

In search for the causal agent of faba bean gall disease in herbarium samples

Theo van der Lee

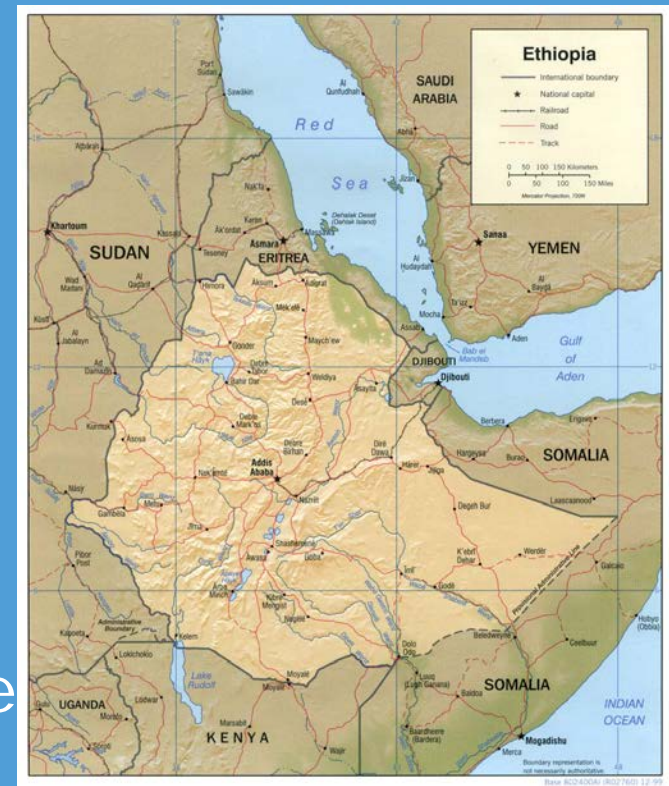
Workshop on the use of NGS technologies for plant pest diagnostics,
Bari, 22th November 2017



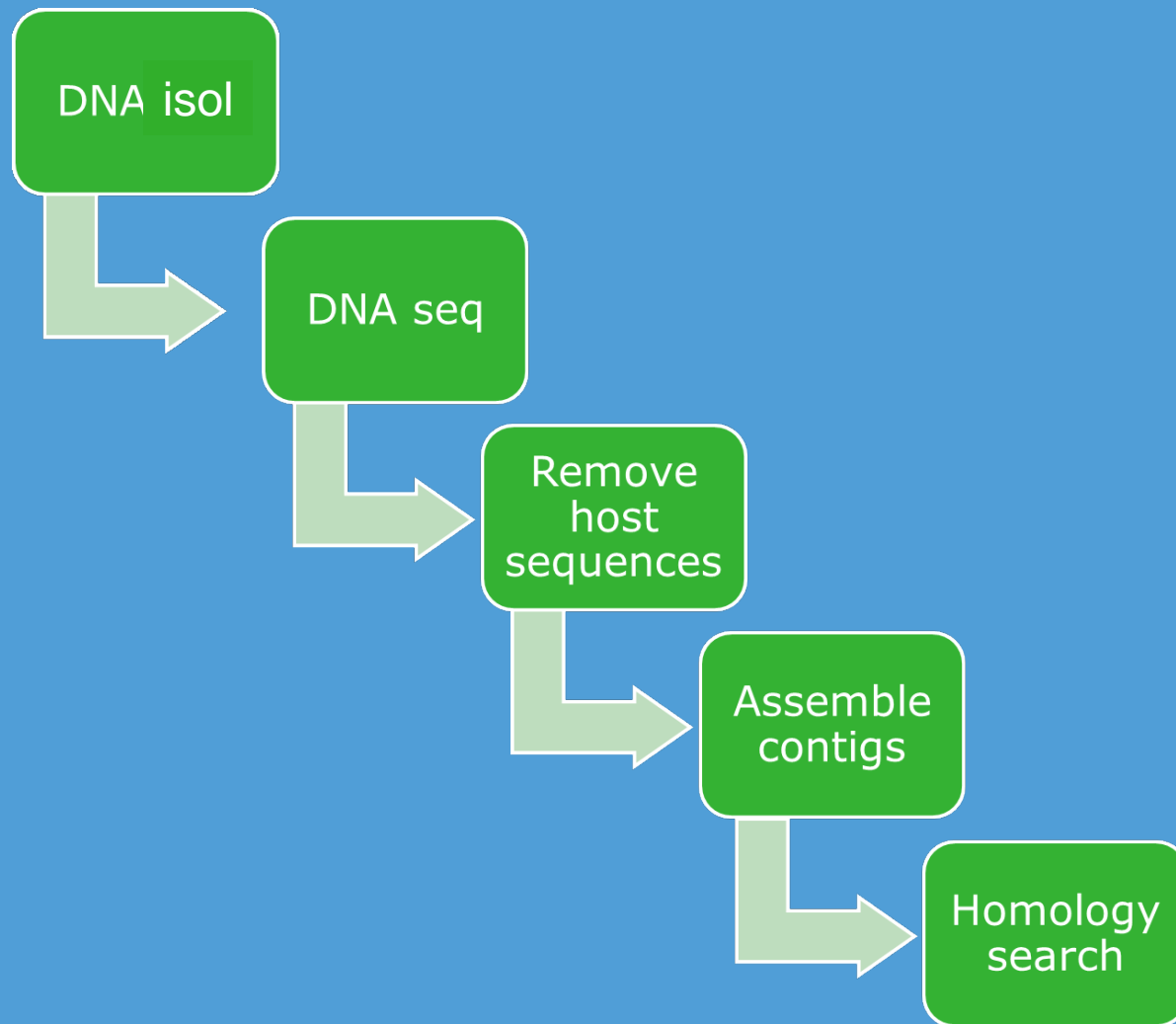
Identification of a potential new pathogen from herbarium samples



Faba bean gall disease



Faba bean gall research strategy



Exp 1 - DNA extraction Faba Bean Gall

13/01/2015

Faba Bean samples Ethiopia

- 002 healthy leaf	→ 2A	= 0.0014 g
- 002 FBG leaf	→ 2B	= 0.0026 g
- 003 healthy leaf	→ 3A	= 0.0016 g
- 003 FBG leaf	→ 3B	= 0.0040 g
- 004 healthy leaf	→ 4A	= 0.0021 g
- 004 FBG leaf	→ 4B	= 0.0033 g

- From each sample we took $\pm 0.5 \text{ cm}^2$ leaf
- For the FBG samples we took 50% clean & 50% FBG symptoms



Protocol 1

DNeasy® Plant Mini Kit

The DNeasy Plant Mini Kit (cat. nos. 69104 and 69106) can be stored at room temperature (15–25°C) for up to 1 year.

For more information, please refer to the *DNeasy Plant Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

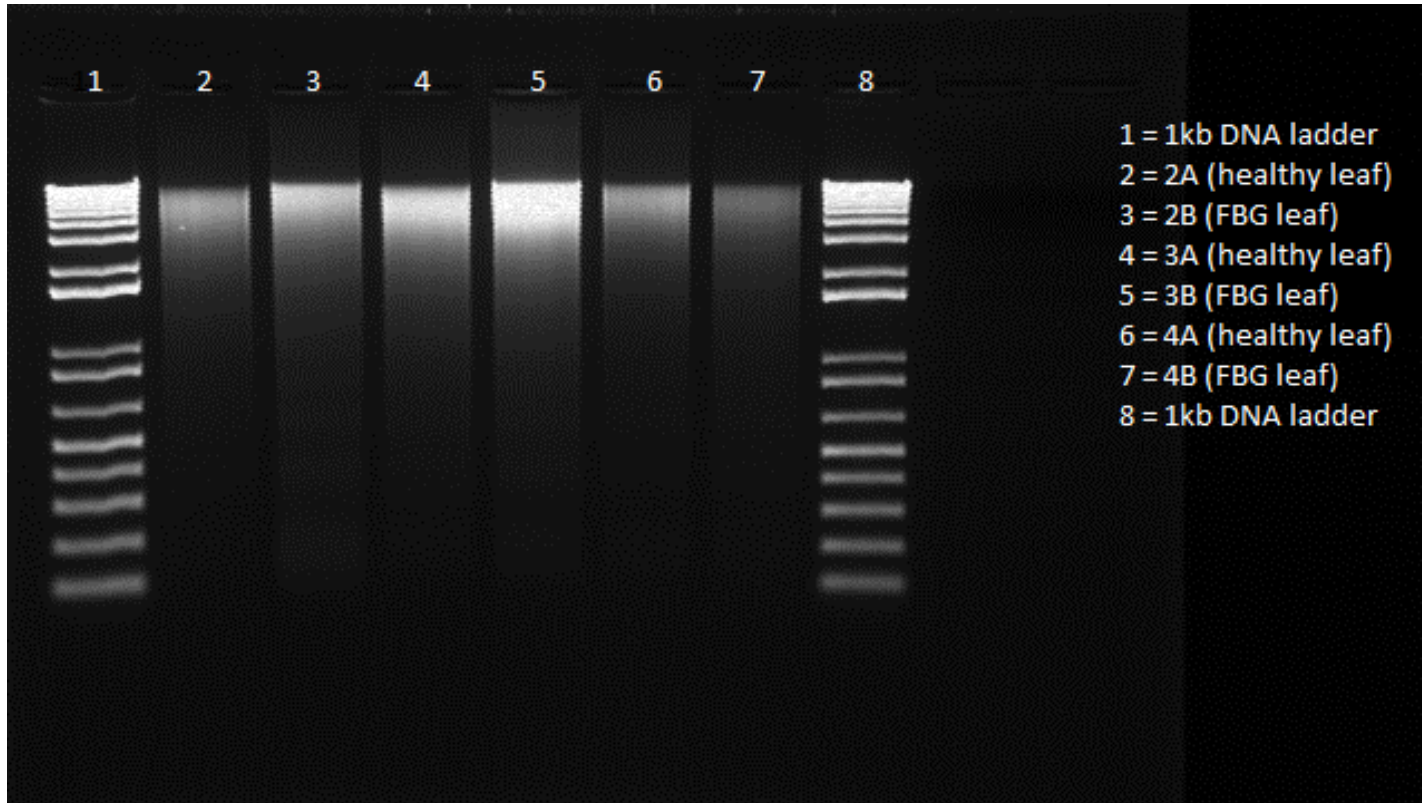
Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
 - If necessary, redissolve any precipitates in Buffer AP1 and Buffer AW1 concentrates.
 - Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
 - Preheat a water bath or heating block to 65°C.
1. Disrupt samples (≤ 100 mg wet weight or ≤ 20 mg lyophilized tissue) using the TissueRuptor®, the TissueLyser II, or a mortar and pestle.
 2. Add 400 μ l Buffer AP1 and 4 μ l RNase A. Vortex and incubate for 10 min at 65°C. Invert the tube 2–3 times during incubation.
Note: Do not mix Buffer AP1 and RNase A before use.
 3. Add 130 μ l Buffer P3. Mix and incubate for 5 min on ice.
 4. **Recommended:** Centrifuge the lysate for 5 min at 20,000 x g (14,000 rpm).
 5. Pipet the lysate into a QIAshredder spin column placed in a 2 ml collection tube. Centrifuge for 2 min at 20,000 x g.
 6. Transfer the flow-through into a new tube without disturbing the pellet if present. Add 1.5 volumes of Buffer AW1, and mix by pipetting.

7. Transfer 650 μ l of the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge for 1 min at ≥ 6000 x g (≥ 8000 rpm). Discard the flow-through. Repeat this step with the remaining sample.
8. Place the spin column into a new 2 ml collection tube. Add 500 μ l Buffer AW2, and centrifuge for 1 min at ≥ 6000 x g. Discard the flow-through.
9. Add another 500 μ l Buffer AW2. Centrifuge for 2 min at 20,000 x g.
Note: Remove the spin column from the collection tube carefully so that the column does not come into contact with the flow-through.
10. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
11. Add 100 μ l Buffer AE for elution. Incubate for 5 min at room temperature (15–25°C). Centrifuge for 1 min at ≥ 6000 x g.
12. Repeat step 11.

Step 1. Disrupt samples using RVS beats in TissueLyser (2 min, 30 hertz)

Results on 1% agarosegel



Results Picogreen

Samples	DNA concentrations (ng/ μ l)	Yield (ng)
2A - healthy leaf	10.16	914
2B - FBG leaf	13.95	1256
3A - healthy leaf	15.00	1350
3B - FBG leaf	24.56	2211
4A - healthy leaf	9.28	835
4B - FBG leaf	6.03	543

Total volume \rightarrow 90 μ l

70 μ l to Greenomics (10/02/2016)

Data received on 09/03/2016

Data no mismatches 07/04/2016

Samplesheet

quotation	2016-02-01_HiSeq_Theo_vd_Lee_6_Faba_bean_gal_disease_DNA_seq_3740090100_quotation			
Full name project leader	Theo van der Lee			
e-mail project leader	theo.vanderlee@wur.nl			
project name	6_Faba_bean_gal_disease_DNA_seq			
project number	3740091100			
samples delivered by	Ilse Houwers			
expected sample(s) delivery date (dd/mm/yy):	12/02/2016			
requested sequencing	HiSeq			
requested read length	125PE			
requested lanes	1			
no. of samples	6			
concentration based on nanodrop/qubit/other	Picogreen			
sample ID	concentration ng/uL	OD 260/280	OD 260/230	volume uL
2A_healthy_leaf	10.16			70
2B_FBG_leaf	13.95			70
3A_healthy_leaf	15.00			70
3B_FBG_leaf	24.56			70
4A_healthy_leaf	9.28			70
4B_FBG_leaf	6.03			70

Data analysis - Workflow

Configure Map Reads to Reference

1. References (Map Reads to Reference)

2. Mapping options (Map Reads to Reference)

Mapping options

Read alignment

- Mismatch cost: 2
- Cost of insertions and deletions: Linear gap cost
- Insertion cost: 3
- Deletion cost: 3
- Insertion open cost: 6
- Insertion extend cost: 1
- Deletion open cost: 6
- Deletion extend cost: 1
- Length fraction: 0.8
- Similarity fraction: 0.8
- Global alignment:
- Color space alignment:
- Color error cost: 3
- Auto-detect paired distances:

Non-specific match handling

- Non-specific match handling: Map randomly

Navigation: Previous, Next, Finish, Cancel

Configure De Novo Assembly

1. Mapping options (De Novo Assembly)

Mapping options

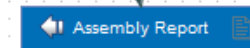
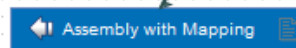
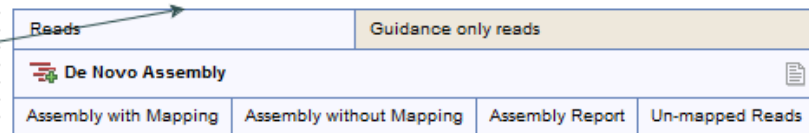
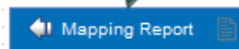
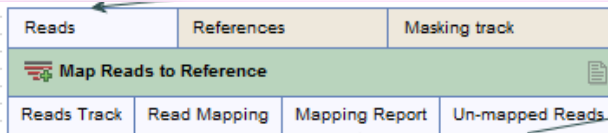
Map reads back to contigs

- Mapping mode: Map reads back to contigs (slow)
- Update contigs:

Mapping options

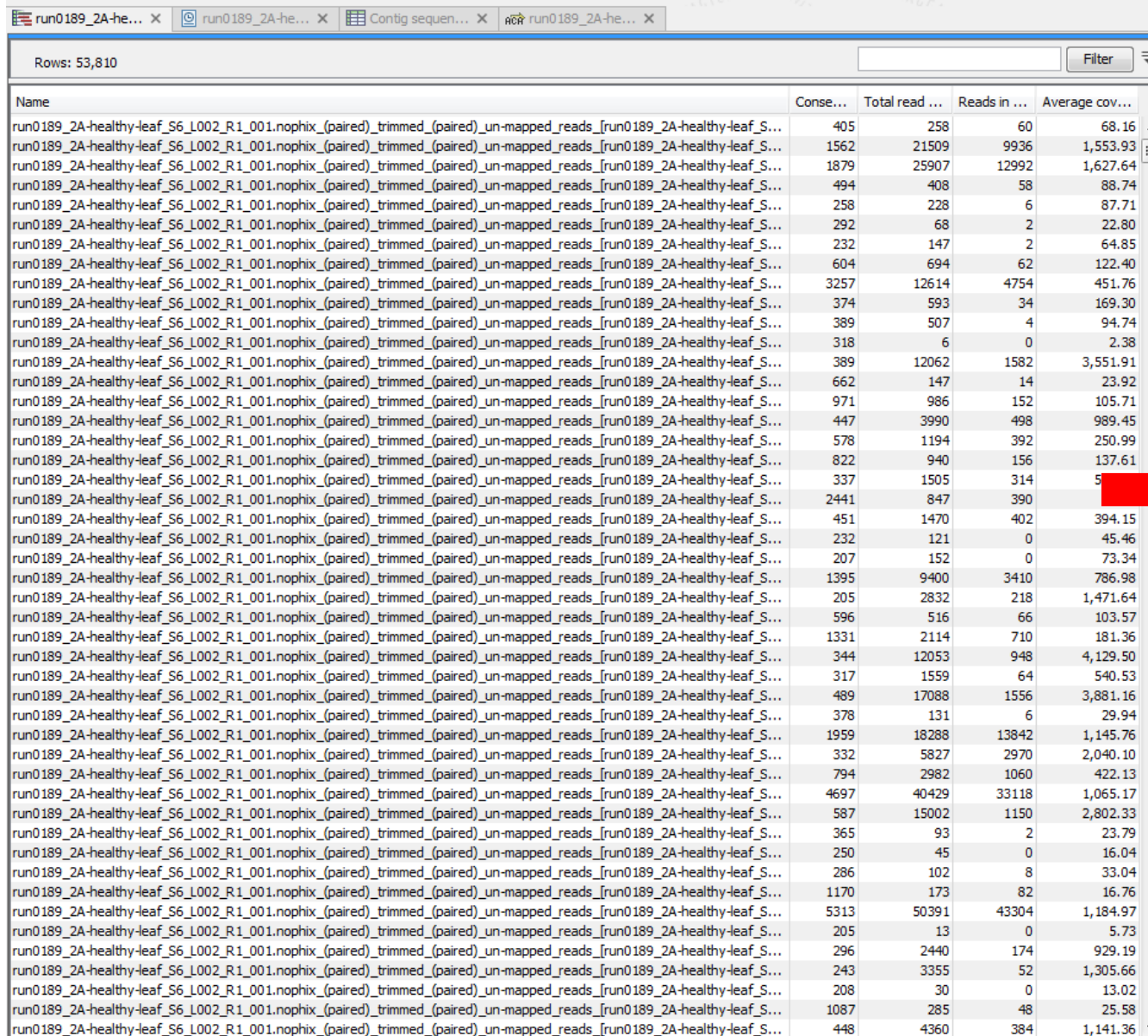
- Mismatch cost: 2
- Insertion cost: 3
- Deletion cost: 3
- Colospace error cost: 3
- Length fraction: 0.5
- Similarity fraction: 0.8
- Colospace alignment:
- Alignment mode: local
- Match mode: random
- Create list of un-mapped reads:

Navigation: Previous, Next, Finish, Cancel



De novo assembly unmapped reads– 2A Healthy leaf

CLC → FBG analysis → Mapping faba bean_assembly unmapped reads_TL



Rows: 53,810

Name	Consequ...	Total read ...	Reads in ...	Average cov...
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	405	258	60	68.16
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1562	21509	9936	1,553.93
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1879	25907	12992	1,627.64
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	494	408	58	88.74
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	258	228	6	87.71
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	292	68	2	22.80
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	232	147	2	64.85
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	604	694	62	122.40
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	3257	12614	4754	451.76
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	374	593	34	169.30
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	389	507	4	94.74
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	318	6	0	2.38
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	389	12062	1582	3,551.91
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	662	147	14	23.92
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	971	986	152	105.71
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	447	3990	498	989.45
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	578	1194	392	250.99
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	822	940	156	137.61
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	337	1505	314	5...
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	2441	847	390	...
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	451	1470	402	394.15
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	232	121	0	45.46
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	207	152	0	73.34
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1395	9400	3410	786.98
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	205	2832	218	1,471.64
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	596	516	66	103.57
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1331	2114	710	181.36
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	344	12053	948	4,129.50
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	317	1559	64	540.53
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	489	17088	1556	3,881.16
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	378	131	6	29.94
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1959	18288	13842	1,145.76
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	332	5827	2970	2,040.10
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	794	2982	1060	422.13
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	4697	40429	33118	1,065.17
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	587	15002	1150	2,802.33
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	365	93	2	23.79
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	250	45	0	16.04
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	286	102	8	33.04
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1170	173	82	16.76
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	5313	50391	43304	1,184.97
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	205	13	0	5.73
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	296	2440	174	929.19
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	243	3355	52	1,305.66
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	208	30	0	13.02
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	1087	285	48	25.58
run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S...	448	4360	384	1,141.36

Extract 1 contig from assembly subset → contig 2

2B FBG leaf

Used references for mapping:

Originates from:

CSVX01.1 (history)

run0189_2A-healthy-leaf_S6_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2A-healthy-leaf_S6_L002_R1_001.nophix]_(single)_contig_2 (history)

De novo assembly unmapped reads:

Name	Consensus	Total read c...	Reads in ...	Average cov...
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	547	3402	746	778.04
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	291	78	6	23.79
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	370	77	20	23.23
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	521	19	8	4.22
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	304	6282	456	2,288.44
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	339	1351	64	442.79
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	324	11826	708	3,980.87
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	422	3776	730	1,087.45
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	262	136	6	51.07
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	367	1796	136	566.39
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	220	552	2	302.23
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	201	1041	60	549.03
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	349	5268	1290	1,558.60
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	296	463	62	178.66
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	947	6463	1852	814.35
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	561	1974	174	434.32
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	254	140	10	61.76
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	341	13441	938	4,611.28
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	401	11232	460	3,268.51
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	424	24512	3756	6,695.15
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	441	650	38	141.88
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	791	5387	1378	806.99
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	987	6277	1822	765.84
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	606	3388	276	689.98
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	289	3384	96	1,117.59
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	463	2149	540	498.34
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	256	15	0	5.88
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	410	762	18	174.71
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	402	6458	566	1,976.87
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	447	6259	1528	1,682.38
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	776	608	82	76.59
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	201	32	2	15.44
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	627	13	8	2.59
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	257	478	40	190.56
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	374	947	22	275.32
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	358	85	2	23.83
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	432	19108	3356	5,337.30
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	207	130	4	68.21
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	227	107	14	45.59
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	1080	1230	196	120.27
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	287	5277	164	2,164.80
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	222	152	0	71.30
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	217	372	2	169.31
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	313	38	2	12.72
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	304	45	8	14.25
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	669	618	126	101.87
run0189_2B-FBG-leaf_S7_L002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_L00...	481	4708	396	1,201.67

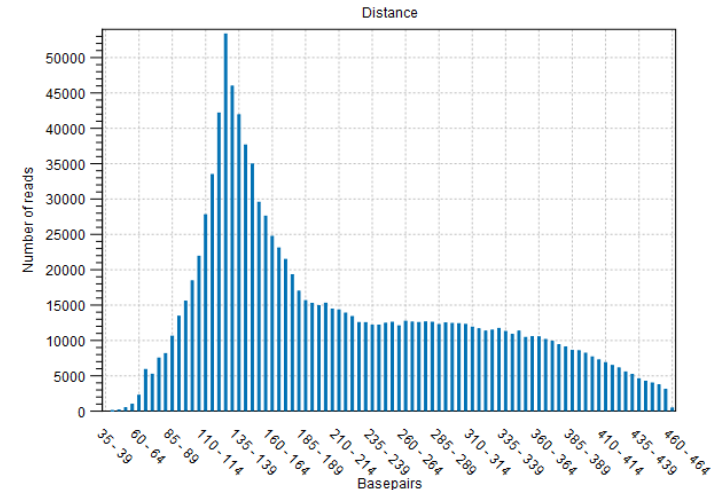
1.2 Contig measurements (including scaffolded regions)

	Length
N75	287
N50	347
N25	444
Minimum	126
Maximum	7,502
Average	348
Count	103,644
Total	36,088,890

1.5 Summary statistics

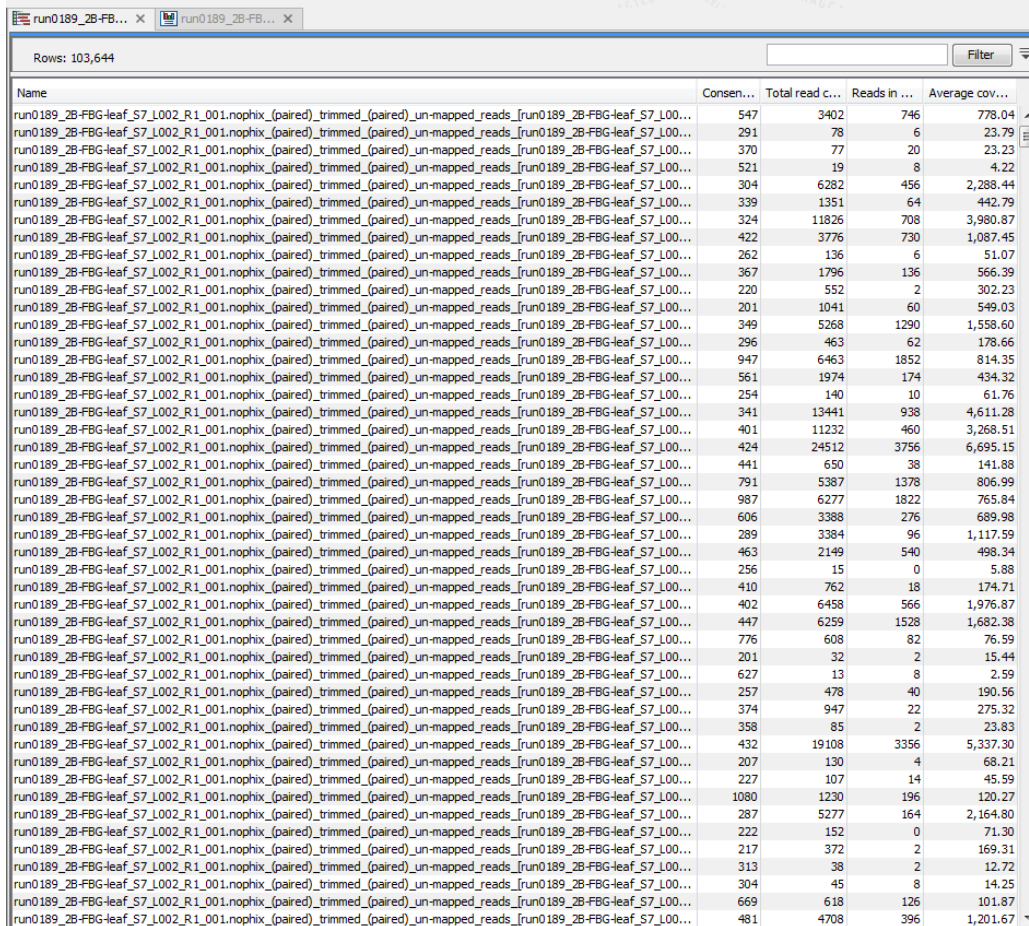
	Count	Average length	Total bases
Reads	45,878,868	125.38	5,752,241,088
Matched	28,218,747	125.37	3,537,779,935
Not matched	17,660,121	125.39	2,214,461,151
Contigs	103,644	348	36,088,890
Reads in pairs	2,353,566	215.37	
Broken paired reads	20,045,284	125.46	

1.9 Paired reads distance distribution



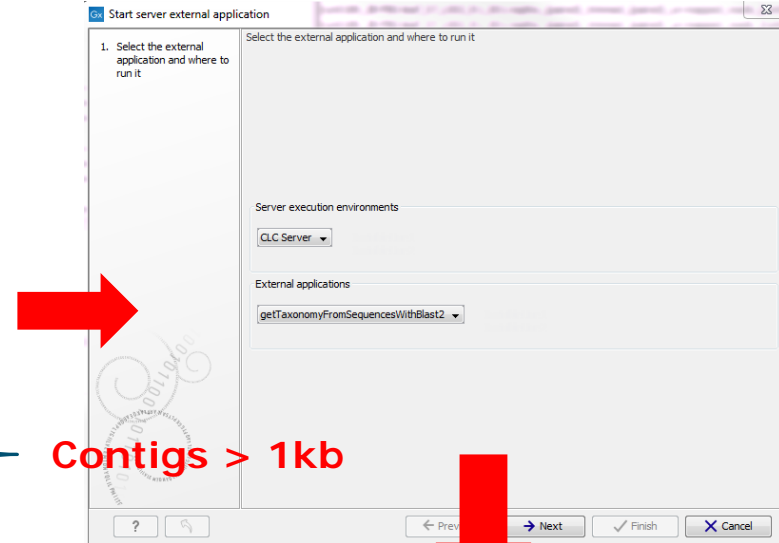
2B FBG leaf

Extract contigs > 1kb



Rows: 103,644

Name	Consen...	Total read c...	Reads in ...	Average cov...
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	547	3402	746	778.04
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	291	78	6	23.79
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	370	77	20	23.23
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	521	19	8	4.22
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	304	6282	456	2,288.44
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	339	1351	64	442.79
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	324	11826	708	3,980.87
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	422	3776	730	1,087.45
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	262	136	6	51.07
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	367	1796	136	566.39
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	220	552	2	302.23
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	201	1041	60	549.03
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	349	5268	1290	1,558.60
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	296	463	62	178.66
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	947	6463	1852	814.35
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	561	1974	174	434.32
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	254	140	10	61.76
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	341	13441	938	4,611.28
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	401	11232	460	3,268.51
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	424	24512	3756	6,695.15
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	441	650	38	141.88
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	791	5387	1378	806.99
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	987	6277	1822	765.84
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	606	3388	276	689.98
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	289	3384	96	1,117.59
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	463	2149	540	498.34
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	256	15	0	5.88
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	410	762	18	174.71
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	402	6458	566	1,976.87
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	447	6259	1528	1,682.38
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	776	608	82	76.59
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	201	32	2	15.44
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	627	13	8	2.59
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	257	478	40	190.56
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	374	947	22	275.32
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	358	85	2	23.83
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	432	19108	3356	5,337.30
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	207	130	4	68.21
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	227	107	14	45.59
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	1080	1230	196	120.27
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	287	5277	164	2,164.80
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	222	152	0	71.30
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	217	372	2	169.31
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	313	38	2	12.72
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	304	45	8	14.25
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	669	618	126	101.87
run0189_2B-FBG-leaf_S7_I002_R1_001.nophix_(paired)_trimmed_(paired)_un-mapped_reads_[run0189_2B-FBG-leaf_S7_I00...	481	4708	396	1,201.67



Start server external application

Select the external application and where to run it

1. Select the external application and where to run it

Server execution environments

GLC Server

External applications

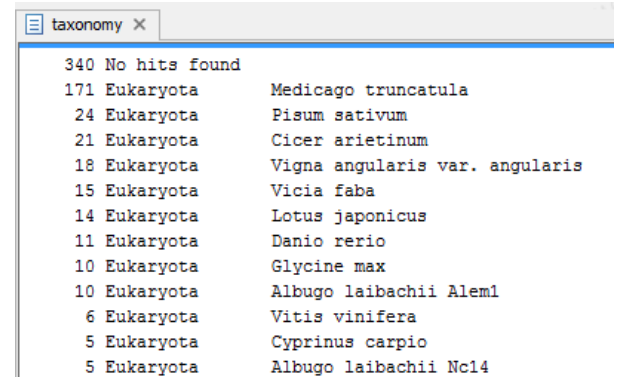
getTaxonomyFromSequencesWithBlast2

Next

Finish

Cancel

Contigs > 1kb



taxonomy

340	No hits found	
171	Eukaryota	Medicago truncatula
24	Eukaryota	Pisum sativum
21	Eukaryota	Cicer arietinum
18	Eukaryota	Vigna angularis var. angularis
15	Eukaryota	Vicia faba
14	Eukaryota	Lotus japonicus
11	Eukaryota	Danio rerio
10	Eukaryota	Glycine max
10	Eukaryota	Albugo laibachii Aleml
6	Eukaryota	Vitis vinifera
5	Eukaryota	Cyprinus carpio
5	Eukaryota	Albugo laibachii Nc14

2A healthy leaf

taxonomy x	
270 Eukaryota	Medicago truncatula
232 No hits found	
81 Eukaryota	Vicia faba
44 Eukaryota	Lotus japonicus
37 Eukaryota	Pisum sativum
31 Eukaryota	Cicer arietinum
30 Eukaryota	Glycine max
24 Eukaryota	Vigna angularis var. angularis
11 Eukaryota	Trifolium repens
9 Eukaryota	Danio rerio
7 Eukaryota	Vitis vinifera
7 Eukaryota	Solanum pennellii
7 Eukaryota	Solanum lycopersicum
7 Eukaryota	Lupinus angustifolius

2B FBG leaf

taxonomy x	
340 No hits found	
171 Eukaryota	Medicago truncatula
24 Eukaryota	Pisum sativum
21 Eukaryota	Cicer arietinum
18 Eukaryota	Vigna angularis var. angularis
15 Eukaryota	Vicia faba
14 Eukaryota	Lotus japonicus
11 Eukaryota	Danio rerio
10 Eukaryota	Glycine max
10 Eukaryota	Albugo laibachii Alem1
6 Eukaryota	Vitis vinifera
5 Eukaryota	Cyprinus carpio
5 Eukaryota	Albugo laibachii Nc14

3A healthy leaf

taxonomy x	
435 Eukaryota	Medicago truncatula
410 No hits found	
99 Eukaryota	Vicia faba
73 Eukaryota	Pisum sativum
63 Eukaryota	Cicer arietinum
54 Eukaryota	Vigna angularis var. angularis
51 Eukaryota	Glycine max
44 Eukaryota	Lotus japonicus
11 Eukaryota	Vitis vinifera
11 Eukaryota	Trifolium repens
9 Eukaryota	Lens culinaris
8 Eukaryota	Solanum lycopersicum
8 Eukaryota	Danio rerio
6 Eukaryota	Solanum pennellii

3B FBG leaf

taxonomy x	
615 No hits found	
481 Eukaryota	Medicago truncatula
96 Eukaryota	Vicia faba
71 Eukaryota	Pisum sativum
67 Eukaryota	Lotus japonicus
54 Eukaryota	Cicer arietinum
49 Eukaryota	Glycine max
39 Eukaryota	Vigna angularis var. angularis
16 Eukaryota	Danio rerio
11 Eukaryota	Vitis vinifera
11 Eukaryota	Solanum lycopersicum
10 Eukaryota	Solanum pennellii
9 Eukaryota	Cyprinus carpio
8 Eukaryota	Albugo laibachii Alem1
7 Eukaryota	Trifolium repens

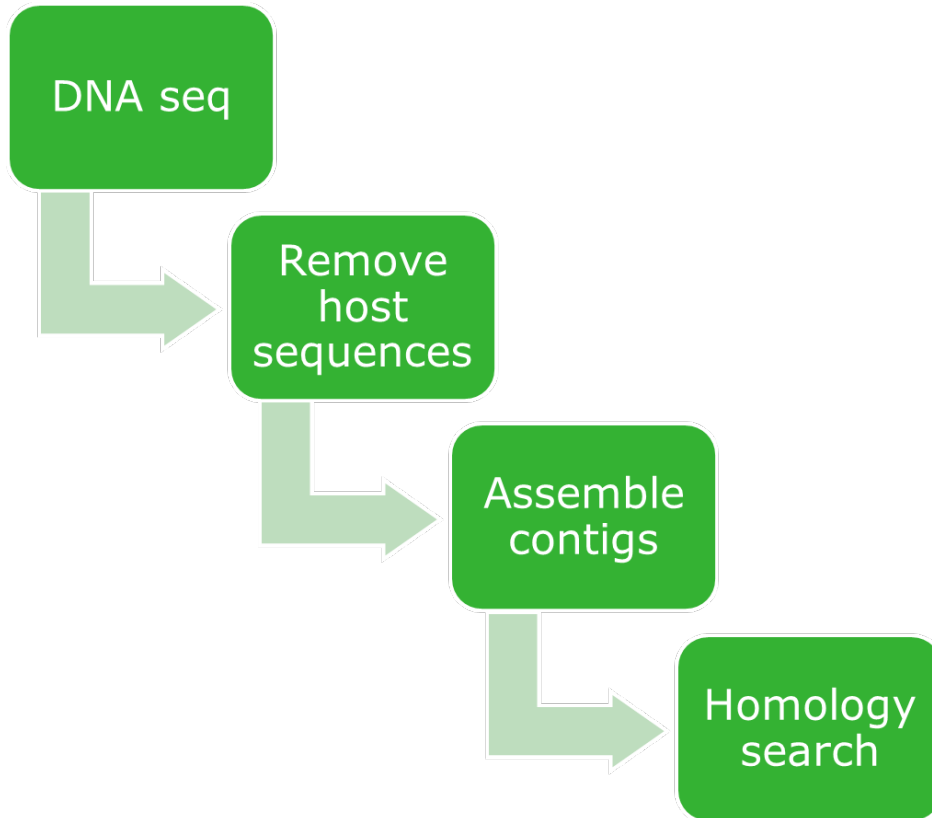
4A healthy leaf

taxonomy x	
369 Eukaryota	Medicago truncatula
309 No hits found	
80 Eukaryota	Vicia faba
54 Eukaryota	Lotus japonicus
46 Eukaryota	Pisum sativum
46 Eukaryota	Cicer arietinum
36 Eukaryota	Vigna angularis var. angularis
23 Eukaryota	Glycine max
14 Eukaryota	Danio rerio
8 Eukaryota	Solanum pennellii
8 Eukaryota	Lens culinaris
6 Eukaryota	Vitis vinifera
6 Eukaryota	Trifolium repens
5 Eukaryota	Lupinus angustifolius
5 Eukaryota	Cyprinus carpio

4B FBG leaf

taxonomy x	
380 No hits found	
328 Eukaryota	Medicago truncatula
82 Eukaryota	Vicia faba
53 Eukaryota	Pisum sativum
43 Eukaryota	Lotus japonicus
40 Eukaryota	Cicer arietinum
38 Eukaryota	Vigna angularis var. angularis
34 Eukaryota	Glycine max
9 Eukaryota	Solanum pennellii
9 Eukaryota	Solanum lycopersicum
9 Eukaryota	Danio rerio
7 Eukaryota	Lens culinaris
7 Eukaryota	Albugo laibachii Nc14
6 Eukaryota	Trifolium repens
6 Eukaryota	Albugo laibachii Alem1

Faba bean gall disease



2A healthy leaf

Count	Kingdom	Species
270	Eukaryota	Medicago truncatula
232	No hits found	
81	Eukaryota	Vicia faba
44	Eukaryota	Lotus japonicus
37	Eukaryota	Pisum sativum
31	Eukaryota	Cicer arietinum
30	Eukaryota	Glycine max
24	Eukaryota	Vigna angularis var. angularis
11	Eukaryota	Trifolium repens
9	Eukaryota	Danio rerio
7	Eukaryota	Vitis vinifera
7	Eukaryota	Solanum pennellii
7	Eukaryota	Solanum lycopersicum
7	Eukaryota	Lupinus angustifolius

2B FBG leaf

Count	Kingdom	Species
340	No hits found	
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15	Eukaryota	Vicia faba
14	Eukaryota	Lotus japonicus
11	Eukaryota	Danio rerio
10	Eukaryota	Glycine max
10	Eukaryota	Albugo laibachii Aleml
6	Eukaryota	Vitis vinifera
5	Eukaryota	Cyprinus carpio
5	Eukaryota	Albugo laibachii Nc14

3A healthy leaf

Count	Kingdom	Species
435	Eukaryota	Medicago truncatula
410	No hits found	
99	Eukaryota	Vicia faba
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63	Eukaryota	Cicer arietinum
54	Eukaryota	Vigna angularis var. angularis
51	Eukaryota	Glycine max
44	Eukaryota	Lotus japonicus
11	Eukaryota	Vitis vinifera
11	Eukaryota	Trifolium repens
9	Eukaryota	Lens culinaris
8	Eukaryota	Solanum lycopersicum
8	Eukaryota	Danio rerio
6	Eukaryota	Solanum pennellii

3B FBG leaf

Count	Kingdom	Species
615	No hits found	
481	Eukaryota	Medicago truncatula
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49	Eukaryota	Glycine max
39	Eukaryota	Vigna angularis var. angularis
16	Eukaryota	Danio rerio
11	Eukaryota	Vitis vinifera
11	Eukaryota	Solanum lycopersicum
10	Eukaryota	Solanum pennellii
9	Eukaryota	Cyprinus carpio
8	Eukaryota	Albugo laibachii Aleml
7	Eukaryota	Trifolium repens

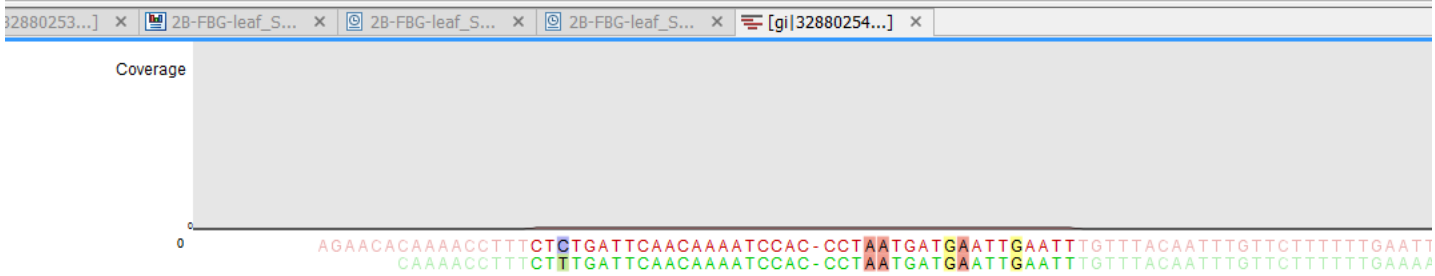
4A healthy leaf

Count	Kingdom	Species
369	Eukaryota	Medicago truncatula
309	No hits found	
80	Eukaryota	Vicia faba
54	Eukaryota	Lotus japonicus
46	Eukaryota	Pisum sativum
46	Eukaryota	Cicer arietinum
36	Eukaryota	Vigna angularis var. angularis
23	Eukaryota	Glycine max
14	Eukaryota	Danio rerio
8	Eukaryota	Solanum pennellii
8	Eukaryota	Lens culinaris
6	Eukaryota	Vitis vinifera
6	Eukaryota	Trifolium repens
5	Eukaryota	Lupinus angustifolius
5	Eukaryota	Cyprinus carpio

4B FBG leaf

Count	Kingdom	Species
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9	Eukaryota	Solanum lycopersicum
9	Eukaryota	Danio rerio
7	Eukaryota	Lens culinaris
7	Eukaryota	Albugo laibachii Nc14
6	Eukaryota	Trifolium repens
6	Eukaryota	Albugo laibachii Aleml

Low homology but best fit to *Albugo laibachii*



Count	Taxonomy
404	Eukaryota <i>Albugo laibachii</i> Alem1
116	Eukaryota <i>Albugo laibachii</i> Nc14; <i>Albugo laibachii</i> Alem1
0	No hits found

Faba bean gall disease candidate

- Albugo species
- White rust
- Not a bacterium, virus or fungus but an oomycete
- Possibly spreads quickly by zoospores
- Possibly seed transmitted
- Likely obligate biotroph so it may not be possible to obtain a pure culture
- Many of our genomic sequences are probably derived of this potential pathogen (estimated >500 contigs). These can be used to develop fast diagnostics and the genome may help to understand the pathogen.



Acknowledgements

Ilse Houwers



Theo van der Lee (PhD)

Contact: theo.vanderlee@wur.nl

Research initiated by

Woubit Dawit (PhD)
Associate Professor
Ambo University, Ethiopia