

Bio Resources at CARC

Center for Advanced Research Computing

University of Southern California

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Research Facilitator in Life Science



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Bio Resources (updated twice a year)

<https://carc.usc.edu/user-information/bio-resources>

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About Services **User Information** Education & Outreach News & Events **User Support**

The CARC strives to provide a complete and comprehensive user support experience.

Getting Started
Accounts And Allocations
System Information
User Guides

CARC User Portal
CARC User Forum
CARC OnDemand
Condo Cluster Program

Bio Resources
Using USC's Cryo-EM Instruments
Frequently Asked Questions

New Collaboration with Amgen

Brings Cryo-EM to USC

Phase 1 of the cryogenic electron microscopy (cryo-EM) project is now complete.

[Read More](#)

Bio Resources (updated twice a year)

User Information

- Getting Started
- Accounts and Allocations
- System Information
- User Guides
- CARC User Portal
- CARC User Forum
- CARC OnDemand
- Condo Cluster Program
- Bio Resources**
- Using USC's Cryo-EM Instruments
- Frequently Asked Questions

Bio Resources

Reference genomes, protein and nucleotide sequences databases, and other bio resources are now available on Discovery and Endeavour. If you need a specific release that is not currently included in the pages below, please [submit a help ticket](#) and we will try to make those resources available to you.

Genomes

A set of ready-to-use reference sequences and annotations for commonly analyzed organisms, sourced from iGenomes.

Genbank

The GenBank sequence database is an open access, annotated collection of all publicly available nucleotide sequences and their protein translations.

Genome Taxonomy Database (GTDB)

The Genome Taxonomy Database (GTDB) is an initiative to establish a standardized microbial taxonomy based on genome phylogeny.

Pfam Database

The Pfam database is a large collection of protein families, each represented by multiple sequence alignments and hidden Markov models (HMMs).

TIGRFAMs

TIGRFAMs is a resource consisting of curated multiple sequence alignments, Hidden Markov Models (HMMs) for protein sequence classification, and associated information designed to support automated annotation of (mostly prokaryotic) proteins.

UniProt

The Universal Protein Resource (UniProt), a collaboration between the European Bioinformatics Institute (EBI), the SIB Swiss Institute of Bioinformatics, and the Protein Information Resource (PIR)

Bio Resources (updated twice a year)

<https://carc.usc.edu/user-information/bio-resources>

- **Genomes** - reference sequences and annotations for commonly analyzed organisms
- **Genbank** - collection of all public nucleotide sequences and their protein translations
- **Genome Taxonomy Database (GTDB)** - an initiative to establish a standardized microbial taxonomy based on genome phylogeny
- **Pfam Database** - large collection of protein families, each represented by multiple sequence alignments and hidden Markov models (HMMs)
- **TIGRFAMs** - curated multiple sequence alignments, Hidden Markov Models (HMMs) for protein sequence classification
- **UniProt** – UniProtKB (curated protein information), UniRef (closely related sequences), UniParc (all protein sequences, consisting only of unique identifiers and sequences)

Bio Resources (through command line)

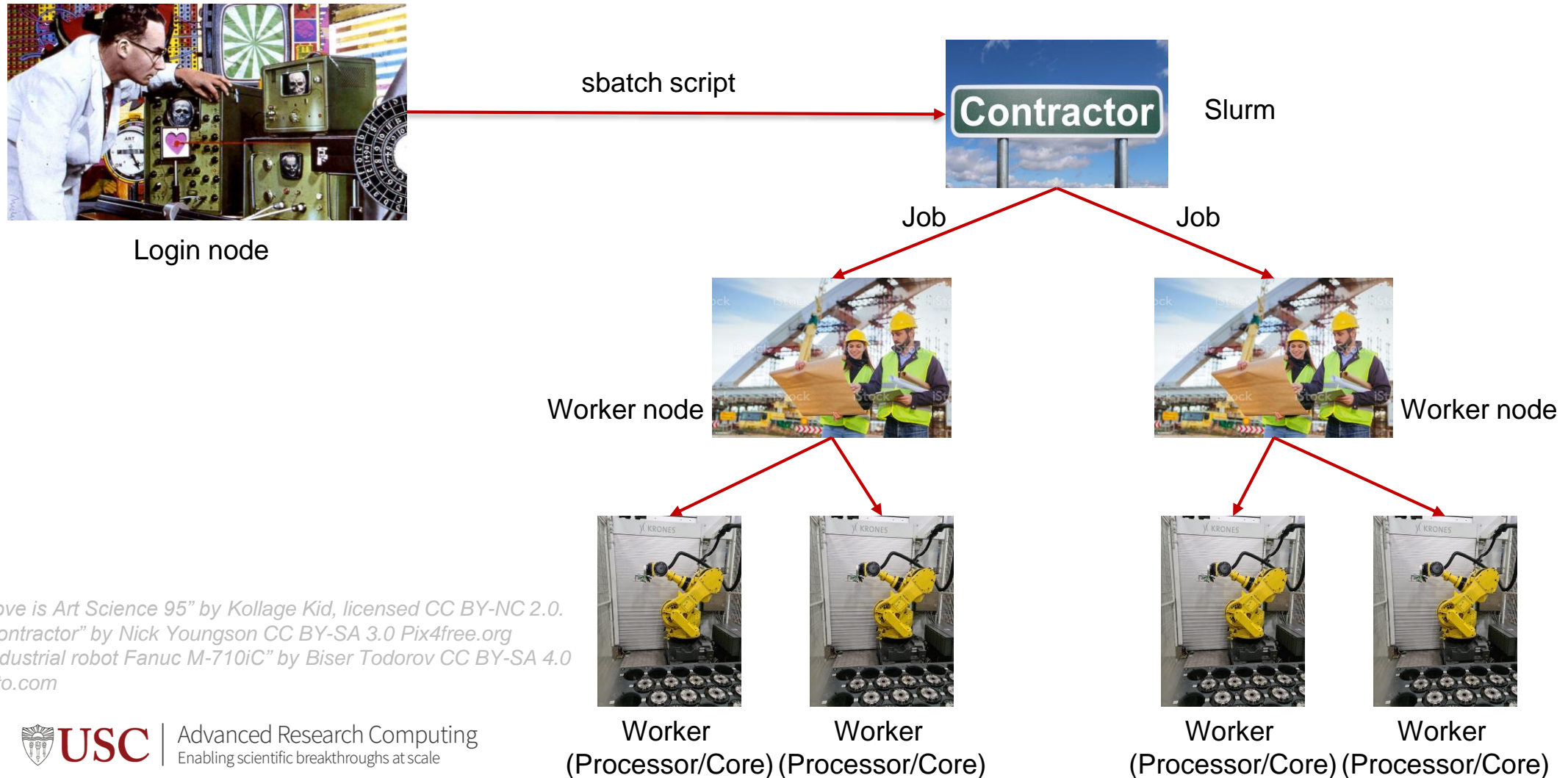
<https://carc.usc.edu/user-information/bio-resources>

- **Biogeotrases** - set of metagenomes, collected under the auspices of the bioGEOTRACES component of the international GEOTRACES program
`/project/biodb/biogeotrases`
- **TaraOceans** - marine microbial metagenomes sampled across space and time
`/project/biodb/taraoceans`
- **Variant Effect Predictor cache** - VEP can use a variety of annotation sources to retrieve the transcript models used to predict consequence types. Cache contains all transcript models, regulatory features and variant data for a species and allows for an offline use of VEP
`/project/biodb/vep-cache`

Some terms

- **Head Node** – The system that controls the cluster
- **Worker (Compute) Node** – Systems that perform the computations in a cluster
- **Login Node** – System that users log into to use a cluster
- **Scheduler** – Software that controls when jobs are run and the node they are run on
- **Shell** – A program that users employ to type commands
- **Script** – A file that contains a series of commands that are executed
- **Job** – A chunk of work that has been submitted to the cluster

How does it work?



What partition should I use?

- **debug** - small, short or test jobs that take less than 30m; short queue
- **main** (default) - most jobs (+older GPUs); up to 48h runtime (2 days)
- **epyc-64** - larger multithreaded jobs; up to 48h (2 days)
- **gpu** – jobs with GPUs (P100, V100, A100, A40); up to 48h (2 days)
- **largemem** - jobs that require huge amount of memory (up to 1TB); up to 168h (7 days)
- **oneweek** - long running jobs; up to 168h (7 days)

Lets get going

- Detailed policies and directions
 - <https://carc.usc.edu/user-information/getting-started>
- Do not install software yourself, contact us
 - <https://carc.usc.edu/education-and-outreach/office-hours> (Tue, 2:30-5:00)
 - Submit a ticket! (<https://carc.usc.edu/user-support/>)
 - When we install software, it is available to everyone
- Program running slow? *Submit a ticket!*
- Don't know what resources to use? *Submit a ticket!*
- Any other questions? *Submit a ticket or visit our forum*

Important Things to Note

- Job length
 - If over 24 hours, can this be split up, can threads be increased?
- Many small files
 - To be avoided!
 - Group into larger units (larger files or jobs) if possible
- Data
 - Save space by removing temp files
 - Archive data as soon as reasonable
 - Let us know if you are adding several TB of data
 - Use /scratch1 or /scratch2 whenever possible for temporary files

Important Things to Note

- Make sure you are not on the login node when you launch an application
 - You can check the system you are on by typing `hostname`
- Make sure you reserve as many processors as you need
 - A mismatch here can increase your runtime or wait time
- Make sure you reserve as much RAM as needed
 - Overestimating increases wait time, underestimating crashes
- Know which resources work the best
 - Sometimes using a debug or epyc-64 is better

Log into CARC OnDemand

- Open the web browser and go to:
 - <https://carc-ondemand.usc.edu>
- Enter your username and password
- Choose an option in Duo-2FA, and confirm your access
- Done. You should see the CARC OnDemand dashboard

CARC OnDemand Dashboard

CARC OnDemand Files ▾ Jobs ▾ Clusters ▾ Interactive Apps ▾ My Interactive Sessions

Help ▾ Logged in as osinski Log Out



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OnDemand provides an integrated, single access point for all of your HPC resources.

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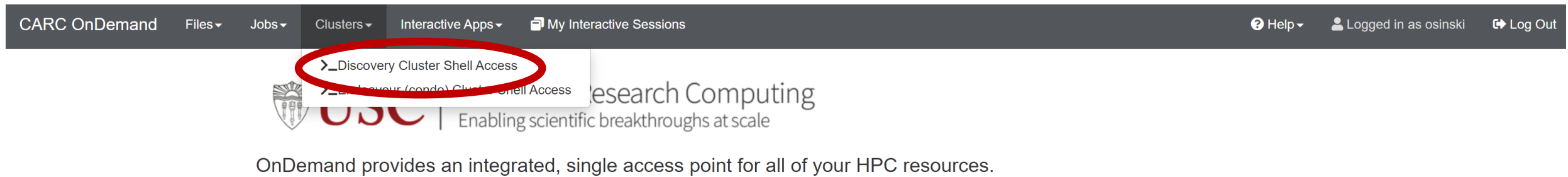
OPEN OnDemand

OnDemand version: v1.8.18



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CARC OnDemand Dashboard



CARC OnDemand Files Jobs Clusters Interactive Apps My Interactive Sessions

Help Logged in as osinski Log Out

Discovery Cluster Shell Access

Environ (conda) Cluster Shell Access

Research Computing

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OnDemand provides an integrated, single access point for all of your HPC resources.

CARC OnDemand Dashboard

```
Duo two-factor login for osinski
Enter a passcode or select one of the following options:

1. Duo Push to XXX-XXX-4132
2. Phone call to XXX-XXX-4132
3. SMS passcodes to XXX-XXX-4132

Passcode or option (1-3): 1
Success. Logging you in...
Last login: Thu Mar 3 15:16:56 2022 from 10.21.74.187
-----
      Welcome to the Center for Advanced Research Computing (CARC)
      at the University of Southern California (USC)
-----

CARC website : https://www.carc.usc.edu
User support : https://www.carc.usc.edu/user-support
User portal  : https://hpcaccount.usc.edu

** Unauthorized use/access is prohibited **

If you log on to this computer system, you acknowledge your awareness of and
concurrency with the USC CARC Acceptable Use Policy. USC will prosecute
violators to the full extent of the law.
-----

[osinski@discovery2 ~]$ █
```

Copy the text below:

git clone <https://github.com/uschpc/workshop-bioresources.git>

and paste it in the terminal by pressing Shift+Ins keys simultaneously, then press Enter

CARC OnDemand Dashboard

```
Duo two-factor login for osinski
Enter a passcode or select one of the following options:

1. Duo Push to XXX-XXX-4132
2. Phone call to XXX-XXX-4132
3. SMS passcodes to XXX-XXX-4132

Passcode or option (1-3): 1
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** Unauthorized use/access is prohibited **

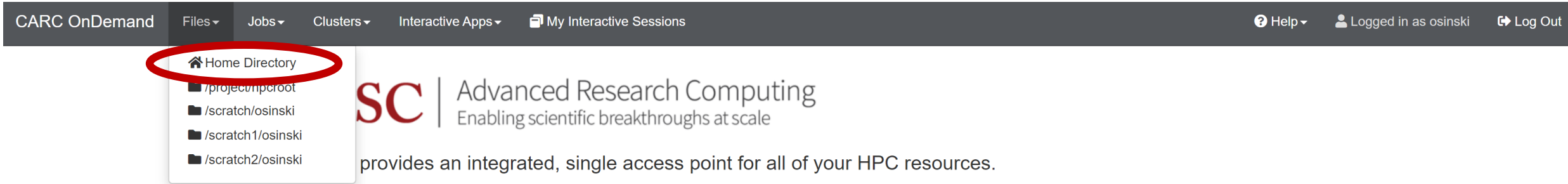
If you log on to this computer system, you acknowledge your awareness of and
concurrency with the USC CARC Acceptable Use Policy. USC will prosecute
violators to the full extent of the law.

-----

[osinski@discovery2 ~]$ git clone https://github.com/uschpc/workshop-bioresources.git
Cloning into 'workshop-bioresources'...
remote: Enumerating objects: 22, done.
remote: Total 22 (delta 0), reused 0 (delta 0), pack-reused 22
Unpacking objects: 100% (22/22), done.
Checking out files: 100% (23/23), done.
[osinski@discovery2 ~]$
```



CARC OnDemand: File Management



The screenshot shows the CARC OnDemand web interface. The top navigation bar is dark grey with the following items: "CARC OnDemand", "Files" (highlighted with a red circle), "Jobs", "Clusters", "Interactive Apps", and "My Interactive Sessions". On the right side of the navigation bar are links for "Help", "Logged in as osinski", and "Log Out". Below the "Files" menu, a dropdown list is visible with the following options: "Home Directory" (highlighted with a red circle), "/project/ncroot", "/scratch/osinski", "/scratch1/osinski", and "/scratch2/osinski". The main content area features the USC logo and the text "Advanced Research Computing" and "Enabling scientific breakthroughs at scale". Below this, a paragraph states: "provides an integrated, single access point for all of your HPC resources."

powered by

OPEN **OnDemand**

OnDemand version: v1.8.18

CARC OnDemand: File Management

The screenshot displays the CARC OnDemand File Management interface. The left sidebar shows the 'Home Directory' tree with various folders. The main pane shows the contents of the directory `/home1/osinski/`. The top toolbar includes buttons for 'Go To...', 'Open in Terminal', 'New File', 'New Dir', 'Upload', 'Show Dotfiles', and 'Show Owner/Mode'. The main pane has a toolbar with 'View', 'Edit', 'Rename/Move', 'Download', 'Copy', 'Paste', and '(Un)Select All'. A table lists the files and directories in the current directory.

name	size	modified date
..	dir	
bin	dir	10/05/2021
bioresources-json-generators	dir	03/01/2022
condor-8.8-bak-end1	dir	11/03/2021
data	dir	03/04/2022
jsons	dir	01/02/2022
lammps-el7	dir	03/15/2022
local_modules	dir	03/12/2021
mhc	dir	09/16/2021
ondemand	dir	07/21/2021
other	dir	03/22/2022
out	dir	11/11/2021
perl5	dir	09/14/2021
relion	dir	03/11/2021
relion-3.1.1	dir	03/11/2021
relion-3.1.1_c10.0.230-k40	dir	07/12/2021
relion-3.1.1_c10.1.243-a60	dir	03/12/2021
relion-3.1.1_c10.1.243-k40x	dir	07/08/2021
slurm-workshop-sep2021	dir	09/17/2021
spack-dev	dir	03/31/2021
venv	dir	06/04/2021

Create the BLAST Job Script

- Replace **swissprot** with the path to the v5 of swissprot db obtained from <https://carc.usc.edu/user-information/bio-resources/genbank>

```
#!/bin/bash
#SBATCH --nodes 1
#SBATCH --ntasks 10
#SBATCH --partition debug
#SBATCH --time 00:05:00
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load blast-plus
echo "Start BLAST Job"
blastp -db swissprot -query blast/query.txt -out results/blast/results.txt -num_threads
$SLURM_NTASKS
echo "Finish BLAST Job"
```

Create the BLAST Job Script

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Genbank

GenBank and its collaborators receive sequences produced in laboratories throughout the world from more than 100,000 distinct organisms. GenBank has become an important database for research in biological fields and has grown in recent years at an exponential rate by doubling roughly every 18 months.

To access the data below, click the format/version of your choice, then select the database from the drop-down list. Click the clipboard icon to copy the on-disk location. You can then paste it in your submission scripts to use in your analysis.

/project/biodb/genbank/2022-01-01/swissprot



Date Downloaded	FASTA	V4	V5	Database
2021-03-01	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	16S_ribosomal_RN ▾
2022-01-01	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	swissprot ▾



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CARC OnDemand: Job Composer



OnDemand provides an integrated, single access point for all of your HPC resources.

CARC OnDemand: Job Creation

CARC OnDemand / Job Composer Jobs Templates

Help

Jobs

+ New Job

☆ Create Template

Edit Files

Job Options

Open Terminal

Submit

Stop

Delete

Show 25 entries

Search:

Created	Name	ID	Cluster	Status
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 1 of 1 entries

Previous 1 Next

Job Details

2420550

Job Name:

(default) Simple Sequential Job

Submit to:

Discovery Cluster

Account:

Not specified

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/1



CARC OnDemand: Job Creation

CARC OnDemand / Job ComposerJobsTemplatesHelp

Jobs

+ New Job

From Default Template

From Template

From Specified Path

From Selected Job

Open Terminal

Submit

Stop

Delete

Create Template

Search:

Created	Name	ID	Cluster	Status
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 1 of 1 entries

Previous1Next

Job Details

2420550

Job Name:

(default) Simple Sequential Job

Submit to:

Discovery Cluster

Account:

Not specified

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/1

Script name:

CARC OnDemand: Job Creation

Create a new job from a path

Path to source (Required)

Source path

Enter the path to a directory on the file system. The contents of this path will be copied to a new workflow.

Job Attributes (Optional)

Name

Script name

Cluster:

Account

Account is an optional field. If not set, the account may be auto-set by the submit filter.

CARC OnDemand: Job Creation

Job was successfully created.

Jobs

+ New Job

☆ Create Template

Edit Files

Job Options

Open Terminal

Submit

Stop

Delete

Show 25 entries

Search:

Created	Name	ID	Cluster	Status
March 22, 2022 8:35pm	blast1		Discovery Cluster	Not Submitted
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 2 of 2 entries

Previous 1 Next

Job Details

Job Name:

blast1

Submit to:

Discovery Cluster

Account:

osinski_703

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/2

CARC OnDemand: Editing a Job

Folder Contents:

- [blast1.job](#)
- [fastqc1.job](#)
- [fastqc2.job](#)
- [fastqc_numbered_array.job](#)
- [fastqc_unnumbered_array.job](#)

Submit Script

blast1.job

Script contents:

```
#!/bin/bash
#SBATCH --nodes 1
#SBATCH --ntasks 10
#SBATCH --partition debug
#SBATCH --time=00:05:00
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load blast-plus
echo "Example blast start"
sleep 20
blastp -db /project/biodb/genbank/2021-03-01/swissprot -query data/blast/query.txt -out
echo "Example blast end"
```

[Open Editor](#) [Open Terminal](#) [Open Dir](#)

CARC OnDemand: Editing a Job

Save

home1/osinski/ondemand/data/sys/myjobs/projects/default/2/blast1.job

Key Bindings

Default

Font Size

12px

```
1 #!/bin/bash
2 #SBATCH --nodes 1
3 #SBATCH --ntasks 10
4 #SBATCH --partition debug
5 #SBATCH --time=00:05:00
6 #SBATCH --account=ttrojan_001
7 #SBATCH --chdir /home1/ttrojan/workshop-bioresources
8 module purge
9 module load gcc/9.2.0
10 module load blast-plus
11 echo "Example blast start"
12 sleep 20
13 blastp -db /project/biodb/genbank/2021-03-01/swissprot -query data/blast/query.txt -out results/blast/results.txt -num_threads $SLURM_NTASKS
14 echo "Example blast end"
15 |
```

CARC OnDemand: Submitting a Job

CARC OnDemand / Job ComposerJobsTemplates

Help

Jobs

+ New Job

☆ Create Template

Edit FilesJob OptionsOpen TerminalSubmitStopDelete

Show 25 entriesSearch:

Created	Name	ID	Cluster	Status
March 22, 2022 8:35pm	blast1		Discovery Cluster	Not Submitted
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 2 of 2 entriesPrevious1Next

Job Details

Job Name:
blast1

Submit to:
Discovery Cluster

Account:
osinski_703

Script location:
/home1/osinski/ondemand/data/sys/myjobs/projects/default/2

Script name:
blast1.job

CARC OnDemand: Submitting a Job

CARC OnDemand / Job ComposerJobsTemplates

Help

Job was successfully submitted.

Jobs

+ New Job

Create Template

Edit FilesJob OptionsOpen TerminalSubmitStopDelete

Show 25 entriesSearch:

Created	Name	ID	Cluster	Status
March 22, 2022 8:35pm	blast1	7825946	Discovery Cluster	Running
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 2 of 2 entriesPrevious1Next

Job Details

7825946

Job Name:

blast1

Submit to:

Discovery Cluster

Account:

osinski_703

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/2

Script name:

CARC OnDemand: Results

CARC OnDemand / Job Composer Jobs Templates Help

Job was successfully submitted. ×

Jobs

+ New Job

☆ Create Template

Edit Files Job Options Open Terminal Submit Stop Delete

Show 25 entries

Search:

Created	Name	ID	Cluster	Status
March 22, 2022 8:35pm	blast1	7825946	Discovery Cluster	Completed
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 2 of 2 entries

Previous 1 Next

Job Details

7825946

Job Name:

blast1

Submit to:

Discovery Cluster

Account:

osinski_703

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/2

Script name:

CARC OnDemand: Results

CARC OnDemand / Job ComposerJobsTemplatesHelp

Job was successfully submitted.

Jobs

+ New Job

Create Template

Edit FilesJob OptionsOpen TerminalSubmitStopDelete

Show25entries

Search:

Created	Name	ID	Cluster	Status
March 22, 2022 8:35pm	blast1	7825946	Discovery Cluster	Completed
February 25, 2021 11:30am	(default) Simple Sequential Job	2420550	Discovery Cluster	Completed

Showing 1 to 2 of 2 entries

Previous1Next

Job Details

7825946

Job Name:

blast1

Submit to:

Discovery Cluster

Account:

osinski_703

Script location:

/home1/osinski/ondemand/data/sys/myjobs/projects/default/2

Script name:

CARC OnDemand: Results

The screenshot shows a web-based file manager interface. The left sidebar displays a tree view of the 'Home Directory' with various folders. The main panel shows the contents of the directory `/home1/osinski/workshop-bioresources/`. At the top of the main panel, there is a toolbar with buttons for 'Go To...', 'Open in Terminal', 'New File', 'New Dir', 'Upload', 'Show Dotfiles', and 'Show Owner/Mode'. Below this, there is another toolbar with buttons for 'View' (circled in red), 'Edit', 'Rename/Move', 'Download', 'Copy', 'Paste', and '(Un)Select All'. The file list below shows the following items:

name	size	modified date
..	dir	
data	dir	03/22/2022
jobs	dir	03/22/2022
presentation	dir	03/22/2022
results	dir	03/23/2022
LICENSE	6.88kb	03/22/2022
slurm-7825946.out	215b	03/23/2022

The file `slurm-7825946.out` is highlighted with a blue bar and is also circled in red.

CARC OnDemand: Results

```
=====
SLURM_JOB_ID = 7825946
SLURM_JOB_NODELIST = e09-18
TMPDIR = /tmp/SLURM_7825946
=====
"Example blast start"
"Example blast end"
```

CARC OnDemand: Results

The screenshot displays the CARC OnDemand file manager interface. The left sidebar shows the 'Home Directory' with a list of folders including 'bin', 'bioresources-json-generators', 'condor-8.8-bak-end1', 'data', 'jsons', 'lammmps-el7', 'local_modules', 'mhc', 'ondemand', 'other', 'out', 'perl5', 'relicon', 'relicon-3.1.1', 'relicon-3.1.1_c10.0.230-k40', 'relicon-3.1.1_c10.1.243-a60', 'relicon-3.1.1_c10.1.243-k40x', 'slurm-workshop-sep2021', 'spack-dev', 'venv', and 'workshop-bioresources'. The main panel shows the current directory path `/home1/osinski/workshop-bioresources/results/blast/` at the top, which is circled in red. Below the path, there is a toolbar with buttons for 'View', 'Edit', 'A-Z Rename/Move', 'Download', 'Copy', 'Paste', and '(Un)Select All'. The 'View' button is also circled in red. The file list below shows a table with columns 'name', 'size', and 'modified date'. The file 'results.txt' is highlighted in blue and circled in red. The file details are as follows:

name	size	modified date
..	dir	
results.txt	3.05kb	03/23/2022

CARC OnDemand: Results

Query=
Length=15

Sequences producing significant alignments:	Score (Bits)	E Value
Q9JK11.1 RecName: Full=Reticulon-4; AltName: Full=Foocen; AltName...	35.0	0.001
Q99P72.2 RecName: Full=Reticulon-4; AltName: Full=Neurite outgrow...	35.0	0.001
Q9NQC3.2 RecName: Full=Reticulon-4; AltName: Full=Foocen; AltName...	33.9	0.004

>Q9JK11.1 RecName: Full=Reticulon-4; AltName: Full=Foocen; AltName: Full=Glut4 vesicle 20 kDa protein; AltName: Full=Neurite outgrowth inhibitor; Short=Nogo protein [Rattus norvegicus]
Length=1163

Score = 35.0 bits (79), Expect = 0.001, Method: Composition-based stats.
Identities = 15/15 (100%), Positives = 15/15 (100%), Gaps = 0/15 (0%)

Query	1	HYLGLANKSVKDAMA	15
		HYLGLANKSVKDAMA	
Sbjct	1135	HYLGLANKSVKDAMA	1149

>Q99P72.2 RecName: Full=Reticulon-4; AltName: Full=Neurite outgrowth inhibitor; Short=Nogo protein [Mus musculus]
Length=1162

Score = 35.0 bits (79), Expect = 0.001, Method: Composition-based stats.

Job Arrays

- A way to run the same commands on many (hundreds, thousands) of datasets/samples.
- A variable called `$SLURM_ARRAY_TASK_ID` is used to determine the element of the array being run.
- `#SBATCH --array=1-1000`
- `$SLURM_ARRAY_TASK_ID` becomes 1 in first job, 2 in second job, etc...

Without Job Arrays – Numbered Files

```
#!/bin/bash
#SBATCH --nodes 1
#SBATCH --ntasks 1
#SBATCH --partition main
#SBATCH --time 00:05:00
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load fastqc
echo "Starting FastQC job"
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_1_50K.fastq
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_2_50K.fastq
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_3_50K.fastq
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_4_50K.fastq
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_5_50K.fastq
fastqc -o results/fastqc-rawseq-ordered raw-seq-ordered/yeast_6_50K.fastq
echo "Finish FastQC job"
```

Job Arrays – Numbered Files

- Here is an example SLURM script for a job array. Save as `fastqc_numbered_array.job`

```
#!/bin/bash
#SBATCH --nodes 1
#SBATCH --ntasks 1
#SBATCH --partition main
#SBATCH --time 00:05:00
#SBATCH --array=1-6
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load fastqc
echo "Starting FastQC job"
sleep 20
fastqc -o results/fastqc-rawseq-ordered-arr raw-seq-
ordered/yeast_${SLURM_ARRAY_TASK_ID}_50K.fastq
echo "Finish FastQC job"
```

Job Arrays – Unnumbered Files

- Start by creating a list of all of the unnumbered filenames
- Then create slurm array script for fastqc jobs that have unnumbered filenames

```
#!/bin/bash
#SBATCH --nodes 1
#SBATCH --ntasks 1
#SBATCH --partition main
#SBATCH --time 00:05:00
#SBATCH --array=1-6
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load fastqc echo "Starting FastQC job"
sleep 20
ls raw-seq/ > unnumbered-filenames.txt
line=$(sed -n -e "$SLURM_ARRAY_TASK_ID p" unnumbered-filenames.txt)
fastqc -o results/fastqc-rawseq-unordered raw-seq/${line}
echo "Finish FastQC job"
```

What is Wrong

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 1
#SBATCH --mem=1g
#SBATCH --nodes 1
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
# -----Commands-----
python3 /home1/ttrojan/script.py
```

What is Wrong

- The module is not loaded

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 1
#SBATCH --mem=1g
#SBATCH --nodes 1
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load python/3.7.6
# -----Commands-----
python3 /home1/ttrojan/script.py
```

What is Wrong II

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 20
#SBATCH --mem=10g
#SBATCH --nodes 1
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load blast-plus
# -----Commands-----
blastn -query fasta.file -db database_name -outfmt 6 \
-num_alignments 1 -num_descriptions 1 -out output_file
```

What is Wrong II

- Number of processors and no working directory

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 20
#SBATCH --mem=10g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load blast-plus
# -----Commands-----
blastn -query fasta.file -db database_name -outfmt 6 num_alignments 1 \ -num_descriptions 1 -out
output_file -num_threads 20
```

What is Wrong II

- Number of processors and no working directory
- Better to use `$SLURM_NTASKS`

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 20
#SBATCH --mem=10g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load blast-plus
# -----Commands-----
blastn -query fasta.file -db database_name -outfmt 6 num_alignments 1 \ -num_descriptions 1 -out
output_file -num_threads $SLURM_NTASKS
```


What is Wrong III

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 1
#SBATCH --mem=200g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/8.3.0
module load R
# -----Commands-----
Rscript /home1/ttrojan/R_example.R
```

What is Wrong III

- Wrong partition/mem requirements too high

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition epyc-64
#SBATCH --ntasks 1
#SBATCH --mem=200g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/8.3.0
module load R
# -----Commands-----
Rscript /home1/ttrojan/R_example.R
```

What is Wrong IV

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --nodes 1
#SBATCH --mem=4g
#SBATCH --ntasks 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/8.3.0
Module load cuda/10.0.130
module load motioncor2
# -----Commands-----
bash /home1/ttrojan/motioncor2.job
```

What is Wrong IV

- GPU resources not specified

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --nodes 1
#SBATCH --mem=4g
#SBATCH --ntasks 1
#SBATCH --gres=gpu:p100:1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/8.3.0
Module load cuda/10.0.130
module load motioncor2
# -----Commands-----
bash /home1/ttrojan/motioncor2.job
```

What is Wrong V

```
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 1
#SBATCH --mem=15g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load samtools
# -----Commands-----
samtools stats example.bam
```

What is Wrong V

- No bash shebang line, `#!/bin/bash`
- Can use long names for SBATCH parameters

```
#!/bin/bash
# -----SLURM Parameters-----
#SBATCH --partition main
#SBATCH --ntasks 1
#SBATCH --mem=15g
#SBATCH --nodes 1
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
# -----Load Modules-----
module purge
module load gcc/9.2.0
module load samtools
# -----Commands-----
samtools stats example.bam
```

Genome mapping and tools: Read mapping

- Aim: to find coordinates of reads in the reference genome.
- Challenges:
 - Millions of short sequences
 - Sequences are often paired
 - Errors are not randomly distributed
- Most popular programs are bowtie and bwa (both use Burrows-Wheeler Transform algorithm). Two-step approach:
 - Create an index for the reference genome (one time for one genome).
 - Map reads to the reference genome using this index

Genome mapping and tools – overview I

- FastQC
 - FastQC is a quality control application for high throughput sequence data
 - Checks the quality of their sequence data
 - Generates an HTML report

<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

Genome mapping and tools – overview II

- bowtie
 - The first version of bowtie [Langmead et al. 2009] is optimal for:
 - short reads (under 50 bp)
 - reads without indels (insertions/deletions)
- bowtie2
 - The second version of bowtie2 [Langmead & Salzberg 2012] is optimal for:
 - long reads (more than 50 bp)
 - reads with indels
 - various alignment options
- Each version has its own index file format (bowtie-build / bowtie2-build tools).
- A popular RNA-seq analysis toolset (tophat, cufflinks) is based on bowtie / bowtie2

<http://bowtie-bio.sourceforge.net>

Genome mapping and tools – overview III

- `bwa`
 - `bwa backtrack` [Li, Durbin 2009]:
 - for short reads (< 100bp)
 - `bwa bwasw` [Li, Durbin 2010]:
 - for long reads (70bp - 1Mbp)
 - short indels
 - `bwa mem` [Li 2013]:
 - for long reads (70bp - 1Mbp)
 - faster and more efficient

Genome mapping and tools – overview IV

- samtools package - A set of utilities for processing SAM/BAM files
- samtools view
 - convert a bam file into a sam file - `samtools view sample.bam > sample.sam`
 - Convert a sam file into a bam file - `samtools view -bS sample.sam > sample.bam`
 - Extract all the reads aligned to the range specified. An index of the input file is required
`samtools view -h -b sample_sorted.bam "chr1:10-13" > tiny_sorted.bam`
- samtools sort
`samtools sort unsorted_in.bam sorted_out`
- samtools index
`samtools index sorted.bam (creates an index file, sorted.bam.bai)`

<http://samtools.sourceforge.net>

Genome mapping and tools – overview IV

- samtools package - A set of utilities for processing SAM/BAM files

- samtools view

- convert a bam file into a sam file - `samtools view sample.bam > sample.sam`

- Convert a sam file into a bam file - `samtools view -bS sample.sam > sample.bam`

- Extract all the reads aligned to the range specified. An index of the input file is required

`samtools view -h -b sample_sorted.bam "chr1:10-13" > tiny_sorted.bam`

- samtools sort

`samtools sort unsorted_in.bam sorted_out`

- samtools index

`samtools index sorted.bam (creates an index file, sorted.bam.bai)`

<http://samtools.sourceforge.net>

-b: output BAM

-S: read SAM

add a proper header

Genome mapping and tools – overview IV

- **samtools flagstat** – report basic statistics

```
samtools flagstat sample.bam
```

An example of output:

```
4198456 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 duplicates
4022089 + 0 mapped (95.80%:-nan%)
4198456 + 0 paired in sequencing
2099228 + 0 read1
2099228 + 0 read2
3796446 + 0 properly paired (90.42%:-nan%)
4013692 + 0 with itself and mate mapped
8397 + 0 singletons (0.20%:-nan%)
167574 + 0 with mate mapped to a different chr
72008 + 0 with mate mapped to a different chr (mapQ>=5)
```

- **samtools faidx** – index a FASTA file

```
samtools faidx ref.fasta (creates an index file ref.fasta.fai)
```

- **samtools merge** – merge several BAM files into one

```
samtools merge out.bam in1.bam in2.bam
```

Genome mapping and tools – overview V

- BedTools package
 - `bamtobed` - Convert BAM alignments to BED (& other) formats
 - `bamtofastq` - Convert BAM records to FASTQ records
 - `bedtobam` - Convert intervals to BAM records
 - `closest` - Find the closest, potentially non-overlapping interval
 - `complement` - Extract intervals `_not_` represented by an interval file
 - `coverage` - Compute the coverage over defined intervals
 - `genomecov` - Compute the coverage over an entire genome
 - `getfasta` - Use intervals to extract sequences from a FASTA file
 - `intersect` - Find overlapping intervals in various ways
 - `shuffle` - Randomly redistribute intervals in a genome
 - `sort` - Order the intervals in a file

Genome mapping and tools – overview V

`bedtools intersect`

- Report the intervals that represent overlaps between your two files:

```
bedtools intersect -a cpg.bed -b exons.bed
```

- Report the original feature in each file:

```
bedtools intersect -a cpg.bed -b exons.bed -wa -wb
```

- How many base pairs of overlap were there?

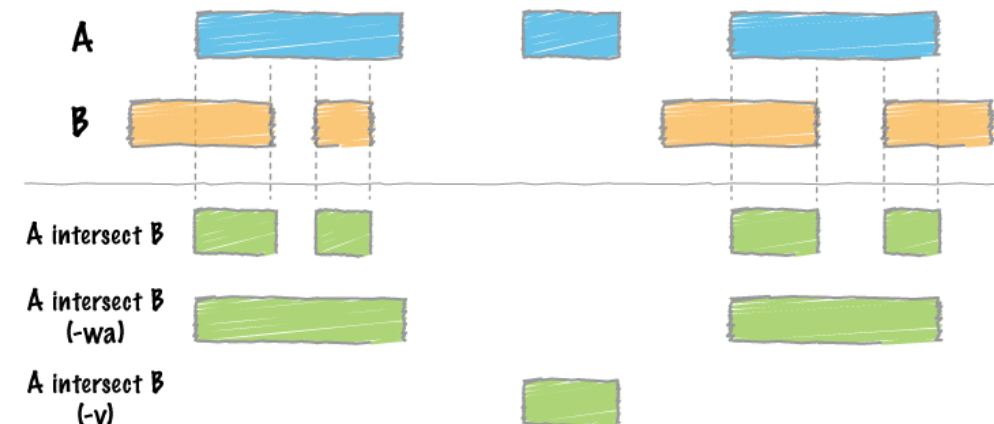
```
bedtools intersect -a cpg.bed -b exons.bed -wo
```

- Counting the number of overlapping features:

```
bedtools intersect -a cpg.bed -b exons.bed -c
```

- Find features that DO NOT overlap:

```
bedtools intersect -a cpg.bed -b exons.bed -v
```



Exercise

- There are paired reads of some DNA sequencing experiment of the human sample:

`bio-bootcamp/R1.fastq.gz`

`bio-bootcamp/R2.fastq.gz`

- You will study some particular region of the human genome
- Map reads to the human reference genome (version hg19 – find path on our Bio Resources)
- Extract reads that map to your region only
- Upload the reads to UCSC genome browser as a custom track
- count the number of insertions and deletions in SAM file

How To: Mapping

- load bowtie2 program:

```
module purge
module load gcc/9.2.0
module load bowtie2
```

- Copy sequence of a chromosome your region is located at as a FASTA file
 - find the path on our website in Homo sapiens > UCSC > hg19 > Chromosome 21
 - <https://carc.usc.edu/user-information/bio-resources/reference-genomes>
 - Add chr*.fa at the end of the path
 - `cp path_above /home1/ttrojan/bio-bootcamp/results/read-mapping`
- Map reads to this chromosome using `bowtie2` with the standard parameters

Don't forget to make an index (`bowtie-build2`) of the chromosome before mapping!

- You will get a SAM file as an output, convert to BAM (`samtools view`)
- Count the number of insertions and deletions in SAM file (use `cut` for field 6, and `grep`)

How To: Extracting reads

- load BedTools:

```
module purge
```

```
module load gcc/9.2.0
```

```
module load bedtools2
```

- Create a tab-delimited BED file with the coordinates of your region:

```
chr21 10000000 20000000
```

- Convert SAM file with mapped reads to BAM file using `samtools view`
- Use `bedtools intersect` to extract the reads from the BAM file

You'll need a BED file to upload the result to UCSC genome browser, so figure out how to make `bedtools intersect` to produce an output in BED format.

How To: UCSC custom track

- Upload the BED file to UCSC genome browser
'Add custom track' button → Choose file → Submit

Thanks to the whole CARC team:

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Reference material:

- HPCBio (Holmes J., Clark L., Drnevicch J., Valizadegan N.)
- CNRG (Davidson D., Leigh J.)
- Skoltech (Khrameeva E.)

Resources

- CARC home page
 - <https://carc.usc.edu>
- Bio Resources at CARC
 - <https://carc.usc.edu/user-information/bio-resources>
- CARC User Forum
 - <https://hpc-discourse.usc.edu/categories>
- SLURM tutorials
 - <https://slurm.schedmd.com/tutorials.html>
- SLURM quick reference
 - <https://slurm.schedmd.com/pdfs/summary.pdf>

Mapping exercise: Answers

```
#!/bin/bash
#SBATCH --partition main
#SBATCH --nodes 1
#SBATCH --ntasks 20
#SBATCH --time 01:00:00
#SBATCH --mem 4g
#SBATCH --account=ttrojan_001
#SBATCH --chdir /home1/ttrojan/workshop-bioresources
module purge
module load gcc/9.2.0
module load bowtie2
module load samtools
module load bedtools2
mkdir results/read-mapping
cp data/R*.gz results/read-mapping
gunzip results/read-mapping/R1.fastq.gz
gunzip results/read-mapping/R2.fastq.gz
cp -v /project/biodb/genomes/Homo_sapiens/UCSC/hg19/Sequence/Chromosomes/chr21.fa results/read-mapping/
bowtie2-build --threads $SLURM_NTASKS results/read-mapping/chr21.fa results/read-mapping/chr21index
bowtie2 --threads $SLURM_NTASKS -x results/read-mapping/chr21index -q results/read-mapping/R1.fastq > results/read-mapping/R1.sam
bowtie2 --threads $SLURM_NTASKS -x results/read-mapping/chr21index -q results/read-mapping/R2.fastq > results/read-mapping/R2.sam
samtools view results/read-mapping/R1.bam | cut -f 6 | grep -c 'D' > results/read-mapping/R1.no_of_deletions.txt
samtools view results/read-mapping/R1.bam | cut -f 6 | grep -c 'I' > results/read-mapping/R1.no_of_insertions.txt
samtools view results/read-mapping/R2.bam | cut -f 6 | grep -c 'D' > results/read-mapping/R2.no_of_deletions.txt
samtools view results/read-mapping/R2.bam | cut -f 6 | grep -c 'I' > results/read-mapping/R2.no_of_insertions.txt
samtools view -bS results/read-mapping/R1.sam > results/read-mapping/R1.bam
samtools view -bS results/read-mapping/R2.sam > results/read-mapping/R2.bam
samtools sort results/read-mapping/R1.bam > results/read-mapping/R1_sorted.bam
samtools sort results/read-mapping/R2.bam > results/read-mapping/R2_sorted.bam
samtools view -h -b results/read-mapping/R1_sorted.bam "chr21:10000000-20000000" > results/read-mapping/R1_sorted_region.bam
samtools view -h -b results/read-mapping/R2_sorted.bam "chr21:10000000-20000000" > results/read-mapping/R2_sorted_region.bam
bamToBed -i results/read-mapping/R1_sorted_region.bam > results/read-mapping/R1_sorted_region.bed
bamToBed -i results/read-mapping/R2_sorted_region.bam > results/read-mapping/R2_sorted_region.bed
bedtools intersect -a results/read-mapping/R1_sorted_region.bed -b results/read-mapping/R2_sorted_region.bed > results/read-mapping/reads.bed
```