	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*		
Title: Creating a consensus DNA sequence from ABI sequence data using Sequencher			
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Authors: Ahmed Gaballa, Jordan Skeens and A. Andrus		Approved by:	

Creating a consensus DNA sequence from ABI sequence data using Sequencher

FILE NAME: 8.4.5 Creating Consensus DNA-Sequencher.docx

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SECTION 1 - INTRODUCTION

Purpose

To create and edit consensus sequence from ABI files generated from Sanger DNA sequencing. The obtained sequence can be used for BLAST analysis, e.g., *rpoB* and *sigB* allelic typing.

Scope

This SOP may be used by all lab members. For automated editing of large number of sequences using SeqTrace, please refer to 8.4.1-Automated Sequence Editing SOP.

Definitions

ABI (or AB1): A file produced by Sanger DNA sequencing instrument that contains sequence chromatogram (also known as electropherogram, or electrophoregram). This file is used to determine DNA bases identity (base calling).

Consensus: a single sequence derived from a set of overlapping DNA segments originating from one genetic source.

SECTION 2 - MATERIALS

1. **Computer.**

2. **Sequence files:** Sequence files obtained from BRC facility (Biotechnology Research Center). Raw data files are in .ab1 format and edited consensus sequences are saved as .fas files.

3. **Sequencher:** DNA editing software. The lab has a license to use Sequencher through Cornell BRC.

SECTION 3- PROCEDURE

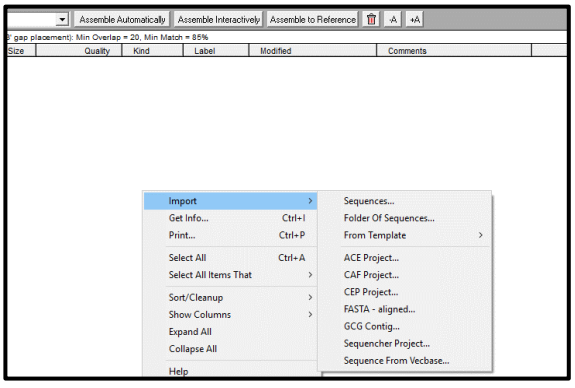
3.1 Installation of Sequencher:

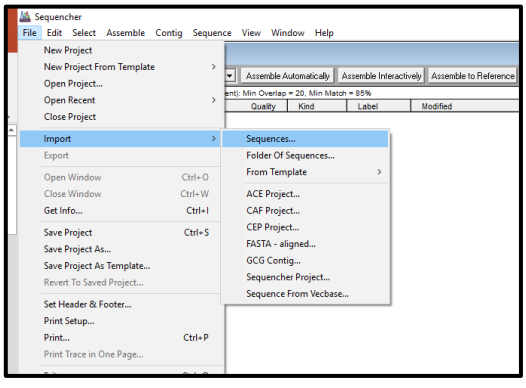
1. Download and install the latest keyserver client for your OS:
   Windows: http://www.sassafras.com/links/K2Client.exe
   MacOS: http://www.sassafras.com/links/K2Client.pkg
   During the install, be sure to enter Keyserver Address : brcks.biotech.cornell.edu
   Please ask a lab member for the username and password (Hint: same username and password as for Geneious).
2. Download Sequencher software: https://www.genecodes.com/free-download
   You may be prompted for a license file. Download and use the file from:
   https://cornell.box.com/s/celf2wufrjt7hpnone8kf3ww9khmnnly

3.2 Sequence editing in Sequencher:

1. Import Raw sequence files:

   a. Log-in to Sequencher.
   b. Create new project: in the toolbar, go to "File → New Project".
   c. To import raw files right click, or use the toolbar menu "File → Import → sequences", browse to the folder containing the raw sequence files.





   d. Select Forward and Reverse files with the .ab1 file extension (not the .seq files) and hit open.

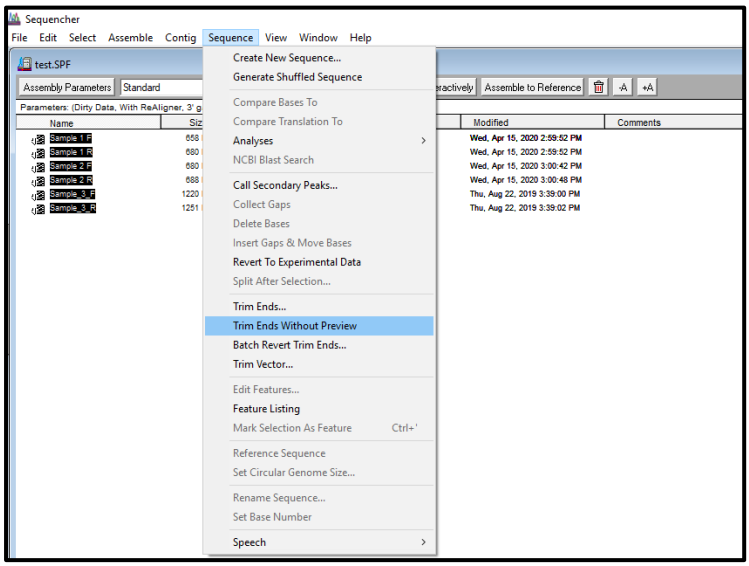
e. Save Project: in the toolbar menu, go to "File → Save project, or Ctrl+S", browse to your sequence-designated folder, choose a file name and click save.

2. Trim ambiguous sequences:

Important note: this step may trim bases that might have been possible to call manually. If the final edited consensus sequence is shorter than the minimum required sequence for a specific application (e.g., 632bp for *rpoB*-based allelic typing of bacillales or 660bp for *sigB*-based allelic typing of *Listeria*), re-analyze your sequence with omitting this step. Attempt to base-call ambiguous sequence manually. If base calling is not possible, re-send the DNA to BRC for sequencing after troubleshooting possible issues related to DNA quality and quantity.

a. Select all sequences.

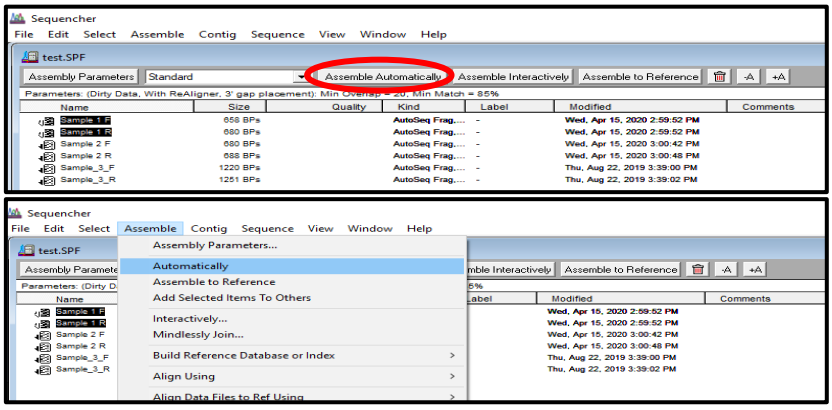
b. In the toolbar menu choose "Sequence → Trim Ends Without Preview".



3. Assemble sequences:

a. Select both forward and reverse files for one sequence (select one file, hold Ctrl key and select the second) and click "Assemble Automatically" radio button (or from the toolbar

go to "Assemble → Automatically").

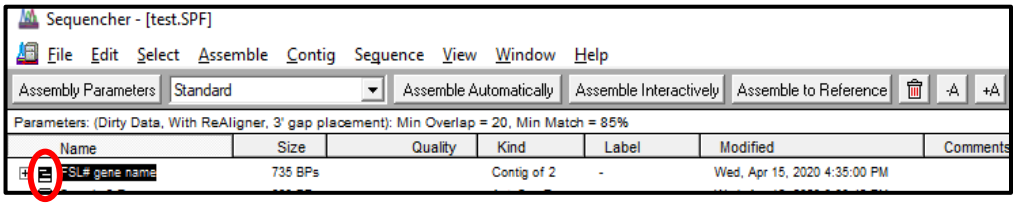


b. Change the name of the Contig, right click on the Contig name and select "Rename contig" from the popup menu or from the toolbar go to "Contig → Rename contig". Choose a unique name to reflect the FSL and the gene you have sequenced.

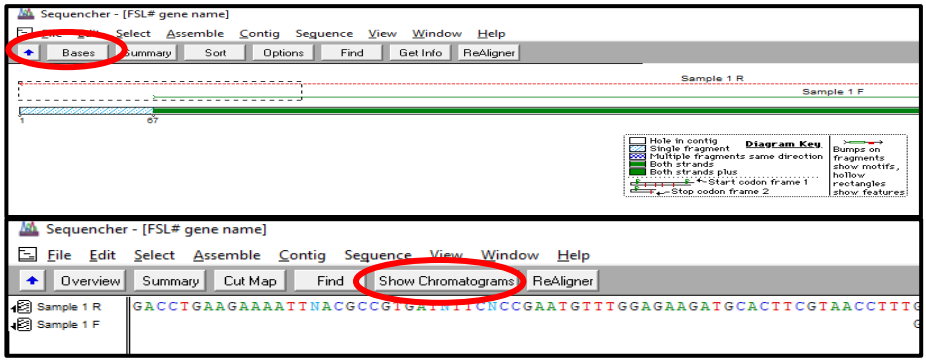
c. To view the forward and reverse sequences in a Contig click the + to the far-left of the contig name.

4. Editing sequences:

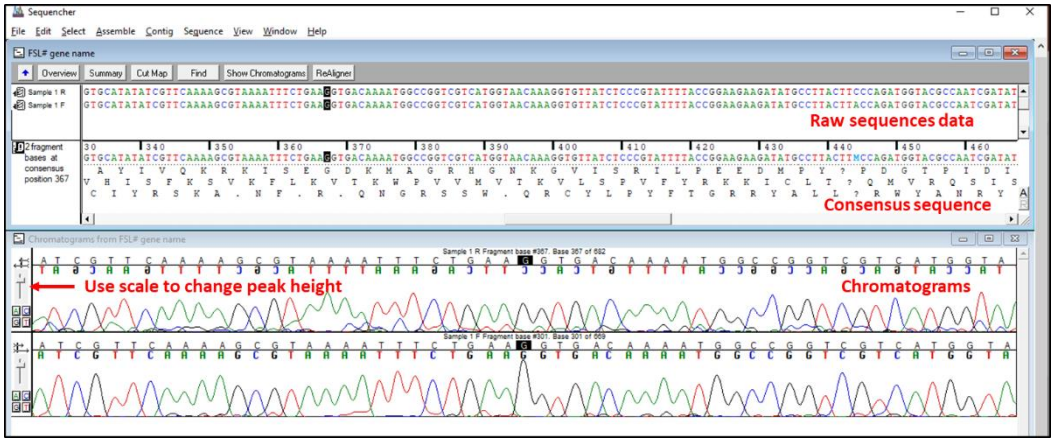
a. Double-click on the symbol to the left of the Contig name.



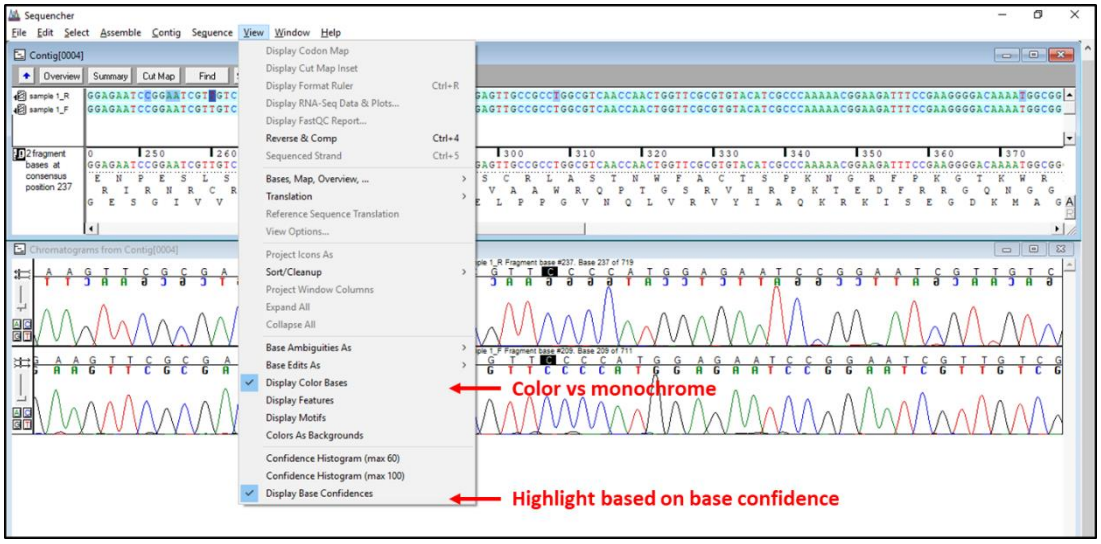
b. When the new window pops up, click the radio button "Bases" then "Show Chromatograms".



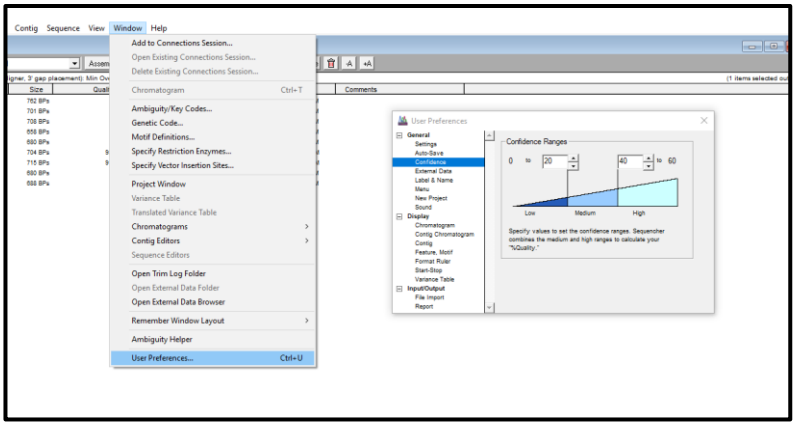
c. The forward and reverse sequences are at the top of the page with the consensus sequence below them. Make edits only to the consensus sequence.



d. You can change how the bases are displayed from the "View" menu. This includes color vs monochrome "View → Display Color Bases"; shading by base confidences values "View → Display base confidences".



You can view and change confidence-shading ranges from the toolbar menu "Window → User preferences (or Ctrl+U)" and select "confidences" in the popup window from the menu on the left.



e. Trim messy sequence from 5’ and 3’ ends by highlighting these bases in the consensus sequence and hit delete.

f. Make edits in the consensus sequence by comparing peaks in the chromatograms. Change base editing mode (i.e., replace base vs insert base) from the toolbar menu “Edit → Replace When Editing or Insert When Editing”.

Hint: 8.4.1-Automated Sequence Editing SOP describes valuable background details about quality (confidence) score and its use in base calling (introduction and 3.7 sections).

g. Undo a change from toolbar menu “Edit → Undo or Ctrl+Z”.

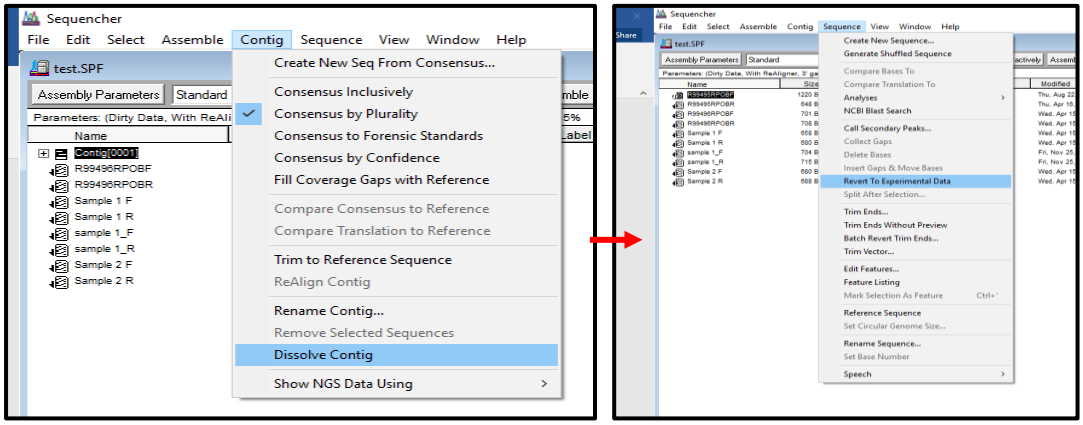
h. Sequencher automatically saves edits, so you only need to save your project.

i. At this step, it is good idea to estimate if you have the minimum sequence length for a specific application (e.g., 632bp for *rpoB*-based allelic typing of bacillales or 660bp for *sigB*-based allelic typing of *Listeria*). However, BLAST analysis will be required to determine if you have the complete sequence of the allelic typing region.

j. Dissolving Contigs and reverting to experimental data:

   i. Undo function can be used only to revert one edit. To revert to the original sequence, select the contig from the main project window then from the toolbar menu choose "Contig → dissolve Contig". This option can also be done by right click on the contig name and select dissolve Contig from the popup menu.

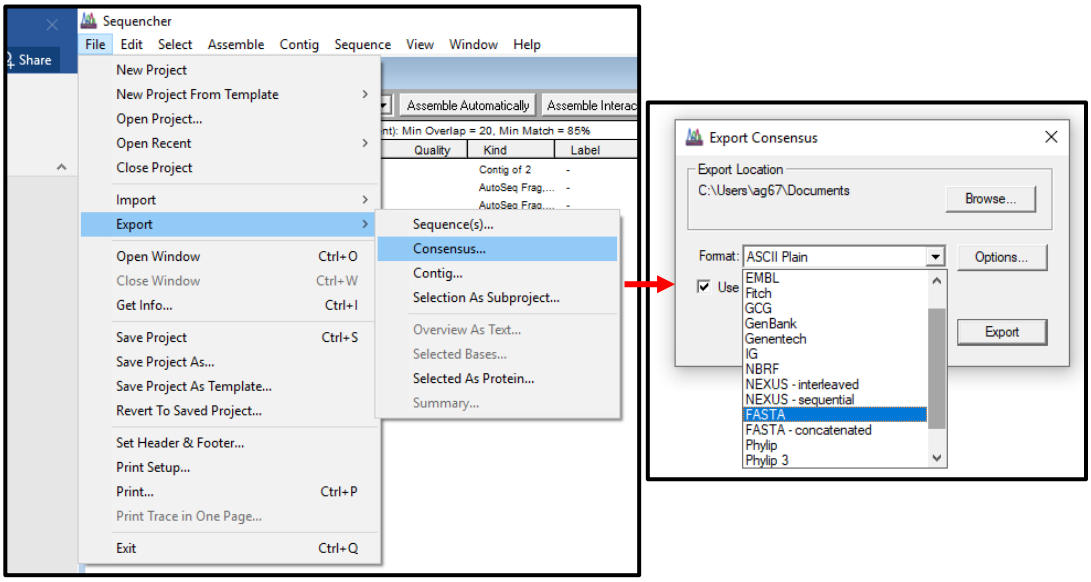
   ii. Select the individual sequence files and from the toolbar menu choose "Sequence → Revert to Experimental Data"



k. Exporting edited Contig as a FASTA (.fas) file:

   i. To export consensus, select the edited contigs. From the toolbar menu choose "File → Export → consensus" or right click on the contig and choose Export, then Consensus from the popup menu.

ii. In the popup window, choose Fasta from the drop menu of "Format". Browse to your designated sequence folder then click OK.



SECTION 4- METHOD REVIEWS & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version: draft	05/30/13	A. Andrus	Draft SOP
Version 1 (SOP 8.1.1.1.21)	4/22/20	Ahmed Gaballa and Jordan Skeens	