

Aligning contigs manually using the Genome Finishing Module

March 17, 2023

Sample to Insight -

 $\label{eq:QIAGEN} QIAGEN \ Aarhus \ \cdot \ Silkeborgvej \ 2 \ \cdot \ Prismet \ \cdot \ 8000 \ Aarhus \ C \ \cdot \ Denmark \\ digitalinsights.qiagen.com \ \cdot \ ts-bioinformatics@qiagen.com \\$



Aligning contigs manually using the Genome Finishing Module

The CLC Genome Finishing Module is a collection of tools that have been developed to help finish microbial genomes.

This tutorial is an introduction to joining, splitting, and extending contigs manually using the **Align Contigs** tool, the **Join Two Contigs** option, the **Analyze Contigs** tool, and the **Extend Contigs** tool of the Genome Finishing Module.

The features demonstrated in this tutorial include:

- Aligning contigs to a reference sequence. This can be used to visualize the orientation and order of contigs by alignment to a reference sequence.
- Manually joining two contigs. This reduces the number of contigs by joining adjacent contigs.
- Splitting a contig into two. This can be used to separate sequences that mistakenly have been assembled into one contig.
- Extending a contig based on the original sequences that form the contig.

Prerequisites For this tutorial, you must be working with CLC Genomics Workbench 12.0 or higher, and have installed the Genome Finishing Module. How to install plugins is described here: http://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Install.html.

Additionally, this tutorial was made with Genome Finishing Module 23.0. If you are working with an earlier version, some elements in this tutorial (such as contig naming) may differ slightly from your local installation.

NB! Important tips for this tutorial

- For newer CLC Genomics Workbench versions, the classic coverage view is no longer enabled per default in the Read Mapping view. To enable this, enable **Alignment info** | **Coverage (classic)** | **Graph** from the Read Mapping view **Side Panel**.
- If at any time your view doesn't line up with the figures in this tutorial, try zooming out or scrolling sideways (particularly for the Read Mapping views).
- If you are unfamiliar with Read Mapping views, using the **Help** button in the lower right corner of the view or pressing F1 while viewing a Read Mapping, will bring up the manual.

Background of the dataset and analysis The dataset available for download contains:

- *E. coli_DH10B*. Reference sequence Escherichia coli K12 substr DH10B.
- paired_illumina_miseq_1. Part one of Illumina MiSeq paired-end whole genome data.
- paired_illumina_miseq_2. Part two of Illumina MiSeq paired-end whole genome data.



• paired_illumina_miseq_tutorial_assembly. Assembly of the paired read data.

In this tutorial we will improve the assembly of paired data (paired_illumina_miseq_1) by using the join, split, and extend contigs functionalities in Genome Finishing Module. Lastly, we will look at how to use the read data by mapping to the modified contigs.

The E. coli dataset used in this tutorial is a subset of a publicly available dataset. The E. coli strain DH10B reads are from https://emea.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/appnote_miseq_ecoli.pdf and the reference sequence is from NCBI (id=NC_010473.1): http://www.ncbi.nlm.nih.gov/nuccore/NC_010473.1?report=genbank.

Importing the data

To get started, we need to download and import the sample data.

- 1. Download the sample data from our website and unzip it in your desired location: http://resources.qiagenbioinformatics.com/testdata/finishing_module_ tutorial.zip
- 2. Start the workbench.
- 3. To import the reference sequence go to:

```
File | Import ((2) | Standard Import ((2)
```

- 4. Choose the files called "E_coli_DH10B.fa" and "paired_illumina_miseq_tutorial_assembly.clc". Leave the Import type set to **Automatic import**.
- 5. Specify where to save the downloaded data. It is a good idea to make a folder for this, for example called "Genome Finishing tutorial". Click **Finish**.
- Next we are going to import the paired read data. This requires simultaneous import of the two files called "paired_illumina_miseq_1.fastq" and "paired_illumina_miseq_2.fastq". Go to:

File | Import (🐴) | Illumina (🚔)

- 7. Select the two files and tick **Paired reads** under **General options**. Set **Minimum distance** at 150 and **Maximum distance** at 450. Click **Next**.
- 8. Specify where to save the downloaded data and click **Finish**.

De Novo Assembly

Normally, we would start our work by creating a de novo assembly. However, due to randomness introduced when assembling, we will not assemble reads and instead use the pre-constructed assembly imported in the previous step. Note: When doing your own analysis, de novo assemblies can always be created by launching **De Novo Assembly** from the **Toolbox**:

De Novo Sequencing (🚘) | De Novo Assembly (🚎)



Annotating potential problems

The **Analyze Contigs** tool is useful for identifying misassembled reads and can be run before aligning the contigs. The tool will only be mentioned briefly in this tutorial, but further information about the Analyze Contigs tool can be accessed at: https://resources.qiagenbioinformatics.com/manuals/clcgenomefinishing/current/index.php?manual=Analyze_Contigs.html.

- 1. To use the Analyze Contigs tool, double click on the tool in the Toolbox (1). This opens up a wizard. Select the assembled data (paired_illumina_miseq_tutorial_assembly).
- 2. Proceed using the default settings and click **Next** until you reach the final window where you can select whether to **Open** or **Save** the result.
- 3. Choose to **Save** the results and click on the button labeled **Finish**. Save the results to the tutorial data location; for example in a subfolder titled "Analyzed Contigs". Note, that by default the tool also generates a report and an annotation table (also called *Contig analysis table*) containing all the new annotations added to the input. As we will not be using these for this tutorial, feel free to untick the respective boxes in the wizard.

Additional annotations have now been added to the read mapping list and can be assessed by clicking on **Show Annotation Table** (E). We will come back to this later.

Running the Align Contigs tool

1. Next, open the Align Contigs tool from the Toolbox:

Genome Finishing Module (\bigcirc) | Align Contigs (\leq)

- 2. Select the assembled data (paired_illumina_miseq_tutorial_assembly) and click on the Next button.
- 3. This takes you to the **Select contig mapping parameters** step shown in figure 1. Select the reference sequence (E_coli_DH10B) by clicking on the folder (). Keep the default settings for the BLAST options and Match options. Click **Next**.
- 4. Choose to Save the result to a new subfolder, for example named "Aligned contigs".

The Align Contigs output

The output file of the **Align Contigs** tool is an aligned **Contig Table** (1), which contains two tables:

1. The **Contig table** summarizes information about the contigs. This table opens per default when clicking on the **Contig Table** file, or can be accessed by clicking on the **Show Contig table** icon ((()) in the bottom of the view (see red box on figure 2). Selecting a contig and clicking on **Show Contigs** will open a **Read Mapping** view of the sequences composing that contig.



Gx Align Contigs	×
 Choose where to run Select contigs 	Select contig mapping parameters
 Select contig mapping parameters <i>Result handling</i> 	Reference(s) Ouse input contigs as references
	(by selected reference(s) References XXX E_coll_DH108 DH0
	BLAST options BLAST word size 20 Maximum BLAST e-value 0.0001
	Minimum match size 100
Help Reset	Previous Next Finish Cancel

Figure 1: Select the contig mapping parameters.

2. The Contig match table lists the matches between the contigs and the reference sequences found by BLAST. This table is opened by clicking on the Show Contig match table icon (i) in the bottom of the view (figure 3 red box). Because this is a BLAST result, each contig may appear multiple time with different hits. A Show Contig Matches button in the Contig match table allows the visualization of the contigs scaffold in a Read mapping view. Change the *Compactness* mode in the Side Panel from *Packed* to *Low* to make the contig names visible next to the contig sequence. Under the reference sequence, a coverage track indicates with peaks the potential overlaps between contigs, and finally the different contigs aligning to the reference sequence. If the coverage graph is not showing, enable it by checking Alignment info | Coverage (classic) | Graph in the Side Panel.



Figure 2: Top: The Contig table. Bottom: Read Mapping view of selected contig.



					Filter to Sel	ection				Filter	1
Reference	Contig	Referen	Referen	Contig start	Contig end	Contig s	Match c	Aligned	Contig p	Identity	
coli DH10B	paired_illumina_miseq contig 113	4132328	4134029	1	1702	1702	1	1702	100.00	99.94	1
coli DH10B	paired illumina miseg contig 65	4134046	4134289	1	244	244	1	244	100.00	100.00	
coli DH10B	paired illumina miseg contig 64	4134390	4137470	3070	1	3070	1	3070	100.00	99.58	
coli DH10B	paired illumina miseq contig 88	4137817	4166347	1	28531	28531	1	28531	100.00	100.00	
coli_DH10B	paired_illumina_miseq contig 55	4166335	4167099	22301	21537	765	1	765	2.38	90.59	1
coli DH10B	paired illumina miseg contig 99	4166458	4167032						100.00	100.00	1
coli DH10B	paired illumina miseg contig 28	4167081	4225732	1	58653	58653	2	58623	57.68	99.97	7
coli DH10B	paired illumina miseg contig 43	4180027	4183405	19598	22973	3376	4	2312	6.15	80.32	
coli DH10B	paired illumina miseg contig 22	4225715	4264037	17732	56055	38324	1	38324	68.37	99.99	
coli_DH10B	paired_illumina_miseq contig 113	4264233	4265934	1	1702	1702	1	1702	100.00	99.88	
Show Cor	Lansa Internet and a second second	4005070	4000100	224		224		224	Refresh Co	100.00	
E_ [E_coli_DH:											_
			19,780 I		19;	800		19,820 I)		
E_COli_DH	108] × 19,780 E_coli_DH108_TTCCTTCATGC	GGATGCT	1	GTAGTAAT	ì		DAACTTAT	ì		GTTCAGA	<u>.</u>
E_COli_DH	10B] ×	GGATGCT	1	GTAGTAAT	ì		CAACTTA	ì		GTTCAG	\ \
E_coli_DH	108] × 19,780 E_coli_DH108_TTCCTTCATGC	GGATGCT	I GAAAAGA GAAAAGA	GTAGTAAT	TGCTGGTA	ATGACTCO	CAACTTA	TTGATAGT	GTTTTAT		

Figure 3: Top: The Contig match table. Bottom: Read Mapping view with Coverage enabled and Compactness set to Low.

These two tables are linked, which means that selecting an item in one table, will automatically select related items in the other. The tables are also linked with their respective Read Mapping views, meaning that selecting a contig in the table and clicking on the **Zoom to selection** () button in the lower left side of the view area will fill the view with the current selection. You can also zoom in and out on the regions of interest by holding down the Ctrl key (Cmd () on Mac) while scrolling with the mouse wheel. It will zoom in the region where you hold the mouse. Additionally, you can use the zoom functions at the bottom of the view.

Joining contigs

We will start with the simple operation of joining contigs using the **Contig match table**.

- 1. Start in the **Contig table** (**E**) and select "paired_illumina_miseq contig 45" in the *Contig* column.
- 2. Switch to the **Contig match table** by clicking on (is in the bottom left corner.
- 3. Contig 45 has been automatically selected in the Contig match table. Here, you will see that this contig has only one hit to the reference sequence and that the hit has 100% identity with the reference sequence and 26133 aligned nucleotides. Click on the Show Contig Matches button to open the Contig match Read Mapping view.
- 5. Remember to shift the *Compactness* mode from *Packed* to *Low* to make the contig names visible next to the contig sequence, and zoom out until contig 84 is visible on the 5' end and contig 58 is visible on the 3'end (figure 4).

In the coverage graph, we can see a number of peaks indicating overlap between these contigs. We will also notice that contig 92 appears twice in this region. By filtering for "92" on the Contig column in the Contig match table we can see that contig 92 has 15 hits with 1195 aligned





Figure 4: Show contig matches for contig 45 and zoom out to visualize the surrounding hits.

nucleotides and an identity close to 100%. This tells us this contig actually covers several regions but was only assembled into one contig due to these regions being repeats.

In the next section, we will focus on connecting the region from contig 84 at the 5' end to contig 58 at the 3' end.

Joining contigs when one of the contig is a repetitive sequence

We will start out by joining contig 84 to contig 92. This can be done directly in the Contig match Read Mapping view by selecting the region you wish to join on the reference sequence.

- 1. Click and drag on the reference sequence to select a region containing the overlap between the contig 84 and contig 92. Note: be careful not to select too much. You only need to select nucleotides within the overlap.
- 2. Right click on the selected region of the reference sequence and click on **Join Two Contigs** (figure 5).



Figure 5: Join two contigs by right clicking on the reference sequence.



3. This opens up the wizard shown in figure 6. When the selected region only contains two contigs, the contigs to join are selected automatically in the wizard. Otherwise it is necessary to manually select the two contigs of interest from a drop down menu in the wizard. Select contig 84 and contig 92.

elect parameters for bining contigs	Select parameters for joining c	ontigs	
	Select first contig match	paired_illumina_miseq contig 84 (Ref pos=1471812) V	
	Select second contig match	paired_illumina_miseq contig 92 (Ref pos=1483463) $ \lor$	
	Old contigs		
	Keep second contig		

Figure 6: Join Contigs Wizard. Select contigs to join and keep contig 92.

- We wish to keep a copy of contig 92 because we, as stated earlier, need to use this contig twice in our region. Therefore check the box to keep the contig corresponding to contig 92 (in figure 6 contig 92 is selected as the second contig). Click **Finish**.
- 5. Joined contig 1 can now be seen in figure 7. We chose to keep contig 92 but contig 84 is gone from the Contig match table as we did not keep this contig. Tip: For a fast way to compare your joined contigs to the previous state, use Ctrl + Z to undo (Cmd () + Z on Mac) and Ctrl + Y (Cmd () + Y on Mac) to redo.

Because this contig was created from a repeat (contig 92), Joined contig 1 appears several times in the Contig match table (). However, only one match represents the full length of the contig. The rest can be ignored (figure 8).



Figure 7: Joined contig 1 along with other overlapping contigs.

Rows: 15 / 38	5						Filter to S	slection joined		Filter
Reference	Contig	Reference start	Reference end	Contig start	Contig end	Contig span length	Match count	Aligned nucleotides	Contig percentage	Identity
E_coli_DH108	Joined contig 1	247283	248477	12722	11526	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	513146	514340	12722			1	1197	9.41	99
E_coli_DH108	Joined contig 1	626406	627600	12722	11526	1197	1	1197	9.41	99
E_coli_DH10B	Joined contig 1	739666	740860	12722	11526	1197	1	1197	9.41	99
E_coll_DH108	Joined contig 1	1471813	1484658	1	12722	12722	2	12722	100.00	99
E_coli_DH10B	Joined contig 1	1515112	1516215	12629	11525	1105	1	1105	8.69	93
E_coli_DH108	Joined contig 1	1521766	1522961	11525	12722	1198	1	1198	9.42	99
E_coli_DH108	Joined contig 1	2067104	2068298	12722	11526	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	2155191	2156386	12722	11525	1198	1	1198	9.42	99
E_coli_DH108	Joined contig 1	2190781	2191975	12722	11526	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	2377929	2379123	12722	11526	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	3170618	3171813	11525	12722	1198	1	1198	9.42	99
E_coli_DH108	Joined contig 1	3225913	3227107	11526	12722	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	3461323	3462517	12722	11526	1197	1	1197	9.41	99
E_coli_DH108	Joined contig 1	3747804	3748998	12722	11526	1197	1	1197	9.41	99

Figure 8: Contig match table shows that Joined contig 1 matches several times in the reference.

6. Repeat the joining procedure by joining Joined contig 1 with contig 45. These two contigs have one true hit in the Contig match table and we therefore do not need to make copies.



Leave both 'Keep' boxes unchecked. This action removes Joined contig 1 and adds **Joined** contig 2.

7. Join contig 120 to contig 92 (figure 9). Make sure to use the better matches (in this case the reverse/red matches) for the two contigs. If any of the contigs have disappeared from view, zoom out or scroll sideways to find them. Here, it becomes clear why we chose to create a copy of contig 92. Otherwise, we would be missing this contig to bridge the gap between contig 120 and contig 58.

Normally, we would make another copy of contig 92, but for this tutorial, we are only focusing on a small region. We therefore do not need to keep contig 92.



Figure 9: Join contig 120 and contig 92 by right clicking on the selected region of the reference sequence.

8. Finally, join the forward (green) **Joined contig 3** to the reverse (red) contig 58.

We have now done as much as we can by simply joining overlapping contigs. Locate **Joined contig 4** in the Contig match table () by filtering. Select the match with highest *Contig percentage* and *Identity* and zoom in to the 5' end.

Observe that there is a gap between Joined contig 4 and Joined contig 2 (figure 10). In the next section, we will cover how to join contigs separated by a gap.

Rows: 32 / 3	156						1	Filter to Selection.	. joined conti	g 4	Filter
Reference	Contig /		Reference st	Reference end	Contig start	Contig end	Contig span I	Match count	Aligned nucl	Contig perce	Identity
E coli DH10B	Joined contig 4		513239	514340	4602	3499	1104	1	1104	12.53	99.83
E coli DH10B	Joined contig 4		626499	627600	4602	3499	1104	1	1104	12.53	99.8
coli DH10B	Joined contig 4		739759	740860	4602	3499	1104	1	1104	12.53	99.83
coli_DH10B	Joined contig 4		1483464	1484565	3499	4602	1104	1	1104	12.53	99.83
_coli_DH10B	Joined contig 4		1510901	1519712	8813	1	8813	1	8813	100.00	99.1
coli DH10B	Joined contig 4		1521766	1522868	3498	4602	1105	1	1105	12.54	99.83
coli_DH10B	Joined contig 4		1722913	1723223	222	532	311	1	311	3.53	95.50
		(400	1,510,600 I		1,510,800 I		1,511,000		1,511,200		1,511,400
	E_coli_DH10E										
	E_coli_DH10E Coverage (classic)	5	ر								
	Coverage (classic)	s 									
aired illumin	Coverage (classic)										
aired_illumin	Coverage (classic)	s 									
aired_illumin	Coverage (classic) Joined contig 2 a_miseq contig 104	0									
aired_illumin	Coverage (classic) Joined contig 2 a_miseq contig 104 Joined contig 4										
aired_illumin	Coverage (classic) Joined contig 2 a_miseq contig 104 Joined contig 4 Joined contig 2										
aired_illumin	Coverage (classic) Joined contig 2 a_miseq contig 104 Joined contig 4 Joined contig 2		,								
aired_illumin	Coverage (classic) Joined contig 2 a_miseq contig 104 Joined contig 4 Joined contig 2 Joined contig 2									e`	

Figure 10: Joined contig 2 and Joined contig 4 are separated by a gap.



Joining contigs separated by a gap

To join two sequences separated by a gap, it is not possible to use the joining option in the right click menu. We will now go through the procedure step by step.

Before joining the contigs you need to take note of the gap size and the orientation of the contigs. An easy way of measuring the gap size is to select the sequence in the gap region. When mousing over the sequence, the size of the highlighted sequence is shown in the bottom right corner "size 123" (figure 11). Check also the suggested orientation of the contigs relative to each other. Here Joined contig 2 should be placed before Joined contig 4.



Figure 11: Measure the size of the gap and see the result in the lower right corner.

2. Go back to the **Contig table** (**E**), find and then select Joined contig 2 and Joined contig 4 (figure 12). Click the **Join contigs...** button at the bottom of the table.

Rows: 2 / 124			Filter to S	election joined	Filter Ţ
Contig /	Contig length	Total read count	Average coverage	Contig matches length	Contig matches count
Joined contig 2	38286	5455	19.92	55525	16
Joined contig 4	8813	2260	35.07	24583	16
Show Contigs	Extract 🛒 Copy Conti	g The Map Reads	Join Contigs	Remove Contigs	

Figure 12: Find and select Joined contig 2 and Joined contig 4 and click Join Contigs.

3. In the Join Contigs wizard (figure 13) check **Manual gap** and specify the gap size. Check the option **Place contig "Joined contig 2" before "Joined contig 4"** and click **Finish**.

In figure 14 you can see the final result of the joining procedures - one long contig (**Joined contig 5**) that replaces the initial contigs 84, 92, 45, 120, and 58. We now have one large contig spanning the same region as the five original contigs. The procedure can be continued with more overlapping contigs but this is not covered in this tutorial. Note: make sure to choose the Joined contig 5 with the highest Contig percentage in the Contig match table (). Because of all the repetitive elements we integrated into Joined contig 5 there are multiple matches from Joined contig 5 which can be ignored.

Now that we are done with the first steps, it is a good time to save our progress. You can save the contig match table by simply clicking Ctrl + S (Cmd (\bigcirc) + S on Mac).





Figure 13: Join Contigs wizard window.

Rows: 353				Fi	lter to Selection	on			🔘 Match any 🔘	Match all	\$
				Reference	~	contains	~		H	Filter	
Reference	Contig /	Referen	Referen	Contig start	Contig end	Contig sp	Match count	Aligned n	Contig percentage	Identity	
E coli DH10B	Joined contig 5	739732	740860	43086	44215	1130	1	1130	2.37	99.82	~
E_coli_DH10B	Joined contig 5	739787	740860	12642	11549	1094	1	1094	2.29	98.17	
coli DH10B	Joined contig 5										
E_coli_DH10B	Joined contig 5	1483464	1484592	44215	43086	1130	1	1130	2.37	99.82	
E_coli_DH10B	Joined contig 5	1515140	1516116	12643	11661	983	1	983	2.06	93.90	
E coli DH10B	Joined contig 5	1521766	1522895	44216	43086		1	1131	2.37	99.82	
E coli DH10B	Joined contig 5	1521767	1522840	11549	12642	1094	1	1094	2,29	98.17	
coli DU100	Joined contin F	2067170	2060200	42096	44015	1120	1	1120	2.22	00.72	Y
₩ 💽 🔛 🖳 [₩ * [E_coli_[1							
	E coli DH10B -										ſ
(E_coli_DH10B -										ĺ
	Coverage (classic)							v	<u> </u>		, ,
	Coverage (classic)				7			v	<u> </u>		
	Coverage (classic) a_miseq contig 71 – Joined contig 5								<u> </u>		-
paired_illumina	Coverage (classic) a_miseq contig 71 – Joined contig 5 Joined contig 5							*	<u> </u>		
oaired_illumina	Coverage (classic) a_miseq contig 71 – Joined contig 5 Joined contig 5 _miseq contig 104							¥			
paired_illumina aired_illumina_	Coverage (classic) a_miseq contig 71 Joined contig 5 Joined contig 5 miseq contig 104 Joined contig 5							¥			
oaired_illumina aired_illumina_ aired_illumina_	Coverage (classic) a_miseq contig 71 Joined contig 5 Joined contig 5 miseq contig 104 Joined contig 105				##			¥	-		
oaired_illumina aired_illumina_ aired_illumina_ aired_illumina_	Coverage (classic) a_miseq contig 71 - Joined contig 5 Joined contig 5 miseq contig 104 Joined contig 105 miseq contig 105 miseq contig 106							V #			
aired_illumina iired_illumina_ iired_illumina_ iired_illumina_	Coverage (classic) a_miseq contig 71 Joined contig 5 Joined contig 5 miseq contig 104 Joined contig 105							#			

Figure 14: Joined contig 5 - the result of joining contigs 84, 92, 45 and 120, and 58

Splitting contigs

We will now look at how to split contigs. For this, we return to the annotations we added to the contigs in the first step of the tutorial. Note: If you missed this step, there is a workaround to add annotations to an aligned **Contig Table** (\leq) in the bottom of this tutorial (section Workaround for adding annotations to an aligned Contig Table). Otherwise, continue on from here.

Open the annotation table by clicking **Show Annotation Table** (B) in the bottom left corner of the aligned **Contig table** (S), then deselect all annotation types except **Unaligned ends** in the Side Panel (figure 15). Of the 5 contigs that contain unaligned ends, we will focus on contig 68.

1. Clicking on contig 68 in the Annotation Table and then switching to the **Contig table** (E) in the lower left corner, will keep the contig highlighted in the view. Open the Read Mapping by either double-clicking contig 68 or clicking **Show Contigs**. Under **Annotation types** in the Read Mapping Side panel tick **Unaligned ends**. We can easily locate the unaligned end by holding down Ctrl (Cmd ()) on Mac) and clicking on Show Annotation Table () in the Read Mapping to open a linked table in split view. Selecting the unaligned end in the Annotation Table will automatically select this region in the Read Mapping (remember to tick **Unaligned ends** in the Annotation Table as well). Alternatively, zoom out to find the



Rows: 5	Filter:		All 🗸		I Annotation Table Settings	
1013.5			2		Shown annotation types	
Sequence	Name	Туре	Region	Qualifiers	Alternatives excluded	
paired_illumina_miseq contig 5	Unaligned ends	Unaligned ends	complement(2533325334)		Broken pair	
					Contigs joined	
paired_illumina_miseq contig 35	Unaligned ends	Unaligned ends	139140		Low coverage	
paired_illumina_miseq contig 68	Unaligned ends	Unaligned ends	126127		Scaffold	
					Single stranded coverage	в
paired_illumina_miseq contig 80	Unaligned ends	Unaligned ends	complement(19581959)		Sudden coverage change	9
paired_illumina_miseq contig 91	Unaligned ends	Unaligned ends	complement(38393840)		Unaligned ends	
					Variation	
					Select All	
					Deselect All	

Figure 15: Result of contig analysis. The filter has been used to identify contigs with unaligned ends.

region containing the unaligned ends (figure 16).

The sudden drop in coverage in the region to the right of the unaligned ends indicates that this is a repetitive region which has not been duplicated the correct number of times by the de novo assembler. To resolve this error we need to split the contig and thus isolate the repetitive region.



Figure 16: Contig 68 contains unaligned ends as a result of a repeat region which has not been duplicated the correct number of times.

- 2. To split the contig select the two nucleotides covered by the Unaligned ends annotation in the Read Mapping view.
- 3. Right-click on the selected nucleotides and select **Split contig...** (figure 17). This opens up a dialog where reads intersecting the split can be distributed between the resulting two contigs. The contigs will be automatically expanded to preserve the alignment of the reads. This means when one or more reads intersect the split, the resulting two contigs will have an overlap. Each read is automatically placed on the contig where it has the best alignment.
- Leave the reads in the default position and click **Finish**. The contig has now been split into two contigs (**contig 68 Split a** and **Split b**) that have replaced contig 68 in the Contig table (I=). The workbench will automatically close the Read Mapping for contig 68 after the split has been performed as it no longer exists.
- 5. In the Contig table (=) locate contig 68 Split a and Split b. The column *Contig matches count* indicates the number of times a contig matches the reference (figure 18). Contig 68 Split b has only one match while contig 68 Split a has 11 matches, another indicator of a repetitive element.







Rows: 2 / 124		F	ilter to Selection sp	split Filter		
Contig	Contig length	Total read count	Average coverage	Contig matches length	Contig matches count	
paired_illumina_miseq contig 68 - Split a	212	147	75.00	1468	11	
paired_illumina_miseq contig 68 - Split b	25059	2871	15.70	24995	1	

Figure 18: Contig split a and b have different Contig match counts.

- 6. Open the Contig match table () and find the entries for contig 68 Split a by filtering and notice that the *Contig percentage* for 10/11 matches is below 60%. We will investigate this in the Contig match Read Mapping view.
- 7. Select the first match of Split a (position 20438 to 20563) and click **Show Contig Matches** in the bottom left. Remember to set *Compactness* to *Low* and notice that a large region of the contig is unaligned (figure 19, faded ends). There is also a small gap between contig 68 Split a and contig 99, meaning we cannot join these directly. Split a also appears to overlap contig 55, but by switching *Compactness* to *Packed* in the Side Panel, we see that the repeat in contig 55 contains a lot of mismatches and is therefore unlikely to belong here.
- 8. Open the Contig table Read Mapping view for contig 68 Split a. Under **Annotation types** in the Side panel tick **Split position** to enable the split annotation and observe that a region starting from position 127 (the split position) has very low coverage. This low coverage region is an artifact from the split and overlaps with contig 68 Split b and can be deleted. Select the whole region starting from position 126, right-click and choose to **Delete selection** (figure 20).
- 9. Save the Read Mapping by clicking Ctrl + S (Cmd (\mathbb{H}) + S on Mac).



					Filte	r to Selection					Filter	1
Reference	Contig		Refere	Refere	Contig start	Contig end	Contig	Match	Aligned	Contig	Identity	Ī
E coli DH10B	paired illumina	misea contia 70	1	15405	15406	1	15406	1	15406	25.83	99.86	5
E coli DH10B		miseq contig 103	15387	16731	1345	1	1345	1	1345	100.00	100.00	j I
E_coli_DH10B	paired illumina	miseq contig 102	16714	19813	3100	1	3100	1	3100	100.00	100.00)
E coli DH10B		miseg contig 55	19796	20559	21538	22301	764	1	764	2.38	90.58	3
E coli DH10B	paired illumina	miseq contig 99	19862	20436	575	1	575	1	575	100.00	100.00)
		_miseq contiq 68	. 20438	20563	1	126	126	1	126	59.43	100.00	đ
E coli DH10B		miseg contig 34	20546	134077	113534	1	113534	1	113534	100.00	100.00	5
E coli DH10B		misea contia 75	134204	150415	16212	1	16212	1	16212	100.00	99.99	
E coli DH10B	paired illumina	misea contia 9	150427	197738	47312	1	47312	1	47312	100.00	100.00)
E coli DH10B		miseq contig 113	197729	199430	1	1702	1702	1		100.00	99.53	
E_coli_DH10B		miseq contig 65	199447	199690	1	244	244	1	244	100.00	100.00	5
E_coli_DH10B		miseg contig 64	199791	202870	3070	1	3070	1	3070	100.00	99.58	
E coli DH10B		miseq contig 51	202968	243885	1	40916	40916	1	40916	100.00	100.00	5
E coli DH10B		miseq contig 11	213202	213445	2688	2931	244	1		8.32	97.95	
\Xi * [E_coli_(DH10B] ×											
₩ [E_coli_[DH10B] ×	20,400 I	20,4 I	50	20,500 I		20,550 I		20,600 I		20,850 I	
╤ * [E_coli_[E_coli_DH10B	20,400 I	20,4 1	50	20,500 I		20,550 I		20,600 I		20,650 I	_
	E_coli_DH10B	20,400 I	20.4 I	50	20,500 I		20,550 I		20,600 I		20,650 I	_
aired_illumina_	E_coli_DH10B miseq contig 55	20,400 I	20,4	50	20,500 1		20,550 I		20,800 I		20,650 I	-
aired_illumina_ aired_illumina_	E_coli_DH10B miseq contig 55 miseq contig 99	20,400 I	20,4	50	20,500 I		20,550 I		20,600 I		20,650 I	_
aired_illumina_ umina_miseq o	E_coli_DH10B _miseq contig 55 _miseq contig 99 contig 68 - Split a	20,400 I	20,4	50	20,500 I		20,550 I		20,600 I		20,650 I	
aired_illumina_ aired_illumina_ umina_miseq o	E_coli_DH10B miseq contig 55 miseq contig 99	20,400 i	20,4	50	20,500 I		20,550 I		20,800 I		20,850 I	
ired_illumina_ ired_illumina_ imina_miseq o	E_coli_DH10B _miseq contig 55 _miseq contig 99 contig 68 - Split a	20.400	20,4	50	20,500 i		20,550		20,800 I		20,850	

Figure 19: Contig match Read Mapping view showing the first match of contig 68 Split a.



Figure 20: Deleting a low coverage region that is no longer needed.

Extending contigs

Next, we will take care of the missing fragment at the 5' end of the repeat.

- 1. In the Contig table Read Mapping for contig 68 Split a scroll to the first position. Observe the arrows on the 5' end of the mapped reads (figure 21). Such reads can be used to extend the contig.
- 2. Ensure that the Contig table Read Mapping window for contig 68 Split a is active by clicking anywhere within it and then select:

```
Genome Finishing Module ( 📄 ) | Extend contigs ( 气 )
```

in the Toolbox. This will automatically add the contig as input to the tool. Alternatively, the contig can be exported, saved in the Navigation Area and used as input for the tool.





Figure 21: Reads pointing out of a contig end that could be used to extend the contig.

- 3. Leave the parameters on default settings and click **Next**. In the next step of the wizard choose to **Save** the results and choose a suitable location, for example a subfolder titled "Extended contig".
- 4. Open the extended contig you just saved and enable **Extended region** by ticking the box under **Annotation types** in the Side panel. The contig should now have a 67bp extension at the 5' end (figure 22).



Figure 22: Contig 68 - Split a after extension.

- 5. Open the aligned Contig table (shows from the Navigation Area and use the **Add/Extract...** button to add the extended contig.
- 6. Delete the old **contig 68 Split a** as it is no longer needed. This is done through the Contig table (=) by selecting the contig in the list and clicking **Remove contigs**.
- 7. The extended contig does not have any reads mapped to it. To change that, click the **Map reads...** button in the Contig table (**E**) and select **Replace all reads**. This will remove all reads from all contigs and map new reads.
- 8. In the wizard select the paired Illumina reads which were imported at the start of this tutorial "paired_illumina_miseq_1 (paired)". These reads are the same set of reads that were mapped to the contigs when the tutorial started. Mapping them again will update the read mapping on all contigs.
- 9. Open the extended contig in Contig table Read Mapping view to see that reads have now been mapped to it, and the extension has decent coverage (figure 23).
- 10. The extended contig now completely spans the gap between contig 99 and contig 34 (figure 24). Using the join functionality as described in section Joining contigs we can close the gap. As the extended contig is needed for 11 gaps, we should remember to tick the "Keep" box for the contig.







Figure 24: The Contig match table Read Mapping view confirms that the extended contig is spanning the gap between contig 99 and contig 34.

We have now gone through how to join, split, and extend contigs. If you wish, you can use these tools to further improve the tutorial assembly. A good assembly is a starting point for several downstream analysis tools found in other plugins. For example, you can use the **Microbial Genomics Module** to find antibiotic resistance: https://resources.giagenbioinformatics.com/manuals/clcmgm/current/index.php?manual=Drug_Resistance_Analysis.html.

Another example is using the tools in the **Whole Genome Alignment** plugin to compare to similar genomes: https://resources.qiagenbioinformatics.com/manuals/wholegenomealignment/current/ index.php?manual=Introduction.html.

Workaround for adding annotations to an aligned Contig Table

If at any point you want to use the Analyze Contigs tool, but have already aligned your contigs, there is a workaround to do so described here:

- Extract all the contigs by saving your contigs to a Read Mapping List. To do so, click on Show contig table (E), make sure the Filter is cleared, and click Ctrl + A (Cmd ()) + A on Mac) to highlight all contigs. Then use the Add/extract... button to extract and save the updated contigs.
- 2. Use the extracted Read Mapping List (ﷺ) from the Navigation Area as input for **Analyze Contigs**, as described above in section Annotating potential problems.
- 3. In the aligned Contig Table (1), select all contigs (Ctrl + A or Cmd (1) + A on Mac), and use the **Remove Contigs** button in the bottom of the view.
- 4. Lastly, use the **Add/extract...** button, choose Add Contigs, and select the just extracted and annotated Read Mapping List (**E**).



