MacVector 17.5.3

for Mac OS X

The online updater for this release is 153.6 MB in size

You must be running MacVector 15.5.4 or later for this updater to work!

If the updater fails, DOWNLOAD THE FULL INSTALLER HERE!

System Requirements

MacVector 17.5 is supported on any Intel Macintosh running **Mac OS X 10.9** (OS X Mavericks) or higher. There are no other specific hardware requirements for MacVector – if your machine can run OS X 10.9 or above, it can run MacVector. A complete installation of MacVector 17.5 uses approximately 280 MB of disk space. Please note this release will NOT run on OS X 10.7 or earlier and is likely to have problems on OS X 10.8.

ASSEMBLER NOTE: If you are performing contig assembly using *MacVector with Assembler*, we recommend you have **at least** 2 GB of **FREE** RAM available on your machine. For any serious NGS work using phrap, velvet, SPAdes, Flye or bowtie, you should have at least 8 GB and preferably 16 GB or more for satisfactory performance.

Installation and License Activation

You can choose to install MacVector in one of two ways; if you want to install MacVector for all users of the computer, simply drag the MacVector folder onto the Applications folder. You will be prompted for a system administrator account and password during this copy. If you don't have administrative privileges, or if you want to install it for just your own use, you can install MacVector in the /Applications/ folder in your own personal home directory. To do that, double-click on the **OpenUserApplicationsFolder** icon to open the folder in a separate window, then drag the MacVector folder into that window.

When you first run MacVector, you must enter a valid license owner, serial number and activation code if one does not already exist on your computer. This information is usually sent by e-mail but is also printed on the inside of the CD sleeve if you opted to receive MacVector on physical media. If you previously installed an earlier version of MacVector and have a serial number with a maintenance end date of February 1st 2020 or later, MacVector 17.5 will automatically use your existing license and you will not be required to enter the details again. NOTE: you can optionally activate a license for your own use without requiring Administrative privileges.

Changes for MacVector 17.5.3

Bug Fixes and Enhancements

There have been a number of changes to increase general stability on macOS 10.13 (High Sierra).

The Align to Reference window no longer scrolls back to the beginning when it is resized.

There is a new right-click (<ctrl>-click) context-sensitive menu item in the *Align to Reference* Editor called Cut Clipped Residues and Re-align. This will remove the clipped (also called "trimmed") residues from the ends of reads, rename then by appending ".r" (Right) or ".l" (Left) and realign them to the reference using the last used settings. This is a great way to explore unusual splicing events such as occurs with SARS-CoV-2.

The maximum **hash** value for *Align to Folder* has been increased to 14. This speeds up searches at the cost of using more memory.

A crash in the MSA **Picture** tab has been fixed.

Edit | Cut is now enabled in the *Align to Reference* Editor.

The threshold for the number of reads that can be in an *Align to Reference* assembly before **Undo** is disabled has been increased to 5000.

Changes for MacVector 17.5.2

Bug Fixes and Enhancements

A hang when attempting to reverse-complement a contig in the *Contig* Editor has been fixed.

The *Align to Reference* cDNA alignment algorithm has been tweaked to do a better job splitting read segments that end at short imperfect repeats.

A bug where you could not copy short sections of sequence from within a single sequence in the MSA Editor window has been fixed.

Some glitches in the Align To Reference Editor have been fixed.

You can now use **Find** | **Replace** to find and replace <space> characters in sequences (make sure to check "Find literal").

Align To Folder now handles interleaved fasta and fastq files.

Translating orfs on the minus strand for the automatic **Scan DNA** | **ORF** items now honors the minus strand.

A bug where fastq paired end reads would sometimes show up as length "1" has been fixed.

A bug where the *Graphics Palette* would not respond correctly to turning on/off RE sites after a full RE analysis has been fixed.

The first exon on the complementary strand of a cDNA alignment in *Align to Reference* no displays correctly.

You can now paste a copied sequence to replace a section of DNA to add new features. i.e., copy an annotated source fragment, **Edit** | **Find** that same sequence on a destination and paste to replace – any new features on the clipboard will be added without changing any existing features on the target sequence.

Enhancements

There is a new **Trimming** button in the *Align to Reference* and *Contig* Editors. You can use this to show/hide the greyed out trimmed (also called "clipped") residues in the alignment.

You can now add features to a sequence by copying an annotated region from one sequence document, selecting the identical sequence in a second sequence document (use Edit->Find to make sure it is identical) then using **Edit->Paste**. This will not change any existing features in the target document but will simply add any new features from the copied source sequence.

MacVector no longer ships with an internal NCBI E-Utils API key. This should improve response when blast searching or using Entrez. However, we do recommend you fill out the form and get your own API Key from the NCBI for optimal performance.

Changes for MacVector 17.5.1

Bug Fixes

A crashing bug affecting user running macOS High Sierra (10.13) has been fixed.

Problems using the **Find Sequencing Primer/Probe** function with sequences that have negative numbering origins has been fixed.

Manipulating **Gibson Assembly/LIC** projects using a 3'exonuclease now correctly honors the minimum overlap setting.

You can now toggle the new domain outlining functionality on/off from the Picture tab.

Changes for MacVector 17.5.0

Outlining Shared Domains in Aligned Sequences

Multiple Sequence Alignments now retain feature information from their individual input sequences and can use this information to outline shared domains in the aligned sequences. To use this feature, first individually annotate the sequences you want to align, make sure the domains/features you are interested in are visible and set the **Fill** color to the color you would like to see in the alignment. Then add the sequences to a multiple sequence alignment document and align in the usual way (or, keep the single sequence documents open and choose **Analyze** | **Align Multiple Sequences Using...**). Then click on the **Mode** toolbar button (shown below) and select **Show Features**.



This turns on a simple feature display mode in the **Editor** tab where you can see the extent and color of the features. When you switch to the **Picture** tab, you will see colored outlines around the shared domains;

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| WP | _122620685.1 | {WP_1226206 | 585} Q | GAP | IAEI | AAR | LHL | SRGT | VRN | YMAT | AIR | KVGA | RNR | VDA | IRI | VQSAG |
| WP | _153176890.1 | {WP_1531768 | 390} Q | GAP | IAEI | AAR | LHL | SRGT | VRN | YLAT | AVR | KVGA | RNR | VDA | I R I | V Q S A G |
| WP | _061441281.1 | {WP_0614412 | 281) Q | GAP | IAEI | AAR | LHL | SRGT | VRN | YLAT | AVR | KVGA | RNR | VDA | I R I | VQSAG |
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de novo Assembly of PacBio and Oxford Nanopore reads with Flye

Flye is an assembler algorithm tuned to assemble poor quality long reads such as those produced by PacBio and Oxford Nanopore sequencers. Because these reads tend to be very error prone, MacVector 17.5 also includes an optional polishing step using *Racon*. With typical bacterial genome assemblies, it is fairly common to be able to assemble reads into a single full-length genome contig, such as with this set of reads from a strain of *E. coli*;

| • | • | | | | | Unt | itled — | Pro | ject | | | | | | |
|--------------|----------------------|----------|-----------|-------|--------------|------------|---------|------|------------|--------|-----|-------|----------------|-------|------|
| Ad | d Reads Add Seqs Ad | dd Ref | Remove | Prefs | ✓ Replica | O Phred | CrossM | atch | 🍑 Phrap | Bowtie | SP/ | Ades | Solution Velve | Flye | Q. |
| | Project I | Propert | ties | Co | overage | | | | | | | | | | |
| | Name | | | | Status | Len | gth 🗸 | # | | ClipL | 0 | ClipR | | Start | Stop |
| | sra_data.fasta | | | | PacBio (raw | v) | 4313 | | 59046 | | | | | | |
| ₩E. | ▼ Flye sra_data - 17 | 7:36 - 、 | Jan 6, 20 | 20 | | | | | | | | | | | |
| ₩E. | contig_1 Conti | g 1 | | | | 4 | 642427 | | 60985 | | 1 | 4642 | 427 | 1 | 46 |
| ₩E. | ▼ Flye sra_data - 17 | 7:52 - 、 | Jan 6, 20 | 20 | | | | | | | | | | | |
| 4 11. | contig_1 Conti | g 2 | | | | 46 | 642444 | | 60981 | | 1 | 46424 | 144 | 1 | 46 |
| ₩. | ▼ Flye sra_data - 18 | 3:25 - 、 | Jan 6, 20 | 020 | | | | | | | | | | | |
| ₩. | contig_1 Conti | g 3 | | | | 46 | 642495 | | 60981 | | 1 | 46424 | 195 | 1 | 46 |
| | | | | | | | | | | | | | | | |

Note that it is important to tell MacVector what type of reads you are assembling - this is easily done by double-clicking on the **Status** item after importing the reads via the **Add Reads** toolbar button.

| Using the file named sra_da Downloads directory. | ata.fasta in the /Users/kendall/ | |
|---|---|---|
| Source of Data: | PacBio (raw) | ٢ |
| Quality Score Encoding: | Automatic (will try to determine from data) | ٢ |
| Paired-end Type: | Unpaired | ٢ |
| | Locate Cancel OK | |

The three jobs above are the result of varying *Flye* parameters. However, all three resulted in a single contig approximately matching the size of the *E. coli* genome - the differences in length reflect different "polishing" strategies with the last one having one round of internal polishing by *Flye* and one additional round of polishing by *Racon*.

| Read pre-processing |
|---|
| Discard reads less than 1,000 nt |
| FLYE Options |
| Expected genome size: 5 Mbp |
| Threads: 11 🗘 |
| Flye polishing iterations: |
| Minimum overlap between reads: 1,000 bp |
| Initial minimum coverage: 50 |
| Run additional consensus polishing with Racon |
| Iterations: 1 C Window width: 500 |
| ? Defaults Cancel OK |

As one of the last steps in *Flye* assembly, MacVector aligns the input reads against each contig consensus using *minimap2* so that you can view the alignments in the *Contig* **Editor**. Because of the noisy nature of Long Read sequence data, MacVector removes additional erroneous inserted residues in each read prior to display to clean up the visual alignments.

Contig and Align to Reference Editor Enhancements

There have been a number of enhancements to these editors, primarily to aid in visualizing edits and quality values and to "clean up" the visual appearance of alignments.

Residue Background Colored by Quality

A **Shading** toolbar button lets you turn on coloring based on the quality value assigned to each residue;



The intensity of the colors indicates the phred-based quality value of each residue. For

individual reads, this ranges from 0 (deep red) through 20 (white) to 40 or above (deep green). The consensus scale is doubled and ranges from 0 (deep red) through 40 (white) to 80 or above (deep green). Gaps are always shown with a white background. As with earlier versions of MacVector, you can "mouse-over" a residue to view the numerical information in a tooltip.

Edited Residues Have a Blue Background

Edited residues are always given a phred quality value of 99 - these residues are given a blue background;

30 40 50 \ATTATGCAAAGAATCAACTTGTATT/

AACCCCATTTATGGTCGCTATGACAG CGTTTTCGTTCCATTGGCCCTCAAAC AAACCCCTTGAACCGTAACATTCAAA AAACCCCGTAAATGCTCTATTTTTAG, TTTTCGTTCCATTGGCCCTCAAACCC

Base Calling with Phred

You can now directly run phred on Sanger sequencing trace files in the *Align to Reference* Editor by clicking on the Basecall toolbar item with the appropriate sequences selected;



GAAATGCACAGGAAGGATTCG

Note that you must do this before aligning the sequences. You can always "reset" (i.e. unalign) and sequence by selecting its name and choosing Reset (un-align) Selected Reads from the right-click (or <ctrl>-click) context-sensitive menu.

Editing Enhancements

There are some new context-sensitive menu items in the Align to Reference Editor tab

Delete Clipped Residues - deletes any greyed-out ("clipped" or "trimmed") residues. While these are ignored by the consensus calculation, some users prefer to delete them for a cleaner looking alignment.

Close Gaps by Deleting Residues - you'll often see gaps in the consensus where one or more reads has an additional erroneous inserted residue. This menu item removes the extra residues from the read, cleaning up the visual appearance of the alignment.

You can now "nudge" reads in the *Align to Reference* Editor tab. Select the name of the sequence you want to nudge and use the left/right arrow keys to move it around. If you have problematic alignments where you need to physically insert residues or gaps, hold down the <option> key when typing to insert the residue or gap before the currently selected residue, then use the nudge function to get the alignment right. The consensus updates in real time.

Miscellaneous Enhancements

There have been a large number of minor enhancements. Some, such as reworking code

behind the scenes to replace deprecated Apple functions and refactoring code for better stability and performance to help ensure that MacVector will continue to work on upcoming releases of macOS and take advantage of improved hardware. There have also been improvements to Dark Mode support in many areas and much better handling of the labels in crowded **Map** views.

Support information

For assistance with MacVector, please contact your local MacVector, Inc office. You will need a current MacVector maintenance contract to be eligible for technical support other than for basic installation or licensing problems. New sales of MacVector include 12 months of support that also entitles you to any upgrades to MacVector released during the maintenance period.

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When contacting Customer Support with a technical problem, please be prepared to give your product serial number as well as a detailed description of your problem and any error messages you encounter. Visit the MacVector Web site for details of any available updates, and any relevant information that could not be added to these release notes in time for publication:

http://www.macvector.com

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Software for Scientists