Mining Microbial Genomes from Datasets on the Sequence Read Archive

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Sequence Read Archive (SRA)

- The Sequence Read Archive (SRA) is a public repository maintained by National Center for Biotechnology (NCBI) to make raw sequence data from diverse projects available to the research community. SRA currently hosts around 11 PB of sequencing data.
- Reusing or adding these datasets to a research project can reduce sequencing costs of a project, generate enough evidence for statistical and computational methods to be applied, and greatly broaden the scope of studies.
- Accessing and retrieving specific datasets from SRA can be challenging, given its size and heterogeneous content.

Mining the SRA

- Using the NCBI SRA website (https://www.ncbi.nlm.nih.gov/sra) allows users to lookup specific datasets using the accession, project name, location, or other metadata.
- Using filters, the users can also generate a list of metagenomics/metatranscriptomic datasets that contain a specific taxa of interest.
- In the case the user is looking for a novel microbial genome/sequences, the search becomes a little more challenging as this would require,

1. Downloading all datasets (complete or subset) to a local storage space to search the query against.
2. Require lots of compute resources for a long time, to align the query sequence against the large number of query datasets.

SearchSRA gateway

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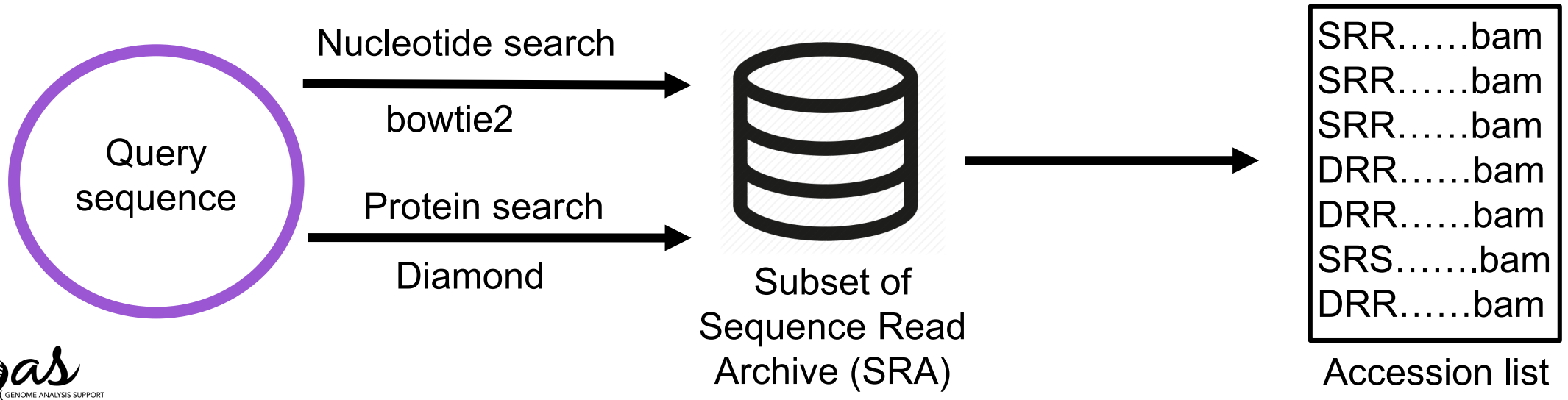
SearchSRA gateway

- Developed by Rob Edwards lab, San Diego State University on XSEDE resources, Jetstream and Wrangler, https://www.searchsra.org/.
- User uploads an input sequence, and waits … while the website spins up multiple virtual machines and searches subsets of the SRA database stored on Wrangler, in parallel. The results are all concatenated and given back to the user as one output.
- For more information- https://edwards.sdsu.edu/research/similarity-searching-the-sra/



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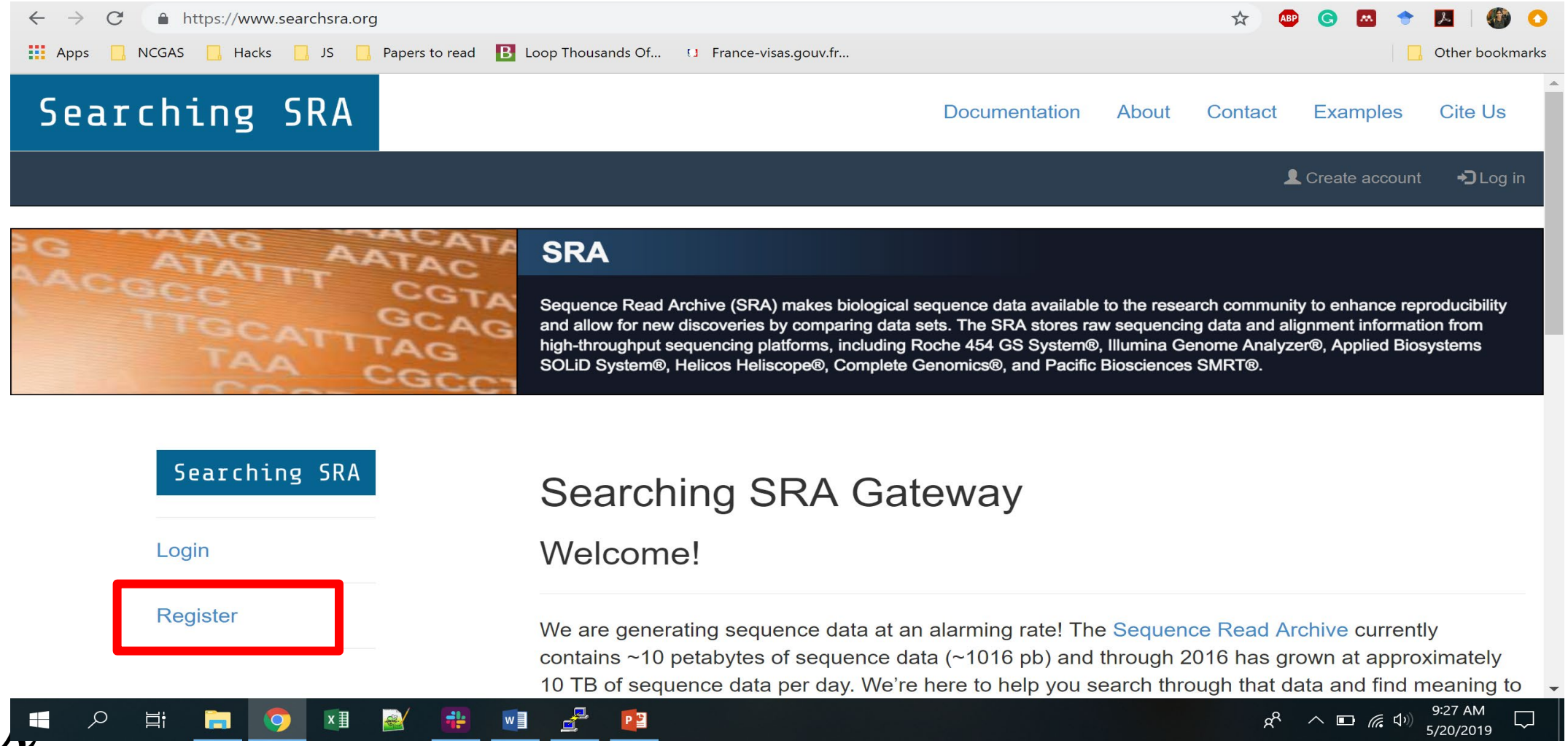
Create an account



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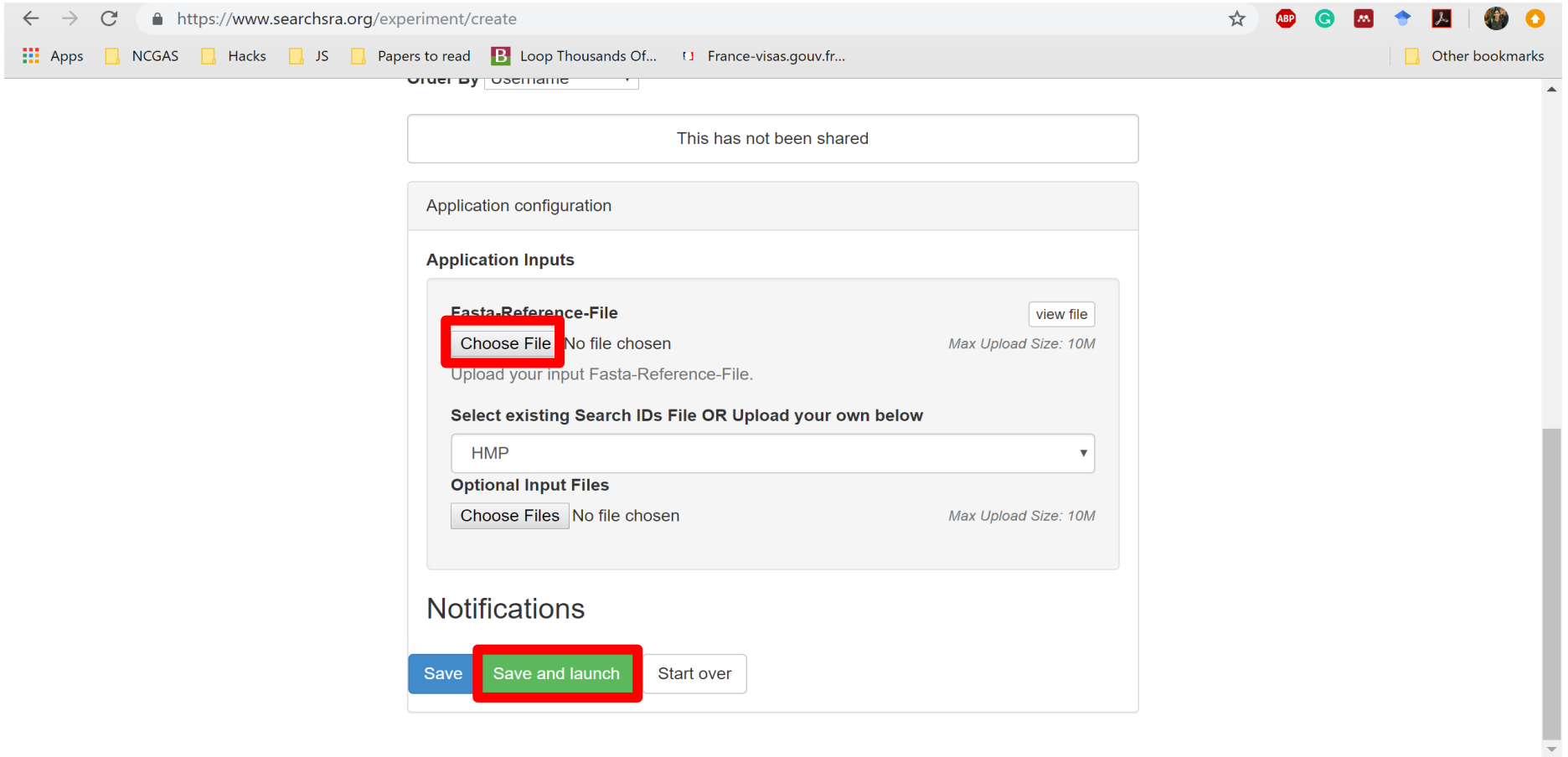
Upload a query sequence



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Results from SearchSRA gateway

Experiment Status	COMPLETED
Job	Name: A1524838725 · ID: 439 · Status: COMPLETE · Creation Time: 02/21/2019, 1:32 PM - GMT-0400 (Eastern Daylight Time)
Notifications To:	
Creation Time	02/21/2019, 1:32 PM - GMT-0400 (Eastern Daylight Time)
Last Modified Time	02/21/2019, 9:11 PM - GMT-0400 (Eastern Daylight Time)
Inputs	Fasta-Reference-File: reference Select existing Search IDs File OR Upload your own below: All-SRA-metagenomes
Outputs	Downloading-Details: report.txt Search-SRA-Standard-Error: search-SRA.stderr Search-SRA-Standard-Out: search-SRA.stdout
Storage Directory	Open
Errors	

Report.txt

results_url = http://149.165.169.158/results/5124a181-38ef-47fe-98be-e2c5a7941b65/results.zip

results_size = 496M
total_compute_hours = **178.64**

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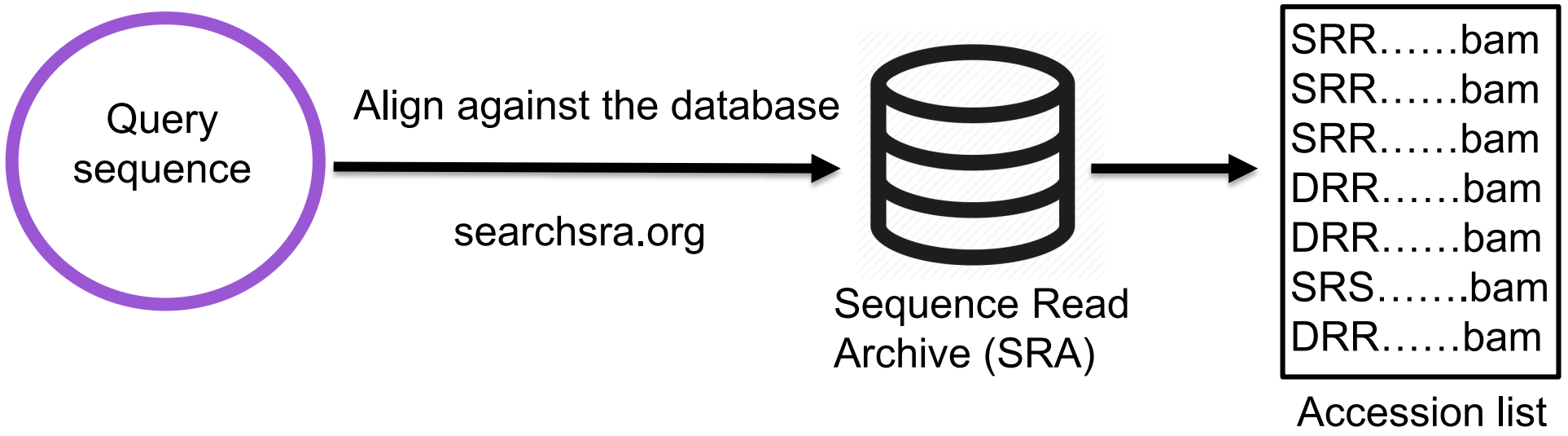
Downloading and filtering the results

Downloading the results

- Download SearchSRA gateway results— a list of bam files that contain information of only the aligned reads from each SRA sample aligned against the query sequence.
- Generates bam files for even those SRA samples that had no hits as well, in this case the bam files are empty

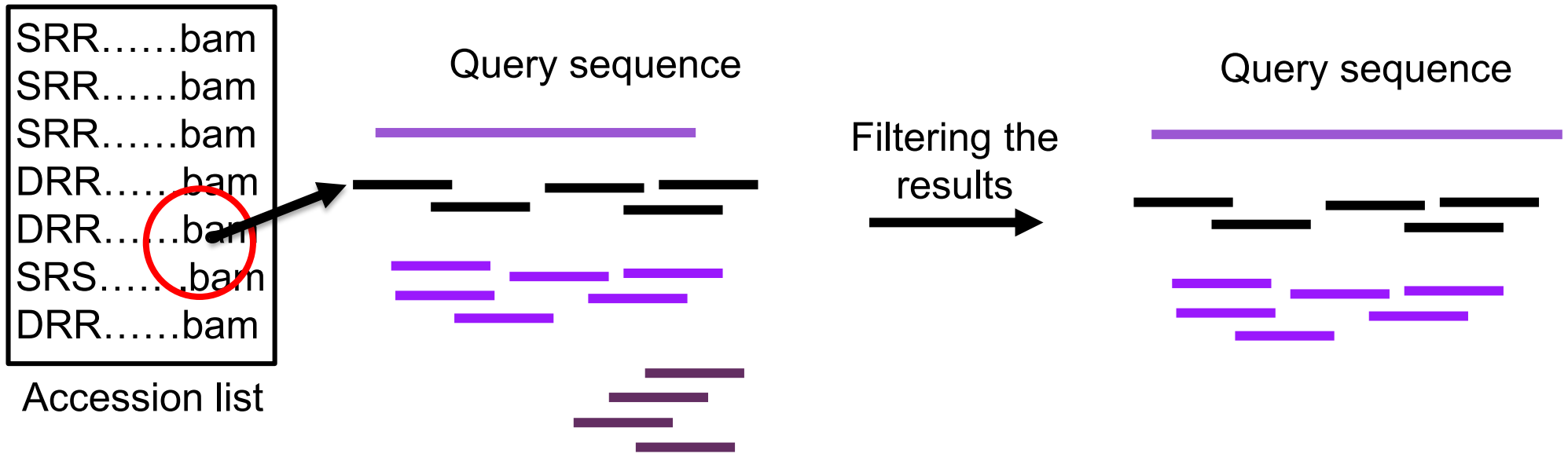


Filtering the results

We run the filtering script that generates a new directory labelled as subset with a list of only those bam files that have,

- alignment length more than 100bp, and
- more than 10 hits mapping to the query sequence per SRA sample

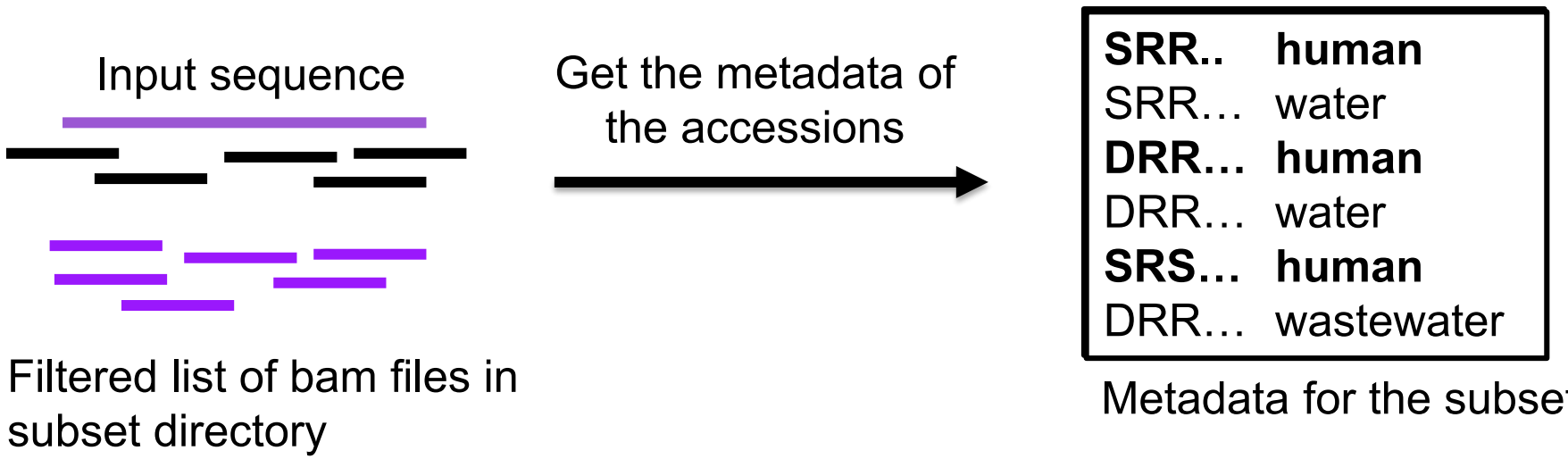
This script will remove both the empty bam files and potential false positives.



Collecting the metadata/run information

Further filtering using metadata

- Using E-utilities toolkit developed by NCBI, we can lookup the metadata associated with the previously filtered datasets to further narrow down the datasets.
- For example, if query sequence was identified in a human gut sample, and in this study we are looking for the prevalence of the genome in other human gut samples – we can narrow down the filtered datasets to only human gut samples.



Visualizationa and further downstream analysis using Anvi'o

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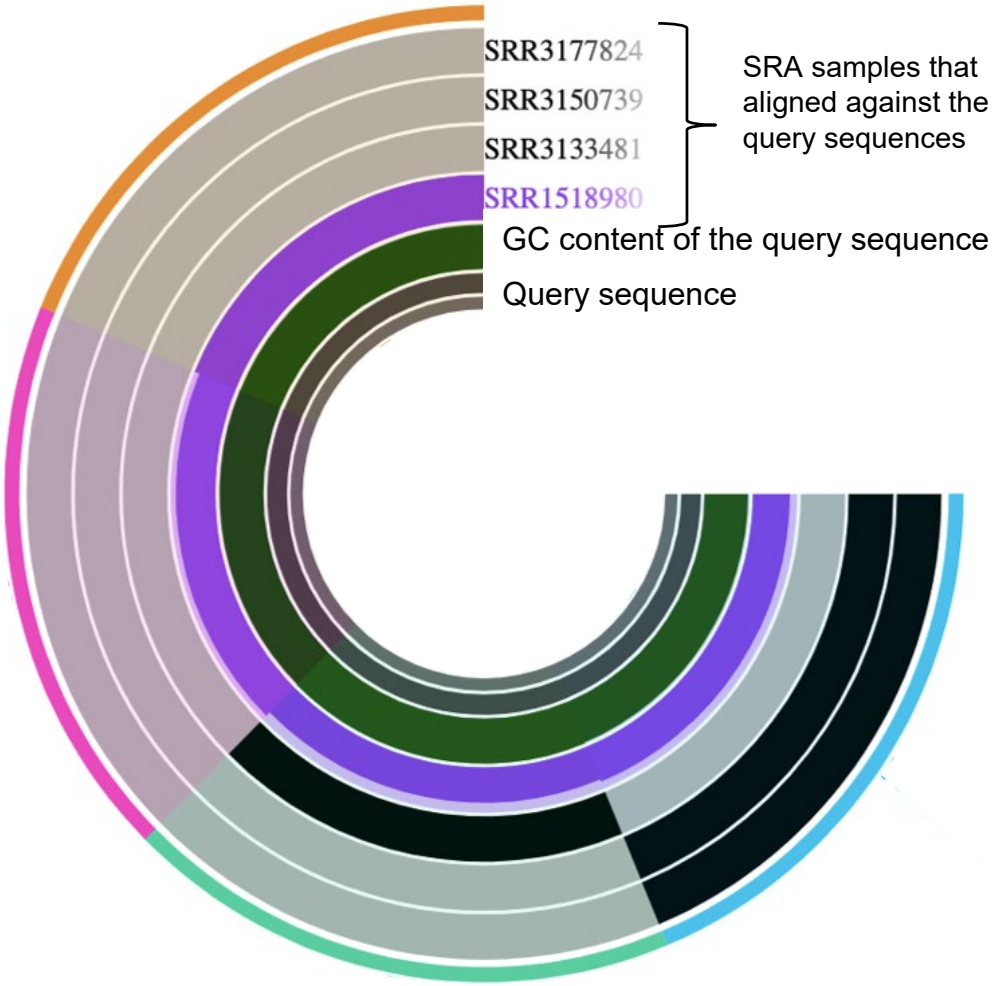
Visualizing the results using Anvi'o

- Anvi'o is an open source tool that allows some really fun visualization and analysis of whole genome metagenomics data.
- In this workflow, we initially use the Anvi'o image for visualization. The figure represents how the reads from filtered SRA samples (four concentric circles labelled as SRR****) align against the query sequence (inner most ring).
- Can look around further using other analysis/workflows available through Anvi'o – documentation available here http://merenlab.org/2016/06/22/anvio-tutorial-v2/



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Overview of the workflow

Query sequence

Align against the database

searchsra.org

Sequence Read Archive (SRA)

SRR……bam
SRR……bam
SRR……bam
DRR……bam
DRR……bam
SRS……bam
DRR……bam

Accession list

Query sequence

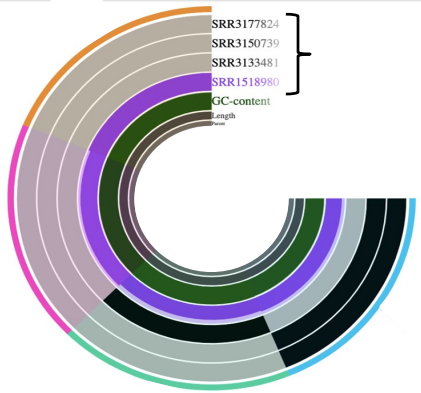
Filtering the results

Query sequence

Get the metadata of the accessions

SRR..	human
SRR…	water
DRR…	**human**
DRR…	water
SRS…	**human**
DRR…	**human**

Metadata for the subset



Visualization

Conclusions

- This workflow was developed and tested using the crAssphage genome that is found in the 90% of the human gut microbiome samples.
- This workflow is transferable to other datasets, and is actively being developed to test and include other features.
- This workflow can identify other environments where the query sequence can be found in, as well as widen the scope of the study by generating enough evidence for statistical and computational methods to be applied.
- The workflow and the scripts are available to the community through GitHub with documentation: https://github.com/NCGAS/CEWiT-REU-Identifying-datasets-in-SRA-using-Jetstream
- The workflow and visualization tools are installed and available as a pre-configured image on the Jetstream cloud computing system (https://use.jetstream-cloud.org/application/images/831). Contact NCGAS (help@ncgas.org) for access to this resource.

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Affiliations:

PERVASIVE TECHNOLOGY INSTITUTE

PITTSBURGH SUPERCOMPUTING CENTER

Collaborator: ABI-1458689 2015, ABI-1759914 2018

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RESEARCH TECHNOLOGIES
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NSF Awards:
DBI-1062432 2011, ABI-1458641 2015, ABI-1759906 2018

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