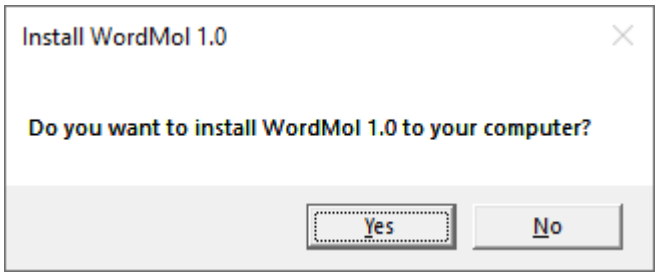
# Contents

**Word Mol 1.0**

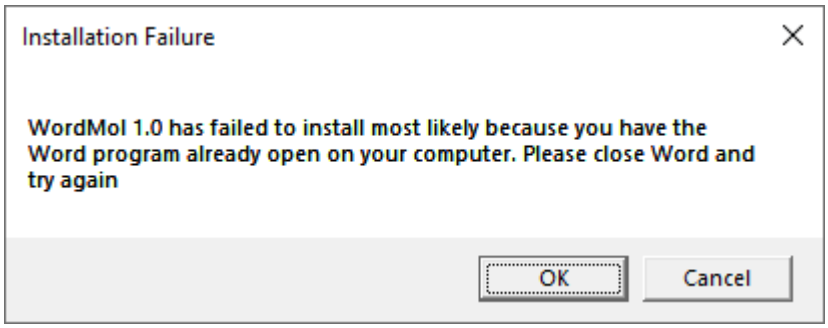
This program is a Word Addin and it allows you to use word to analyse basic DNA Sequences. The best way to learn the program is simply to use it.

## 1 Installing WordMol 1.0

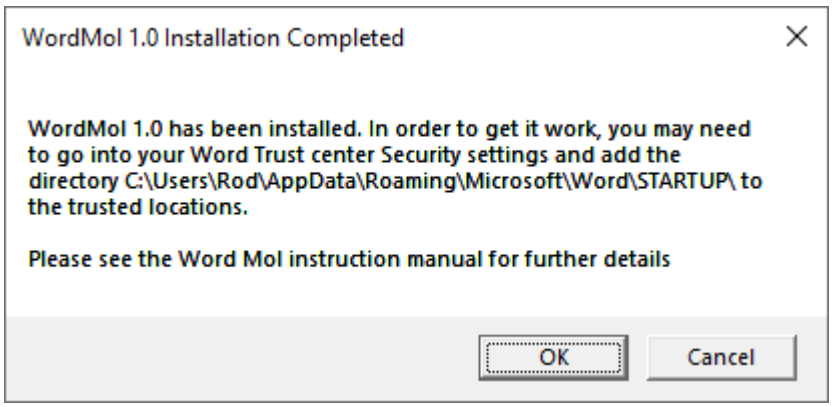
After you have Extracted all the files from the WordMol.zip file you are ready to install program. After double clicking Install WordMol 1.0.vbs the following prompt appears:




Please make sure your Word program is closed before clicking Yes to the above question box or else if Word is open, you will receive the following installation error




Assuming Word is not currently running on your computer the program instantly installs and the final message box will be displayed:

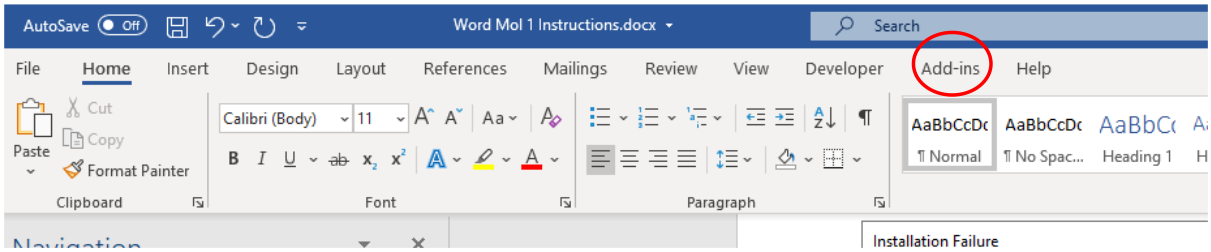



You should make a note of the directory path that is showing in this window because you will need this information shortly. In my example above the directory path I will need to add to my Word trusted location is c:\users\Rod\AppData\Roaming\Microsoft\Word\STARTUP.

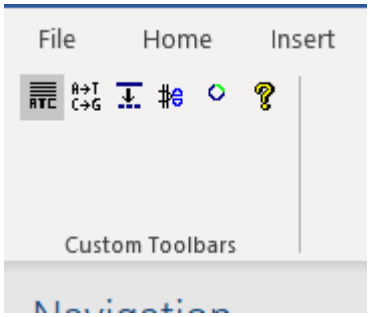
Note the program also adds a folder in your default documents folder called WordMol Materials. In this folder it will copy this manual along with several Word documents which

contain example DNA sequences that form part of a learning tutorial that you can use with this manual to obtain a good understanding of the full functionality of this program.

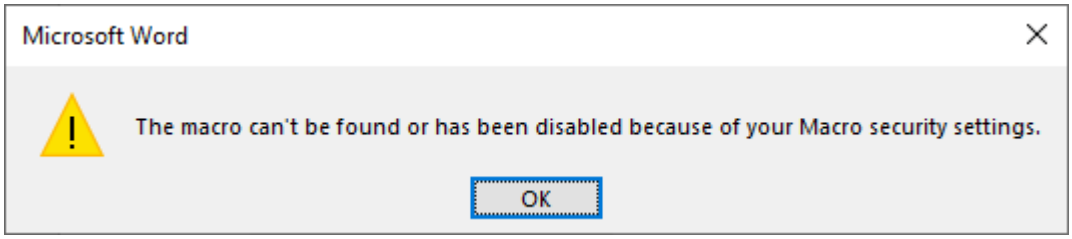
Now restart Word and you should see a new item in the menu strip called Add-ins



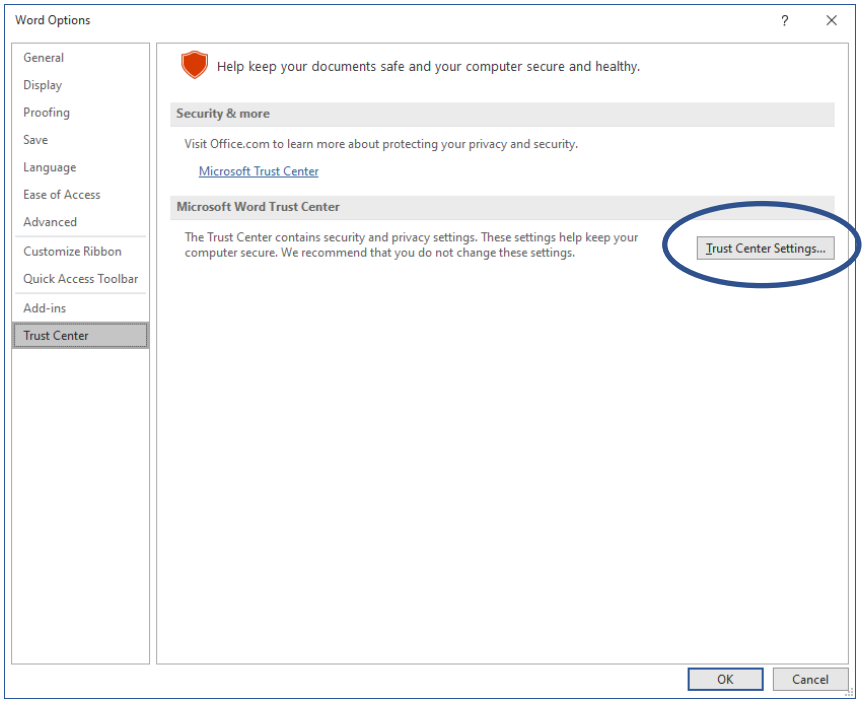

Click on Add-ins and you will see the WordMol 1.0 Toolbar



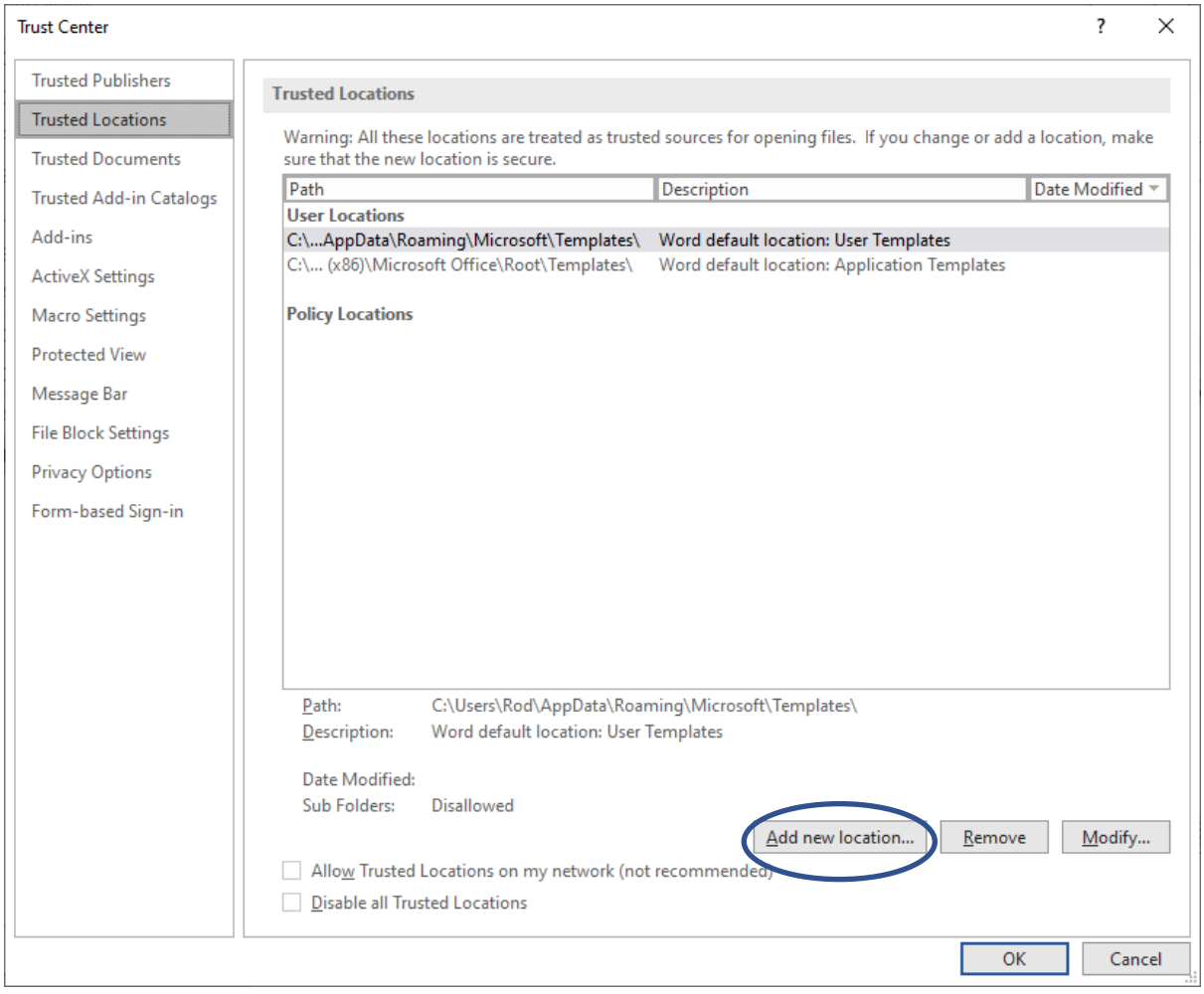

Click on the yellow question mark key and if you get the following error then you need to do the following to finish installing this software.



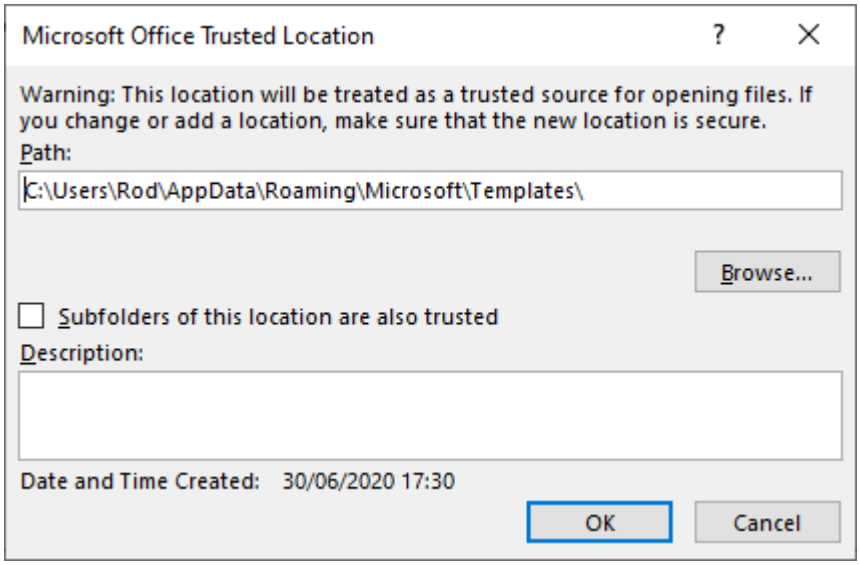

Click on File Options > Trust Center to open the following window




Now click Trust Center Settings and go to <Trusted Locations>



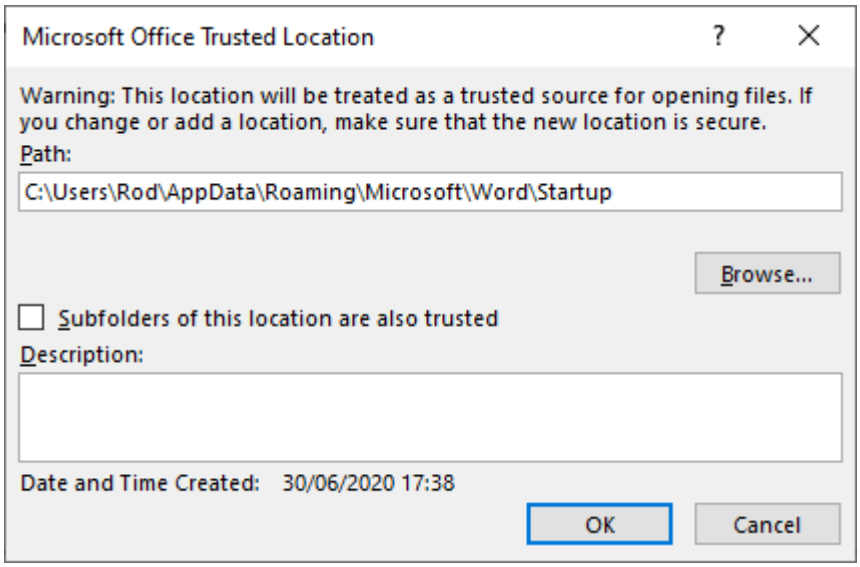

Now Click <Add new location> and a similar window to one below should now be displayed



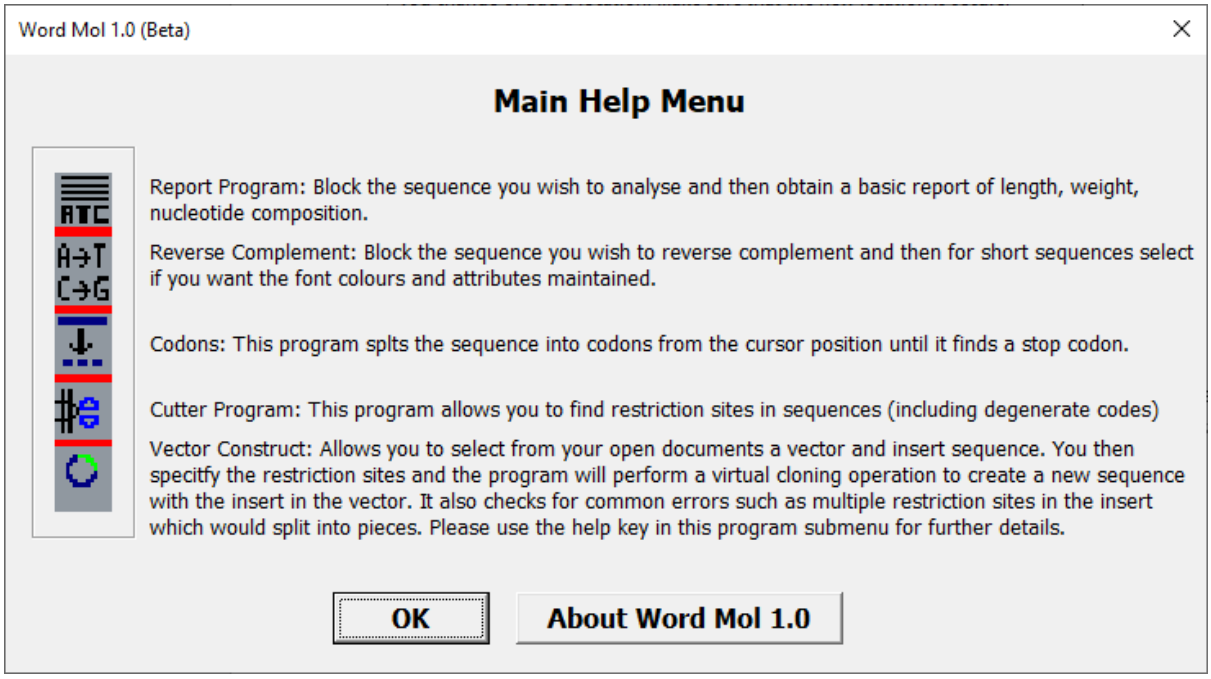

Your own computer will have C:\users\<Your login name>\AppData\Roaming\Microsoft\Templates

You need to take this path and change it to

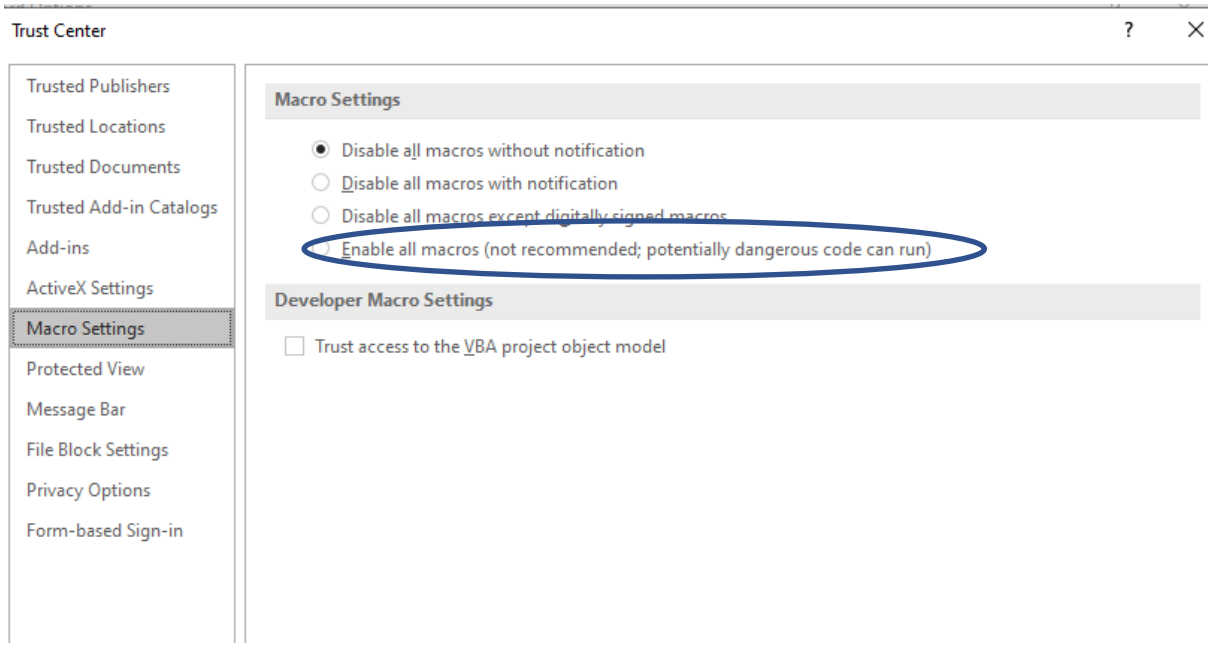
C:\users\<Your login name>\AppData\Roaming\Microsoft\Word\Startup. If you're not sure what the correct path is, you can always run the install program again to see what path you need to enter.




Once you have added this location. Click OK and dismiss the Trust Center box. Now exit word completely and restart the program. If you have successfully defined the WordMol 1.0 program as safe, when you now push the yellow question mark the following screen should be displayed.




If you still get Macro security error then go back to the trust center but this time select Macro settings and change the disable all macros without notification to Enable all macros. This option should not be necessary but if you do need to make this change, due to an old version of Word, then please be very careful about opening Word documents downloaded from the internet with the extension .docm or .doc.




## 2 Using WordMol 1.0

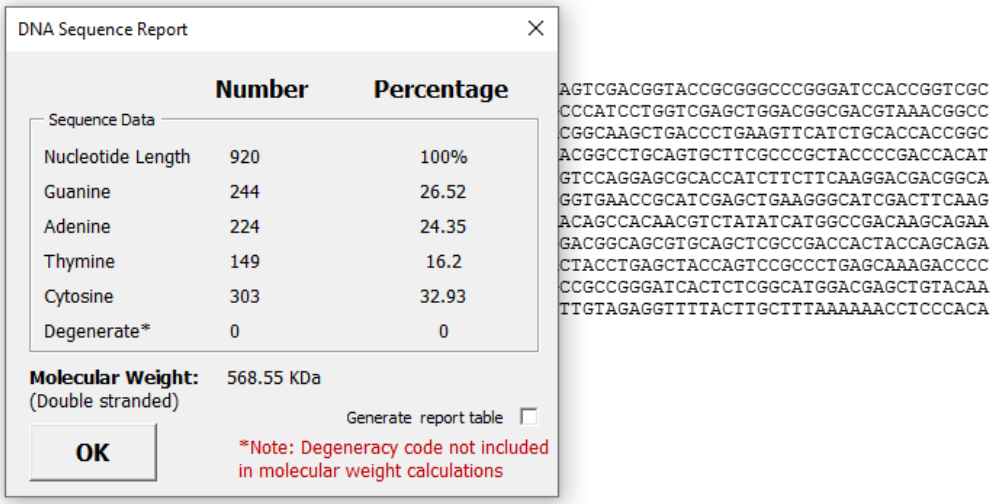
The best way to learn the program is to use the several tutorial sequences to see how the program works. Most of the functions are very simple to use with the virtual cloning function possessing the most functionality.

The manual will now briefly document each function.

## 2.1 Sequence Report

Go to your documents folder and open the WordMol Materials folder where you will find all the demo sequences required to follow the examples shown in this manual below.

Now open Demo Sequence 1 and click place the cursor at the beginning of the sequence and push the sequence report button and the following little window will appear




If you want the report, simply tick this option push OK and a report appears under the sequence.

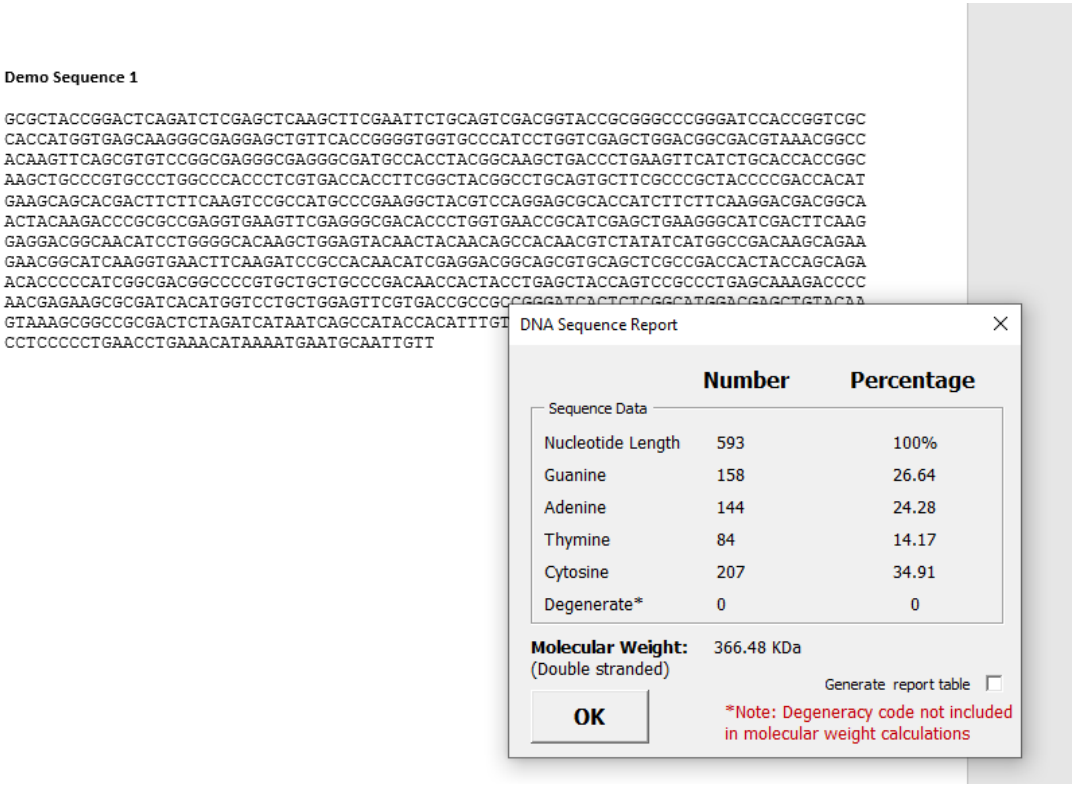
**Demo Sequence 1**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGC
CACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC
AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACAT
GAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA
ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAA
GAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA
ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCC
AACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA
GTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACA
CCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

**Report for Blocked Sequence**

| Nucleotide | Number | Percentage |
|---|---|---|
| Nucleotide Length | 920 | 100% |
| Adenine | 224 | 24.35 |
| Thymine | 149 | 16.2 |
| Cytosine | 303 | 32.93 |
| Guanine | 244 | 26.52 |
| Degenerate* | 0 | 0 |
| Molecular Weight is 568.55 KDa | | |

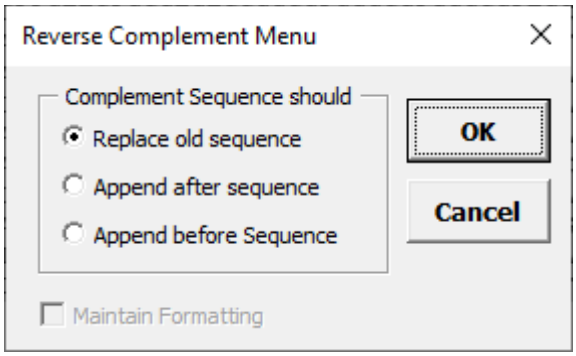
Note you can also just block a little bit of the sequence to just get the nucleotide report for that part you have selected



## 2.2 Reverse Complement

You must select the sequence you want to reverse complement. Using Demo sequence 1, I have selected the whole sequence and pushed the Reverse Complement button

The following menu now appears:




You can either replace the sequence, place the reverse complement under the sequence or before the sequence. In this example, I shall place the reverse complement under the original sequence:

**Demo Sequence 1**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGC
CACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC
AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACAT
GAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA
ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAA
GAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA
ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCC
AACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA
GTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACA
CCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

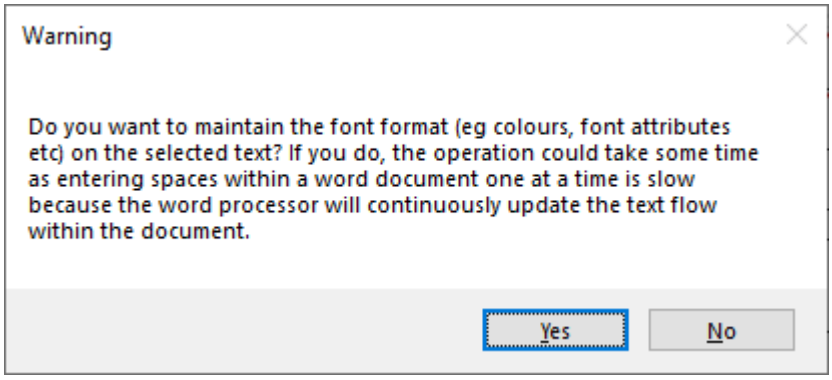
*AACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAAT
GTGGTATGGCTGATTATGATCTAGAGTCGCGGCCGCTTTACTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCG
GTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGTAGCTCAGGTAGTG
GTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCT
CGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTG
TAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAG
GGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGT
AGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGGCGAAGCACTGCAGGCCGTAG
CCGAAGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTA
GGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCA
CCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGGTGGCGACCGGTGGATCCCGGGCCCGCGGTACCGTCGACTGC
AGAATTCGAAGCTTGAGCTCGAGATCTGAGTCCGGTAGCGC

*Figure 1: Demo 1 Sequence after the reverse complement function has been applied. Note WordMol 1.0 also supports the degeneracy code and will reverse complement it.*

## 2.3 Putting a Sequence into Codons 

To demonstrate this option open Demo sequence 2.docx

Wherever the cursor is within the sequence this is where it will start to place the sequence into 3 letter codons until it reaches a stop codon TAG, TAA or TGA. If your cursor is at the beginning of the sequence you will get this message




If you select No, the program copies the sequence into its memory processes it and then replaces the whole sequence with the codon sequence in a single step so is very fast. If however you select <Yes>, then you will see a progress bar appear because the program then steps through the sequence adding each space one at a time.

If you block select only a part of the sequence, or you put the cursor midway through the sequence then only that part of the sequence is processed.

Note in Figure 2 the block of nucleotides I selected led to an early stop codon in the sequence.

**Demo Sequence 2**

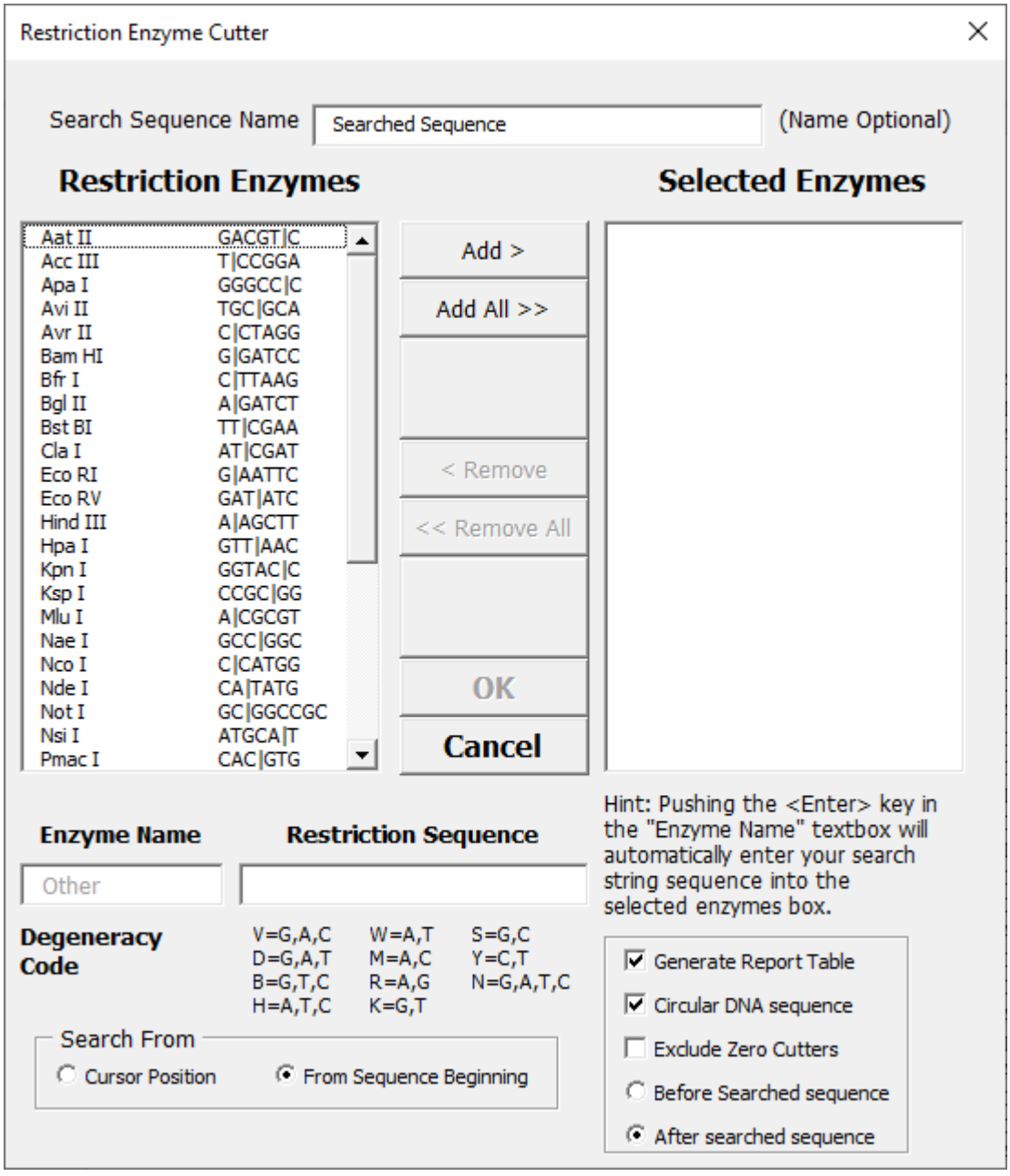
GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGC
CACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACA AGT TCA GCG TGT CCG GCG AGG GCG AGG GCG ATG CCA CCT ACG GCA AGC TGA
CCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAG
TGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCG
CACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCA
TCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAAC
GTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGT
GCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCT
ACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC
ACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTT
TTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

*Figure 2: In this example I have run the WordMol codon creator on a subblock of Demo Sequence 2. The codon generator starts the three letter grouping from the first nucleotide in the block. It just happened in the example I used, that the reading frame led to an early stop codon (TGA) ending the codon sequence early.*

## 2.4 Cutter Program

In order to demonstrate this program please open Demo Sequence 3 file. Place the cursor at the beginning of the sequence and then push the cutter program button in the Wordmol addin menu.

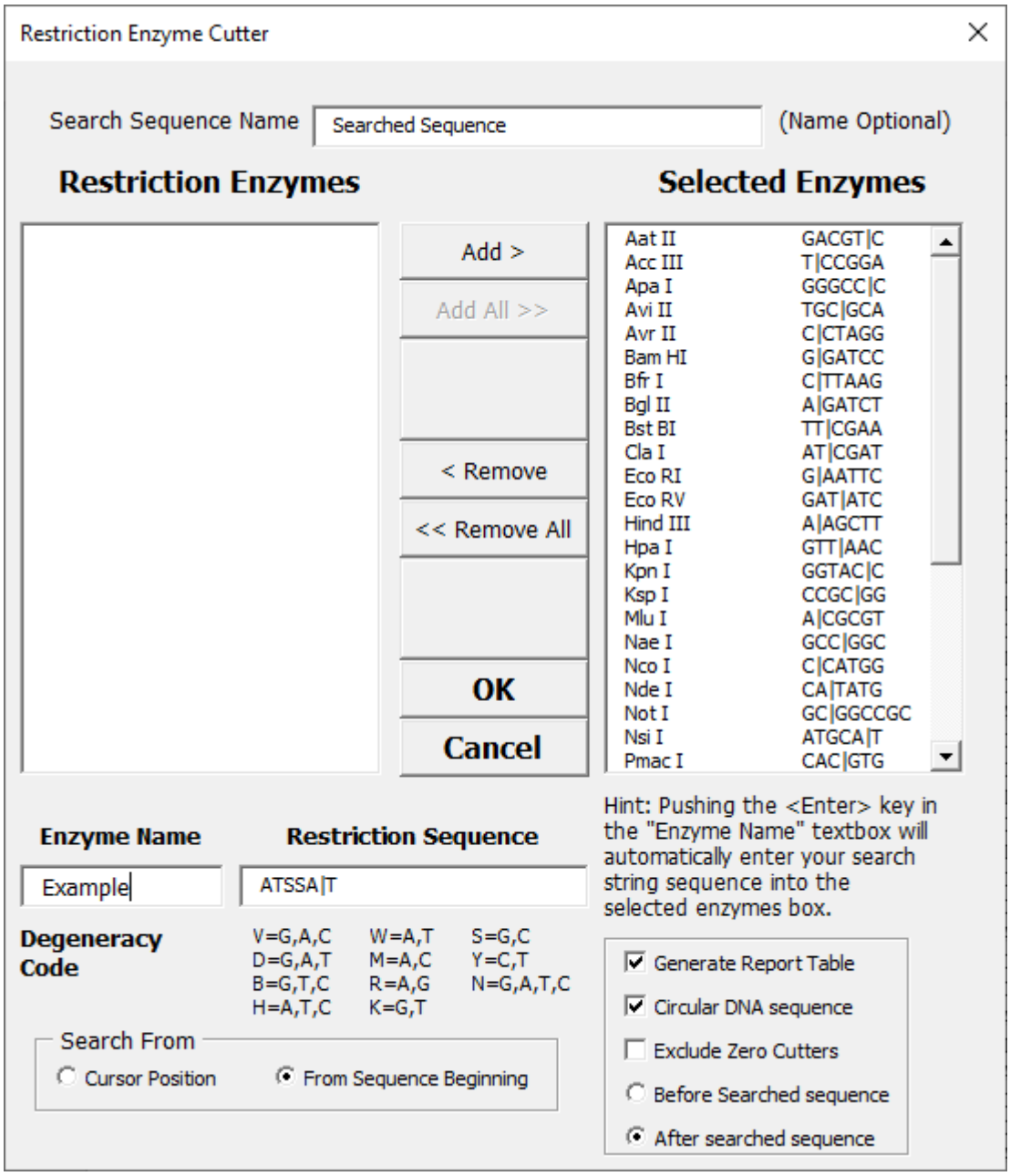
The following menu appears




The program has a list of common restriction enzymes used in molecular biology. You can also enter multiple user defined enzymes by typing the restriction site into the restriction site and the corresponding enzyme name in the Enzyme name box. You indicate the cut point with the pipe symbol (|). You can also enter degenerate code into the search box.

Let's first click the Add All>> button to search for all the default WordMol 1 enzymes. Next we shall add a degenerate sequence. For example a restriction enzyme that cuts ATSSA|T (where S can be G or C) could be entered in the restriction sequence as shown in the screenshot on the next page.

You can quickly enter multiple enzymes this way by pushing the Enter key or the Add > button.




The program now offers several choices. If the cursor position is somewhere in the middle of the sequence you can tell it to search from that position or from the beginning of the sequence. If you have blocked the sequence this option will be greyed out because the cutter program will search only the inside the selected block of text.

The option box allows you to produce a report table either before or after the sequence (bottom radio button selection) and exclude enzymes from the table that do not cut sequence to make the table less cumbersome. Note the option of assuming the DNA sequence is circular only affects the report table not the actual restriction enzyme search in that it simply assumes one less DNA fragment if the DNA sequence was circular. The program is not advanced enough to detect a search sequence that bridges the beginning and the end of the sequence which could occur if the DNA sequence was circular.

For our example let's put the report table after the search sequence, exclude zero cutters and assume that the DNA sequence is linear not circular. We now push OK

The sequence will now look something like Figure 3


*Figure 3: Part of Demo Sequence 3 after running the cutter program*

And the report table will look similar to Figure 4. In general, you will probably want to use the cutter program with fewer enzymes to search out possible restriction sites for cloning strategies. Once you have a cloning strategy, WordMol allows you to check it using its final function, virtual molecular cloning.

**Searched Sequence (3996 bp)**

| Enzyme Name | Enzyme Sequence | No. of Cuts | Cut Positions |
|---|---|---|---|
| Acc III | T\|CCGGA | 1 | 3133 |
| Apa I | GGGCC\|C | 4 | 63, 983, 1903, 3147 |
| Bam HI | G\|GATCC | 4 | 66, 986, 1906, 3206 |
| Bfr I | C\|TTAAG | 1 | 3052 |
| Bgl II | A\|GATCT | 4 | 15, 935, 1855, 3518 |
| Bst BI | TT\|CGAA | 4 | 33, 953, 1873, 3992 |
| Eco RI | G\|AATTC | 5 | 35, 955, 1875, 3043, 3218 |
| Hind III | A\|AGCTT | 7 | 28, 948, 965, 1868, 1885, 3055, 3199 |
| Kpn I | GGTAC\|C | 4 | 55, 975, 1895, 3772 |
| Ksp I | CCGC\|GG | 3 | 58, 978, 1898 |
| Nae I | GCC\|GGC | 1 | 3166 |
| Nco I | C\|CATGG | 4 | 83, 1003, 1923, 3063 |
| Not I | GC\|GGCCGC | 5 | 807, 1727, 2647, 3225, 3240 |
| Pst I | CTGCA\|G | 6 | 44, 293, 1213, 2133, 3033, 3929 |
| Pvu II | CAG\|CTG | 2 | 2815, 3268 |
| Rsa I | GT\|AC | 10 | 53, 514, 796, 973, 1434, 1716, 1893, 2354, 2636, 3770 |
| Sac I | GAGCT\|C | 3 | 26, 946, 1866 |
| Sal I | G\|TCGAC | 1 | 45 |
| Sau 3A | \|GATC | 24 | 15, 66, 584, 732, 770, 820, 935, 986, 1504, 1652, 1690, 1740, 1855, 1906, 2424, 2572, 2610, 2660, 3092, 3206, 3408, 3518, 3653, 3841 |
| Sma I | CCC\|GGG | 3 | 64, 984, 1904 |
| Spe I | A\|CTAGT | 1 | 3022 |
| Xba I | T\|CTAGA | 4 | 817, 1737, 2657, 3247 |
| Xho I | C\|TCGAG | 4 | 19, 939, 1859, 3607 |
| Example | ATSS\|AT | 1 | 3409 |

| Resulting Fragment(s) Generated From | Assuming DNA is | No. of Fragments | Fragment Length(s) (bp) |
|---|---|---|---|
| Above Enzymes | Linear | 98 | 15, 4, 7, 2, 5, 2, 9, 1, 8, 2, 3, 5, 1, 2, 17, 210, 221, 70, 148, 38, 26, 11, 10, 3, 115, 4, 7, 2, 5, 2, 10, 8, 2, 3, 5, 1, 2, 17, 210, 221, 70, 148, 38, 26, 11, 10, 3, 115, 4, 7, 2, 5, 2, 10, 8, 2, 3, 5, 1, 2, 17, 210, 221, 70, 148, 38, 26, 11, 10, 3, 155, 207, 11, 10, 9, 3, 8, 29, 41, 14, 19, 33, 7, 12, 7, 15, 7, 21, 141, 109, 89, 46, 117, 2, 69, 88, 63, 4 |

*Figure 4:* The report table after running the cutter program. Note it found our example enzyme cut at one place nucleotide 3409.

## 2.5 Virtual Molecular Cloning

This function allows you to check a cloning strategy in terms of placing a DNA insert into a DNA plasmid or vector.

Therefore, the program requires two DNA sequence on which to work, one is defined as the vector the other is defined as the insert. These sequences might be in different Word documents or they may both be in same Word document. Because of this, when you click

this button the first thing Word does is search through all your open documents in Word looking for possible DNA sequences and loading them into a menu for you to select. Therefore, before you run this program you should make sure you have closed all unnecessary documents and have only documents open documents that contain your relevant sequence. In order to demonstrate some of the functionality of this part of the program, we are going to work through several sequences.

First please open from the WordMol folder, “Cloning Demo Create Codons.docx”

Now click the molecular cloning button and the following menu will be displayed.

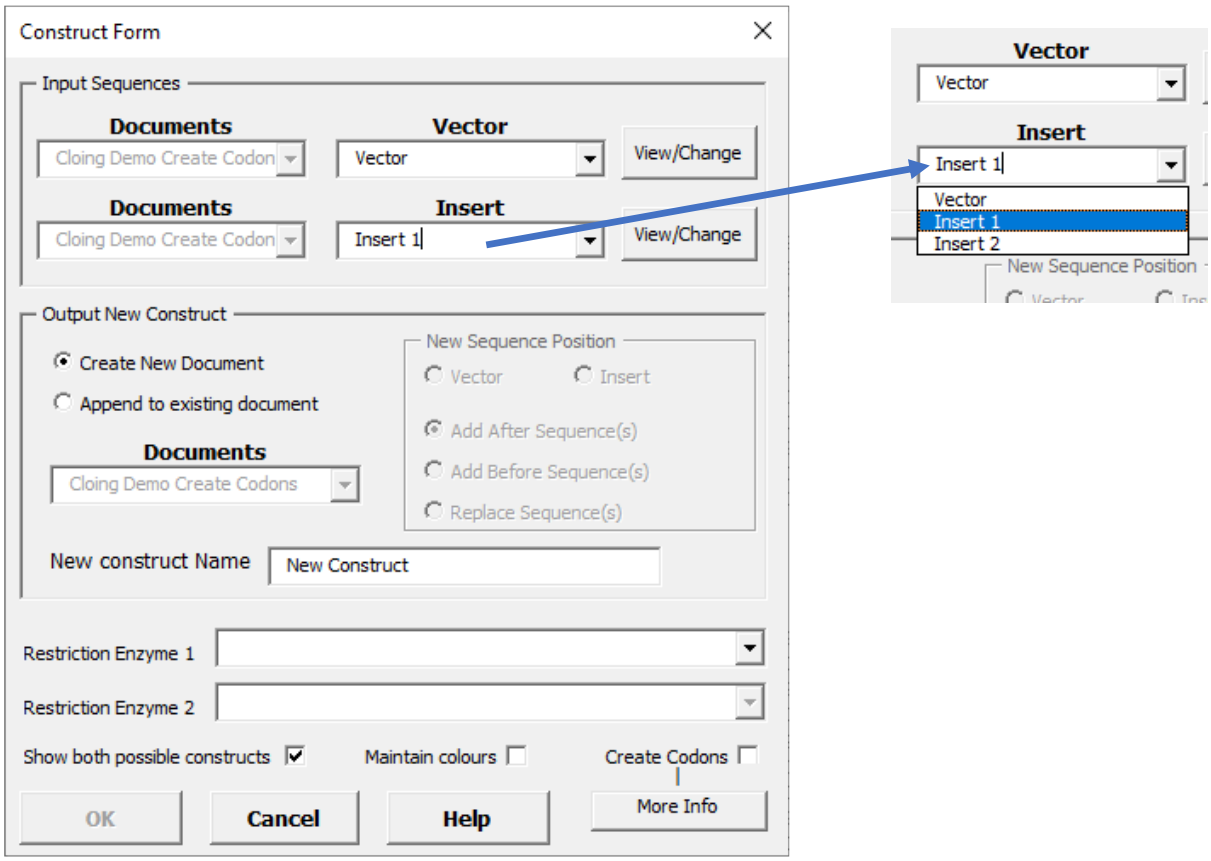



*Figure 5: How the cloning program loads various sequence into its memory space for the user to select using the drop menu to select any document that is open in word that contains sequences and also the sequences that were found in the document. In our example because we only have a single document open the Documents dropdown menu is not active.*

Because we only have one document only, the documents dropdown menu is greyed and inactive. The three sequences in the document have been loaded in both the vector and insert dropdowns as shown so you can quickly select your desired sequence. In this first demonstration let’s leave the vector sequence in the vector dropdown and Insert 1 in the Insert dropdown as shown in Figure 5.

Now select the first two restriction enzymes in the dropdown menu Aat II and Acc III. Let’s for now leave both maintain colours and create codons unticked and just click the OK button (Figure 6) and the following window will be displayed (Figure 7).

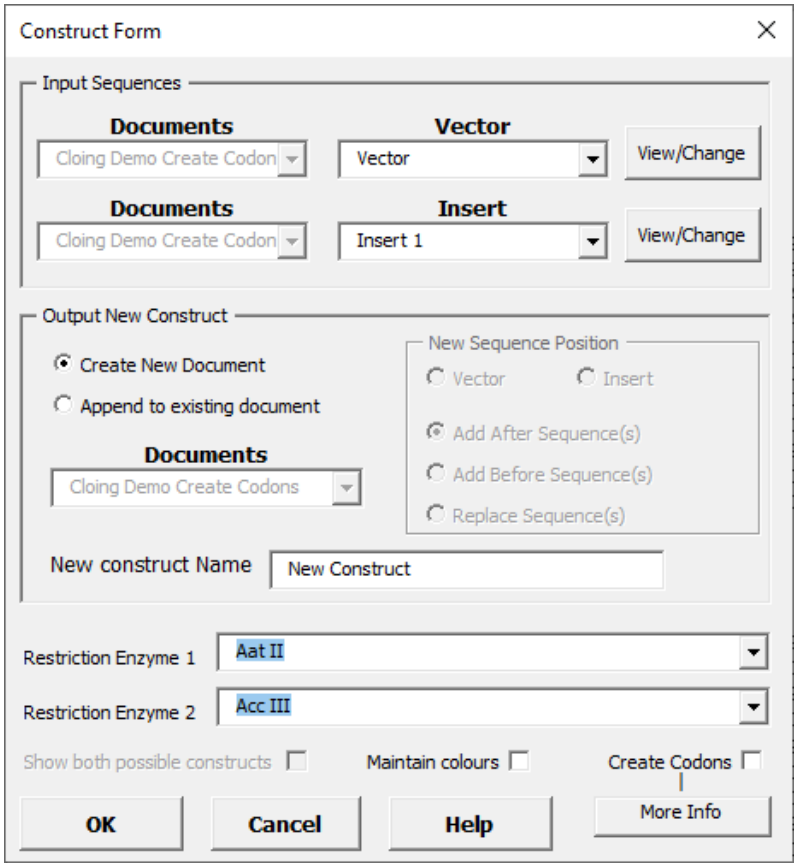



*Figure 6: Aat II and Acc III are the enzymes you should select for your first demonstration of the cloning program.*

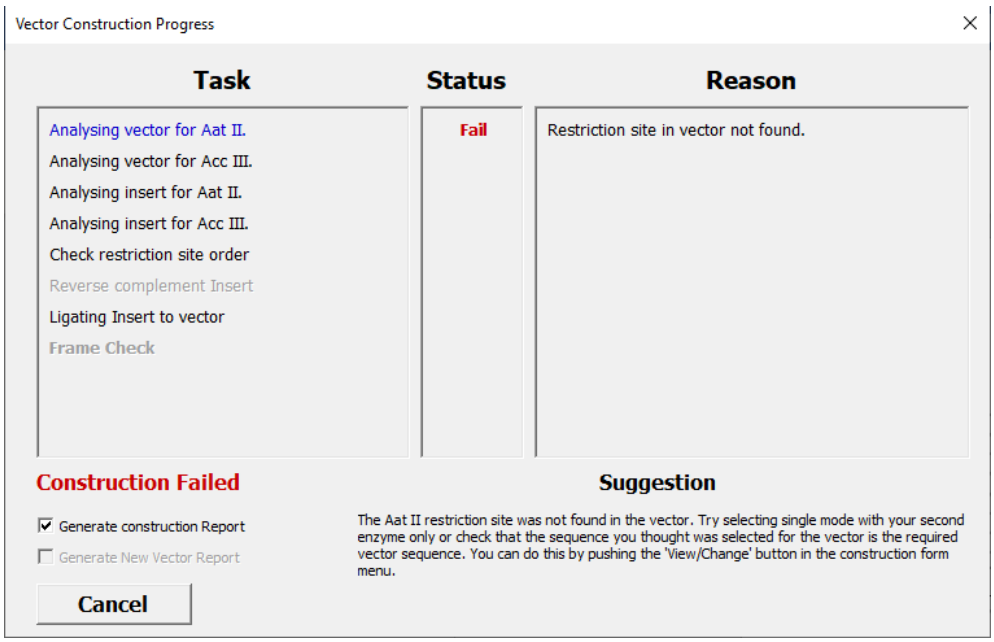



*Figure 7: The result of trying to insert any insert from my demonstration files into any vector from my demonstration files using the restriction site AaII or Acc III; neither of which are found in my example vectors and so the cloning strategy fails a the first step.*

The cloning strategy quickly failed because Aat II was not found in the vector. You can select to generate the report if you want to but if you do, close the document after you have seen it before running the cloning program a second time. Alternatively, just untick the Generate Construction Report button and push the cancel key to return to your original document containing your vector and insert sequences.

### *2.5.1 Create Codons Option*

Now let's repeat the process but this time we shall select the correct enzymes Bam H1 and Xho I as shown in Figure 8. This time we shall also tick create codons and now click OK.

After clicking OK you will get the following window in Figure 9. This time the program found both restriction sites in the vector and the insert but they were in the opposite order in the vector to the insert. The program will dutifully make the resultant cloned product but the insert will be the reverse complement sequence to the one that is in your Word document and so it is this sequence that WordMol places inside your vector (Figure 10)

Once you have examined this document, close it without saving it. Let's now select an insert sequence that has the BamH1 and Xho I sequence in the matching order to the vector (Insert 2).

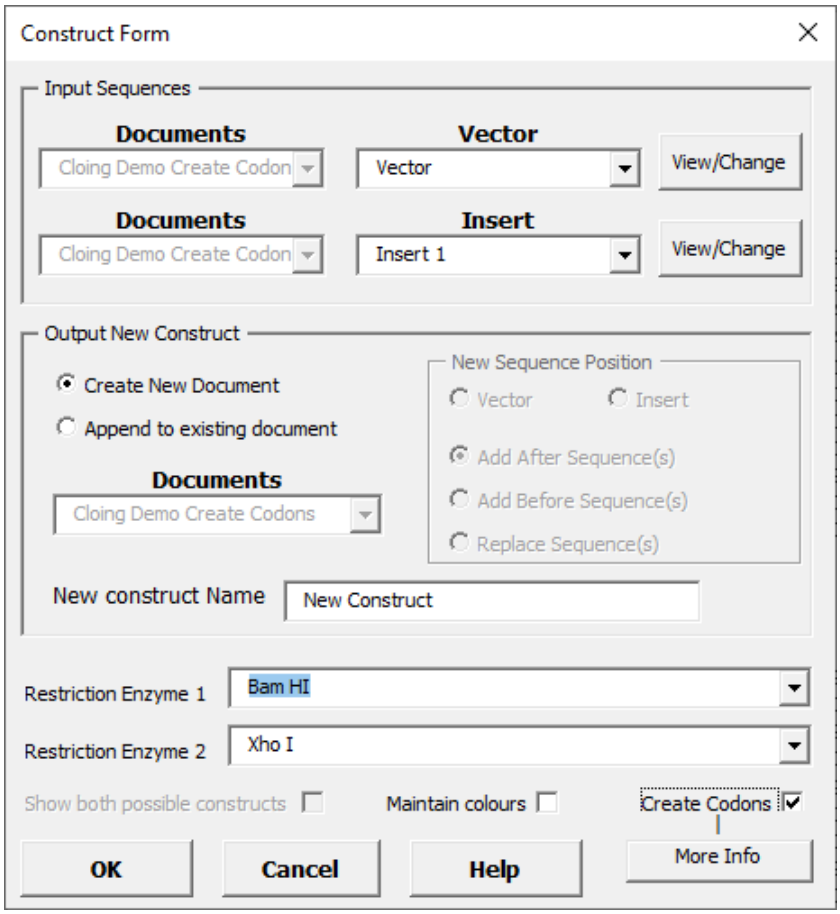



*Figure 8: The menu setting you should select for the second demonstration of the virtual WordMol cloning program.*

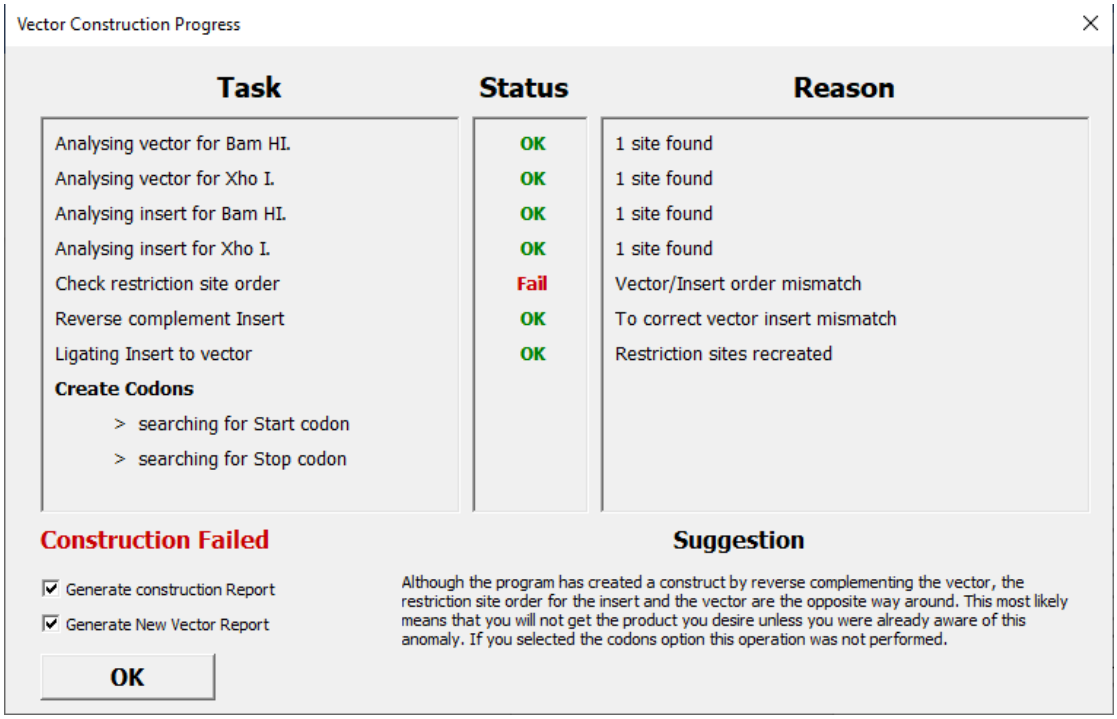
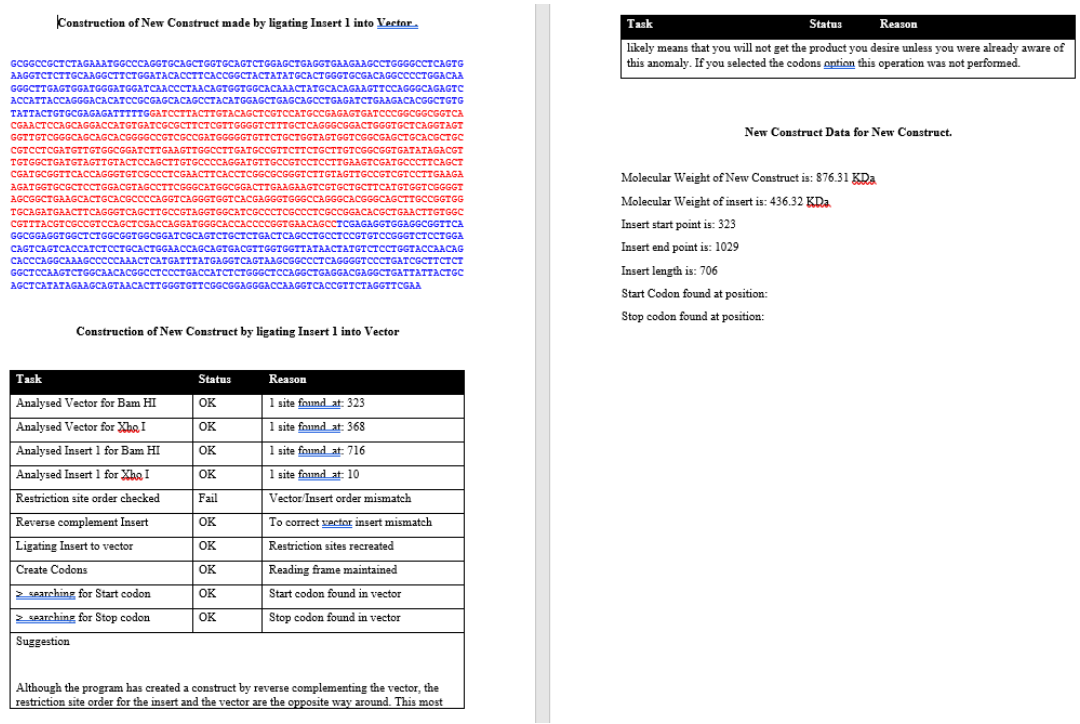



*Figure 9: The report window after trying selecting Insert 1 and Vector one from the Cloning Demo Create Codons Word Document. The problem with Insert 1 is the restriction sites BamH1 and xhoI are in the wrong 5' to 3' order compared to the vector.*



**Construction of New Construct made by ligating Insert 1 into Vector .**

GCGGCCGCTCTAGAAATGGCCCAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
AAGGTCTCTTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAA
GGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAAGTTCCAGGGCAGAGTC
ACCATTACCAGGGACACATCCGCGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAAGACACGGCTGTG
TATTACTGTGCGAGAGATTTTTGGATCCTTACTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCA
CGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGT
GGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGC
CGTCCTCGATGTTGTGGCGGGATCTTGAAGTTGGCTTGATGCCGTTCTTCTGCTTGTCGGCGGTGATATAGACGT
TGTGGCTGATGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCT
CGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGA
AGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGT
AGCGGCTGAAGCACTGCACGCCCCAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGG
TGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGC
CGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCCTCGAGAGGTGGAGGCGGTTCA
GGCGGAGGTGGCTCTGGCGGTGGCGGATCGCAGTCTGCTCTGACTCAGCCTGCCTCCGTGTCCGGGTCTCCTGGA
CAGTCAGTCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAG
CACCCAGGCAAAGCCCCCAAACTCATGATTTATGAGGTCAGTAAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCT
GGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGC
AGCTCATATAGAAGCAGTAACACTTGGGTGTTCGGCGGAGGGACCAAGGTCACCGTTCTAGGTTCGAA

**Construction of New Construct by ligating Insert 1 into Vector**

| Task | Status | Reason |
|---|---|---|
| Analysed Vector for Bam HI | OK | 1 site found_at: 323 |
| Analysed Vector for Xho I | OK | 1 site found_at: 368 |
| Analysed Insert 1 for Bam HI | OK | 1 site found_at: 716 |
| Analysed Insert 1 for Xho I | OK | 1 site found_at: 10 |
| Restriction site order checked | Fail | Vector/Insert order mismatch |
| Reverse complement Insert | OK | To correct vector insert mismatch |
| Ligating Insert to vector | OK | Restriction sites recreated |
| Create Codons | OK | Reading frame maintained |
| > searching for Start codon | OK | Start codon found in vector |
| > searching for Stop codon | OK | Stop codon found in vector |
| Suggestion | | |
| Although the program has created a construct by reverse complementing the vector, the restriction site order for the insert and the vector are the opposite way around. This most | | |

| Task | Status | Reason |
|---|---|---|
| likely means that you will not get the product you desire unless you were already aware of this anomaly. If you selected the codons option this operation was not performed. | | |

**New Construct Data for New Construct.**

Molecular Weight of New Construct is: 876.31 KDa

Molecular Weight of insert is: 436.32 KDa

Insert start point is: 323

Insert end point is: 1029

Insert length is: 706

Start Codon found at position:

Stop codon found at position:

*Figure 10: The report produced from trying to place Insert 1 into Vector 1 from the document Cloning Demo Create Codon's Word Document. The program has created a new construct but the insert is the reverse complement from the insert you were trying to put into the vector. Note because this is not the correct insert the colour code of the construct is blue for the vector and red for the insert. If the cloning strategy was correct, then the insert would be colour coded green see Figure 13.*

Click the cloning button and make sure your menu looks like Figure 11.

In this example, I have also requested that the sequence report and new sequence is not placed in a new document but placed inside the document that contains the original sequences. This then activates the New Sequence Position submenu. I have indicated that I want the sequence to be placed After the insert which means it will appear after Insert 2 which is the last sequence in Cloning Demo Create Codons Word Document.

This time when I run the program, I get a successful construct as indicated in the construction status window Figure 12.

The default setting when the construct has been successful is not to bother generating the full Construction report but just the new vector report so let's go with this default. After pushing OK, the new vector will be placed after Insert 2 in the "Cloning Demo Create Codons" Word document along with a short report as shown in Figure 13.

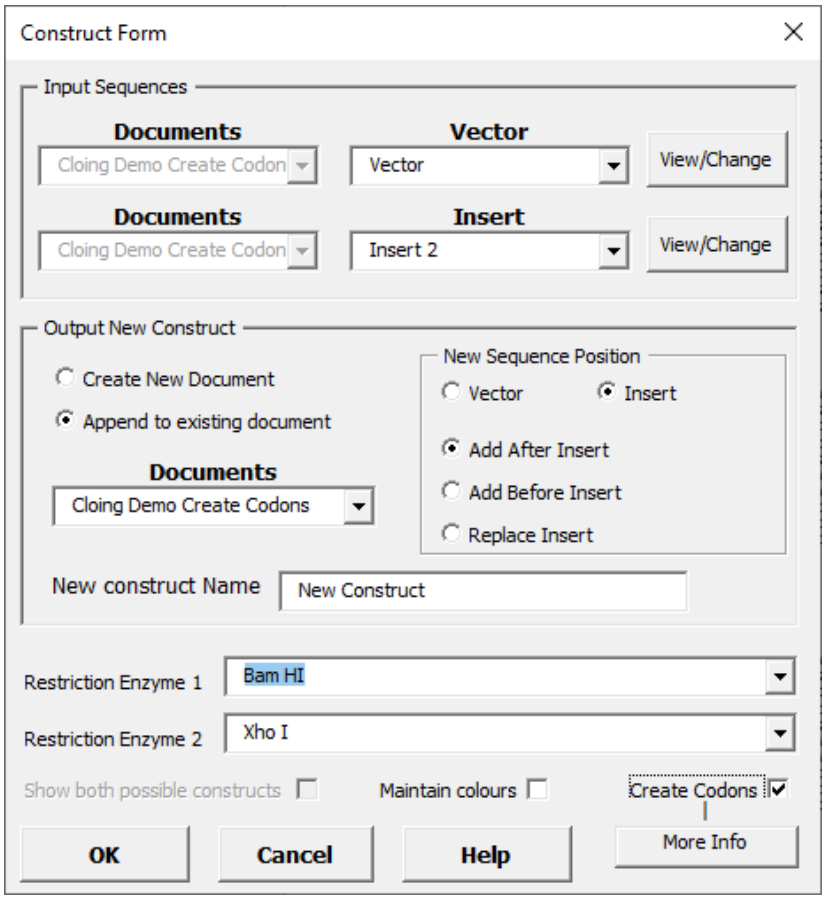



*Figure 11: Please make sure your menu looks like this before running the next demonstration.*

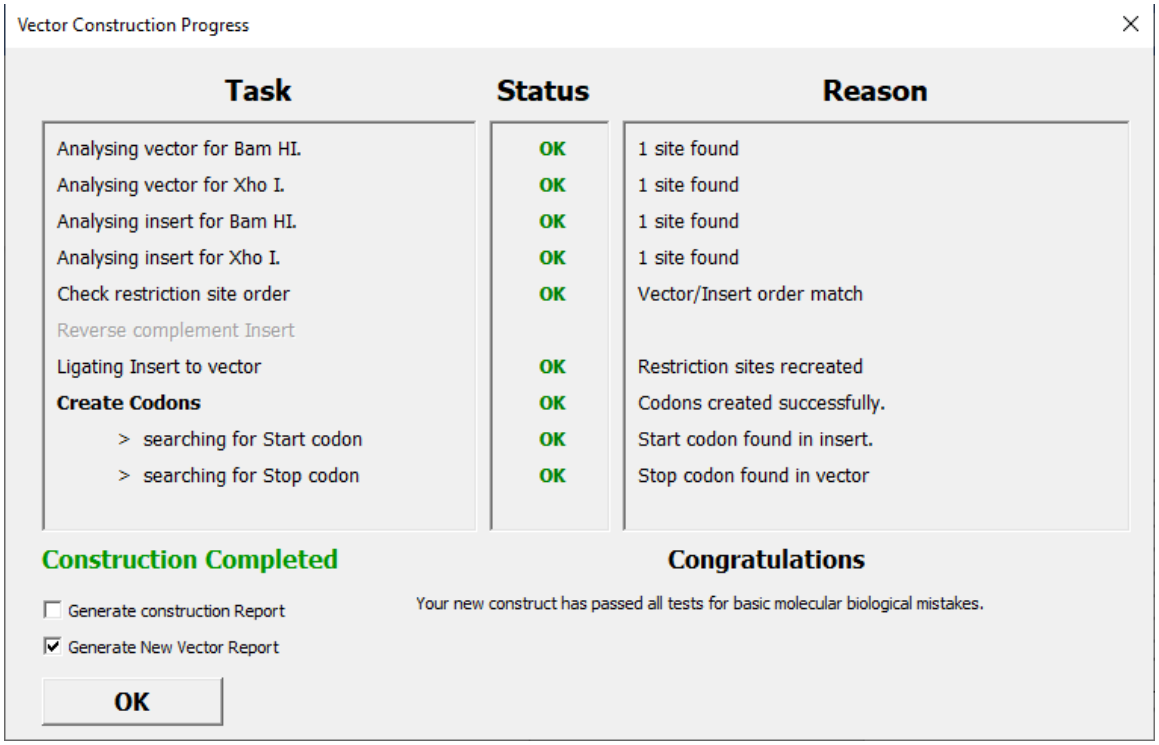



*Figure 12: With the setting from Figure 11, the report window will this time show a successful construct.*

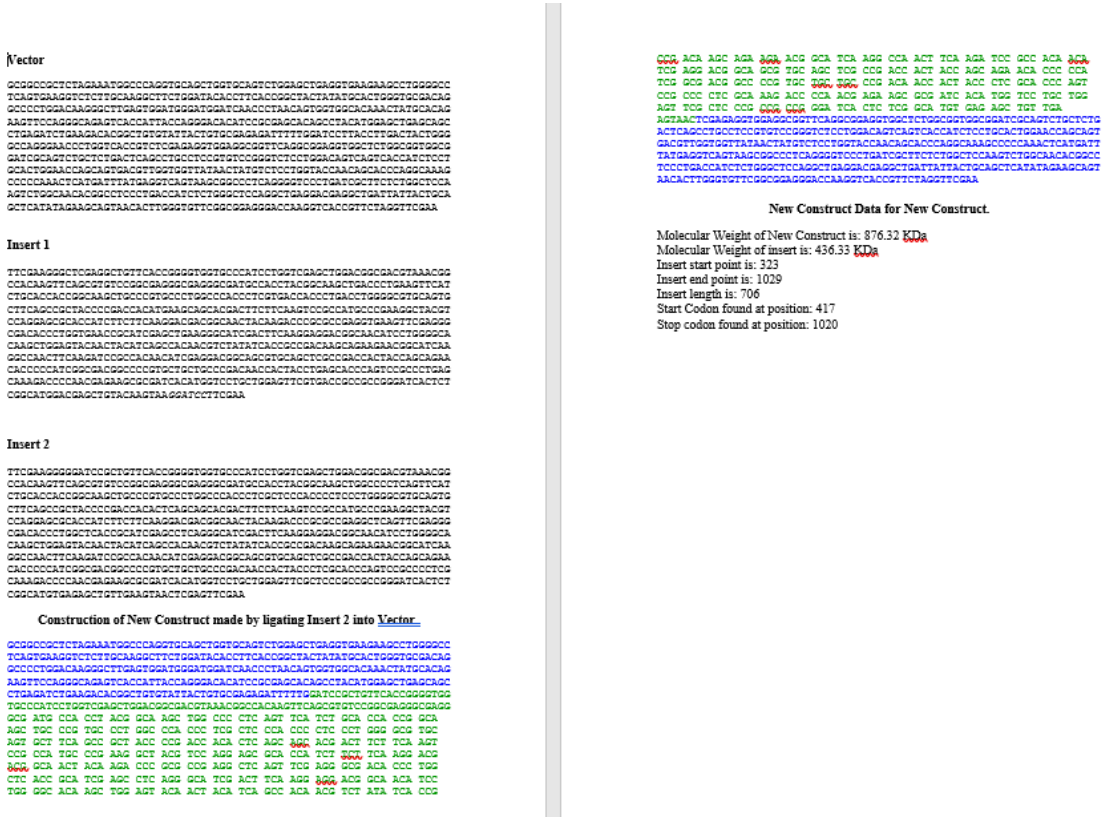



*Figure 13: The result of using the virtual molecular cloning program to place Insert 2 into Vector 1 from the document Cloning Demo Create Codons Word Document.. A successful*

*cloning strategy will place the vector in blue and the insert in green. Note because we did not tick the "Generate Construction Report option" the contents of the construction report window (Figure 12) is not transferred to the Word document in the form of a table.*

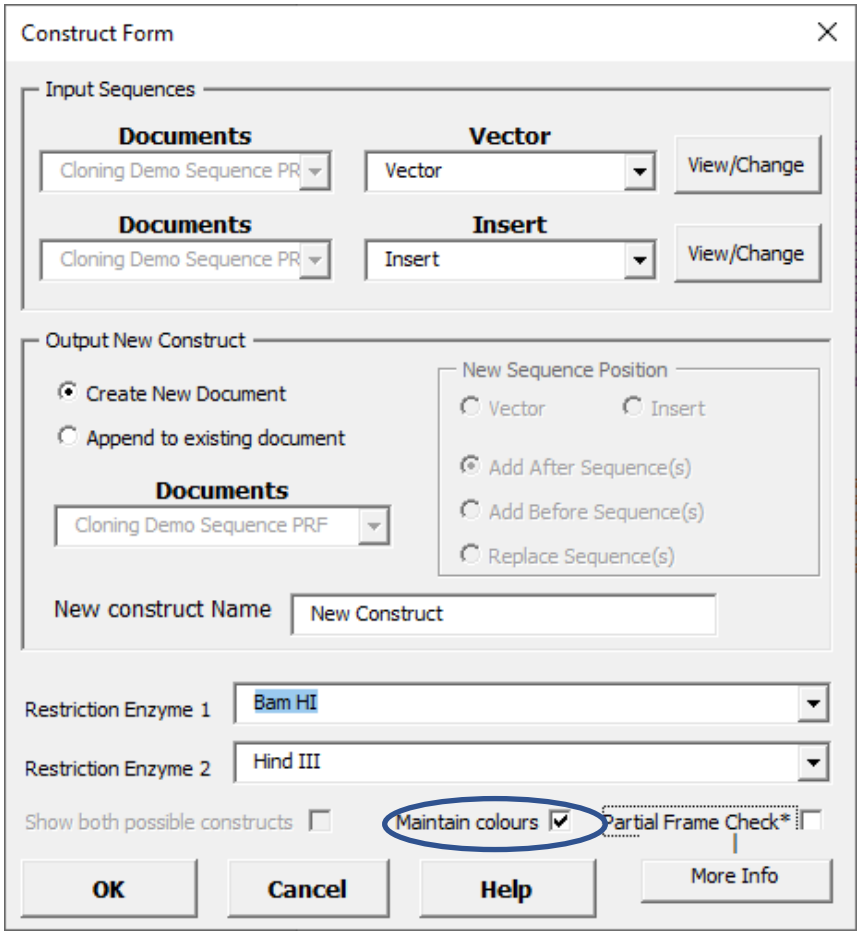
Note because we selected the create codon option, the program has attempted to place the insert sequence into codons. The method it uses to do this as follows:

It first looks for a start codon (ATG) in the first half of the insert. If it fails to find one it then searches backwards from the position where the insert was placed into the vector in a 5' direction until it encounters its first ATG start codon in the vector. Once it has identified that codon, it will then place the sequence into codons until it reaches a stop codon.

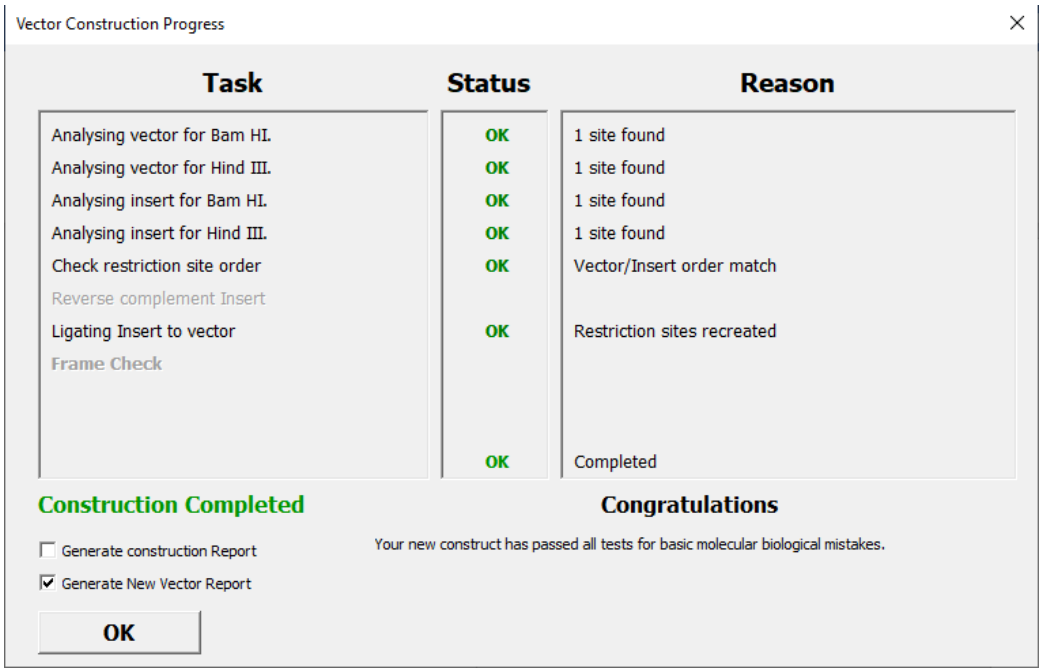
Obviously, while this is a sensible strategy it still may not result in the correct reading frame for your vector/insert construct so although you can use this functionality, you will need to confirm that the codons WordMol has created really are correct in relation to the information you have about your vector and insert sequences.

### *2.5.2 Partial Frame Check Option*

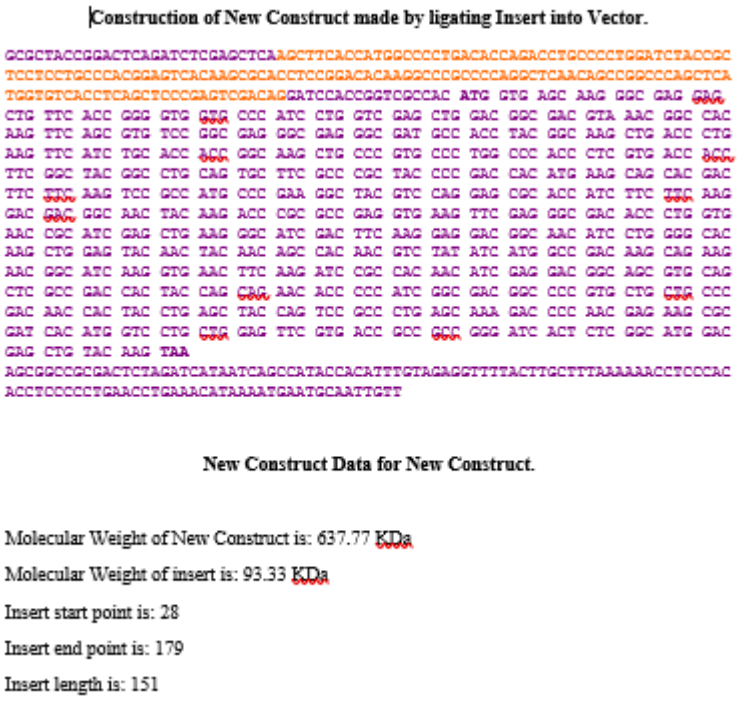
Now close all the open document and this time open the Word document Cloning Demo PRF. Click the virtual cloning button and the following window will appear (next page). This time we shall demonstrate the maintain colour option so please tick this box as shown in the window (circled) on the next page.



You will see that because the vector has its codon spacing but the insert has no codon spacing the Create codons option has now changed to Partial Frame Check*. For now we shall leave this unticked and simply click OK. The following window will appear showing a successful cloning strategy




The resultant new document will look like:



**Construction of New Construct made by ligating Insert into Vector.**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCACCATGGCCCCTGACACCAGACCTGCCCCTGGATCTACCGC
TCCTCCTGCCCACGGAGTCACAAGCGCACCTCCGGACACAAGGCCCGCCCCAGGCTCAACAGCCGGCCCAGCTCA
TGGTGTCACCTCAGCTCCCGAGTCGACAGGATCCACCGGTCGCCAC ATG GTG AGC AAG GGC GAG GAG
CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC
AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG
AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC
TTC GGC TAC GGC CTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC
TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG
GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG
AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC
AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG
AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG
CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC
GAC AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC
GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC
GAG CTG TAC AAG TAA
AGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCAC
ACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

**New Construct Data for New Construct.**

Molecular Weight of New Construct is: 637.77 KDa

Molecular Weight of insert is: 93.33 KDa

Insert start point is: 28

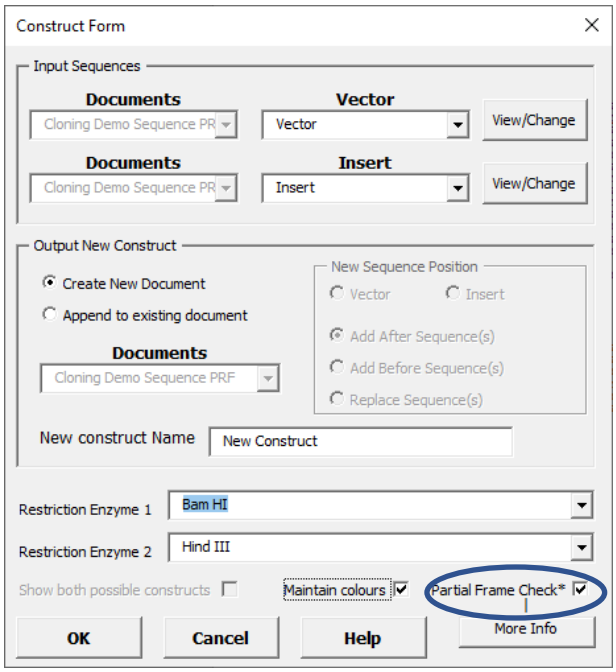
Insert end point is: 179

Insert length is: 151

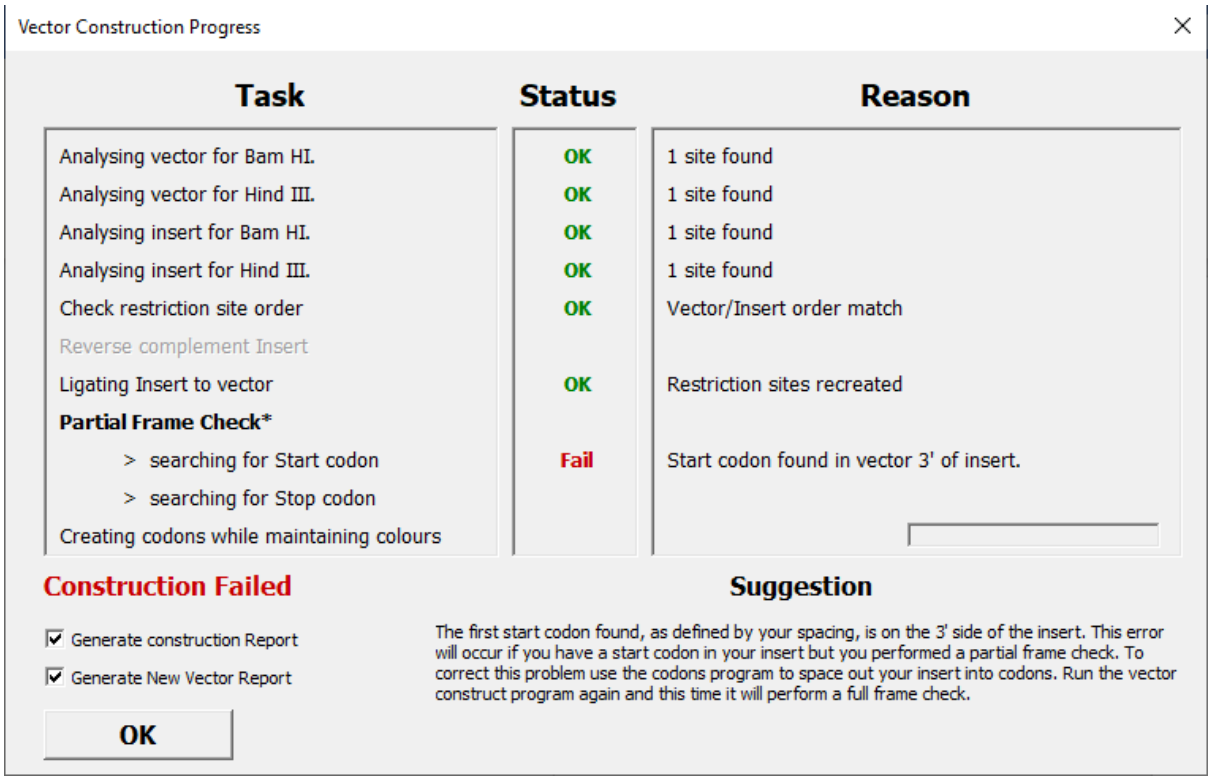
The first thing to note is that this short insert has gone into the vector before the start codon in the vector. If we had not selected the maintain colours option then the vector

would be in its standard blue colour with the insert in green (see Figure 13). However, because we had already coloured our vector and insert different colours in our original document, these colours are now transferred to the new document.

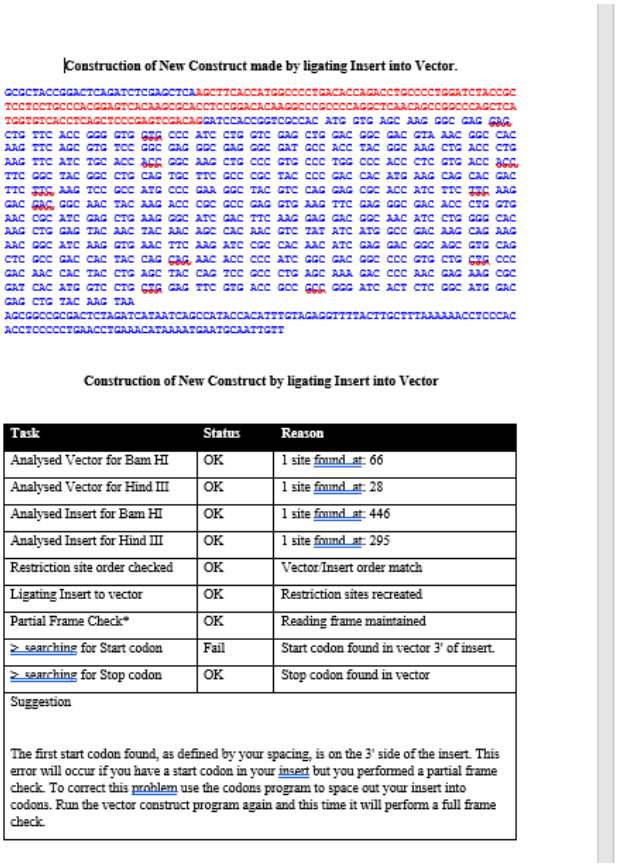
Let's now close this new document and run the virtual cloning strategy again, this time selecting the partial frame check option:




The following report window is displayed



This time the construct has failed because the Start codon of the vector is located 3' of the insert which may suggest a potential problem. Because the cloning strategy has failed, the maintain colours option has been ignored and the insert is now coded in red.



**Construction of New Construct made by ligating Insert into Vector.**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCACCATGGCCCCTGACACCAGACCTGCCCCTGGATCTACCGC
TCCTCCTGCCCACGGAGTCACAAGCGCACCTCCGGACACAAGGCCCGCCCCAGGCTCAACAGCCGGCCCAGCTCA
TGGTGTCACCTCAGCTCCCGAGTCGACAGGATCCACCGGTCGCCAC ATG GTG AGC AAG GGC GAG GAG
CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC
AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG
AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC
TTC GGC TAC GGC CTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC
TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG
GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG
AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC
AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG
AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG
CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC
GAC AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC
GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC
GAG CTG TAC AAG TAA
AGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCAC
ACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

**Construction of New Construct by ligating Insert into Vector**

| Task | Status | Reason |
|---|---|---|
| Analysed Vector for Bam HI | OK | 1 site found..at: 66 |
| Analysed Vector for Hind III | OK | 1 site found..at: 28 |
| Analysed Insert for Bam HI | OK | 1 site found..at: 446 |
| Analysed Insert for Hind III | OK | 1 site found..at: 295 |
| Restriction site order checked | OK | Vector/Insert order match |
| Ligating Insert to vector | OK | Restriction sites recreated |
| Partial Frame Check* | OK | Reading frame maintained |
| >..searching for Start codon | Fail | Start codon found in vector 3' of insert. |
| >..searching for Stop codon | OK | Stop codon found in vector |

Suggestion

The first start codon found, as defined by your spacing, is on the 3' side of the insert. This error will occur if you have a start codon in your insert but you performed a partial frame check. To correct this problem use the codons program to space out your insert into codons. Run the vector construct program again and this time it will perform a full frame check.

**New Construct Data for New Construct.**

Molecular Weight of New Construct is: 637.77 KDa

Molecular Weight of insert is: 93.33 KDa

Insert start point is: 28

Insert end point is: 179

Insert length is: 151

Error - No start codon was found!

The partial Frame check only operates if your vector has codon spacing. If your vector has no codon spacing but the insert does then in this instance, all the Word Mol functions to do with creating codons or checking reading frames are disabled because you have already defined your codon spacing in your insert and there is no reading frame in the vector to check it against.

If you want to see an example of this, open the word document Cloning Demo Sequence No Create Codon Option which looks like this:

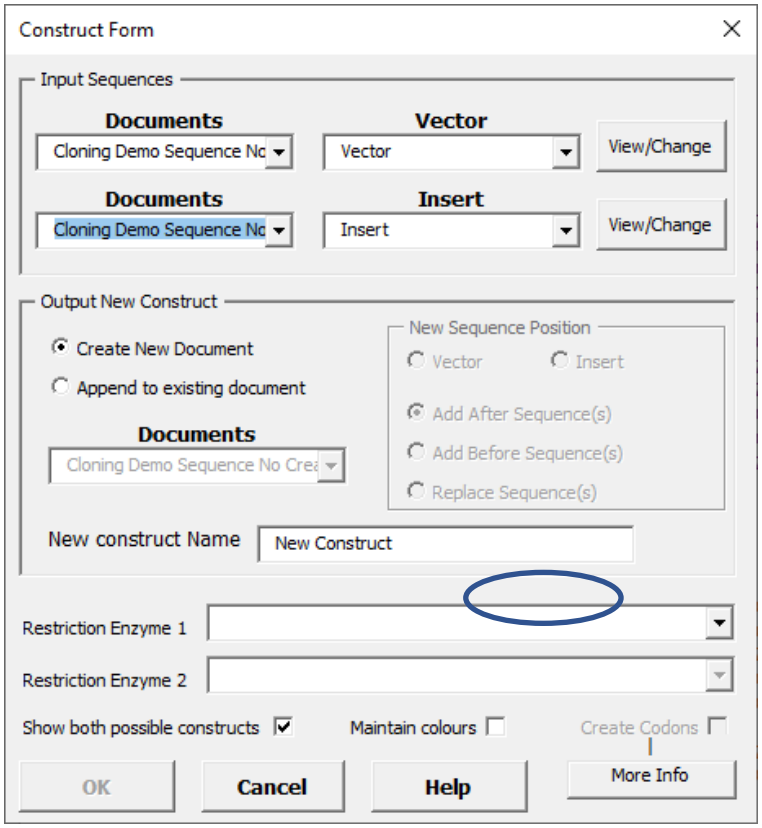
Vector

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGC
CACATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCA
AGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATG
AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAA
CTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGG
AGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAG
AACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA
CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCA
ACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
TAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACAC
CTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

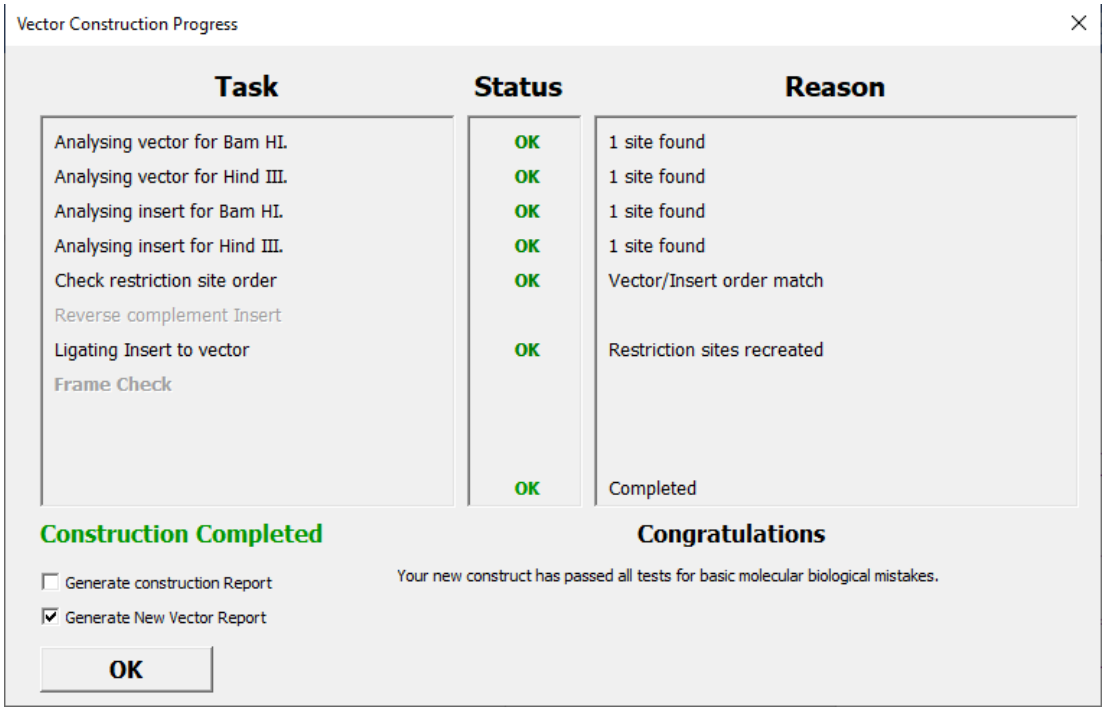
Insert

AAGCTT ATG AGC GCC CAA TAC GCA AAC CGC CTC TCC CCG CGC GTT GGC CGA TTC ATT AAT
GCA GCT GGC ACG ACA GGT TTC CCG ACT GGA AAG CGG GCA GTG AGC GCA ACG CAA TTA ATG
TGG GTT AGC TCA CTC ATT AGG CAC CCC AGG CTT TAC ACT TTA TGC TTC CGG CTC GTA TGT
TGT GTG GAA TTG TCA GCG GAT AAC AAT TTC ACA CAG GAA ACA GCT ATG ACC ATG ATT ACG
CCA AGC TCA GAA TTA ACC CTC ACT AAA GGG ACT AGT CCT GCA GGT TTA AAC GAA TTC GCC
CTT CAC CAT GGC CCC TGA
CACCAGACCTGCCCCTGGATCTACCGCTCCTCCTGCCCACGGAGTCACAAGCGCACCTCCGGACACAAGGCCCGCCCCAG
GCTCAACAGCCGGCCCAGCTCATGGTGTCACCTCAGCTCCCGAGTCGACAGGATCCAAGGGCGAATTCGCGGCCGCTAAA
TTC

Now run the virtual cloning program and the following window will appear




The Create Codons box is de-selected and not operational and if you run the program the following window will appear showing the program had not used any of its frame checking capabilities in this particular virtual cloning experiment.




The resultant construct from this virtual cloning exercise is as follows:

**Construction of New Construct made by ligating Insert into Vector.**

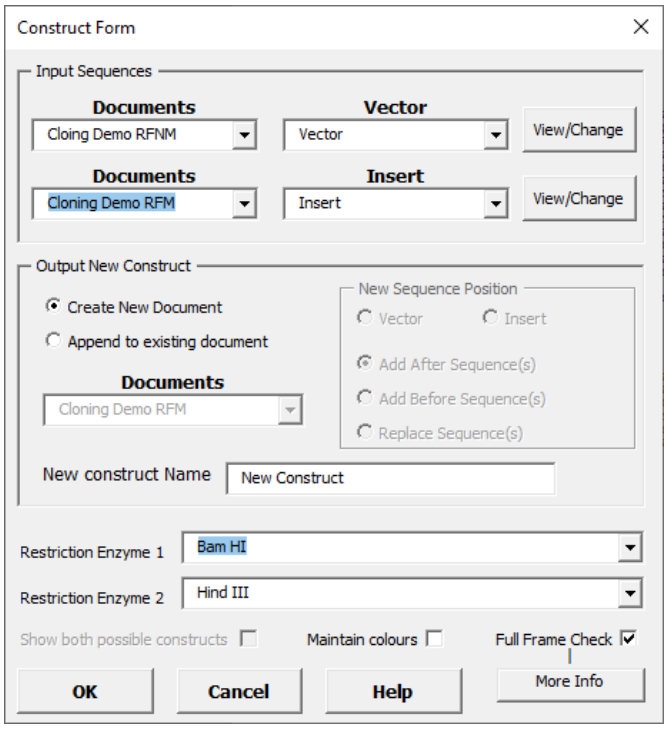
GCGCTACCGGACTCAGATCTCGAGCTCAAGCTT ATG AGC GCC CAA TAC GCA AAC
CGC CTC TCC CCG CGC GTT GGC CGA TTC ATT AAT GCA GCT GGC ACG
ACA GGT TTC CCG ACT GGA AAG CGG GCA GTG AGC GCA ACG CAA TTA
ATG TGG GTT AGC TCA CTC ATT AGG CAC CCC AGG CTT TAC ACT TTA
TGC TTC CGG CTC GTA TGT TGT GTG GAA TTG TCA GCG GAT AAC AAT
TTC ACA CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TCA GAA
TTA ACC CTC ACT AAA GGG ACT AGT CCT GCA GGT TTA AAC GAA TTC
GCC CTT CAC CAT GGC CCC TGA
CACCAGACCTGCCCCTGGATCTACCGCTCCTCCTGCCCACGGAGTCACAAGCGCACCTCCGG
ACACAAGGCCCGCCCCAGGCTCAACAGCCGGCCCAGCTCATGGTGTCACCTCAGCTCCCGAG
TCGACAGGATCCACCGGTCGCCAC**ATG**GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGT
GCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGG
GCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTG
CCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTA
CCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGG
AGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG
GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACAT
CCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGC
AGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG
CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAA
CCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGG
TCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG**TAA**

AGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTA
AAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

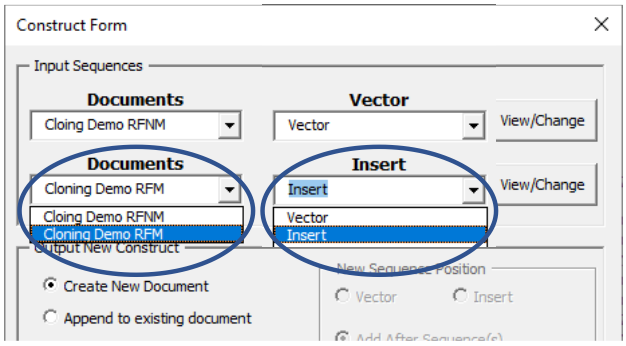
### *2.5.3 Full Frame Check*

The final functionality of this program to demonstrate is its full frame check feature. This operates when codon spacing is defined both for the vector and for the insert. In order to get an idea of how this functionality operates close all documents and then open from the Word Mol folder Coding Demo RFNM.docx and Coding Demo RFM.docx.

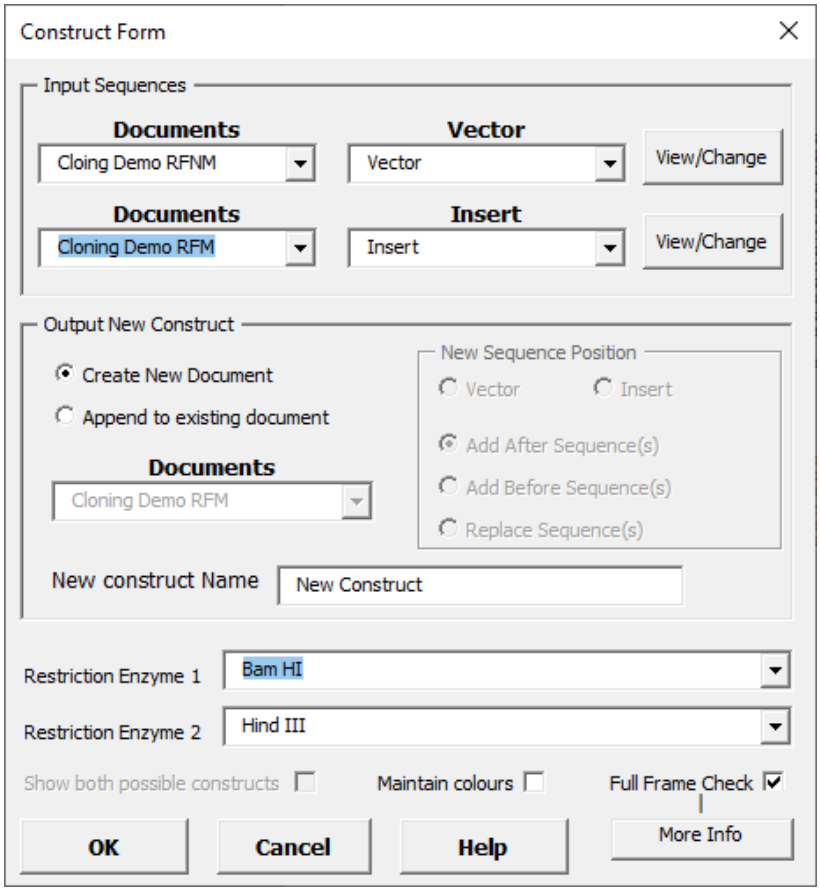
Now click the molecular cloning button and the following menu will be displayed.



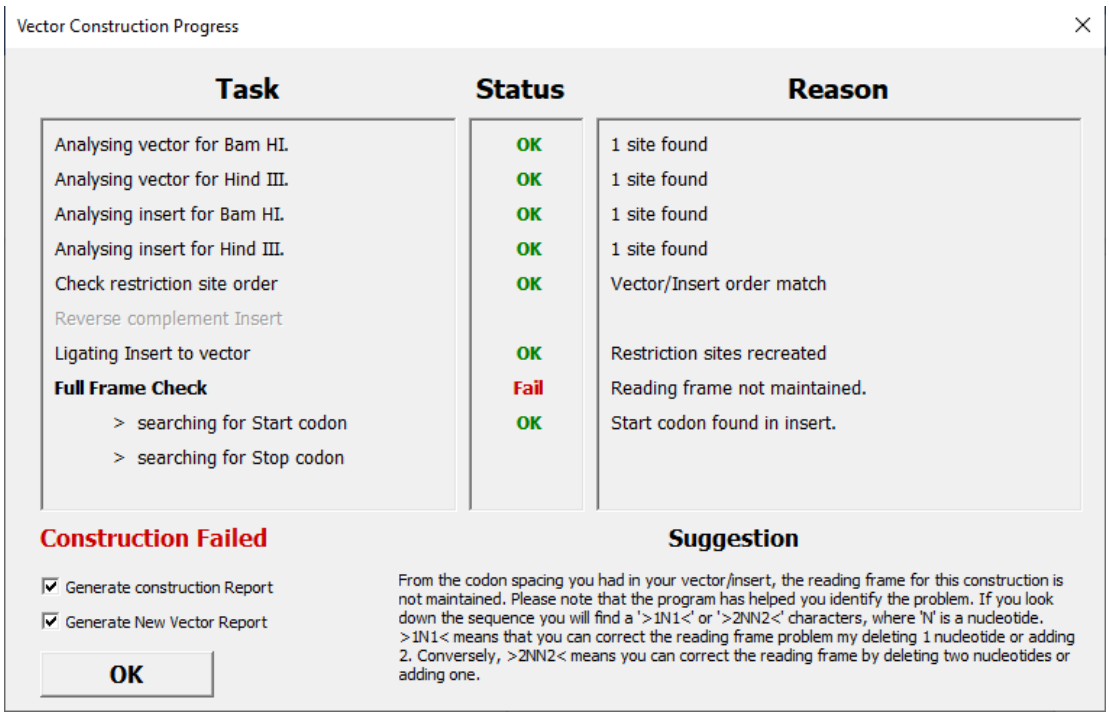

In this example, because we have two documents open, the documents dropdown menus in the input sequences are now both operational. In all, there are four possible sequences we could select: 2 in the Word document Cloning Demo RFNM and two in the Word Document Cloning Demo RFM. We can get to any one of the four using the two active drop-down menus in the Construct Form menu (circled below).



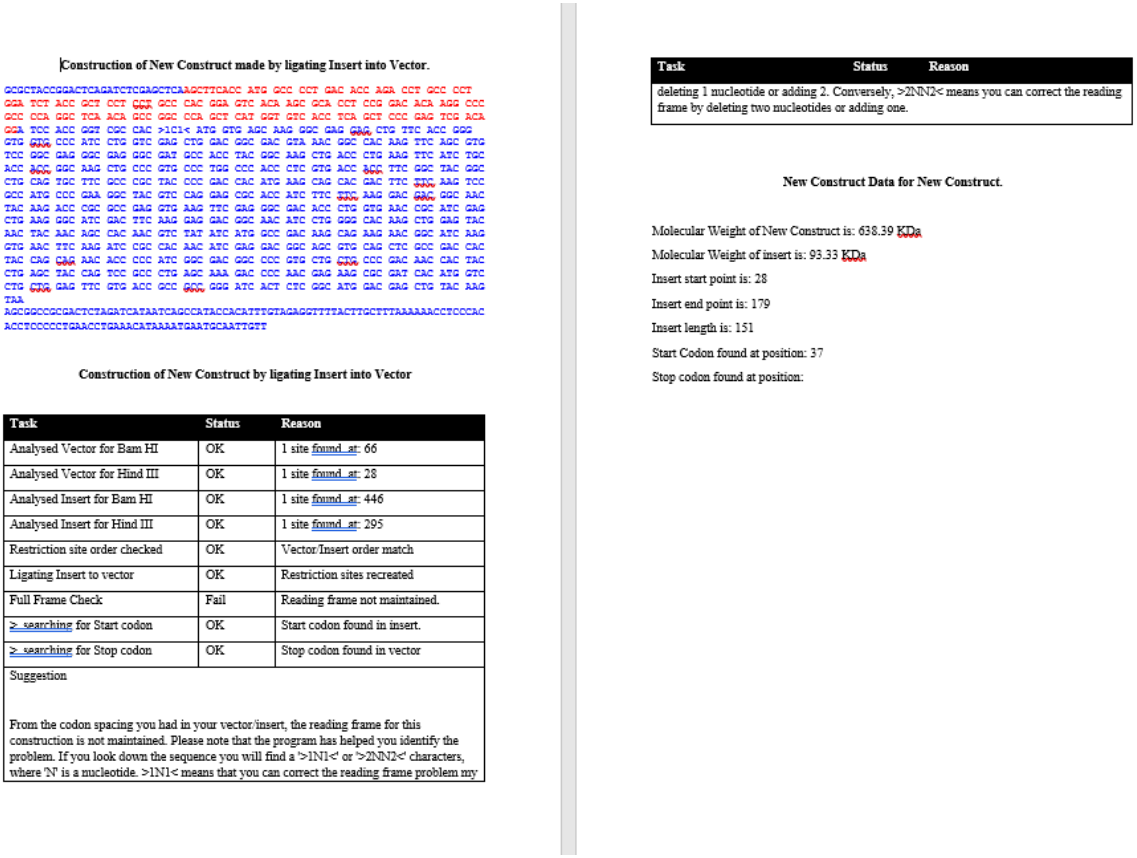

Now in this first example let's select the following:




You will see this time we are selecting the insert from the RFM file and the vector from the RFNM document. In fact, the inserts in both documents are identical so this is exactly the same as if we selected the Vector and Insert from the Cloning Demo RFNM document only. Now click OK and the following construct report is produced



This time the cloning strategy has failed because the reading frame of the vector is not maintained as it enters the insert. Click OK to produce the failure file:



**Construction of New Construct made by ligating Insert into Vector.**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCACC ATG GCC CCT GAC ACC AGA CCT GCC CCT
GGA TCT ACC GCT CCT CCT GCC CAC GGA GTC ACA AGC GCA CCT CCG GAC ACA AGG CCC
GCC CCA GGC TCA ACA GCC GGC CCA GCT CAT GGT GTC ACC TCA GCT CCC GAG TCG ACA
GCA TCC ACC GGT CGC CAC >1C1< ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG
GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG
TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC
ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC TTC GGC TAC GGC
CTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC
GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC
TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG
CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC
AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG
GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC
TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC
CTG AGC TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC
CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG
TAA
AGCGGCCGCGAACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCAC
ACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

**Construction of New Construct by ligating Insert into Vector**

| Task | Status | Reason |
|---|---|---|
| Analysed Vector for Bam HI | OK | 1 site found..at: 66 |
| Analysed Vector for Hind III | OK | 1 site found..at: 28 |
| Analysed Insert for Bam HI | OK | 1 site found..at: 446 |
| Analysed Insert for Hind III | OK | 1 site found..at: 295 |
| Restriction site order checked | OK | Vector/Insert order match |
| Ligating Insert to vector | OK | Restriction sites recreated |
| Full Frame Check | Fail | Reading frame not maintained. |
| >..searching for Start codon | OK | Start codon found in insert. |
| >..searching for Stop codon | OK | Stop codon found in vector |
| Suggestion<br><br>From the codon spacing you had in your vector/insert, the reading frame for this construction is not maintained. Please note that the program has helped you identify the problem. If you look down the sequence you will find a '>1N1<' or '>2NN2<' characters, where 'N' is a nucleotide. >1N1< means that you can correct the reading frame problem my | | |

| Task | Status | Reason |
|---|---|---|
| deleting 1 nucleotide or adding 2. Conversely, >2NN2< means you can correct the reading frame by deleting two nucleotides or adding one. | | |

**New Construct Data for New Construct.**

Molecular Weight of New Construct is: 638.39 KDa

Molecular Weight of insert is: 93.33 KDa

Insert start point is: 28

Insert end point is: 179

Insert length is: 151

Start Codon found at position: 37

Stop codon found at position:

If you look carefully at the report you will see that in the failed construct the program has helpfully placed a single nucleotide into arrow delineators (**in this example >1C<**). This is telling you that the reading frame is failing because of a shift of one nucleotide between the vector and insert reading frames.

Let's now repeat the exercise this time Selecting the document Demo Cloning RFM: Vector (for the vector) and Demo Cloning RFM: Insert (for the insert) as shown in Figure 14.

As an extra exercise, we are also going to demonstrate the last bit of functionality in the Construct form menu by renaming the vector from its standard name "Vector" to the name "HB322". To do this we click on the View/Change button and the submenu in panel B appears. From here we can change the name of the vector or push the Define Vector button. For now, we shall simply change the name of vector as shown in Figure 14B. However, if we also wanted to change the text that has been automatically selected for the vector, we could push the define vector button and the currently selected text, inside the appropriate document for the selected vector, would be displayed. We could then simply navigate to a different point in the document and select a different DNA sequence to define our vector should we wish to do that.

Once we have altered the text to HB322 and our two restriction enzymes we then click the OK button and the following construction report window appears (Figure 15).

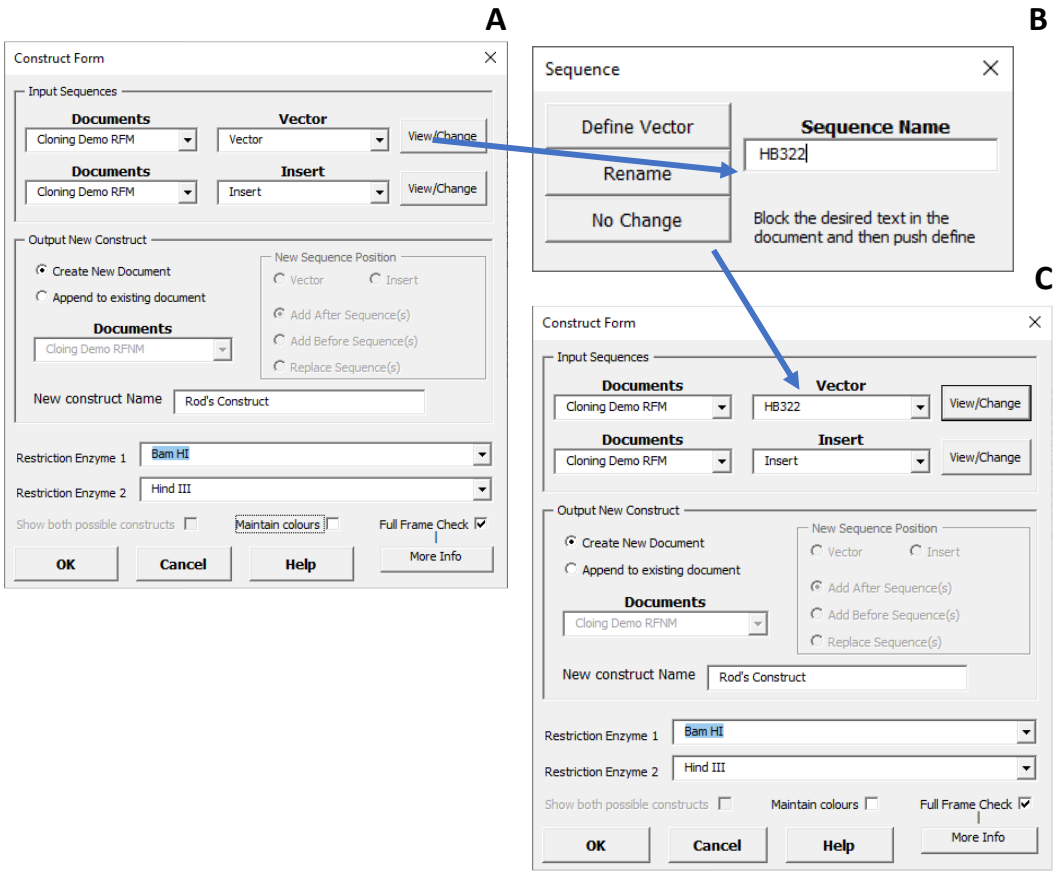



*Figure 14: Using the View/Change buttons to manually change either the vector insert name or the DNA sequence text that is selected for the program to operate on. In our demonstration we are only changing the name so after entering the new name we push the Rename button. If we pushed the Define Vector we would be taken to the document that contains the currently selected sequence where we could block select a different sequence in that document.*

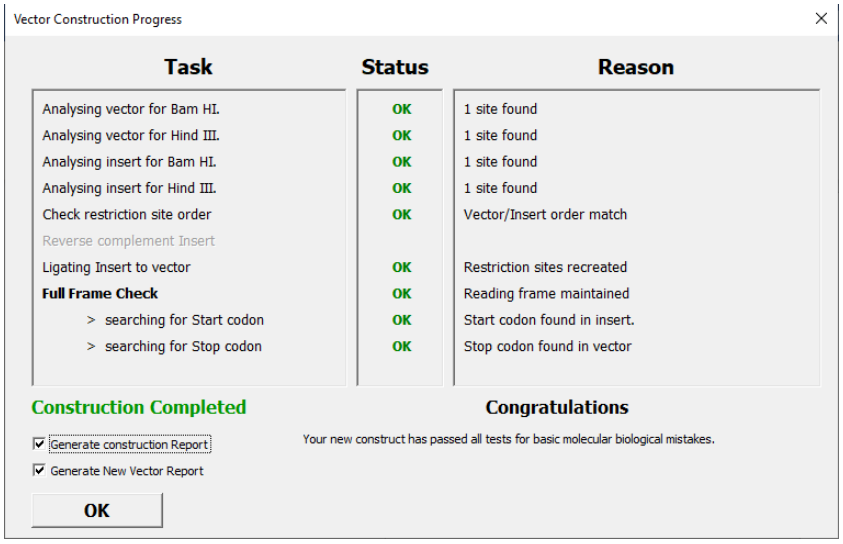
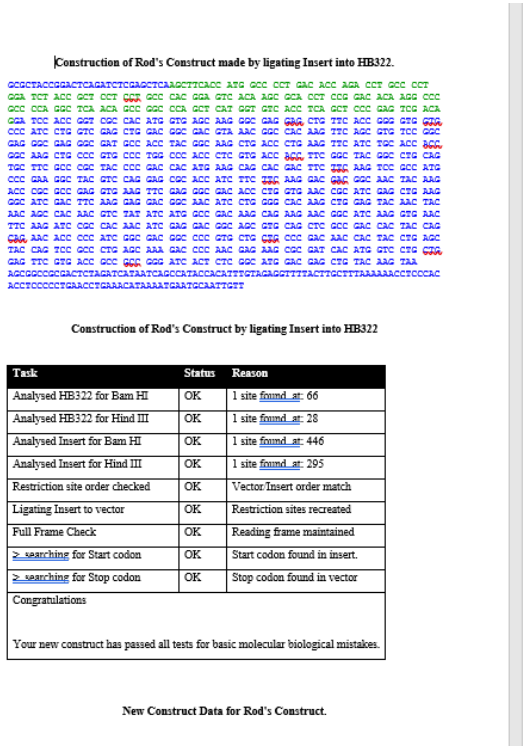



*Figure 15: A successful construct using the full frame check capability of WordMol 1.0. In this example we have also elected for WordMol 1.0 to copy this report to the document file in the form of a construction table by selecting the Generate Construction Report Option.*

This time the frame check has been maintained so virtual cloning strategy has been completed successfully. In our example, we shall select this time to also generate the cloning report table and click the OK button to produce the following report.



**Construction of Rod's Construct made by ligating Insert into HB322.**

GCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCAGC ATG GCC CCT GAC ACC AGA CCT GCC CCT
GGA TCT ACC GCT CCT CCT GCC CAC AGA GTC ACA AGC CCA CCT CCG GAC ACA AGG CCC
GCC CCA GGC TCA ACA GCC GGC CCA GCT CAT GGT GTC ACC TCA GCT CCC GAG TCG ACA
GCA TCC ACC GGT CGC CAC ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG
CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC
GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC
GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC TTC GGC TAC GGC CTG CAG
TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG
CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG
ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG
GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC
AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC
TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG
CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC
TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG
GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG TAA
AGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCAC
ACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT

**Construction of Rod's Construct by ligating Insert into HB322**

| Task | Status | Reason |
|---|---|---|
| Analysed HB322 for Bam HI | OK | 1 site found. at: 66 |
| Analysed HB322 for Hind III | OK | 1 site found. at: 28 |
| Analysed Insert for Bam HI | OK | 1 site found. at: 446 |
| Analysed Insert for Hind III | OK | 1 site found. at: 295 |
| Restriction site order checked | OK | Vector/Insert order match |
| Ligating Insert to vector | OK | Restriction sites recreated |
| Full Frame Check | OK | Reading frame maintained |
| >. searching for Start codon | OK | Start codon found in insert. |
| >. searching for Stop codon | OK | Stop codon found in vector |
| Congratulations<br><br>Your new construct has passed all tests for basic molecular biological mistakes. | | |

**New Construct Data for Rod's Construct.**

Molecular Weight of Rod's Construct is: 637.77 KDa
Molecular Weight of insert is: 93.33 KDa
Insert start point is: 28
Insert end point is: 179
Insert length is: 151
Start Codon found at position: 37
Stop codon found at position: 913

*Figure 16: The final new construct created by WordMol 1.0 using the demonstrations files included with this program. Note because we did not select the Maintain Colours from the Virtual Cloning menu, the vector is in its standard blue colour and insert is coded green indicating a successful cloning strategy.*

### *2.5.4 Further Notes on Full Frame Check*

As discussed above, Full frame check only operates when codon spacing is defined for both the **insert and the vector**. If there is a change in reading frame between the insert and the vector then the construction fails. Also the full frame check feature will check that it reaches a stop codon at some point in the sequence. If it fails to find a stop codon, then it will also fail the construct.

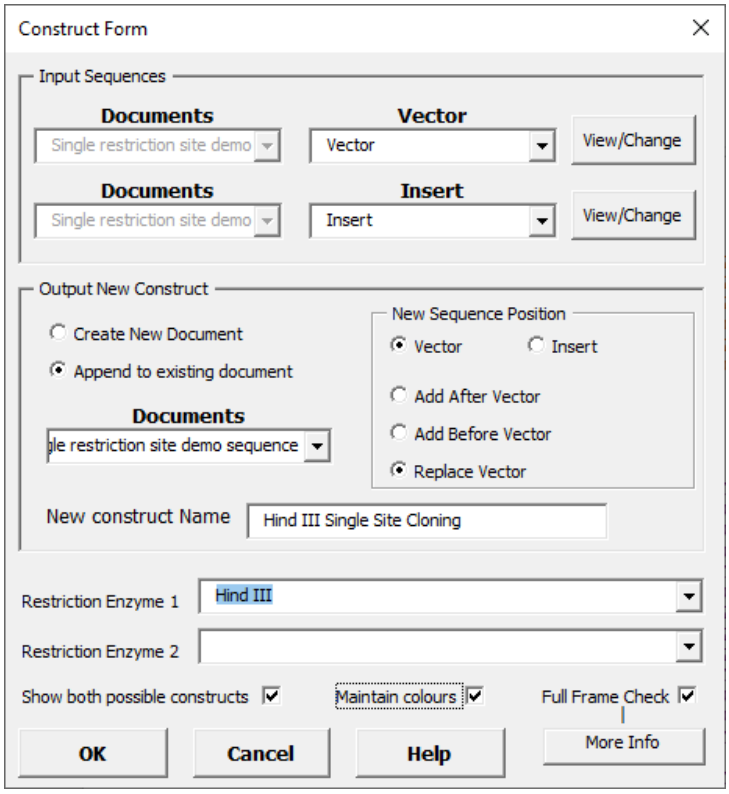
### *2.5.5 Single Restriction Site Cloning.*

One final possibility is the insert and vector contain duplicate restriction sites leading to the creation of multiple constructs depending on whether it is the insert or its reverse complement that is ligated into the vector. If you want to see an example of how Word Mol 1.0 deals with his situation open the final demo file Single Restriction Site Demo Sequence.docx.

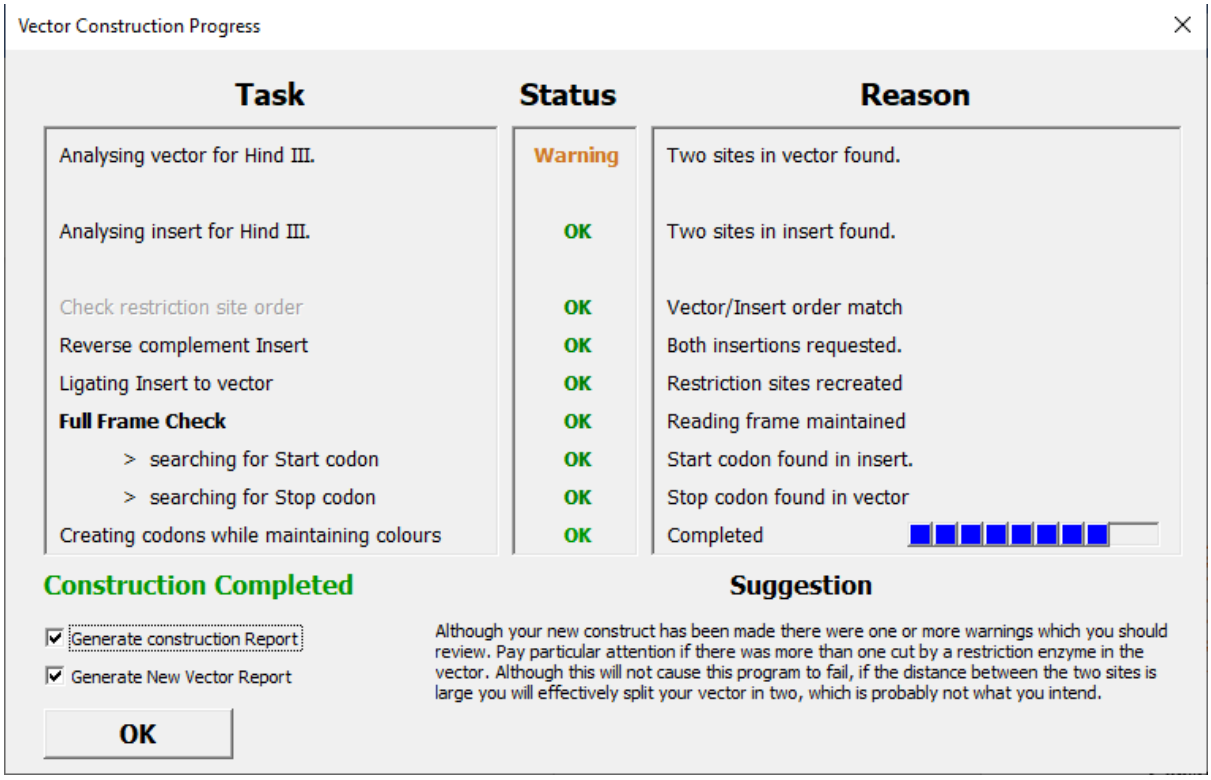
Click the molecular Cloning button and the first thing you will notice is this time the vector has the Insert selected while the Insert has the vector selected. This is simply because in this document, I have placed the insert first in the document whereas in all the other documents the vector was placed first. Ultimately the first sequence WordMol finds will be placed in the Vector menu and the second sequence in the insert menu. It does not intelligently try to

work out which is which. So the first thing we need to do is use our drop down menus to place the vector in the vector dropdown box and the insert in the Insert Dropdown box as shown below.

In our example we shall also place the constructs after the vector sequence by selecting Append to existing document and we'll opt to **replace the vector** as shown in the construct from window below. The single restriction site is Hind III so select this for restriction enzyme 1 and this time let's give the construct the name "Hind III Single Site Cloning. We shall do a full frame check and also click the Maintain Colours Option. Finally, we will also select the default option of showing both possible constructs from this cloning strategy.

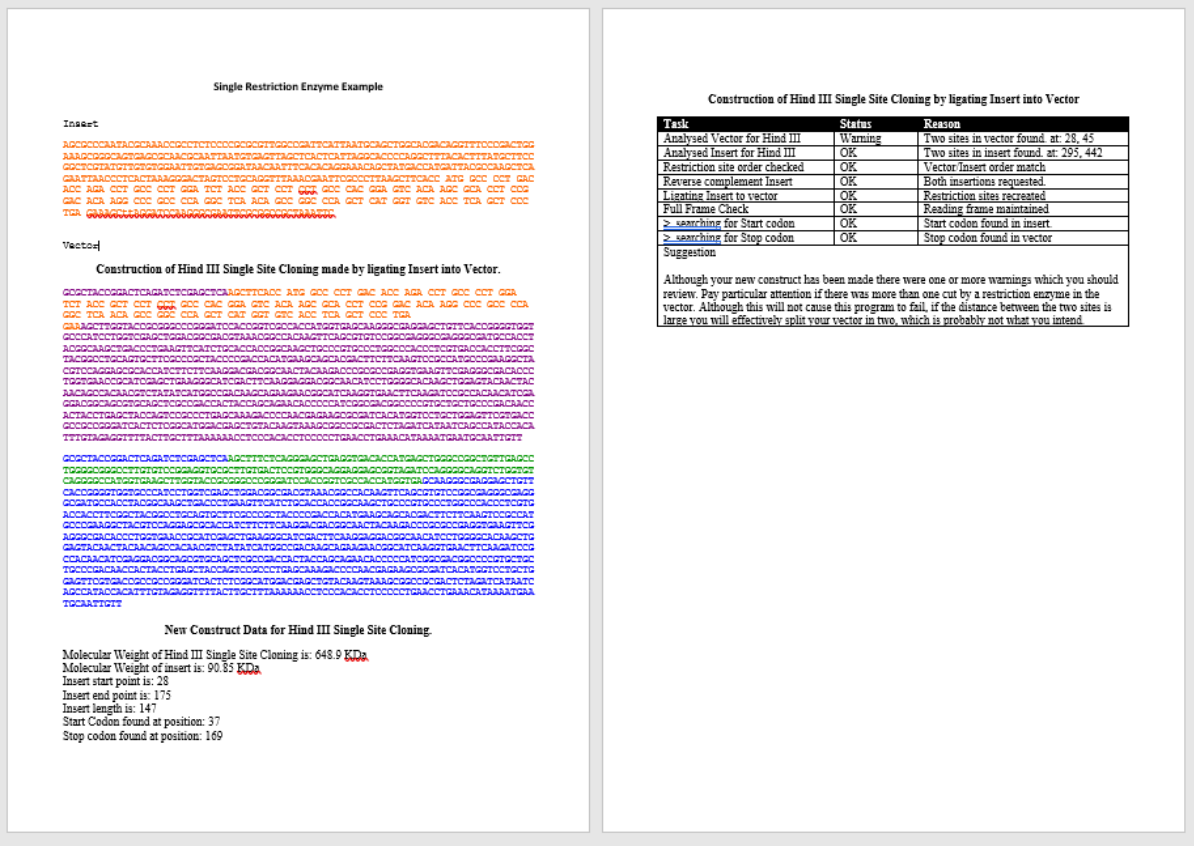



Click OK and the following construction window will appear.




We want to see the construction report so keep both options ticked and click OK.

The following information will be replace the original vector sequence producing the following document:



Single Restriction Enzyme Example

Insert

Vector

Construction of Hind III Single Site Cloning made by ligating Insert into Vector.

New Construct Data for Hind III Single Site Cloning.

Molecular Weight of Hind III Single Site Cloning is: 648.9 KDa
Molecular Weight of insert is: 90.85 KDa
Insert start point is: 28
Insert end point is: 175
Insert length is: 147
Start Codon found at position: 37
Stop codon found at position: 169

Construction of Hind III Single Site Cloning by ligating Insert into Vector

| Task | Status | Reason |
|---|---|---|
| Analysed Vector for Hind III | Warning | Two sites in vector found. at: 28, 45 |
| Analysed Insert for Hind III | OK | Two sites in insert found. at: 295, 442 |
| Restriction site order checked | OK | Vector/Insert order match |
| Reverse complement Insert | OK | Both insertions requested. |
| Ligating Insert to vector | OK | Restriction sites recreated |
| Full Frame Check | OK | Reading frame maintained |
| > searching for Start codon | OK | Start codon found in insert. |
| > searching for Stop codon | OK | Stop codon found in vector |

Suggestion

Although your new construct has been made there were one or more warnings which you should review. Pay particular attention if there was more than one cut by a restriction enzyme in the vector. Although this will not cause this program to fail, if the distance between the two sites is large you will effectively split your vector in two, which is probably not what you intend.

You will see that the construct with your insert sequence is placed first with the original colours of the vector and insert maintained as we requested the program do that. The other possible construct is also shown this time with the reverse complement of our insert placed in the vector this time in the standard blue green colours. The new construct report refers only to the first construct. Because we opted for the construction report to also be included this appears as a table after the second vector sequence indicating that because this construct was made with a single restriction enzyme two possible vectors have been produced from our ligation reaction.

This completes the tutorial on Word Mol 1.0. I hope you find this program a useful addition to your repertoire of programs that help you achieve your cloning objectives.

## 3 Conclusion

If you do find this program useful, please consider leaving a positive review here and promoting my website www.bensonium.com to your research colleagues. You might also like to check out my other programs which are also available on the website here.