



sRNA Detection Results

Summary:

This tutorial explains how to submit the output of small RNA (sRNA) deep sequencing experiments (sRNA-seq) into the [sRNA Detection module](#) of Oasis. sRNA detection is the first analysis module of Oasis, and it examines sample qualities, as well as quantifies known and novel sRNAs for each submitted sample. This module supplies valuable information on the quality of samples, and returns count files that can be submitted to Oasis' [DE Analysis module](#) (differential sRNA expression analysis) or the [Classification module](#) (biomarker detection). This tutorial shows how to interpret the results of the sRNA Detection module, using example data from a [psoriasis demo dataset](#) (Joyce et al., 2011).

NOTE: We would like to stress the importance of thoroughly assessing the quality of your sRNA-seq samples, since keeping low-quality samples in downstream analyses ([DE Analysis](#) and [Classification](#)) will generate poor results.

Part 0: Accessing Analysis Results

We start by giving you a brief introduction on how to open the sRNA Detection output in your local computer.

1. Once the sRNA Detection module finishes your analysis, you will receive an e-mail notification with a link to download your results as a .zip file. Click on the link to save the file to your local hard-drive.
2. Decompress the zip file to obtain a directory with all your results.
3. Next, you can open the decompressed folder and click on the web-report file 'summary.html' (Fig. 1). This will open the main sRNA Detection results page in your default browser, allowing you to interactively examine your data (Fig. 2). More information regarding the directory contents can be found in **Part 3**.


 data	Date modified: 26-Jan-16 12:05
 summary	Date modified: 26-Jan-16 12:04
 std.err Type: ERR File	Date modified: 25-Jan-16 11:05 Size: 83.7 KB
 std.out Type: OUT File	Date modified: 25-Jan-16 11:05 Size: 39.3 KB
 summary	Date modified: 25-Jan-16 11:05 Size: 15.3 KB

Fig. 1 Structure of the results folder including the 'summary.html' file.



Fig. 2: Browser view of the 'summary.html' file

Part 1: Overall Quality Control (QC)

This part will guide you how to assess the quality of your data from the sRNA Detection analysis. Various plots and metrics in this output will provide you with insight into the overall technical and biological quality of your samples.

The first thing you will see when opening the page is the **Summary Statistics** table, containing information on the total number of reads, percentage of trimmed reads (adapter trimming), and percentage of uniquely aligned reads per sample (Fig. 3). In general, low percentages of trimmed and/or uniquely mapped reads indicate problematic samples. If there are only few samples with such low quality indicators, they can be treated as outliers and therefore be removed from downstream analyses. Nevertheless, when many/all of your samples show low percentages of trimmed or mapped reads, the adapter or reference genome you selected, respectively, may have been non-corresponding for the input samples. Alternatively, it is possible that your sample was contaminated with foreign genomic material, or it might have a lot of repetitive short sequences.

Sample	Initial number of reads	% trimmed reads	% of uniquely mapped reads
SRR330857	2173425	71.5%	51.35%
SRR330860	8289696	96.9%	5.29%
SRR330858	8341507	84.2%	52.73%
SRR330862	10450125	79.5%	28.57%
SRR330859	11922572	91.0%	33.92%
SRR330863	10890612	89.0%	52.80%

Fig. 3: Summary statistics table of the Oasis sRNA Detection

In addition to the summary statistics table, you will notice a menu below it, showing multiple buttons (Fig. 2, red box). Pressing each of the buttons will show you a

different set of results.

1. **Quality control:** pressing this button will show you various interactive plots of overall sample qualities:

- a. **Principal component analysis (PCA) plot:** the PCA plot gives you an overview of the sample similarities by showing how well samples cluster with each other based on their sRNA expression. In brief, the PCA plot shows two leading principal components (PCs) of the data in the x and y axes, with the percentages of variance “explained” by each of them next to the equivalent PC. The PCA plot can be used to detect outlier samples, which can negatively affect the detection of differentially expressed (DE) sRNAs (increasing the variance). As mentioned before, such outliers should be removed for downstream analyses. As there are no clear outliers in the PCA plot for the psoriasis data (Fig. 4), we can conclude this data has good quality in terms of general variance between the samples. In order to show you what an outlier in the PCA plot looks like, Fig. 5 shows the PCA plot of our [Alzheimer’s demo dataset](#) (Leidinger et al., 2013), where samples SRR837503, SRR837506 and SRR837453 are clear outliers.

The PCA plot in the output also has an interactive feature, where the sample name appears for a particular point when the mouse is moved unto it.

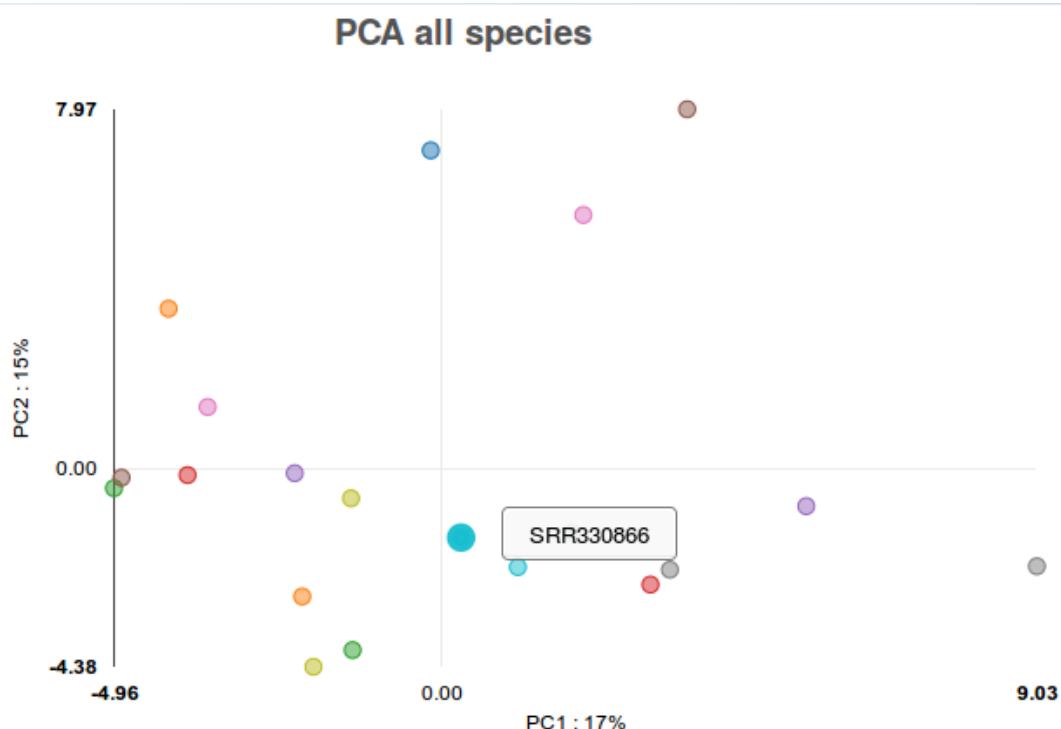


Fig. 4: PCA plot for psoriasis demo data

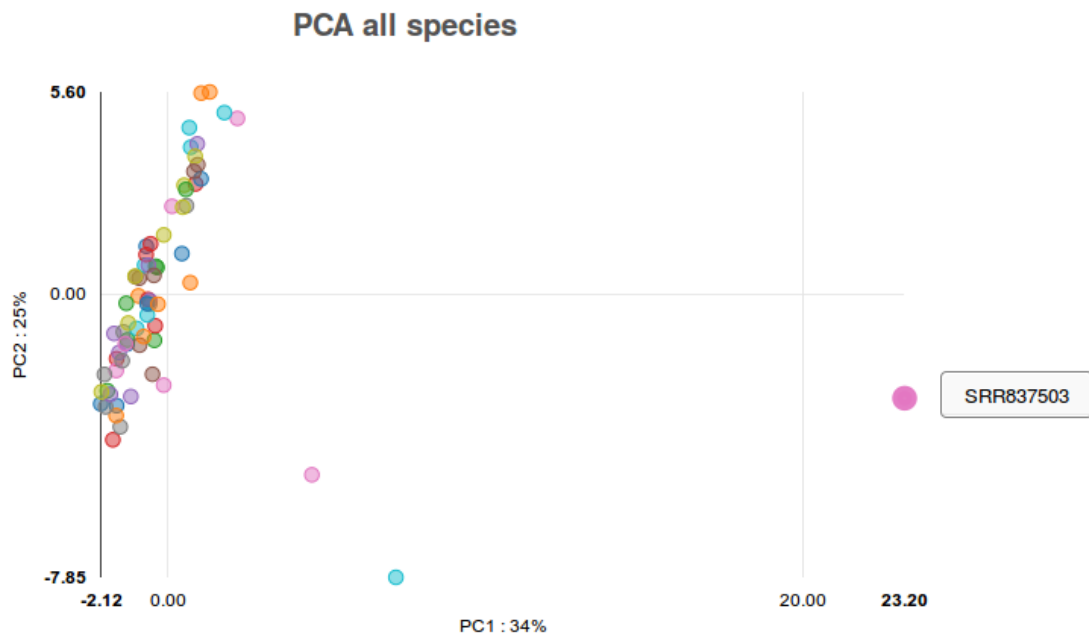


Fig. 5: PCA plot for Alzheimer's demo data

- b. **Trimmed reads:** these interactive bar plots show how many reads are filtered for being too short or too long. By pressing the different buttons at the top, the bars can be shown (solid button) or hidden (hollow button). In addition, when you move the mouse onto a bar, the plot shows the approximate number of reads that are too long or short for a particular sample (using “k” and “M” to indicate thousands and millions of reads, respectively). For the psoriasis data, the minimum read length used was 15 bp and the maximum read length used was 40 bp, and while many reads were filtered out for being shorter than 15 bp, no reads were filtered for being longer than 40 bp (Fig. 6).



Fig. 6: number of reads filtered after trimming for psoriasis data

- c. **Reads per species:** sRNAs are grouped into various species, including micro RNA (miRNA), piwi RNA (piRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA) and ribosomal RNA (rRNA).

As such, the reads may be associated with any of those sRNA species, and the equivalent plot shows the percentage of reads associated with each of the species. Similar to the “trimmed reads” barplot, bars can be shown or hidden, and the information of each bar is shown when the mouse is moved unto it. For the psoriasis data, the samples have a larger proportion of miRNA reads than reads associated with the other species (Fig. 7).

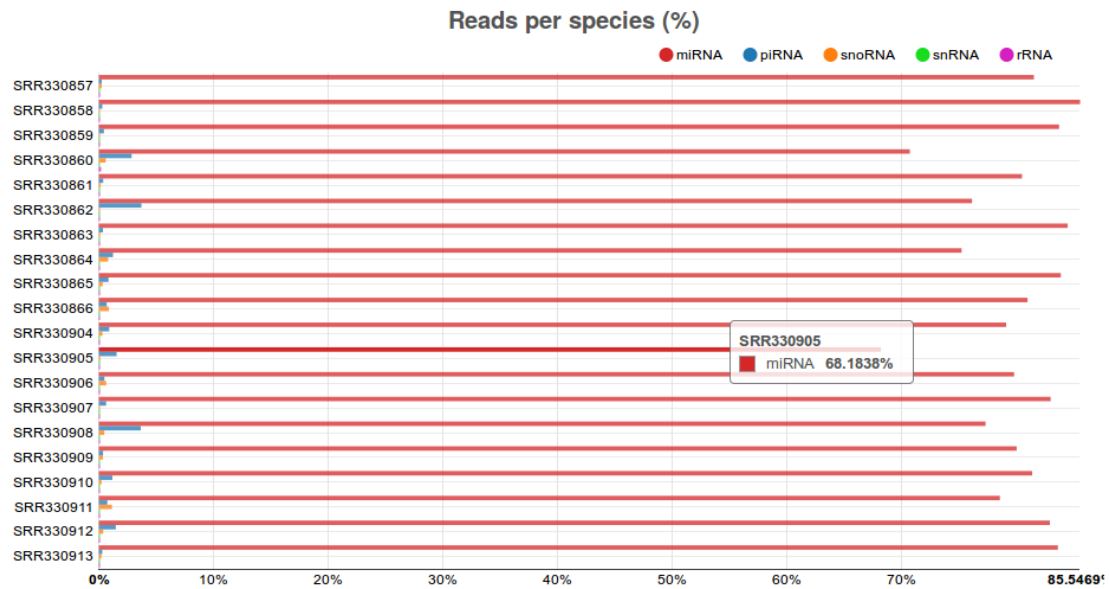


Fig. 7: number of reads per sRNA species in psoriasis data

- d. **Mapped reads:** this barplot shows how many reads are initially present for each sample, how many are kept after trimming and length filtering, and how many are uniquely mapped to the reference genome. Similar to the “trimmed reads” barplot, bars can be shown or hidden, and the information of each bar is shown when the mouse is moved unto it. The psoriasis data shows a relatively small difference between the initial number of reads and the reads kept after filtering, indicating few reads have been discarded after trimming. However, the number of mapped reads is much smaller compared to the initial number of reads. Nevertheless, sRNA-seq samples are known for having considerably small mapping rates, and for such samples, such patterns are reasonable (Fig. 8).

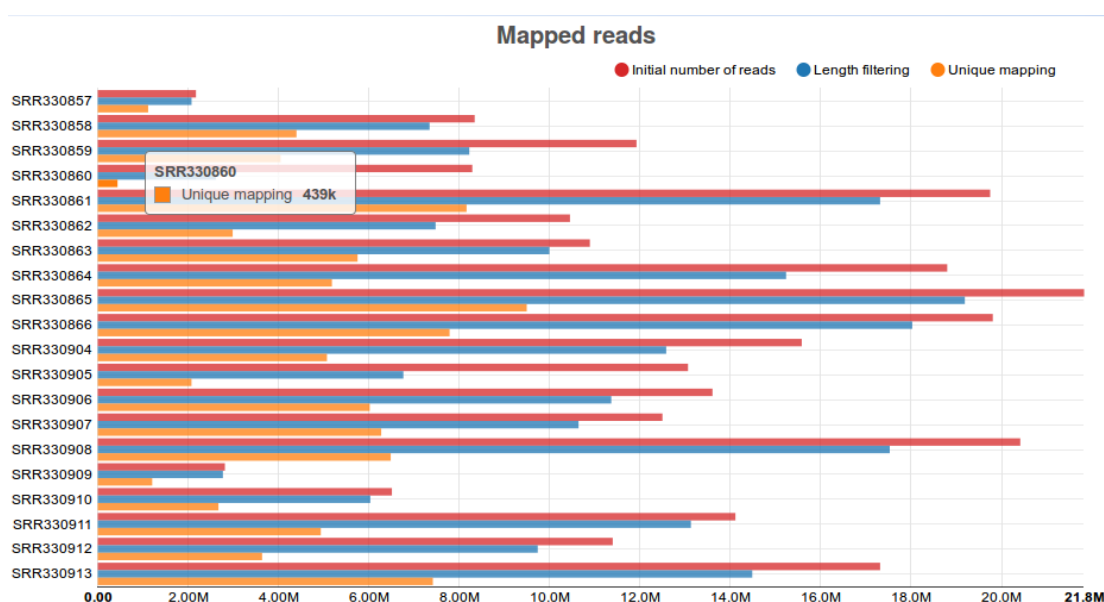


Fig. 8: number of reads as different steps of the sRNA detection analysis on psoriasis data

2. **Show other PCA plots:** pressing this button will show you PCA plots for different sRNA species. While they are not interactive, they work on the same principle as the previous PCA plot, but using expressions of reads belonging to particular sRNA species.
3. **Description:** pressing this button will show you an online version of this tutorial.
4. **Novel miRNA:** pressing this button will show you information on novel miRNAs that have been predicted during the sRNA Detection analysis. This section contains a table with a provisional ID, a score given by the miRDeep2 software, and information regarding the number of reads involved and the consensus sequence. Clicking on the provisional ID opens a PDF file with additional information, including an image of the folded miRNA structure and the sequences contributing to its consensus sequence.

Part 2: Individual Sample Quality Control

The output of Oasis' sRNA Detection module also allows you to look at each sample in more detail. By clicking on a sample name in the summary statistics table (for example, SRR330857 in Fig. 3), you will access detailed information on

the sample in a new browser window (Fig. 8). This part of the tutorial will explain the different plots and tables for the individual samples as you would access by clicking on a sample name.

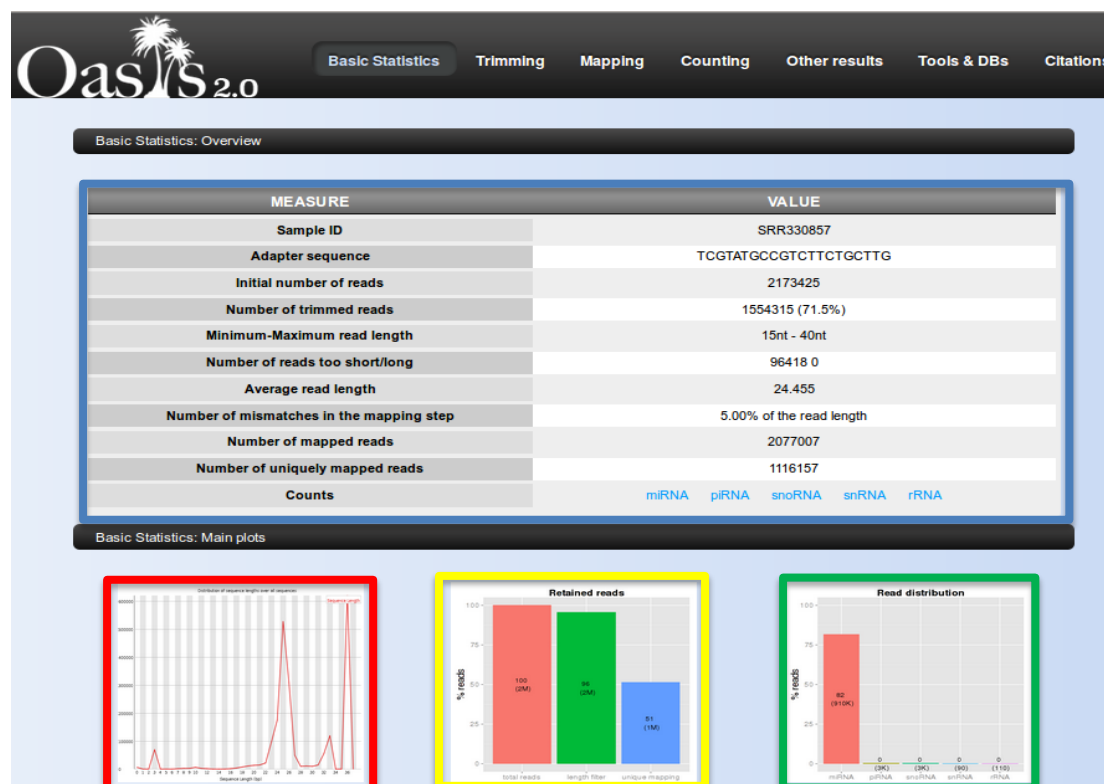


Fig. 9: individual sample overview page

1. **Basic statistics Overview:** the single sample QC output gives the most important information on its entry page, namely a table with expanded statistics including initial number of reads, number of trimmed reads (adapter removal), maximum and minimum read lengths (used for length filtering of reads, as set in the [sRNA Detection analysis](#)), number of reads too short or long (depending on maximum and minimum lengths), average read length (after filtering), number of mismatches allowed for mapping (as set in the [sRNA Detection analysis](#)), number of all mapped reads, and number of uniquely mapped reads (Fig. 8, blue box).
2. **Read-length distribution plot:** this plot shows the size distribution of reads after adapter removal and before length filtering. This plot should contain a peak centered at 19-25 bp that represents miRNAs and other small RNAs like piRNAs. An additional peak at the maximum length is also possible. In the optimal case, you will see a strong peak at 19-25 bp and little to no reads below the minimum length or above the maximum length. In the case of the psoriasis sample SRR330857, no reads have length above 36, and few reads have length below 15, so it is a relatively high quality sample.

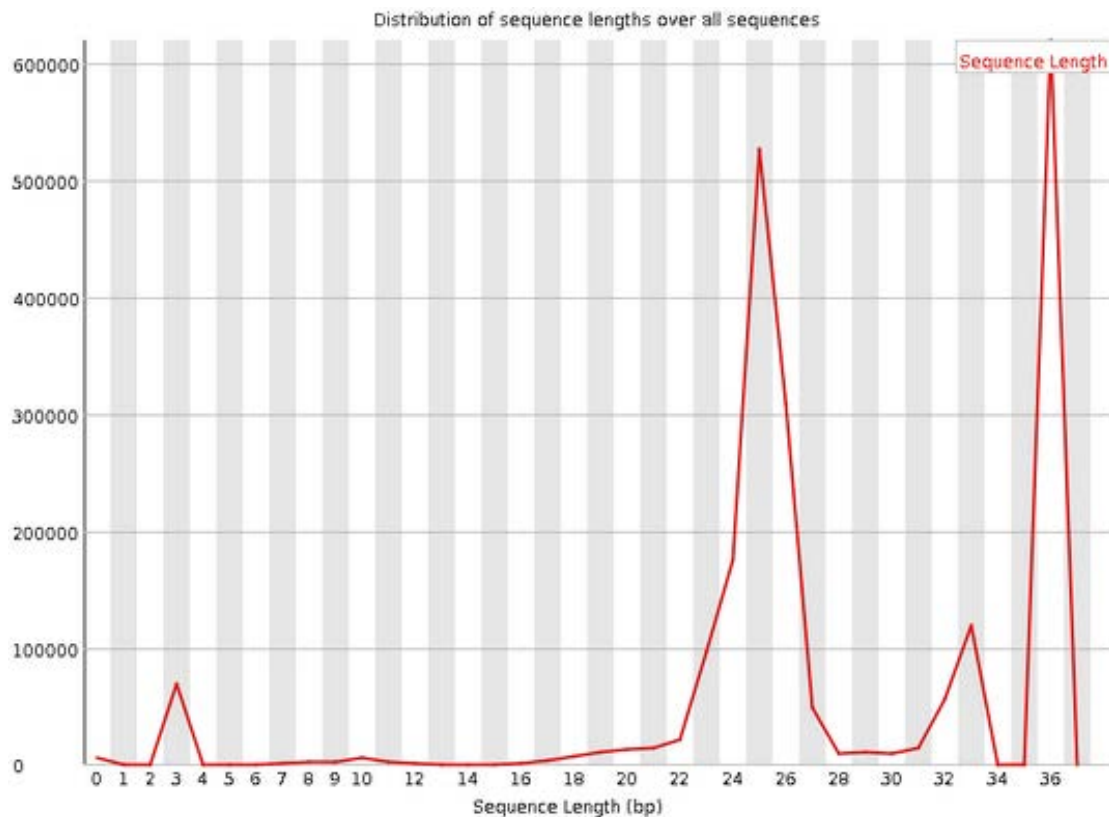


Fig. 10: read-length distribution plot

3. **Filtering barplot:** this plot shows the total numbers and percentages of initial input reads (total reads), reads left after length filtering (length filter) and reads that map uniquely to the genome (unique mapping) (Fig. 10). For each bar, the percentage appears in the middle of the bar and the number shown in brackets is the number of reads in thousands ('k') or in millions ('M'). The "length filter" bar shows reads left after adapter removal (trimming) and filtering for reads that are too short or too long. The "unique mapping" bar shows reads that are uniquely map to the genome. High length filter and unique mapping percentages indicate good sample quality in principle, but those numbers can still vary greatly depending on the organism, tissue, cell-type, and protocols used.

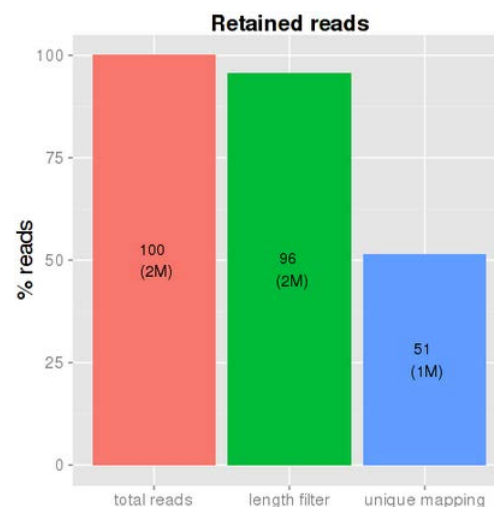


Fig. 11: read filtering barplot

4. **sRNA species barplot:** this plot shows the percentage (and total number) of reads that have been assigned to specific sRNA species (Fig. 12). Since miRNAs are prominently the most prevalent species, results will usually show a high number of miRNAs and few of the other sRNA species. Nevertheless, the exact distribution of reads over the different sRNA species will depend on the organism, tissue, cell-type, or protocols used, and can vary greatly.

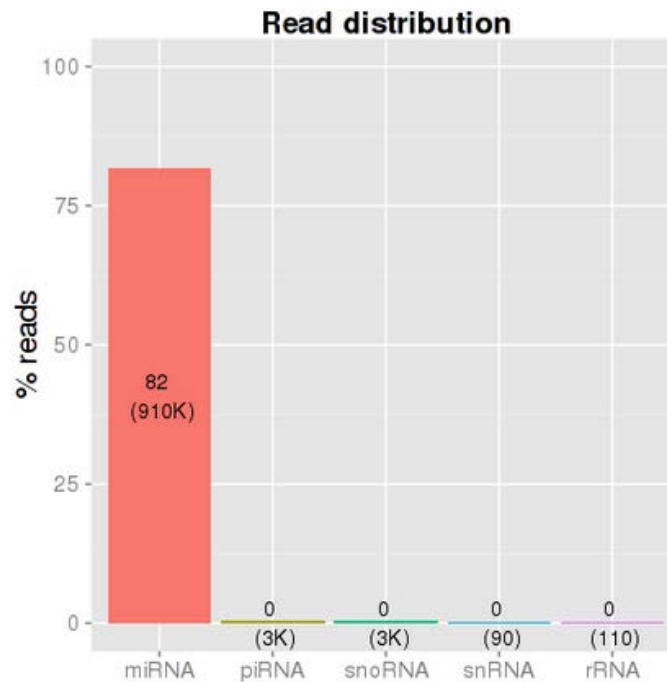


Fig. 12: sRNA classes barplot

Part 3: Count files and subsequent analyses

Within the output directory (Fig. 1), you will notice a directory “data”. Within this directory, you will find a directory “counts”, which contains text files with the sample names and the ending “allspeciesCounts”, indicating those are count files for all known sRNA species, as well as novel, predicted miRNAs, found for each sample (Fig. 13). Each count file contains a particular sRNA ID and the number of reads associated with it, and those files are uploaded to the downstream analyses modules, i.e. the [differential analysis](#) or the [classification](#). As mentioned before, having the quality module separated from the downstream analyses gives you an opportunity to look at the sample qualities, determine if any of them are of bad quality, and leave their corresponding count files out of the downstream analysis.
























 species	26-Jan-16 12:05	File folder	
 SRR491337_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491338_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491339_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491340_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491341_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491342_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491343_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491344_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491345_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491346_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491347_NRC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491348_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491349_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491350_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491351_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491356_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491357_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491358_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491359_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491363_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491364_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB
 SRR491365_ccRCC_allspeciesCounts	25-Jan-16 11:05	Text Document	465 KB

Fig. 13: count directory

Part 4: Other information

If you are interested in the details of the sRNA Detection analysis, please read the original publication (Capece et al., 2015). If you use the original Oasis publication, please cite it, as citations are our currency and allow us drive the development of Oasis further.

In case you want to give us feedback, please send an e-mail to oasis@dzne.de. We are happy to receive your criticism and suggestions as this will make Oasis a better analysis tool.

With this we leave you to your analysis and wish you god-speed from the Oasis team.

References

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- Leidinger, P., Backes, C., Deutscher, S., Schmitt, K., Mueller, S. C., Frese, K., ... Keller, A. (2013). A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biology*, 14(7), R78. <http://doi.org/10.1186/gb-2013-14-7-r78>