NCBI tools for microbial genomics

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U.S. National Library of Medicine
National Center for Biotechnology Information

Instructor information

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Outline

- Annotation in the biological analysis workflow
- What is the stand-alone Prokaryotic Genome Annotation Pipeline (PGAP)?
- Start-to-finish annotation with PGAP
- The Read Assembly and Annotation Pipeline Tool (RAPT) = assembly + annotation
- Start-to-finish assembly and annotation with RAPT

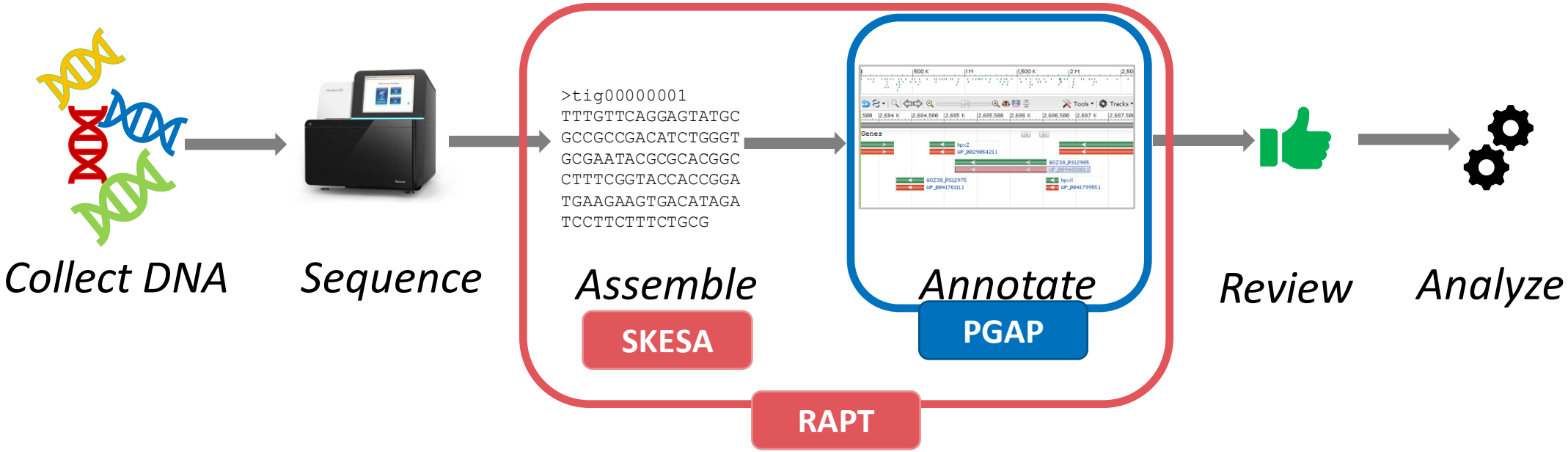
https://github.com/ncbi/pgap

https://github.com/ncbi/rapt

Importance of gene annotation in whole-genome analyses

- Determination of gene location and function
- Precursor of downstream analyses, including
  - Comparative genomics
  - Phylogenetic trees
  - Exploration of biochemical pathways
  - Virulence factor discovery
  - …
- Assessment of sample or assembly quality

PGAP and RAPT in a typical workflow

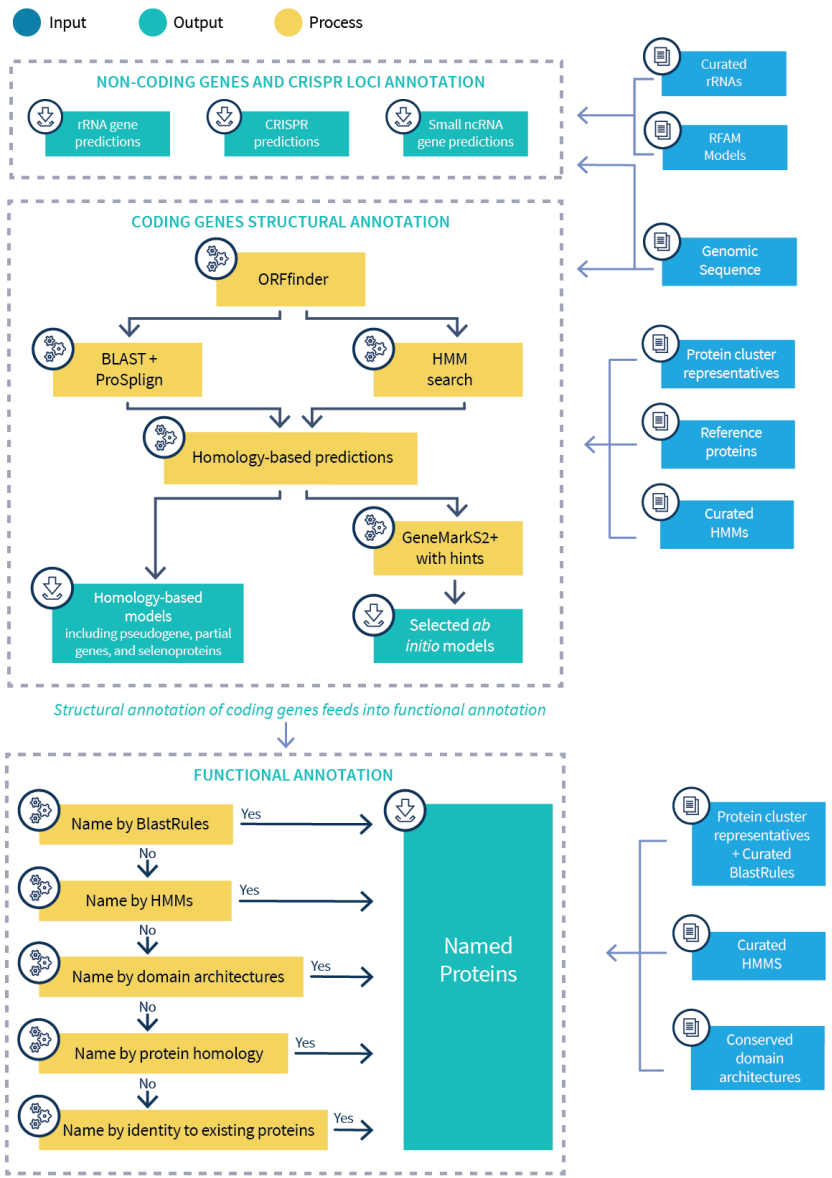


What does PGAP do?

- Annotates the genome with PGAP, the pipeline used to annotate RefSeq genomes

What does RAPT do?

- Assembles a genome from **Illumina** short reads sequenced from a bacterial or archaeal isolate, using SKESA
- Annotates the genome with PGAP, the pipeline used to annotate RefSeq genomes



PGAP in brief

RefSeq annotation pipeline

- Automated pipeline
- Protein-coding gene prediction with:
  - Protein homology
  - Ab initio calls (GeneMarkS2+)
  - Protein profile hidden Markov models
- Non-coding gene prediction with:
  - tRNAscan-SE
  - RFAM
- Functional annotation with:
  - hidden Markov models
  - BlastRules
  - CDD architectures
  - Protein homology

Why run PGAP yourself?

- Proprietary genomes
- Quick characterization of starting material
- Assembly QC
- Large volume
- Comparison to PGAP-annotated RefSeq assemblies

What is stand-alone PGAP?

- Our goal
- PGAP releases
- Systems requirements

How is stand-alone PGAP packaged?

A PGAP release is:

- A Docker image in dockerhub containing:
  - The pipeline in CWL (= the “glue” between the applications)
  - The binaries (pgap-utils)
  - cwltool, the reference runner for CWL
- The reference data on AWS
  - Custom Blast databases
  - HMM models
  - Taxonomy database
  - etc…

What is stand-alone PGAP?

- How does it work?
- PGAP releases
- Systems requirements

Hardware and software requirements

- Linux, Windows, Mac OSX
- Python (version 3.6 or higher)
- Docker, Singularity or podman
- 100 GB disk free space
- 8-core CPU, 32 GB memory recommended

Start-to-finish PGAP annotation

- Download pgap.py
- Download the Docker image and reference data
- Prepare the inputs
  - Input file format
  - FASTA
  - Metadata
- Run the annotation in the container
- Review the results

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pgap.py, the PGAP command line interface

- Choose where to run PGAP
  - Locally
  - On a remote machine
  - In the cloud

- Download the convenience script, pgap.py

```
curl -OL https://github.com/ncbi/pgap/raw/prod/scripts/pgap.py
  % Total    % Received % Xferd  Average Speed   Time    Time     Time  Current
                                 Dload  Upload   Total   Spent    Left  Speed
100   130  100   130    0     0   1203      0 --:--:-- --:--:-- --:--:--  1203
100 24389  100 24389    0     0   187k      0 --:--:-- --:--:-- --:--:--  187k
```

- Make pgap.py executable

```
chmod +x pgap.py
```

Start-to-finish PGAP annotation

- Download the pgap.py
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Download the Docker image and the reference data

```
./pgap.py --taxcheck --update
The latest version of PGAP is 2020-09-24.build4894, you have nothing
installed locally.
Downloading (as needed) Docker image ncbi/pgap:2020-09-24.build4894
2020-09-24.build4894: Pulling from ncbi/pgap
Digest:
sha256:d2d57c18a3cbcf51179b17c34f349752737edbd21edca9a9a078e5d181c42008
Status: Image is up to date for ncbi/pgap: 2020-09-24.build4894
Installing PGAP reference data version 2020-09-24.build4894
Downloading and extracting tarball:
https://s3.amazonaws.com/pgap/input-2020-09-24.build4894.tgz
Downloaded 16485112395 of 16485112395 bytes (100.00%)
```

Reference data:

- input-2020-09-24.build4894.tgz

Example genomes (including *Mycoplasma genitalium* G37)

- test_genomes-2020-09-24.build4894.tgz

Start-to-finish PGAP annotation

- Download pgap.py
- Download the Docker image and reference data
- Prepare the inputs
  - Generic YAML
  - FASTA
  - Metadata
- Run the annotation in the container
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Let's annotate a Klebsiella pneumoniae genome

The generic YAML file: a pointer to two other files:

- Multifasta of the assembly
- Metadata (organism, contact info, author(s))

```
fasta:
    class: File
    location: K_pneu.fasta
submol:
    class: File
    location: K_pneu_meta.yaml
```

K_pneu.yaml

```
>tig00000001
TTTGTTCAGGAGTATGCGCCGCCGACATCTGGGTGCGAATACGCGCACGGCCTTTCGGTACCACCGGATGAAGAAGTGAC
ATAGATCCTTCTTTCTGCGGCTCGCGGGCAAAGTTCTGCGCCACCACCGCCTCGCCCAGCATCACCGGAATGATGGCGTG
GTCGGCTCGGCCAGCGTGAAGCCTGCCGCGGTCATTTTTCGCGGAACAGACGCGCGTTAGCCCACAGGCGATCGCGCAGG
TCTACCAACCATCTCCAGCACTTTGATGGAGGCGGCCACGATAATGGCCGGCGCCAGAGAGTTGGAGAACAGATACGGAC
AGGGCGCTGCGCATCAACCACCTCTTTGCGCGCGGCGGTGTAGCCGCCGAGCGCCCAGCGCAACGTGCCGGTGATGATAT
CGGCGCGCCCCATCACATCGCAGTATTCGTAGAACCGCGGCCGTTTCGCCGACAGAAGCCGACGGCGTGGGAGTCATCGA
```

Start-to-finish PGAP annotation

- Download pgap.py
- Download the Docker image and reference data
- Prepare the inputs
  - Input file format
  - FASTA
  - Metadata
- Run the annotation in the container
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Generic YAML file: a pointer to two other files

```
fasta:
    class: File
    location: K_pneu.fasta
submol:
    class: File
    location: K_pneu_meta.ya
```

K_pneu.yaml

```
topology: 'linear'
organism:
    genus_species: 'Klebsiella pneumoniae'
contact_info:
    last_name: 'Thibaud-Nissen'
    first_name: 'Francoise'
    email: 'thibaudf@ncbi.nlm.nih.gov'
    organization: 'NCBI prok group'
    department: 'Department of Microbiology'
    street: '9000 Rockville Pike'
    city: 'Bethesda'
    postal_code: '20845'
    state: 'Maryland'
    country: 'USA'
authors:
- author:
    first_name: 'Peter'
    last_name: 'Cooper'
```

Required only if submitting to GenBank

Start-to-finish PGAP annotation

- Download the pgap.py
- Download the Docker image and reference data
- Prepare the inputs
  - Input file format
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Annotate your genome

```
$ ./pgap.py -r -o K_pneu_results K_pneu.yaml
PGAP version 2020-09-24.build4894 is up to date.
Output will be placed in: /home/thibaudf/K_pneu_results
PGAP completed successfully.
```

-r : allows transmitting to NCBI that the execution has started and stopped

-o : path to the output directory

Tip: use `screen`, `tmux` or `nohup` if running remotely

```
$ nohup ./pgap.py -r -o K_pneu_results K_pneu.yaml &
$ disown <pid>
```

Start-to-finish PGAP annotation

- Download the pgap.py
- Download the Docker image and reference data
- Prepare the inputs
  - Input file format
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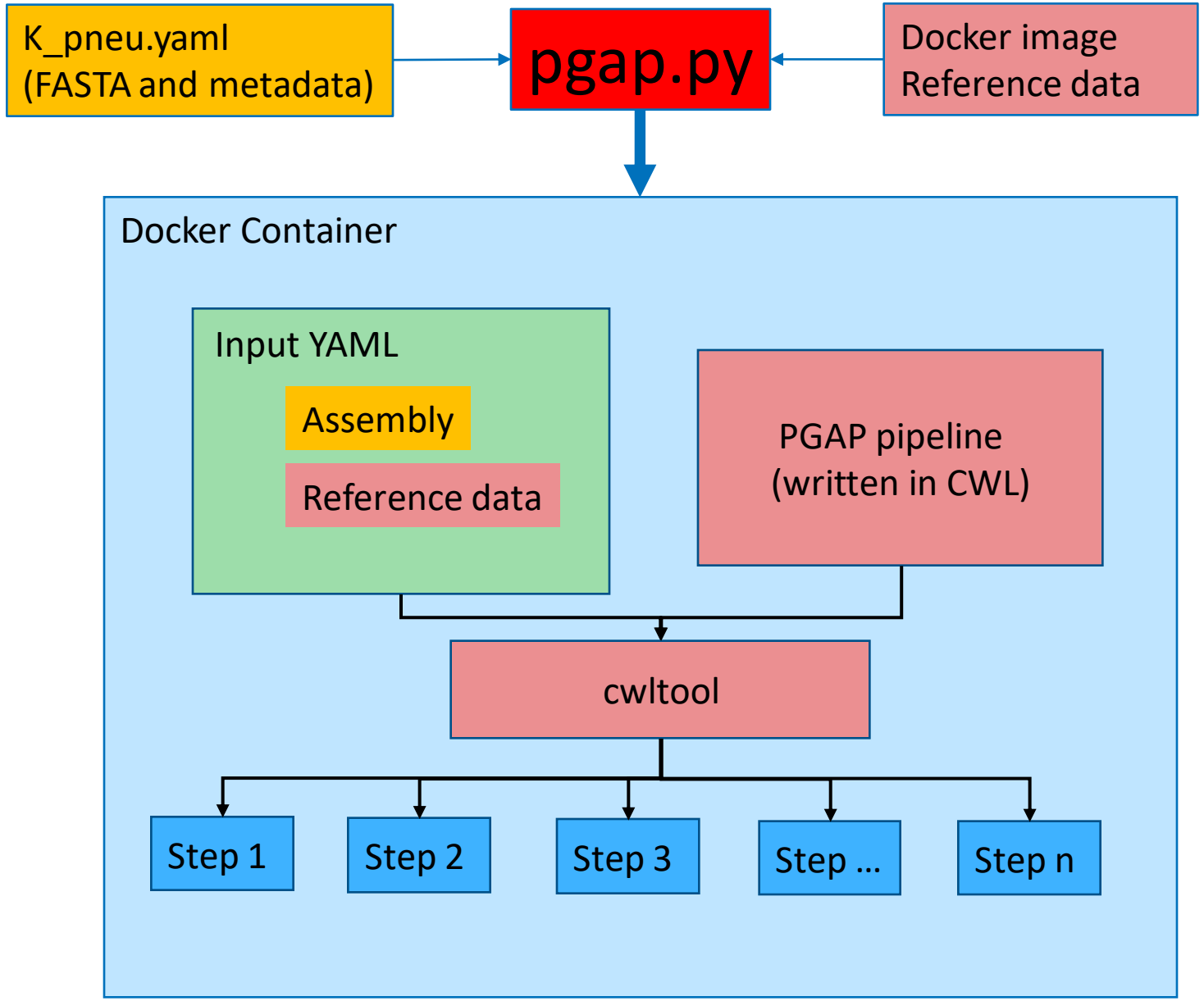
Execution of PGAP in a container



Review the results

- Output formats
  - FASTA
  - GFF
  - ASN.1
  - GenBank
- Metadata
  - Topology
  - Species
  - Submitter
  - Submitter info
- Annotation summary
  - Run information
  - Many frameshifts!

Output

- The annotation in a variety of formats
  - annot.faa – annotated proteins (fasta format)
  - annot.sqn – annotated genomic sequence in ASN format (use for submission)
  - annot.gff – annotated genomic sequence in GFF3 format
  - annot.gbk – annotated genomic sequence in GenBank flat file format
- Vector or adaptor sequences in tab-delimited format
  - calls.tab: Coordinates of vector or adaptor sequences in tab-delimited format.
- (Taxonomic assignment verification files. Only produced if using the flag --taxcheck or --taxcheck-only)
  - ani-tax-report.txt: Results of the taxonomy check in text format.
  - ani-tax-report.xml: Results of the taxonomy check in xml format.

More about input QA in the hands-on activities!

Run PGAP on draft assemblies

"Pre-annotation"

Get a sense of the assembly quality early in your workflow

With the flag `--ignore-all-errors`

```
./pgap.py -r \
--ignore-all-errors \
-o K_pneu_results \
K_pneu.yaml
```

- Ignores the genome size check
- Ignores invalid sequences due to
  - Vector and adaptor sequences
  - Stretches of Ns at the end of sequences
- Ignores the taxcheck results if –taxcheck is used

⚠ Results may not comply with GenBank quality criteria

More about this in the hands-on activities!

Review the results

- Output formats
  - FASTA
  - GFF
  - ASN.1
  - GenBank
- Metadata
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Info provided on input in the output

```
LOCUS       tig00000001          5364382 bp    DNA     linear   BCT 26-OCT-2020
DEFINITION  Klebsiella pneumoniae, whole genome shotgun sequence.
ACCESSION
VERSION
KEYWORDS    WGS.
SOURCE      Klebsiella pneumoniae
  ORGANISM  Klebsiella pneumoniae
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Enterobacteriaceae; Klebsiella.
REFERENCE   1  (bases 1 to 5364382)
  AUTHORS   Cooper,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-DEC-2019) Department for Microbiology, National
            Center for Biotechnology Information, 9000 Rockville Pike,
            Bethesda, Maryland 20845, USA
COMMENT     The annotation was added by the assembly submitters using the NCBI
            Prokaryotic Genome Annotation Pipeline (PGAP). Information about
            stand-alone PGAP can be found here: https://github.com/ncbi/pgap/
```

Review the results

- Output formats
  - FASTA
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  - Many frameshifts!

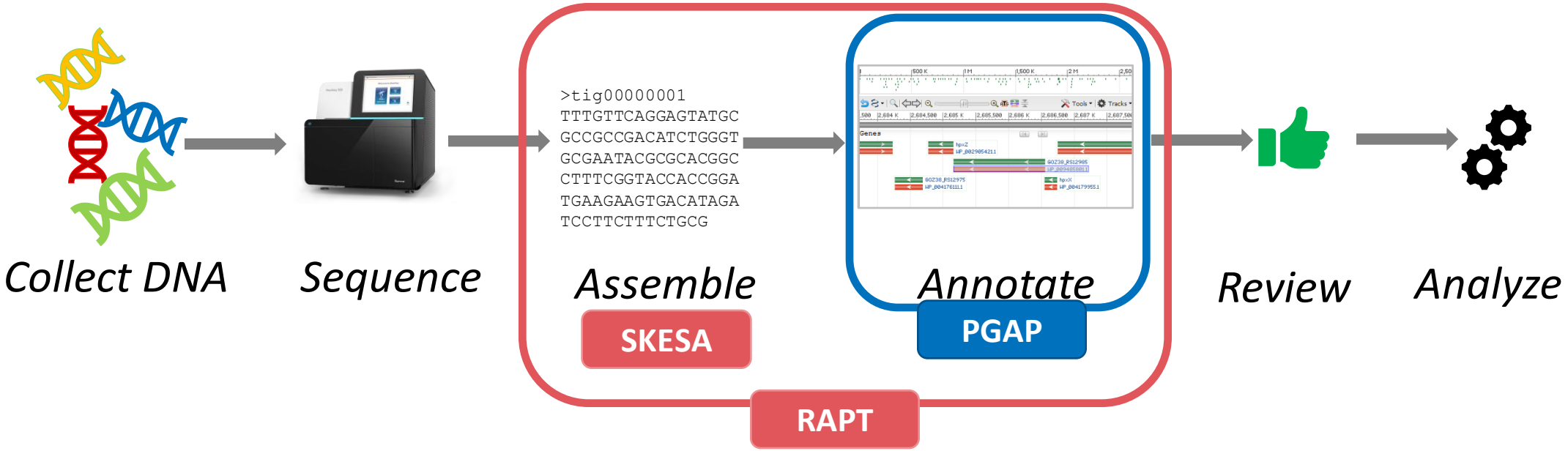
Annotation summary

```
##Genome-Annotation-Data-START##
Annotation Provider                    :: Organization
Annotation Date                        :: 10/26/2020 13:35:31
Annotation Pipeline                    :: NCBI Prokaryotic Genome Annotation
                                           Pipeline (PGAP)
Annotation Method                      :: Best-placed reference protein set;
                                           GeneMarkS-2+
Annotation Software revision           :: 2020-09-24.build4894
Features Annotated                     :: Gene;CDS;rRNA;tRNA;ncRNA;repeat_regior
Genes (total)                          :: 6,316
CDSs (total)                           :: 6,194
Genes (coding)                         :: 2,172
CDSs (with protein)                    :: 2,172
Genes (RNA)                            :: 122
rRNAs                                  :: 9, 8, 8 (5S, 16S, 23S)
complete rRNAs                         :: 9, 8, 8 (5S, 16S, 23S)
tRNAs                                  :: 87
ncRNAs                                 :: 10
Pseudo Genes (total)                   :: 4,022
CDSs (without protein)                 :: 4,022
Pseudo Genes (ambiguous residues)      :: 0 of 4,022
Pseudo Genes (frameshifted)            :: 3,875 of 4,022
Pseudo Genes (incomplete)              :: 252 of 4,022
Pseudo Genes (internal stop)           :: 104 of 4,022
Pseudo Genes (multiple problems)       :: 205 of 4,022
##Genome-Annotation-Data-END##
```

Run information

Annotation results

The Read Assembly and Annotation Pipeline Tool (RAPT): An extension of PGAP



What does PGAP do?

- Annotates the genome with the pipeline used to annotate RefSeq genomes

What does RAPT do?

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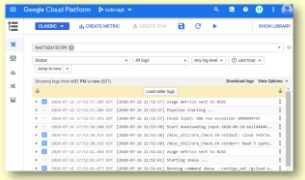
GCP RAPT Workflow Overview

From the Google Console Shell

Starts a virtual machine

Downloads and instantiates the RAPT Docker container



Sends execution logs to Google Stackdriver

Copies results to Google storage bucket

Shuts down the virtual machine

Requirements

- GCP account and project with a quota of at least 16 CPU in a given region
- Google Life Sciences API enabled
- A Google storage bucket

GCP RAPT Start-to-finish

- Download the RAPT interface
- Explore RAPT
- Run RAPT
  - Starting with an SRA run
  - Starting with reads fasta
- Follow the progress
- Review the results

run_rapt_gcp.sh, the command line interface for RAPT

Simply copy and paste these two commands at your Cloud Shell prompt, as specified in https://github.com/ncbi/rapt/

```
$ curl -sSLo rapt.tar.gz
https://github.com/ncbi/rapt/releases/download/v0.2.0/rapt
-v0.2.0.tar.gz

$ tar -xzf rapt.tar.gz && rm -f rapt.tar.gz
```

```
$ ls
CHANGELOG.md README.txt release-notes.txt
run_rapt_gcp.sh run_rapt.py
```

GCP RAPT Start-to-finish

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Explore GCP RAPT

This command will provide instructions and options for running RAPT:

```
$./run_rapt_gcp.sh help
./run_rapt_gcp.sh help
Usage: run_rapt_gcp.sh  <command>  [options]
Job creation commands:
     submitacc <sra_acxn> <-b|--bucket URL> [--label LABEL]
            [--skesa-only] [--no-usage-reporting] [--machine-type TYPE]
            [--boot-disk-size NUM] [--timeout SECONDS]
            Submit a job to run RAPT on an SRA run accession (sra_acxn).
     submitfastq <fastq_uri> <--organism "Genus species"> [--strain "ATCC xxxx"]
            <-b|--bucket URL> [--label LABEL] [--skesa-only]
            [--no-usage-reporting] [--machine-type TYPE] [--boot-disk-size NUM]
            [--timeout SECONDS]
            Submit a job to run RAPT on Illumina reads in FASTQ or FASTA format.
            fastq_uri is expected to point to a google cloud storage (bucket).

            The --organism argument is mandatory. It is the binomial name or, if the
            species is unknown, the genus for the sequenced organism. This identifier
            must be valid in NCBI Taxonomy. The --strain argument is optional.
[…]
          Common options:
          ==================
            -b|--bucket URL
              Mandatory. Specify the destination storage location to store results
                   and job logs.
            --label LABEL
              Optional. Tag the job with a custom label, which can be used to filter jobs
```

GCP RAPT Start-to-finish

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Using an SRA run as input to RAPT

```
$./run_rapt_gcp.sh submitacc SRR11675939
-b gs://mybucket --label Mbovis_NADC67
```

submitacc SRR11675939 - input SRA run
-b gs://mybucket – bucket where output will be copied
--label Mbovis_NADC67 – optional tag

GCP RAPT Start-to-finish

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Using fastq files as input to RAPT

To run RAPT with a fastq or fasta file, use the `submitfastq` command, point to the location of the fastq file, and use the -b option to point to your Google bucket for the output location.

Here is an example of using an input file stored locally:

```
$./run_rapt_gcp.sh submitfastq
./Mp_ATCC_25960_local.fastq --organism "Mycoplasma
pirum" --strain "ATCC 25960" -b gs://mybucket --
label Mp_25960_l
```

(Please note that the quotes are required for the organism and strain options.)

Here is an example of using an input file in a Google bucket:

```
$./run_rapt_gcp.sh submitfastq
gs://friendbucket/Mp_ATCC_25960.fastq --organism
"Mycoplasma pirum" -b gs://mybucket --label
Mp_25960_b
```

GCP RAPT Start-to-finish

- Download the RAPT interface
- Explore RAPT
- Run RAPT
  - Starting with an SRA run
  - Starting with reads fasta
- Follow the progress
- Review the results

Launch the job…

```
$./run_rapt_gcp.sh submitfastq gs://friendbucket/Mp_ATCC_25960.fastq -b
gs://mybucket --label Mp_25960_b --organism "Mycoplasma pirum"

RAPT job has been created successfully.
-----------------------------------------------------
Job-id:                 5541b09bb9
Output storage:         gs://mybucket/5541b09bb9
GCP account:            1111111111111-compute@developer.gserviceaccount.com
GCP project:            example
-----------------------------------------------------

[**Attention**] RAPT jobs may take hours to finish. Progress of this job can be
viewed in GCP stackdriver log viewer at:

        https://console.cloud.google.com/logs/viewer?project=strides-
documentation-testing&filters=text:5541b09bb9

For current status of this job, run:

        run_rapt_gcp.sh joblist | fgrep 5541b09bb9

For technical details of this job, run:

        run_rapt_gcp.sh jobdetails 5541b09bb9
$
```

GCP RAPT Start-to-finish

- Download the RAPT interface
- Explore RAPT
- Run RAPT
  - Starting with an SRA run
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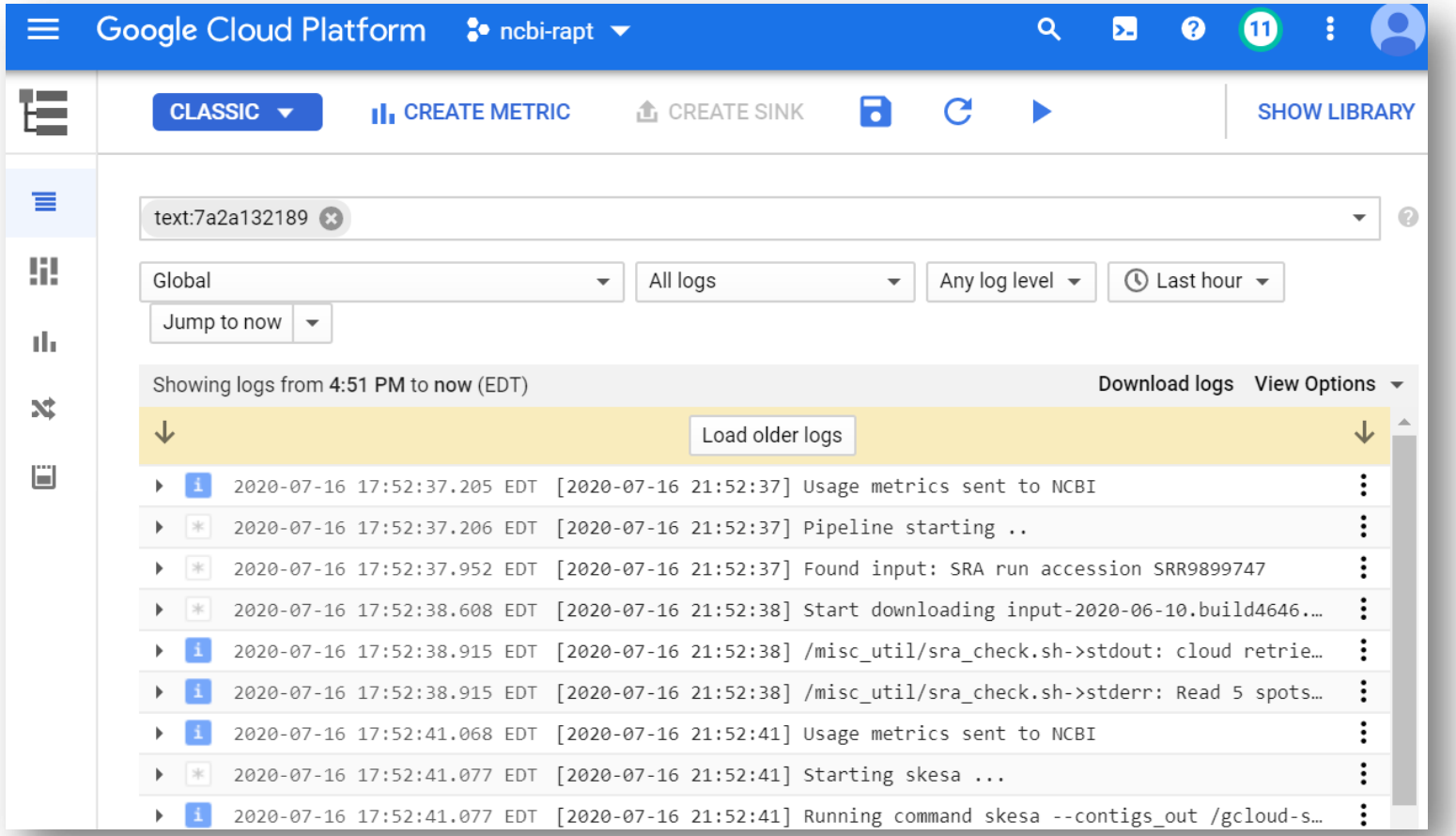
- Follow the progress
- Review the results

Checking progress

Execution information is available in the Google Console

Follow the link provided in the standard output to view the logs



Check the status of all jobs executed in this project

Start-to-finish

- Download the RAPT interface
- Explore RAPT
- Run RAPT
  - Starting with an SRA run
  - Starting with reads fasta
- Follow the progress
- Review the results

```
$./run_rapt_gcp.sh joblist
```

```
$./run_rapt_gcp.sh joblist
GCP Account: [111111111111-compute@example.gserviceaccount.com]
Project: [example]
JOB_ID     USER    LABEL   SRR    STATUS  START_TIME   END_TIME   OUTPUT_URI
53002b6616 shlu Done 2020-11-10T20:20:28 2020-11-10T20:43:52 gs://ncgas-test-shlu/53002b6616
eacac6f98d shlu Done 2020-11-10T20:16:40 2020-11-10T20:38:54 gs://ncgas-test-shlu/eacac6f98d
```

GCP RAPT Start-to-finish

- Download the RAPT interface
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Review the output files

Files are in the selected output gs bucket (i.e. gs://mybucket/5541b09bb9/output.tar.gz)

- View the output of the run in the Google storage bucket

```
$ gsutil ls gs://mybucket/5541b09bb9/
output.tar.gz
run.log
```

- Copy the output of the run in the Google storage bucket, and untar

```
$ gsutil cp -r gs://mybucket/5541b09bb9/ .
$ cd 5541b09bb9
$ tar -xzvf output.tar.gz
```

Same files as for PGAP
+ skesa.out.fa: multifasta file of the assembly produced by SKESA
+ verbose.log
+ concise.log

Coming next….

- RAPT and PGAP are on github

  https://github.com/ncbi/pgap
  https://github.com/ncbi/rapt

- Subscribe and stay informed of new releases

- Soon….
  - Performance improvements in PGAP and RAPT (Dec 2020)
  - A web service for RAPT (Jan 2021)

We want your feedback !

- Will these tools be useful to your research? Yes/No
- What other tools would help you reach your goals?
- How do you use annotated genomes?
- What computing environment do you have access to?

- Are you interested in getting sneak previews of new tools or features, and in becoming a tester? ✋

Talk to us during this workshop or later!

- prokaryote-tools@ncbi.nlm.nih.gov
- thibaudf@nih.gov

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