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Transcriptome annotation in the cloud: complexity, best practices and cost.

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Abstract:	Background The NIH Science and Technology Research Infrastructure for Discovery, Experimentation, and Sustainability (STRIDES) initiative provides NIH-funded researchers cost-effective access to industry-leading commercial cloud providers, such as Amazon Web Services (AWS; Seattle, WA, USA) and Google Cloud Platform (GCP; Mountain View, CA, USA). These cloud providers represent an alternative for the execution of large computational biology experiments like transcriptome annotation which is a complex analytical process that requires the integration of multiple biological databases and several advanced computational tools. The core components of annotation pipelines published since 2012 are BLAST sequence alignments using annotated databases of both nucleotide or protein sequences almost exclusively with networked on premises compute systems. Findings We present a comparative study of multiple BLAST sequence alignments using two public cloud providers: AWS and GCP. We have prepared several Jupyter Notebooks with all the code required to submit computing jobs to the batch system on each cloud provider. We consider the consequence of the number of query transcripts in input files and the effect on cost and processing time. We tested compute instances with 16, 32 and 64 vCPUs on each cloud provider. Four classes of timing results were collected: the total run time, the time for transferring the BLAST databases to the instance local solid state disk drive (SSD), the time to execute the Common Workflow Language (CWL) script and the time for the creation, setup and release of an instance. This study aims to establish an estimate of the cost and compute time needed for the execution of multiple BLAST runs in a cloud environment. Conclusions We demonstrate that the public cloud providers are a practical alternative for the execution of advanced computational biology experiments at low cost. Using our cloud recipes, the BLAST alignments required to annotate a transcriptome with ~500,000 transcripts can be processed in less than 2 hours with a compute cost of about 200-250 USD. In our opinion, for BLAST based workflows, the choice of cloud platform is not dependent on the workflow but, rather, on the specific details and requirements of the cloud provider (e.g. NCBI maintains updated copies of the very large genetic sequence databases, such as nr, RefSeq and SRA, on both GCP and AWS). These choices include the accessibility for institutional use, the technical knowledge required for effective use of the platform services, and the availability of open-source frameworks such as application programming interfaces (APIs) to deploy the workflow.
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Response to Reviewers:	Reviewer reports (see cover letter for figure added: Response: We thank the editors and referees for the comments and for their time dedicated to this manuscript. Editor: Your manuscript "Transcriptome annotation in the cloud: complexity, best practices and cost." (GIGA-D-20-00202R1) has been assessed again by our re-reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in GigaScience, once you have carried out some final very minor revisions suggested by our re-reviewers. We require code and test data snapshots in our GigaDB repository and I've cc'd our curators to help you with this. As you discuss a CWL workflow I'd suggest including this as a figure and linked to the permalink in the legends so readers can interact with it: https://view.commonwl.org/workflows/github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl Response: We added a new paragraph and a new figure 3 to the manuscript with the workflow schema and the interactive link was added to the figure caption like: Figure 3 shows the scheme of the transcriptome annotation workflow used in this study, interactive link is available in the caption of the figure. Figure 3: Transcriptome Annotation workflow schema, https://view.commonwl.org/workflows/github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl Reviewer #1: I appreciate the authors' responses to the reviewer comments and their improvements in the manuscript. Overall, I do not find any further critical issues to be solved in the content. Below are a few minor suggestions: 1. Pricing of cloud usage: Both AWS and GCP officially announces that they continue their efforts to reduce the computing cost regularly. It would be better to mention that the pricing of cloud instances may change in the future as well as the instance selections, and that may lead to the change of the study's conclusion. Table 1 also should have information about the date and the region. Response: We added two columns (Region and Last used Date) to Table 1 as recommended by the referee. WE also modified the Table caption to: Table 1:Machine types with resources in each cloud. Prices and instance type may change in the future as is common practice of cloud providers 2. The container image registry In the experiments with both vendors, the authors used the same docker image hosted in the Google Container Registry. It means that the GCP has an advantage in the transfer of the container image, which may take some time during the instance setup. It would be fair to mention it or mention that the pulling time too short to be worth consideration. Response: We agreed with the referee that we should mention that the docker image was hosted in the Google Container Registry. However, this fact does not represent any limitation for AWS as the download of the image don't affect the performance of AWS instances. We added these sentences to the Result and Discussion section:

	The AWS platform is more efficient than the GCP during the instance creation, setup and release, see Figure 4b. This step is only 0.1% of the total cost despite the docker image used in the study being hosted in the Google Container Registry. I am looking forward to the publication of this manuscript, which may give many insights into practical cloud usage to the readers.
Additional Information:	
Question	**Response**
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	Yes
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?	Yes
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically	Yes

appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?	

Transcriptome annotation in the cloud: complexity, best practices and cost.

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Abstract

Background

The **NIH Science and Technology Research Infrastructure for Discovery, Experimentation, and Sustainability (STRIDES) initiative** provides NIH-funded researchers cost-effective access to industry-leading commercial cloud providers, such as Amazon Web Services (AWS; Seattle, WA, USA) and Google Cloud Platform (GCP; Mountain View, CA, USA). These cloud providers represent an alternative for the execution of large computational biology experiments like transcriptome annotation which is a complex analytical process that requires the integration of multiple biological databases and several advanced computational tools. The core components of annotation pipelines published since 2012 are BLAST sequence alignments using annotated databases of both nucleotide or protein sequences almost exclusively with networked on premises compute systems.

Findings

We present a comparative study of multiple BLAST sequence alignments using two public cloud providers: AWS and GCP. We have prepared several Jupyter Notebooks with all the code required to submit computing jobs to the batch system on each cloud provider. We consider the consequence of the number of query transcripts in input files and the effect on cost and processing time. We tested compute instances with 16, 32 and 64 vCPUs on each cloud provider. Four classes of timing results were collected: the total run time, the time for transferring the BLAST databases to the instance local solid state disk drive (SSD), the time to execute the Common Workflow Language (CWL) script and the time for the creation, setup and release of an instance. This study aims to establish an estimate of the cost and compute time needed for the execution of multiple BLAST runs in a cloud environment.

Conclusions

We demonstrate that the public cloud providers are a practical alternative for the execution of advanced computational biology experiments at low cost. Using our cloud recipes, the BLAST alignments required to annotate a transcriptome with ~500,000 transcripts can be processed in less than 2 hours with a compute cost of about 200-250 USD. In our opinion, for BLAST based

workflows, the choice of cloud platform is not dependent on the workflow but, rather, on the specific details and requirements of the cloud provider (e.g. NCBI maintains updated copies of the very large genetic sequence databases, such as nr, RefSeq and SRA, on both GCP and AWS). These choices include the accessibility for institutional use, the technical knowledge required for effective use of the platform services, and the availability of open-source frameworks such as application programming interfaces (APIs) to deploy the workflow.

Background

The **NIH Science and Technology Research Infrastructure for Discovery, Experimentation, and Sustainability (STRIDES) initiative** (https://cloud.cit.nih.gov/) permits NIH supported scientists to explore the use of cloud environments and provides cost-effective access to industry-leading commercial cloud providers. The NIH's STRIDES cloud provider partners, at the time of this study, were Amazon Web Services (AWS; Seattle, WA, USA) and Google Cloud Platform (GCP; Mountain View, CA, USA). Cloud computing offers an *on-demand* model where a user can dynamically allocate "unlimited" compute resources and then release them as soon as the analysis is complete [1]. They offer a reduced cost of compute resources and a friendly user interface that makes cloud computing accessible for large computational biology experiments.

As part of the STRIDES initiative, NIH-funded institutions began to upload and compute data in the cloud. Public biological databases like the Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra/docs/sra-cloud/) and computational tools like BLAST (https://github.com/ncbi/blast_plus_docs), from the National Center for Biotechnology Information (NCBI), were migrated and are available for public use on AWS and GCP. In addition, NIH-funded researchers are contributing to the NIH's STRIDE initiative not only migrating data analysis workflows to the cloud but also disseminating the suitability of the cloud computing for computational biology experiments.

The annotation of RNA transcripts with functional and biological processes is an important step in developing an understanding of the biological complexity of an organism. Annotation is a challenging process that requires the integration of multiple biological databases and several computational tools to accurately assign a function to an RNA product. Available public information on a target organism is the main limitation of the annotation of non-model organisms. The NCBI Genome database, for instance, contains 54,049 genome-sequencing projects by organism [2]. This includes 12,204 eukaryotes genomes for more than 1,000 species or strains at different assembly levels (95 complete genomes, 1,872 chromosomes, 7,743 scaffolds, and 2,494 contigs (https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/), accessed on June 30, 2020. Although these data include an important group of organisms, there is a lack of annotation of several species that have significant public health and economic importance. Significantly, in the plant kingdom, *Viridiplantae*, only 3 complete genomes, 331 chromosomes, 625 scaffolds, and 394 contigs are annotated. The advances in next-generation sequencing technologies and the decrease in the cost of sequencing a complete transcriptome is driving a new era in which annotation will be increasing, important and productive.

A review of published manuscripts since 2012 [3-11] reveals that many developed pipelines have a common core component and use the NCBI BLAST tools [12] to align assembled transcriptomes against annotated databases of nucleotides or proteins to identify similarity and infer function. After an assembly, these alignments are the initial step to identify close and/or distant homologous genes, proteins, and functional domains that could be cross-referenced with

other public databases, such as Gene Ontology [13], to generate new annotations of query sequences. As the number of transcripts assembled per study increases, the computing power and storage required to align these transcripts to the BLAST databases also increases. On premises computer infrastructures (including server farms) have been used mainly for the computation of sequence alignments using BLAST. Many laboratories, however, are not equipped with the compute power required for the analysis of increased transcriptome sequencing results. Although a minimum infrastructure could be easy to build and maintain, it may be unnecessary and less financially burdensome with the advent of cloud computing and its utilization in computational biology.

The utilization of cloud environments for computational biology experiments is increasing [14-17]. However, little has been published estimating cloud costs and implementation best practices. A recent work published by Ohta *at al.* [18] presents a tool named CWL-metrics that collects runtime metrics of Docker containers and workflow metadata to analyze workflow resource requirements. This study presents a cost estimation for the execution on the cloud for AWS EC2 instances but does not mention the cloud batch system for users to submit thousands of jobs to the cloud.

Modern cloud providers offer "unlimited" compute resources that can be accessed *on-demand*. An *instance*, as the virtual machines are named in the cloud environment, is deployed using a variety of operating systems like GNU/Linux or Microsoft Windows. Users pay only for the time that the instance is running plus the cost of other resources such as network egress and/or the size of network storage devices. A workflow can be deployed on a manually created instance but this is not cost efficient as the instance will need to be manually reconfigured with workflow dependencies. It will also remain active once the analysis is completed which wastes resources. Private genomic cloud providers, for instance DNAnexus (www.dnanexus.com), DNAstar (www.dnastar.com), Seven Bridges (www.sevenbridges.com) and SciDAP (scidap.com), and others, also offer cloud-based genomics frameworks. These commercial cloud providers make the execution of computational biology experiments easier by offering command line and web-based interfaces designed for genomic data analysis.

Most cloud providers offer a batch system that can do the configuration automatically for users to submit several parallel jobs. The batch system makes the process of instance creation, setup and termination fully automatic.

Batch processing is a technique for processing data as a single large collection of iterative steps instead of individually. It reduces user interactivity to process submissions by automating the remaining steps. Modern cloud providers offer a batch system that can be personalized to process many different workflows. Figure 1 shows the component of a generic cloud batch system. It is comprised of a *batch queue* to which users submit the *tasks*. Each *task* uses a *job definition* to create a *job* where all computational resources and the workflow steps are outlined. Then, an *instance* is automatically created with the resources requested by the *job*. Since all the data for the analysis is in the cloud, the *instance* downloads the input data from the *cloud storage system* and, after successfully completing the workflow, uploads the results, releasing all computational resources.

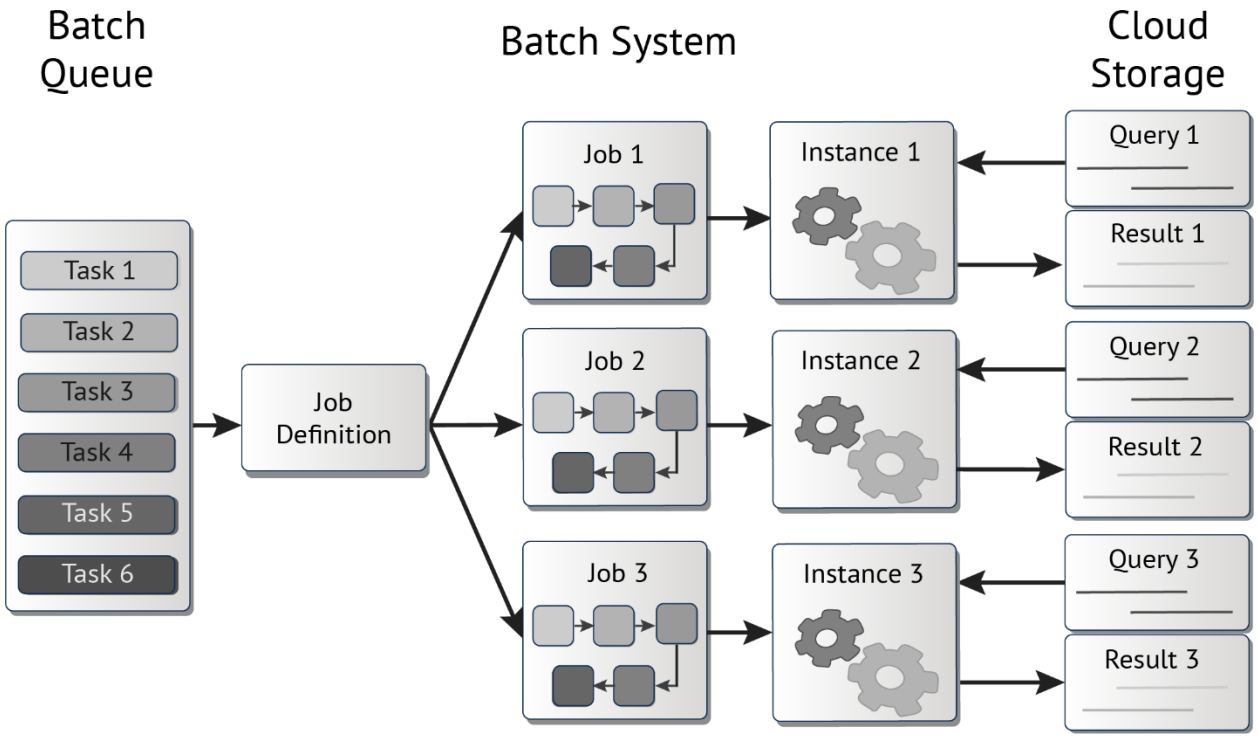

Figure 1: Basic components in a cloud-based batch system

In this manuscript, we present a comparative study of multiple BLAST searches and alignments required to annotate transcriptome data. This study aims to establish an estimation of the cost and time needed for the execution of multiple BLAST searches on the cloud. Our recommendation on best practices for deploying computational biology workflows in the cloud are also presented.

Methods

Transcriptome Annotation Workflow

This study focuses only on the many BLAST alignments which are the most compute-demanding core of a transcriptome annotation process. BLAST alignments require considerable compute resources which generate intermediate results that are used to complete the annotation process. The remaining part of the annotation pipeline is excluded from our study as it can be executed on a workstation and does not require an extensive use of the cloud.
The input for the workflow is a transcriptome in FASTA format. First, TransDecoder (RRID:SCR_017647)[19] is executed to generate all open reading frames (ORFs) from the input file. Then, BLASTP and RPS-BLAST are executed on the TransDecoder output files generating a list of homologous proteins and conserved protein domains (BLASTP uses the BLAST ***nr*** database, and RPS-BLAST uses the NCBI Conserved Domain Database (CDD) [20]). The transcriptome files are also used as inputs for BLASTN and RPST-BLASTN which are executed using the BLAST ***nt*** database and the NCBI CDD database, respectively. These processes generate a list of homologous genes and a list of conserved domains, see Figure 2. The workflow was implemented using the Common Workflow Language (CWL) [21] and is freely available at:

https://github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl
The workflow uses as input a FASTA file, which we named *query*, and includes multiple transcripts to be processed. The number of transcripts to be included in a *query* is another parameter that merits an analysis. The size of the *query* affects the workflow processing time as a complete transcriptome could be comprised of thousands to hundreds of thousands of transcripts assembled from a next-generation sequencing (NGS) experiment [22].
Our analysis is based on the execution of the workflow with a batch system provided by each cloud platform. This approach keeps the compute time, and therefore the cost, to a minimum. It also limits the user interaction with the jobs to only the submission step.

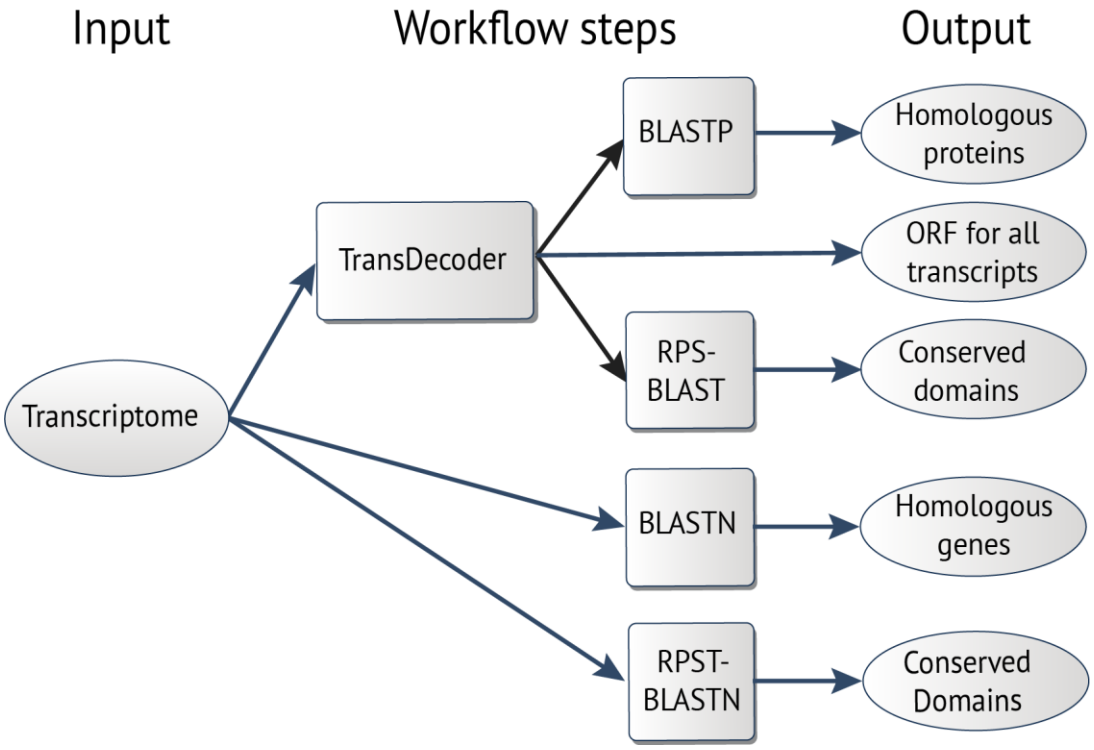

Figure 2: Schema of the transcriptome annotation workflow

Containerized workflows

Containerizing a workflow involves bundling it with all its dependencies and configuration files so that it can be executed across different computing environments. The workflow dependencies in the container uses the same version and compiled libraries when it is executed in any computing infrastructure which would make the process highly reproducible. In this study, we use Docker as the container engine. Docker permits the creation of container images that can be used on a personal laptop or on a cloud platform. The workflow container image generated is freely available from the Google Container registry (https://cloud.google.com/container-registry) with name: *gcr.io/cbb-research-dl/transannot-cloud-cmp*
All files used to generate this image are available at: https://github.com/ncbi/cloud-transcriptome-annotation/tree/master/config/gcp/docker

Common Workflow Language

Common Workflow Language (CWL) [21] is an open standard workflow language used to describe and implement complex pipelines which uses interchangeable blocks. The resulting product is portable and scalable. It can be executed across a variety of hardware environments as dissimilar as personal laptops or the cloud.
Workflow managers are tools that simplify the execution of workflows in multiple computational environments. Some have been developed to manage and execute CWL workflows like Toil[23], CWL-Airflow[24], Arvados (https://arvados.org/) and REANA (http://reanahub.io/). Others, however, use their own workflow languages like Nextflow [25], and SnakePipes [26]. All provide a unified interface to users to choose the compute environment to process jobs. Users can configure the workflow manager to submit jobs to a high-performance compute cluster or to a cloud provider. Nevertheless, all these workflow managers use the cloud batch system to submit jobs for computing in the cloud.
In this study, we aim to estimate the minimum cost of executing a transcriptome annotation pipeline in the cloud. We selected CWL because it is the workflow language with many available workflow managers. Also, CWL provides a reference implementation runner: ***cwltool*** (https://github.com/common-workflow-language/cwltool) (cwltool, RRID:SCR_015528). This runner can be executed on the command line inside a GCP or AWS job definition minimizing all dependencies for processing a workflow. We intentionally avoided the use of workflow managers to be able to quantify runtime for the workflow steps as precise as possible.
Figure 3 shows the scheme of the transcriptome annotation workflow used in this study, interactive link is available in the caption of the figure.

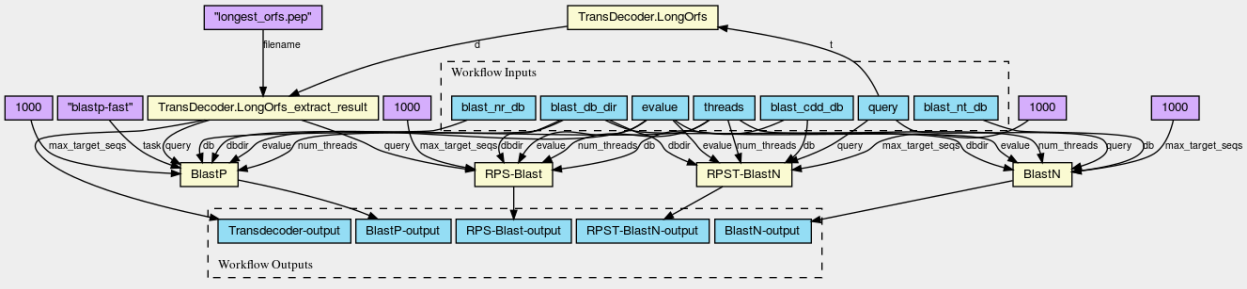

Figure 3: Transcriptome Annotation workflow schema, https://view.commonwl.org/workflows/github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl

GCP

The Google Cloud Platform (GCP) offers a batch system specifically designed for life sciences, the Cloud Life Sciences (https://cloud.google.com/life-sciences). This system was initially Google Genomics but has evolved to allow the scientific community to process biomedical data at scale.
Cloud Life Sciences offers an Application Program Interface (API) implemented for users to develop their own workflow in JSON format using three main attributes: *actions*, *environments* and *resources*. *Actions* are the list of *commands* to execute using a defined container image. They also include statements to mount local solid-state drives (SSD) or network storage devices, defined in *resources*. *Environments* define the environment variables available inside the container. Finally, *resources* define the instance type and the local SSD or network storage devices.

The API, using the JSON described in Box 1, automatically creates instances on-demand, following the requirements defined in the *resources* section of the job JSON file. GCP also provides a customized container image where the instance interacts with other GCP products like Google Storage where data is stored. In addition, GCP creates the instances using a customized Linux operating system that formats and mounts the instance local disks making them available for the jobs.

Box 1: Brief extract of the GCP pipeline definition JSON file

```
{
  "actions": [

    ...,

    {
      "commands": [
        "/bin/bash",
        "-c",
        "cwltool --no-container --on-error continue --tmpdir-prefix /data/ --tmp-outdir-prefix /data/ --outdir /data/${SAMPLE}
https://raw.githubusercontent.com/ncbi/cloud-transcriptome-annotation/master/bin/cwl-ngs-workflows-
cbb/workflows/Annotation/transcriptome_annotation.cwl

 --blast_db_dir /data --threads ${CPUs} --evalue 1e-5 --blast_nt_db nt --blast_nr_db nr --blast_cdd_db split-cdd --fasta
/data/${SAMPLE}.fa >> /data/pipeline.log 2>&1"
      ],
      "imageUri": "gcr.io/cbb-research-dl/transannot-cloud-cmp",
      "mounts": [
        {
          "disk": "gcloud-shared",
          "path": "/data"
        }
      ]
    },

    ...,

    "environment": {

    "CPUs": "64"
  },

    "resources": {
    "virtualMachine": {
      "bootDiskSizeGb": 60,
      "bootImage": "projects/cos-cloud/global/images/family/cos-stable",
      "disks": [
        {
          "name": "gcloud-shared",
          "sizeGb": 600,
          "type":"local-ssd"
        }
      ],
      "machineType": "n1-standard-64",

      ...,

    }
  }

}
```

Box 1 shows a brief extract of the pipeline used in GCP. We show only the main activity where the *command* attribute defines the command line to execute the CWL workflow. *ImageUri* attribute defines the container image used to run the command. In this case, our previously created Docker image. Finally, the *mounts* attribute defines the paths in the container to mount the disks created in the *resources* attribute.

The *VirtualMachine* attribute defines the resources used to create the job instance. In this attribute, users can define instance boot disk size, operating system, extra disks and the machine type. The complete JSON file is available at: https://github.com/ncbi/cloud-transcriptome-annotation/blob/master/config/gcp/pipeline.json

AWS

AWS Batch (https://aws.amazon.com/batch/) is the batch system provided by Amazon Web Services. It is comprised of *compute environments*, *job queues* and *job definitions*. The *compute environment* defines the computational resources to be used by the batch jobs. It is connected to the Amazon Elastic Container Service (ECS) which is a fully managed service that creates and manages computer clusters inside the Amazon cloud environment. The resources defined by the compute environment are used by the ECS to create and setup instances in which the workload is distributed. *Job queues* are used as an intermediate service to associate submitted jobs with the compute environments. Lastly, the jobs use a *job definition*, in JSON format, which defines specific information for the job, like container images, commands, number of vCPUs, RAM memory, environment variables and local or remote folder to mount on the container.

Box 2: Brief extract of the AWS job definition JSON file

```
{
  ...,
  "containerProperties": {
    "image": "gcr.io/cbb-research-dl/transannot-cloud-cmp",
    "vcpus": 64,
    "memory": 131072,
    "command": [
      "/usr/envs/transannot/bin/aws-pipeline.sh"
    ],
    "volumes": [
      {
        "host": {
            "sourcePath": "/data"
        },
        "name": "data"
      }
    ],
    "environment": [
      {
        "name": "CPUs",
        "value": "32"
      }
    ],
    "mountPoints": [
      {
        "containerPath": "/data",
        "sourceVolume": "data"
      }
    ],
    ...,
  }
}
```

Box 2 shows a brief extract of the job definition JSON script used in AWS. The *containerProperties* attribute defines the job properties. *Image* defines the container image, in this case our Docker image. *Command* defines the command to be executed inside the container.

In the case of AWS, a single command can be outlined in the job definition, thus, complex pipelines with multiple steps can be encapsulated in a BASH script. This script can be stored inside the container image or the container can download it at runtime. For simplicity, we have included this script inside the Docker image.
The AWS Batch system automatically creates all infrastructure, network components and compute instances, following the requirements of the compute environments. The default configuration of the Amazon Machine Image (AMI) used for the instances, however, is not configured to use local SSD disks available on certain machine types. This limits the default options on the AWS Batch system to certain types of workflows. Workflows that use intensive disk IO operations will have improved performance and efficiency if local SSD disks are used. Thus, a modified AMI capable of use the instance local disks is required for our study. We create a customized AMI for our study that is freely available in the AWS zone *us-east1* with ID: *ami-0dac0383cac1dc96e*. This AMI creates an array with the local SSD disks in the instance using the Linux utility *mdadm*. The array is formatted with XFS filesystem and mounted in a folder named */data*.
To improve the default AWS Batch options, Amazon offers a Virtual Private Cloud (VPC) that provides an extra layer of isolation for the resources used by the AWS Batch system. This VPC logically isolates all resources used in a defined virtual network improving the security. It is customizable for each compute problem.
The templates used in our study to create all the components of the AWS Batch system are available at: https://github.com/ncbi/cloud-transcriptome-annotation/tree/master/config/aws. All resources are created in the Jupyter notebook in: "02 - AWS-Batch".

GCP and AWS batch system limitations

Bioinformatics best practices for pipeline execution require the containerization of each tool included in the analysis. Projects such as Bioconda [27] and Biocontainers [28] provide standard containerized images for thousands of bioinformatics tools. However, the batch system for both tested cloud providers requires that all tools used in the workflow to be included in a single container. Each action in the cloud job definition has associated a single Docker image that is used to execute the action task. Docker-in-Docker, the process to execute docker containers inside another Docker container is not permitted in both GCP and AWS. This limitation constrains users to containerize all tools involved in a workflow, into a single Docker image. Hence, knowledge on how to create Docker images is a requirement for the migration of workflows to the cloud.

GCP and AWS transitory instances

Both GCP and AWS offer access to transitory instances which are spare compute capacity at a reduced cost. These instances are called SPOT in AWS and Preemptible in GCP. The transitory instances at reduced cost results from the fact that the cloud provider might terminate the instance at any time. Preemptible prices in GCP are fixed but not in AWS. The cost of the SPOT instances has a minimum but can be increased to the normal EC2 price if the demand for resources increases.
Transitory instances for workflow execution require extra processing steps to identify terminated jobs for resubmission. This is a reasonable option to reduce the cost of the analysis but requires a flexible timeframe to complete all analyses. Users need to be aware of this caveat.

Jupyter Notebooks

Jupyter notebooks are an open-source web application framework for the creation and sharing of documents that contain live code (RRID:SCR_018315)[29]. It is a standard way to share scientific code for ease of reproducibility and reuse [30]. The implementation of our study was fully developed in Jupyter notebooks. Readers can reproduce our results and figures using the notebooks that are available at the project GitHub repository. The notebooks create all cloud resources and submit the jobs to the batch systems. They also retrieve the job logs in JSON format and create the figures automatically from those logs. Each notebook includes a description about its purpose and is named using a numeric prefix to highlight the execution order.

The notebooks implemented in this study are designed to be executed on a local laptop or a workstation. Both interact asynchronously with the cloud providers using the command line APIs provided. In the case of GCP, we used the Google Cloud SDK (https://cloud.google.com/sdk). For AWS, we used the AWS Command Line Interface (https://aws.amazon.com/cli/). In these notebooks, the workflow input files are created, these are uploaded to each cloud provider storage space, the cloud batch systems are configured, the jobs are submitted and the results are retrieved. The notebooks interact with the cloud batch system to process jobs and retrieve results and logs stored in the ***results/PRJNA320545*** folder.

Table 1:Machine types with resources in each cloud. Prices and instance type may change in the future as it it is common practice of cloud providers.

Provider	Machine type	vCPU	Memory (GB)	Instance Local SSD (GB)	Network Bandwidth (Gbps)	Region	Last used Date	USD/Hour
AWS	m5d	16	64	2 x 300	Up to 10	us-east-1	Nov 12, 2020	0.904
AWS	m5d	32	128	2 x 600	10	us-east-1	Nov 12, 2020	1.808
AWS	m5d	64	256	4 x 600	20	us-east-1	Nov 12, 2020	3.616
AWS	m5dn	16	64	2 x 300	Up to 25	us-east-1	Nov 12, 2020	1.088
AWS	m5dn	32	128	2 x 600	25	us-east-1	Nov 12, 2020	2.176
AWS	m5dn	64	256	4 x 600	75	us-east-1	Nov 12, 2020	4.352
GCP	n1	16	60	24 x 375	32	us-east1-c	Oct 30, 2020	0.861
GCP	n1	32	120	24 x 375	32	us-east1-c	Oct 30, 2020	1.393
GCP	n1	64	240	24 x 375	32	us-east1-c	Oct 30, 2020	2.475
GCP	n2	16	64	24 x 375	32	us-east1-c	Oct 30, 2020	0.951
GCP	n2	32	128	24 x 375	32	us-east1-c	Oct 30, 2020	1.572
GCP	n2	64	256	24 x 375	32	us-east1-c	Oct 30, 2020	2.816

Results and Discussion

In this study, we present an analysis of the complexity, cost and best practices for executing the core components of a transcriptome annotation workflow in the cloud. For our experiments, we used the two cloud provider partners of the NIH's STRIDES Initiative: GCP and AWS. For each cloud provider, similar compute instances were tested using 16, 32 and 64 vCPUs. The machine

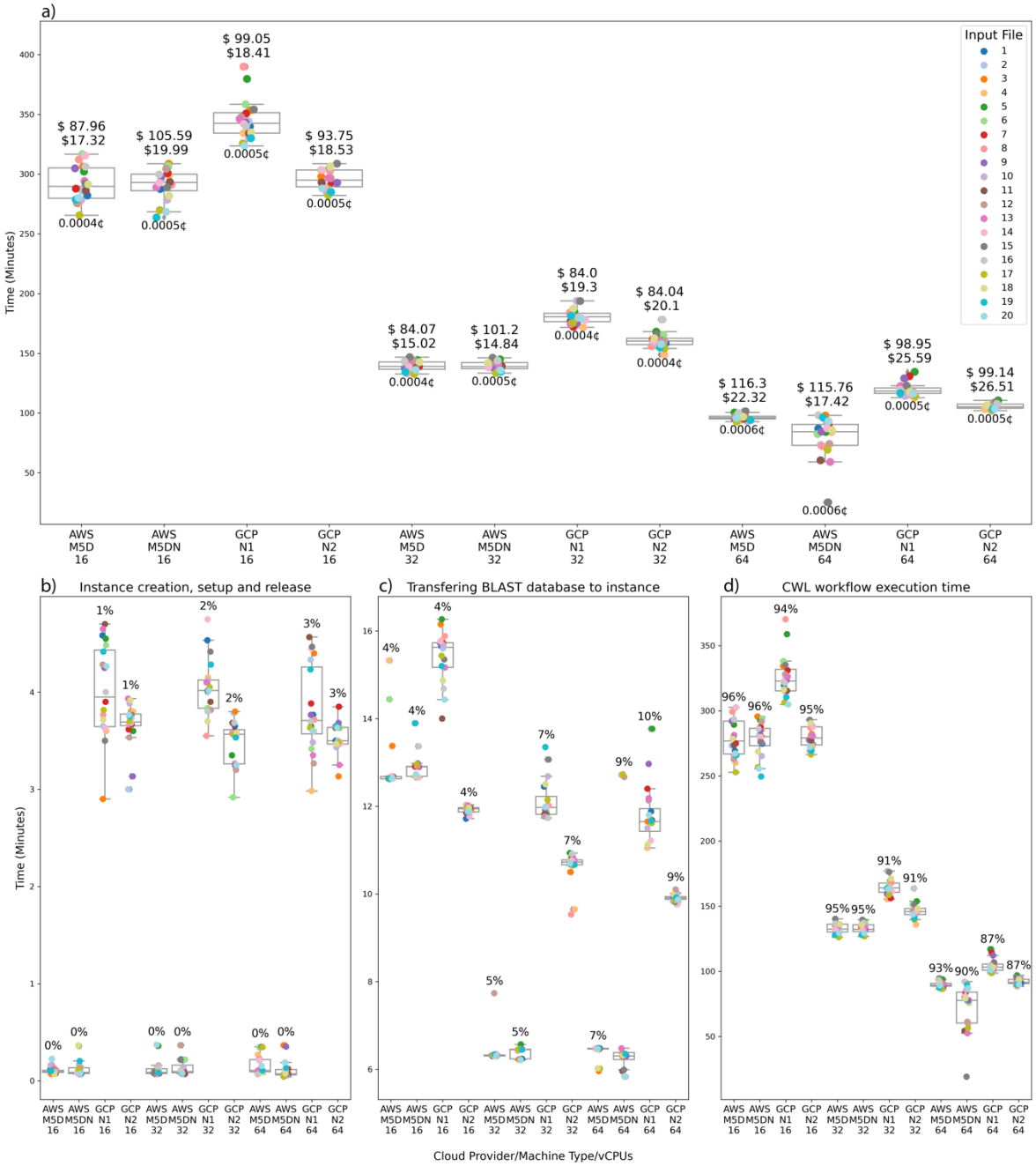

Figure 4: Time and cost for the 10,000 query size files. a) Total time for each input file for each configuration (Cloud provider/Machine Type/vCPUs). The total cost of processing the 20 input files (200,000 transcripts in total) is at the top of each box using normal and transitory instances. The cost of processing one transcript is at the bottom of each box. b) Time and percent of the total cost for instance creation, setup and release. c) Time and percent of the cost for transferring the BLAST databases to the instance from the cloud storage bucket (S3 in AWS and Cloud Storage in GCP). d) Time and percent of the cost for the CWL workflow execution.

types and their resources are described in Table 1. We used the transcriptome assembled from a public BioProject with ID PRJNA320545 for the organism *Opuntia streptacantha* (prickly pear cactus). The transcriptome includes 474,563 transcripts generated with Trinity [31], and is available in ***data/PRJNA320545/transcriptome.fasta.gz***. The transcriptome length distribution and statistical metrics are available in the 01 - Data Partitioning notebook.
From the *Opuntia* pool of transcripts, we analyzed three sizes of *query* files: 2,000, 6,000, and 10,000 transcripts in each input *query* file. Two experiments were executed. First 20 FASTA files (input files for the workflow) for each query size were randomly created, see notebook "01 - Data Partitioning". Each of these files were submitted independently as jobs to the batch systems on each cloud provider. For the second experiment, 120,000 transcripts were randomly selected and then partitioned in files with 2,000, 6,000, and 10,000 transcripts to analyze the relationship between query size, runtime and cost.
Jobs were submitted to each cloud platform using the notebook "02 - Google Cloud Platform" and "02 - AWS-Batch". In each notebook, the input files created for each experiment were copied to the respective cloud storage system, followed by job submissions for each configuration of machine type/CPU.

Four times were collected from the jobs:

1. the total run time
2. the time to transfer the BLAST databases to the instance local SSD disk
3. the time executing the CWL workflow
4. the time for creation, setup and release of the instance

Figure 4 shows the collected times for the 10,000-query size. In Figure 4a the total run time for each input file (each containing 10,000 transcripts) for a total of 200,000 transcripts processed for each cloud provider, machine type and the number of vCPUs.
In addition, each box in Figure 4a shows the total cost for the 20 files using normal and transitory instances (top) and the cost of processing one transcript (bottom). The bottom row with three plots shows the remaining three times collected from the jobs.
The total running time for the 10,000-query sized files are similar for the same number of vCPUs notwithstanding the cloud provider. Furthermore, this example shows how the running time can be reduced by more than a half by increasing the number of vCPUs. Unfortunately, this time reduction does not decrease the total cost of the project as the price per hour for machines with more vCPUs increases as well.
The AWS platform is more efficient than the GCP during the instance creation, setup and release, see Figure 4b. This step is only 0.1% of the total cost despite the docker image used in the study being hosted in the Google Container Registry. The GCP cost for this stage goes from 1.5% to 4.5% on bigger machines. The differences are due to the Amazon Elastic Container Service (ECS) which allocates new jobs on existing instances as soon as the instance gets free without releasing them, whereas GCP creates, sets up, and releases an instance for each job.
Transferring the BLAST databases from each cloud storage (S3 in AWS and Cloud Storage in GCP) bucket, Figure 4c, (current size is 342GB), to the instance local SSD disk is a crucial step in reducing the cost of the analysis. Initially, we tested the default parameters in both cloud providers which use network storage devices taking an average of 1 hour which is about 30 % of the total cost of the analysis and takes more time than the CWL workflow execution. After customizing both batch systems to use the instance local SSD disks, the time was reduced to a range of 4 % to 11 % of the total cost in the 10,000 query size.

As expected, the CWL workflow execution time is the most time-consuming part of the job, Figure 4d. All configurations show similar times for executing the CWL workflow. The GCP N1 machine type spent more time on the CWL workflow than the other machine types in all configurations because the GCP N1 is the Google first generation machine type with slower vCPUs.
Figure 5 shows the time and cost of processing 120,000 transcripts using second generation 64 vCPUs instances on each cloud provider. Reducing the number of transcripts per input file reduces the total run time but will also increase the cost of the analysis as more instances will be used. BLAST databases are transferred to more instances spending, on average, 10 minutes for each instance. For example, our experiment with the 10,000-query size processes all transcripts in about 105 minutes with a total cost of 59.37USD using 12 instances (GCP, N2, 64 CPU). Processing the same number of transcripts with a query size of 2,000 costs122.36USD with all transcripts processed in 43 minutes using 60 instances (GCP, N2, 64 CPU).

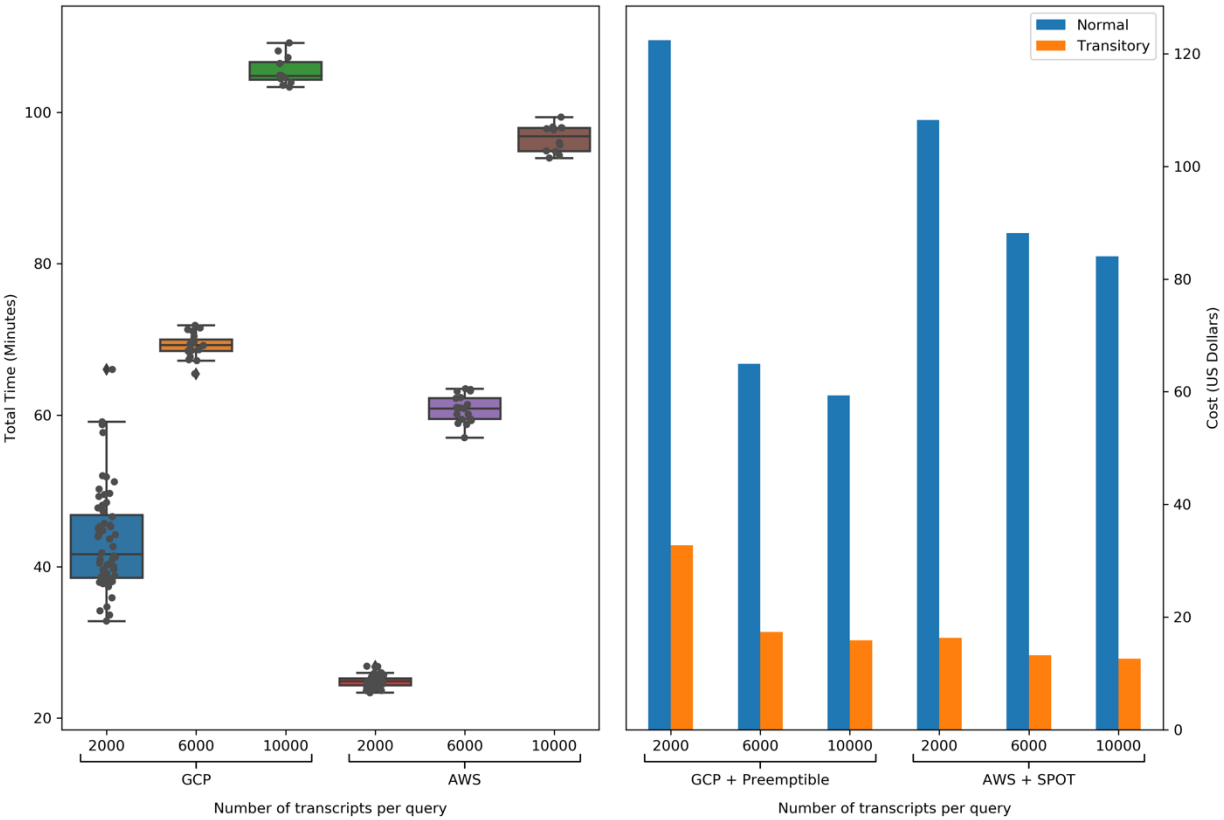

Figure 5: Left plot shows the total processing time for 120,000 transcripts using different query sizes. Right plot shows the total cost using normal compared to transitory instances.

We have determined that a transcriptome with ~500,000 transcripts can be processed in less than 2 hours with a compute cost ranging from 200USD to 250USD using normal instances. For transitory instances (SPOT in AWS and preemptible in GCP) the total cost could be reduced to 50USD for the complete analysis. However, the processing of all transcripts requires a flexible timeframe due to the availability of the transitory instances and the number of terminated jobs that require resubmission. In our opinion, these are reasonable costs that make the transcriptome annotation process in the cloud accessible to any genomic laboratory without access to an on-premise computational infrastructure.

Best practices

Our recommendation for best practices using public cloud providers for computational biology experiments are:

1. For reproducibility, write the pipeline using a workflow language.
   We recommend CWL because the resulting product is portable and scalable, and it can be executed across a variety of computational environments as dissimilar as personal laptops or the cloud. As mentioned above, CWL is the workflow language with many workflow managers available and they can be directly executed in a container using the **cwltool** runner.
2. Containerize the CWL workflow with Docker.
   Use Conda/Bioconda to install all Bioinformatics tools in the container image.
3. Use Jupyter Notebooks for coding and documenting each step during experiments.
4. Use the cloud provider batch system for deploying jobs.
5. Cloud computing behaves differently than local workstations or on-premise clusters.
   Users should define and execute small tests with their data and workflow before submitting large jobs. Testing different cloud services and configurations could help to reduce the runtime and cost for the whole analysis.
6. Use the instance local disks for computing instead the default network devices.
7. Use transitory instances to reduce the cost ONLY if there are no timeframe restrictions for completing the analysis.

Conclusion

Despite differences in the configuration and setup of batch systems between GCP and AWS, the cost and processing time are similar for the type of workflow we designed for our experiment. In our opinion, for BLAST-based workflows, the choice of a cloud platform is not dependent on the workflow but, rather, on the specific details of the cloud provider. These specific details are related to the accessibility of each cloud platform for institutional use, the technical knowledge of the specific platform services, and/or the availability of open-source frameworks to deploy the workflows on a specific cloud provider.
We found that GCP is easier to use as it only requires a JSON file for batch processing whereas AWS needs a complete setup of all batch system components. GCP is more suitable for daily data analysis work in research laboratories. On the other hand, AWS, once properly configured, is more efficient in terms of machine creation, setup and release. The ECS can reuse instances reducing the cost for large data analysis projects. AWS is more suitable for large data analysis groups to establish a set of queues and compute environments for multiple pipelines.

Availability of supporting source code and requirements

Project name: Cloud comparison for Transcript-Annotation data analysis pipeline
Project home page: https://github.com/ncbi/cloud-transcriptome-annotation
Operating system(s): Linux and MacOS
Programming languages: Python, BASH
Other requirements: Conda/Bioconda, Jupyter Notebook
CWL workflow:

https://github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl
CWL Viewer:
https://w3id.org/cwl/view/git/0d8650062673c8af2c1139c557afc4c3d6a1b53c/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl
RRID:

Data Availability

Snapshots of the GitHub archive are available in the *GigaScience* GigaDB repository [32].

Abbreviations

AMI: Amazon Machine Images (AMI)
API: Application Program Interface
AWS: Amazon Web Services
CDD: Conserved Domain Database
CWL: Common Workflow Language
ECS: Amazon Elastic Container Service
GCP: Google Cloud Platform
NCBI: National Center for Biotechnology Information
ORFs: Open Reading Frames
SSD: Solid State Disk
VPC: Virtual Private Cloud

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RVA, LMR and DL contributed to the design of the annotation workflow and the manuscript preparation. RVA designed, implemented and executed all cloud environments, configurations and experiments. All authors read and approved all versions of the manuscript.

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NCBI Cloud and System Group: Al Graeff, Brian Koser, Andrew Arensburger, Brad Plecs, Ron Patterson and Dima Beloslyudtsev for their support with the cloud platforms.

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Figure 1

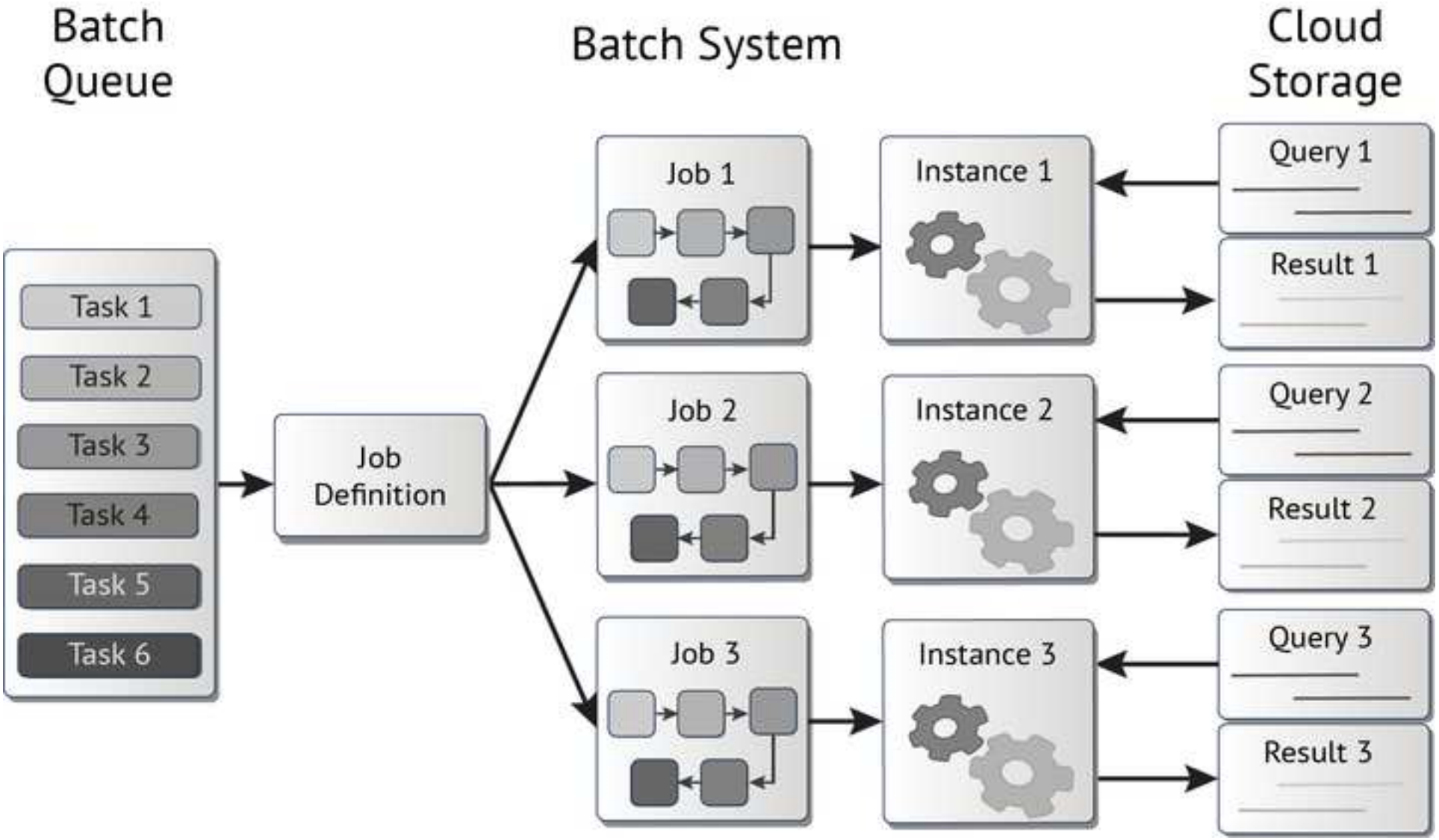

Figure 2

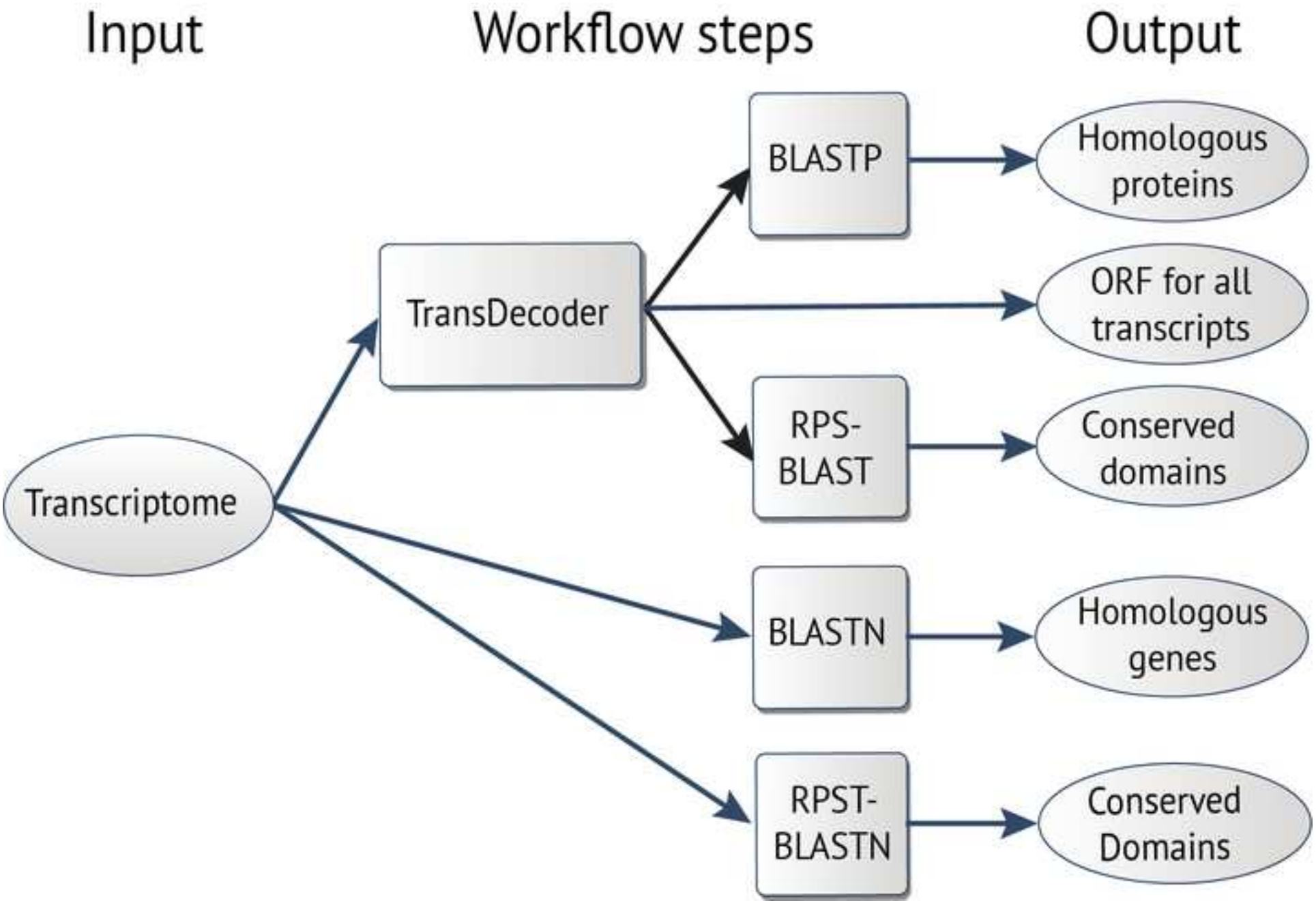

Figure 3

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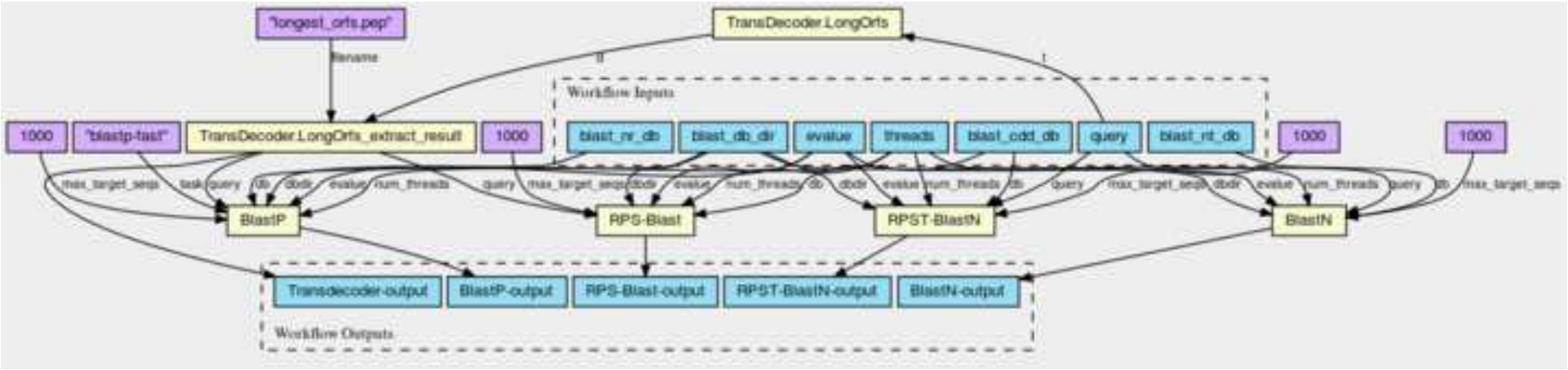

Figure 4

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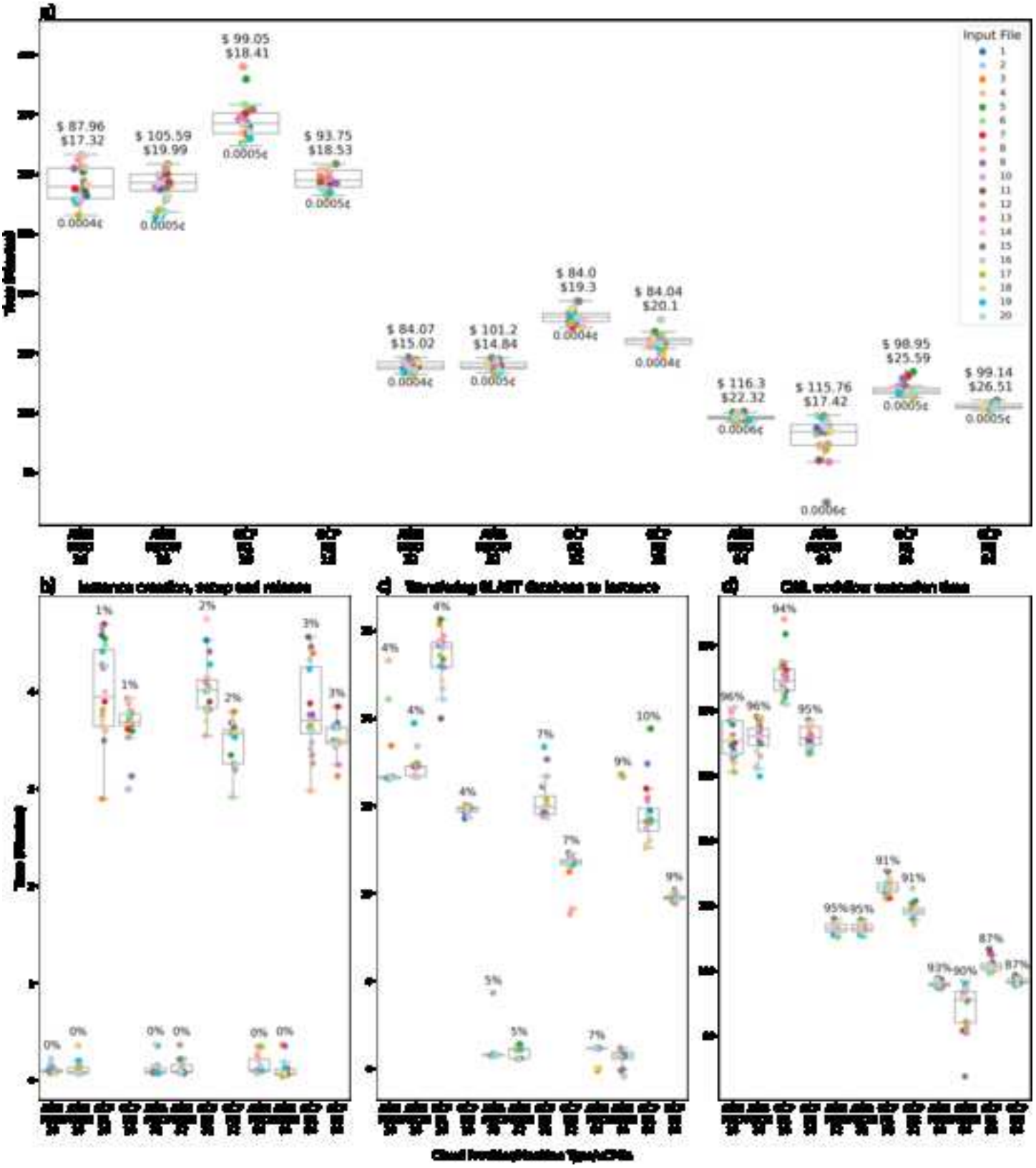
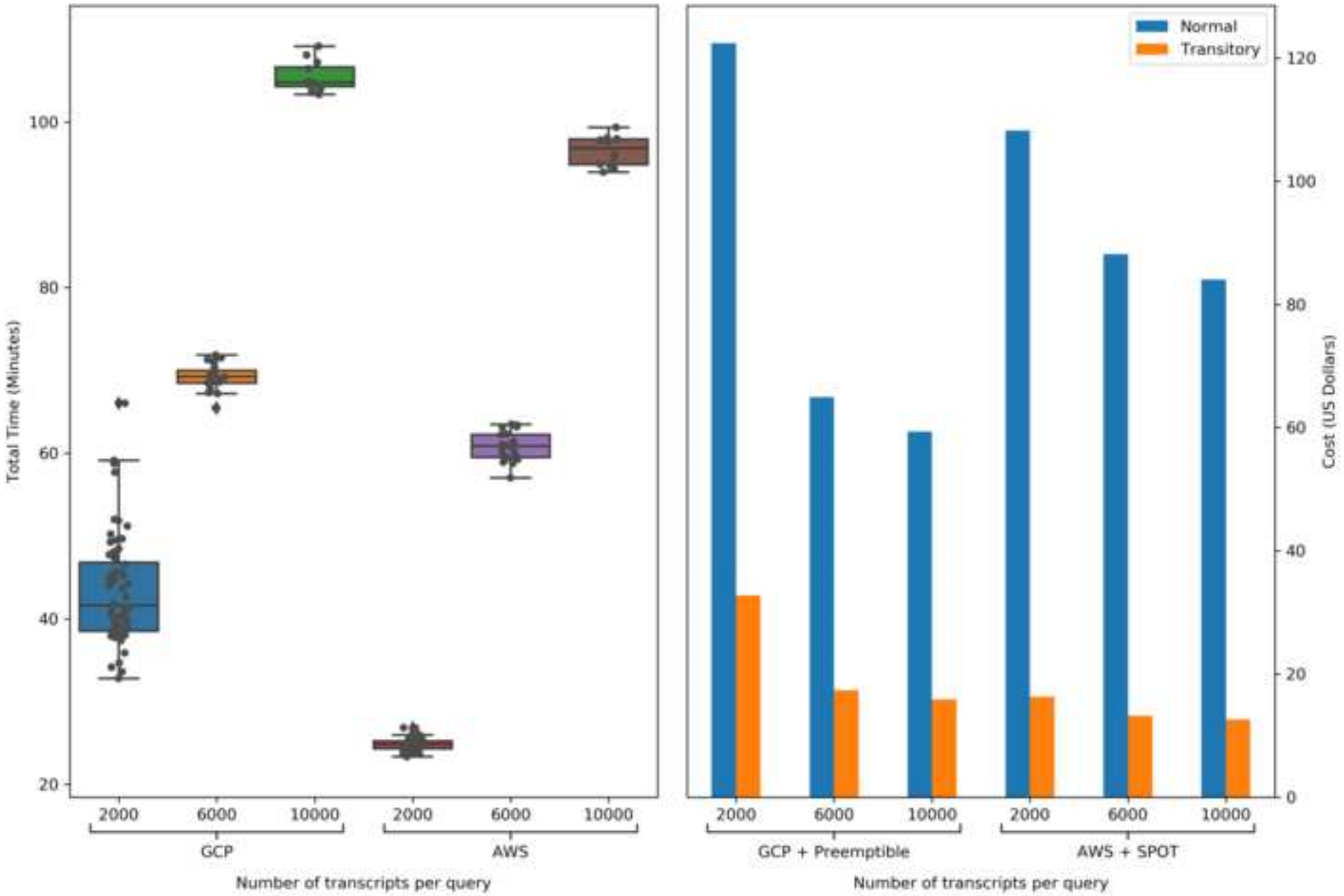


Figure 5



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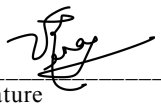
Name of publication: GigaScience



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December 7, 2020

Dear Editors,

We would like to resubmit our manuscript entitled "Transcriptome annotation in the cloud: complexity, best practices and cost" for your consideration for publication in *GigaScience as a Technical Note*.

We thank the editors and referees for the considerable time dedicated to our manuscript. We added a new figure with the workflow interactive schema and cloud instances region and accessed date as suggested by the editors and referees.

In this manuscript, we present a comparative study of multiple BLAST alignments using two public cloud providers: Amazon Web Services (AWS; Seattle, WA, USA) and Google Cloud Platform (GCP; Mountain View, CA, USA). These are the cloud providers partners of the **NIH Science and Technology Research Infrastructure for Discovery, Experimentation, and Sustainability (STRIDES) initiative**. We have prepared several Jupyter Notebooks with all the code required to submit BLAST jobs to the batch system on each cloud provider in order to reproduce or extend our results. We demonstrate that the public cloud providers are a practical alternative for executing advanced computational biology experiments at quite low cost. Using our cloud recipes, the BLAST alignments required to annotate a transcriptome with ~500,000 transcripts can be processed in less than 2 hours with a computing cost of about US$ 200-250.

All code used during this study is freely available at: https://github.com/ncbi/cloud-transcriptome-annotation

Thank you very much for your consideration of our manuscript. We look forward to your reply on this submission.

Sincerely,

Roberto Vera Alvarez [1,a], Leonardo Mariño-Ramírez [1,2,b] and David Landsman [1,c]

[1] Computational Biology Branch, National Center for Biotechnology Information, National Library of Medicine, Natioanl Insitutes of Health, Bethesda, MD, USA.
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Response to reviewers:

Response: We thank the editors and referees for the comments and for their time dedicated to this manuscript.

Editor:

Your manuscript "Transcriptome annotation in the cloud: complexity, best practices and cost." (GIGA-D-20-00202R1) has been assessed again by our re-reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in GigaScience, once you have carried out some final very minor revisions suggested by our re-reviewers. We require code and test data snapshots in our GigaDB repository and I've cc'd our curators to help you with this. As you discuss a CWL workflow I'd suggest including this as a figure and linked to the permalink in the legends so readers can interact with it:

https://view.commonwl.org/workflows/github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl

Response: We added a new paragraph and a new figure 3 to the manuscript with the workflow schema and the interactive link was added to the figure caption like:

Figure 3 shows the scheme of the transcriptome annotation workflow used in this study, interactive link is available in the caption of the figure.

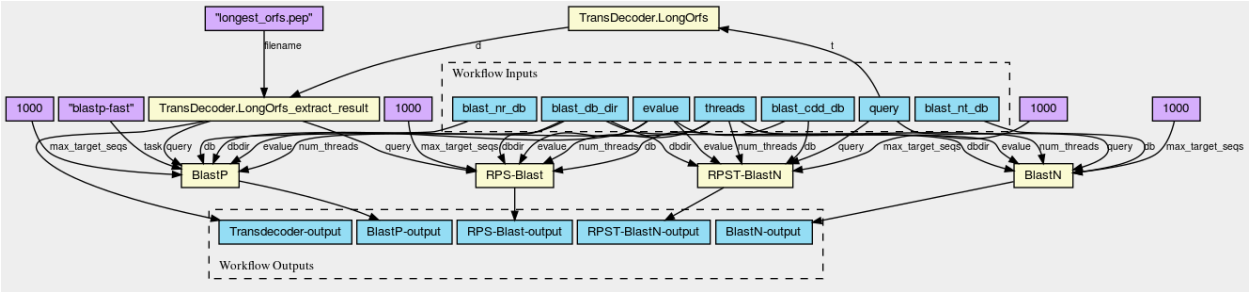

Figure 3: Transcriptome Annotation workflow schema, https://view.commonwl.org/workflows/github.com/ncbi/cloud-transcriptome-annotation/blob/master/bin/cwl-ngs-workflows-cbb/workflows/Annotation/transcriptome_annotation.cwl

Reviewer #1: I appreciate the authors' responses to the reviewer comments and their improvements in the manuscript. Overall, I do not find any further critical issues to be solved in the content. Below are a few minor suggestions:

1. Pricing of cloud usage:

Both AWS and GCP officially announces that they continue their efforts to reduce the computing cost regularly. It would be better to mention that the pricing of cloud instances may change in the future as well as the instance selections, and that may lead to the change of the study's conclusion. Table 1 also should have information about the date and the region.

Response: We added two columns (Region and Last used Date) to Table 1 as recommended by the referee. WE also modified the Table caption to:

Table 1:Machine types with resources in each cloud. Prices and instance type may change in the future as is common practice of cloud providers

2. The container image registry

In the experiments with both vendors, the authors used the same docker image hosted in the Google Container Registry. It means that the GCP has an advantage in the transfer of the container image, which may take some time during the instance setup. It would be fair to mention it or mention that the pulling time too short to be worth consideration.

Response: We agreed with the referee that we should mention that the docker image was hosted in the Google Container Registry. However, this fact does not represent any limitation for AWS as the download of the image don't affect the performance of AWS instances. We added these sentences to the Result and Discussion section:

The AWS platform is more efficient than the GCP during the instance creation, setup and release, see Figure 4b. This step is only 0.1% of the total cost despite the docker image used in the study being hosted in the Google Container Registry.

I am looking forward to the publication of this manuscript, which may give many insights into practical cloud usage to the readers.