaatcgccttg gcggtatttcacaccgcatatggtgcactctcagt	Reverse Translate Protein
ctgcatgtgtcagaggttttcaccgtcatcaccgs	
tttttctaaatacattcaaatatgtatccgctcat aaacgctggtgaaagtaaaagatgctgaagatcag	Edit Genetic Code
atcccgtattgacgccgggcaagagcaactcggto gccaacttacttctgacaacgatcggaggaccgas	DNA Statistics
caacgttgcgcaaactattaactggcgaactactt	Protein Statistics

The new document looks like this:



 SeqBuilder Pro – Use Sequence > New Sequence > Translate. For more information, see the SeqBuilder Pro User Guide topic <u>Create a sequence based on another sequence</u>.



The new document looks like this:

	10
Ν	APNTQTASPRALA
0	****
0	

Select and edit a genetic code

To select a genetic code:

- EditSeq Use Goodies > Genetic Codes and choose a code from the list.
- SeqBuilder Pro Use Sequence > Select Genetic Code and choose a code from the list. For more information, see the SeqBuilder Pro User Guide topic <u>Choose the genetic code</u>.

To edit a genetic code:

- EditSeq Use Goodies > Edit Genetic Code.
- SeqBuilder Pro Use Sequence > Edit Genetic Code. For more information, see the SeqBuilder Pro User Guide topic Modify the genetic code.

Though the command is in a different menu, the genetic code editor is the same for both applications.

Genetic Code Edito	r	×
Description Sta	ndard Genetic Code	
Starts: AUG		RNA DNA
Genetic Code S	tart Codons	
A Ala GCN C Cys UGY D Asp GAY E Glu GAR F Phe UUY G Gly GGN H His CAY I Ile AUH K Lys AAR L Leu YUN M Met AUG N Asn AAY P Pro CCN Q Gln CAR R Arg MGN S Ser WSN T Thr ACN	GCA GCG GCU UGC UGU GAC GAU GAA GAG UUC UUU GGA GGG AUA AUC AUA AUC AUA AUC AUA AUC AUA CUC AUG CUU ACA AAC AAC ACG AGA AGG AGA AGG AGA AGG AGA AGG AGA AGG AGC AGG AGC AGG AGC AGG AGA AGG AGC ACG ACG ACC	1
V Val GUN W Trp UGG X ??? Y Tyr UAY . ter URR	GUA GUG GUC GUU UGG UAC UAU UAA UAG UGA	
	III : Code: drag codons to desired anslation: Alt/Option-click the	
	ОК	Cancel

Back translate a protein sequence

To create a new document containing the back translation of a selected portion of protein sequence:

• EditSeq – Use Goodies > Reverse Translate Protein.

	EditSeq					
	File Edit Search Speech Features	Goo	dies	Net Search	Window	Help
ſ	I10K_PLAKN.pro : PROTEIN Selection: 1 -> 40 = 40			erse Complem erse Sequence	ent	
	L10 20 30		Tran	Islate DNA		
	fnsnmlrgsvceedvslmtsidnmieeidf		-			
	enteomveevadhitodmidevahhvldni		Reve	erse Translate I	Protein	
	emteqmveevadhitqdmidevahhvldni avekvkhiveteetqktvepeqieetqntv			erse Translate I etic Codes	Protein	•
			Gen			۲
	avekvkhiveteetqktvepeqieetqntv etqktvepeqteeaqktvepeqteetqktv		Gen Edit	etic Codes		۲

The new document looks like this:

🔰 🚺 🕴 Position: 1				
10 2	0 30 	40 l	50 ll	60 l
TTYAAYWSNAAYATGYTNMG	NGGNWSNGTNTGYGA	RGARGAYGTN	USNYTNATGA	CNWSN
ATHGAYAAYATGATHGARGA	RATHGAYTTYTAYGA	RAARGARATH	TAYAARGGNW	SNCAY

• SeqBuilder Pro – Use Sequence > New Sequence > Back Translate. For details, see the SeqBuilder Pro User Guide topic <u>Create a sequence based on another sequence</u>.

🕃 Se	qBui	ilder P	ro											
File	Edit	t Fea	atures	Enzymes	Seq	uence	Cloning	Priming	Format	Vi	ew	Net Search	Tools	Window
1 1	110K		(N.gbk	:		New S	Sequence			۲		Back Trans	late	
		Selecti	ion: Fo	orward 14		ORF [etection O	ptions				Forward Reverse Co	mnleme	ent
				10)RF Style lation Displ	201				Reverse	mpierrie	
		N o		nmlrgsv			Genetic Co	-				New Origin	1	
		0		++++++			ienetic Cod			•	Γ	110	kDa antiş	gen
	×	o N	vha	lsgdvtq		Linea	r				en	eqieetqn	tvep	eateeta
3			-	++++++		Circu	lar				117.	+++++++++++++++++++++++++++++++++++++++		
		0				DNA					E		kDa antiş	gen
		0				RNA					egi	on Repetit	tive regio	n Repe
C.A		o				Statis	tics		Ctrl-T					

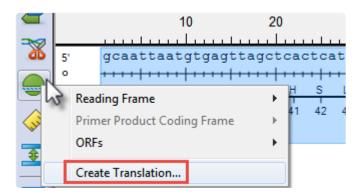
The new document looks like this:

10 	20 ll	30 l	40 	50 ll	60 l
TTYAAYWSNAAYATGY	TNMGNGGNW	SNGTNTGYG	ARGARGAYGTN	WSNYTNATGA	CNWSN
ATHGAYAAYATGATHG	ARGARATHG	AYTTYTAYG	ARAARGARATH	TAYAARGGNW	SNCAY

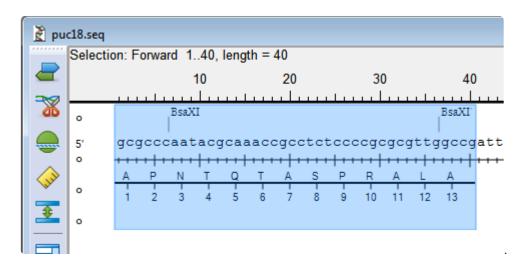
Display a sequence and its translation simultaneously

To display the translation below the nucleotide sequence:

- EditSeq Not available.
- SeqBuilder Pro Select part or all of the sequence, click on the Translations and ORFs () tool and choose Create Translation. For more information, see the SeqBuilder Pro User Guide topic <u>Translations</u>.



The translation appears below the DNA/RNA sequence.



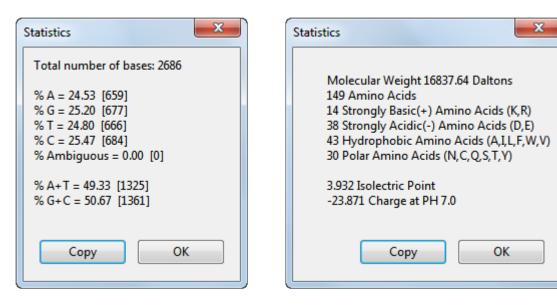
View sequence statistics

To view sequence statistics:

• EditSeq – Use Goodies > DNA Statistics or Goodies > Protein Statistics.

```
- 0 ×
B DNA Statistics
Sequence Info about pucl8.seq(1,2686)
       Total number of bases is 2686
       % A = 24.53
                              [659]
      % G = 25.20
                              [677]
      % T = 24.80
                              [666]
       % C = 25.47
                              [684]
       % Ambiguous = 0.00
                               [0]
       % A+T = 49.33
                              [1325]
       % C+G = 50.67
                              [1361]
BASE COUNT
              659 a 684 c 677 g
                                       666 t
       Davis, Botstein, Roth Melting Temp C. 85.49
       Wallace Temp C
                                        8094.00
A Protein Statistics
                                    - C ×
Protein Info about Human Calmodulin.pro(1,149)
                                               .
       Molecular Weight 16837.64 Daltons
       149 Amino Acids
       14 Strongly Basic(+) Amino Acids (K,R)
       38 Strongly Acidic(-) Amino Acids (D,E)
       43 Hydrophobic Amino Acids (A,I,L,F,W,V)
       30 Polar Amino Acids (N,C,Q,S,T,Y)
       3.932 Isolectric Point
       -23.871 Charge at PH 7.0
•
```

 SeqBuilder Pro – Use Sequence > Statistics. For more information, see the SeqBuilder Pro User Guide topic <u>Sequence Statistic</u>.



Work with features

Many feature-related commands are the same in EditSeq and SeqBuilder Pro, including **Features > New Feature**, **Features > New Feature with Translation**, and **Features > New Translation**. The EditSeq command **Features > Join Segment** changes to **Features > Join to Feature** in SeqBuilder Pro, but functions identically.

However, SeqBuilder Pro's "Features" view offers feature-related functionality that is not available in EditSeq. For instance, you can reverse the strand the feature is on, add and edit qualifier fields, change the lengths of segments or features, select text to use as the feature name, and much more. For details, see the SeqBuilder Pro User Guide topic Edit information about a feature.

	0	Туре	Name		Range	Strand	Length	Description
~	0	CDS		=	1001452			
		/dnas_tit	e = pr	otein #3				
		/function	- nc	on-functional				
		/experim	ent = ex	perimental evidence, no additio	nal details recorded			
		/note		Thymidine at position 1442 cause functional product identical to the		which prem	naturely t	erminates the protein at this point. Ochre suppressing strains allow readthrough and express
		/citation	- [4]				
		/codon_s	tart = 1					
		/transl ta	ble = 11	n				
		/product	= pr	otein #3				
		/protein	- 1 / H & B & B & B & B & B & B & B & B & B &	A73388.1				
		/db_xref		:405967				
		/translati	SI			ATSRLRM	IGSMMSN	GAYRFYRNPNV SAEAIRKAGAMQTVKLAQEFPELLAIEDTT SL SYRHQVAEELGKLG SIQDK SRGWWVI VIAVCDREADIHAYLQDRLAHNERFVVR SKHPRKDVE SGLYLIDHLKNQPELGGYQI SIPQKGVVDKRGKI
	0	CDS		NRPARKA SL SLRSGRITLKQGNIT EAEHVE SQ SAETVLTPDECQLLG				RTGIASWGALW
~	0	CDS CDS	KE protein #4	NRPARKA SL SLR SGRITLKQGNITI EAEHVE SQ SAETVL TPDECQLLG	LDKGKRKRKEKAGSLQWA		GGFMDSK	RTGIASWGALW
~ ~			KE protein #4	IRPARKASLSLRSGRITLKQGNITI EAEHVE SQ SAETVLTPDECQLLG oside-3'-O-phosphotransferase	2651452		GGFMD SK	RTGIASWGALW
	0	CDS	KE protein #4 aminoglyce bleomycin	IRPARKASLSLRSGRITLKQGNITI EAEHVE SQ SAETVLTPDECQLLG oside-3'-O-phosphotransferase	2651452 15592353		GGFMD SK 1188 795	RTGIASWGALW A Thymidine at position 1442 causes an ochre stop codon which prematurely terminates the prote
V	0	CDS CDS	KE protein #4 aminoglyce bleomycin	IRPARKASL SLR SGRITLKQGNIT AEHVE SQ SAETVL TPDECQLLGV oside-3'-O-phosphotransferase resistance	2651452 15592353 23742754		GGFMD SK 1188 795 381	RTGIASWGALW A Thymidine at position 1442 causes an ochre stop codon which prematurely terminates the prote
V	0	CDS CDS CDS	KE protein #4 aminoglyco bleomycin streptomyc	IRPARKASL SLR SGRITLKQGNIT AEHVE SQ SAETVL TPDECQLLGV oside-3'-O-phosphotransferase resistance	2651452 15592353 23742754 27933593	YMAIARL	GGFMD SK 1188 795 381 801	RTGIASWGALW A Thymidine at position 1442 causes an ochre stop codon which prematurely terminates the prote
v v	0	CDS CDS CDS CDS CDS	KE protein #4 aminoglyce bleomycin streptomyce protein #1 protein #2	IRPARKA SL SLRSGRITLKQGNITI AEHVESQ SAETVLTPDECQLLGV oside-3'-O-phosphotransferase resistance in phosphotransferase	2651452 15592353 23742754 27933593 complement (43055735)	YMAIARL	GGFMDSK 1188 795 381 801 1431	RTGIASWGALW A Thymidine at position 1442 causes an ochre stop codon which prematurely terminates the prote This protein confers streptomycin resistance in some species of Gram-negative bacteria, but is cry
~	0	CDS CDS CDS CDS CDS CDS	KE protein #4 aminoglyce bleomycin streptomyc protein #1 protein #2 A missing '	IRPARKASL SLRSGRITLKQGNIT AEHVESQSAETVLTPDECQLLG oside-3'-O-phosphotransferase resistance in phosphotransferase C' in the sequence published	2651452 15592353 23742754 27933593 complement (43055735) complement (43055570)	YMAIARL	GGFMDSK 1188 795 381 801 1431	LRVIDIYTHRWRIEEFHKAWKTGAGAERQRMEEPDNLERMV SIL SFVAVRLLQLRE SFTLPQALRAQGLL RTGIASWGALW A Thymidine at position 1442 causes an ochre stop codon which prematurely terminates the prote This protein confers streptomycin resistance in some species of Gram-negative bacteria, but is cry A missing 'C' in the sequence published by Auerswald et al.(1981) is corrected within that publishe Ac651 primer with clamp

Save or export a sequence

To save or export a sequence:

- EditSeq offers the following options:
 - To save in Lasergene (.seq or .pro) file format, use File > Save As.
 - To export to Genbank (.gbk), FASTA (.fas), or GCG (.gcg) file formats, use File > Export .
 - To export multiple sequences into a single file with a Genbank (.gbk), FASTA (.fas or .fap), or DNA Multiseq (.mseq) file format, use File > Export all as one.
- **SeqBuilder Pro** offers the following options:
 - To save in SeqBuilder Pro (.sbd), Lasergene (.seq or .pro), GenBank (.gbk), FASTA (.fas or .fap), ABI (.abi) or EMBL (.embl) file formats, use File > Save As. For details, see the SeqBuilder Pro User Guide topic <u>Save</u>.
 - To export sequences in .sbd, .seq, .gbk, .embl, or .fas. formats, use File > Export Sequence(s)
 From Project. For details, see Export sequences to a file.
 - To export sequences to GenVision (.gnv) format, use File > Export As GenVision Project. For details, see Export sequences to GenVision.

Transitioning from PrimerSelect to SeqBuilder Pro

As of Lasergene 16.0, SeqBuilder Pro completely replaced PrimerSelect. Compared to its co-predecessor, PrimerSelect, SeqBuilder Pro features a modern, colorful user interface and <u>greatly increased functionality</u>.

This short video is an overview of the SeqBuilder Pro application:

For an illustrated comparison showing how to perform an PrimerSelect task in SeqBuilder Pro, click any of the links below:

- Trim sequence ends
- Locate primers
- View dimers, pair dimers and hairpins
- <u>View general primer statistics</u>
- <u>View information on primer composition and amplfication</u>
- Edit primers
- Import primers from a catalog
- <u>Nominate new primers to a catalog</u>
- Save a primer catalog
- Select and edit a genetic code

Make SeqBuilder Pro resemble PrimerSelect

To make the SeqBuilder Pro layout look similar to PrimerSelect, press the **Primer Design Layout** tool on the left of SeqBuilder Pro's document window. SeqBuilder Pro will display the Primer Design view in the upper half of the Document window, and the Primers view in the lower half.

PrimerSelect application layout:

📄 PrimerSelect - [Upp	er Primer WorkBench]				- 0	×
🧮 File Edit Condit	ons Locate Log Report Optic	ons Net Search Window Help				_ 8 ×
Length = 24, Tm = 66.	3					
Sites	* * * * * * * * * * * * *			AACGTTCCAGTA	ACCGGGCA	
	i j		nnn '			
Comp 3'LAUICA	CTAAAAAGAGACCAGGG	GGCGTAGGTATGGCGGT	CAACAAAT GGGAGT G	TIGCAAGGICAT	reeccoer	
Frame 1 ter Val Primer Frame 2 Glu te Primer Frame 3 ₃u Ser Primer	Ar r Phe Phe Ser Gly Pro Asp Phe Ser Leu Val Pro	a Arg lle His Thr Ala Se Va Ala Ser lle Pro Pro V Va Ala Ser lle Pro Pro V	√al Val Tyr Pro His , √al Val Leu Phe Thr Leu Thr	Asn Val Pro Val	Thr Gly His	¥
						^
Priming Sites	500 1000	1500 2000	2500 3000	3500	4000	
No dimers > 2 bp					Hairpin 2 bp, 0.6	ikc/m
				5 3' TT	GCCGCATO	ç
<						>
	Name:	Note:	ОК	Cancel		

SeqBuilder Pro application using **Primer Design Layout** tool:

Z000 4000 Par Name Score Pred Tm Prid Tm Ta OPT Pred Stm Langth Orientation: Forward 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 pair: Forward pair: 1 dnas_title: Forward pair: 1																		22.seq	br3
s cotgagtgatttttototggocgocgocatocatacogocagttgtttacoctcacacagttocagta s cotgagtgatttttototggocgocgocatocatacogocagttgtttacoctcacacagttocagta s gccGCGATCCATACCGCCAGTT 4 s ggactcactaaaaagagaccagggoggocgtaggtatggoggtcaaaaatgggaggtgtgcaaaggtoat R R R H H T AP S C P P H H T AP S C P P H H P Y R O L G C G Y R W N R R M W Y A L O A A D M G G T T s correction forward (1765. 1783) P C More selection Feature Selection Feature Selection Feature Type: PCR.prin Location: Forward current: 1 o correction: Forward current: 1 o domas >2 20 0 P P Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prod dTm Length 2000 - Pir Name Score Prind Tm Tis OPT Prind Tm		ies	Enzymes	🎯 Style 🍯			100	. 1	1790				d: Length=21	Pair "Forwar	Working	175	ATC	k 🍌	[
Coccentration of the state of the stat	-	ght: 1 ~	Weight:	Line														•	1
B drug 1000 2000 2000 4000 Primer 1000 2000 2000 4000 B drug 1000 2000 2000 4000 Primer 1000 2000 2000 4000 Primer 2 200 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pred dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pri dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pri dTim Larget 2 000 4000 Par Name Score Pred Tim Pri dTim Ta OPT Pri dTim Ta OPT Pri dTim Core				Fill:															
y ggactactaaaaagagacagggoggogtaggatggeggtaaaaaagggtgtggagttggaaggtatg y ggactaactaaaaagagacagggoggogtaggatggeggtaaaaaaagggatgttgaaaggtatt A R A H T A P S C A R A H T A P S C P P P A H P Y R Q L A R A A D M G G T T 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0										+++++++	+++++						++++		
A A S I P				Shape:	5	gtcatt	tgcaag	gagtgt	acaaatgg			gcgtagg	agggcgg	gagacca	taaaaa	tcact	ggac		
G C G V R W N R R M W V A L Q A A D M G G T T Backer Prome: Booking 1000 2000 4000 Prome: Booking 1000 2000 4000 Prome: Prome: Booking 1000 2000 4000 Prome: Prom	~	ost ~	oked Goalpost	Stro					с		H T	AS	A						
Constraints and the second secon		tails	s 🕕 Details	र्ट्ने Settings						ALO		C G W	G R R					4 5	
Prime 1000 2000 3000 4000 Binding 1000 2000 3000 4000 No dimes > 2 bp Most state pair dimer 6 bp. 4.8 kolm (BAO) 5 0C. AFCORMOUTLY Haspin 2 bp. 0.6 kolm 5 0C. AFCORMOUTLY Feature Information: 2000 4000 Par Name 5 000 TriffectoreATA-/ 0 0 TriffectoreATA-/ 0 0 Orientation: Forward current: 1 dinas_title: 4 0 0 0 0 0 0 0		ction	re selecti	Featur					gace	1783)	1 (17661	Forward			m pSC101	from	_	0	1
No dimers > 2 bp Most stable are dimer (b = 0.3 kcm (BAD)) S CC. ACCONSTATT 3 - CGB > 7 TTTTTCCCCGATATT 3 - CGB > 7 TTTTTCCCCCGATATT 3 - CGB > 7 TTTTTCCCCCGATATT 3 - CGB > 7 TTTTTCCCCCCGATATT 3 - CGB > 7 TTTTTCCCCCCCATATT 3 - CGB > 7 TTTTCCCCCCCATATT 3 - CGB > 7 TTTTCCCCCCCATATT 3 - CGB > 7 TTTCCCCCCCATATT 3 - CGB > 7 TTCCCCCCCATATT 3 - CGB > 7 TTCCCCCCCCATATT 3 - CGB > 7 TTCCCCCCCCATATT 3 - CGB > 7 TTCCCCCCCCATATT 3 - CGB > 7 TTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC					-	00	40		3000			2000		1000				Binding	
3 1 1 1 1 1 1 1 1 6 1 1 1 1 6 1	imer					bp, 0.6 kc/m	Hairpin 2		0	9.8 kc/m (BAD)	ner 6 bp9	stable pair dim	Most				> 2 bp		-
Current: 1 des		1766178				1.11					11 1	1 11111							
Current: 1 des	ł	Forward	m:	Orientatio	- 31														ŀ
< >> pair:		1								Score Prod	lame S	JO Pair N	400		2000				- L
	1 (1/66	Forward (£	_	>													<	
Circular 🛱 Linear 🚰 Sequence 🚰 Primer Design	CATCC	GCCGCA		primer:						gn	ier Desi	De Prim	uence	RCG Seq	Linear	_	Circular	0	
Show v Active O Set Name Name F/R Primer Sequence Score Length Tn V A 19.05 [4]		19.05 [4]			Tn ^	Length	Score		Sequence	Primer	F/R		Name	et Name	© \$		Active	Show	
					62. 🗸	21		GCCAGTT	ATCCATACC	GCCGC	F	7661783)	Forward (1		Θ			1	
% Ambiguous 0.00 [0]					>													<	
😢 Features 👔 Primers 💭 Comment 🧮 Minimap 🛄 Site Summary 😜 History 💼	>	12 on 141 1					listory	• •	te Summary	Si	linimap	M	omment	s 📮 (Primers		Features	B I	

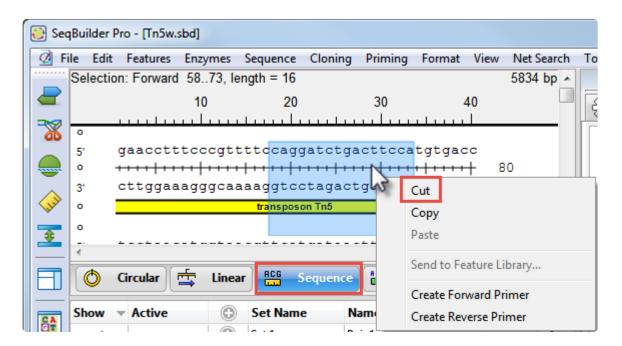
Trim sequence ends

To trim sequence ends:

• PrimerSelect – Use Conditions > Sequence Positions and Limits. In the dialog box, choose either Manually or From Feature Table to open another dialog in which you can specify the cut points.

e 1	PrimerSelect	- [Un	titled]									
	File Edit	Con	ditions	Locate Log Report Op	ptions Net							
			Sequer	ce Positions and Limits	Ctrl+=							
7			Initial (Initial Conditions								
∆G			Primer	Characteristics	Ctrl+K							
Sec	quence Posit	tions	and Lim	its								
	Set Sequence	e Po	sitions									
	Sequence:	T	n5w	•								
	Starts at	1		in template and extends to	5834							
	Set Sequence Limits											
	Set Sequence Limits Manually From Feature Table											
	2			ОКС	ancel							

 SeqBuilder Pro – Select a portion of an already-open sequence in the the Circular, Linear or Sequence views and use Edit > Cut; or right-click on the selection and choose Cut. For more information, see the SeqBuilder Pro User Guide topic <u>Use editing commands</u>.



Locate primers

To locate primer pairs automatically:

Both the classic and modern applications provide multiple ways to find primers, but there are subtle differences between the commands. The images show the results of using each command when the same sequence feature is selected in each of the applications. A short video is available at the bottom of this topic.

PrimerSelect – To search both template strands for primers or probe sequences meeting specified conditions, use Locate > Primers & Probes (left image). To limit the search to only primers contained with the "primer catalog," use Locate > Only Catalogued Primers. To search for compatible pairs required for PCR amplification, use Locate > PCR Primer Pairs (right image).

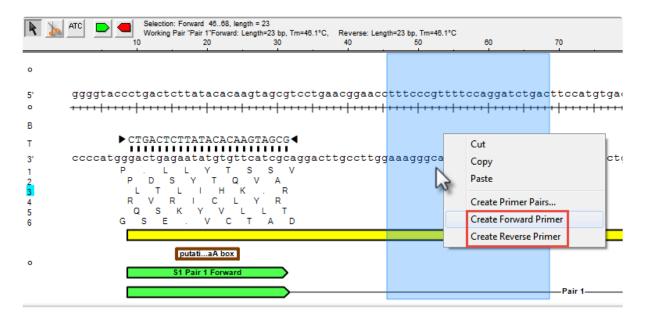
		🗮 Located Primer Pairs		
		10 24 Primary Pairs, 91 Located, 15 Alternates		
	1	🗾 10po 20po 30po 40po 50po 🗌		් Length
				580bg 🔺
🗮 Located Primers				516bp
✓ Start End Length Tm dG dProfile Name				404bp 570bp
Upper Primers: 121 Located				479bp 406bp
✓ 1671 1692 22-mer 58.2 -42.6 36.7 ✓ 1575 1593 19-mer 52.8 -37.6 43.5				-
✓ 1592 1610 19-mer 64.7 -45.2 189.2 ✓ 1592 1611 20-mer 65.4 -46.8 191.0	-	Atemate Pairs:		578bp
Lower Primers: 122 Located				579bp 581bp
✓ 4159 4176 18-mer 52.3 -36.1 59.8 ✓ 4166 4182 17-mer 51.4 -35.5 61.0	^			578bp 580bp
✓ 4275 4292 18-mer 66.3 -45.1 250.7 ✓ 4276 4292 17-mer 61.9 -41.5 207.5				579bp
	-		26.3 2.6 17.9	577bp 👻
 • 	► at	<		▶

 SeqBuilder Pro – For detailed information on primer design, see the SeqBuilder Pro User Guide topic <u>Primers</u>. In brief, to create pairs based around a selected feature or a sequence range, use Priming > Create Primer Pairs to open an options dialog (left image). Select the desired options and click OK to display the list of primer pairs (right image).

Create Primer Pairs
Locations
Primers end exactly at selection
Choose optimal primer location
Stay within 0 bp of selection
Display at most 1 primer pairs
Amplify 5' - 9 to 5826 - 3'
► Conditions
Primer Characteristics
OK Cancel Defaults Help

	gBuilder Pro - [Tn5w.sbd]
	ile Edit Features Enzymes Sequence Cloning Priming Format View Net Search Tools
2	ATC Selection: Forward 9.5826, length = 5818 5834 bp Working Pair 'Pair 1'Forward: Length=23 bp, Tm=46.1°C, Reverse: Length=23 bp, Tm=46.1°C, 10 Reverse: Length=23 bp, Tm=46.1°C, 40 60
*	o
-	5' ggggtacctgactcttatacacaagtagcgtcctgaacggaacctttccc
(III)	в
	T CCCCatgggactgagaatatgtgttcatcgcaggacttgccttggaaaggg
	1 P.LLYTSSV 2 PDSYTQVA 3 LTLIHK.R
CA	4 R V R I C L Y R 5 Q S K Y V L L T 6 G S E . V C T A D
	transposon Tn5
	o S2 Pair 1 Forward
U	Pair
$\overline{\mathbf{k}}$	Primer Binding 2000 4000 Sites
e	Most stable dimer 3 bp, -3.5 kc/m 5' CTGACTCTTATACACAASTAGCG 3' 5' CTGACTCTT CAASTAGCG 3' 5' CTGACTCTTATA 111
	3 GOGATGAACACATATTCTCAGTC 5' 3' GOGATGAA ATTCTCAGTC 5' 3' GOGATGAACAC
	5000 Pair Name Score Prod Tm Pri dTm Ta OPT Prod dTm Length Pair 1 1.000 84.4 0.0 58.0 38.3 5818

To locate forward & reverse primers separately based around a selected feature or a sequence range, use **Priming > Create Forward Primer** or **Priming > Create Reverse Primer** or the corresponding right-click commands. Both options are only available after using **Priming > Create Primer Pairs** at least once. (In the image below, note the already-found primers to the left of the current selection.)

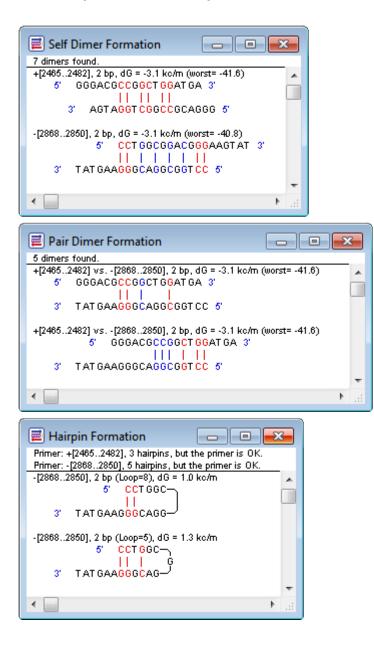


The following video is a brief introduction to primer design in SeqBuilder Pro. For another video on the same topic, see: <u>Primer Design in SeqBuilder Pro</u>.

View dimers, pair dimers and hairpins

To view information about dimers, pair dimers, hairpins, and false priming sites:

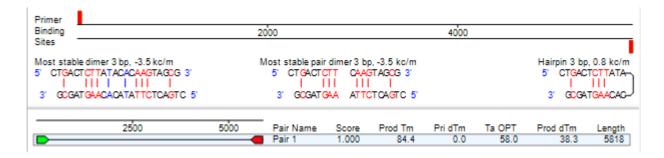
- **PrimerSelect** Do either of the following:
 - Select a primer pair and choose Report > Primer Self Dimers, Report > Primer Pair Dimers, and/or Report > Primer Hairpins



 Use Edit > Work on Upper Primer or Edit > Work on Lower Primer to display the primer Workbench. Then use the tools on the left to display self-dimers, hairpins and/or false priming sites.

📕 Upper Primer WorkBe	ench				3
Primer	CT GAAGCGGGA GCGGGA IIIIII	AGGGACT GGCT AGGGACT GGCT IIIIIIIIIIII TCCCT GACCGA	GCTATTG	• • • • • + • 1846 ◀	^
1 1 1 1					-
					^
Primin;	1000 2000) 3000	4000	5000	
Nodimers ≻ 2 bp				Hairpin 2 bp, 1.2 kc/m	
		3	5' 8' GTG	GCGGGAAG TCGGTCAGG	
1					Ŧ
•				, t	
Name:	Note:			OK Cancel	

SeqBuilder Pro – This information is displayed automatically in the Primer Design view. To learn how
to interpret this information, see the SeqBuilder Pro User Guide topic <u>Primer Design view</u>.



View general primer statistics

To view general statistics about located primer pairs:

• PrimerSelect – Use Report > Located Primers & Probes to open a window with these statistics.

	Loca	ted	Primer	5					
\checkmark	Start	End	Length	Tm	dG	dProfile	Name		
Up	per Prin	ners:	121 Loca	nted					
\checkmark	1437	1453	17-mer	52.8	-37.5	150.0			
~	1571	1592	22-mer	58.2	-42.6	36.7			-
~	1575	1593	19-mer	52.8	-37.6	43.5		L	_
1	1592	1610	19-mer	64.7	-45.2	189.2			
~	1592	1611	20-mer	65.4	-46.8	191.0			Ŧ
Lou	ver Prin	ners:	122 Loca	ted					
$\overline{}$	4159	4176	18-mer	52.3	-36.1	59.8			
1	4166	4182	17-mer	51.4	-35.5	61.D			
1	4275	4292	18-mer	66.3	-45.1	250.7		r	
2	4276	4292	17-mer	61.9	-41.5	207.5		-	-
1									Ŧ
4								h.	

• **SeqBuilder Pro** – Primer statistics are displayed automatically in the Primers view. To learn how to interpret this information, see the SeqBuilder Pro User Guide topic <u>Primers view</u>.

Show	Active	•	Set Name	Name	F/R	Primer Sequence	Score	Length	Tm	dTm	Ta OPT	dG	*
~	•	\bigcirc	Set 2	Pair 1		<ctgactcttatacacaagtagcg><cgctacttg< th=""><th>1.000</th><th>5818</th><th>84.4</th><th>38.3</th><th>58.0</th><th></th><th></th></cgctacttg<></ctgactcttatacacaagtagcg>	1.000	5818	84.4	38.3	58.0		
			Set 2	S2 Pair 1 Forward	F	CTGACTCTTATACACAAGTAGCG		23	46.1	0.0		-37.1	
			Set 2	S2 Pair 1 Reverse	R	CTGACTCTTATACACAAGTAGCG		23	46.1	0.0		-37.1	
		•	Set 1	Pair 1		<ctgactcttatacacaagtagcg><cgctacttg1< td=""><td>1.000</td><td>5818</td><td>84.4</td><td>38.3</td><td>58.0</td><td></td><td></td></cgctacttg1<></ctgactcttatacacaagtagcg>	1.000	5818	84.4	38.3	58.0		
•													
8	Feature	es 📔	Primers	Comment		finimap 🔢 Site Summary 🚯 History							

View information on primer composition and amplification

To view information about primer composition and amplification:

 PrimerSelect – Select a primer pair and choose Report > Amplification Summary or Report > Composition Summary.

🗐 Am	plificati	on Sum	mary	,				x	
	-								
			_						
	Upper Primer: 18-mer 5' GGGACGCCGGCTGGATGA 3' Lower Primer: 19-mer 5' CCTGGCGGACGGGAAGTAT 3'								
DNA 25	i0 pM, Sal	t 50 mM		Upper Primer Lower P			er Primer		
	Tm Overall St Location	ability		61.3 ℃ -41.6 kc 246524	/m	56 -40 286			
Primers	Tm - Prin Tm Diffe Annealing		ture	28.7 °C 5.1 °C 61.3 °C					
Product				404 bp 84.8 °C 64.9% 106.4 °C					
	Produc	t Melting 1	ſempe	rature (%G	C Meth	nod)		-	
	Salt			For	mamio	de		1	
mM	XSSC	XSSPE	0%	10 %	2	0%	50%		
1 10 50	10 0.051 0.062			6 50.1 2 66.7 8 78.3	6	3.6 D.2 1.8	24.1 40.7 52.3		
165 330 500	165 0.846 1.031 9 330 1.692 2.062 9		93./ 98./ 101.	4 86.9 4 91.9	81 81	D.4 5.4 8.4	60.9 65.9 68.9		
1000	2.564 5.128	3.125 6.250	106.		-	8.4 3.4	68.9 73.9	-	
•							1	ы	

Upper Primer: 18-mer 5' GGGACGCCGGCTGGATGA 3' Lower Primer: 19-mer 5' CCTGGCGGACGGGAAGTAT 3'							
Primers	Upper Prime	er Primer					
Single Strand Mr Extinction Coefficient 1/E	5.7 k 5.71 nMVA26 32.5 µg/A26i	0 5.38	6.0 k nMVA260 µg/A260				
Product	Composition	Quantity	Per Cent				
Upper Strand Mr 124.7 k	А	64	15.8				
Lower Strand Mr 125.0 k	С	134	33.2				
Both Strands Mr 249.7 k	G	128	31.7				
Length 404 bp	т	78	19.3				
Tm (% GC Method)84.8 °C	I	D	0.0				
Tm at 6xSSC 106.4 °C	A+T	142	35.1				
GC Content 64.9%	G+C	262	64.9				

• **SeqBuilder Pro** – Double-click on a primer in the Primer Design view to view its composition statistics in the Details panel. For more information, see the SeqBuilder Pro User Guide topic <u>Details panel</u>.

Settings

Feature selection

👔 Details

Feature Information:	
Feature Type:	PCR_product
Location:	95826
Orientation:	Forward
current:	1
pair:	Pair 1
score:	1.000
set:	Set 2
Statistics:	
% A	19.34 [1125]
% A+T	39.91 [2322]
% Ambiguous	0.00 [0]
% C	29.55 [1719]
% G	30.54 [1777]
% G+C	60.09 [3496]
% T	20.57 [1197]
% diCG	9.18 [534]
Total number of bases	5818
% AA	4.14 [241]
% AC	4.66 [271]
% AG	5.69 [331]
% AT	4.85 [282]
% CA	6.10 [355]
% CC	7.74 [450]
% CG	9.18 [534]
% CT	6.53 [380]
% GA	6.74 [392]
% GC	10.88 [633]
% GG	8.54 [497]
% GT	4.37 [254]
% TA	2.36 [137]
% TC	6.26 [364]
% TG	7.13 [415]
% TT	4.83 [281]

Amplification information is not available in SeqBuilder Pro.

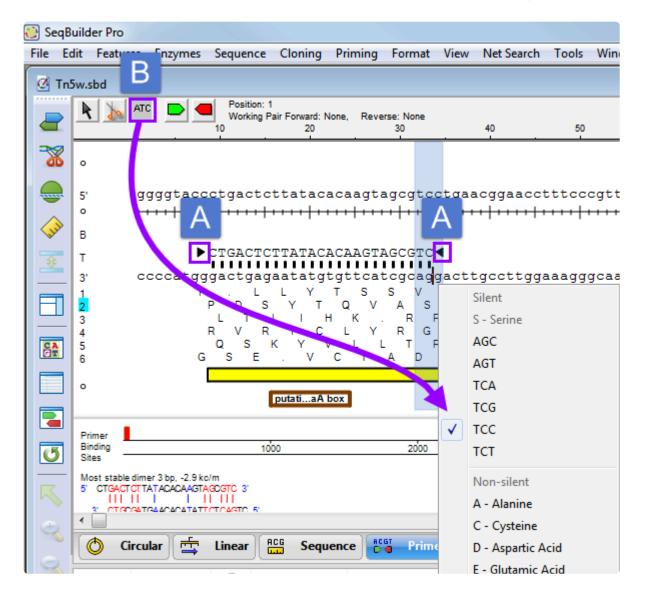
Edit primers

To edit primers:

• **PrimerSelect** – Use **Edit** > **Work on Upper Primer** or **Edit** > **Work on Lower Primer**. Drag the black triangles left or right to change the size and location of the primer.

	E Upper Primer WorkBench								
000	Length	= 24, Tm = 65.4							
	Sites	The set of							
T T	Seq	CAGCT GT GCT CGACGTT GT CACT GAAGCGGGAAGGGACT GGCT GCT AT T GGGCGAAGT GCC GGGGCAGGAT CT CCT GT CA							
2	Primer								
	Comp Frame	PGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGT Ser Cys Ala Arg Arg Cys His ter Ser Gly Lys Gly Leu Ala Ala Ile Gly Arg Ser Ala Gly Ala Gly Ser Pro Val Ile							
	Primer Frame : Primer Frame : Primer	Ala Gly Arg Asp Trp Leu Leu Gln Leu Cys Ser Thr Leu Ser Leu Lys Arg Glu Gly Thr Gly Cys Tyr Trp Ala Lys Cys Arg Gly Arg Ile Ser Cys His Lys Arg Glu Gly Thr Gly Cys Tyr Trp							
	Frame Primer Frame	Leu Gin Ala Arg Arg Gin ter Gin Leu Pro Phe Pro Ser Ala Ala Ile Pro Arg Leu Ala Pro Ala Pro Asp Giy Thr Met Leu Pro Phe Pro Ser Ala Ala Ile Pro Ala Thr Ser Ser Thr Thr Val Ser Ala Pro Leu Ser Gin Ser Ser Asn Pto Ser Thr Giy Pro Cys Ser Arg Arg Asp A:	•						
	Priming		6						
	Sites	1000 2000 3000 4000 5000							
	No dime	s > 2 bp Hairpin 2 bp,1 2 kc/m 5' GC GGGAAG 11 1 3' GT GT CGGT CAGG	•						
4									
		Name: Note: OK Cancel	41						

 SeqBuilder Pro – In the Primer Design view, either A) drag the black triangles left or right to change the size and location of the primer; or B) use the mutation/codon change tool to select a silent or nonsilent mutation for any codon in the primer. For more information, see the SeqBuilder Pro User Guide topic Edit primers.



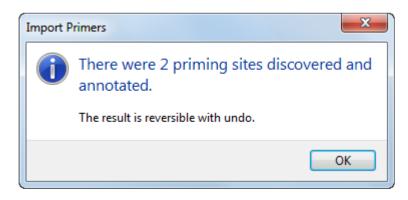
Import primers from a catalog

To import primers from a primer catalog:

• **PrimerSelect** – Use **File** > **Open** and change the file type to **Primer Catalog (.pri)**. After opening a file with a .pri extension, the primer catalog is displayed as a list.

🗐 common_primers.pri									
🗸 » Name	Length	Tm	GC	Sequence					
✓ T7 Promotor	18	32.3 °C	38.9 %	AATACGACTCACTATAGG					
✓ » T7 Terminator	19	47.9 °C	52.6 %	GCTAGTTATTGCTCAGCGG					
✓ » T3	18	37.8 ° C	38.9 %	ATTAACCCTCACTAAAGG					
✓ SP6	19	32.4 °C	36.8 %	GATTTAGGTGACACTATAG					
✓ M13F(-21)	18	48.6 °C	50.0 %	TGTAAAACGACGGCCAGT					
✓ » M13F(-40)	17	41.7 °C	52.9 %	GTTTTCCCAGTCACGAC					
✓ » M13R Reverse	18	41.9 °C	50.0 %	CAGGAAACAGCTATGACC					
✓ » AOX1 Forward	21	51.2 °C	47.6 %	GACTGGTTCCAATTGACAAGC					
✓ » A0X1 Reverse	21	54.3 °C	47.6 %	GCAAATGGCATTCTGACATCC					
pGEX Forward (GST 5, pGEX 5')	23	67.9 °C	65.2 %	GGGCTGGCAAGCCACGTTTGGTG					
✓ » pGEX Reverse (GST 3, pGEX 3')	23	64.6 °C	65.2 %	CCGGGAGCTGCATGTGTCAGAGG					
<				► a					

SeqBuilder Pro – After entering a sequence, use File > Import Primers from a Catalog and select a file with a .pri extension. SeqBuilder Pro attempts to locate each primer in the catalog on the current sequence by searching for sites on the template that match at least the 12-mer at the 3' end of the primer. Those that are found are annotated as PCR_primer features and added to the Primers view. If primers were located, a message confirming the new primers will appear.



Otherwise, a message appears stating "There were no priming sites discovered." For details on importing primers, see the SeqBuilder Pro User Guide topic <u>Import primers</u>.

Nominate new primers to a catalog

To nominate new primers to an unsaved primer catalog:

PrimerSelect – Use File > Enter New Primer and type in the primer information. After clicking OK, the primer is added to the primer catalog, which is displayed with the new entry. To view the catalog at any other time, choose Log > Primer Catalog.

	Primer Catalog								
\checkmark	»	Name	Length	Tm	GC	Sequence			
\sim	»	Primer C	10	na	60.0 %	AGGTCCTGAC	*		
~	»	Primer E	11	9.5 °C	45.5 %	CTATTCCGACT			
	_						Ŧ		
•						Þ			

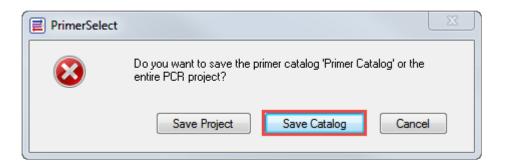
• **SeqBuilder Pro** – Before saving a catalog (below), ensure that only primers you wish to save are displayed in the Primers view.

Show	 Active 	•	Set Name	Name	F/R	Primer Sequence
V	•	٠	Set 1	Pair 1		<atgattgaacaagatggactacacgc< td=""></atgattgaacaagatggactacacgc<>
 		Θ	Set 2	S4 Pair 1 Reverse:Set 4:Set	R	GGAGGTCACATGGAAGTCAGAT
~		Θ	Set 2	S4 Pair 1 Reverse:Set 4:Set	F	GGAGGTCACATGGAAGTCAGAT
						F.
	Features	Prime	ers 🤍 Comr	nent Minimap	Sit	e Summary 🕒 History 🗧

Save a primer catalog

To save the primer catalog:

• PrimerSelect – Use File > Save. When prompted, choose Save Catalog.



 SeqBuilder Pro – Use File > Save Primer Catalog to save the primer catalog in .txt, .fas or .pri format. For details, see the SeqBuilder Pro User Guide topic Export primers.

Select and edit a genetic code

To select a genetic code:

- PrimerSelect Use Options > Genetic Codes and choose a code from the list.
- SeqBuilder Pro Use Sequence > Select Genetic Code and choose a code from the list. For more information, see the SeqBuilder Pro User Guide topic <u>Choose the genetic code</u>.

To edit a genetic code:

- PrimerSelect Use Options > Edit Genetic Code.
- SeqBuilder Pro Use Sequence > Edit Genetic Code. For more information, see the SeqBuilder Pro User Guide topic <u>Modify the genetic code</u>.

Though the command is in a different menu, the genetic code editor is the same for both applications.

Genetic Code E	Genetic Code Editor							
Description	Standard Genetic Code							
Starts: AUG	Starts: AUG DNA							
Genetic Code	Start Codons							
A Ala GCI C Cys UGY D Asp GAY E Glu GAF F Phe UUY G Gly GGI H His CAY I Ile AUF K Lys AAR L Leu YUN M Met AUC N Asn AAY P Pro CCI Q Gin CAF R Arg MGI S Ser WSI T Thr ACN V Val GUI W Trp UGA X ??? Y Y Tyr UAY	Y UGC UGU GAC GAU GAC GAU GAC GAU GAC GAU GAC GAU Y UUC UUU N GGA GGG GGC GGU Y CAC CAU A AUA AUC AUU A AUA AUC AUU A AAA AAG N CUA CUG CUC CUU UUA UUG AAC AAU N CCA CCG CCC CCU A CAA CAG N AGA AGG CGA CGG CGC CGU N AGC AGU UCA UCG UCC UCU A ACA ACG ACC ACU N GUA GUG GUC GUU G UGG							
. ter URI	R UAA UAG UGA							
Edit Genetic Code: drag codons to desired AA. Edit Back Translation: Alt/Option-click the codons.								
OK Cancel								

Transitioning from MegAlign to MegAlign Pro

As of Lasergene 16.0, MegAlign Pro completely replaced MegAlign. Compared to its predecessor, MegAlign, MegAlign Pro features a modern, colorful user interface and <u>greatly increased functionality</u>.

This short video is an overview of the MegAlign Pro application:

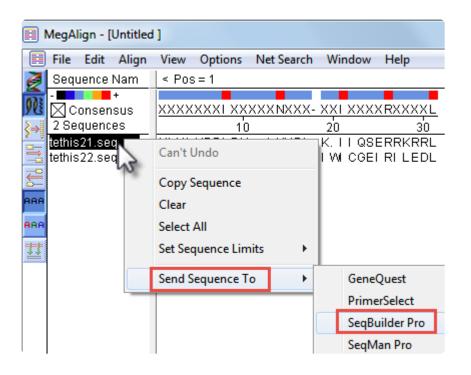
For an illustrated comparison showing how to perform an MegAlign task in MegAlign Pro, click any of the links below:

- Edit a sequence
- Trim sequence ends
- Rename a sequence
- <u>View sequence information</u>
- <u>Translate or back translate a sequence</u>
 - Select and edit a genetic code
- Perform a pairwise alignment
 - View pairwise alignment results
- Perform a multiple alignment
 - View multiple alignment results
 - View sequence distances
 - View phylogenetic tree
 - Create a subalignment
- Find a position
- Locate gaps and disagreements
- <u>Copy and export</u>

Edit a sequence

To edit a sequence:

 MegAlign – After entering sequences, select a sequence and use File > Send Sequence to SeqBuilder Pro. Edit the sequence and save it in SeqBuilder Pro to automatically update it in MegAlign. There is no need to reload the sequence into MegAlign.



• **MegAlign Pro** – Sequences must be manually opened in SeqBuilder Pro and edited there before entering them in MegAlign.

Trim sequence ends

To trim sequence ends:

 MegAlign – Use Options > Set Sequence Limits > From Feature Table (left) or Options > Set Sequence Limits > By Coordinates (right)

E Set Sequence Ends	
Set Sequence Ends	
Feature List tethis21.seq	
Tetrahymena thermophila source H2B-1 mRNA mRNA H2B-1 mRNA mRNA histone H2B-1 CDS	
Segments of Tetrahymena thermophila	Set Ends
I (1 > 906)	tethis21.seq(1>906)
-	J J J J J Lazat AATT TAAAT Z
	Length: 906 bp Range: 906 bp
Next Change the Rest OK Cancel	Next Cancel OK

• **MegAlign Pro** – Sequences must be manually opened in SeqBuilder Pro and edited there before entering them in MegAlign.

Rename a sequence

To rename a sequence:

- **MegAlign** Click for about two seconds on the name of the sequence to allow editing. Then type in a new name.
- **MegAlign Pro** There are two options for renaming sequences: a manual option, and an automatic option that can rename all selected sequences simultaneously using a specified naming convention.
 - To manually rename a selected sequence, choose Edit > Rename. For details, see the MegAlign Pro User Guide topic <u>Rename sequences manually</u>.

Choose Label								
What label would you like to display in this application for the selected sequence?								
Display: Custom label								
Label: NP_014555								
OK Cancel								

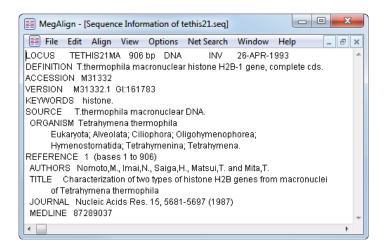
To rename one or more selected sequences using specified data fields, use Edit > Rename with Fields. For details, see the MegAlign Pro User Guide topic <u>Rename sequences</u> automatically based on specified data fields. The left pane shows available fields, and the right pane shows fields that will be displayed as part of the sequence name. Initially, **Default name** is the only field listed in the right pane. An example showing the appearance of the name using the specified field(s) appears in the **Example** box at the bottom of the dialog. Select and organize the fields you wish to display as part of the sequence name

Choose Label	
Display: One or more fields	in this application for the selected sequences?
Available fields Accession number Data file name Data file path Data file time Data specification Description Organism Sequence length Sequence shape Sequence version	Display fields in this order
Separator: - Example: AAC41757	
	OK Cancel

View sequence information

To view information about a selected sequence:

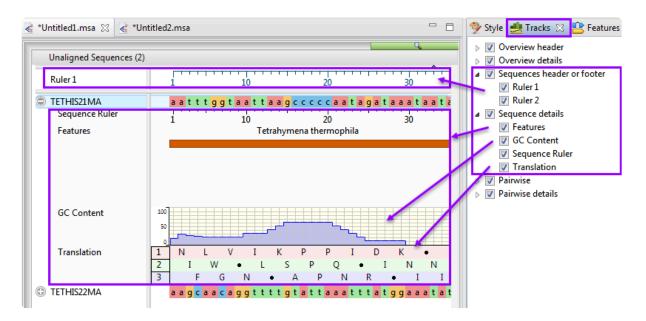
• MegAlign – Use View > Sequence Information.



- MegAlign Pro Do either or both of the following:
 - Select a sequence in any view to display its information in the Details panel. To learn more about this panel, which varies according to the current selection, see the MegAlign Pro User Guide topic <u>Details panel</u>.

🕕 Details 🔀								
TETHIS21MA	TETHIS21MA ⑦							
Organism: T.thermophila macronuclear DNA. Description: T.thermophila macronuclear histone H2B-1 gene, complete cds.								
Sequence type: DNA Residues: 906								
▼ Properties								
Accession number: Sequence shape: Sequence version:								
▼ Data								
Data specification: tethis21.seq Data file time: 10/19/04 12:53 PM Data file path: C:\Users\Public\Documents\DNASTAR\Lasergene 15 Data\Demo MegAlign\Histone Sequences\tethis21.seq								

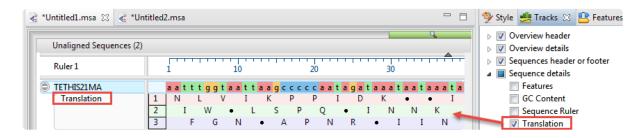
Use the Tracks panel to apply tracks to the sequence in the Overview and/or Sequences view.
 For details, see the MegAlign Pro User Guide topics <u>Tracks panel</u> and <u>Tracks</u>. Track types vary according to whether the sequence is nucleotide or protein, and whether the sequence is part of a pairwise or multiple assembly, or is still unassembled. Tracks may include information about GC content, gap fraction, protein translation, or Sashimi data.



Translate or back translate a sequence

To translate or back translate a sequence:

- **MegAlign** Use the **Show as DNA** (2) and **Show as Protein** (2) tools to toggle between how sequences are displayed on the Worktable. Each command is applied to all sequences on the Worktable.
- MegAlign Pro To display a translation of a nucleotide sequence in MegAlign Pro, use the Tracks panel to apply the Translation track. For details, see the MegAlign Pro User Guide topic <u>Translation</u> <u>track</u>.



To instead use the translated or back-translated version of a sequence in a MegAlign Pro alignment, first open the sequence in SeqBuilder Pro and change it there there, Then enter the revised sequence in MegAlign.

Select and edit a genetic code

To select a genetic code:

- MegAlign Use Options > Genetic Codes and choose a code from the list.
- MegAlign Pro You can choose an alternate genetic code only when mapping features from one sequence to another using Features > Map Features. In the Options screen, check the box next to Override genetic code, then select the desired code from the drop-down list. See the MegAlign Pro User Guide topic Options screen for details.

Map Features	
Options	
Choose desired opti	ions.
 Features Options Output 	Mapping Minimum feature coverage: 80 🔿 %
	Translation Image: Override genetic code: NCBI: 1 (Standard Code)
	Feature report Include specific sequence changes Unmapped interval report
	Cenerate report of intervals without mapped features Minimum length of interval to report: 1
?	< Back Next > OK Cancel

To see feature mapping in action, see the video Copying annotations between genomes.

To edit a genetic code:

• MegAlign – Use Options > Edit Genetic Code.

• MegAlign Pro – Not available.

Perform a pairwise alignment

To perform a pairwise alignment:

 MegAlign – Select two sequences in the Worktable and use Align > One Pair > Wilbur and Lipman, Align > One Pair > Martinez-NW, Align > One Pair > Lipman-Pearson, or Align > One Pair > DotPlot. In the ensuing dialog, make any desired changes to alignment parameters.

DNA Wilbur-Lipman							
Wilbur-Lipman Parameters							
tethis22.seq(1>905) vs. tethis21.seq(1>906)							
Ktuple:	3						
Gap Penalty:	3						
Window:	Window: 20						
Set Defaults OK Cancel							

MegAlign Pro – Select two sequences any view and use Align > Pairwise or press the Align tool (

(Local: Smith-Waterman, Global: Needleman-Wunsch or Semi-Global: Needleman-Wunsch) and make any changes to alignment parameters. For details, see the MegAlign Pro User Guide topic Perform a Pairwise Alignment.

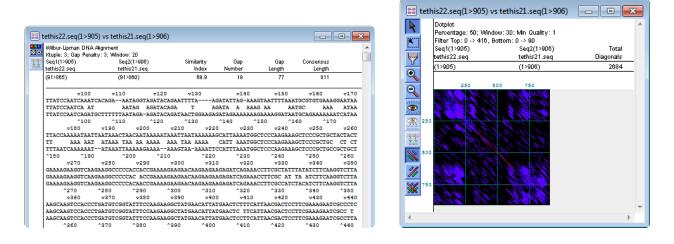
K Align Pairwise	×
	The two selected sequences .ocal: Smith-Waterman (?)
Local: Smith-Waterman options	
Substitution matrix:	NUC44 •
Gap open penalty:	10
Gap extension penalty:	1
Require minimum word match:	7
Reset to Default	OK Cancel

The following brief video is an overview of pairwise alignment in MegAlign Pro.

View pairwise alignment results

To view pairwise alignment results

• **MegAlign** – Depending on the pairwise alignment method used, results open automatically in the Alignment or DotPlot views.



MegAlign Pro – Results open in the Pairwise view, whose appearance is controlled by the Style panel's Pairwise Alignment section. Tracks, if present, are controlled by which tracks have been checked in the Tracks panel. If desired, use the drop-down menus and tools in the top of the view to open additional Pairwise alignment using different combinations of sequences and alignment methods. For more information, see the MegAlign Pro User Guide topics Pairwise view / Pairwise Alignment section and Tracks.

MegAlign Pro - Untitled1.msa									
File Edit View Align Featu	ures Sequences Ov	erview Windo	w Help						
💰 *Untitled1.msa 🛛 🔌 *Untitled2.msa 🖓 🗖									
Align: TETHIS22MA - with TETHIS21MA - 🌐 🔛 📑									
						9			
Local: Smith-Waterman DNA	alignment [Matrix: '	NUC44" Gap p	enalty: 10 (Gap extensi	on penalty: 1]				
TETHIS22MA T 1>905 1		y %Gaps Iden	Gap tical Count		Score Lenath				
Alignment 1>902 1		6 19.2%	671 46						
Ruler	1 1	0	20		30	40			
C TETHIS22MA	aag <mark>c</mark> a	a <mark>cagg</mark> ttt	tg ta tt	aaatt	ta tggaa	a - tatg			
© TETHIS21MA						 2 + + 2 + -			
	TETHIS21MA a a g c c c c a a t a - g a t a a a t a a t a a a t a a a t t a t - E								
Ruler	50		60	7	ⁱ o	80	- 11		
C TETHIS22MA	g <mark>c</mark> aaattga	atgaagta	gatttg		gtggaga	aatccg			
C TETHIS21MA	- <mark>c c a a</mark> ttt <mark>a</mark>	IIII a <mark>aaat</mark> a	aatt	- <mark>atc</mark> -	<mark>c</mark>	aatcag			

For a brief introduction to using the Tracks panel, see the following video:

Perform a multiple alignment

To perform a multiple alignment:

 MegAlign – Select the sequences to align and use Align > By Jotun Hein Method, Align > By Clustal V Method, or Align > By Clustal W Method. To change parameters prior to alignment, use Align > Method Parameters, choose the tab of interest and make changes, then press the Now button.

Multiple Alignment Parameters of Untitled							
Jotun Hein Clustal V Clusta	al W						
Multiple Alignment Par	ameters	Pairwise Alignment Parameters					
Gap Penal	lty: 10.00	Fast-Approx.	Slow-Accurate				
Gap Length Pena	lty: 0.20	Gap Penalty:	10.00				
Delay Divergent Seqs()	%): 30	Gap Length:	0.10				
DNA Transition Weig	ht: 0.50						
Use Negative Matrix							
Protein Weight I	Matrix	Protein Weight Matrix					
BLOSUM Series	PAM Series	BLOSUM 30	PAM 350				
 Gonnet Series 	Identity Matrix	Gonnet 250	⊘ Identity				
DNA Weight N	fatrix	DNA Weight Matrix					
IUB	CLUSTALW	IUB	© CLUSTALW				
Set to Defaults		Align: Later	Now Cancel				

To edit a multiple alignment, select a portion of sequence in the Worktable and use the **Straighten**

Columns (), Shuffle Right () or Shuffle Left () tools.

MegAlign Pro – Select the sequences to align and use Align > Align Using Clustal Omega, Align > Align Using Clustal W, Align > Align Using MAFFT, or Align > Align Using MUSCLE.

Alternatively, press the **Align** tool (**Der**) and choose the option of the same name. To change alignment options, instead use **Align > Align with Options** or press the **Align** tool and choose the option of the same name. Select the desired method using the drop-down menu, then edit the settings and press **Align**. For more information, see the MegAlign Pro User Guide topic <u>Perform an initial multiple alignment</u>.

Align Sequences	Align: All sequences Vsing: ClustalW							
ClustalW options								
Scoring matrix: BLOSUM								
Iteration method: None	▼ Maximum Iterations: 1							
Multiple alignment param	eters							
Gap open penalty: Default: 10								
Gap extend penalty:	Default: 0.20							
Delay divergent sequences	: Default: 30	%						
Pairwise alignments for guide tree Slow, accurate Gap open penalty: Default: 10								
Gap extend penalty: De	fault: 0.1							
Reset to Default	Align Can	cel						

The following brief video is an overview of multiple alignment in MegAlign Pro.

Additional videos highlighting multiple alignment functionality in MegAlign Pro include: <u>Merging and</u> realigning sequences, <u>Aligning genomes using Mauve</u> and <u>Aligning multi-segment files</u>.

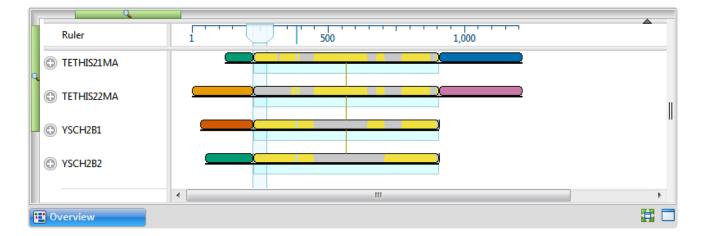
View multiple alignment results

To view multiple alignment results:

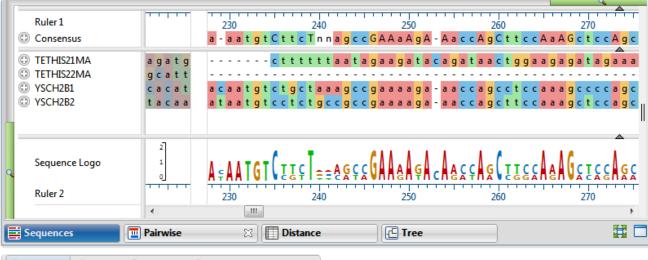
• MegAlign – Use View > Alignment Report.

Majority	MADQLTEEQI AEFI	KEAFSLFDKDG	DGXI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLXLM	ARKMKDT	
	10	20	30	40	50	60	70	80	
arley Calmodulin.pro	MADQLTDDQI AEFI	KEAFSLFDKDGI	DGCI TTKEL	TVMRSLGQNP	TEAELQDMI	NEVDADGNGTI	DFPEFLNLM	ARKMKDT	80
llack Mold Calmodulin.pro	MADSLTEEQVSEY	KEAFSLFDKDGI	DGQI TTKEL	TVMRSLGQNP	SESELQDMI I	NEVDADNNGTI	DFPEFLTMM	ARKMKDT	80
Chicken Calmodulin.pro	MADQLTEEQI AEFI	KEAFSLFDKDGI	DGTI TTKEL	TVMRSLGQNP	FEAELQDMI I	NEVDADGNGTI	DFPEFLTMM	ARKMKDT	80
Cilliate Calmodulin.pro	MADNLTEEQI AEFI	KEAFSLFDKDGI	DGTI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLSLM	ARKMKDT	80
ectric Eel Calmodulin.pro	MADQLTEEQI AEFI	KEAFSLFDKDGI	DGTI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLTMM	AKKMKDT	80
luman Calmodulin.pro	MADQLTEEQI AEFI	KEAFSLFDKDGI	DGTI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLTMM	ARKMKDT	80
ily Calmodulin.pro	MADQLTDDQI SEFI	KEAFSLFDKDGI	DGCI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLNLM	ARKMKDT	80
aramecium Calmodulin.pro	MAEQLTEEQI AEFI	KEAFALFDKDGI	DGTI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLSLM	ARKMKEQ	80
Potato Calmodulin.pro	MAEQLTEEQI AEFI	KEAFSLFDKDG	DGCI TTKEL	TVMRSLGQNP	TEAELQDMI :	SEADADQNGTI	DFPEFLNLM	ARKMKDT	80
Rat Calmodulin.pro	MADQLTEEQI AEFI	KEAFSLFDKDGI	DGTI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLTMM	ARKMKDT	80
Red Bread Mold Calmodulin.pro	MADSLTEEQVSEF	KEAFSLFDKDG	DGQI TTKEL	TVMRSLGQNP	SESELQDMI I	NEVDADNNGTI	DFPEFLTMM	ARKMKDT	80
Red Bryony Calmodulin.pro	MADQLTDDQI SEFI	KEAFSLFDKDG	DGCI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLNLM	ARKMKDT	80
Rice Calmodulin.pro	MADQLTDDQI AEFI	KEAFSLFDKDG	DGCI TTKEL	TVMRSLGQNP	TEAELQDMI I	NEVDADGNGTI	DFPEFLNLM	ARKMKDT	80

MegAlign Pro – Results are displayed in the Overview and Sequences view, whose contents and appearances are controlled through the Style panel's Overview and Sequence sections, respectively. Tracks, if present, are controlled by which tracks have been checked in the Tracks panel. For more information, see the MegAlign Pro User Guide topics Overview / Overview section, Sequences view / Sequence section, and Tracks.



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A Overview										
Alignment layout										
Spacing:		1	1 1	1 1						
Presentation	Presentation									
Font: Segoe UI				9 🌲						
Track names for	ont:									
Segoe UI			-	9						
Multiple block display										
✓ Show connect	ing lines									
Reference:				-						



🦻 Style 🔀 連 Tracks 🔛 Features 🢡 Places 🛛 🖓 🗖
♥ Overview
▲ Sequence
Presentation
Font: Segoe UI 🔹 9 🚔
Track names font:
Segoe UI 🗸 🥑 🛓
Color sequence foreground:
Color by chemistry
Color sequence background:
• 0

For a brief introduction to using the Tracks panel, see the following video:

To view multiple alignment results:

									Identi	ŕ							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Ľ	1		81.9	90.6	90.6	89.9	90.6	99.3	86.6	91.9	90.6	82.6	98.7	99.3	98.7	1	Barley Calmodulin.pro
1	2	20.8		84.6	83.2	83.9	84.6	82.6	81.2	80.5	84.6	99.3	81.9	81.9	83.2	2	Black Mold Calmodulin.pro
	3	10.1	17.3		90.6	99.3	100.0	89.9	88.6	90.6	100.0	85.2	89.9	89.9	90.6	3	Chicken Calmodulin.pro
2	4	10.1	19.0	10.1		89.9	90.6	89.9	92.6	89.3	90.6	83.9	89.3	89.9	90.6	4	Cilliate Calmodulin.pro
	5	10.8	18.2	0.7	10.8		99.3	89.3	87.9	89.9	99.3	84.6	89.3	89.3	89.9	5	Electric Eel Calmodulin.pro
- 6	6	10.1	17.3	0.0	10.1	0.7		89.9	88.6	90.6	100.0	85.2	89.9	89.9	90.6	6	Human Calmodulin.pro
1	7	0.7	19.9	10.8	10.8	11.6	10.8		85.9	91.3	89.9	83.2	99.3	98.7	99.3	7	Lily Calmodulin.pro
8	8	14.8	21.7	12.4	7.8	13.2	12.4	15.7		88.6	88.6	81.9	85.2	85.9	86.6	8	Paramecium Calmodulin.pro
9	9	8.5	22.6	10.1	11.6	10.8	10.1	9.3	12.4		90.6	81.2	91.3	91.3	91.9	9	Potato Calmodulin.pro
1	0	10.1	17.3	0.0	10.1	0.7	0.0	10.8	12.4	10.1		85.2	89.9	89.9	90.6	10	Rat Calmodulin.pro
1	1	19.9	0.7	16.5	18.2	17.3	16.5	19.0	20.8	21.7	16.5		82.6	82.6	83.9	11	Red Bread Mold Calmodulin.pr
1	2	1.4	20.8	10.8	11.6	11.6	10.8	0.7	16.5	9.3	10.8	19.9		98.0	98.7	12	Red Bryony Calmodulin.pro
1	3	0.7	20.8	10.8	10.8	11.6	10.8	1.4	15.7	9.3	10.8	19.9	2.0		98.0	13	Rice Calmodulin.pro
1	4	1.4	19.0	10.1	10.1	10.8	10.1	0.7	14.8	8.5	10.1	18.2	1.4	2.0		14	Soybean Calmodulin.pro
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		

• MegAlign – Use View > Sequence Distances.

 MegAlign Pro – Results are displayed in the Distance view, whose contents and appearance are controlled through the Style panel's Distance section. For more information, see the MegAlign Pro User Guide topics <u>Distance view</u> / <u>Distance section</u>.

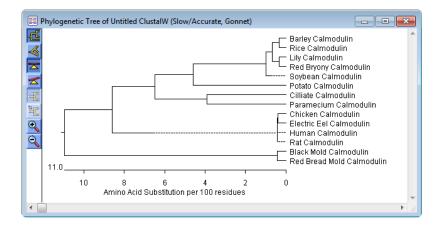
м	etric: Uncorrected Pairwise Distance	Sequ	uences	14	Global	gap re	moval	Resid	dues co	nsider	ed: 149)			
		Α	В	С	D	E	F	G	н	I	J	K	L	М	N
Α	Barley Calmodulin.pro		0.18	0.09	0.09	0.10	0.09	0.01	0.13	0.08	0.09	0.17	0.01	0.01	0.01
В	Black Mold Calmodulin.pro	0.18		0.15	0.17	0.16	0.15	0.17	0.19	0.19	0.15	0.01	0.18	0.18	0.17
С	Chicken Calmodulin.pro	0.09	0.15		0.09	0.01	0.00	0.10	0.11	0.09	0.00	0.15	0.10	0.10	0.09
D	Cilliate Calmodulin.pro	0.09	0.17	0.09		0.10	0.09	0.10	0.07	0.11	0.09	0.16	0.11	0.10	0.09
Ε	Electric Eel Calmodulin.pro	0.10	0.16	0.01	0.10		0.01	0.11	0.12	0.10	0.01	0.15	0.11	0.11	0.10
F	CALM_HUMAN	0.09	0.15	0.00	0.09	0.01		0.10	0.11	0.09	0.00	0.15	0.10	0.10	0.09
G	Lily Calmodulin.pro	0.01	0.17	0.10	0.10	0.11	0.10		0.14	0.09	0.10	0.17	0.01	0.01	0.01
н	Paramecium Calmodulin.pro	0.13	0.19	0.11	0.07	0.12	0.11	0.14		0.11	0.11	0.18	0.15	0.14	0.13
I	Potato Calmodulin.pro	0.08	0.19	0.09	0.11	0.10	0.09	0.09	0.11		0.09	0.19	0.09	0.09	0.08
J	Rat Calmodulin.pro	0.09	0.15	0.00	0.09	0.01	0.00	0.10	0.11	0.09		0.15	0.10	0.10	0.09
Κ	Red Bread Mold Calmodulin.pro	0.17	0.01	0.15	0.16	0.15	0.15	0.17	0.18	0.19	0.15		0.17	0.17	0.16
L	Red Bryony Calmodulin.pro	0.01	0.18	0.10	0.11	0.11	0.10	0.01	0.15	0.09	0.10	0.17		0.02	0.01
М	Rice Calmodulin.pro	0.01	0.18	0.10	0.10	0.11	0.10	0.01	0.14	0.09	0.10	0.17	0.02		0.02
Ν	Soybean Calmodulin.pro	0.01	0.17	0.09	0.09	0.10	0.09	0.01	0.13	0.08	0.09	0.16	0.01	0.02	
	Sequences 📃 🛅 Pairw	ise		23		Distan	ce		2	Tree					

🦻 Style 🛛 🛃	Tracks 🖺 Features Places		
∓ Overview			
∓ Sequence			
🗧 Multiple Align	ment		
🗧 Pairwise Align	ment		
Distance			
Parameters			
Metric:	Uncorrected Pairwise Distance	•	?
Gap treatment:	Global gap removal	•	?
Presentation			
Font:	Segoe UI 🔹	9	* *
Decimal places:	2		-

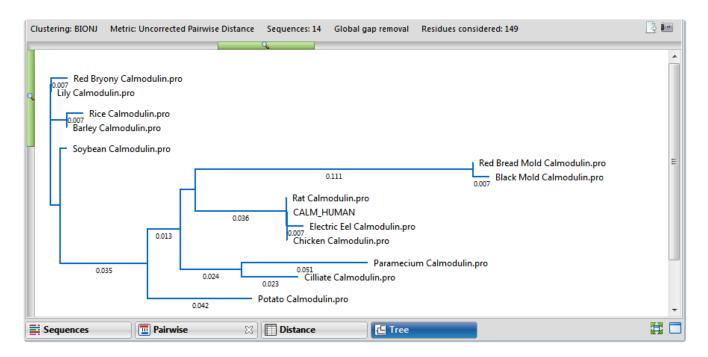
View phylogenetic tree

To view multiple alignment results:

• **MegAlign** – Use **View** > **Phylogenetic Tree**. The appearance and calculations used in this window are controlled via the tools on the left.



 MegAlign Pro – Results are displayed in the Tree view, whose contents and appearance are controlled through the Style panel's Tree section. For more information, see the MegAlign Pro User Guide topics <u>Tree view</u> / <u>Tree section</u>.



🦻 Style 🕺 連 1	Fracks 💾 Features 🥊 Places		
Overview			
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Fairwise Alignr	nent		
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ት ሥ ሐ 🔁			
Leaves			
Font:	Segoe UI 🔹	9	*
🔲 Background:			
Branches			
Color:			
Width:	2		*
Show branch	length		
Decimal places:	3		* *

The following short video shows how to create and edit phylogenetic trees in MegAlign Pro.

Create a subalignment

To create a subalignment:

- MegAlign After performing a multiple alignment, select a portion of the consensus sequence in the Worktable and use Align > Create Alignment From Selection. After choosing a name for the new project, the subalignment is opened in a new MegAlign project window.
- MegAlign Pro After performing a multiple alignment, display a Consensus track and select a portion of the consensus sequence in the Sequence view. Then use Align > Realign Subsequences. Choose the desired alignment type and parameters, then press Align. The results are shown in the current MegAlign Pro project rather than in a new project window. For more information, see the MegAlign Pro User Guide topic Subalign sequences.

Find a position

To find a position:

• MegAlign – Use Edit > Go To Position.

Go To Position	
Go To Position In:	the Consensus
Single Residue	149
Range	
9	OK Cancel

MegAlign Pro – Use Edit > Go To (Pairwise) Position (left). For more information, see the MegAlign
Pro User Guide topic <u>Search</u>. To return to a sequence location or range you have visited before, use
the Places panel (right), discussed in the topic <u>Places panel</u>.

Go to Sequence Position								
Please enter the sequence position (1-149):								
OK Cancel								
🦻 Style 进 Tracks 😩 Features 🦞 Places 🕱 👘 🖳								
A Favorites								
284	60							
50 Alcohol dehydrogenase gene	1							
265>282	€							
▲ Recent								
284	6							
50	4							
300	~							

The following video shows how to bookmark favorite locations using the Places panel:

Locate gaps and disagreements

To find gaps or areas of disagreement in an alignment:

 MegAlign – Use Edit > Find Disagreement and choose whether to search for a disagreement or a gap

Find Disagreement
Disagreement within: Level 4 - orange
Find Next Find Previous
Cancel

Or, to make facets of the alignment stand out in the Alignment Report, add one or more decorations to the Worktable using **Options > New Decoration**.

Decoration			
Alignment Decoration Name			
Decoration #1			
Decoration Parameters			
Box 💽 residues that match	the Consensus		
	by 0 distance units		
Show on Report			
2	OK Cancel		
Alignment Report of Untitled ClustalW (Slow/	Accurate, Gonnet)		
Majority	MADQLTEEQIAEFKEAFSLFDKDODG	(I TTKELGTVMRSLGQNPTEAELQDM	I NEVDADGNGTI DEPERLALMARKMKDT
	10 20	30 40 50	60 70 80
Barley Calmodulin.pro Black Mold Calmodulin.pro	MADQLTDDQIAEFKEAFSLFDKDGDG MADSLTEEQVSEMKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM TTKELGTVMRSLGQNP SES ELQDM	
Chicken Calmodulin.pro	MADQLTEEQI AEFKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM	INEVDADGNGTIDFPEFLTMMARKMKDT 80
Cilliate Calmodulin.pro Electric Eel Calmodulin.pro	MADNLTEEQIAEFKEAFSLFDKDGDG MADQLTEEQIAEFKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM TTKELGTVMRSLGQNPTEAELQDM	INEVDADGNGTIDFPEFLISEMARKMKDT 80
Human Calmodulin.pro	MADQLTEEQI AEFKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM	INEVDADGNGTIDFPEFLTMMARKMKDT 80
Lily Calmodulin.pro	MADQLTDDQIBEFKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM	
Paramecium Calmodulin.pro Potato Calmodulin.pro	MAEQLTEEQIAEFKEAFALFDKDGDG MAEQLTEEQIAEFKEAFSLFDKDGDG	TTKELGTVMRSLGQNPTEAELQDM TTKELGTVMRSLGQNPTEAELQDM	
Rat Calmodulin.pro	MADQLTEEQI AEFKEAFSLFDKD0D0		INEVDADGNGTIDFPEFLITMARKMKDT 80
Red Bread Mold Calmodulin.pro			INEVDAD NGTIDFPEFLTMMARKMKDT 80
Red Bryony Calmodulin.pro Rice Calmodulin.pro			INEVDADGNGTIDFPEFLNLMARKMKDT 80
	MADE ID MI ACT NEAT SET DEDODO	TIRELOT WIRALOUNFIEXELUDM	
			►

 MegAlign Pro – Use the Style panel's Pairwise Alignment or Multiple Alignment sections to specify how to display similarities or differences. See the MegAlign Pro User Guide topics <u>Pairwise</u> <u>Alignment section</u> and <u>Multiple Alignment section</u> for details.

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Align: Barley Calmodulin.pro	with Black Mold Calmodulin.pro	Overview Overview
Local: Smith-Waterman Protein alignment [Matrix: "BLO		▲ ₹ Sequence ₹ Multiple Alignment
Barley Calmodulin.pro Black Mold Calmodul 1>149 1>149	%Identity %Similar %Gaps Identical Similar Count Leng	Layout
Alignment 1>149 1>149	81.9% 94.6% 0.0% 122 141 0	0 651 149 Automatic wrapping
		Comparison
Ruler 1 10 C Barley Calmodulin.pro MA D Q L T D D Q I A E F	20 30 40 KEAFSLFDKDGDGCITTKELGTVMRSLGQNPTE	
③ Black Mold Calmodulin : : : . : MADSLTEEQVSEY		S E L Q DM I N E V D A D N
		Match Bar
Ruler 70	80 90 100	110 Color background: Same as guery ?
	IA R KM K D T D S E E E <mark>L K</mark> E A F <mark>R</mark> V F D K D Q N G F I S A A E L F	RHVMTNLGEKLTDE
Black Mold Calmodulin NG T I D F P E F L T MM	AKKMKUTUSEEEIKEAFKVFDRDNNGFISAAELI	RHVMTSIGEKLTDD

Copy and export

To copy a sequence:

- MegAlign To copy any selected text as it appears in a view, use Edit > Copy. To copy part or all of the consensus sequence after performing an alignment, select the sequence range and use Edit > Copy Consensus.
- MegAlign Pro To copy any selected text as it appears in a view, use Edit > Copy. To copy the selected text or data in FASTA format, use Edit > Copy As FASTA. For details, see the MegAlign Pro User Guide topic Copy. Paste and Delete.

To export a sequence:

- MegAlign Use File > Export Sequences to export selected sequences from the Worktable or File
 > Export Consensus to export the consensus sequence from the Worktable. Files can only be exported to Lasergene DNA (.seq) or protein (.pro) format.
- MegAlign Pro Use any of over a dozen commands to export data to a number of formats. Among many other choices, you can export all the sequences (File > Export Data > Sequences), or data from the Distance view (File > Export Data > Distance Matrix) or Tree view (File > Export Data > Tree). For more information, see the MegAlign Pro User Guide topics Export data to a file and Export a tree to a tree viewer.

To export an image:

- **MegAlign** Not available.
- MegAlign Pro To export an image showing the contents of a view, use File > Export Image > (View Name). For details, see the MegAlign Pro User Guide topic Export an image of the view. Once you have exported the image, you can edit it in PowerPoint as shown in this brief video:

Transitioning from SeqMan Pro to SeqMan Ultra

SeqMan Ultra was introduced in Lasergene 17.0 as the modern replacement for SeqMan Pro, now considered a legacy application. Both applications will be included in Lasergene for a short time, but we strongly encourage all users to switch to SeqMan Ultra as soon as possible.

SeqMan Ultra is a 64-bit application, which means faster project opening, better performance for analyzing large files, increased capacity, and compatibility with macOS 10.15. Compared to its predecessor, SeqMan Pro, SeqMan Ultra features a modern, colorful user interface and <u>greatly increased functionality</u>.

For an illustrated comparison showing how to perform a SeqMan Pro task in SeqMan Ultra, click any of the links below:

- Get acquainted with the SeqMan Ultra interface
- Create a new assembly
- Open an existing assembly
- Select and work with contigs
- <u>View contigs, consensuses and reads graphically</u>
- View and work with features
- View and work with variants
- <u>View information about a project or selection</u>
- Search for sequences online

Get acquainted with the SeqMan Ultra interface

SeqMan Ultra uses the same type of graphic user interface as DNASTAR's MegAlign Pro, GenVision Pro, SeqNinja, Protean 3D and SeqBuilder Pro. This style of interface has one or more large "views" on the left, and a narrower set of expandable "panels" on the right. The panels are used for specifying the appearance of the views, selecting what is included in the views, showing details about a selection, etc.

tranded-Brain-2017.assembly	🔀 📵 Welcome	- 0	🔗 Explorer 🎐 Style 🛛 🎂 Tracks
NC_000001		l 🛛 👻 🧕	✿ Sequence
		Q	Presentation
Ruler		88,000 40,389,000	Font: Segoe UI 🗸 9 📮
Coverage	10		Track names font:
			Segoe UI 🗸 🥥 🗘
Split reads			Color sequence foreground:
NC_000001(1>248961879 -	gene: SMAP2		Color by chemistry
Features	gene smarz		Color sequence background:
		~	Taylor
)_39121954_r	H		Alignment style pane may override these colors
_12346742_f	No. 1		Augment sigle pune may overnue these colors
_7784328_r_dup[2]	•	~	Alignment
<		>	Editing: No editing allowed permanently
NC_000001 ×		📰 🗖	Layout reads:
NC 000001	L	₽⊫Q ȘI L 💽 ∀¤	Color only in targeted coverage regions
NC_000001	Length=248,961,879		Quality: Show scores
Ruler 15 560 120 15 560 120 15 560 120 15 560 120			Alignment coloring
Consensus t	15,560,120 15,560,130 15,560,140 A G G C C C G A G G T A T G A A G A A G C A A A T C A T T G A C	15,560,150 15,560,160 G A C T T C A T C A C C C G A A A C	Color matches and differences
Coverage 15			Match: 🗹 🛓 🗆 🖌
	. .	U.S. C.S. C.S. C.S. C.S. C.S. C.S. C.S.	Difference: 🗌 🛕 🗹 🚄
ID 46236232 f du ← 4	•	GACTICATCACCCGAAAC	Bases ignored in consensus calling
	AGGCCCGAGGTATGAAGAAGCAAATCATTGAC		Sases ignored in consensus calling F Strategy
	A G G C C C G A G G T A T G A A G <mark>G</mark> A G C A A A T C A T T G A C		2.
ID_46132660_r → 4	AGGCCCGAGGTATGAAGAAGCAAATCATTGAC		🕦 Details 💷 Jobs 🔀 🛛 🔘 🗡 🖻
ID 31216784 r du → 4	AGGCCCGAGGTATGAAGAAGCAAATCATTGAC	GACTICATCACCCGAAAC	Job N Status Started

We think you'll find the new **SeqMan Ultra** interface to be very intuitive. However, we would like to highlight a few items that might not be readily apparent.

Changing the layout and number of views:

- SeqMan Pro Views, tables and reports appear as separate windows that can be resized manually.
- SeqMan Ultra Graphic, tabular and text data is each contained in a named "view." You can display
 from 1-3 views at a time. To specify the number and layout of views, use the View > Change layout
 command or the Change layout tool (image varies) in the bottom right corner of each view. The
 following options are available:

- **One One** To show only the current view and cause it to occupy all of the view "real estate."
- Two Horizontal (Two Horizontal) To show the current view on top and one additional view below it.
- **Two Vertical** (**Two Vertical**) To show the current view on the left, and one additional view to the right.
- **Three Horizontal** (Three Horizontal) To show the current view on top and two additional views below it.
- Three Left (Three Left) To show the current view on the left and two additional views stacked one on top of the other on the right.
- **Three Right** (Three Right) To show the current view on the right and two additional views stacked one on top of the other on the left.
- **Three Top** (Three Top) To show the current view on the top and two additional views side-by-side on the bottom.
- **Three Bottom** (Three Bottom) To show the current view on the bottom and two additional views side-by-side on the top.

Changing parameters:

• SeqMan Pro – Assembly and display parameters are changed using Project > Parameters.

SeqMan Parameters	
Loading Files Preassembling End Trimming Contaminant Screening Assembling Clustal Subalignment Consensus Calling Strategy Viewing & Coverage Pair Specifier Conflict Split Variant Discovery Primer Walking Editing & Color Vector Catalog Servers Internet	Loading Files Sequence Files Preserve case when adding Trace Files Copy trace data into project ABI Trace Files Use edited data if it exists
Restore	OK Cancel

• **SeqMan Ultra** – Assembly parameters are now specified during project setup in SeqMan NGen. Display customization is now handled through the Style panel and Tracks panel.

🛞 Explorer	🦻 Style	🛙 🍰 Tr	acks			8
∓ Sequence	2					0
🏝 Alignmer	nt					
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Layout reads						
Color onl	y in targete	d coverag	e regions			
Quality:	Show sco	ores			\sim	
Alignment	coloring-					
Color mat	ches and di	fferences			~	
Match: Difference						
Bases igno	red in cons	ensus call	ing			
Negated w	eights: 🗌 🛛	A 🗸 🦯				
Trimmed:		A 🛛 🖉				
▲ Strategy						
– Read visibi	lity					
Show:	All					
Unpa			-		-	
			, –	single con	tig	
	nsistent (sin	igle contig		+ +	*	
🗹 Incor	nsistent (mi	ulti contig) grou		grout	
🛞 Explorer	🦻 Style	<u>⊭</u> Tracks	23			8
✓ ■ Strateg						0
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	Features					
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Remaining parameters are accessed through **Project > Project Parameters**.

🗮 Project Parameters	×
Strategy Viewing and Cove Strategy Viewing and Coverage settings	rage impact coverage graph coloring and coverage report.
 Consensus Strategy Viewing and Coverage Pair Specifier 	Maximum expected coverage:100Coverage threshold:4Minimum number on each strand:3
?	OK Cancel

Creating a new assembly:

- SeqMan Pro Launch the application. If you have closed all the windows in the document—use File
 New. Then add files, change any options, and press Assemble.
- SeqMan Ultra Launch the application and click New Assembly.

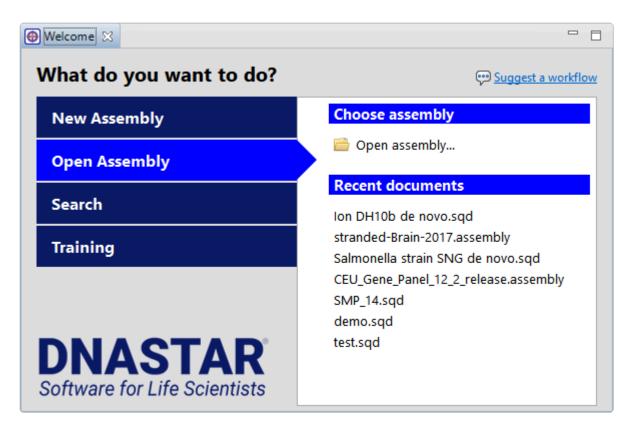
⊕ Welcome 🛛	- 8
What do you want to do?	💬 <u>Suggest a workflow</u>
New Assembly	Molecular biology
Open Assembly	 Sanger/ABI de novo assembly Sanger/ABI variant analysis Sanger/ABI reference-guided
Search	
Training	Genomics De novo genome assembly and editing
DNASTAR	 Hybrid reference-guided/de novo genome assembly Metagenomic and heterogenous samples Short read polishing of a long read draft genome Variant analysis and resequencing Variant Call Format (VCF) analysis
Software for Life Scientists	Transcriptomics ChIP-seq De novo transcriptome assembly and annotation miRNA quantitation and discovery RNA-Seq

Select a workflow from the list to launch the SeqMan NGen wizard with the workflow already selected. Follow the wizard prompts and choose to assemble locally or on the cloud.Once the assembly is finished, you will see options for downstream analysis, including opening the assembly in SeqMan Ultra.

Open an existing assembly

Opening an assembly:

- SeqMan Pro Use drag & drop, the File > Open command or the File > Recent Documents command. Only one assembly at a time can be open.
- SeqMan Ultra supports all of the methods listed above, but supports multiple assemblies by having a different tab for each assembly. In addition, SeqMan Ultra features a "Welcome" tab. By pressing this tab and then pressing Open Assembly, you can open an assembly at any time, even if you already have other assemblies open.



Select and work with contigs

Selecting, renaming, scaffolding, and reordering contigs:

In both applications, contigs and scaffolds appear in a table. The functions above can be accomplished by typing into a table cell, or by using menu commands or right-click commands. The main difference is the location of the table.

• SeqMan Pro – The table appears in the Project Summary window:

🛄 Untitled.sqd				• 💌
Name	Length	Seqs	Pos Con	flict Split 🚊
Contig 178	3,759	583	0	^
Contig 179	4,585	746	0	
Contig 180	7,056	1,456	0	
Contig 181	3,565	669	0	
Contig 182	1,558	289	0	
Contig 183	1,592	232	0	
Contig 184	1,477	222	0	
Contig 185	940	131	0	
Contig 186	508	135	0	
Contig 187	1,256	221	0	
▼Scaffold 1			0	_
Contig 47	31,013	9,437	0	
Contig 93	54,592	15,662	0	
Contig 117	16,728	4,902	0	
Contig 128	17,796	4,134	0	
Contig 135	18,724	4,345	0	
				`
1 Contig 104				~
2 Contig 67				
2 Contig 67				
3 Contig 56				
3 Contig 56				×

SeqMan Ultra, it appears in the Explorer panel on the upper right of the SeqMan Ultra window. If not visible, click on the Explorer tab to bring it to the front or use View > Explorer:

🛞 Explorer 🙁	🦻 Style 🚦	Tracks			
Name	Length	Sequences	Position	^	
Contig 359	835	306	0		
Contig 360	1,337	541	0		Ħ
Contig 361	1,180	447	0		8
Contig 362	406	116	0		8
Contig 363	315	90	0		
Contig 364	306	94	0		
Contig 365	865	290	0		
Contig 366	322	85	0		
Contig 367	307	93	0		
 Scaffold 1 			0		
Contig 9	6,838	2,594	0		
Contig 25	45,665	18,258	0		
Contig 55	367	207	0		
Contig 87	290	217	0		
Contig 104	667	227	0	¥	

View contigs, consensuses and reads graphically

Viewing contigs and constituent sequences graphically:

In both applications, graphical information about contigs and constituent sequences is shown in the Alignment and Strategy views.

- SeqMan Pro These views are accessed using Contig menu commands or by double-clicking on a contig in the Project Summary window.
- SeqMan Ultra The views are accessed using View menu commands, by double-clicking on an item in the Explorer panel or by clicking the view tools to the right of the Explorer panel.

🛞 Explorer 🖾 🕯	🦻 Style 🚦	Tracks			
Name	Length	Sequences	Position	^	
 All Referenc 			0		\rightarrow
NC_000001	248,961	2,331,301	0		-
NC_000002	242,197	1,487,731	0		8
NC_000003	198,298	1,221,578	0		Å
NC_000004	190,216	739,050	0		
NC_000005	181,541	1,056,056	0		
NC 000006	170.000	1 010 661	•		

New features in SeqMan Ultra include the ability to:

- Open and compare views for two or more contigs simultaneously. For instance, you could open the Alignment view for Contig 3 and Contig 7 and compare them side-by-side.
- Use the Tracks panel to specify which data tracks to generate. These can include items such as the consensus translation, consensus and read features, rulers, trace data; and coverage, split read and pair consistency graphs. Within the views, you can then choose whether to show or hide the tracks using the plus/minus sign to the left of each track. You can also reorder tracks by dragging them to the desired position and dropping them there.

🕀 Welcome 📄 stranded-	-Brain-2017.assembly 🕅		🛞 Explorer Style 🛃 Tracks 🔀
₩ NC_000001	9	2	✓ ✓ Strategy header ✓ Contig
Ruler Coverage Split reads	65,350,000 65,400,000 65,450,000	65,550,000 65,550,000	 Coverage ⊘ Coverage ⊘ Pair Consistency ♥ Reference ∅ Features ♡ Ruler ♥ Split reads ▼ ✓ Alignment header
NC_000001(1>248961879 Features	CDS: DNAJC6 [8] CDS: DNAJC6 [8] CDS: DN	mRNA: LEP gene: LEPR	 ✓ Consensus ✓ Consensus translation ✓ Coverage ✓ Meterence ✓ Features
Pair Consistency	mRNA: DNAJC6 [8] mR mRNA: DNI gen see o o	mRNA: LEPF	 ✓ Ruler ✓ Alignment reads ✓ Features ✓ Traces

• Customize track color schemes, fonts, spacing, and much more using the Style panel.

🛞 Explorer	🦻 Style	🔀 🍰 Tra	cks	
🌲 Sequence	2			
Presentatio	'n			
Font: Sego	oe Ul		~ 9	▲ ▼
✓ Track na	mes font:			
Sego	oe Ul		~ 9	•
Color se	quence fo	reground:		
Colo	or by chem	istry		• ?
Color se	quence ba	ckground:		
Colo	or by chem	istry		• ?
Alignment	style pane	may overrid	le these colors	
â Alignmer	_			
Editing:		ing allowed	permanently	\sim
Layout reads				
Quality:	Show so	ed coverage	regions	
Alignment		ores		~
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Difference				
_		sensus callin	ig	
Negated we	-			
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Read visibil	lity			
Show:	All			•
🗹 Unpa	ired		`	
✓ Incor	nsistent (si	ngle contig)	single contig	
🗹 Incor	nsistent (m	ulti contig)	group 1	oup 2
Cons	stent (sin	a ^l e contig)	single contig	<u> </u>

View and work with features

Viewing and working with features in a graphical display:

- **SeqMan Pro** Reference or consensus features are displayed by default in the Strategy view and by expanding the arrow next to the reference sequence name in the Alignment view.
- **SeqMan Ultra** Feature "tracks" for the reference, consensus, or individual reads can be displayed or hidden from the Alignment and Strategy views using the Tracks panel.

stranded-Brain-2017.assemb	oly 🔀 🚯 Welcome					🛞 Explorer 🦻 Style 🛃 Tracks 🖇
Ruler Coverage Split reads		40,750,000	40,800,000	2 9 40,850,000		 Strategy header Contig Coverage Pair Consistency Reference Features
NC_000001(1>248961879 Features	gene: RIM	CDS: NFYC [10] 	CDS: KCNQ4 [5]	ge d + nc	-	 ✓ Ruler ✓ Split reads ✓ Alignment header ✓ Consensus
	mRNA: RI	CDS: NFYC [5]	CDS: KCNQ4 [4]	i		Consensus translation

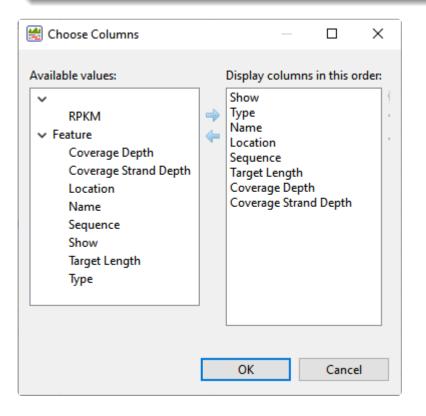
Viewing and working with features in a tabular display:

- SeqMan Pro Open the Features table by selecting a contig in the Project window and choosing Features > Show Feature Table. Table columns can be added or removed by right-clicking on the table and choosing Show/Hide Column. The types of features included in the table are controlled using Feature menu commands. Additional commands from this menu let you add new features or edit existing features.
- SeqMan Ultra Open the Features view by selecting a contig in the Explorer panel and choosing
 View > Features or by using the tool to the right of the Explorer panel.

🛞 Explorer 🔀 🦻 Style 🎂 Tracks 🛛 🗖						
Name	Length	Sequences	Position	^ ≣		
 All Referenc 			0	멅		
NC_000001	248,961	2,331,301	0			
NC_000002	242,197	1,487,731	0			
NC_000003	198,298	1,221,578	0	Ä		
NC_000004	190,216	739,050	0			
NC_000005	181,541	1,056,056	0			
NC 000006	170.000	1 010 661	•	_		

From within the Features view, table columns can be added or removed using the "gear" tool in the top right of the view.

NC_	000010 All Fe	atures	 21,381 features 	₩ 🖹	Ξ \boxtimes
ow	Туре	Name	Location	Sequence	^
	source	Homo sapiens	1>133797422	NC_000010	
~	assembly_gap	10000	1>10000	NC_000010	
~	ncRNA	LOC102723376	11721>12029,12798>130	NC_000010	



As in SeqMan Pro, the types of features included in the table are controlled using **Feature** menu commands. Unlike in SeqMan Pro, SeqMan Ultra does not yet support creating or editing features.

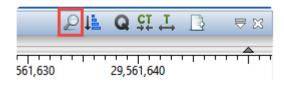
View and work with variants

Viewing and working with variants in a graphical display:

- SeqMan Pro Variants are displayed as colored nucleotides in the Alignment view, and can be spotted more easily using Variant > Show Variants.
- **SeqMan Ultra** Variants can be displayed in the Alignment view using a variety of foreground and background color schemes. These displays are customized using the Style panel.

🛃 SeqMan Ultra		- 🗆 X
File Edit View Project Contig Sequence Features	Variants Search Window Help	
Welcome stranded-Brain-2017.assembly 🔀		🖉 Explorer 🦻 Style 😒 🛃 Tracks 🗖 🗖 🖶
NC_000001 Length=248,961,879	₽₽ Q \$1 ↓ 🖻 🛎 🛪	Sequence Presentation
Find: Variant Variant	~	Presentation Font: Segoe UI
Ruler		Track names font:
Consensus T C C T G	21,346,340 C T C C C A G C C	Segoe UI 9
© Coverage		Color sequence foreground:
		Solid
© NC_000001(1>248961879 T C C C T G	СТСССАGСС	
ID_23042280_f → T C C C T G	СТСССА G С	Color sequence background:
ID_41766929 ← T C C C T G	CTCCCAGC	Color by chemistry
$ID_5618964_r \leftarrow T C C C G G$ $ID_13778240_r \leftarrow T C C C T G$	C T C C C A G C C C T C C C A G C C	Alignment style pane may override these colors
ID_23042280_r ← T C C C T_G	СТССАВСС	Alignment
ID_12986562_r ← T C C C <mark>G</mark> G	СТСССА ССС	Editing: No editing a lowed permanently \sim
		Layout reads:
		Color only in targeted coverage regions
		Quality: Show scores
		Alignment coloring
		Color only differences from consensus
		Using color options in Sequence style
		Bases ignored in consensus calling
		Negated weights: 🗌 🔺 🗹 🖉
٩		Trimmed:
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Project Report 🔀 🧮 NC_000001 🛛 🗙		₹ Strategy

Unlike SeqMan Pro, SeqMan Ultra provides a fast method of moving from one variant to the next along the sequences. Start by clicking the magnifying glass tool in the upper right of the Alignment view:



From the Search menu, choose **Variant**; then use the green arrows to search for variants upstream and downstream of the current cursor position.

≣ N	C_000010	
Find:	Variant 🗸 🔶	•
	Consensus Consensus With Ambiguity Variant	TTT
0	Coverage Reference Features Go to Position) a g c

Viewing and working with variants in a tabular display:

- SeqMan Pro Open the Variants table by selecting a contig in the Project window and choosing Variant > Variant Report. Table columns can be added or removed by right-clicking on the table and choosing Show/Hide Column. The types of features included in the table are controlled using the Filter button. SNPs can be marked as putative/confirmed/rejected by clicking in the SNP column.
- SeqMan Ultra Open the Variants view by selecting a contig in the Explorer panel and choosing View > Variants or by using the tool to the right of the Explorer panel.

5	Position	
	0	井
331,301	0	-
487,731	0	<u> </u>
221,578	0	Ă
739,050	0	8
056,056	0	

As in SeqMan Pro, SNPs can be marked as putative/confirmed/rejected by clicking in the SNP column.

From within the Variants view, table columns can be added or removed using the "gear" tool in the top right of the view.

A Cnt → Ref ID Cons Pos B Cnt Cons Pos Type 113 p.Q103 C Cnt Type Type 190 p.A397 B Cnt Called Base Genotype p.V1966 K Cnt Homopolymer P.V0166 721 p.K2574 M Cnt Q Call SNP % Feature Type 721 p.S2690 N Cnt Q Call SNP % Feature Type 7221 p.K2574 M Cnt Q Call SNP % Feature Type Feature Name 7721 p.S2690 V Cnt Q Call SNP % Feature Name 7721 p.S2690 2882 p.89610 V Variant Amino Acid Change Acnt Coll p.82434 9651 p.51984 V Variant Acnt C Cnt G Cnt 7721 p.S2690 Codon Cooling Feature Distance G Cnt T Cnt 7721 p.S11209 Depth Deletion Depth Feature Name Feature Name 520 p.S11209 Scol p.pt114 De	🛃 Choose Columns			_		×		
✓ Residue Count A Cnt A Cnt Ref ID C Cnt Cons Pos D Cnt G Cnt G Cnt Genotype H Cnt Genotype M Cnt P Not Ref N Cnt Genotype N Cnt SNP K Cnt Monopolymer N Cnt P Not Ref Q Call SNP % Feature Type Feature Type Y Cnt Y Cnt V Variant Amino Acid Change Coss Pos Depth Deletion Depth Depth Feature Name Feature Name Fcature Name Feature Type V1140 OK Cancel	Columns Options						8	° ∰ %
A Cnt B Cnt C Cnt D Cnt G Cnt C Cnt D Cnt G Cnt C Cnt C Cnt D Cnt G Cnt C Call SNP % Feature Type Feature Type Feature Name C CoSMIC C Coll C Cnt C C	Available values:			Display columns in	this order:			_
A Cnt ■ Ref ID Cons Pos B Cnt Cons Pos Type 113 p.Q103 C Cnt Type Type 190 p.A397 B Cnt Called Base Called Base 721 p.K2574 M Cnt P Not Ref Q Call 2882 p.89610 N Cnt Q Call SNP % Feature Type 52690 N Cnt Q Call SNP % Feature Name 7721 p.K2574 T Cnt Transcript ID DNA Change Qalled Base Qalled Dase 2882 p.89610 V Variant Amino Acid Change Acnt Called Base 721 p.K2574 CoSMIC Coding Feature Distance Cont T Cnt 722 p.44250 Codon Codon Cont D Cof Cnt 721 p.51002 Deletion Depth Deletion A Cnt 717 p.0573 Depth Feature Name Feature Name 520 p.51002 Tort OK Cancel 092 p.66988 <td< td=""><td>✓ Residue Count</td><td>^</td><td>]</td><td></td><td></td><td></td><td></td><td>Amino</td></td<>	✓ Residue Count	^]					Amino
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D CntRef BaseG CntCalled BaseG CntGenotypeH CntImpactH CntHomopolymerM CntP Not RefQ CallSNP %S CntFeature TypeT CntFeature TypeV CntDNA ChangeW CntDepthY CntDepthCodonC CntCodonG CntCodonC CntCodonC CntCodonC CntCodonC CntDepthFeature TypeFeature TypeVitatPottameT CntCodonC CntCodonC CntCodonC CntDepthFeature NameFeature TypeVitatCodonColonDepthFeature TypeFeature TypeVitatCodonColonDepthFeature TypeFeature TypeVitatCodonColonDepthFeature TypeFeature TypeVitatFeature TypeVitatOKCancel	C Cnt						-	•
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N Cnt Homopolymer M Cnt P Not Ref Q Call SNP % S Cnt Feature Type T Cnt Transcript ID V Cnt DNA Change W Cnt Deletion A Cnta G Cnt COSMIC G Cnt Coding Feature Distance C Cnt Codon G Cnt Deletion T Cnt Deletion Soft. P.S2690 2882 P.S0610 TT>T] V Cnt DNA Change Deltion A Cnt C Coding Feature Distance G Cnt Codon Codon Depth P.Vac273 S421 p.V114' OK Cancel	H Cnt							•
M Crit P Not Ref Q. Call 2882 p. 89610 R Crit S. Cont Feature Type 2882 p. 89610 T Crit Transcript ID DD DD 7C > C] p. 82430 W Crit DNA Change Amino Acid Change 965T p. 06551 Y Crit Depth Deletion 2138 p. N713 Amino Acid Change C Crit G Crit 3625 p. 11209 Coding Feature Distance C Codin G Crit 3625 p. 11209 Codon Coding Feature Name Feature Name p. 66988 Feature Name Feature Name p. 66988 Feature Name Feature Name p. 66988 S20 p. 66988 392 p. 66988 S20 p. 66988 392 p. 11990 OK Cancel S G] p. S127,	K Cnt						7721	p.K2574
R Cnt SNP % Feature Type Feature Name T Cnt V Cnt V Cnt V Cnt V Cnt Amino Acid Change COSMIC Coding Feature Distance Codon Codon Codng Feature Distance Codon Depth Feature Name Feature Name Feature Type V Cnt Codon Coding Feature Distance Codon Depth Feature Name Feature Type V M Com Codon C	M Cnt							p.S2690
S Cnt S Cnt T Cnt V Cnt W Cnt V Cnt V Cnt V Cnt V Cnt V Cnt V Cnt V Cnt V Cnt Codsmic Codsmic Codon Coding Feature Distance Codon Depth Feature Name Feature Name Feature Name Feature Name Feature Name Feature Name Codsmic Codon Coding Feature Distance Codon Depth Feature Name Feature Name F				-			2882	p.R9610
Feature NameTCntTCntTranscript IDVCntDNA ChangeYCntDepthVariantAmino Acid ChangeCOSMICCotCalled BaseCotCodonG CntCodonTCntDNA ChangeDeletionDNA ChangeDeletionCodonTCntDeletionTCntDNA ChangeDeletionCodonG CntDNA ChangeTCntDepthFeature TypeFeature TypeCotOKCancel							'1T>T]	p.L124F
T Cnt V Cnt Transcript ID V Cnt DNA Change W Cnt Amino Acid Change Y Cnt Depth V Variant Acnt Amino Acid Change Cot COSMIC C Cnt Coding Feature Distance G Cnt Codon T Cnt Deletion 77.2 C] Deletion Acnt Codon G Cnt Dona Change T Cnt Deletion 70.2 C] Codon Coding Feature Distance Codon T Cnt Deletion 70.2 C] Depth 70.2 C] Feature Name 70.2 C] Feature Type V OK Cancel							42C>	p.A1810
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A Cnt COSMIC COSMIC Called Base Coding Feature Distance Codon Codon Cons Pos DNA Change Deletion Depth Feature Name Feature Type v OK Cancel								•
Amino Acid Change C Cnt p.74425 COSMIC G Cnt 3625 p.11209 Called Base T Cnt 3005 p.S1002 Codon T Cnt 1717 p.Q573 Codon DNA Change 144T p.S3826 Deletion Depth 5020 p.R5076 Feature Name ✓ SC <c]< td=""> p.A722 OK Cancel S>G] p.S127,</c]<>							-	
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Coding Feature Distance 717 p.Q573 Codon 717 p.Q573 Cons Pos 717 p.Q573 DNA Change 717 p.Q573 Deletion 717 p.Q573 Depth 717 p.G698 Feature Name 717 p.G698 SCO p.R5076 502 SCO p.R5076 502 SCO p.G698 520 SOK Cancel S92							-	
Codon 3421 p.V1143 Cons Pos 024 p.A3423 Deletion 144T p.S382F Depth 5020 p.G698 Feature Name V 5020 p.G698 SC>C] p.A72V elins p.G698 SC>C] p.A72V elins p.G698 SS20 p.G698 5020 p.G698 SS20 p.G198 5020 p.G698 SS20 SS20 p.S1217 5020				TCnt			-	•
Cons Pos p.V114 Cons Pos p.V114 DNA Change p.A342 Deletion p.G698 Depth 520 Feature Type SC>C] OK Cancel							1717	p.Q573
DNA Change 024 p.A342 Deletion 144T p.S3826 Depth 9.6698 1520 Feature Name 5C > C] p.A72V Elins p.G698 1520 DOK Cancel D							3421	p.V1141
Deletion 1441 p.S3824 Depth 2092 p.G698 Feature Name 5C>C] p.A72V Feature Type 0K Cancel							1024	p.A342
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Feature Name 1520 p.R5070 Feature Type 5C>C] p.A72V elins p.G6980 S592 p.P11980 OK Cancel							2092	p.G698F
Feature Type 5C>C] p.A72V, elins p.G444 2092 p.G698 3592 p.P1198 OK Cancel 5>G] p.S12T,	· ·						1520	p.R507ł
elins p.G444 2092 p.G698 3592 p.P1198 OK Cancel 5>G] p.S12T,		~						•
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OK Cancel 5>G] p.S12T,							-	-
			Г	OK	Consel		-	-
			L	UK	Cancel		5>G]	p.S121, p.V342L

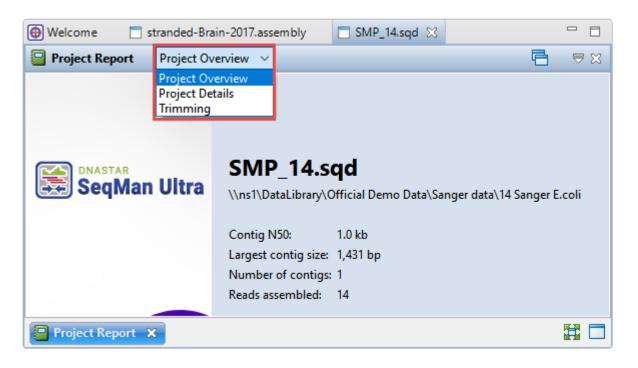
To filter variants shown in the view, use the "filter" tool in the top right of the view to open a filter dialog.

Variant Filter Criteria	×	
Туре	5	? 🌐 %
Substitution Indel		
Min variant size: 0 bp		Amino
	376	p.A626T
Genotype: Any ~	13	p.Q1038
	90	p.A397V
Functional impact	2A>	
Non-coding Max: bp from coding feature		p.V1965
Synonymous Splice sites: Anywhere V	721	p.K2574
✓ Non-synonymous		p.S26901
	382	p.R961C
	T> T]	p.L124R,
🗹 Nonsense 🗹 No-stop 🗹 Frameshift	2C>	p.A181G
Al	C>C]	p.R243C
Alignment	65T	p.D655E
P not ref: 90 %	951	p.S1984
Q call: 0.00	38	p.N713S
SNP min: 15.00 % SNP max: 100.00 %	274	p.A425G
	525	p.11209V
Depth min: 20 Depth max:	005	p.S1002
Include homopolymer length discrepancies	717	p.Q573E
	421	p.V1141
Databases)24	p.A342T,
dbSNP: All ~	44T	p.S382P,
VCF SNP: All)92	p.G698R
	520	p.R507H
COSMIC: All ~	C>C]	p.A72V,
GERP Score	ins	p.G444E
)92	p.G698R
In targeted regions only	i92	p.P1198
In angelea regions only	> G]	p.S12T, p
		p.V342L
Reset to Default Apply OK Can		p.P470T,
		p.K3123

View information about a project or selection

Viewing project reports:

- SeqMan Pro Use the Project > Statistics, Project > Summary, Project > Report or Project > Trim Report commands.
- SeqMan Ultra Use the View > Project Overview, View > Project Details or View > Trimming commands. Once any of the reports is open, you can also open a different report by selecting it from the drop-down menu on the top left of the view.



Viewing details about a selection:

- SeqMan Pro This functionality does not exist.
- SeqMan Ultra Make a selection (e.g. a feature, read, range of sequence, track, etc.) and see a definition or other information in the Details panel. The Details panel may also include Action links that act as shortcuts to additional functions.

🕕 Details 🔀	I Jobs		
Coverage			?
Summary: The	Coverage track displays the coverage	as a plot	
▼ Actions			

Order contigs and close gaps for de novo assembly

To order contigs and close gaps in a de novo .sqd assembly:

- SeqMan Pro Provides a primer walking feature (Contig > Primer Walk) to locate primers that can drive the closure of gaps or to fill in low coverage areas. Results appear in the Alignment view and the Primer Walking report.
- SeqMan Ultra Uses a three step process to create an enhanced template that can be used in future SeqMan NGen assemblies.
 - 1. Order contigs into scaffolds using **Contig > Order Contigs** or **Contig > New Scaffold**.
 - 2. Look for BLAST sequence matches using **Search > Search**.
 - 3. Use **Contig > Add Sequences to Close Gap** to align a sequence match with the existing assembly to close a gap between two contigs.

Add Sequences	Name	Limits	▼ Туре	Add
				Remove
				Remove
	- Alignment op	tions:		
	Minimum mate) %	
		Match size: 12	bp	
		Gap penalty: 0.00)	

For step-by-step instructions, watch the video below or see this help topic.

Search for sequences online

Searching for sequence matches using BLAST or text matches using Entrez:

- SeqMan Pro Use Net Search > BLAST Selection to search NCBI's BLAST database for a sequence match; or Net Search > New Text Search to search NCBI's Entrez database for a text match. Once the search is complete, use the buttons at the top of the search window or additional commands in the Net Search menu to add matches to the project, save them, etc.
- SeqMan Ultra Use Search > Search for BLAST or Search > Search for Text In for Entrez. In both cases, a Search wizard opens. Customize the search as needed, then click Run or Run Now to initiate the search.

😹 Search Online		—		×
Search Choose how to s	search with your 1 nucleotide query sequence.			
✓ Query ✓ Search	Search for: Nucleotides Proteins			
Options	Using: Highly similar sequences (megablast)			\sim
🥪 Job	In: Non-redundant nucleotides (nr)			\sim
	Run Now			
? 💬 🗨	< Back Next >	Run	C	ancel

Monitor the progress of the job in the Jobs panel.

0	Details	III Jobs	: 23		(0 X - 8
_	Job Nar	ne		Status	Started 🔹	Elapsed
P	NC_000	010(7	<	9,357 matches (in 519 sequences)	2/4/20 5:48 PM	18m
P	All Field	ls alo		Expiring at 2/5/20 12:15 PM	1/29/20 12:15	24h 33m

When the search results are ready to review, a link will appear in the **Status** column. Clicking the link opens a new window containing the Text, Table and Pairwise views. Search results are available in the Job panel for 7 days, and (as shown above), a warning message shows when results will expire.

The Table view is similar to the BLAST Search Results window in SeqMan Pro and shows the match as a graphic histogram.

🛃 SeqN	/an Ultra - NC_000010(74862	>213724)					-		×
1: NC_00	0010(74862>21 🗸 🔶 📥		9,357 r	natches	(in 100 se	quences)		7	BB
Acce	Description	Score 🔻	E-Val	% C	% Id	Map			^
AL71	Human DNA sequence f	191321	0	75	100				
NG_0	Homo sapiens zinc finge	155600	0	61	100				
AL58	Human DNA sequence f	65118	0	25	100				
L3906	Homo sapiens interleuki	16420	0	6.6	99				
NG_0	Homo sapiens tubulin b	12178	0	4.7	100				
NG_0	Homo sapiens interleuki	11949	0	4.7	100				
AC21	MACACA MULATTA BA	11074	0	6.1	91				
AC21	MACACA MULATTA BA	10158	0	5.2	92				
AC21	MACACA MULATTA BA	8182	0	4.2	92				
CP03	Eukaryotic synthetic con	7410	0	3.6	94				
Tab		7174	0	2.0	100			-	₩ ■

The Text view provides the same information and additional information in text format.

# BLASTN 2.10.0+																						^
# Query: NC_000010(74862	2>213724)																					
# RID: 3M0UK467016																						603 S
<pre># Database: nt_v5</pre>																						
# Fields: query acc.ver,	, subject ac	c.ver, %	identit	y, a	lignment	length,	mismate	ches, ga	p ope	ns, q.	star	t, q. en	d, s.	start,	s. end	d, eva	lue, b	it sco	re, qu	ery se	q, subj	ect :
# 9357 hits found																						
NC_000010(74862>213724)	AL713922.12	100.000	103604	0	0 1	103604	60802	164405	0.0	1.913	e+05		Huma	DNA :	sequen	ce fro	om clor	e RP11	-631M2	1 on 0	hromoso	me 1(
NC_000010(74862>213724)	AL713922.12	93.508	3666	208	14 440	78 477	14 274	425 23	761	0.0 5	424	TTT-TT	ATTATA	CTTTAA	GTTTTA	GGGTAC	ATGTG	ACAACG	TGCAGG	TTTGT	ACATATG	TATAC
NC_000010(74862>213724)	AL713922.12	90.756	2023	178	6 761	19 781	33 22:	176 24	197	0.0 2	691	ACTCCA	ACAGAC	CTGCAG	CTGAGG	STCCTO	ACCGTT	AGAAGG	AAAACT	AACAAA	CAGAAAG	GACA"
<pre>wc_000010(74862>213724)</pre>	AL713922.12	83.514	2123	259	43 443	22 464	14 17	315 15	254	0.0 1	897	TGGTTT	TTTGTC	CTTGCG	ATAGTT	GCTGA	GAATAA	TGGTTT	CCATTT	TCATCO	ATGTCCC	TACA
<pre>wc_000010(74862>213724)</pre>	AL713922.12	95.747	964 36	1	132725	133683	25707	26670	0.0	1548	CA	AGGAATGT	GAAGGA	CTCTT	CAAGGA	SAACTA	CAAACO	ACTGCT	CAATGA	AATAA	AGAGGAT	ACAA
<pre>WC_000010(74862>213724)</pre>	AL713922.12	93.100	1000	62	6 440	52 450	56 47:	186 46	189	0.0 14	458	TTTATA	TATATA	TATATT	TTATT	ATACTT	TAAGTT	TTAGGG	TACATG	TGCAC/	ACGTGCA	GGTT
NC_000010(74862>213724)	AL713922.12	92.012	964 48	5	132725	133683	106571	105632	0.0	1327	CA	AGGAATGT	GAAGGA	CTCTT	CAAGGA	SAACTA	CAAACO	ACTGCT	CAATGA	AATAA	AGAGGAT	ACAA
NC_000010(74862>213724)	AL713922.12	93.686	776 40	7	131962	132728	27456	26681	0.0	1153	TT	ATTTTTA	T-TT-T	TTTA-	ATTTTTA	T-TATT	ATTATT	ATACTT	TAAGTT	TTAGGO	TACATGT	GCAC/
				-																		E .31
🔟 Pairwise 🛛 🚟 Text																						🛊 E

The Pairwise view allows you to compare a match to the consensus by aligning them pairwise.

Align: 1: NC_0C ~ with AL	L713922.12(60802>164405):	Human DNA sequence	e from clone RP11-631M2	1 on chromosome 10,	complete seque 🗸 :e
DNA alignment [Matrix: "N		Gap extension penalty:	1]		A
NC_000010(74862 1>103604	2>213724) AL713922.12 60802>164405	%ldentity %Gaps ld	Gap Gap dentical Count Length	Score Length	
Alignment 1>103604	60802>164405	100.0% 0.0% 1	103,604 0 0	518,020 103,604	
Ruler	120	130	140	150	160
Inc_000010(74862>2137					
© AL713922.12	AAACAATTCTCC	TGCCTCAGCCT	TCCCGAGTAGCTG	GGATTACAGG	TGCGCGCCACC
		_	:		
Pairwise 🖉 Text					