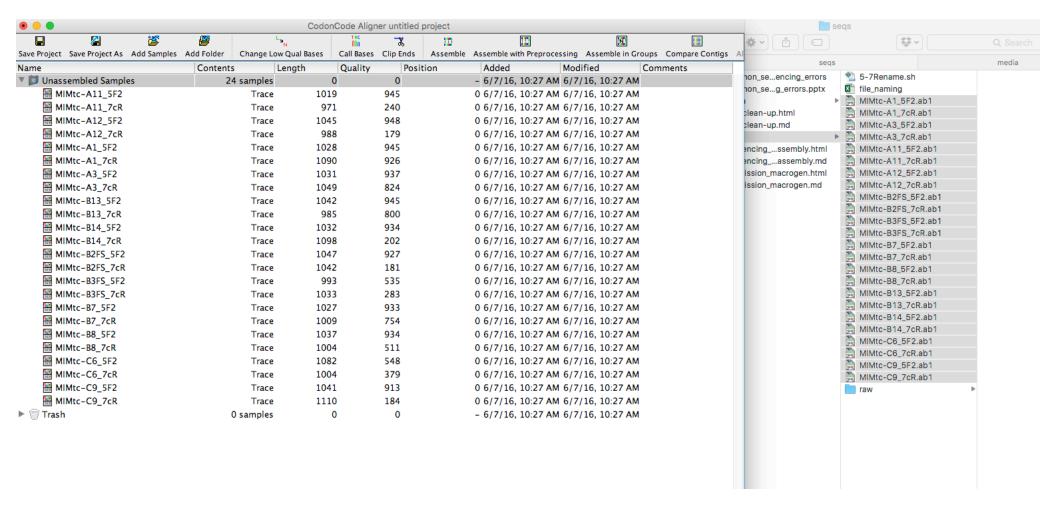
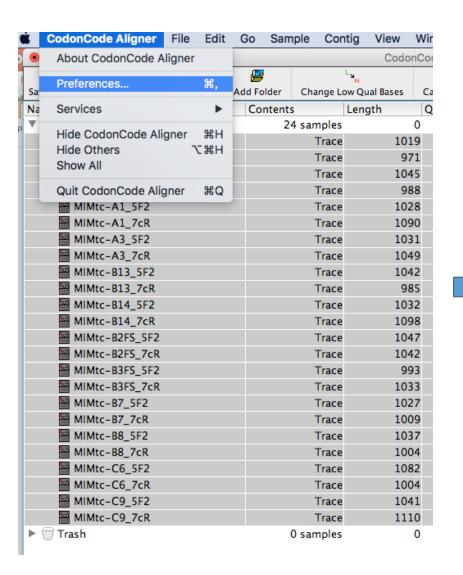
Sequence assembly with Codoncode aligner

Open files in codoncode aligner or you can also drag the file





Select **preferences** to adjust parameters as needed (usually default parameters are fine):

- Clipping parameters (removed bad sequences)
- Assembly parameters
- Other adjustments

Assembly parameters

Assembly	
Algorithm:	End to end alignments 😊
Min. percent identity:	70.0
Min. overlap length:	25 💲
Min. score:	20 💠
Max. unaligned end overlap:	50.0 ‡
Bandwidth (max. gap size):	30 🗘
Word length:	12 🗘
Max. successive failures:	50 💲
Match score:	1 🗘
Mismatch penalty:	-2 🗘
Gap penalty:	-2 🗘
Additional first gap penalty:	-3
Defaults	

Clipping parameters

d clipping					
 Maximize region with e 	0.05				
Use separate criteria fo	r start an	d end:			
im from start until					
✓ Error rate is below	0.1	in a	25	base window	
There are fewer than	3	bases	s with qua	ality	
below	20	in a	25	base window	
rim from end until					
✓ Error rate is below	0.1	in a	25	base window	
There are fewer than	3	bases	s with quality		
below	20	in a	25	base window	
fter end clipping					
Move all sequences	ian	25	bases to trash.		
✓ Move all sequences	with fewe	r than	50		
Phred 20 o bas	ses to tras	sh.			

Phred quality score

$$Q=-10\,\log_{10}P$$

or

$$P=10^{rac{-Q}{10}}$$

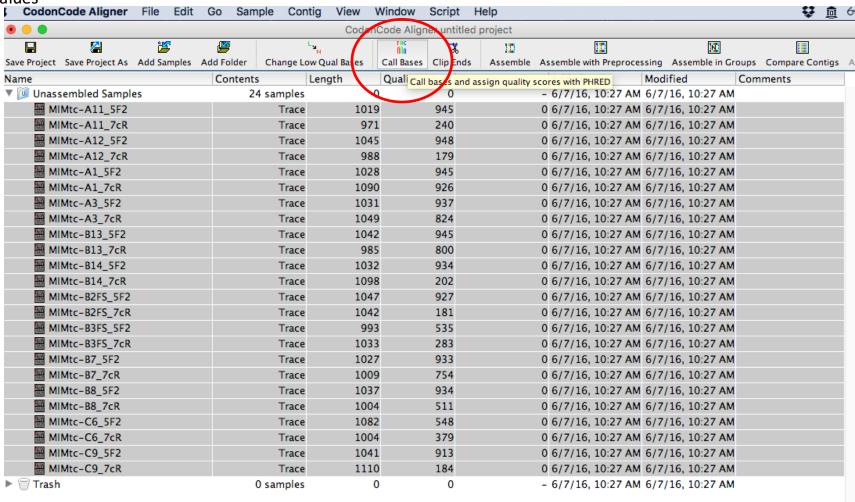
For example, if Phred assigns a quality score of 30 to a base, the chances that this base is called incorrectly are 1 in 1000.

Phred quality scores are logarithmically linked to error probabilities

Phred Quality Score	Probability of incorrect base call	Base call accuracy			
10	1 in 10	90%			
20	1 in 100	99%			
30	1 in 1000	99.9%			
40	1 in 10,000	99.99%			
50	1 in 100,000	99.999%			
60	1 in 1,000,000	99.9999%			

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
33
               59 64
                                                      126
0......26...31......40
                   0.....9......40
                    3.....9..................40
0.2.....41
S - Sanger
          Phred+33, raw reads typically (0, 40)
          Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
  with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
  (Note: See discussion above).
L - Illumina 1.8+ Phred+33, raw reads typically (0, 41)
```

Click on **call bases**, if necessary. files will be removed and new files will be generated with the corrected quality values



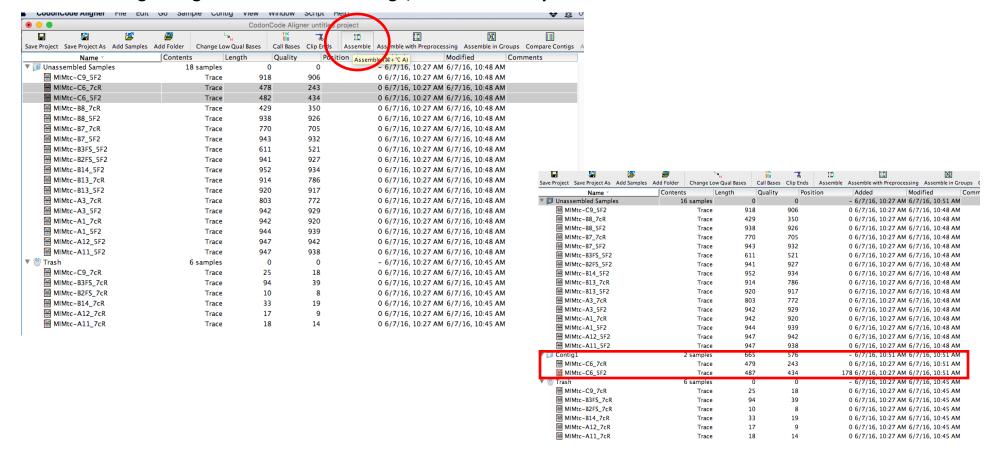
Then select all the sequences and click on clip bases N. Save Project Save Project As Add Samples Add Folder Change Low Qual Bases Call Bas Clip Ends mble Assemble with Preprocessing Assemble in Groups Compare Contigs Contents Length Quality Position Modified Name Added Comments ▼ I Unassembled Samples 24 samples 0 - 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A11_5F2 1019 945 Trace 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A11_7cR 971 240 Trace 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A12_5F2 1045 948 Trace 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A12_7cR Trace 988 179 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A1 5F2 Trace 1028 945 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A1_7cR Trace 1090 926 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A3_5F2 Trace 1031 937 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-A3 7cR 1049 824 Trace 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-B13_5F2 Trace 1042 945 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-B13_7cR Trace 985 800 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-B14_5F2 1032 934 Trace 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM MIMtc-B14_7cR Ti 🔵 🔾 /16, 10:27 AM 6/7/16, 10:27 AM Clipping Preview MIMtc-B2FS_5F2 /16, 10:27 AM 6/7/16, 10:27 AM Preview of clipping results: MIMtc-B2FS 7cR /16, 10:27 AM 6/7/16, 10:27 AM 24 Number of samples clipped MIMtc-B3FS_5F2 /16, 10:27 AM 6/7/16, 10:27 AM 1033 Average length before clipping MIMtc-B3FS 7cR /16, 10:27 AM 6/7/16, 10:27 AM 625 Average length after clipping MIMtc-B7_5F2 /16, 10:27 AM 6/7/16, 10:27 AM Average number of 5' bases clipped 192 MIMtc-B7_7cR /16, 10:27 AM 6/7/16, 10:27 AM 215 Average number of 3' bases clipped MIMtc-B8 5F2 /16, 10:27 AM 6/7/16, 10:27 AM MIMtc-B8_7cR Number of samples moved to Trash /16, 10:27 AM 6/7/16, 10:27 AM MIMtc-C6_5F2 /16, 10:27 AM 6/7/16, 10:27 AM MIMtc-C6_7cR Cancel Change parameters Clip /16, 10:27 AM 6/7/16, 10:27 AM MIMtc-C9_5F2 U 6/7/16, 10:27 AM 6/7/16, 10:27 AM Trace 1041 MIMtc-C9_7cR Trace 1110 184 0 6/7/16, 10:27 AM 6/7/16, 10:27 AM 0 0 0 samples - 6/7/16, 10:27 AM 6/7/16, 10:27 AM

Some sequences did not pass the quality threshold, so they were moved to the trash

	2	æ	\(\rightarrow\)	L _N N		TRC NNn	*	ш		111		ENE	
Save Project	Save Project As	Add Samples	Add Folder	Change Low Qual Base	es (Call Bases	Clip Ends	Assemble	Assemble wit	h Preprocess	ing Asse	emble in Grou	ps Compare C
Name			Contents	s Length		Quality	Posi	tion	Added	N	1 odified	C	omments
▼ Dnass	embled Sample	es	1	8 samples	0		0		- 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-A11_5F2			Trace	947		938		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-A12_5F2			Trace	947		942		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-A1_5F2			Trace	944		939		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-A1_7cR			Trace	942		920		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-A3_5F2			Trace	942		929		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-A3_7cR			Trace	803		772		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-B13_5F2			Trace	920		917		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
SEP MII	Mtc-B13_7cR			Trace	914		786		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-B14_5F2			Trace	952		934		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
∰ MII	Mtc-B2FS_5F2			Trace	941		927		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
₩ MII	Mtc-B3FS_5F2			Trace	611		521		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-B7_5F2			Trace	943		932		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-B7_7cR			Trace	770		705		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-B8_5F2			Trace	938		926		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
	Mtc-B8_7cR			Trace	429		350		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
₩ MII	Mtc-C6_5F2			Trace	482		434		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
₩ MII	Mtc-C6_7cR			Trace	478		243		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
∰ MI	Mtc-C9_5F2			Trace	918		906		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
▼ 🗑 Trash				6 samples	0		0		- 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
MII	Mtc-A11_7cR			Trace	18		14		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
₩ MII	Mtc-A12_7cR			Trace	17		9		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
∰ MII	Mtc-B14_7cR			Trace	33		19		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
∰ MII	Mtc-B2FS_7cR			Trace	10		8		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
🚻 MII	Mtc-B3FS_7cR			Trace	94		39		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	
₩ MII	Mtc-C9_7cR			Trace	25		18		0 6/7/16,	10:27 AM 6	7/16, 1	10:45 AM	

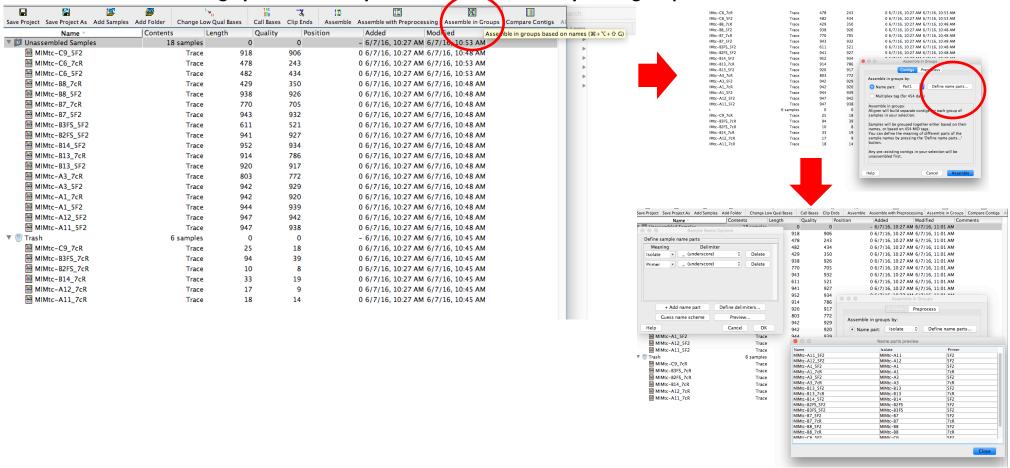
To assemble sequences there are two options, the first is to assemble manually:

- 1. Select the corresponding sequences
- 2. Hit assemble and it will generate a contig
- 3. The resulting contig will be labeled as contig1, make sure that you rename to the actual isolate name



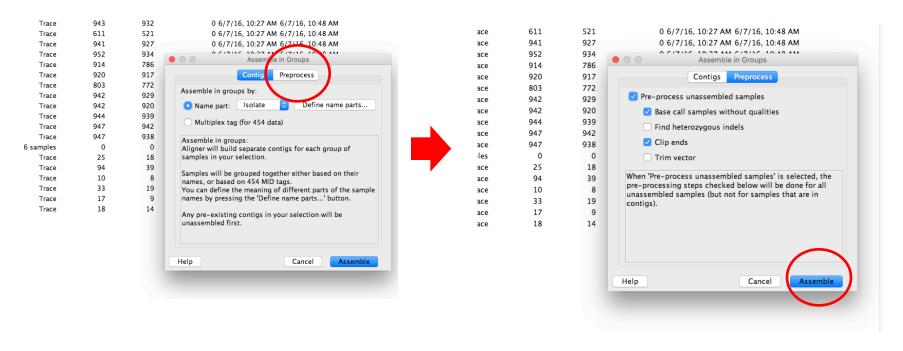
To assemble sequences there are two options, the first is to assemble manually:

- 1. Select assemble in groups
- 2. A window will pop up and the select define name parts to establish your naming system
- 3. Define the naming system and verify it before you continue by clicking on preview

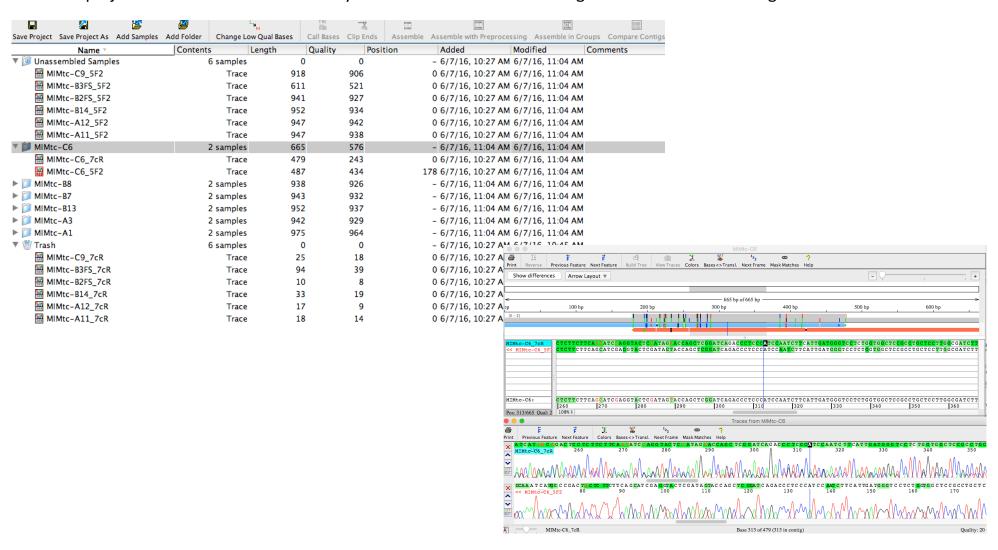


To assemble sequences there are two options, the first is to assemble manually:

- 1. Make sure that the "name part" is the correct one
- 2. There is an option for pre-processing that does what it was done in the previous steps
- 3. Click assemble and you will get contigs named with the name part selected



The project will look like this and the you can double click the contig folder to see chromatograms



Then you can go to **file** then select **export** and pick the **consensus sequences** and save it as fasta for further analysis

