High-Performance Computing & Biology

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slides: https://github.com/perugolate/hpc

I might use terms interchangeably but:

- a computing cluster is a type of HPC
- bioinformatics is type of data science

sorry if I confuse anyone

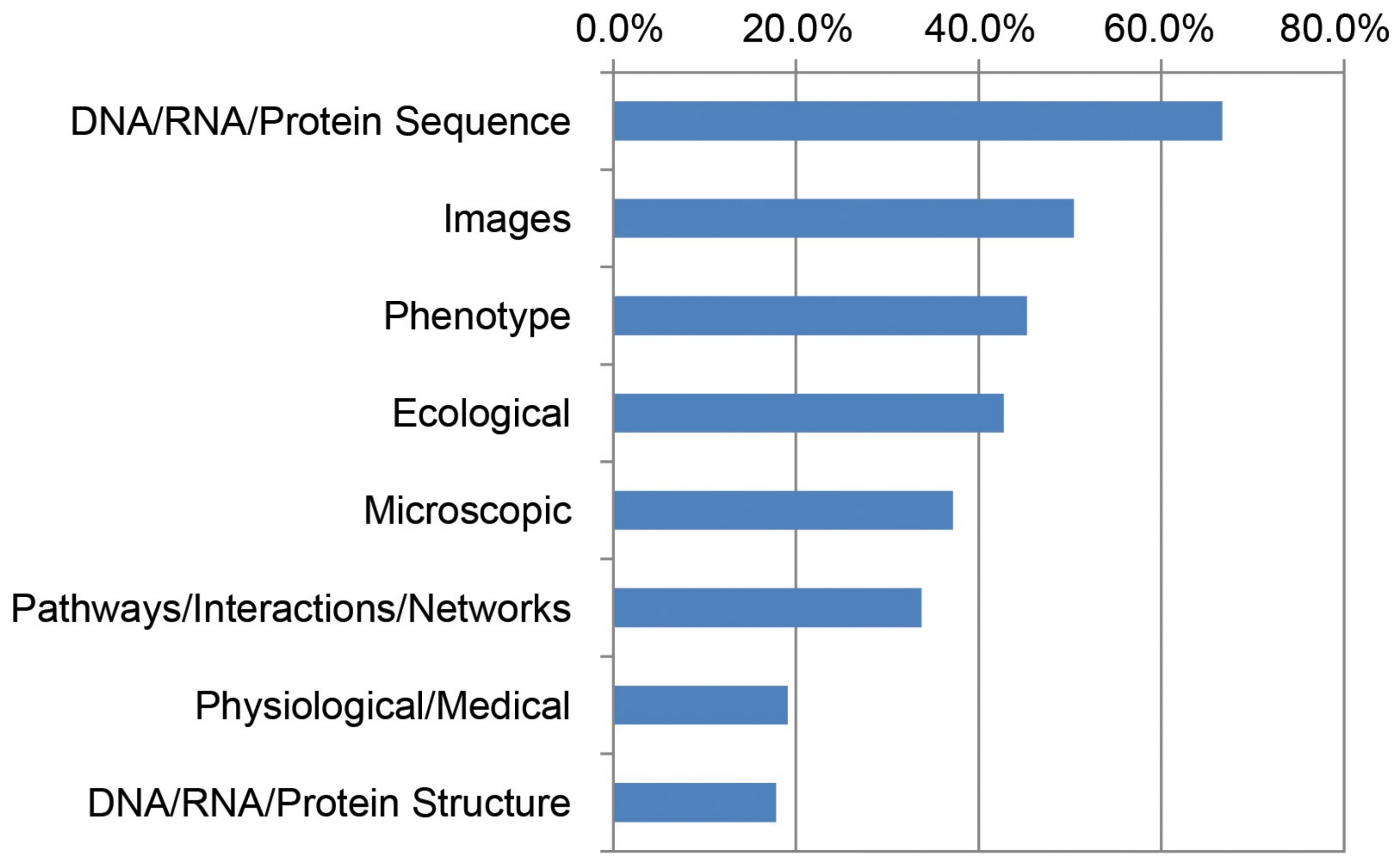
94% of students/faculty/researchers use large data sets or will in the near future (n = 1,097)

47% rated their bioinformatics skill level as “beginner,” (n = 608)

58% felt their institutions do not provide all the computational resources needed for their research (n = 1,024)

https://doi.org/10.1371/journal.pcbi.1005755

Biologists receive little training in data science...

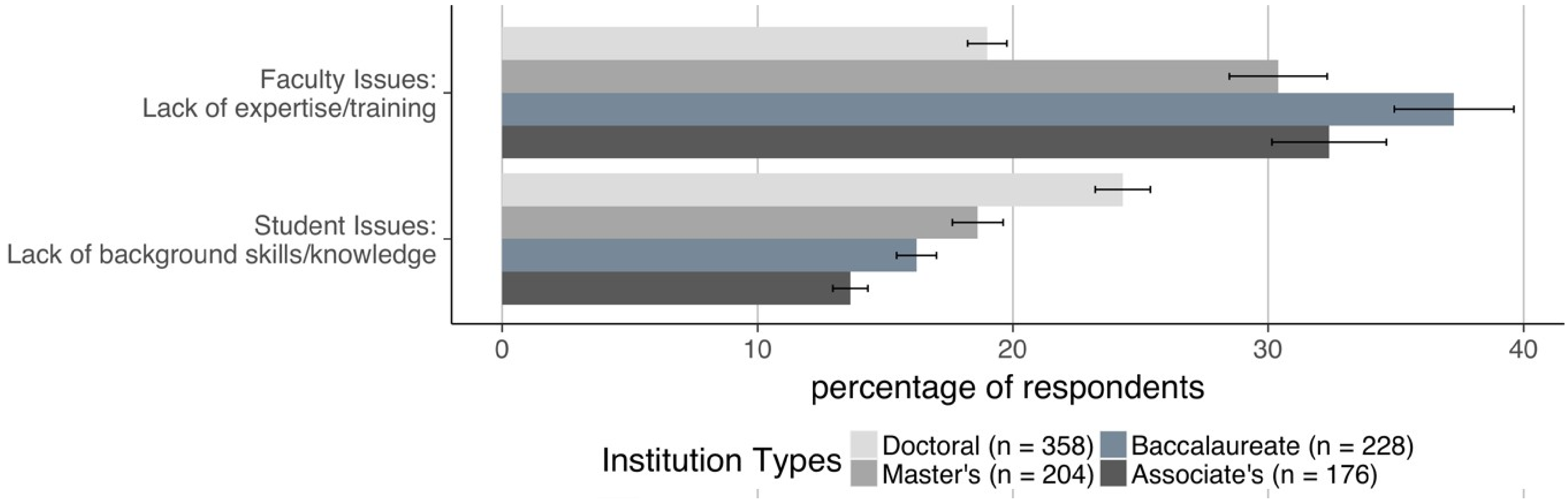


but most projects require it..

https://doi.org/10.1371/journal.pcbi.1005755

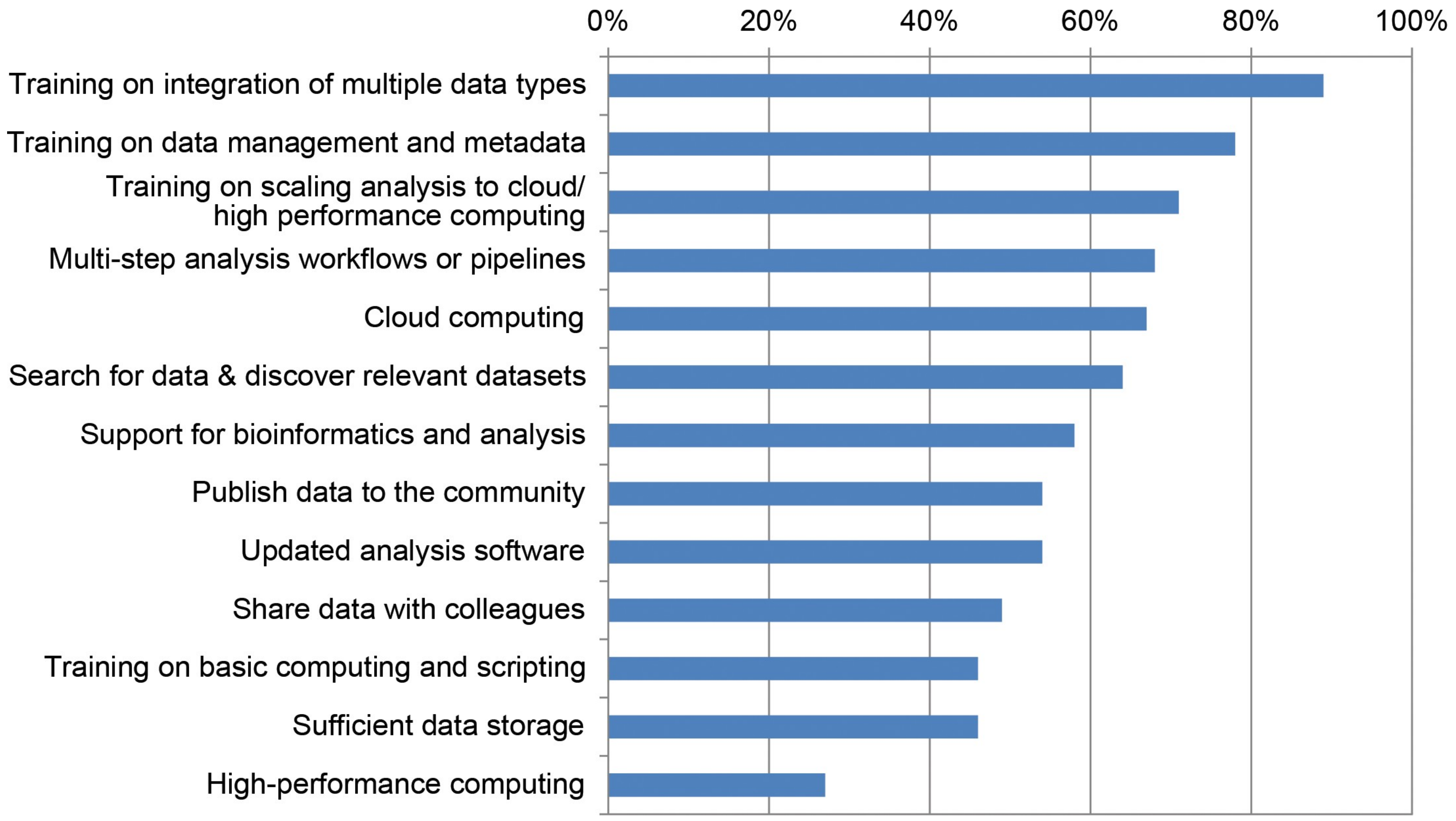
Barriers to teaching bioinformatics

Decade of Highest Degree Earned	Formal Bioinformatics Training (%)	Faculty Integrating Bioinformatics (%)
1980–1989	8.4	35.4
1990–1999	11.3	41.9
2000–2009	35.1	41.7
2010–2016	48.3	25.2



https://doi.org/10.1371/journal.pone.0224288

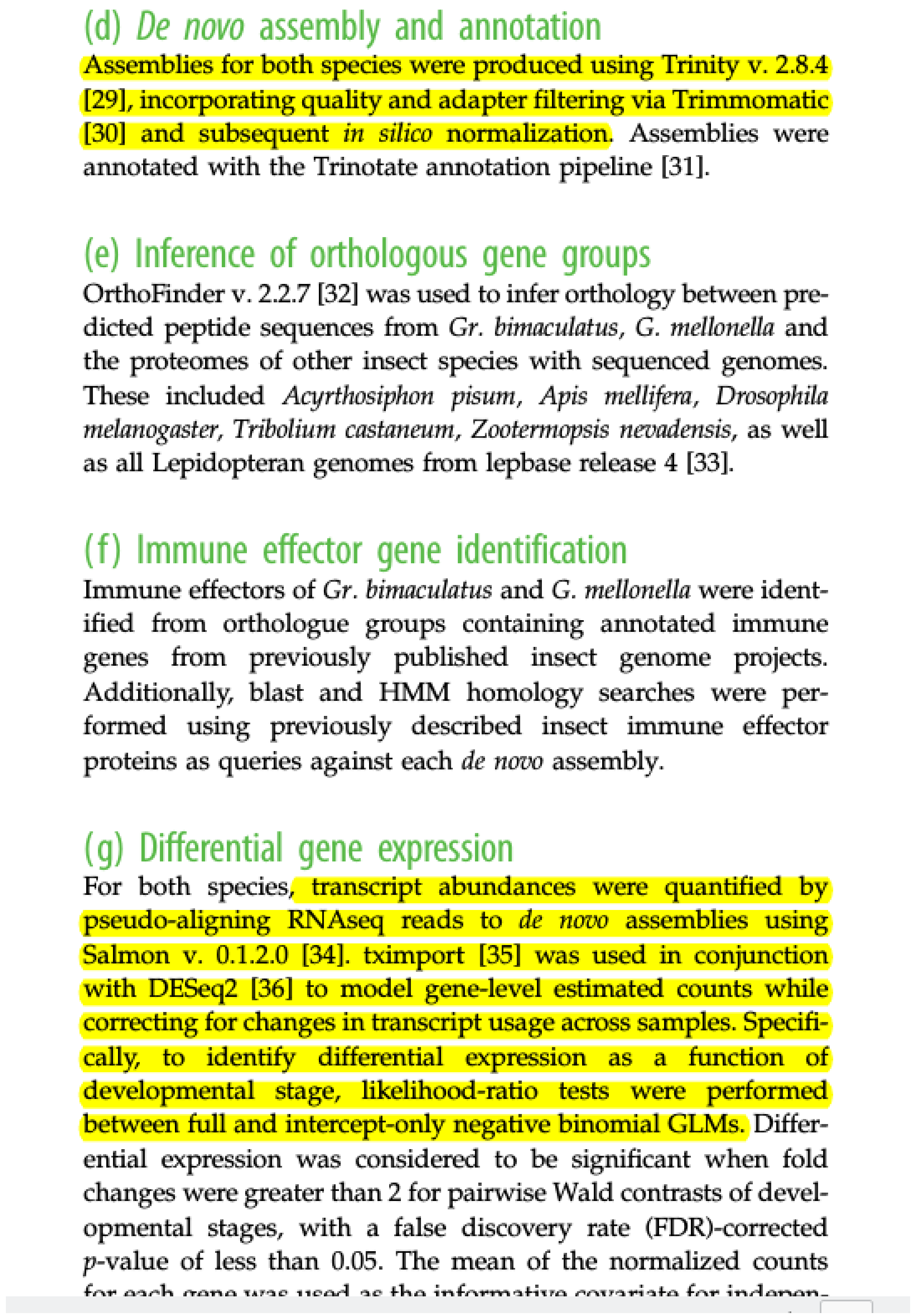
Unmet needs in bioinformatics



https://doi.org/10.1371/journal.pcbi.1005755

“These studies suggest a scenario of big data inundating unprepared biologists.”

https://doi.org/10.1371/journal.pcbi.1005755



(d) *De novo* assembly and annotation

Assemblies for both species were produced using Trinity v. 2.8.4 [29], incorporating quality and adapter filtering via Trimmomatic [30] and subsequent *in silico* normalization. Assemblies were annotated with the Trinotate annotation pipeline [31].

(e) Inference of orthologous gene groups

OrthoFinder v. 2.2.7 [32] was used to infer orthology between predicted peptide sequences from *Gr. bimaculatus*, *G. mellonella* and the proteomes of other insect species with sequenced genomes. These included *Acyrthosiphon pisum*, *Apis mellifera*, *Drosophila melanogaster*, *Tribolium castaneum*, *Zootermopsis nevadensis*, as well as all Lepidopteran genomes from lepbase release 4 [33].

(f) Immune effector gene identification

Immune effectors of *Gr. bimaculatus* and *G. mellonella* were identified from orthologue groups containing annotated immune genes from previously published insect genome projects. Additionally, blast and HMM homology searches were performed using previously described insect immune effector proteins as queries against each *de novo* assembly.

(g) Differential gene expression

For both species, transcript abundances were quantified by pseudo-aligning RNAseq reads to *de novo* assemblies using Salmon v. 0.1.2.0 [34]. tximport [35] was used in conjunction with DESeq2 [36] to model gene-level estimated counts while correcting for changes in transcript usage across samples. Specifically, to identify differential expression as a function of developmental stage, likelihood-ratio tests were performed between full and intercept-only negative binomial GLMs. Differential expression was considered to be significant when fold changes were greater than 2 for pairwise Wald contrasts of developmental stages, with a false discovery rate (FDR)-corrected *p*-value of less than 0.05. The mean of the normalized counts for each gene was used as the informative covariate for indepen-

- The fine details of the computation aren’t reported (memory, storage, time etc)
- it’s easy for others to underestimate the required resources
- computing skill set is also unclear

Storage problems

RNAseq of 35 samples

- one lane of NovaSeq
- 35 compressed fastq files
- >400 GB (compressed)

Time problems

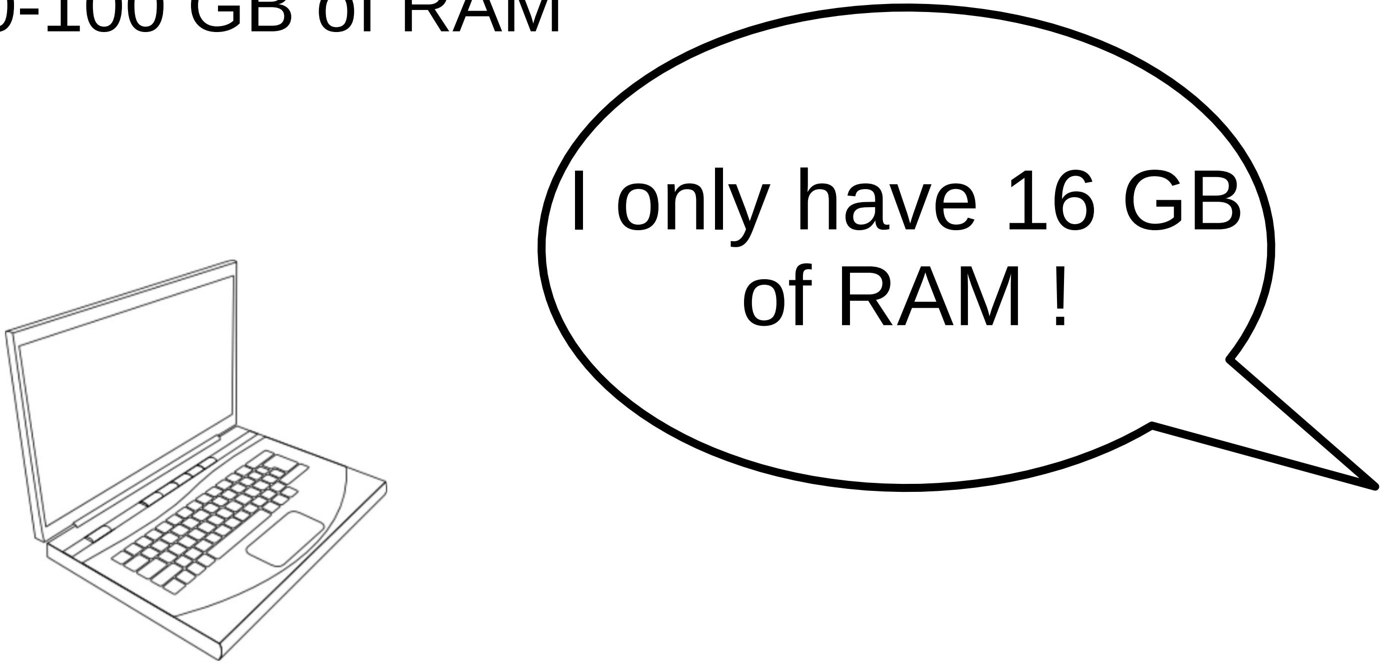
alignment of 35 RNAseq samples to genome

- Each alignment takes 5 hours
- Will run for 7 days
- Will use all resources

Memory problems

Quantification of 35 RNAseq samples gives

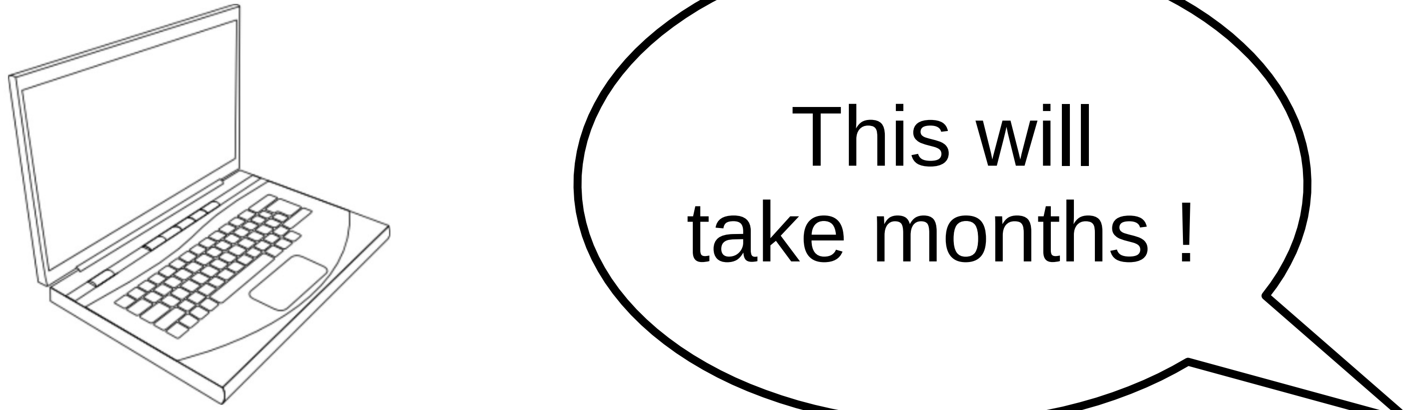
- a matrix of 10,000s of counts x 35
- need to fit 10,000s of models
- needs 20-100 GB of RAM



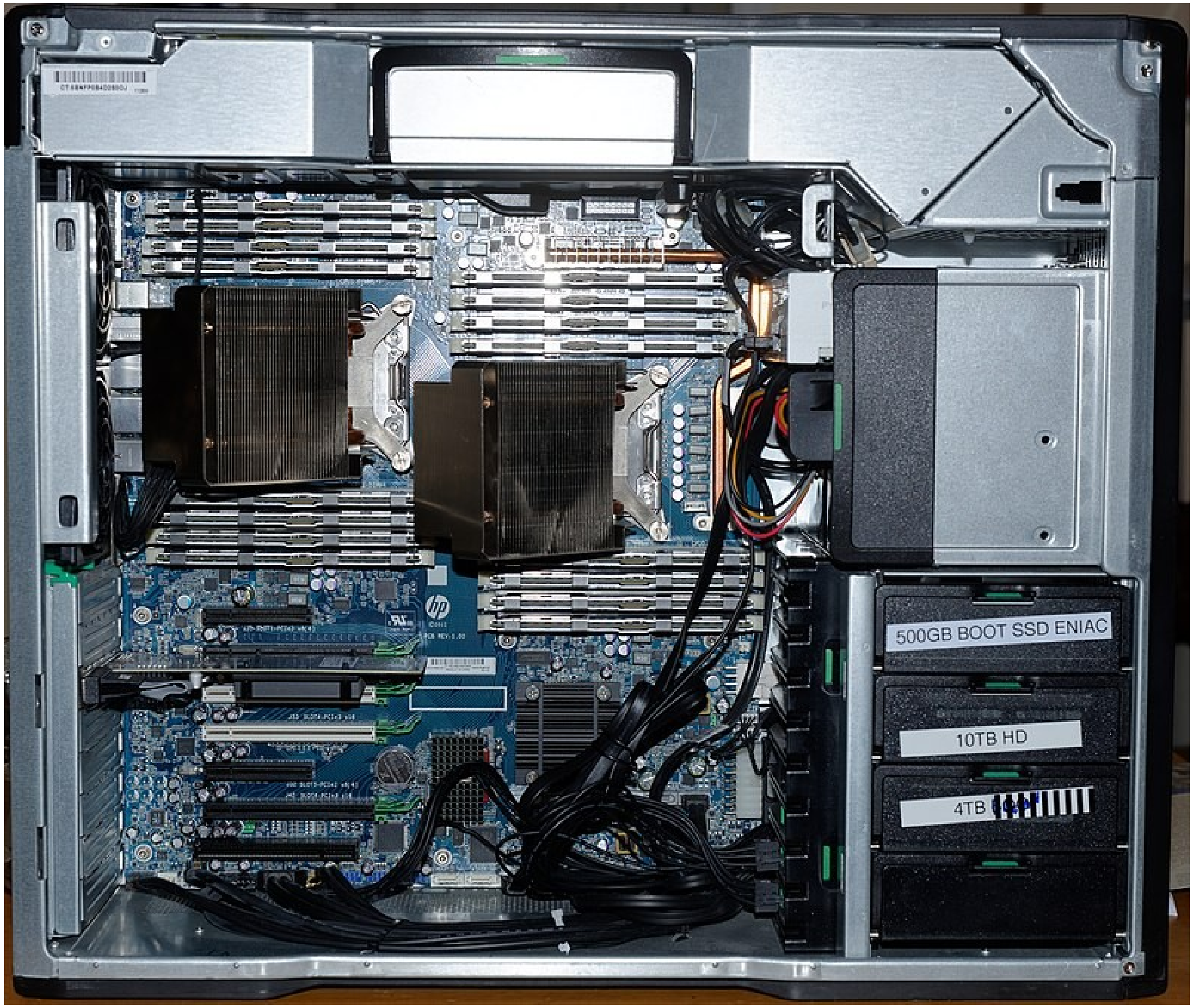
Lots of problems

de novo assembly of 35 RNAseq samples

- needs >100 GB RAM, runs for many days
- needs many CPUs, generates TBs of files
- repeat the assembly many times with different parameters



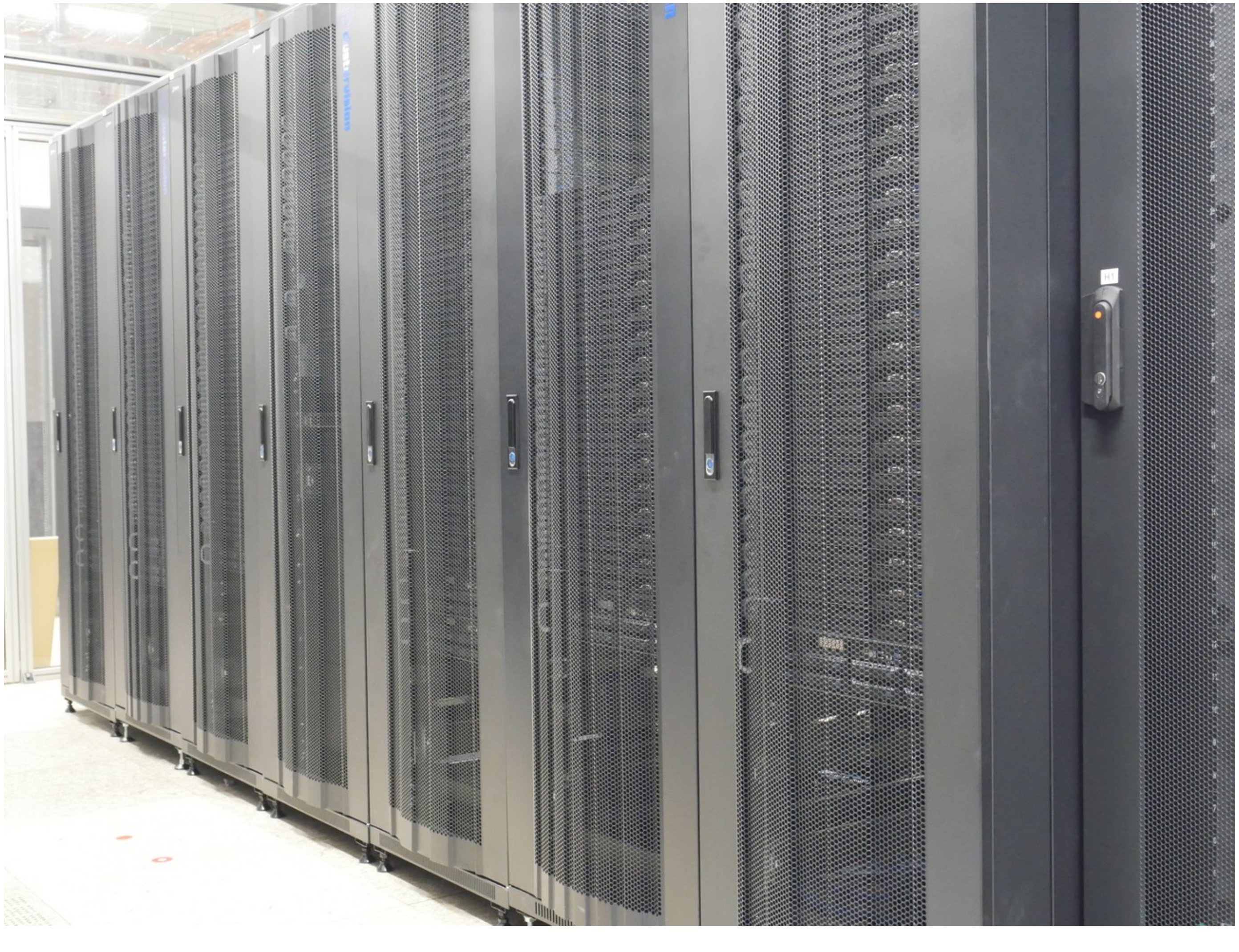
Workstation (1,000s €)



Server (10,000s €)

- e.g. 36,000€
  - 1.5 TB RAM
  - 40 cores
  - 48 TB storage
- Plus energy, cooling, rack etc.

Computing cluster (HPC)
100,000s €



Important points

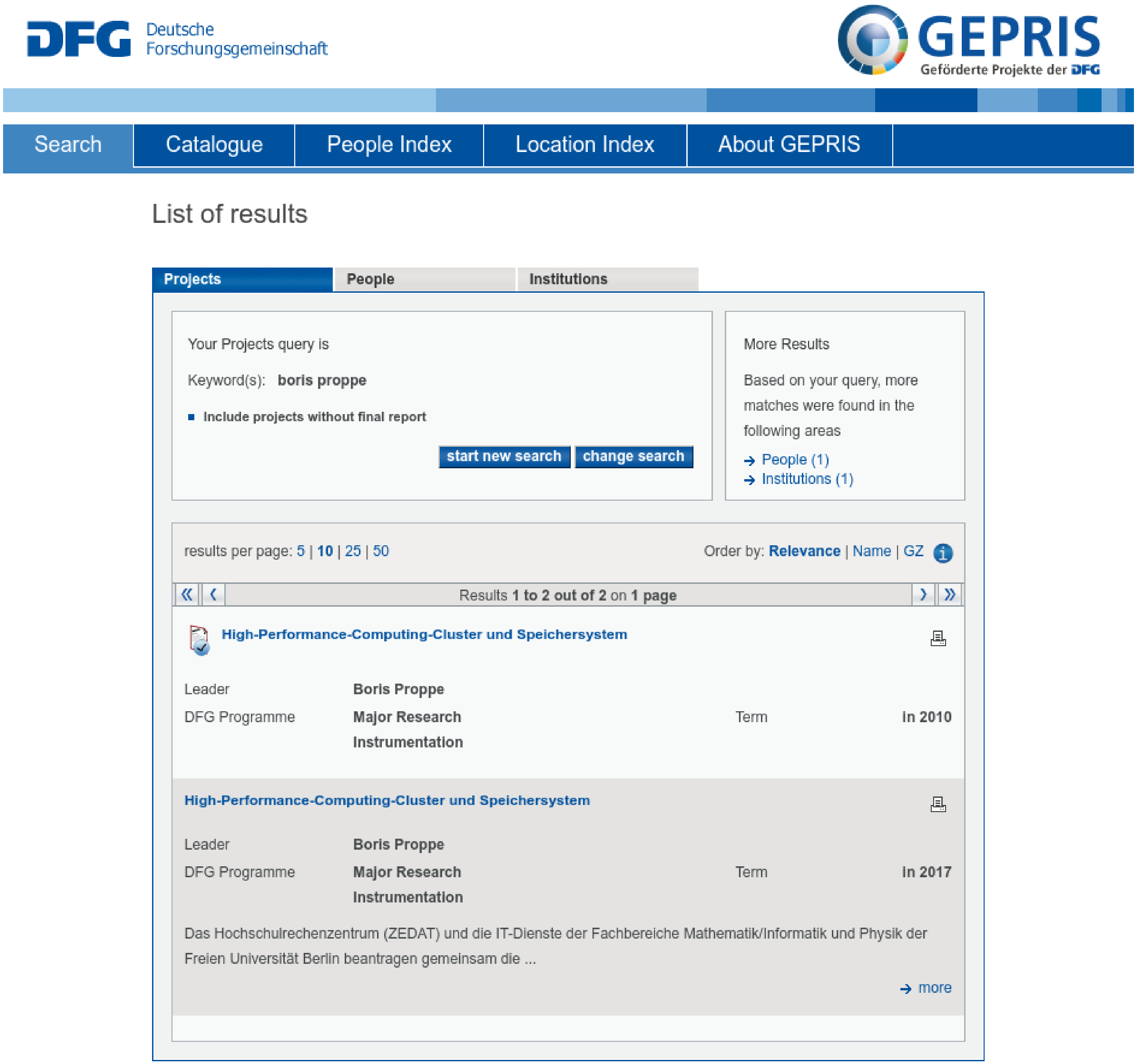
Every university has (access to) a cluster

Usage is generally free or extremely cheap

There are university employees to provide support

The system is highly robust and stable

i.e. it’s there and it works now


DFG Deutsche Forschungsgemeinschaft

GEPRIS Geförderte Projekte der DFG

List of results

Your Projects query is

Keyword(s): **boris proppe**

- **Include projects without final report**

More Results

Based on your query, more matches were found in the following areas

→ People (1)
→ Institutions (1)

results per page: 5 | **10** | 25 | 50

Order by: **Relevance** | Name | GZ

Results **1 to 2 out of 2** on **1 page**

High-Performance-Computing-Cluster und Speichersystem

Leader	**Boris Proppe**		
DFG Programme	**Major Research Instrumentation**	Term	**in 2010**

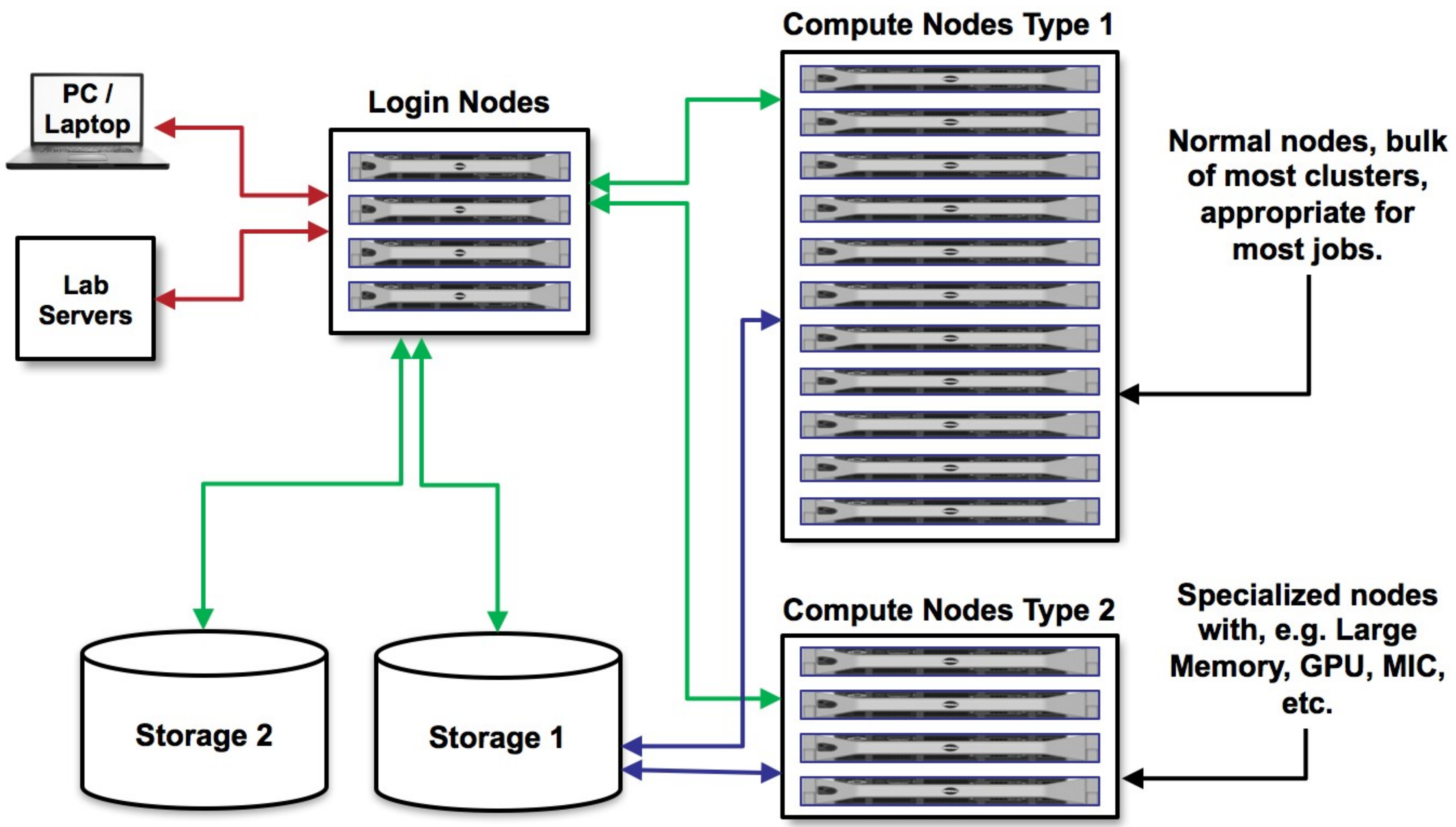
High-Performance-Computing-Cluster und Speichersystem

Leader	**Boris Proppe**		
DFG Programme	**Major Research Instrumentation**	Term	**in 2017**

Das Hochschulrechenzentrum (ZEDAT) und die IT-Dienste der Fachbereiche Mathematik/Informatik und Physik der Freien Universität Berlin beantragen gemeinsam die ...

→ more

Typical cluster


Compute Nodes Type 1
PC / Laptop
Login Nodes
Normal nodes, bulk of most clusters, appropriate for most jobs.
Lab Servers
Compute Nodes Type 2
Specialized nodes with, e.g. Large Memory, GPU, MIC, etc.
Storage 2
Storage 1

Terminology

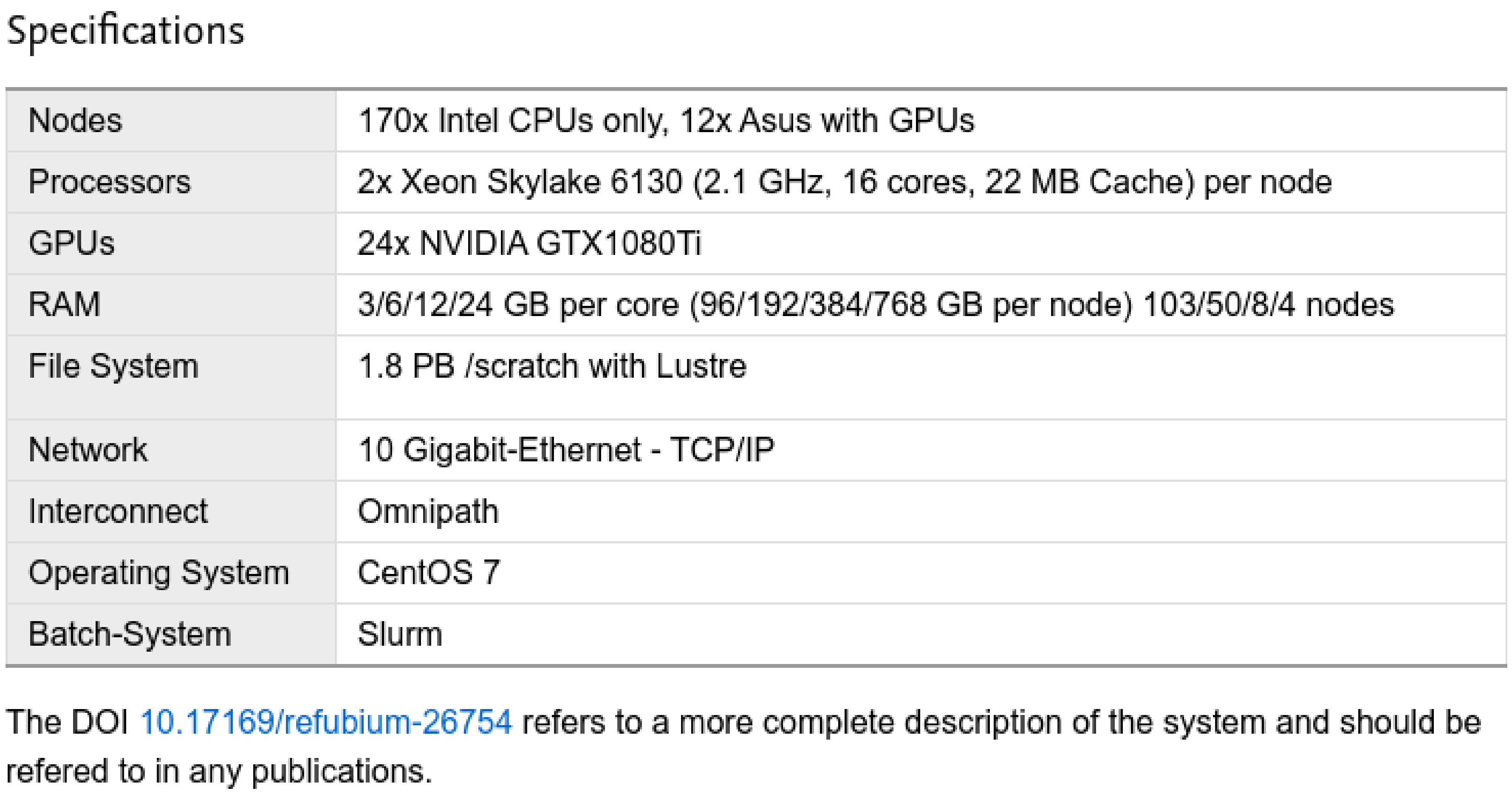
Job: reservation to run commands

Node: physical machine, part of cluster

Core/CPU: processing unit, nodes contain many CPUs

Partition: nodes may be organized into partition
    e.g. begendiv bought large-memory nodes

Curta specifications



Specifications

Nodes	170x Intel CPUs only, 12x Asus with GPUs
Processors	2x Xeon Skylake 6130 (2.1 GHz, 16 cores, 22 MB Cache) per node
GPUs	24x NVIDIA GTX1080Ti
RAM	3/6/12/24 GB per core (96/192/384/768 GB per node) 103/50/8/4 nodes
File System	1.8 PB /scratch with Lustre
Network	10 Gigabit-Ethernet - TCP/IP
Interconnect	Omnipath
Operating System	CentOS 7
Batch-System	Slurm

The DOI 10.17169/refubium-26754 refers to a more complete description of the system and should be refered to in any publications.

http://dx.doi.org/10.17169/refubium-26754

Connecting to the cluster

```
# remember to use your zedat username
ssh USERNAME@curta.zedat.fu-berlin.de
```

Connecting to the cluster

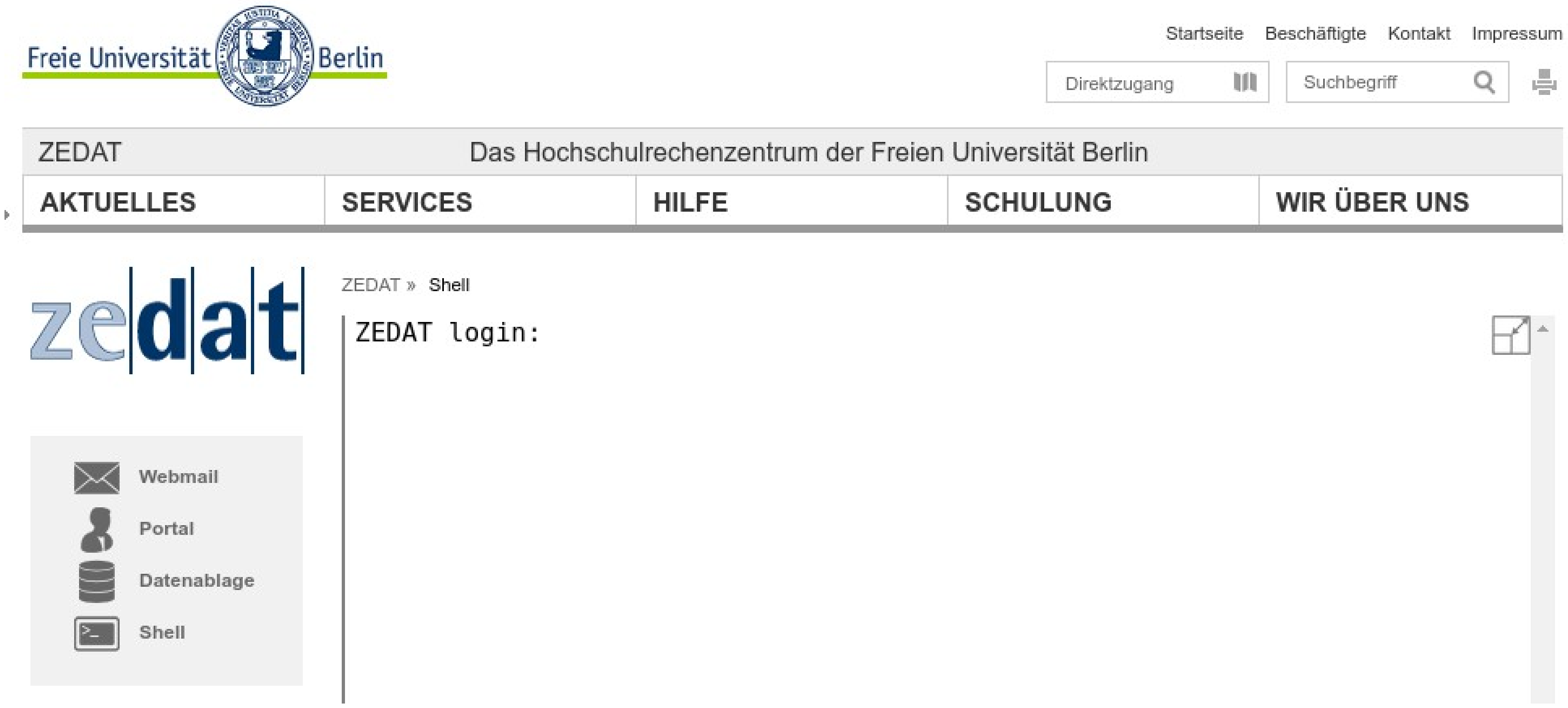
```
# remember to use your zedat username
ssh USERNAME@curta.zedat.fu-berlin.de
```

- must be inside FU network (or use VPN)
- if the VPN doesn’t work on your system then you can first connect to the zedat login server

```
# connect to login server
ssh USERNAME@login.zedat.fu-berlin.de
# you are now connected to the login server
# from here you can connect to the cluster
ssh USERNAME@curta.zedat.fu-berlin.de
# you should now be connected to the cluster (curta)
```

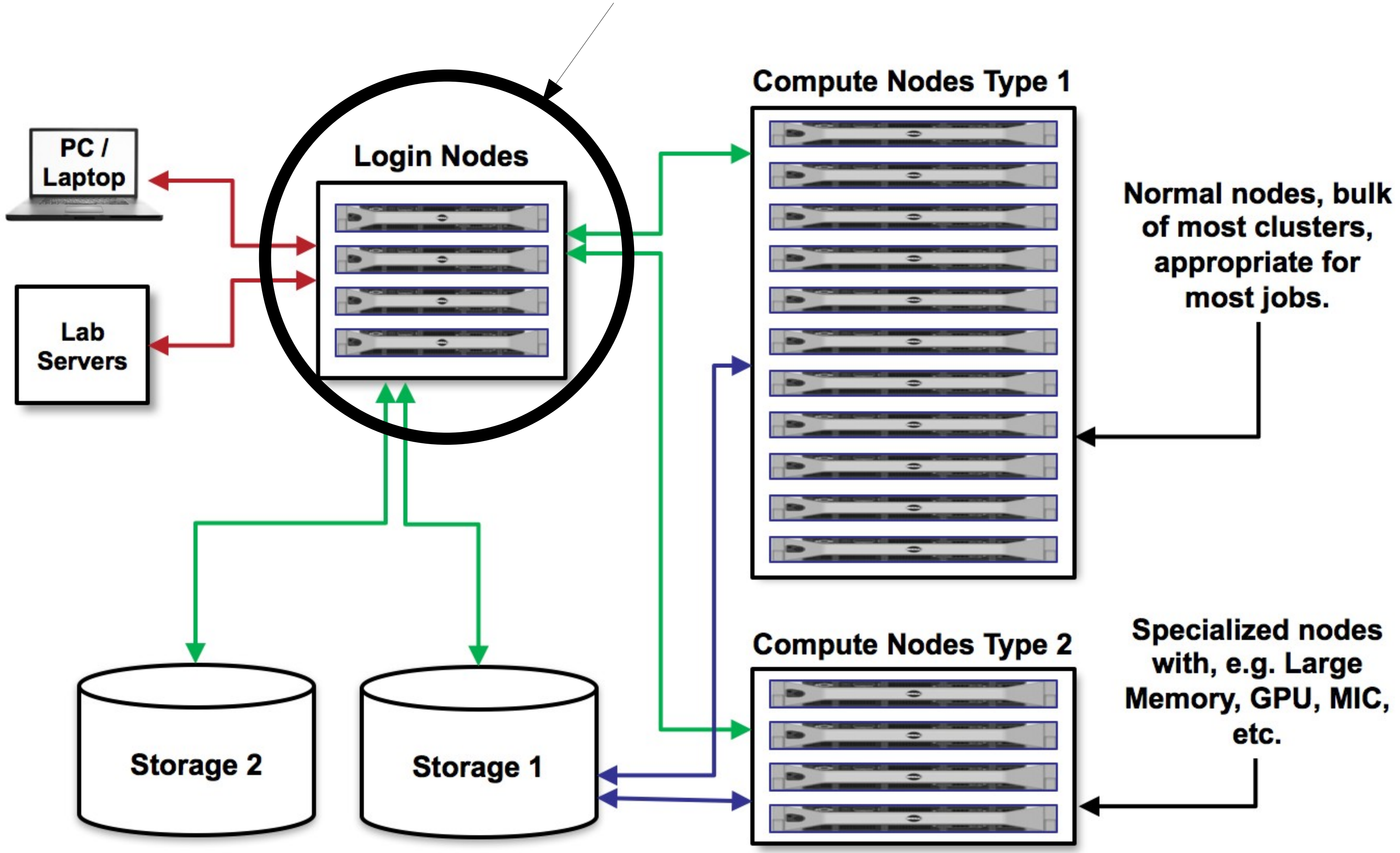
Connecting to the cluster

- zedat also provides a shell here:
  https://www.zedat.fu-berlin.de/Shell



```
ZEDAT login:
```

You are now on one of these nodes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Storage 2
Storage 1

Login nodes

These are for basic tasks:

- Uploading data	# scp, rsync, wget, etc
- Managing files	# cp, mv, gunzip
- Compiling software	# configure, make
- Editing scripts	# nano, vim
- Checking/managing jobs	# squeue

Important note

The login nodes are for setting things up and submitting jobs to the compute nodes

Running commands on the login nodes is bad

- You would get an annoyed email from admin
- It slows/crashes the node for other users

Compute nodes

Job scripts are sent here to run

- resources allocated by workload manager (Slurm)

- other workload managers/schedulers exist
  e.g. PBS, SGE, LFS

- resources are allocated according to:
  system load
  fair share algorithms

Basic conventions

```
# remember to use your zedat username
ssh USERNAME@curta.zedat.fu-berlin.de
```

/home/$USER/	- limited space - backed up nightly - store software, config files
/scratch/$USER/	- lots of storage space - no backup - default working space

Different cultures exist in different clusters

e.g. precise location of scratch, backup policy

First job on the cluster

```
# remember to use your zedat username
ssh USERNAME@curta.zedat.fu-berlin.de
# enter the following command
echo “Hello World”
# “Hello World” should print to your screen (the standard output)
```

First job on the cluster

```
# remember to use your zedat username
ssh USERNAME@curta.zedat.fu-berlin.de
# enter the following command
echo “Hello World”
# “Hello World” should print to your screen (the standard output)
```

We just ran a command on the login node

(this is OK because it is not intensive)

Now lets submit it as a job to a compute node

First job on the cluster

```
# we should work in our scratch directory
# so navigate to your scratch directory
cd /scratch/$USER/
# now make a directory with a sensible name to work in
# yearmonthday_sensible_name is a good naming convention
mkdir 20221130_lvdatascieeb
# navigate inside the new directory
cd 20221130_lvdatascieeb
# copy the example job script from my scratch directory to yours
cp /scratch/perugolate/20221130_lvdatascieeb/20221130_hello_world.sh .
# have a look at the script
cat 20221130_hello_world.sh
```

Example job script

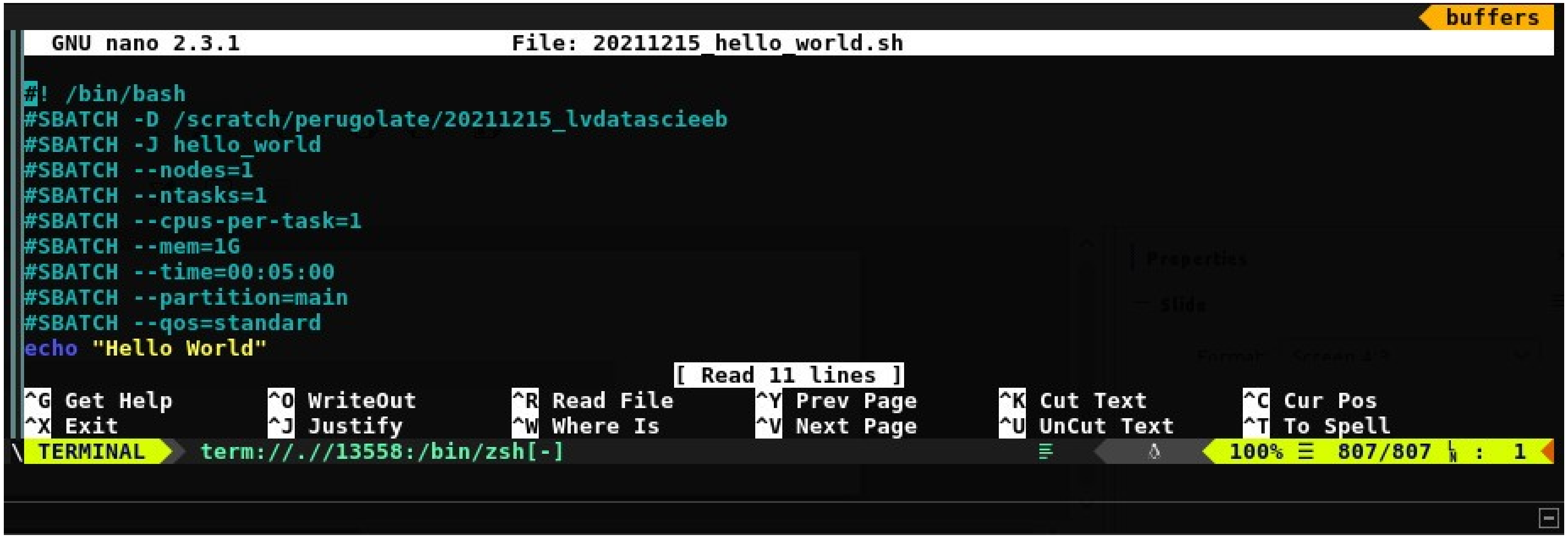
```
#! /bin/bash
#SBATCH -D /scratch/USERNAME/20221130_lvdatascieeb      # directory
#SBATCH -J hello_world           # name of job
#SBATCH --nodes=1                # requested nodes
#SBATCH --ntasks=1               # number of tasks in job
#SBATCH --cpus-per-task=1        # requested CPUs
#SBATCH --mem=1G                 # requested RAM
#SBATCH --time=00:05:00          # requested time
#SBATCH --partition=main         # partition where job will run
#SBATCH --qos=standard           # complicated
echo "Hello World"
```

- line 2 specifies the directory where the job will run

- we need to edit it

Example job script

```
# open the job script in the text editor ‘nano’
nano 20221130_hello_world.sh
# navigate with arrow keys to USERNAME on line 2
# delete and enter your username
# when finished editing, press control+X to exit
# then press shift+Y to save changes
# (or maybe shift+J if your language is set to german, I’m not sure)
```



```
  GNU nano 2.3.1                  File: 20211215_hello_world.sh

#! /bin/bash
#SBATCH -D /scratch/perugolate/20211215_lvdatascieeb
#SBATCH -J hello_world
#SBATCH --nodes=1
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --mem=1G
#SBATCH --time=00:05:00
#SBATCH --partition=main
#SBATCH --qos=standard
echo "Hello World"
                                [ Read 11 lines ]
^G Get Help     ^O WriteOut     ^R Read File    ^Y Prev Page    ^K Cut Text     ^C Cur Pos
^X Exit         ^J Justify      ^W Where Is     ^V Next Page    ^U UnCut Text   ^T To Spell
\ TERMINAL  term://.//13558:/bin/zsh[-]                              100% ☰  807/807 : 1
```

Submit the job

```
# submit the job script using
sbatch 20221130_hello_world.sh
```

sbatch is a command from the slurm workload/schedule managing software

squeue is used to monitor the queue (or squeue --me)

```
squeue
             JOBID PARTITION     NAME     USER ST       TIME  NODES NODELIST(REASON)
           8695474  agkeller     KF-2   heif89  R 1-06:23:29      1 g014
           8694500  agkeller    LiI-3   heif89  R 1-06:50:38      1 g013
           8717240  begendiv jamesTes  james94  R    3:07:30      1 b004
           8717994  begendiv GEP.merq ddepanis  R       6:30      1 b002
           8717993  begendiv GEP.merq ddepanis  R      34:32      1 b003
           8717992  begendiv GEP.merq ddepanis  R      37:32      1 b004
           8717991  begendiv GEP.merq ddepanis  R      46:58      1 b001
           8717989  begendiv GEP.busc ddepanis  R      53:25      1 b002
           8717983  begendiv GEP.busc ddepanis  R    1:36:20      1 b003
```

Submit the job

```
# submit the job script using
sbatch 20221130_hello_world.sh
```

The job should complete instantly

You will notice “Hello World” was not printed to your screen

The standard output was redirected to a file slurm-[jobID].out

Job output

```
# slurm has also added some usage stats to the end of the file
cat slurm-*.out
Hello World

== Epilog Slurmctld
==================================================

Job ID: 8722119
Cluster: curta
User/Group: perugolate/agrolff
State: COMPLETED (exit code 0)
Cores: 1
CPU Utilized: 00:00:00
CPU Efficiency: 0.00% of 00:00:01 core-walltime
Job Wall-clock time: 00:00:01
Memory Utilized: 0.00 MB (estimated maximum)
Memory Efficiency: 0.00% of 1.00 GB (1.00 GB/node)

========================================================
==============
```

Software

The cluster works just like a linux computer

- you can download/compile software

- you can install a package manager e.g. conda

- the HPC staff can help/advise on installation

- common software is available via “module load”
    - multiple versions of python/perl/R
    - bioinformatics software

Second job

Let’s adapt one of Lynn’s R exercises to the cluster

We need to:

- create a copy of the R script in a local directory
- make some edits to the R script
- create a job script to run the R script

Create copy of script/data

Create a new working directory :

```
# navigate to your scratch directory
cd /scratch/$USER/
# create a new working directory inside
mkdir 20230118
# navigate inside new working directory
cd 20230118/
# you should now be in /scratch/$USER/20230118/
```

Copy Lynn’s script from my scratch directory:

```
# copy Lynn’s script from my scratch
cp /scratch/perugolate/CodeToRun.R ./
# copy Lynn’s data from my scratch
cp /scratch/perugolate/Jacob_Legrand_phenotype_dat.txt ./
```

Prepare the data/script

We are going to prepare the code for the first few models and plots (the first 22 lines) as a script

the file paths ("C:\\Users\\govaert\...) show the script was likely not prepared on a unix computer. Lets fix the line endings first:

```
# if a file was prepared on windows or Mac (prior to OS X) the line endings
# will often be (invisibly) different, which can cause all sorts of problems
dos2unix CodeToRun.R
```

```
# copy first 22 lines to a new file with a sensible name
head -n 22 CodeToRun.R > 20230118.R
# we now have new file (20230118.R) which we will run as a job
```

Modify script

```
# open the R script in the text editor nano
nano 20230118.R
# you can use your arrow keys to move your cursor around inside nano
```

Modify line 2 to read:

```
dat. <- read.table("Jacob_Legrand_phenotype_dat.txt", header = TRUE)
```

Save the changes

```
# when finished editing, press control+X to exit
# then press shift+Y and then enter to save changes
# (or maybe shift+J if your language is set to german, I’m not sure)
```

Make a job script

We can reuse the sbatch parameters from our first job script

Lets copy the first 10 lines to a new script file:

```
# copy the sbatch parameter lines from first job script
head -n10 /scratch/perugolate/20221130_lvdatascieeb/20221130_hello_world.sh > 20230118.sh
# open the new file in nano for editing
nano  20230118.sh
```

Make a job script

I have made the following edits in nano

- changed the working directory on line 2 (use your own username here)
- changed job name on line 3 from “hello_world” to “ex_1”
- added 2 commands to the bottom (in bold)

```
#! /bin/bash
#SBATCH -D /scratch/USERNAME/20230118
#SBATCH -J ex_1
#SBATCH --nodes=1
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --mem=1G
#SBATCH --time=00:05:00
#SBATCH --partition=main
#SBATCH –qos=standard
module load R/4.1.0-foss-2021a
Rscript 20230118.R
```

Make a job script

Many versions of R are already installed on the cluster

It is also possible to
- download and compile it yourself
- install it using a package manager such as conda

Here, we have loaded R using:

```
module load R/4.1.0-foss-2021a
```

This is a safe option because it has been compiled properly by the HPC staff

You can see the other software available by using:

```
module avail
```

Submit the job script

```
sbatch 20230118.sh
```

The job should complete within a few seconds

Again nothing is printed to the screen. This has been redirected to a text file slurm-[jobID].txt

```
head slurm-8726728.out
             Df    Sum Sq    Mean Sq F value   Pr(>F)
Sp            4   1186843    296711   33.51    < 2e-16 ***
Temp          1    489807    489807   55.32    2.83e-13 ***
Residuals   743 6578235      8854
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
               Df  Sum Sq   Mean Sq F value   Pr(>F)
Sp              4   1186843  296711    42.66     < 2e-16 ***
Temp            1   489807   489807    70.42     2.42e-16 ***
Sp:Temp         4   1438442  359611    51.70     < 2e-16 ***
```

Job output

R was not run in interactive mode
  - i.e. there was no window to display the plots

In this case Rscript redirects the plots to Rplots.pdf

[normally we would write plots to explicitly with functions e.g. pdf()]

You will need to transfer the file to your computer in order to view it

Open a new terminal on your computer and type:

```
# replace USERNAME with your username
scp username@curta.zedat.fu-berlin.de:/scratch/USERNAME/20230118/Rplots.pdf ./
# you can open Rplots.pdf using your systems pdf viewer
```