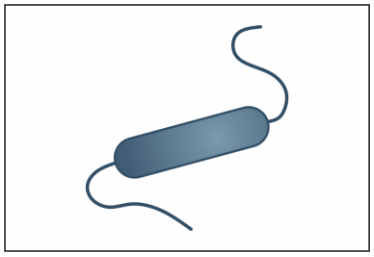
User Guide for CampyBrowse



CONTENT OVERVIEW

Contact information

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1. General information

The CampyBrowse database was set up to allow browsing of deep sequencing datasets (*e.g.* dRNA-seq and Ribo-seq) generated in our lab using *C. jejuni* strain NCTC11168. It is also aimed at collecting annotation information/updates (transcription start sites, sRNAs, small ORFs, ORF modifications) based on these datasets. CampyBrowse uses the open-source platform JBrowse (Buels *et al.*, 2016).

A general JBrowse user guide is available at: https://jbrowse.org/jb2/docs/user_guide/

a. Reference genome

CampyBrowse uses the reference genome for *C. jejuni* NCTC11168 from NCBI (NC_002163.1; ASM908v1 (Parkhill *et al.*, 2000)).

b. Annotation files

i. **Genome annotation:** The genome annotation was retrieved from NCBI (Refseq: GCF_000009085.1; 2014-03-20) (Gundogdu *et al.*, 2007). To this, we have added 5'UTRs and sRNAs based on dRNA-seq (Dugar *et al.*, 2013). Download options for this custom annotation can be found under Section 6 (Accession numbers).

ii. **Novel CJsORFs:** A separate annotation file for novel CJsORFs (small open reading frames, ≤70 amino acids) based on our translatomics (Ribo-seq, TIS (translation initiation site), and TTS (translation termination site)) data (Froschauer, Svensson, *et al.* submitted).

iii. **Additional ORF re-annotations:** re-annotations of the *C. jejuni* NCTC11168 genome (*e.g.*, additions, start codon changes), also based on our translatomics data.

iv. **RBS motif:** A track with locations of a minimal *C. jejuni* RBS (ribosome binding site) motif "AAGG" based on dRNA-seq and translatomics data.

2. Layout of JBrowse and display options

Here, we provide a short intro to JBrowse and our CampyBrowse. For detailed information on JBrowse functions, follow the link to the User Guide:
https://jbrowse.org/jb2/docs/user_guide/

In general, the JBrowse portal is divided into three areas (**Fig. 1A**). Search, orientation, and zoom options for the reference genome are available in a section at the top. Available files/tracks/multitracks are listed in the panel along the right side. These files can be loaded into the third main area (centre) by checking the adjacent box. In this main area, coordinate or expression information for selected tracks is displayed.

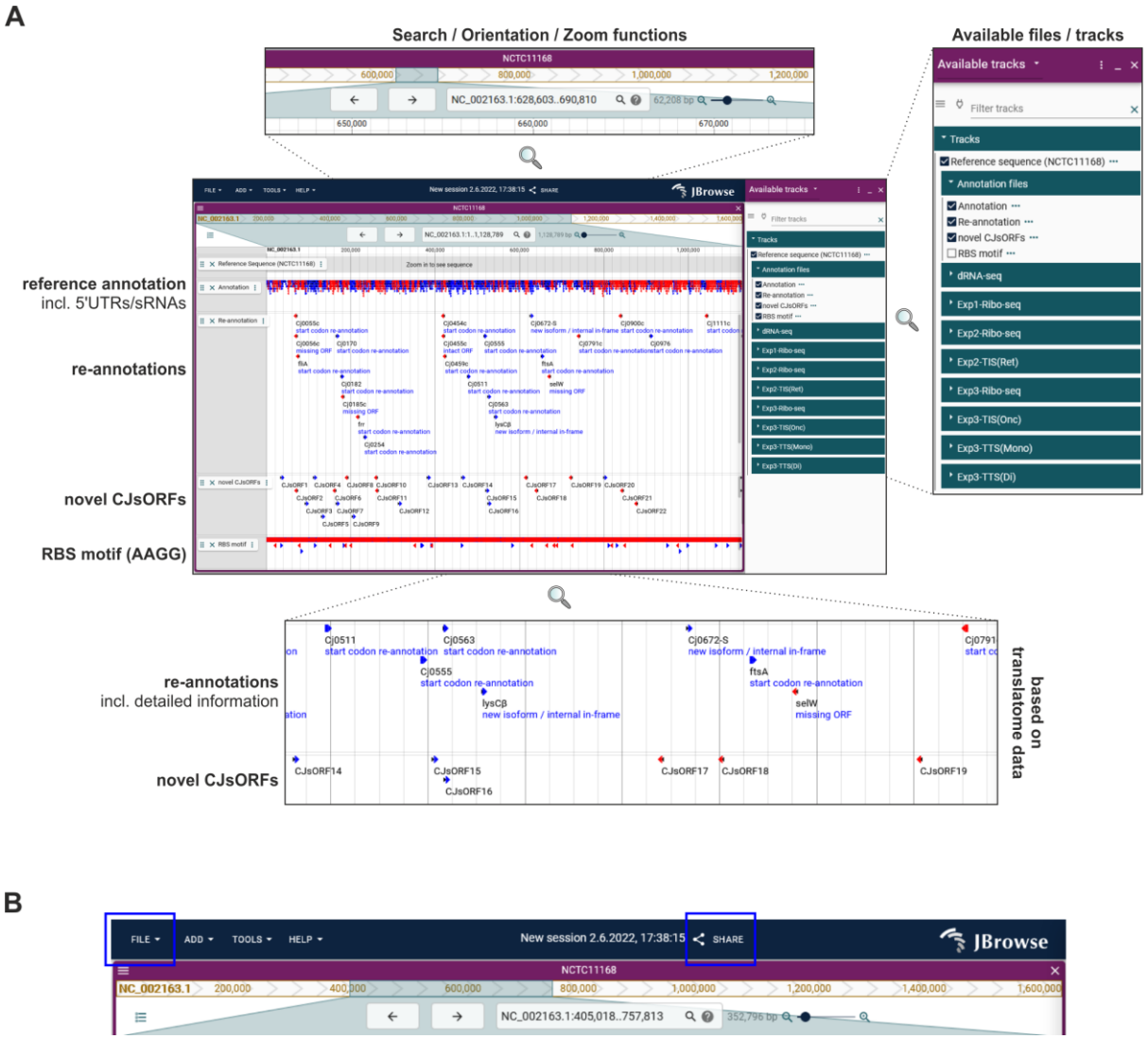

Figure 1. Overview of basic functions and areas of the CampyBrowse portal. (A) Main areas for navigating through the browser, track selection and updated genome annotation based on translatome data are highlighted. **(B)** "File" dropdown menu allows *e.g.,* export of sessions or important additional tracks/libraries. The "SHARE" button creates a link of the current JBrowse session to share it with other people. Both are indicated by the blue rectangle.

Additional tracks (*e.g.*, own RNA-seq data) can also be added from local storage via the dropdown menu “FILE” (top left) for personal/local viewing only (**Fig. 1B**). Currently loaded tracks can be saved as a reloadable “session” for quick access. Sessions can also be shared via a generated link (“SHARE” button) (**Fig. 1B**).

An important consideration when comparing libraries is that their y-axis scale needs to be the same. However, JBrowse automatically adapts the y-axis scale depending on the coverage in the displayed region. This can potentially lead to different scales for, *e.g.*, RIBO and RNA libraries (**Fig. 2**). The y-axis height can be manually modified for each track by clicking the three dots to reveal the option “Set min/max score”. To avoid this issue, we organized the tracks that need to be compared in so-called multitracks, where JBrowse scales all libraries included in one multitrack together. However, for uploaded libraries from local storage, please consider the above-mentioned requirement to use the same scaling for libraries that are being compared.

The three horizontal white lines (see red box in **Fig. 2**) opens another menu where features like searching for a nucleotide sequence or exporting a screenshot of your current JBrowse view can be found.

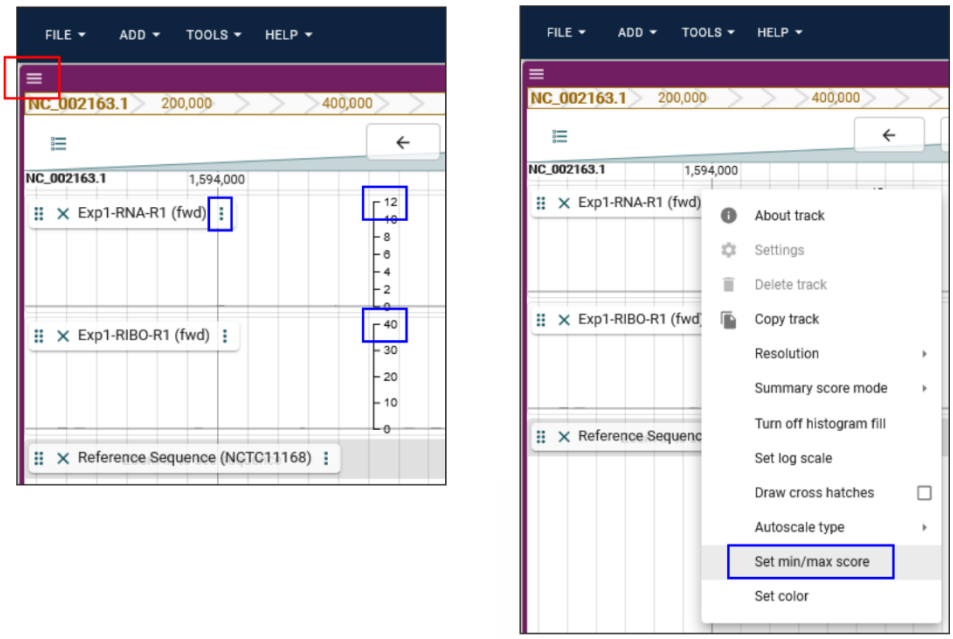

Figure 2: **Scaling of the y-axis.** JBrowse automatically sets the y-axis scale for a certain region independently for each track. To compare, *e.g.,* RNA vs. RIBO coverage the y-axis scale needs to be the same. The three vertical dots after each track name allow opening of different track settings, *e.g.,* “Set min/max score” for y-axis scaling.

3. Available datasets

The currently available libraries in the CampyBrowse track selector are described briefly below (also summarized in **Table 1**). The libraries are grouped in the track selector based on the Experiment they were generated in. In addition, multiple mapping approaches are provided for some libraries (*e.g.*, full-read, single-nucleotide). Please also see the corresponding original publication for additional information, including experimental details.

For quick use, we have provided 3 pre-set JBrowse environments, which display selected libraries from the different experiments. The most important/relevant tracks are already loaded for inspection of dRNA-seq, Ribo-seq, TIS profiling, and/or TTS profiling (**Section 4**). However, modifications (*e.g.,* additional tracks) are also possible, as outlined above (**Fig. 1A & Fig. 1B**).

For most Ribo-seq (and dRNA-seq) experiments, expression in at least two tracks of the same experiment is usually required (*e.g.,* dRNA-seq: plus vs. minus TEX; Ribo-seq: Ribo vs. RNA; TIS profiling: TIS vs. Ribo). As noted above, we grouped the tracks that should be compared in so-called multitracks. This guarantees that the y-axis scale is set to the same height for relevant libraries, and also helps to visually distinguish between the different sets of tracks.

Transcriptome experiments

i. dRNA-seq

(Dugar *et al.*, 2013)

The dRNA-seq dataset was used to identify transcription start sites (TSS) under standard growth conditions: log phase (OD_{600} ~0.3 - 0.4, grown in rich media (Brucella broth), microaerobic atmosphere (see (Dugar *et al.*, 2013) for details). Coverage files include +TEX (terminator exonuclease) treated and -TEX (mock/untreated) libraries for two independent biological replicates (R1 and R2). Coverage tracks are split into forward (fwd) and reverse (rev) strands and show coverage normalized to the minimum number of mapped reads calculated over all libraries. For background on interpreting dRNA-seq data, please see (Sharma and Vogel, 2014).

Translatome experiments

(Froschauer, Svensson *et al.* submitted)

Unless otherwise stated, all translatome experiments were performed with *C. jejuni* NCTC11168 wild-type under standard growth conditions (log phase, rich media (Brucella broth), microaerobic atmosphere) (see Froschauer, Svensson, *et al*. submitted for details). Coverage tracks are split into forward (fwd) and reverse (rev) strands and display relative read coverage normalized to one million reads in the library. For a summary of the details described below, see also **Table 1**.

i. Exp1 – Ribo-seq

Classical Ribo-seq using chloramphenicol (Cm) captured translating ribosomes to identify translated ORFs (three independent biological replicates; R1-R3). A parallel fragmented total RNA library is used to compare transcription vs. translation of a gene of interest. Tracks display coverage based on full-read mapping for each library.

ii. Exp2 – Ribo-seq and translation initiation site (TIS) profiling

In addition to classical Ribo-seq (no drug) and corresponding total RNA-seq data, this dataset includes data from parallel TIS profiling libraries using the antibiotic retapamulin (Ret) for identification of start codons. Ret stalls initiating ribosomes, while elongating ribosomes run off the transcript. For this experiment only, a *C. jejuni* Δ*cmeB* strain (an efflux pump deletion mutant) was used to increase susceptibility to retapamulin. Two sets of paired files are available in the track selector for Exp2:

Exp2 – Ribo-seq includes full-read coverage files for total RNA-seq as well as Ribo-seq libraries (three independent biological replicates R1-R3).

Exp2 – TIS(Ret) includes coverage files for Ribo-seq and TIS(Ret) libraries from three independent biological replicates (R1-R3). Tracks show coverage for 3'-end positions of reads only, and TIS peaks are expected at ~16 nt downstream of start codons (**Fig. 3**). Only reads of length 31+32 nt were used to generate the files, based on metagene analysis, to avoid loss of resolution at TIS peaks due to imprecise trimming by micrococcal nuclease. Note that the Ribo-seq track is derived from the same library as in Exp2-Ribo-seq, but uses single-nt mapping and includes a read-length filter.

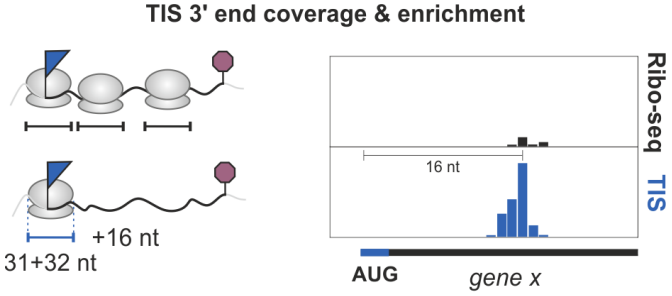

Figure 3. Schematic of TIS-enriched peaks (*e.g.*, Ret - Experiment 2). For optimal coverage files (3' ends of 31+32 nt reads), an enriched peak (TIS/Ribo) is expected at approximately 16 nt downstream of the first nt of the start codon. For Onc parameters (Experiment 3), see **Table 1**.

iii. Exp3 – Ribo-seq, TIS, and TTS (Translation termination site) profiling

This experiment includes five different types of libraries for identification of translated genes, start codons, and stop codons (**Fig. 4A**):

- total RNA-seq,
- Ribo-seq,
- TIS profiling using oncocin [TIS(Onc)],
- two TTS profiling libraries from apidaecin (Api) treatment: monosomes [TTS(Mono)] and disomes [TTS(Di)].

While Api stalls terminating ribosomes at stop codons, it also appears to affect initiating ribosomes in some contexts. Therefore, the TIS(Onc) library also serves as a control to reveal peaks resulting from ribosomes stalled by Api at start codons (**Fig. 4B & Fig. 4C**).

Api-stalled ribosomes at stop codons also cause queuing/collisions upstream. We hypothesized that this might lead to partial resistance to micrococcal nuclease digestion in between and therefore exploited this by also sequencing disome footprints in addition to monosome footprints. Due to stop codon read-through upon Api treatment, peaks can sometimes be located further downstream than expected (**Fig. 4C**).

Four sets of paired files are available in the track selector for Exp3:

Exp3 – Ribo-seq includes full-read coverage files from total RNA-seq and Ribo-seq libraries (three independent biological replicates; R1-R3).

Exp3 – TIS(Onc) includes coverage files from Ribo-seq and TIS(Onc) libraries (three independent biological replicates; R1-R3). Tracks show coverage for read 3'-end positions only. As only 3'-end coverage of reads is displayed, TIS peaks are expected at ~17 nt downstream of start codons (**Fig. 4A & Fig. 4B**). Coverage was calculated using only, *e.g.*, 32 nt reads (R1), based on metagene analysis, to increase resolution of TIS peaks. Note that the Ribo-seq track is derived from the same library as in Exp3 – Ribo-seq but uses single-nucleotide mapping and a read-length filter.

E3 – TTS(Mono) includes coverage files from Ribo-seq, TIS(Onc), and TTS(Mono) libraries (three independent biological replicates; R1-R3). Tracks show coverage for 3'-end positions of reads only, and TTS peaks are expected at ~13 nt downstream of stop codons (**Fig. 4A & Fig. 4B**). Coverage files were calculated using only, *e.g.*, 29 nt reads (R1), based on metagene analysis, to increase resolution. Note that the Ribo-seq and TIS(Onc) tracks are derived from the same library as in Exp3 – Ribo-seq and Exp3 – TIS(Onc), but use single-nt mapping and include a read-length filter.

Exp3 – TTS(Di) displays coverage files from TTS(Di) libraries (three independent biological replicates; R1-R3). Tracks show coverage for read 5'- or 3'-end positions only. As only 5'- or 3'-end coverage is displayed, TTS peaks are expected at, *e.g.*, ~45/13 nt (R1) up-/downstream of stop codons, respectively (**Fig. 4A & Fig. 4B**). Coverage was calculated using only 58 nt reads, based on metagene analysis, to increase resolution.

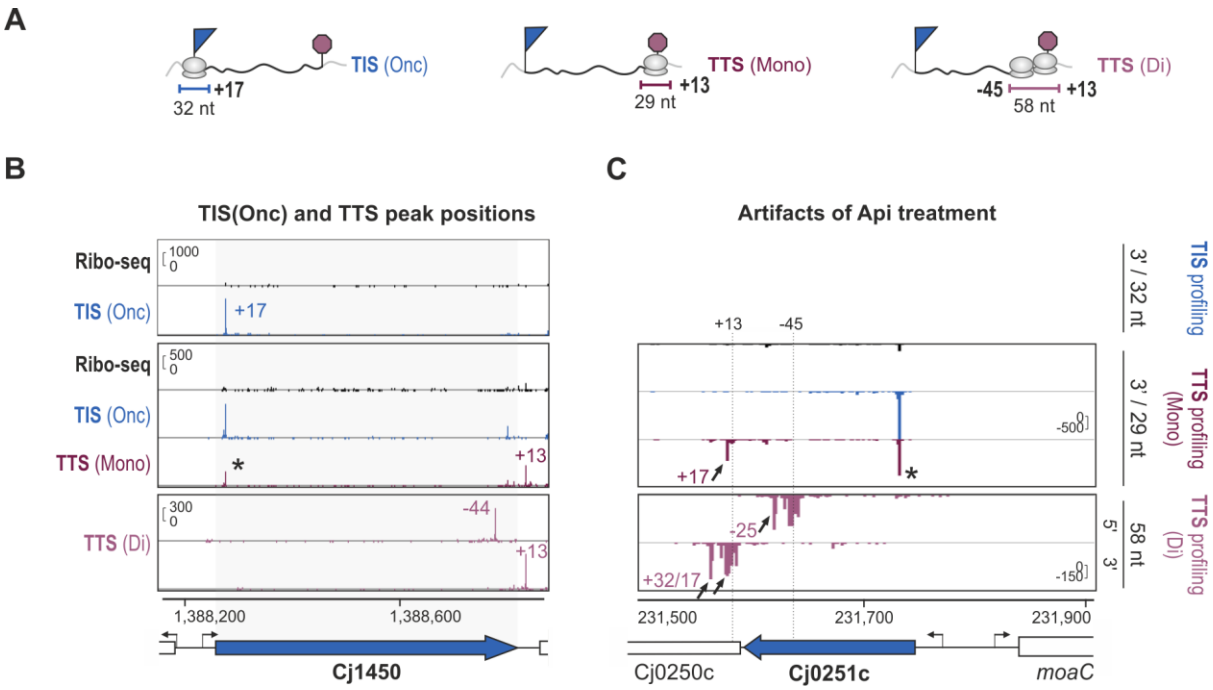

Figure 4. Schematic of TTS (and TIS) signals expected for Experiment 3. (A) Overview of parallel TIS and TTS profiling. Optimal coverage files and peak locations are shown. For a full list of parameters, see **Table 1**. **(B)** Example screenshot of TIS/TTS offsets for 5' or 3' end coverage. **(C)** Two artifacts of Api treatment that should be considered during manual inspection: stop codon read-through (arrow) and enrichment of ribosomes at start codons (*).

Table 1: Summary of available libraries and mapping approaches in the CampyBrowse track selector.

Sequence features		
Name of feature	**Description**	**Reference**
Reference sequence	Genome reference sequence; *C. jejuni* strain NCTC11168	NCBI: NC_002163.1; ASM908v1 (Parkhill *et al.*, 2000)
Custom Annotation	*C. jejuni* strain NCTC11168; Coordinates of ORFs, sRNAs, and 5'UTRs	NCBI Refseq: GCF_000009085.1, updated with 5'UTR and sRNA annotations (20214-03-20) (Gundogdu *et al.*, 2007; Dugar *et al.*, 2013)
Re-annotation	ORF modifications/additions based on Ribo-seq/TIS/TTS	(Froschauer, Svensson, et al. (doi: https://doi.org/10.1101/2022.11.09.515450))
Novel CJsORFs	New ORFs ≤ 70 aa based on Ribo-seq/TIS/TTS	(Froschauer, Svensson, *et al*. (doi: https://doi.org/10.1101/2022.11.09.515450))
RBS (ribosome binding site) motif	Minimal motif (AAGG) based on genome-wide analysis	(Dugar *et al.*, 2013); (Froschauer, Svensson, *et al.* (doi: https://doi.org/10.1101/2022.11.09.515450))

Coverage files							
	Name of library	**Description**	**Rep**	**normal-ization**	**mapping**	**read length**	**Grouped multitracks**
dRNA-seq							
dRNA-seq	-TEX-R1 (fwd/rev)	dRNA-seq (mock-treated control)	R1	min	full-read	all	dRNA-seq R1 (fwd/rev)
	+TEX-R1 (fwd/rev)	dRNA-seq (TEX-digested)	R1	min	full-read	all	
	-TEX-R2 (fwd/rev)	dRNA-seq (mock-treated control)	R2	min	full-read	all	dRNA-seq R2 (fwd/rev)
	+TEX-R2 (fwd/rev)	dRNA-seq (TEX-digested)	R2	min	full-read	all	
Experiment 1							
Ribo-seq	Exp1-RNA-R1 (fwd/rev)	total RNA-seq	R1	mil	full-read	all	Exp1 Ribo-seq R1 (fwd/rev)
	Exp1-RIBO-R1 (fwd/rev)	Ribo-seq	R1	mil	full-read	all	
	Exp1-RNA-R2 (fwd/rev)	total RNA-seq	R2	mil	full-read	all	Exp1 Ribo-seq R2 (fwd/rev)

	Exp1-RIBO-R2 (fwd/rev)	Ribo-seq	R2	mil	full-read	all	
	Name of library	**Description**	**Rep**	**normal-ization**	**mapping**	**read length**	**Grouped multitracks**
Ribo-seq	Exp1-RNA-R3 (fwd/rev)	total RNA-seq	R3	mil	full-read	all	Exp1 Ribo-seq R3 (fwd/rev)
	Exp1-RIBO-R3 (fwd/rev)	Ribo-seq	R3	mil	full-read	all	
Experiment 2							
Ribo-seq	Exp2-RNA-R1 (fwd/rev)	total RNA-seq	R1	mil	full-read	all	Exp2 Ribo-seq R1 (fwd/rev)
	Exp2-RIBO-R1 (fwd/rev)	Ribo-seq	R1	mil	full-read	all	
	Exp2-RNA-R2 (fwd/rev)	total RNA-seq	R2	mil	full-read	all	Exp2 Ribo-seq R2 (fwd/rev)
	Exp2-RIBO-R2 (fwd/rev)	Ribo-seq	R2	mil	full-read	all	
	Exp2-RNA-R3 (fwd/rev)	total RNA-seq	R3	mil	full-read	all	Exp2 Ribo-seq R3 (fwd/rev)
	Exp2-RIBO-R3 (fwd/rev)	Ribo-seq	R3	mil	full-read	all	
TIS profiling	Exp2-RIBO-R1_3' (fwd/rev)	Ribo-seq	R1	mil	3' end	31+32 nt	Exp2 TIS(Ret) profiling 3' R1 (fwd/rev)
	Exp2-TIS(Ret)-R1_3' (fwd/rev)	TIS (Ret)	R1	mil	3' end	31+32 nt	
	Exp2-RIBO-R2_3' (fwd/rev)	Ribo-seq	R2	mil	3' end	31+32 nt	Exp2 TIS(Ret) profiling 3' R2 (fwd/rev)
	Exp2-TIS(Ret)-R2_3' (fwd/rev)	TIS (Ret)	R2	mil	3' end	31+32 nt	
	Exp2-RIBO-R3_3' (fwd/rev)	Ribo-seq	R3	mil	3' end	31+32 nt	Exp2 TIS(Ret) profiling 3' R3 (fwd/rev)
	Exp2-TIS(Ret)-R3_3' (fwd/rev)	TIS (Ret)	R3	mil	3' end	31+32 nt	
Experiment 3							
Ribo-seq	Exp3-RNA-R1 (fwd/rev)	total RNA-seq	R1	mil	full-read	all	Exp3 Ribo-seq R1 (fwd/rev)
	Exp3-RIBO-R1 (fwd/rev)	Ribo-seq	R1	mil	full-read	all	
	Exp3-RNA-R2 (fwd/rev)	total RNA-seq	R2	mil	full-read	all	Exp3 Ribo-seq R2 (fwd/rev)

	Exp3-RIBO-R2 (fwd/rev)	Ribo-seq	R2	mil	full-read	all	
	Name of library	**Description**	**Rep**	**normal-ization**	**mapping**	**read length**	**Grouped multitracks**
Ribo-seq	Exp3-RNA-R3 (fwd/rev)	total RNA-seq	R3	mil	full-read	all	Exp3 Ribo-seq R3 (fwd/rev)
	Exp3-RIBO-R3 (fwd/rev)	Ribo-seq	R3	mil	full-read	all	
TIS(Onc) profiling	Exp3-RIBO-R1_3' (fwd/rev)	Ribo-seq	R1	mil	3' end	32 nt	Exp3 TIS(Onc) profiling 3' R1 (fwd/rev)
	Exp3-TIS(Onc)-R1_3' (fwd/rev)	TIS (Onc)	R1	mil	3' end	32 nt	
	Exp3-RIBO-R2_3' (fwd/rev)	Ribo-seq	R2	mil	3' end	32+33 nt	Exp3 TIS(Onc) profiling 3' R2 (fwd/rev)
	Exp3-TIS(Onc)-R2_3' (fwd/rev)	TIS (Onc)	R2	mil	3' end	32+33 nt	
	Exp3-RIBO-R3_3' (fwd/rev)	Ribo-seq	R3	mil	3'end	32+33 nt	Exp3 TIS(Onc) profiling 3' R3 (fwd/rev)
	Exp3-TIS(Onc)-R3_3' (fwd/rev)	TIS (Onc)	R3	mil	3' end	32+33 nt	
TTS(Mono) profiling	Exp3-RIBO-R1_3'_29nt (fwd/rev)	Ribo-seq	R1	mil	3' end	29 nt	Exp3 TTS(Mono) profiling 3' R1 (fwd/rev)
	Exp3-TIS(Onc)-R1_3'_29nt (fwd/rev)	TIS (Onc)	R1	mil	3' end	29 nt	
	Exp3-TTS(Mono)-R1_3'_29nt (fwd/rev)	TTS (Monosome)	R1	mil	3' end	29 nt	
	Exp3-RIBO-R2_3'_31+32nt (fwd/rev)	Ribo-seq	R2	mil	3' end	31+32 nt	Exp3 TTS(Mono) profiling 3' R2 (fwd/rev)
	Exp3-TIS(Onc)-R2_3'_31+32nt (fwd/rev)	TIS (Onc)	R2	mil	3' end	31+32 nt	
	Exp3-TTS(Mono)-R2_3'_31+32nt (fwd/rev)	TTS (Monosome)	R2	mil	3' end	31+32 nt	
	Exp3-RIBO-R3_3'_30-32nt (fwd/rev)	Ribo-seq	R3	mil	3' end	30-32 nt	Exp3 TTS(Mono) profiling-3' R3 (fwd/rev)
	Exp3-TIS(Onc)-R3_3'_30-32nt (fwd/rev)	TIS (Onc)	R3	mil	3' end	30-32 nt	

	Exp3-TTS(Mono)-R3_3'_30-32nt (fwd/rev)	TTS (Monosome)	R3	mil	3' end	30-32 nt	
	Name of library	**Description**	**Rep**	**normal-ization**	**mapping**	**read length**	**Grouped multitracks**
TTS(Di) profiling	Exp3-TTS(Di)-R1_5' (fwd/rev)	TTS (Disome)	R1	mil	5' end	58 nt	Exp3 TTS(Di) profiling R1 (fwd/rev)
	Exp3-TTS(Di)-R1_3' (fwd/rev)	TTS (Disome)	R1	mil	3' end	58 nt	
	Exp3-TTS(Di)-R2_5' (fwd/rev)	TTS (Disome)	R2	mil	5' end	57+58 nt	Exp3 TTS(Di) profiling R2 (fwd/rev)
	Exp3-TTS(Di)-R2_3' (fwd/rev)	TTS (Disome)	R2	mil	3' end	59+60 nt	
	Exp3-TTS(Di)-R3_5' (fwd/rev)	TTS (Disome)	R3	mil	5' end	58+59 nt	Exp3 TTS(Di) profiling R3 (fwd/rev)
	Exp3-TTS(Di)-R3_3' (fwd/rev)	TTS (Disome)	R3	mil	3' end	58+59 nt	

4. Summary of provided pre-set JBrowse environments

JBrowse pre-set 1: dRNA-seq & Ribo-seq

- **Annotation:** ORFs (NCBI), sRNAs, 5'UTRs
- **Annotation updates** (Novel CJsORFs and Re-annotations)
- **dRNA-seq** (+/-TEX), R1
- **Ribo-seq + RNA-seq coverage:** Exp1, full-read coverage, R1

JBrowse pre-set 2: Ribo-seq & TIS profiling:

- **Annotation:** ORFs (NCBI), sRNAs, 5'UTRs
- **Annotation updates** (Novel CJsORFs and Re-annotations)
- **dRNA-seq**: +/-TEX, R1
- Paired **Ribo-seq + RNA-seq:** Exp2 and Exp3, R1
- Paired **Ribo-seq + TIS(Ret)** libraries: Exp2, 3' ends of 31+32 nt reads only, R1
- Paired **Ribo-seq + TIS(Onc)** libraries: Exp3, 3' ends of 32 nt reads only, R1

JBrowse pre-set 3: Ribo-seq, TIS profiling, & TTS profiling:

- **Annotation:** ORFs (NCBI), sRNAs, 5'UTRs
- **Annotation updates** (Novel CJsORFs and Re-annotations)
- **dRNA-seq**: +/-TEX, R1
- Paired **Ribo-seq + RNA-seq:** Exp3, full-read coverage, R1
- Paired **Ribo-seq, TIS(Onc), and TTS(Mono):** Exp3, 3' ends of 29 nt reads only, R1
- **TTS(Di):** Exp3, 5' and 3' ends of 58 nt reads only, R1

5. How to cite our datasets

dRNA-seq:

Dugar G, Herbig A, Förstner KU, Heidrich N, Reinhardt R, et al. (2013) High-Resolution Transcriptome Maps Reveal Strain-Specific Regulatory Features of Multiple *Campylobacter jejuni* Isolates. PLOS Genetics 9(5): e1003495. https://doi.org/10.1371/journal.pgen.1003495

Ribo-seq/TIS-profiling/TTS-profiling (Exp1 - Exp3):

Froschauer K*, Svensson SL*, Gelhausen R, Fiore E, Kible P, Klaude A, Kucklick M, Fuchs S, Eggenhofer F, Engelmann S, Backofen R, & Sharma CM. Complementary Ribo-seq approaches map the translatome and provide a small protein census in the foodborne pathogen *Campylobacter jejuni*. *Submitted.* doi: https://doi.org/10.1101/2022.11.09.515450

6. Accession numbers (GEO, https://www.ncbi.nlm.nih.gov/geo/)

Transcriptomics:

dRNA-seq: GSE38883

Translatomics:

Exp1-3 - Ribo-seq/TIS/TTS: GSE208756

Download options for annotation files:

The custom annotation file used for analysis of translatomics datasets can be downloaded from GEO (GSE208756) or from the CampyBrowse main area. There also an updated annotation including all re-annotations based on translatomics is available.

7. References

Buels, R., Yao, E., Diesh, C.M., Hayes, R.D., Munoz-Torres, M., Helt, G., *et al.* (2016) JBrowse: a dynamic web platform for genome visualization and analysis. *Genome Biol* **17**: 66.

Dugar, G., Herbig, A., Förstner, K.U., Heidrich, N., Reinhardt, R., Nieselt, K., and Sharma, C.M. (2013) High-resolution transcriptome maps reveal strain-specific regulatory features of multiple *Campylobacter jejuni* isolates. *PLoS Genet* **9**: e1003495.

Gundogdu, O., Bentley, S.D., Holden, M.T., Parkhill, J., Dorrell, N., and Wren, B.W. (2007) Re-annotation and re-analysis of the *Campylobacter jejuni* NCTC11168 genome sequence. *BMC Genomics* **8**: 162.

Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., *et al.* (2000) The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**: 665–668.

Sharma, C.M., and Vogel, J. (2014) Differential RNA-seq: the approach behind and the biological insight gained. *Curr Opin Microbiol* **19**: 97–105.