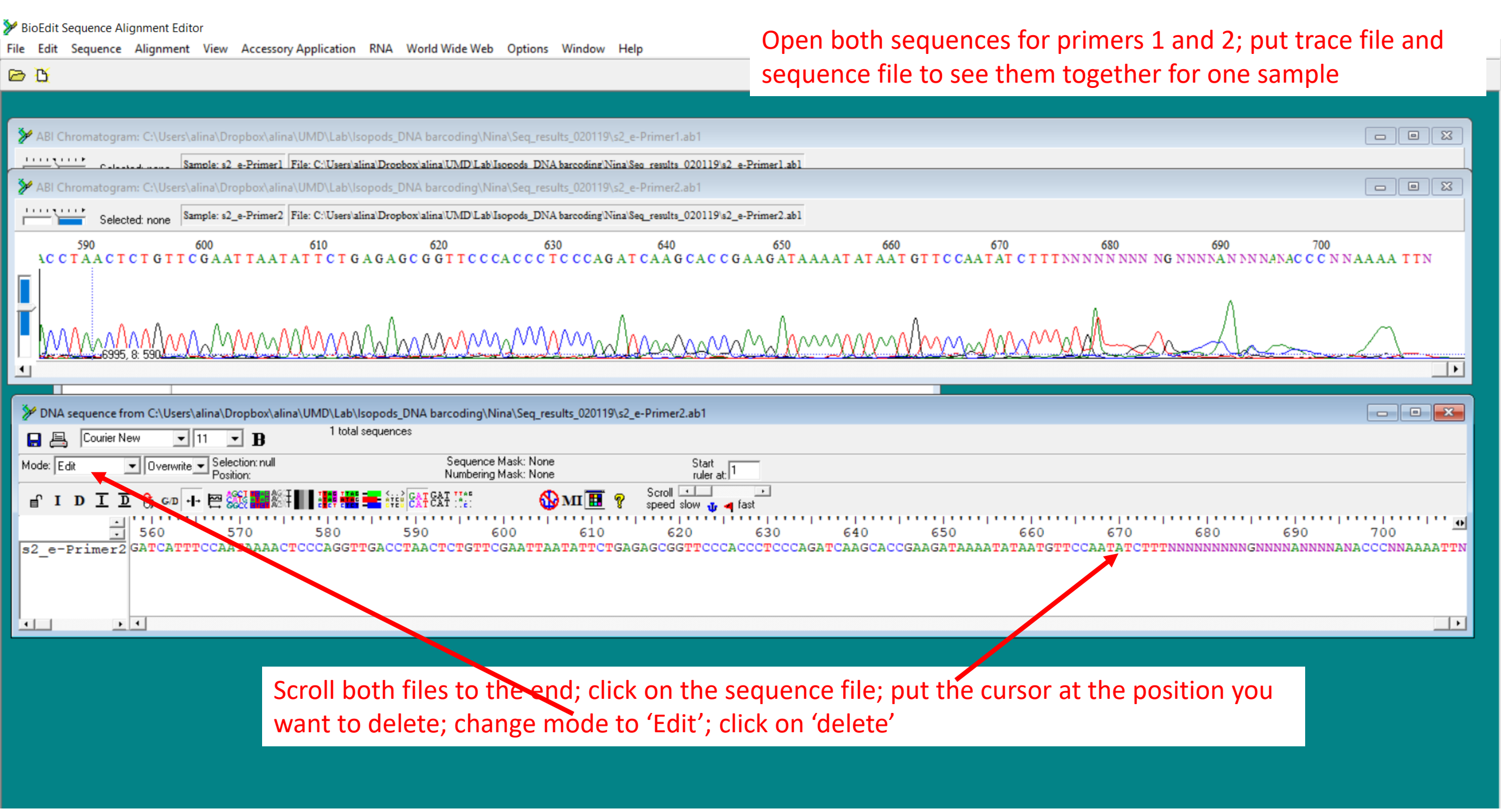
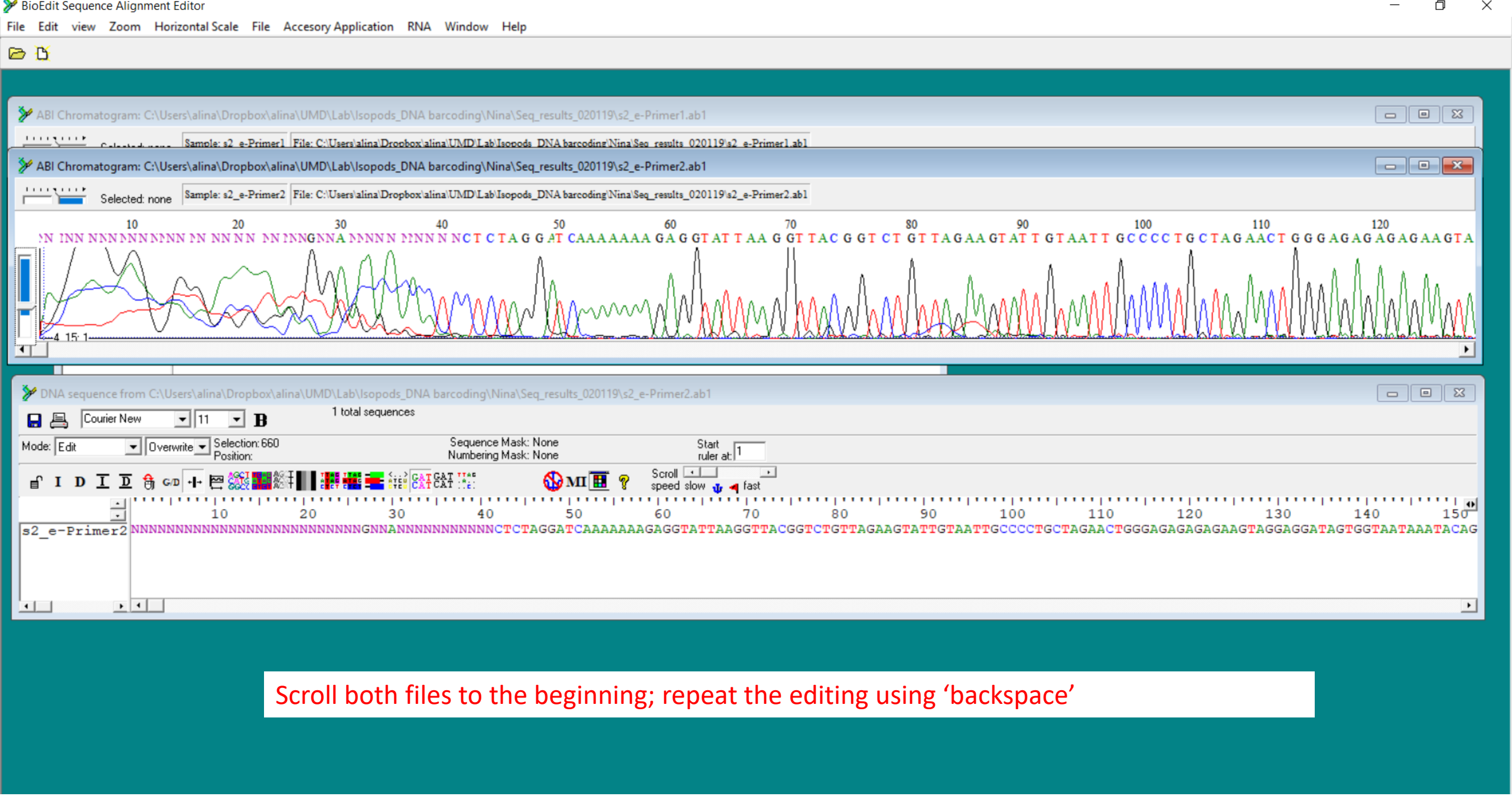
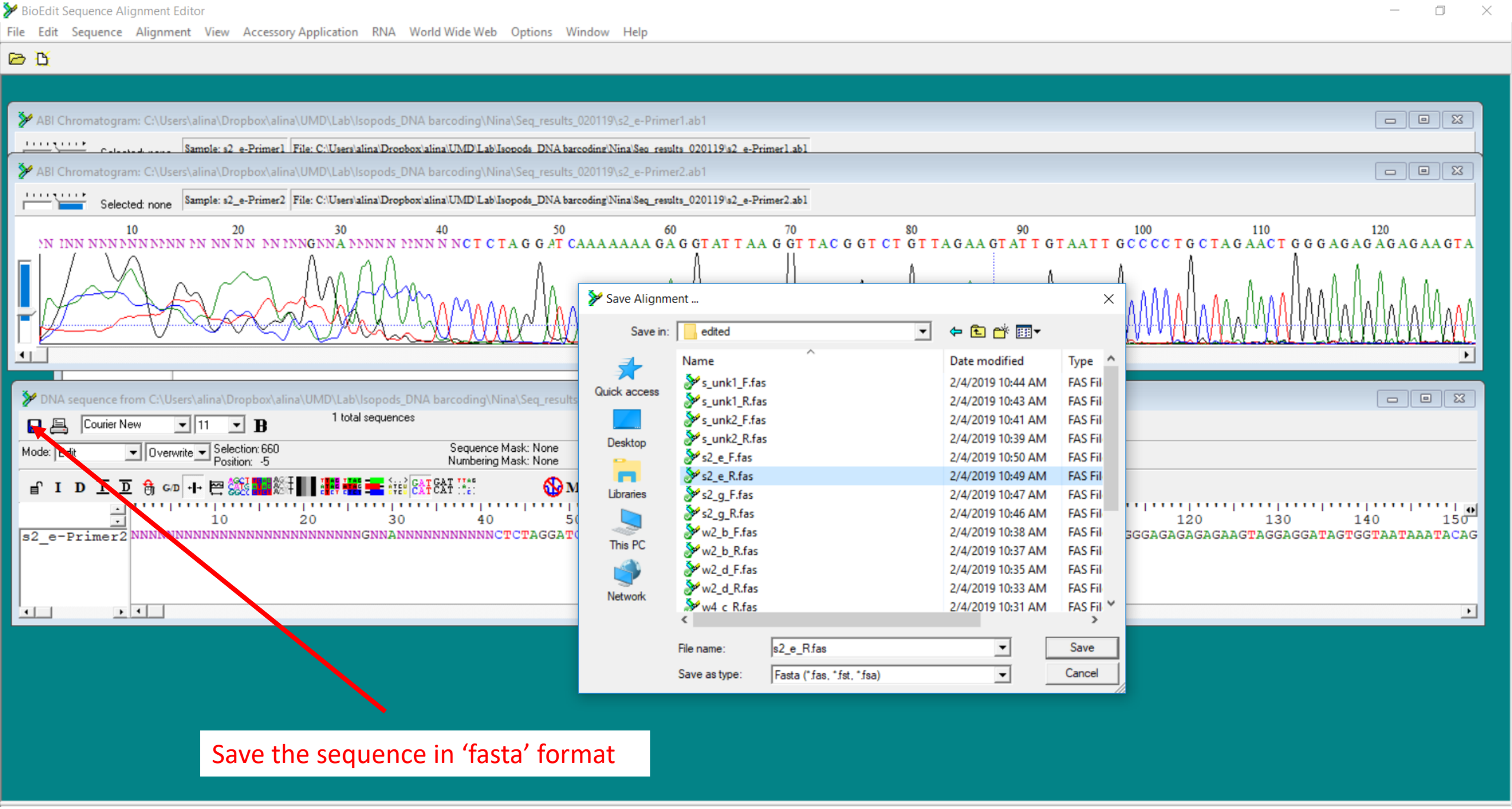

BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
Open both sequences for primers 1 and 2; put trace file and sequence file to see them together for one sample
ABI Chromatogram: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer1.ab1
ABI Chromatogram: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer2.ab1
Selected: none
Sample: s2_e-Primer2
File: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer2.ab1
590 600 610 620 630 640 650 660 670 680 690 700
ACCTAACTCTGTTCGAATTAATATTCTGAGAGCGGTTCCCACCCTCCCAGATCAAGCACCGAAGATAAAATATAATGTTCCAATATCTTTNNNNNNNNNNGNNNNANNNNANACCCNNAAAATTN
6995, 8: 590
DNA sequence from C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer2.ab1
Courier New
11
1 total sequences
Mode: Edit
Overwrite
Selection: null
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
560 570 580 590 600 610 620 630 640 650 660 670 680 690 700
s2_e-Primer2
GATCATTTCCAATAAAACTCCCAGGTTGACCTAACTCTGTTCGAATTAATATTCTGAGAGCGGTTCCCACCCTCCCAGATCAAGCACCGAAGATAAAATATAATGTTCCAATATCTTTNNNNNNNNNNGNNNNANNNNANACCCNNAAAATTN
Scroll both files to the end; click on the sequence file; put the cursor at the position you want to delete; change mode to 'Edit'; click on 'delete'



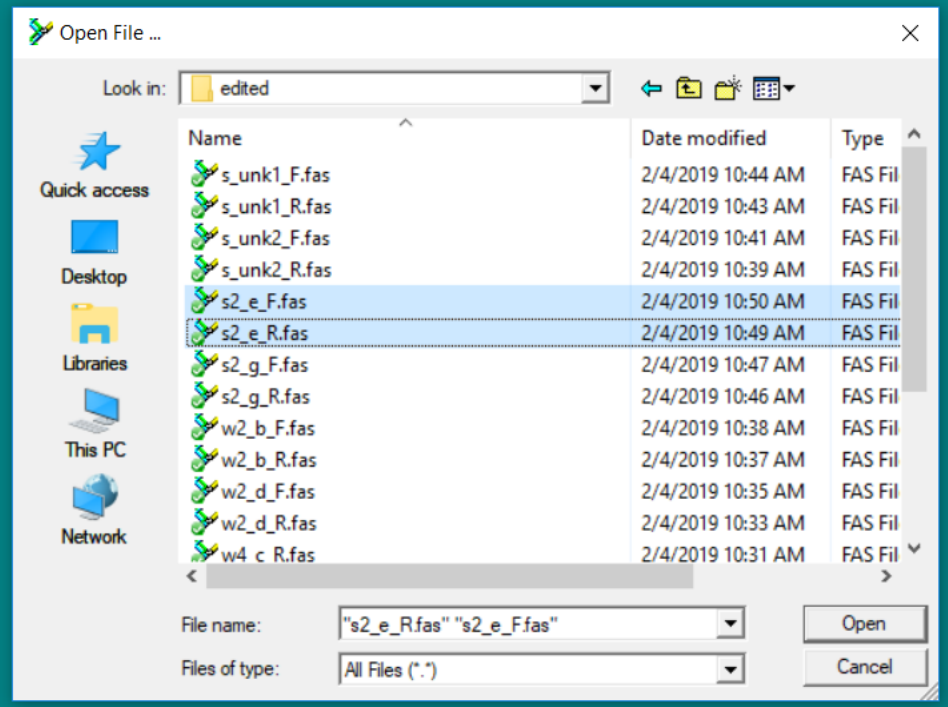
Scroll both files to the beginning; repeat the editing using ‘backspace’


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
ABI Chromatogram: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer1.ab1
ABI Chromatogram: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer2.ab1
Selected: none
Sample: s2_e-Primer2
File: C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\s2_e-Primer2.ab1
DNA sequence from C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results
Courier New
11
1 total sequences
Mode: Edit
Overwrite
Selection: 660
Position: -5
Sequence Mask: None
Numbering Mask: None
s2_e-Primer2
Save Alignment ...
Save in: edited
Quick access
Desktop
Libraries
This PC
Network
Name
Date modified
Type
s_unk1_F.fas 2/4/2019 10:44 AM FAS Fil
s_unk1_R.fas 2/4/2019 10:43 AM FAS Fil
s_unk2_F.fas 2/4/2019 10:41 AM FAS Fil
s_unk2_R.fas 2/4/2019 10:39 AM FAS Fil
s2_e_F.fas 2/4/2019 10:50 AM FAS Fil
s2_e_R.fas 2/4/2019 10:49 AM FAS Fil
s2_g_F.fas 2/4/2019 10:47 AM FAS Fil
s2_g_R.fas 2/4/2019 10:46 AM FAS Fil
w2_b_F.fas 2/4/2019 10:38 AM FAS Fil
w2_b_R.fas 2/4/2019 10:37 AM FAS Fil
w2_d_F.fas 2/4/2019 10:35 AM FAS Fil
w2_d_R.fas 2/4/2019 10:33 AM FAS Fil
w4_c_R.fas 2/4/2019 10:31 AM FAS Fil
File name: s2_e_R.fas
Save as type: Fasta (*.fas, *.fst, *.fsa)
Save
Cancel
Save the sequence in ‘fasta’ format

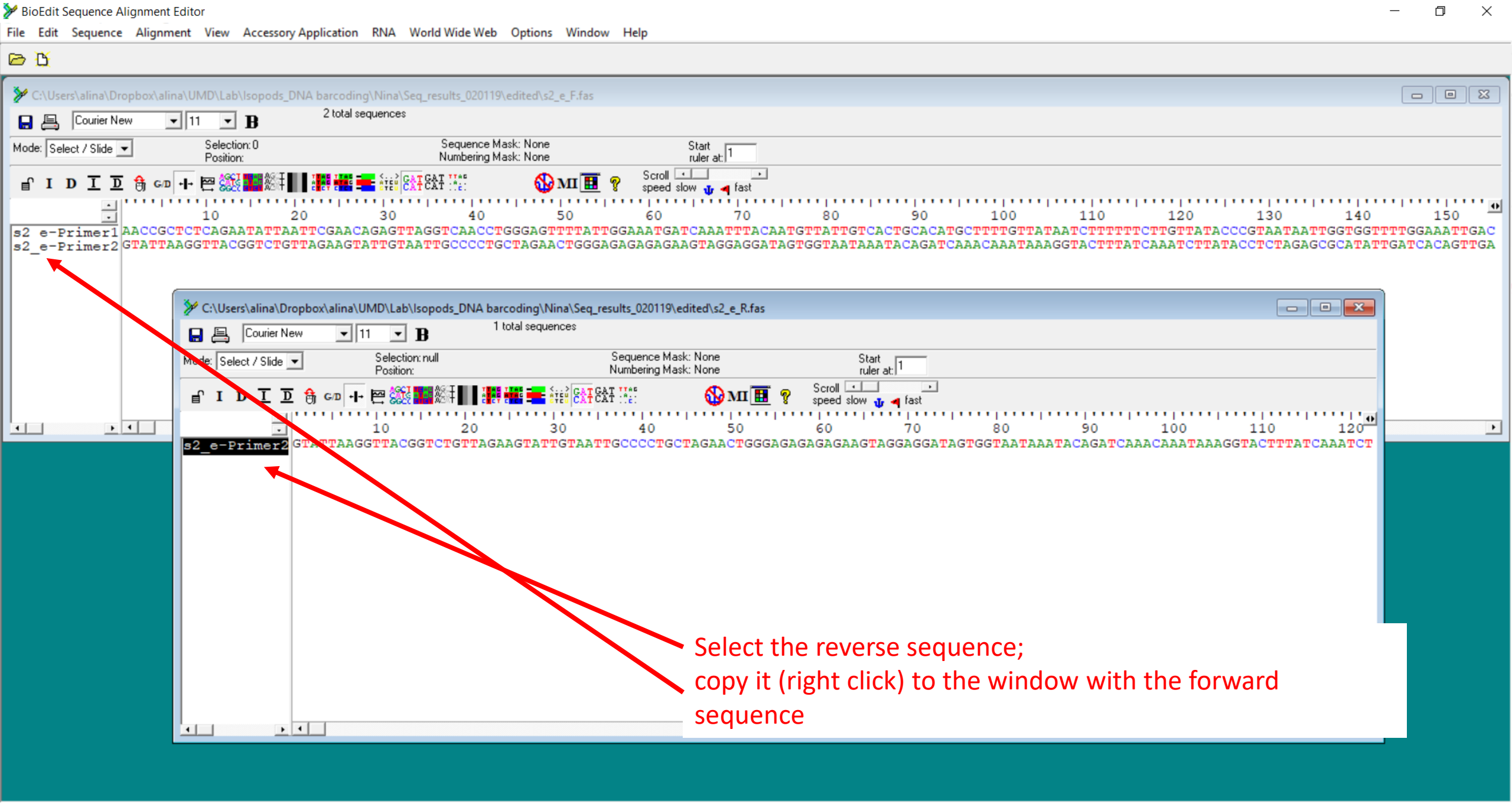
BioEdit Sequence Alignment Editor

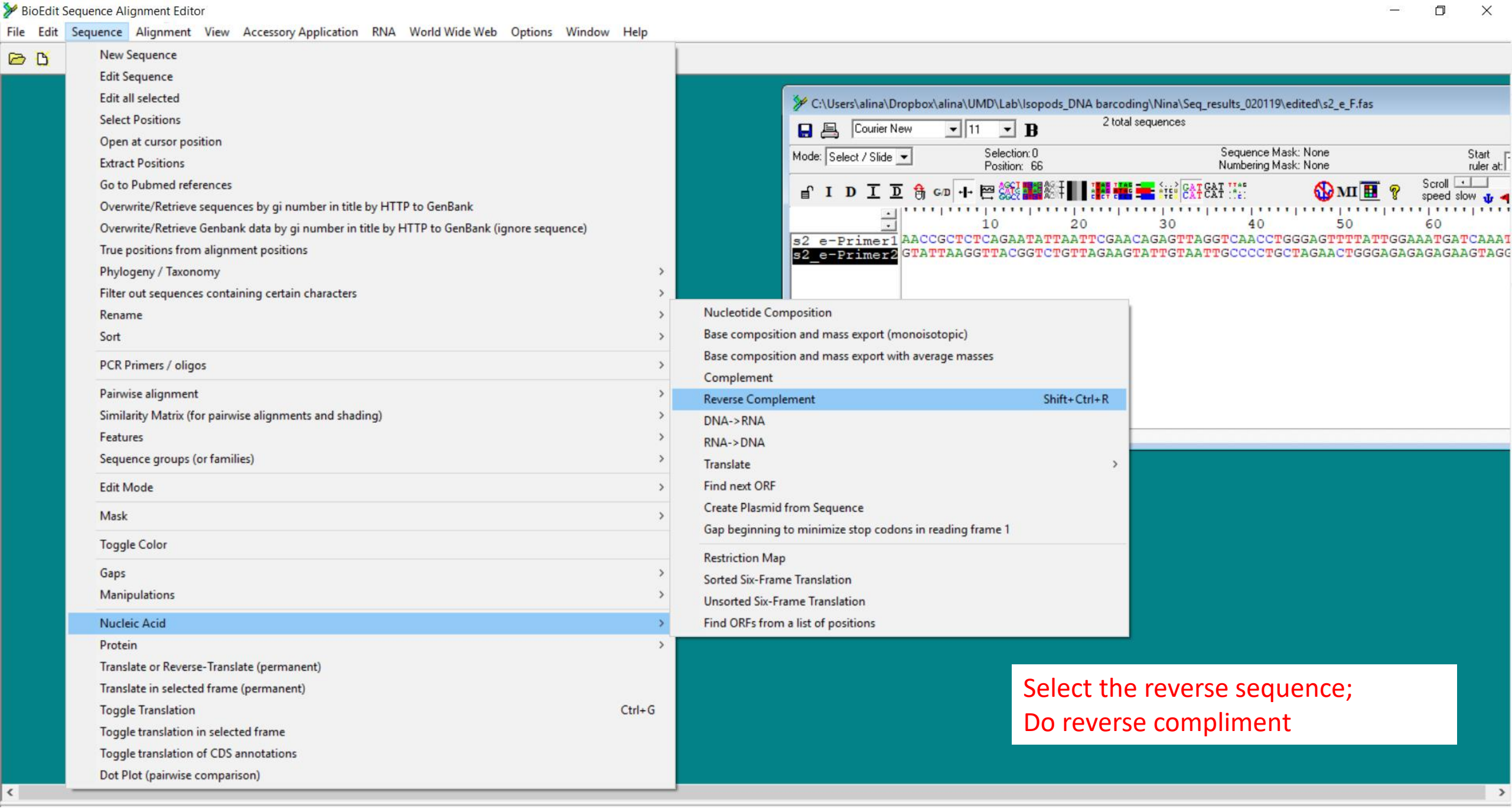
File Accesory Application RNA World Wide Web Options Window Help

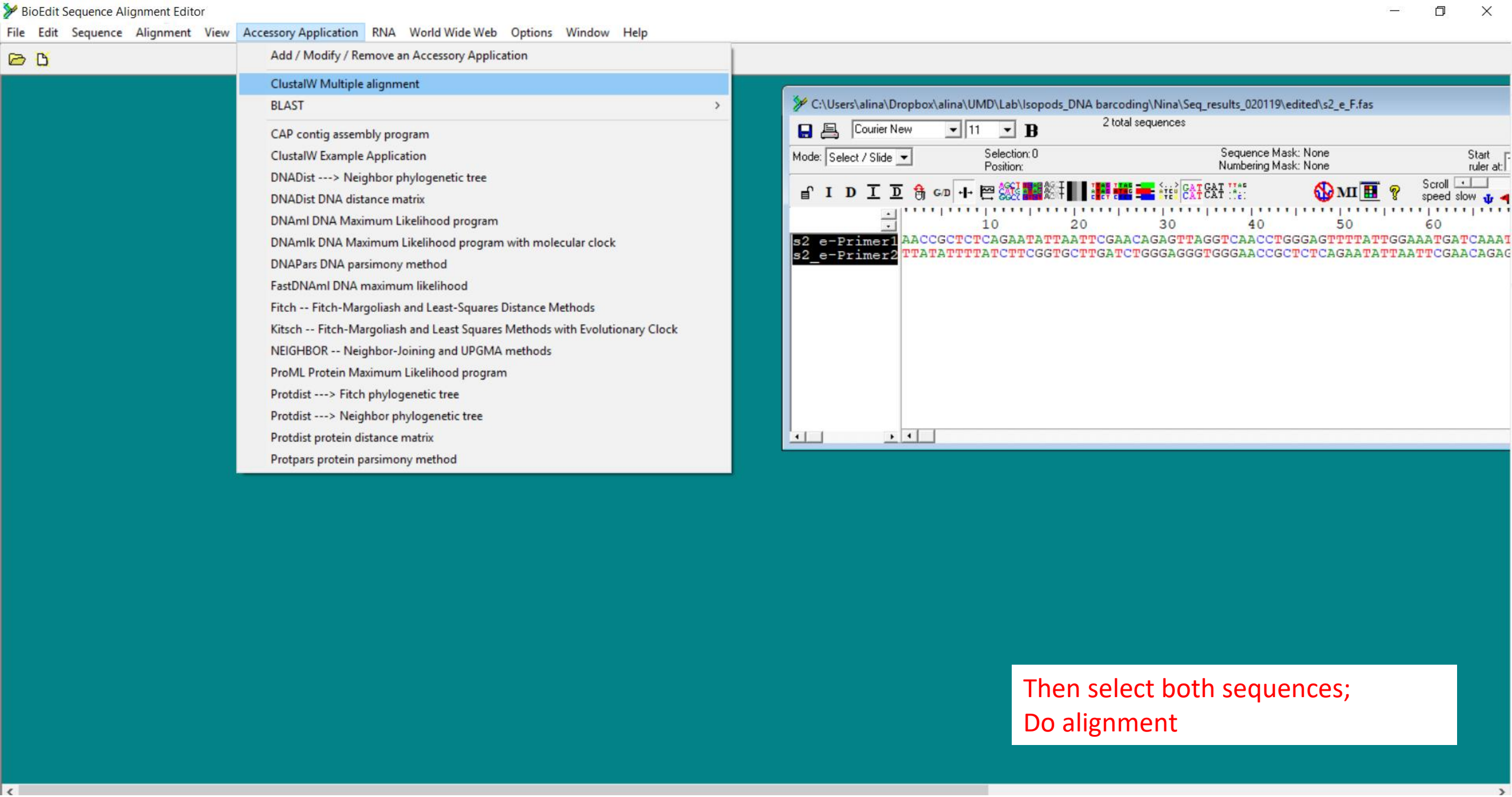
Do the editing for the other sequence; then close all the windows

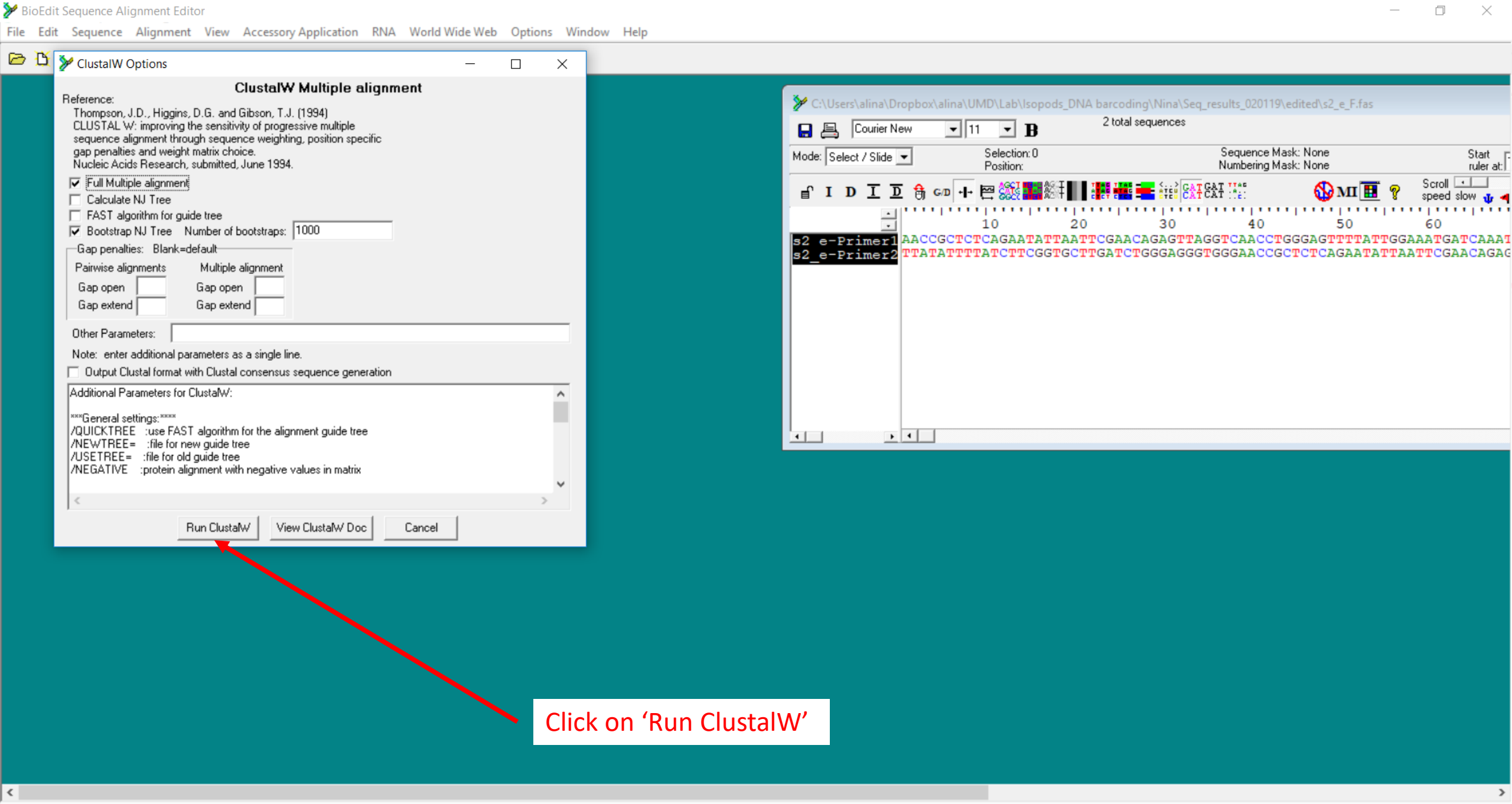


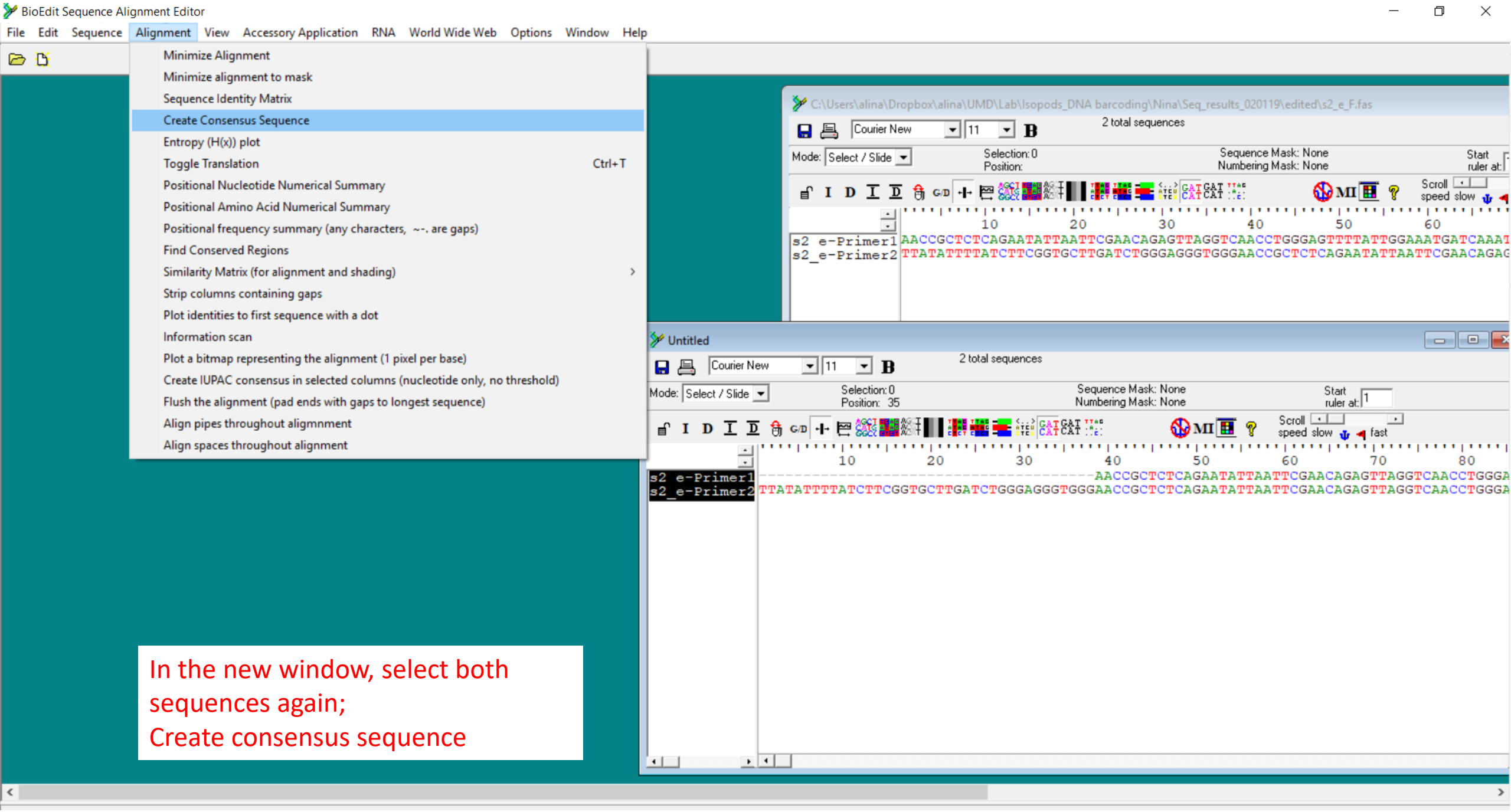
Then, open both forward and reverse edited sequences

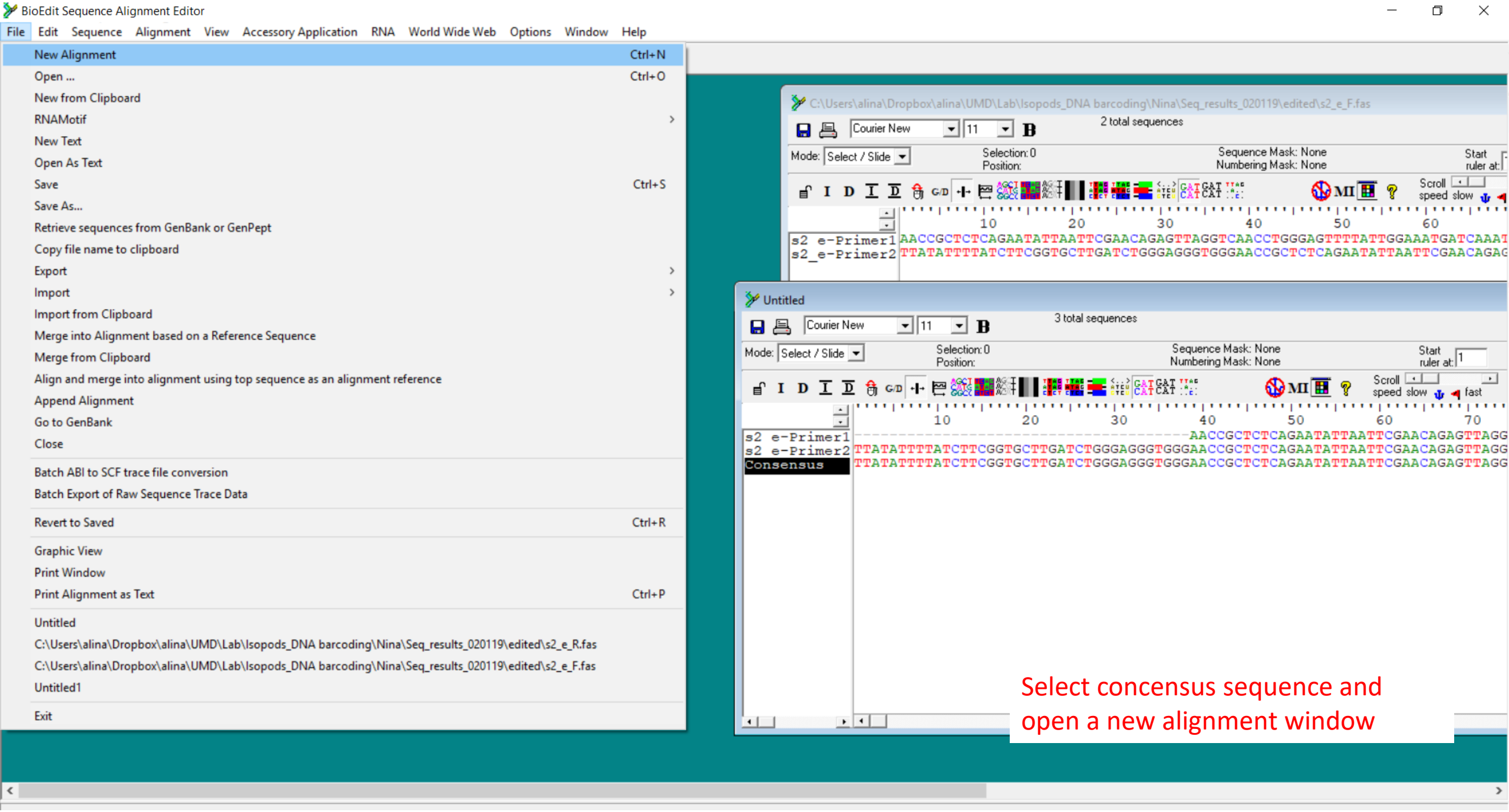

BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
2 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
s2_e-Primer1
s2_e-Primer2
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_R.fas
1 total sequences
Selection: null
Position:
Select the reverse sequence;
copy it (right click) to the window with the forward sequence

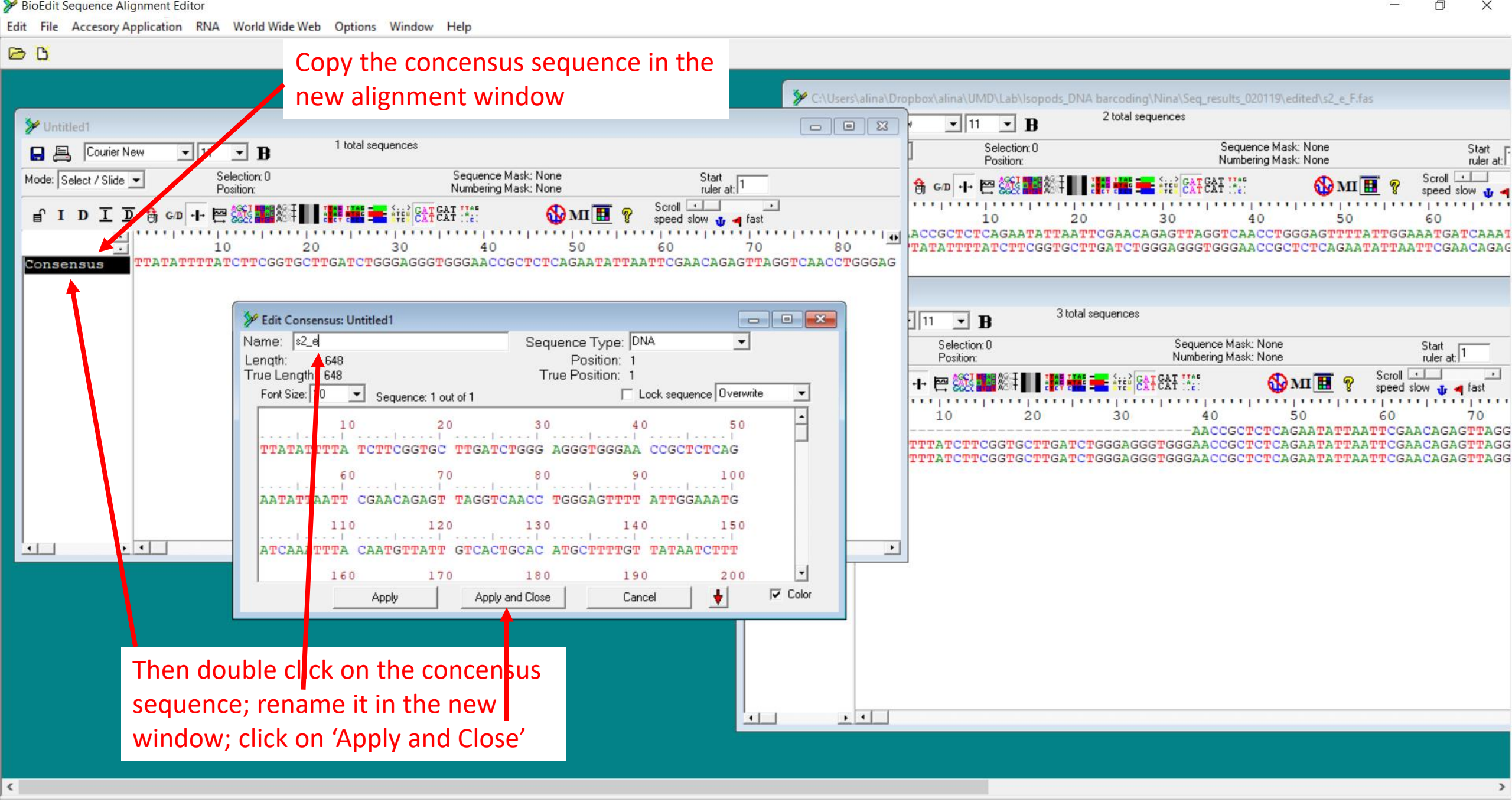

BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
New Sequence
Edit Sequence
Edit all selected
Select Positions
Open at cursor position
Extract Positions
Go to Pubmed references
Overwrite/Retrieve sequences by gi number in title by HTTP to GenBank
Overwrite/Retrieve Genbank data by gi number in title by HTTP to GenBank (ignore sequence)
True positions from alignment positions
Phylogeny / Taxonomy
Filter out sequences containing certain characters
Rename
Sort
PCR Primers / oligos
Pairwise alignment
Similarity Matrix (for pairwise alignments and shading)
Features
Sequence groups (or families)
Edit Mode
Mask
Toggle Color
Gaps
Manipulations
Nucleic Acid
Protein
Translate or Reverse-Translate (permanent)
Translate in selected frame (permanent)
Toggle Translation Ctrl+G
Toggle translation in selected frame
Toggle translation of CDS annotations
Dot Plot (pairwise comparison)
Nucleotide Composition
Base composition and mass export (monoisotopic)
Base composition and mass export with average masses
Complement
Reverse Complement Shift+Ctrl+R
DNA->RNA
RNA->DNA
Translate
Find next ORF
Create Plasmid from Sequence
Gap beginning to minimize stop codons in reading frame 1
Restriction Map
Sorted Six-Frame Translation
Unsorted Six-Frame Translation
Find ORFs from a list of positions
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
2 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position: 66
Sequence Mask: None
Numbering Mask: None
Start ruler at:
Scroll speed slow
10 20 30 40 50 60
s2_e-Primer1 AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAGTTTTATTGGAAATGATCAAAT
s2_e-Primer2 GTATTAAGGTTACGGTCTGTTAGAAGTATTGTAATTGCCCCTGCTAGAACTGGGAGAGAGAGAAGTAGG
Select the reverse sequence;
Do reverse compliment

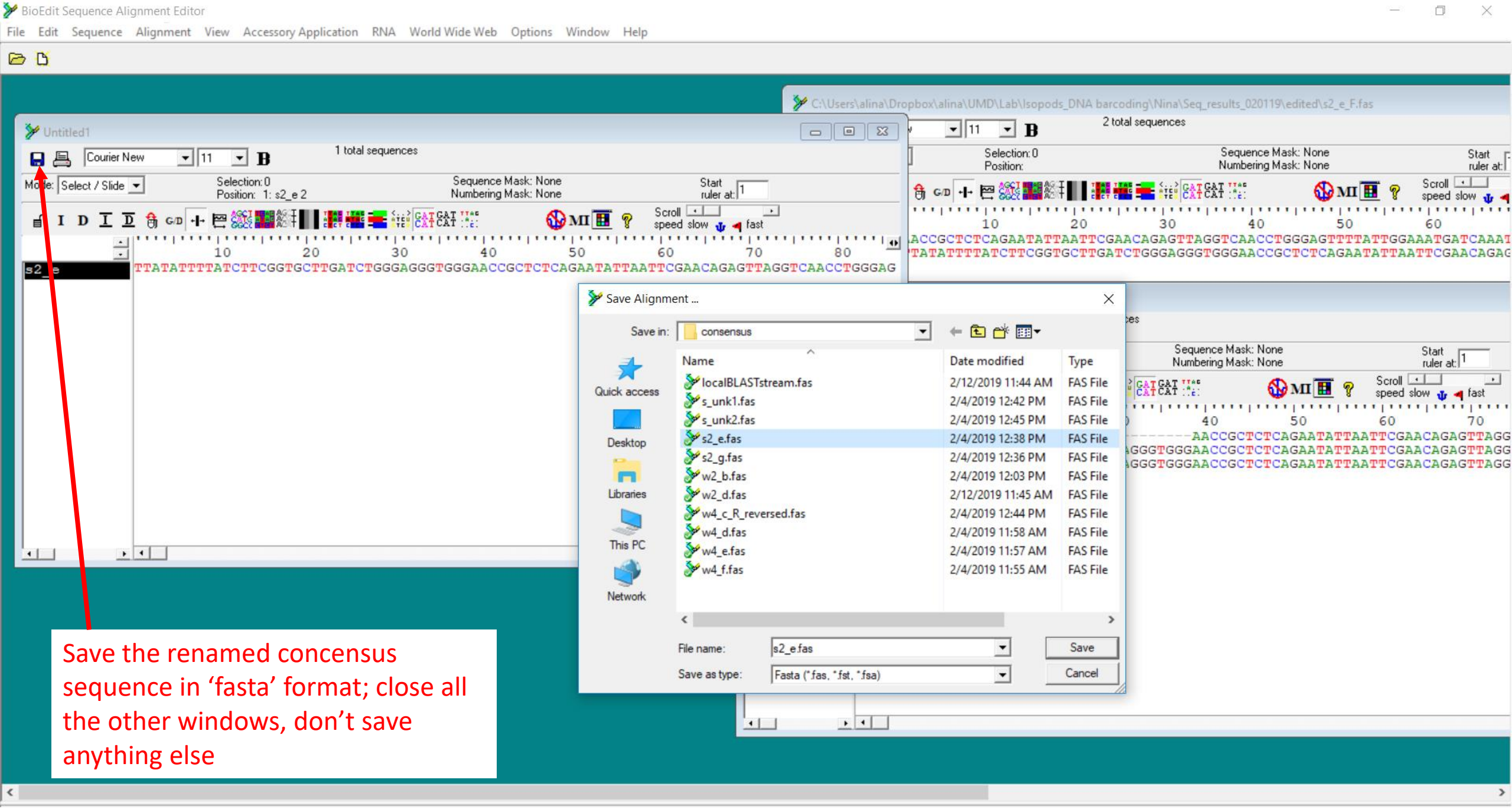

BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
Add / Modify / Remove an Accessory Application
ClustalW Multiple alignment
BLAST
CAP contig assembly program
ClustalW Example Application
DNADist ---> Neighbor phylogenetic tree
DNADist DNA distance matrix
DNAml DNA Maximum Likelihood program
DNAmlk DNA Maximum Likelihood program with molecular clock
DNAPars DNA parsimony method
FastDNAml DNA maximum likelihood
Fitch -- Fitch-Margoliash and Least-Squares Distance Methods
Kitsch -- Fitch-Margoliash and Least Squares Methods with Evolutionary Clock
NEIGHBOR -- Neighbor-Joining and UPGMA methods
ProML Protein Maximum Likelihood program
Protdist ---> Fitch phylogenetic tree
Protdist ---> Neighbor phylogenetic tree
Protdist protein distance matrix
Protpars protein parsimony method
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
Courier New
11
2 total sequences
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at:
Scroll speed slow
10 20 30 40 50 60
s2_e-Primer1 AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAGTTTTATTGGAAATGATCAAAT
s2_e-Primer2 TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAG
Then select both sequences;
Do alignment


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
ClustalW Options
ClustalW Multiple alignment
Reference:
Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994)
CLUSTAL W: improving the sensitivity of progressive multiple
sequence alignment through sequence weighting, position specific
gap penalties and weight matrix choice.
Nucleic Acids Research, submitted, June 1994.
Full Multiple alignment
Calculate NJ Tree
FAST algorithm for guide tree
Bootstrap NJ Tree Number of bootstraps: 1000
Gap penalties: Blank=default
Pairwise alignments
Gap open
Gap extend
Multiple alignment
Gap open
Gap extend
Other Parameters:
Note: enter additional parameters as a single line.
Output Clustal format with Clustal consensus sequence generation
Additional Parameters for ClustalW:
General settings:*
/QUICKTREE :use FAST algorithm for the alignment guide tree
/NEWTREE= :file for new guide tree
/USETREE= :file for old guide tree
/NEGATIVE :protein alignment with negative values in matrix
Run ClustalW
View ClustalW Doc
Cancel
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
Courier New
11
2 total sequences
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at:
Scroll speed slow
10 20 30 40 50 60
s2_e-Primer1 AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAGTTTTATTGGAAATGATCAAAT
s2_e-Primer2 TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAG
Click on ‘Run ClustalW’


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
Minimize Alignment
Minimize alignment to mask
Sequence Identity Matrix
Create Consensus Sequence
Entropy (H(x)) plot
Toggle Translation Ctrl+T
Positional Nucleotide Numerical Summary
Positional Amino Acid Numerical Summary
Positional frequency summary (any characters, ~-. are gaps)
Find Conserved Regions
Similarity Matrix (for alignment and shading)
Strip columns containing gaps
Plot identities to first sequence with a dot
Information scan
Plot a bitmap representing the alignment (1 pixel per base)
Create IUPAC consensus in selected columns (nucleotide only, no threshold)
Flush the alignment (pad ends with gaps to longest sequence)
Align pipes throughout aligmnment
Align spaces throughout alignment
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
2 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at:
Scroll speed slow
s2_e-Primer1
s2_e-Primer2
AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAGTTTTATTGGAAATGATCAAAT
TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAG
Untitled
2 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position: 35
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
s2_e-Primer1
s2_e-Primer2
AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGA
TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGA
In the new window, select both sequences again;
Create consensus sequence


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
New Alignment Ctrl+N
Open ... Ctrl+O
New from Clipboard
RNAMotif
New Text
Open As Text
Save Ctrl+S
Save As...
Retrieve sequences from GenBank or GenPept
Copy file name to clipboard
Export
Import
Import from Clipboard
Merge into Alignment based on a Reference Sequence
Merge from Clipboard
Align and merge into alignment using top sequence as an alignment reference
Append Alignment
Go to GenBank
Close
Batch ABI to SCF trace file conversion
Batch Export of Raw Sequence Trace Data
Revert to Saved Ctrl+R
Graphic View
Print Window
Print Alignment as Text Ctrl+P
Untitled
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_R.fas
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
Untitled1
Exit
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
2 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at:
Scroll speed slow
10 20 30 40 50 60
s2_e-Primer1 AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAGTTTTATTGGAAATGATCAAAT
s2_e-Primer2 TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAG
Untitled
3 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
10 20 30 40 50 60 70
s2_e-Primer1 AACCGCTCTCAGAATATTAATTCGAACAGAGTTAGG
s2_e-Primer2 TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAGTTAGG
Consensus TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAGTTAGG
Select concensus sequence and
open a new alignment window


BioEdit Sequence Alignment Editor
Edit File Accesory Application RNA World Wide Web Options Window Help
Copy the concensus sequence in the new alignment window
Untitled1
1 total sequences
Consensus
Edit Consensus: Untitled1
Name: s2_e
Sequence Type: DNA
Length: 648
True Length: 648
Position: 1
True Position: 1
Font Size: 10
Sequence: 1 out of 1
Lock sequence
Overwrite
Apply
Apply and Close
Cancel
Color
2 total sequences
3 total sequences
Then double click on the concensus sequence; rename it in the new window; click on 'Apply and Close'


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
Untitled1
1 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position: 1: s2_e 2
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
s2_e
TTATATTTTATCTTCGGTGCTTGATCTGGGAGGGTGGGAACCGCTCTCAGAATATTAATTCGAACAGAGTTAGGTCAACCTGGGAG
C:\Users\alina\Dropbox\alina\UMD\Lab\Isopods_DNA barcoding\Nina\Seq_results_020119\edited\s2_e_F.fas
2 total sequences
Selection: 0
Position:
Save Alignment ...
Save in: consensus
Quick access
Desktop
Libraries
This PC
Network
Name
Date modified
Type
localBLASTstream.fas 2/12/2019 11:44 AM FAS File
s_unk1.fas 2/4/2019 12:42 PM FAS File
s_unk2.fas 2/4/2019 12:45 PM FAS File
s2_e.fas 2/4/2019 12:38 PM FAS File
s2_g.fas 2/4/2019 12:36 PM FAS File
w2_b.fas 2/4/2019 12:03 PM FAS File
w2_d.fas 2/12/2019 11:45 AM FAS File
w4_c_R_reversed.fas 2/4/2019 12:44 PM FAS File
w4_d.fas 2/4/2019 11:58 AM FAS File
w4_e.fas 2/4/2019 11:57 AM FAS File
w4_f.fas 2/4/2019 11:55 AM FAS File
File name: s2_e.fas
Save as type: Fasta (*.fas, *.fst, *.fsa)
Save
Cancel
Save the renamed concensus sequence in ‘fasta’ format; close all the other windows, don’t save anything else