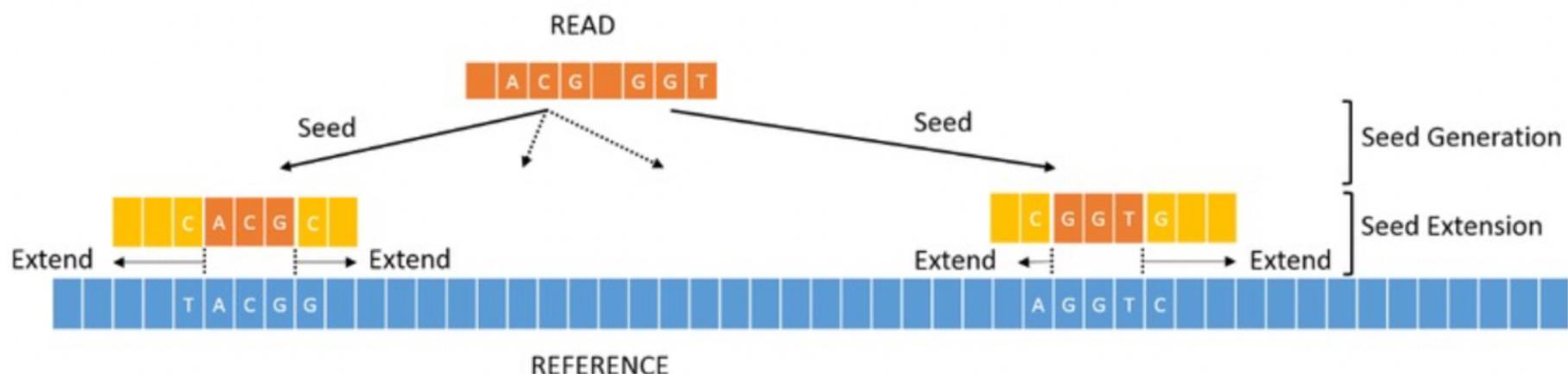


Align reads with BWA-MEM

Burrows-Wheeler Aligner Maximal Exact Matches (BWA-MEM)

BWA is a software package for mapping DNA sequences against a large reference genome



```
bwa mem ref.fa read1.fq read2.fq > aln-pe.sam
```

Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v2 [q-bio.GN].

Galaxy Australia

Workflow Visualize Shared Data Help User Run Tool History Using 1%

Tools

bwa-mem

Upload Data Show Sections

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

BWA-MEM2 - map medium and long reads (> 100 bp) against reference genome

WORKFLOWS

All workflows

Map with BWA-MEM

- map medium and long reads (> 100 bp) against reference genome (Galaxy Version 0.7.17.2)

Tool Parameters

Will you select a reference genome from your history or use a built-in index?

Use a built-in genome index

Built-ins were indexed using default options. See 'Indexes' section of help below

Using reference genome *

A. mellifera 04 Nov 2010 (Amel_4.5/apiMel4) (apiMel4)

Select genome from the list

Single or Paired-end reads

Paired

Select between paired and single end data

Select first set of reads *

4: rsv.reference.fasta

Select second set of reads *

4: rsv.reference.fasta

Specify dataset with forward reads

Specify dataset with reverse reads

Enter mean, standard deviation, max, and min for insert lengths. - optional

History

deleted:false visible:any

RSV_assembly

15.9 MB

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

6: rsv.primers.bed

5: rsv.reference.gff3

4: rsv.reference.fasta

Tools

bwa-mem

Upload Data

Show Sections

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

BWA-MEM2 - map medium and long reads (> 100 bp) against reference genome

WORKFLOWS

All workflows

Map with BWA-MEM
- map medium and long reads (> 100 bp) against reference genome
(Galaxy Version 0.7.17.2)

Tool Parameters

Will you select a reference genome from your history or use a built-in index?

1 Use a genome from history and build index

Built-ins were indexed using default options. See 'Indexes' section of help below

Use the following dataset as the reference sequence *

2 4: rsv.reference.fasta

You can upload a FASTA sequence to the history and use it as reference

Algorithm for constructing the BWT index *

Auto. Let BWA decide the best algorithm to use

(-a)

Single or Paired-end reads

3 Paired Collection

Select between paired and single end data

Select a paired collection *

4 3: P90006023_RSVA_Illumina.fastq

See help section for an explanation of dataset collections

Enter mean, standard deviation, max, and min for insert lengths. - optional

History

deleted:false visible:any

RSV_assembly

15.9 MB

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

6: rsv.primers.bed

5: rsv.reference.gff3

4: rsv.reference.fasta



Tools

bwa-mem



Upload Data

Show Sections

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

BWA-MEM2 - map medium and long reads (> 100 bp) against reference genome

WORKFLOWS

All workflows

Map with BWA-MEM

- map medium and long reads (> 100 bp) against reference genome
(Galaxy Version 0.7.17.2)

Run Tool

Set read groups information?

Do not set

Specifying read group information can greatly simplify your downstream analyses by allowing combining multiple datasets.

Select analysis mode

1.Simple Illumina mode

BAM sorting mode *

Sort by chromosomal coordinates

The 'Not sorted' option can extend the run time of the tool significantly (cause it requires running on only a single thread).

Additional Options

Email notification

 No

Send an email notification when the job completes.

Run Tool

Help

What is does

From <http://arxiv.org/abs/1303.3997>:

History

deleted:false visible:any

RSV_assembly

15.9 MB

6

6



12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

a pair with 2 datasets

7: FastQC on collection 3: Webpage

a pair with 2 datasets

6: rsv.primers.bed



5: rsv.reference.gff3



4: rsv.reference.fasta



Tools

bwa-mem

Upload Data

Show Sections

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

BWA-MEM2 - map medium and long reads (> 100 bp) against reference genome

WORKFLOWS

All workflows



Started tool **Map with BWA-MEM** and successfully added 1 job to the queue.

It produces this output:

- 13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

deleted:false visible:any

RSV_assembly

23.8 MB

7 6

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

a pair with 2 datasets

7: FastQC on collection 3: Webpage

a pair with 2 datasets

6: rsv.primers.bed

Sequence Alignment/Map Format Specification (SAM)

```
Coor      12345678901234 5678901234567890123456789012345
ref       AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGGCCAT

+r001/1    TTAGATAAAGGATA*CTG
+r002    aaaAGATAA*GGATA
+r003    gcctaAGCTAA
+r004    ATAGCT.....TCAGC
-r003    ttagctTAGGC
-r001/2    CAGCGGCAT
```

```
@HD VN:1.6 SO:coordinate
@SQ SN:ref LN:45
r001 99 ref 7 30 8M2I4M1D3M = 37 39 TTAGATAAAGGATACTG *
r002 0 ref 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *
r003 0 ref 9 30 5S6M * 0 0 GCCTAAGCTAA * SA:Z:ref,29,-,6H5M,17,0;
r004 0 ref 16 30 6M14N5M * 0 0 ATAGCTTCAGC *
r003 2064 ref 29 17 6H5M * 0 0 TAGGC * SA:Z:ref,9,+,5S6M,30,1;
r001 147 ref 37 30 9M = 7 -39 CAGCGGCAT * NM:i:1
```

<https://samtools.github.io/hts-specs/SAMv1.pdf>

Galaxy Australia Using 1%

Tools

samtools sort

Upload Data

Show Sections

Samtools sort order of storing aligned sequences

WORKFLOWS

All workflows

Samtools sort order of storing aligned sequences

Tool Parameters

BAM File *

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

Primary sort key

coordinate

Minhash collation

No

Use minimiser for clustering unaligned/unplaced reads. (-M)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Sort alignments by leftmost coordinates, or by read name when -n is used. An appropriate @HD-SO sort order header tag will be added or an existing one updated if necessary.

Ordering Rules

History

deleted:false visible:any

RSV_assembly

23.8 MB

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

6: rsv.primers.bed

Galaxy Australia Using 1%

Tools

samtools sort

Upload Data

Show Sections

Samtools sort order of storing aligned sequences

WORKFLOWS

All workflows

Workflow Visualize Shared Data Help User

Samtools sort order of storing aligned sequences (Galaxy Version 2.0.4)

Tool Parameters

BAM File *

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

Primary sort key

coordinate

Minhash collation

No

Use minimiser for clustering unaligned/unplaced reads. (-M)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Sort alignments by leftmost coordinates, or by read name when -n is used. An appropriate @HD-SO sort order header tag will be added or an existing one updated if necessary.

Ordering Rules

History

deleted:false visible:any

RSV_assembly

23.8 MB

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

6: rsv.primers.bed

Tools

samtools sort

Samtools sort order of storing aligned sequences

WORKFLOWS

All workflows



Started tool **Samtools sort** and successfully added 1 job to the queue.

It produces this output:

- 14: Samtools sort on data 13

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History	+ ↗ ⌂ ⌂
deleted:false visible:any	⌄ ⌂ ⌂
RSV_assembly	edit
31.9 MB	location 8 ⌂ 6 ⌂
<input checked="" type="checkbox"/>	⚙️
14: Samtools sort on data 13	edit delete
13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)	edit delete
12: FastQC on data 2: RawData	edit delete
11: FastQC on data 2: Webpage	edit delete
10: FastQC on data 1: RawData	edit delete
9: FastQC on data 1: Webpage	edit delete
8: FastQC on collection 3: RawData a pair with 2 datasets	edit delete
7: FastQC on collection 3: Webpage a pair with 2 datasets	edit delete

Galaxy Australia Using 1%

Tools

samtools flagstat

Upload Data

Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Tool Parameters

BAM File to report statistics of *

14: Samtools sort on data 13

Output format *

txt

(--output-fmt)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Uses samtools flagstat command to print descriptive information for a BAM dataset. Here is an example of such information:

```
200 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
25 + 0 mapped (12.50%:nan%)
200 + 0 paired in sequencing
100 + 0 read1
100 + 0 read2
```

History

deleted:false visible:any

RSV_assembly

31.9 MB

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

Galaxy Australia Using 1%

Workflow Visualize Shared Data Help User

Tools

Samtools flagstat

Upload Data

Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Samtools flagstat tabulate descriptive stats for BAM dataset (Galaxy Version 2.0.4)

Tool Parameters

BAM File to report statistics of *

1 14: Samtools sort on data 13

Output format *

2 tsv

(--output-fmt)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Uses samtools flagstat command to print descriptive information for a BAM dataset. Here is an example of such information:

200 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
25 + 0 mapped (12.50%:nan%)
200 + 0 paired in sequencing
100 + 0 read1
100 + 0 read2

History

deleted:false visible:any

RSV_assembly

31.9 MB 8 6

14: Samtools sort on data 13

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12: FastQC on data 2: RawData

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9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

Tools

samtools flagstat

 Upload Data

 Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Column 1	Column 2	Column 3
189546	0	total (QC-passed reads + QC-failed reads)
188152	0	primary
0	0	secondary
1394	0	supplementary
0	0	duplicates
0	0	primary duplicates
159354	0	mapped
84.07%	N/A	mapped %
157960	0	primary mapped
83.95%	N/A	primary mapped %
188152	0	paired in sequencing
94076	0	read1
94076	0	read2
157080	0	properly paired
83.49%	N/A	properly paired %
157662	0	with itself and mate mapped
298	0	singletons
0.16%	N/A	singletons %
0	0	with mate mapped to a different chr
0	0	with mate mapped to a different chr (mapQ>=5)

History

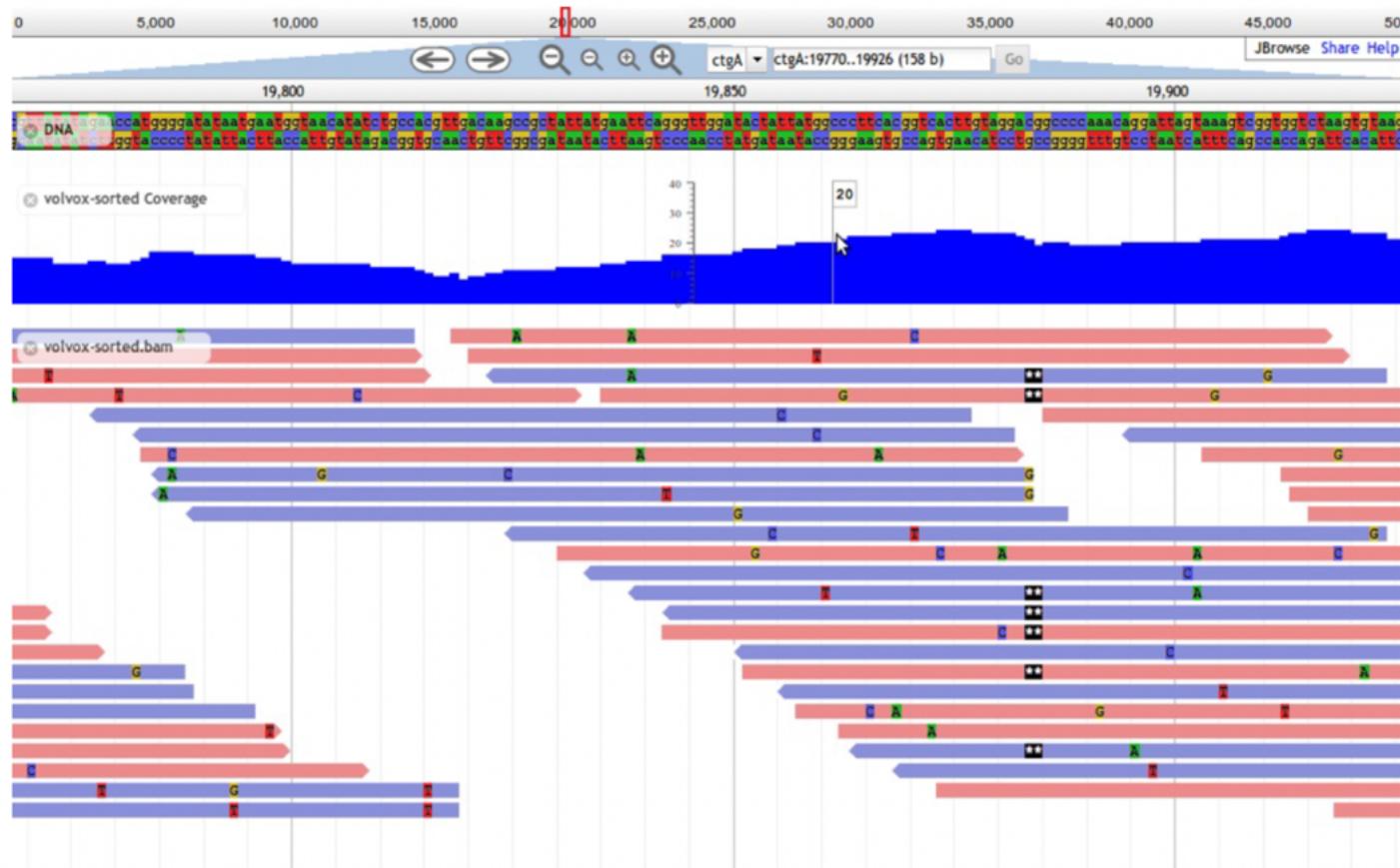
deleted:false visible:any

RSV_assembly

- 15: Samtools flagstat on data 14   
- 14: Samtools sort on data 13  
- 13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)  
- 12: FastQC on data 2: RawData   
- 11: FastQC on data 2: Webpage   
- 10: FastQC on data 1: RawData   
- 9: FastQC on data 1: Webpage   
- 8: FastQC on collection 3: RawData  

a pair with 2 datasets
- 7: FastQC on collection 3: Webpage  

Visualize mapping with JBrowse



<https://jbrowse.org/jb2/>

Galaxy Australia

Workflow Visualize Shared Data Help User Run Tool Using 1%

Tools

jbrowse

Upload Data Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone

upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

All workflows

JBrowse genome browser (Galaxy Version 1.16.11+galaxy1)

Tool Parameters

Reference genome to display

Use a built-in genome

Built-in references

Select a reference genome *

A. mellifera 04 Nov 2010 (Amel_4.5/apiMel4) (apiMel4)

If your genome of interest is not listed, contact the Galaxy team

Output JBrowse *

Minimal for viewing (Documentation removed)

Genetic Code *

1. The Standard Code

JBrowse-in-Galaxy Action

New JBrowse Instance

Track Group

+ Insert Track Group

General JBrowse Options [Advanced]

Plugins

History

deleted:false visible:any

RSV_assembly

31.9 MB

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

a pair with 2 datasets

7: FastQC on collection 3: Webpage

Galaxy Australia Using 1%

Workflow Visualize Shared Data Help User

Tools

jBrowse

Upload Data Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone
upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

All workflows

JBrowse genome browser (Galaxy Version 1.16.11+galaxy1)

Tool Parameters

Reference genome to display

1 Use a genome from history

Built-in references

2 Select the reference genome *

4: rsv.reference.fasta

Output JBrowse *

Minimal for viewing (Documentation removed)

Genetic Code *

1. The Standard Code

JBrowse-in-Galaxy Action

New JBrowse Instance

Track Group

3 + Insert Track Group

General JBrowse Options [Advanced]

Plugins

History

deleted:false visible:any

RSV_assembly

31.9 MB

15: Samtools flagstat on data 14

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13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

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10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

7: FastQC on collection 3: Webpage

Tools

jbrowse

Upload Data

Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone

upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

All workflows

JBrowse genome browser (Galaxy Version 1.16.11+galaxy1)



Run Tool

Track Group

1: Track Group



Track Category *

1 Sequencing & Variation

Organise your tracks into Categories for a nicer end-user experience. You can use #date# and it will be replaced with the current date in 'yyyy-mm-dd' format, which is very useful for repeatedly updating a JBrowse instance when member databases / underlying tool versions are updated.

Annotation Track

1: Annotation Track



Track Type

2 BAM Pileups



BAM Track Data *



3 14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)



Autogenerate SNP Track



No

Not recommended for deep coverage BAM files

Maximum size of BAM chunks *

History

deleted:false visible:any

RSV_assembly

31.9 MB

9

6



15: Samtools flagstat on data 14



14: Samtools sort on data 13



13: Map with BWA-MEM on data

2, data 1, and data 4 (mapped re
ads in BAM format)

12: FastQC on data 2: RawDa

ta



11: FastQC on data 2: Webpa

ge



10: FastQC on data 1: RawDat

a



9: FastQC on data 1: Webpag

e



8: FastQC on collection 3: RawData

a pair with 2 datasets



7: FastQC on collection 3: Webpage

||



Tools

jbrowse

Upload Data

Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone

upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

All workflows

JBrowse genome browser (Galaxy Version 1.16.11+galaxy1)

Run Tool

Maximum size of BAM chunks *

4 10000000

Maximum size in bytes of BAM chunks that the browser will try to deal with. When this is exceeded, most tracks will display 'Too much data' message.

JBrowse Custom Track Config [Advanced]

Track Visibility *

Off for new users

Override Apollo Plugins *

No - Do not Override

Disable the apollo plugin for this track, this disables the ability to make an annotation from this feature.

Override Apollo Draggability *

No - Do not Override

Disable apollo's drag-to-create feature functionality.

+ Insert Annotation Track

+ Insert Track Group

General JBrowse Options [Advanced]

Plugins

Additional Options

History

deleted:false visible:any

RSV_assembly

43.6 MB

10

6

16: JBrowse on data 14 and data 4 - minimal

Add Tags

1.5 KB

format html, database ?

INFO:jbrowse:Processing Sequencing X

Variation / Samtools sort on data 13

WARNING: No feature names found for

HTML file

15: Samtools flagstat on data 14

10

6

14: Samtools sort on data 13

10

6

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

10

12: FastQC on data 2: RawDa

10

6



Tools

jbrowse



Upload Data

Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone

upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

All workflows

JBrowse genome browser (Galaxy Version 1.16.11+galaxy1)



Run Tool

Additional Options

Email notification

 No

Send an email notification when the job completes.

Run Tool

Help

JBrowse-in-Galaxy

JBrowse-in-Galaxy offers a highly configurable, workflow-compatible alternative to Trackster.

Overview

JBrowse is a fast, embeddable genome browser built completely with JavaScript and HTML5.

The JBrowse-in-Galaxy (JiG) tool was written to help build complex JBrowse installations straight from Galaxy, taking advantage of the latest Galaxy features such as dataset collections, sections, and colour pickers. It allows you to build up a JBrowse instance without worrying about how to run the command line tools to format your data, and which options need to be supplied and where. Additionally it comes with many javascript functions to handle colouring of features which would be nearly impossible to write without the assistance of this tool.

The JBrowse-in-Galaxy tool is maintained by the Galaxy IUC, who you can help you with missing features or bugs in the tool.

Options

The first option you encounter is the **Fasta Sequence(s)**. This option now accepts multiple fasta files, allowing you to build JBrowse instances that contain data for multiple genomes or chromosomes (generally known as "landmark features" in gff3 terminology.) Up to 30 will be shown from the dropdown selector within JBrowse, this is a known issue.

Standalone Instances enable you to have either a complete JBrowse instance in a dataset, or just the data directory without JBrowse (e.g. for Apollo). Currently Galaxy copies the entire JBrowse directory in order to have a complete, downloadable file that contains a ready to go JBrowse instance. This is obviously an anti-feature because users don't want

History

deleted:false visible:any



RSV_assembly

31.9 MB



9

6



15: Samtools flagstat on data 14



14: Samtools sort on data 13



13: Map with BWA-MEM on data

2, data 1, and data 4 (mapped re
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12: FastQC on data 2: RawDa

ta



11: FastQC on data 2: Webpa

ge



10: FastQC on data 1: RawDat

a



9: FastQC on data 1: Webpag

e



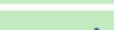
8: FastQC on collection 3: RawData

a pair with 2 datasets



7: FastQC on collection 3: Webpage

||



Galaxy Australia

Workflow Visualize Shared Data Help User Share Using 1%

Tools

- jbrowse
- Upload Data
- Show Sections

JBrowse genome browser

JBrowse - Data Directory to Standalone
upgrades the bare data directory to a full JBrowse instance

WORKFLOWS

- All workflows

Available Tracks

- filter tracks
- Reference sequence (1)
 - Reference sequence
- Sequencing X Variation (1)
 - Samtools sort on data 13

Genome Track View Help Tools Share

0 2,000 4,000 6,000 8,000 10,000 12,000 14,000

7,250 7,500

rsv_reference:7105..7651 (547 b)

Reference sequence Zoom in to see sequence Zoom in to see

Samtools sort on data 13

History

deleted:false visible:any

RSV_assembly

55.3 MB 11 6

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

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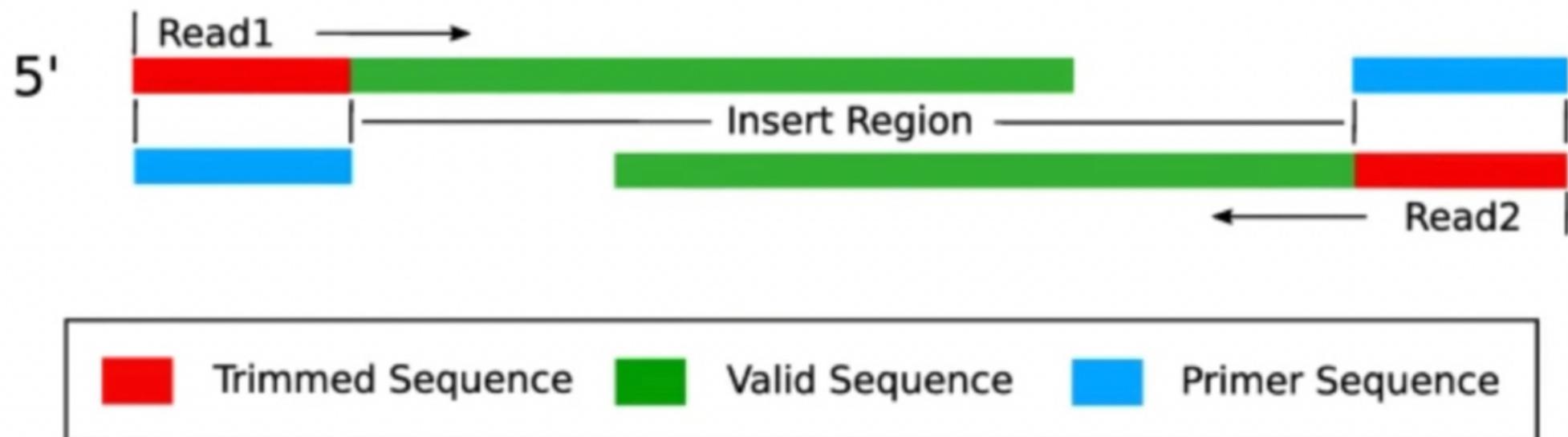
11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

Trim primers

Remove the primer parts of mapped reads, as they reflect the primer that was added and not the actual sample.



Galaxy Australia Using 1%

Tools

ivar trim

Upload Data

Show Sections

ivar trim Trim reads in aligned BAM

WORKFLOWS

All workflows

ivar trim Trim reads in aligned BAM (Galaxy Version 1.4.2+galaxy0)

Tool Parameters

Bam file *

14: Samtools sort on data 13

Aligned reads, to trim primers and quality (-i)

Source of primer information

History

BED file with primer sequences and positions *

6: rsv.primers.bed

(-b)

Filter reads based on amplicon info

No, allow reads to extend beyond amplicon boundaries

When you select Yes, reads that are not fully contained in any amplicon will be dropped before primer trimming. This option is currently marked as [Experimental] in ivar, but nevertheless recommended here. Info on amplicons can be computed from suitable primer BED files (see tool help below) or provided by the user.

Wiggling room for read ends relative to primer binding sites *

0

Reads that occur at the specified offset positions relative to primer positions (as annotated in the primer information dataset) will also be trimmed (default: 0) (-x)

Include reads not ending in any primer binding sites?

History

deleted:false visible:any

RSV_assembly

55.3 MB

11 6

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

Tools

ivar trim



Upload Data

Show Sections

ivar trim Trim reads in aligned BAM

WORKFLOWS

All workflows

ivar trim Trim reads in aligned BAM (Galaxy Version 1.4.2+galaxy0)



Run Tool

Tool Parameters

Bam file *



14: Samtools sort on data 13



Aligned reads, to trim primers and quality (-i)

Source of primer information

History

BED file with primer sequences and positions *



6: rsv.primers.bed



(-b)

Filter reads based on amplicon info

No, allow reads to extend beyond amplicon boundaries

When you select Yes, reads that are not fully contained in any amplicon will be dropped before primer trimming. This option is currently marked as [Experimental] in ivar, but nevertheless recommended here. Info on amplicons can be computed from suitable primer BED files (see tool help below) or provided by the user.

Wiggling room for read ends relative to primer binding sites *

0

Reads that occur at the specified offset positions relative to primer positions (as annotated in the primer information dataset) will also be trimmed (default: 0) (-x)

Include reads not ending in any primer binding sites?

History



deleted:false visible:any



RSV_assembly



55.3 MB

11

6



4

17: JBrowse on data 14 and data 4 - minimal



16: JBrowse on data 14 and data 4 - minimal



15: Samtools flagstat on data 14



14: Samtools sort on data 13



13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)



12: FastQC on data 2: RawData



11: FastQC on data 2: Webpage



10: FastQC on data 1: RawData



9: FastQC on data 1: Webpage



Tools

ivar trim

Upload Data

Show Sections

ivar trim Trim reads in aligned BAM

WORKFLOWS

All workflows

ivar trim Trim reads in aligned BAM (Galaxy Version 1.4.2+galaxy0)

Run Tool

Include reads not ending in any primer binding sites?

 Yes

(-e)

Minimum quality threshold for sliding window to pass *

20

(-q)

Width of sliding window *

4

(-s)

Require a minimum length for reads to retain them after any trimming?

Yes, and determine required length threshold automatically from input (-m -1)

The default automatic setting will determine the threshold as 50% of the mean length of the first 1000 raw input reads, which may or may not be adequate for your data. You can always make the outcome of filtering more predictable by specifying the threshold explicitly. You can also opt to retain reads independently of their trimmed length, but typically this only makes sense if you are performing additional read filtering with additional tools in your analysis pipeline.

Additional Options

Email notification

 No

Send an email notification when the job completes.

Run Tool

History

deleted:false visible:any

RSV_assembly

55.3 MB

11

6

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

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11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage



Tools

ivar trim



Upload Data

Show Sections

ivar trim Trim reads in aligned BAM

WORKFLOWS

All workflows

ivar trim Trim reads in aligned BAM (Galaxy Version 1.4.2+galaxy0)



Run Tool

Additional Options

Email notification

 No

Send an email notification when the job completes.

Run Tool

Help

iVar uses primer positions supplied in a BED file to soft clip primer sequences from an aligned and sorted BAM file. Following this, the reads are trimmed further based on a quality threshold.

Primer and Amplicon info

The tool requires information about primers and their binding sites in 6-column BED format. The information from this file is used to decide whether any mapped read in the BAM input ends with a primer sequence and should, thus, be soft-clipped.

Optionally, the tool can also discard reads that do not fully map to within any amplicon. Such reads are likely to be wet-lab or mapping artefacts and removing them can increase variant calling precision. To calculate the extent of expected amplicons the tool needs to know which primers work together to form an amplicon. The tool can try to deduce this info from the names of the primers found in the primer info dataset. This will require a primer naming scheme following the regex pattern:

.*_(<?P<amplicon_number>\d+).*_(<?P<primer_orientation>L(?:EFT)?|R(?:IGHT)?)

i.e., the following schemes will work (and get parsed as):

- nCoV-2019_1_LEFT (forward primer of amplicon 1)
- 400_2_out_R (reverse primer of amplicon 2)
- QIAseq_163-2_LEFT (forward primer of amplicon 163)

Alternatively, you can specify the amplicon information explicitly through a dataset that lists the names of primers that

History

deleted:false visible:any



RSV_assembly

55.3 MB

11

6

17: JBrowse on data 14 and data
4 - minimal16: JBrowse on data 14 and data
4 - minimal

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data
2, data 1, and data 4 (mapped re
ads in BAM format)12: FastQC on data 2: RawDa
ta11: FastQC on data 2: Webpa
ge10: FastQC on data 1: RawDat
a

9: FastQC on data 1: Webpag

Tools

ivar trim

Upload Data

Show Sections

ivar trim Trim reads in aligned BAM

WORKFLOWS

All workflows



Started tool **ivar trim** and successfully added 1 job to the queue.

It produces this output:

- 18: ivar trim on data 6 and data 14 Trimmed bam

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

deleted:false visible:any

RSV_assembly

60.7 MB 12 6

18: ivar trim on data 6 and data 14 Trimmed bam

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

Galaxy Australia Using 1%

Tools

samtools sort

Upload Data

Show Sections

Samtools sort order of storing aligned sequences

WORKFLOWS

All workflows

Samtools sort order of storing aligned sequences (Galaxy Version 2.0.4)

Tool Parameters

BAM File *

18: ivar trim on data 6 and data 14 Trimmed bam

Primary sort key

coordinate

Minhash collation

No

Use minimiser for clustering unaligned/unplaced reads. (-M)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Sort alignments by leftmost coordinates, or by read name when -n is used. An appropriate @HD-SO sort order header tag will be added or an existing one updated if necessary.

Ordering Rules

History

deleted:false visible:any

RSV_assembly

60.7 MB 12 6

18: ivar trim on data 6 and data 14 Trimmed bam

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

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12: FastQC on data 2: RawData

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Tools

samtools sort

Upload Data

Show Sections

Samtools sort order of storing aligned sequences

WORKFLOWS

All workflows

Workflow Visualize Shared Data Help User

Samtools sort order of storing aligned sequences (Galaxy Version 2.0.4)

Tool Parameters

BAM File *

18: ivar trim on data 6 and data 14 Trimmed bam

Primary sort key

coordinate

Minhash collation

No

Use minimiser for clustering unaligned/unplaced reads. (-M)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Sort alignments by leftmost coordinates, or by read name when -n is used. An appropriate @HD-SO sort order header tag will be added or an existing one updated if necessary.

Ordering Rules

History

deleted:false visible:any

RSV_assembly

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18: ivar trim on data 6 and data 14 Trimmed bam

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Galaxy Australia Using 1%

Tools

samtools flagstat

Upload Data

Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Samtools flagstat tabulate descriptive stats for BAM dataset (Galaxy Version 2.0.4)

Tool Parameters

BAM File to report statistics of *

19: Samtools sort on data 18

Output format *

txt

(--output-fmt)

Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

What it does

Uses samtools flagstat command to print descriptive information for a BAM dataset. Here is an example of such information:

```
200 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
25 + 0 mapped (12.50%:nan%)
200 + 0 paired in sequencing
100 + 0 read1
100 + 0 read2
```

History

deleted:false visible:any

RSV_assembly

66.3 MB 13 6

19: Samtools sort on data 18

18: ivar trim on data 6 and data 14 Trimmed bam

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

Tools

samtools flagstat



Upload Data

Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Samtools flagstat tabulate descriptive stats for BAM dataset (Galaxy Version 2.0.4)



Run Tool

Tool Parameters

BAM File to report statistics of *



19: Samtools sort on data 18



Output format *

tsv

(--output-fmt)

Additional Options

Email notification



Send an email notification when the job completes.

Run Tool

Help

What it does

Uses samtools flagstat command to print descriptive information for a BAM dataset. Here is an example of such information:

```
200 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
25 + 0 mapped (12.50%:nan%)
200 + 0 paired in sequencing
100 + 0 read1
100 + 0 read2
```

History

deleted:false visible:any



RSV_assembly

66.3 MB

13

6



19: Samtools sort on data 18

18: ivar trim on data 6 and data
14 Trimmed bam17: JBrowse on data 14 and data
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ta11: FastQC on data 2: Webpa
ge

Galaxy Australia

Workflow Visualize Shared Data Help User

Using 1%

Tools

- samtools flagstat
- Upload Data**
- Show Sections

Samtools flagstat tabulate descriptive stats for BAM dataset

WORKFLOWS

All workflows

Column 1	Column 2	Column 3
147033	0	total (QC-passed reads + QC-failed reads)
146813	0	primary
0	0	secondary
220	0	supplementary
0	0	duplicates
0	0	primary duplicates
147033	0	mapped
100.00%	N/A	mapped %
146813	0	primary mapped
100.00%	N/A	primary mapped %
146813	0	paired in sequencing
74140	0	read1
72673	0	read2
146020	0	properly paired
99.46%	N/A	properly paired %
146566	0	with itself and mate mapped
247	0	singletons
0.17%	N/A	singletons %
0	0	with mate mapped to a different chr
0	0	with mate mapped to a different chr (mapQ>=5)

History

deleted:false visible:any

RSV_assembly

66.3 MB 14 6

20: Samtools flagstat on data 19

19: Samtools sort on data 18

18: ivar trim on data 6 and data 14 Trimmed bam

17: JBrowse on data 14 and data 4 - minimal

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15: Samtools flagstat on data 14

14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

Identify variants

Variant Call Format (VCF)

```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1>Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1>Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A>Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1>Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0>Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0>Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1>Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1>Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1>Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2>Type=Integer,Description="Haplotype Quality">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001 NA00002 NA00003
20 14370 rs6054257 G A 29 PASS NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:..
20 17330 . T A 3 q10 NS=3;DP=11;AF=0.017 GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3 0/0:41:3
20 1110696 rs6040355 A G,T 67 PASS NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2 2/2:35:4
20 1230237 . T . 47 PASS NS=3;DP=13;AA=T GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20 1234567 microsat1 GTC G,GTCT 50 PASS NS=3;DP=9;AA=G GT:GQ:DP 0/1:35:4 0/2:17:2 1/1:40:3
```

https://en.wikipedia.org/wiki/Variant_Call_Format

Variant Call Format (VCF)

	Name	Brief description (see the specification for details).
1	CHROM	The name of the sequence (typically a chromosome) on which the variation is being called. This sequence is usually known as 'the reference sequence', i.e. the sequence against which the given sample varies.
2	POS	The 1-based position of the variation on the given sequence.
3	ID	The identifier of the variation, e.g. a dbSNP rs identifier, or if unknown a ". ". Multiple identifiers should be separated by semi-colons without white-space.
4	REF	The reference base (or bases in the case of an indel) at the given position on the given reference sequence.
5	ALT	The list of alternative alleles at this position.
6	QUAL	A quality score associated with the inference of the given alleles.
7	FILTER	A flag indicating which of a given set of filters the variation has failed or PASS if all the filters were passed successfully.
8	INFO	An extensible list of key-value pairs (fields) describing the variation. See below for some common fields. Multiple fields are separated by semicolons with optional values in the format: <key>=<data>[,data] .
9	FORMAT	An (optional) extensible list of fields for describing the samples. See below for some common fields.
+	SAMPLEs	For each (optional) sample described in the file, values are given for the fields listed in FORMAT

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Tools

ivar variants

Upload Data

Show Sections

ivar variants Call variants from aligned BAM file

WORKFLOWS

All workflows

ivar variants Call variants from aligned BAM file

Tool Parameters

Bam file *

19: Samtools sort on data 18

Aligned reads, to trim primers and quality

Reference *

4: rsv.reference.fasta

Minimum quality score threshold to count base *

20 (-q)

Minimum frequency threshold *

0.03 (-t)

Output format

Tabular (native tool output)

Coordinates of ORFs for optional variant effect prediction - optional

Nothing selected

Provide a GFF3 dataset with annotated ORFs to have the rightmost 5 columns of the tabular output populated with mutation information at the residue level. (-g)

Additional Options

History

deleted:false visible:any

RSV_assembly

66.3 MB 14 6

20: Samtools flagstat on data 19

19: Samtools sort on data 18

18: ivar trim on data 6 and data 14 Trimmed bam

17: JBrowse on data 14 and data 4 - minimal

16: JBrowse on data 14 and data 4 - minimal

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13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

12: FastQC on data 2: RawData

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Workflow Visualize Shared Data Help User

Tools

ivar variants

Upload Data

Show Sections

ivar variants Call variants from aligned BAM file (Galaxy Version 1.4.2+galaxy1)

Tool Parameters

Bam file *

1 19: Samtools sort on data 18

Aligned reads, to trim primers and quality

Reference *

2 4: rsv.reference.fasta

Minimum quality score threshold to count base *

20 (-q)

Minimum frequency threshold *

0.03 (-t)

Output format

Tabular (native tool output)

Coordinates of ORFs for optional variant effect prediction - optional

3 5: rsv.reference.gff3

Provide a GFF3 dataset with annotated ORFs to have the rightmost 5 columns of the tabular output populated with mutation information at the residue level. (-g)

Additional Options

History

deleted:false visible:any

RSV_assembly

66.3 MB 14 6

20: Samtools flagstat on data 19

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Galaxy Australia

Workflow Visualize Shared Data Help User Using 1%

Tools

ivar variants ▼ X

Upload Data

Show Sections

ivar variants Call variants from aligned BAM file

WORKFLOWS

All workflows

Started tool **ivar variants** and successfully added 1 job to the queue.

It produces this output:

- 21: ivar variants tabular output on data 5, data 4, and data 19

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

deleted:false visible:any ▼ X

RSV_assembly

66.3 MB ▼ X

21: ivar variants tabular output on data 5, data 4, and data 19 ▼ X

20: Samtools flagstat on data 19 ▼ X

19: Samtools sort on data 18 ▼ X

18: ivar trim on data 6 and data 14 Trimmed bam

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16: JBrowse on data 14 and data 4 - minimal

15: Samtools flagstat on data 14 ▼ X

14: Samtools sort on data 13 ▼ X

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)



Galaxy Australia

Workflow Visualize Shared Data Help User Using 1%

Tools

- ivar variants ▼ X
- Upload Data**
- Show Sections

ivar variants Call variants from aligned BAM file

WORKFLOWS

All workflows

REGION	POS	REF	ALT	REF_DP	REF_RV	REF_QUAL	ALT_DP	ALT_RV	ALT_QUAL	ALT_FREQ	TOTAL_DP
rsv_reference	91	T	A	0	0	0	710	118	43	1	716
rsv_reference	147	T	C	0	0	0	1142	226	48	1	1142
rsv_reference	230	A	G	3	0	37	1352	414	44	0.997786	1355
rsv_reference	524	T	C	1	0	37	1939	1108	43	0.999485	1946
rsv_reference	583	G	A	0	0	0	1868	1195	40	1	1868
rsv_reference	615	C	T	1	1	37	1902	1277	40	0.999475	1903
rsv_reference	619	G	T	0	0	0	1903	1295	40	1	1903
rsv_reference	693	G	A	1	1	37	1971	776	40	0.99848	1974
rsv_reference	723	C	T	0	0	0	1934	776	40	1	1934
rsv_reference	729	C	T	1	0	37	1895	724	40	0.998946	1897
rsv_reference	741	C	T	1	0	37	1872	738	41	0.999466	1873
rsv_reference	813	C	T	0	0	0	1653	807	41	1	1653
rsv_reference	876	T	A	0	0	0	1801	1106	41	0.997784	1805
rsv_reference	903	C	T	2	0	55	1792	1036	41	0.998329	1795

<https://usegalaxy.org.au/datasets/56e389a98c2d16788c61cbc2b553c221/outputs>

History

deleted:false visible:any ▼ X

RSV_assembly Edit

66.3 MB 15 locations 6 versions

More

21: ivar variants tabular output on data 5, data 4, and data 19 Edit Delete

20: Samtools flagstat on data 19 Edit Delete

19: Samtools sort on data 18 Edit Delete

18: ivar trim on data 6 and data 14 Trimmed bam Edit Delete

17: JBrowse on data 14 and data 4 - minimal Edit Delete

16: JBrowse on data 14 and data 4 - minimal Edit Delete

15: Samtools flagstat on data 14 Edit Delete

14: Samtools sort on data 13 Edit Delete

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format) Edit Delete

Galaxy Australia

Workflow Visualize Shared Data Help User Using 1%

Tools

- ivar variants x

Upload Data

Show Sections

ivar variants Call variants from aligned BAM file

WORKFLOWS

All workflows

REGION	POS	REF	ALT	REF_DP	REF_RV	REF_QUAL	ALT_DP	ALT_RV	ALT_QUAL	ALT_FREQ	TOTAL_DP
rsv_reference	91	T	A	0	0	0	710	118	43	1	716
rsv_reference	147	T	C	0	0	0	1142	226	48	1	1142
rsv_reference	230	A	G	3	0	37	1352	414	44	0.997786	1355
rsv_reference	524	T	C	1	0	37	1939	1108	43	0.999485	1946
rsv_reference	583	G	A	0	0	0	1868	1195	40	1	1868
rsv_reference	615	C	T	1	1	37	1902	1277	40	0.999475	1903
rsv_reference	619	G	T	0	0	0	1903	1295	40	1	1903
rsv_reference	693	G	A	1	1	37	1971	776	40	0.99848	1974
rsv_reference	723	C	T	0	0	0	1934	776	40	1	1934
rsv_reference	729	C	T	1	0	37	1895	724	40	0.998946	1897
rsv_reference	741	C	T	1	0	37	1872	738	41	0.999466	1873
rsv_reference	813	C	T	0	0	0	1653	807	41	1	1653
rsv_reference	876	T	A	0	0	0	1801	1106	41	0.997784	1805
rsv_reference	903	C	T	2	0	55	1792	1036	41	0.998329	1795
rsv_reference	962	A	C	0	0	0	1021	1022	40	0.998065	1023

History

deleted:false visible:any

RSV_assembly

66.3 MB 15 6

21: ivar variants tabular output on data 5, data 4, and data 19

Add Tags

281 lines

format tabular, database ?

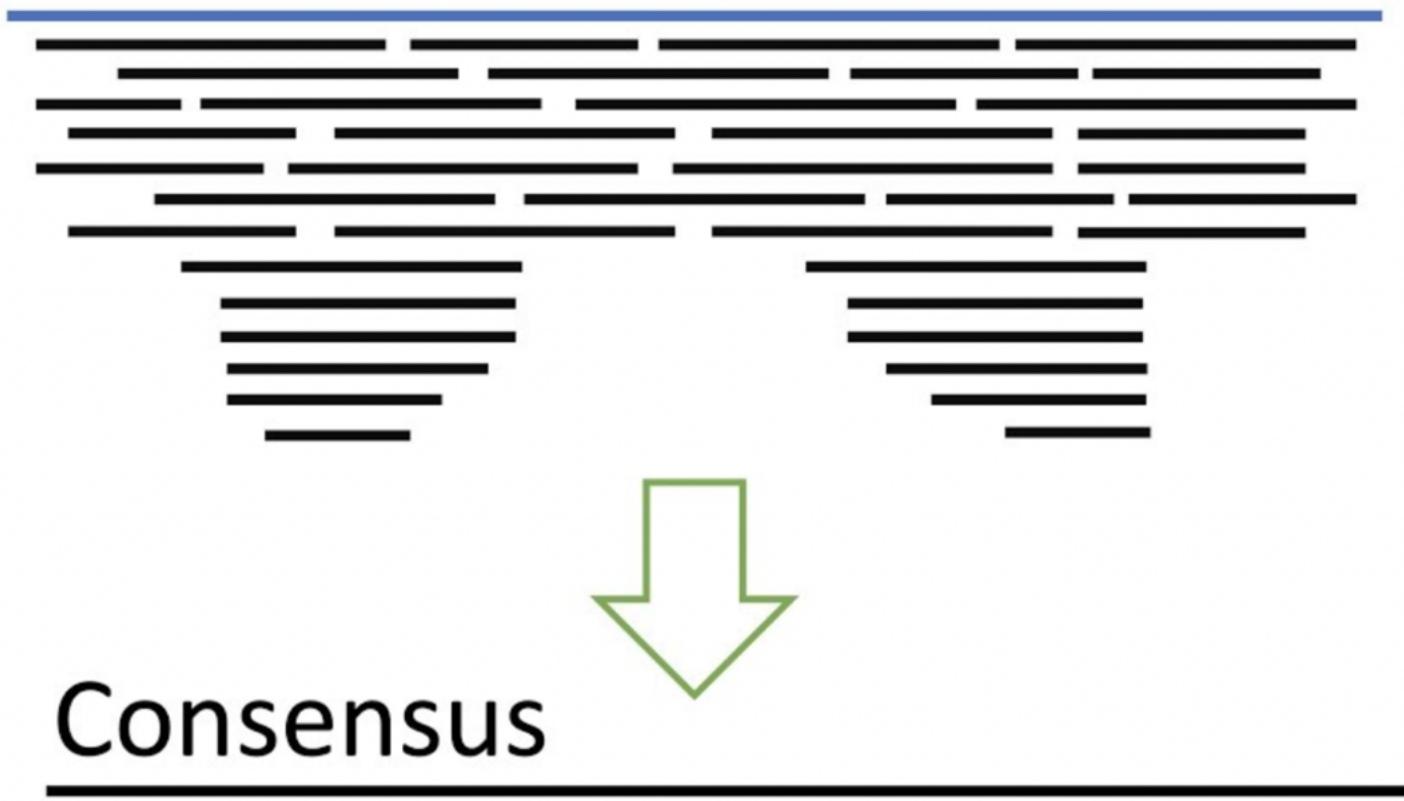
[mpileup] 1 samples in 1 input files
[mpileup] Max depth set to maximum value (2147483647)

Click to download

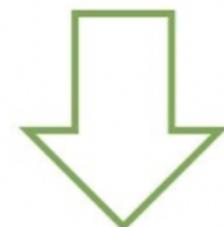
20: Samtools flagstat on data 19

19: Samtools sort on data 18

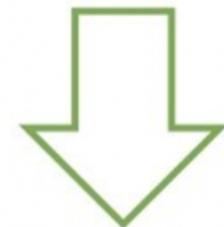
Generate consensus sequence



Fastq



Bam



Consensus

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Tools

ivar consensus

Upload Data

Show Sections

ivar consensus Call consensus from aligned BAM file

WORKFLOWS

All workflows

ivar consensus Call consensus from aligned BAM file (Galaxy Version 1.4.2+galaxy0)

Tool Parameters

Bam file *

19: Samtools sort on data 18

Aligned reads, to trim primers and quality

Minimum quality score threshold to count base *

20 (-q)

Minimum frequency threshold *

0.0

0 - Majority or most common base
0.2 - Bases that make up atleast 20% of the depth at a position
0.5 - Strict or bases that make up atleast 50% of the depth at a position
0.9 - Strict or bases that make up atleast 90% of the depth at a position
1 - Identical or bases that make up 100% of the depth at a position. Will have highest ambiguities (-t)

Minimum indel frequency threshold *

0.8

Same meaning as the "Minimum frequency threshold" above, but applied to indels. (-c)

Minimum depth to call consensus *

10 (-m)

How to represent positions with coverage less than the minimum depth threshold *

Represent as N (-n N)

History

deleted:false visible:any

RSV_assembly

66.3 MB 15 6

21: ivar variants tabular output on data 5, data 4, and data 19

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Tools

ivar consensus

Show Sections

ivar consensus Call consensus from aligned BAM file

WORKFLOWS

All workflows

ivar consensus Call consensus from aligned BAM file (Galaxy Version 1.4.2+galaxy0)

Tool Parameters

Bam file *

1 19: Samtools sort on data 18

Aligned reads, to trim primers and quality

Minimum quality score threshold to count base *

20

(-q)

Minimum frequency threshold *

2 0.75

0 - Majority or most common base
0.2 - Bases that make up atleast 20% of the depth at a position
0.5 - Strict or bases that make up atleast 50% of the depth at a position
0.9 - Strict or bases that make up atleast 90% of the depth at a position
1 - Identical or bases that make up 100% of the depth at a position. Will have highest ambiguities (-t)

Minimum indel frequency threshold *

0.8

Same meaning as the "Minimum frequency threshold" above, but applied to indels. (-c)

Minimum depth to call consensus *

3 20

(-m)

How to represent positions with coverage less than the minimum depth threshold *

Represent as N (-n N)

- History
- deleted:false visible:any
- RSV_assembly
- 66.3 MB 15 6
- 21: ivar variants tabular output on data 5, data 4, and data 19
- 20: Samtools flagstat on data 19
- 19: Samtools sort on data 18
- 18: ivar trim on data 6 and data 14 Trimmed bam
- 17: JBrowse on data 14 and data 4 - minimal
- 16: JBrowse on data 14 and data 4 - minimal
- 15: Samtools flagstat on data 14
- 14: Samtools sort on data 13
- 13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

Tools

ivar consensus



Upload Data

Show Sections

ivar consensus Call consensus from aligned BAM file
aligned BAM file

WORKFLOWS

All workflows

ivar consensus Call consensus from aligned BAM file (Galaxy Version 1.4.2+galaxy0)

Run Tool

How to represent positions with coverage less than the minimum depth threshold *

Represent as N (-n N)

Additional Options**Email notification**

Send an email notification when the job completes.

Run Tool

Help

To generate a consensus sequence iVar uses the output of samtools mpileup command. The mpileup output must be piped into iVar consensus

The command formed by this wrapper is :

```
samtools mpileup -A -a -d 0 -Q 0 sorted.bam | ivar consensus [options]
```

There are five parameters that can be set:

- **Minimum quality**: the minimum quality of a base to be considered in calculations of variant frequencies at a given position
- **Minimum frequency threshold**: the minimum frequency that the most likely base must surpass to be called as the consensus base at a position.
- **Minimum indel frequency threshold**: has the same meaning as the previous threshold, but gets applied to indels specifically. Setting this threshold higher than the threshold applied to SNVs helps reduce indel artefacts in the generated consensus.
- **Minimum depth to call consensus**: the minimum required depth to call a consensus base
- **How to represent positions with coverage less than the minimum depth threshold**: for positions for which the above minimum depth to call a consensus base is not reached, you can choose one of three different actions:

History

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**RSV_assembly**

66.3 MB

15

6



21: ivar variants tabular output on data 5, data 4, and data 19

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Tools

ivar consensus ▼ X

Upload Data

Show Sections

ivar consensus Call consensus from aligned BAM file

WORKFLOWS

All workflows

✓ Started tool **ivar consensus** and successfully added 1 job to the queue.

It produces this output:

- 22: ivar consensus on data 19 Consensus

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History + ↻ ⌂

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RSV_assembly

66.3 MB 16 ↺ 6 ⌂

✓ + ⚙️

22: ivar consensus on data 19 Co nsensus 🔗 🔍 🗑

21: ivar variants tabular output o n data 5, data 4, and data 19 🔗 🔍 🗑

20: Samtools flagstat on data 19 🔗 🔍 🗑

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14: Samtools sort on data 13 🔗 🔍 🗑

13: Map with BWA-MEM on data 🔗 🔍 🗑



Tools

ivar consensus

 Upload Data

 Show Sections

ivar consensus Call consensus from aligned BAM file

WORKFLOWS

All workflows

```
>Consensus_Samtools_sort_on_data_18_threshold_0.75_quality_20
NNNNNNNNNNNNNNNNNNNNNNNNNNNTGCGTAACCAAAAAATGGGCAATAAGAATTGATAAGTACCACTTAAATTAACTCCAT
TGGTTAGAGATGGCAGCAACTCATGGAGTAGATAAAAGTTAGATTGACAACTGTGTTGACATGATGAAGTAGCATGTTAAATAAC
ATGCTATACTGACAAATTAACTACAGTTAACATGCTTGGCTAACGGCAGTTACATACATAACATCAAATTGAATGGCATTTGTCATG
TTTACAACTAGTGATTTGCCCTAAATAATATTGAGTAGTAACTTCAACATGCCAAATTGACAACTGCCAGTATACAAATTGAGGTTATATA
TGGGAAATGAGGAAATTACACACTGCTCAACCTAACTGCCCTAAAGATGACAACTGTGAAATTAAATTCTCCAAAACAATGATTC
AAACATGACCAATTATATGAATCAATTATCTGAATTACTTGGATTGACCTCAATCCATAAAATCATAAATAATCAACTAGCAAAATCAATG
TCACTAACACCATTAGTTAATATAAACTTAACAGAAGATAAAAATGGGCAAAATAATCAATTCTACGCCAACATGGACACAACACA
CAATGATACACACACCACAAAGACTGATGATCACAGACATGAGCATTATCGCTTGAGACTATAAATACATCTAACTAGAGATATCATAA
CACATAATTATACCTGATAAATCATGAATGCACTGAGAAAATCTGATGAAAGACGCCACATTACATTTCTGTCACACTATGAA
ATGAAATCTTGCACAAAGTGGGAAAGCACTTAAATAATAAAAATACAGAATACACACAAAATGGCATTTCATGCCAAATTATTTAT
CAATCATGATGGGTTCTAGAATGCAATTGGCTTACAGCCTACAGCACACCCATAATACAGTATGATCTCAATCCATGAATATCAA
ACCAAGACCCAAACATCCGAAATAACAACTTATGCATAATCACACTCCATAATCAAATGGAGCCTGAAATTATAGTTATTTAAATTAA
AGGAAAGACATAAGATGAAAGATGGGCAAAACAAAAATGGCTTCTAGCAAAGTCAGTTGAAATGATACACTCAACAAAGATCAACTTCA
TCATCCACGAAATATACCATCCAACGGAGCACAGGAGACAGCATTGACACTCCTAATTATGATGTCAGAAACACATTAAATAAGTTATGTTG
CATGTTATTAAATCACAGAAGATGCTTAATCATAAATTCACTGGGTTAATAGGTATGTTATGCTAGATTAGGAAGAGAACACCA
TAAAATACTCAAAAGATGCAAGGATATCATGTTAAAGCAAAATGGAGTGTAAACACACATGCCAGAACAGATCAATGGGAAAGAAATGAAA
TTGAAGTGTAACTTAGCAAAACTTAACAACTGACATTCAACATTGAGATAGAATCTAGAAAATCTACAAAAAAATGCTAAAGA
AATGGGAGGGTGGCTCAGAATCACAGGCGTACTCTCTGATTGGGAGTATAATTATGATAGCAGCTTAACTTAAAGGTTTATTACCC
CAGCAGGAGATAGTCAGGCTTACAGCTGTGATTAGGAGAGCTAATAATGCTCTAAAATGAAATGAAACGTTAAAGGTTTATTACCC
AAGGATATAGCCAACAGCTTCTATGAAGTGTGTTGAAAATATCCTCACTTATAGTGTGTTGTCATTGGTATAGCACAATCTTCTAC
CAGAGGTGGCAGTAGAGTTGAAGGGATTTTTCAGGATTCTTATGAATGCCATGCTGCAGGCCAAGTGATGTTACGGTGGGGGTTCTAG
CAAATCAGTCAAAACATTATGTTAGGACAGCCTAGTGTACAAGCAGAAATGGAACAGTGTGGAGGTGATGAGTATCTCAGAAATG
GGTGGAGAACAGGATTCTACCATATTGAAACACCCAAAAGCATACTATTCTTGTACTTCTCCTACTTCTAGTGTAGTTG
GGCAATCTGCTGGCTCAGGCTAAATGGGAGAATCACAGGATCACCAAGGAATCAAGATTATGATGTCGAAACAGCATATGCTGAC
AACTCAAAAGAAAATGGTGTGATTAACACTACAGTGTATTAGATTGACAGCAGAAGAACTAGAGGCTATCAAACATCAGCTTAACTCAAAGAT
AATGATGTAGAGCTTGAGTTAATAAAAAGTGGGCAAAATAAGCATCATGGAAAAGTTGCTCTGAATTCTCATGGAGAAGATGCAACAA
ACAGAGCCACCAATTCTAGAATCATAAAGGCAATTACATCACCCAAAGATCCAAAGAAAAAGATAGTATCATATCTGTCACACTCA
ATAGATATAGAAGTAAACCAAAAGAACGCCATAACATCAACCTTAAACCAATTCAACCTTAAACCAATAATGAGACAGATGATCTGAGGAAACAA
GCCAAATTATCAAAGAACGCCCTAGTAAAGTTCAAGAACAGGCCCTATGCCAGTGATAATCTTCTTCTAAACTACAAAGAACCATAG
AAACATTGATAATATGAAAGAACATCTAGTATTCATATGAAGAAATAATGATCAGACAAACGATAATATAACAGCAAGATTAGATGG
ATTGATGAGAAAATTAAGTGAATACTAGGAATGCTTACACATTAGTAGTGTACAAGTGTGGAGGCCACATCTGCTGGGATGTTATAAGAGA
TGCCATGGTTGGTTAAAGAGAAGAAATGATGAGAAAATCAGAACTGACAGCTTAAATGACCAATGACAGACTAGAAGCTATGGCAAGACTCA
GGAATGAAGAAAAGTGGCAAAAGACATCAGATGAAAGTGTCTCAATCCAACATCAGAGAAAATGAAACACCTGTTGAAAGGG
AATGATACTGACAATGATCTACTTGAAAGATTCTGATTAGCTACAAACTGTCATCAAAACACAAACCAATGAAAACCAACAAACAA
ACCAACTCACTCATCCAAACACATCTACCTGCTGATCAGGCCAAACAGGCCAAAACACAGGCCATCCTACCGACTCTGGAAA
AAATGCTGATACTATGTTACAAAAAAATGGGCAAAATATGGGAAACATCTGTAATAACCTTCAGGCTTACACACAGCTGCTGTTCA
ATACAATCTCTGAGAAAAGACGATGTCATCTCATACTTACATTAACATGGGTCCTGTTCAATCATCTGAGCATCTACTCATTA
AAGAACTAGCCAATGTCATATACTAGTGAACAAATATCCACACCAAGGGACCTCTTAAAGAGTCATGATAAAACTCAAGAAGTGTGG
CTAGCACAATGCCAGCAATTACATGTTGCTAATGTGCTTGGATGAAAGAAGCAAGCTAGCATATGATGTAACCAACCCCTGTGA
AATTAGGCATGCACTTACATGCCAAATCAACAGCTACAGCTACAGGCTTCAACAAACGATCTACATGAAACACTCAACCCAAACACATG
ACATCATGCTTATGTAATTGAAATATGTAACATCAAAAGACTCATATAACCAACATACCTAAGATCTATCAGCTCAGAAATAAA
GATCTGACACACTTGAAGAAAATACAACCAACTGAAATTCAAAATGCCATTACAAATGCAAAATCATCCCTACTI Download CTGTTAGT
CATCACAGTGTGACAACAAAGGAGCATTCAAAATCATAAAGGCCAAACAGTCATCTAGTAGTGTGTTGGCTACUATGAGAAAAGAAA
GTATATATTGTTACACAAATGGGAGCACACAGCTACAGCTACAGGCTTCAACAAACCCCATGGAAGGTTAACCTTTCTTCTACATCAATG
AGTAGATTCTACAAACTTCTAATCACATTCTCACTTACATCAATCATAATCAGGAAACCCCTCTGAGTTCAATCAATCAATCAAACACTCATC
```

History

deleted:false visible:any

RSV_assembly

 66.3 MB

22: ivar consensus on data 19 Co

Add Tags

1 sequences

format fasta, database

Minimum Quality: 20

Threshold: 0.75

Minimum depth: 20

1/1

5



21: ivar variants tabular output
n data 5, data 4, and data 19

20: Samtools flagstat on data 19

Galaxy22-[ivar_consensus_on_data_19_Consensus].fasta — Edited

```
>Consensus_Samtools_sort_on_data_18_threshold_0.75_quality_20
NNNNNNNNNNNNNNNNNNNNNNNNNNNTCGTAAACCAAAAAATGGGCAAATAAGAATTGATAAGTACCACTAAATTAA
ACTCCATTGGTTAGAGATGGCAGCAACTCATTGAGTATGATAAAAAGTTAGATTGCAAATCTGTTGACAATGATGAAGTAGCA
TTGTTAAAATAACATGCTAATCTGACAAATTAAACAGTTAACAATGCTTGGCTAAGGCAGTTACATACATAATCAAATTGA
ATGGCATTGTATTTGTCATGTTATTAAAGTAGTGATATTGCCCTAATAATAATATTGATGAAATCCAATTTCACAACAAT
GCCAGTATTACAAATGGAGGTTATATGGGAAATGATGAACTAACACACTGCTCTAACCTAATGGCTAATAGATGACAAT
TGTGAAATTAAATTCTCAAAAAACTAAGTGATTCAACAATGACCAATTATATGAATCAATTATCTGAAATTACTGGATTTGACC
TCAATCCATAAATCATATAAAATATCAACTAGCAAATCAATGTCACTAACACCATTAGTTAATAAAACTAACAGAAGATAAA
AATGGGCAAATAATCAATTCTCGACCCAAACCATGGACACAACACAATGATACCAACACCACAAAGACTGATGATCACAGA
CATGAGACCATTATCGCTTGAGACTATAAAACATCTTAACAGAGATATCATAACACATAAATTATATACTTGATAAATCAT
GAATGCGATAGTGAGAAAACCTTGATGAAAGACAGGCCACATTACATTCTGGTCAACTATGAAATGAAACTATTGACAAAGTGG
GAAGCACTAAATATAAAAATATACAGAATAACACACAAATATGGCACTTTCTATGCCAATATTATCAATCATGATGGGTT
CTTAGAAATGCTTGGCATTAAGCCTACCAAGCACACACCCATAATATACAAGTATGATCTCAATCCATGAATATCAAACCAAGAC
CCAAACAATCGAAATAACAACCTTATGCATAATCACACTCCATAATCCAATGGAGCCTGAAAATTATAGTTATTTAAATTAA
GGAAAGACATAAGATGAAAGATGGGCAAATACAAAAATGGCTTCTAGCAAAGTCAAGTTGAATGATACTCAACAAAGATCAA
CTTCTATCATCCAGCAAATATACCATCCAAACGGAGCACAGGAGACAGCATTGACACTCTTAATTATGATGTGAGAAACACATTA
ATAAGTTATGTGGCATGTTAATCACAGAAGATGCTAATCATAAATTCACTGGGTTAATAGGTATGTTATATGCTATGCTAG
ATTAGGAAGAGAAGACACCAAAAAACTCAAAAGATGCACTGGGAGAGGTGGCTCCAGAATACAGGCATGACTCTCTGATTGTGGGAT
CAAGACATCAATGGGAAAGAAAATGAAATTGAGTGTAACTTAGCAAACACTGACATTCAAACATTGAGATAG
AATCTAGAAAATCTACAAAAAAATGCTAAAGAAATGGGAGAGGTGGCTCCAGAATACAGGCATGACTCTCTGATTGTGGGAT
GATAATATTATGTATAGCAGCATTAGTAATAACCAAATTAGCAGCAGGAGATAGTCAGGTCTACAGCTGTGATTAGGAGAGCT
AAATATGCTCTAAAAATGAAATGAAACGTTATAAAGGTTATTACCCAAAGGATATGCCAACAGCTTCTATGAAGTGTGTTGAA
AAATATCCTCACTTTAGATGTTTTGTTCATTTGGTATAGCACAATCTCTACCAGAGGTGGCAGTAGAGTTGAAGGGATTT
TGCAGGATTGTTATGAATGCCATGGTCAAGTGTGTTACGGTGGGGGTCTAGCAAATCAGTCAAACATTATG
TTAGGACACGCTAGTGACAAGCAGAAATGGAACAAGTTGTGGAGGTGTAGAGTATGCTCAGAAATTGGGTGGAGAAGCAGGAT
TCTACCATATATTGAAACAACCCAAAAGCATCACTATTATCTTGACTCAATTCTCCTACCTCTAGTGTAGTATTGGGCAATGC
TGCTGGCCTAGGCATAATGGGAGAATACAGAGGTACACCAAGGAATCAAGATTATATGATGCTGCAAAGCATATGCTGAACAA
CTCAAAGAAAATGGTGTGATTAACTACAGTGTATTAGATTGACAGCAGAAGAACTAGAGGCTATCAAACATCAGCTTAATCCAA
AAGATAATGATGTAGAGCTTGAGTTAATAAAAAAGTGGGCAATAAGCATCATGGAAAAGTTGCTCTGAATTCCATGGAG
AAGATGCAAACAAACAGAGCCACCAATTCTAGAATCAATAAAAGGCAAATTACATCACCCAAAGATCCAAGAAAAAGATAG
TATCATATCTGTCAACTCAATAGATATAGAAGTAACCAAGAAAGCCCTATAACATCAAATTCAACCCATTATAAACCCAAATAAT
GAGACAGATGATACTGTAGGGACAAGCCAATTCAAGAAAGCCTCTAGTAAGTTCAAAGAAGACCCATTGCCAGTGATA
ATCCTTTCAAAACTATACAAAGAAACCATAGAAACATTGATAATAATGAAGAAGAATCTAGCTATTGATGAAAGAAATAAA
```

Save workflow



Tools

search tools

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FASTQ Quality Control

SAM/BAM

BED

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The Genome Lab has just launched! This corner of the Galaxy is dedicated to genome ...

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Galaxy AUSTRALIA

Outage Notification

Galaxy Australia is planning a full day outage on **Wednesday 25th October** for maintenance. Please plan accordingly and check the usegalaxy.org.au website for any updates.

Job submission will be switched off at midnight and any running jobs will be stopped at 06:00.

We estimate that Galaxy will be unavailable from 06:00 to 18:00 on Wednesday 25th October AEST.

If you have any questions or concerns regarding this outage, please get in contact with us at help@genome.edu.au.

We thank you for your patience and apologise for any inconvenience this may cause.

Galaxy Australia Admins.



Thanks for making the Galaxy Community Conference great!

91 in-person participants
40 virtual attendees
20 countries represented
many koalas hugged!

316 authors
48 talks
49 posters
14 training workshops



Stay in touch: tinyurl.com/biocommunity-news

History + ⌛ ⌂ ⌄

You have 1 histories.

Show Histories Side-by-Side

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JBrowse on data 14 and data 4 - minimal

JBrowse on data 14 and data 4 - minimal

Samtools flagstat on data 14

Samtools sort on data 13

Map with BWA-MEM on data



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The following list contains each tool that was run to create the datasets in your current history. Please select those that you wish to include in the workflow.

Tools which cannot be run interactively and thus cannot be incorporated into a workflow will be shown in gray.

Workflow name

RSV_assembly_workflow

**Tool**

Dataset Collection Creation

Dataset collection created in a way not compatible with workflows

Data Fetch

This tool cannot be used in workflows

Data Fetch

This tool cannot be used in workflows

Data Fetch

This tool cannot be used in workflows

FastQC

Include "FastQC" in workflow

History items created

3 P90006023_RSVA_Illumina.fastq

 Treat as input dataset P90006023_RSVA_Illu

4 rsv.reference.fasta

 Treat as input dataset rsv.reference.fasta

5 rsv.reference.gff3

 Treat as input dataset rsv.reference.gff3

6 rsv.primers.bed

 Treat as input dataset rsv.primers.bed

7 FastQC on collection 3: Webpage

8 FastQC on collection 3: RawData

Historysearch datasets

RSV_assembly

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22: ivar consensus on data 19 Co nsensus

21: ivar variants tabular output o n data 5, data 4, and data 19

20: Samtools flagstat on data 19

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14: Samtools sort on data 13

13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped reads in BAM format)

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Tools

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Name Tags Updated Sharing Bookmarks Run

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Edit Copy Invocations Download Rename Share Export View Delete

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RSV_assembly_workflow

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search tools

Name RSV_assembly_workflow

Version 1: Oct 20th 2023, 14 : ▾

Annotation Genome assembly for Illumina NGS data

These notes will be visible when this workflow is viewed.

License Specify a license for this workflow.

Creator Add a new creator - either a person or an organization.

Tags Add Tags

Apply tags to make it easy to search for and find items with the same tag.

- 75% +

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13: Map with BWA-MEM on data 2, data 1, and data 4 (mapped re ads in BAM format)

Thank you !

clyde.dapat@influenzacentre.org



WHO Collaborating Centre
for Reference and
Research on Influenza
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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

