Protocol For Identification of rpoB Allelic Types

- Edit sequences. Remember to remove primer ends. Sequences need to be at least 632 base pairs.
- Do an alignment of edited sequences in Megalign. Use reference alignment ATCC10987_AEO17194_rpoB located in MilkQual-Molecular work.
- Select the region from 2455 to 3086 as follows: Go to edit, select go to position and then range and then enter 2455 and 3086. This will highlight the region that you want. Note: Remember to delete reference alignment before saving alignment.
- Go to align and select create alignment from selection. Save as the order number that is on your BRC results. This will save the alignment as a Megalign file. Then save this file as a Fasta file. Note: it is helpful to have a file for alignments and then another for the Bioedit results when you have them.
- Open the Fasta file in Bioedit. Hit control A to select all. Select Accessory Application-BLAST-local blast. A confirmation box will pop up asking if you want to do a batch job, select yes. The search screen will come up. For Nucleotide database choose 10-8-10rpob_ATdatabase.fas. This is the most recent database of all the rpob AT types collected so far. As new types are found the database will be updated. Change box for Max number of hits to 1. Change box for Max number of alignments to show to 1. Change box for Threshold for extending hit to 850 (This is the approximate number of AT types that the alignments will be queried against. Hit Do Search.
- The database will match each query sequence against the sequences in the database and show Sequences producing significant alignments (In this case only one alignment). It will show the AT type and the isolate that is the representative for that type. The identities should = 632/632 (100%). If it does then it is that allelic type. If they do not match 100% you will need to identify the base pair(s) that do not match and check them against the sequence to make sure that it is not an editing or alignment error. If after double checking the sequence you have determined that it is not an error, then this is a new allelic type.
- Example:
  Sequences producing significant alignments:

  AT015_F4129   1253   0.0

     Score = 1253 bits (632), Expect = 0.0
     Identities = 632/632 (100%)
     Strand = Plus / Plus
  This sequence would be AT 015.
- To check sequences for isolates that do not match, first find the base pair that doesn't match. Identify the position on the query sequence. Open the file for that isolate in Seqman and find the position in the alignment. It is a good idea to look at a section of the alignment before and after the base pair that you are looking for to make sure you are looking at the right one. If the difference is due to an editing error, correct the sequence and re-save it. In that case it would be the AT type identified by Bioedit. If the difference is not due to an editing error, then this would be a new allelic type.