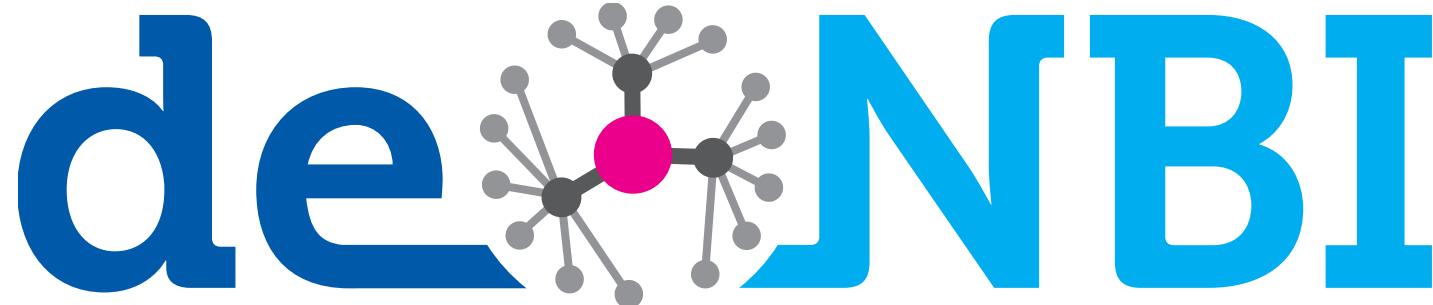


Functional microbiome research – bioinformatics section

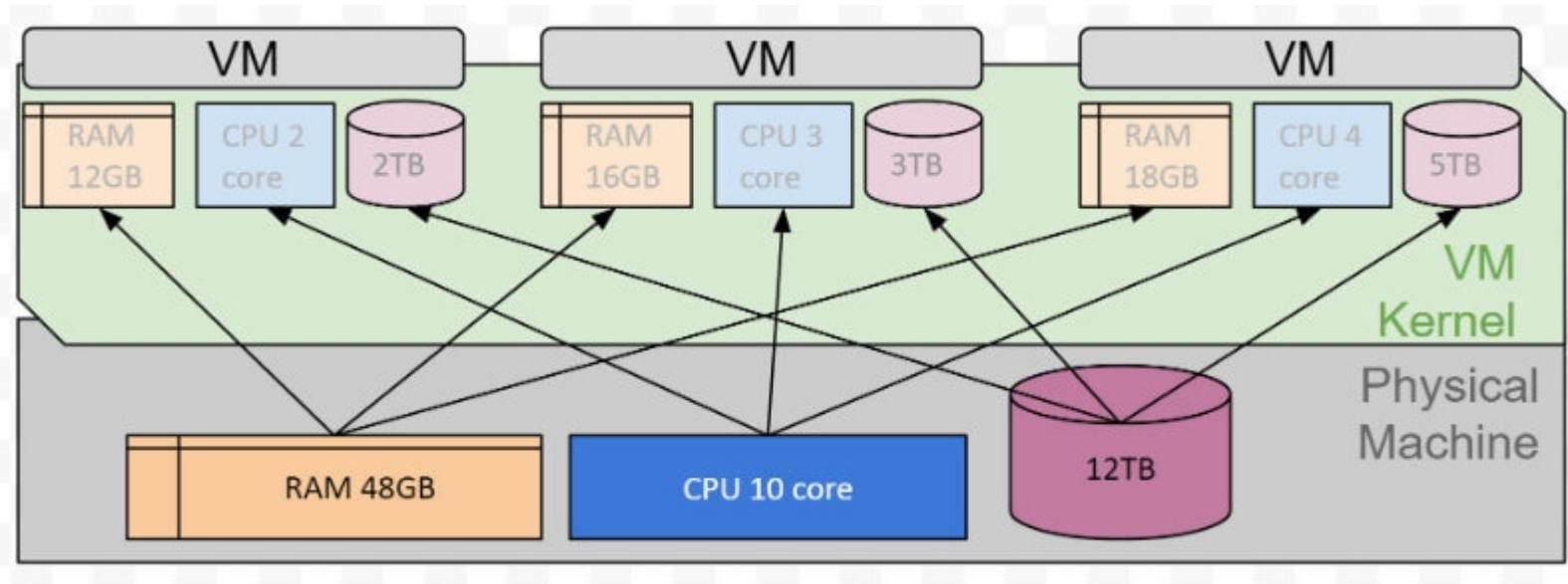
Session 1 – intro to linux



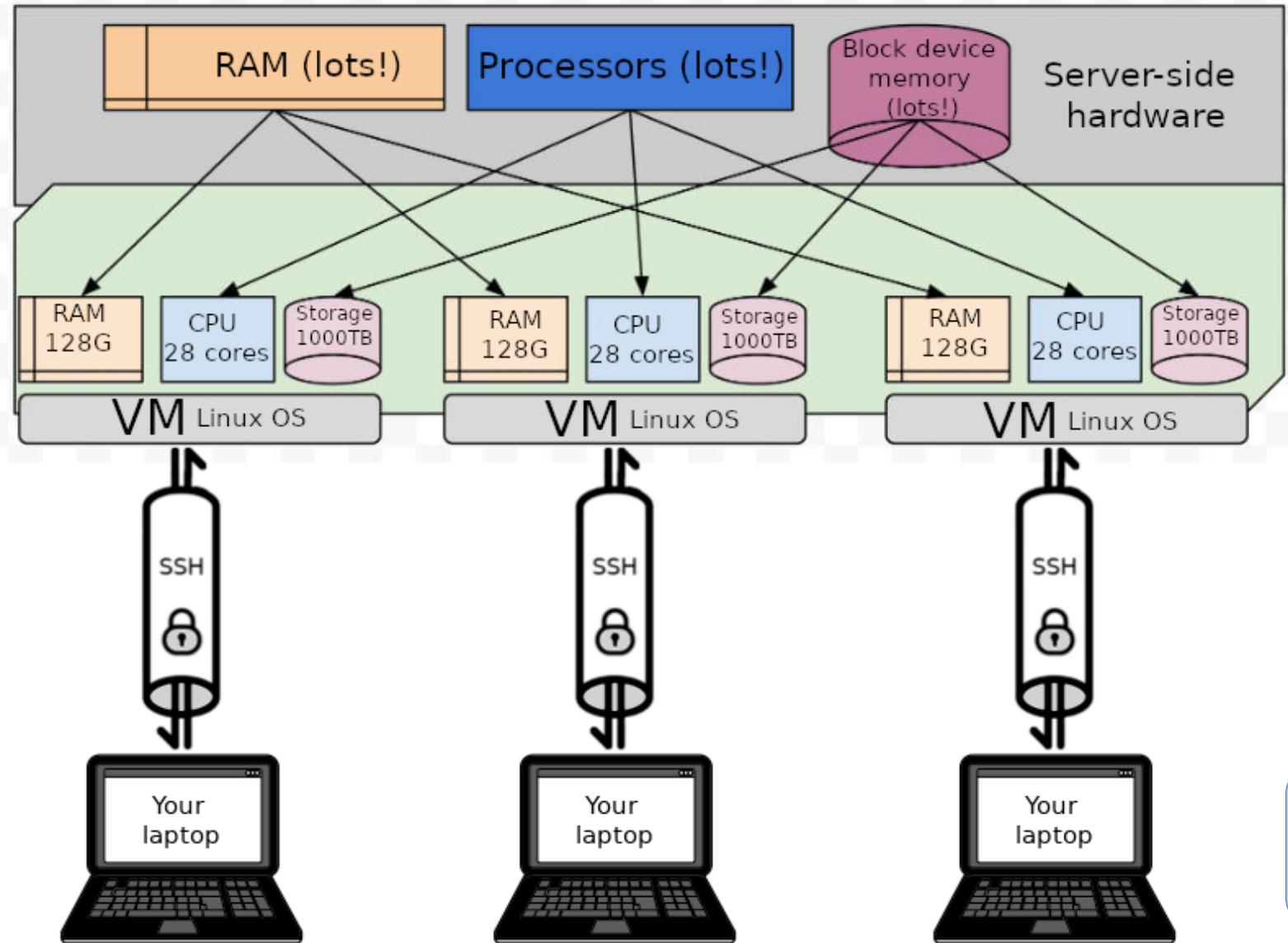
GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

First, thanks to de.NBI. They are amazing.

Distributed computing and compute clusters are essential tools for scientists.



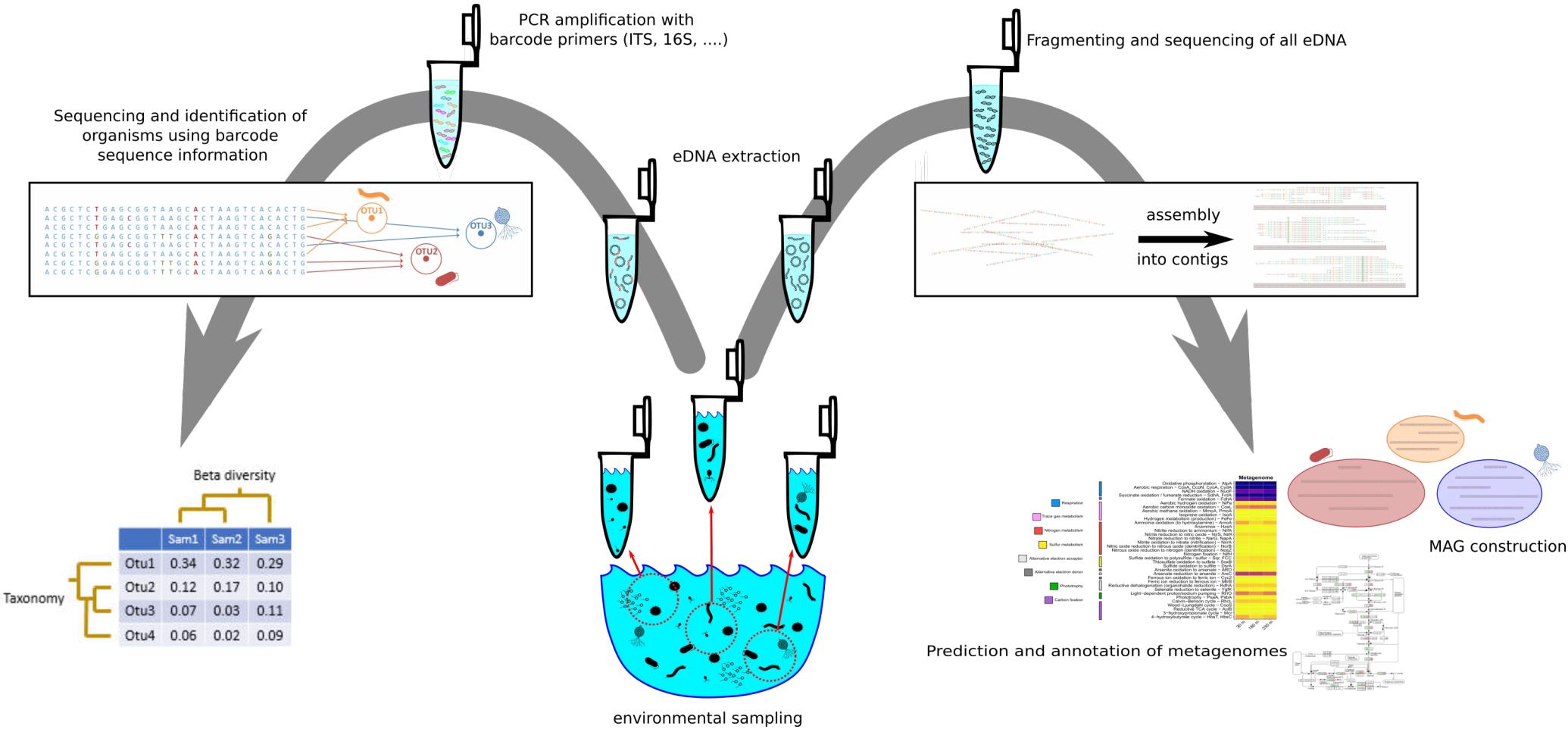
We use our local computers to talk to shared remote servers that have lots of high-powered processors, and storage and RAM.



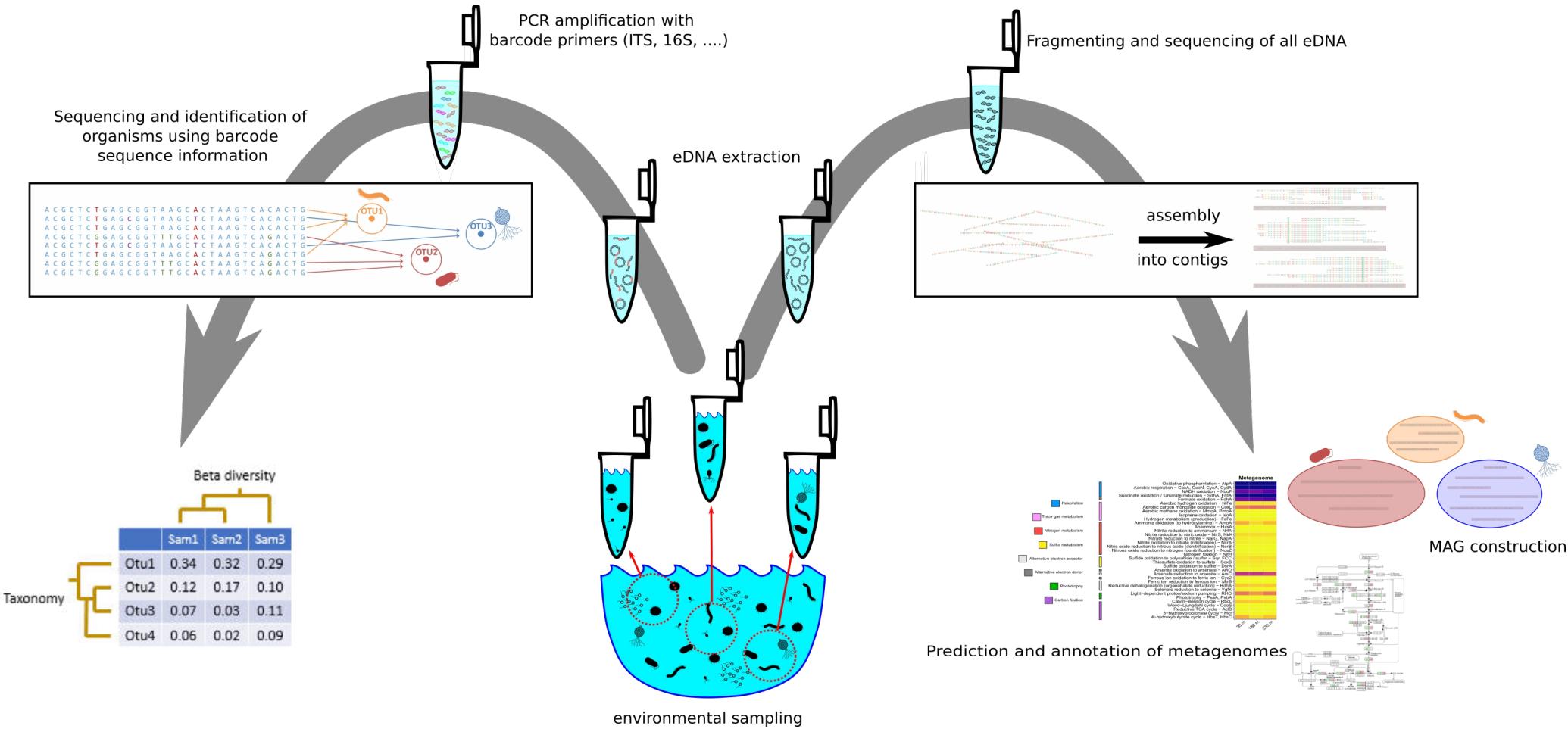
de.NBI / Universität
Bielefeld

Us.

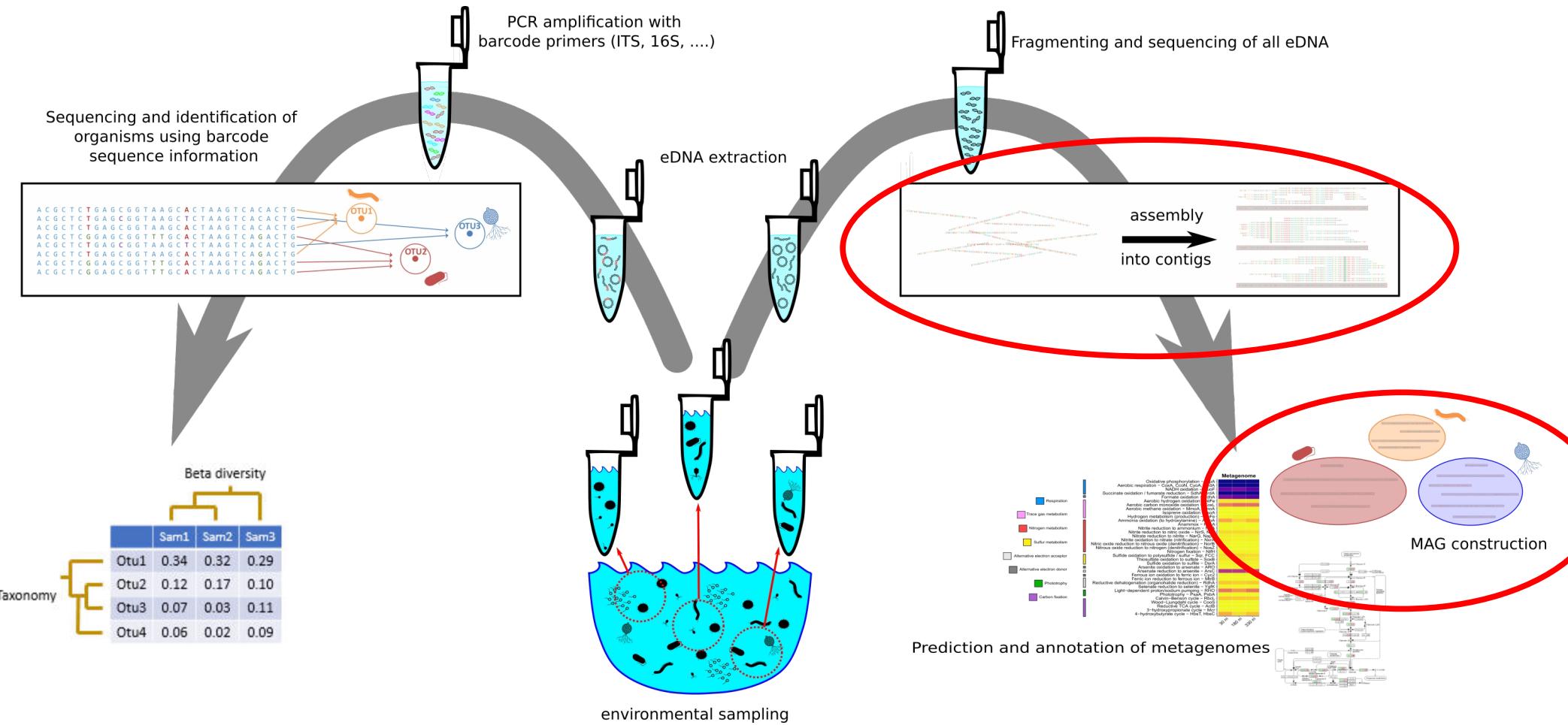
Review – what do we do with DNA sequence data?



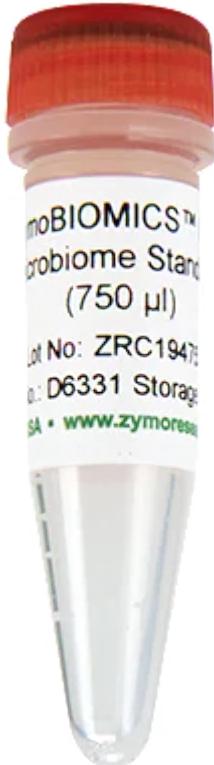
Why do we need distributed computing to do this?



Because some steps take ~100 gig of RAM (or more!) and lots of processors.



Dataset 1: ZymoBIOMICS HMW DNA Standard.



Species	Theoretical Composition (%)				
	Genomic DNA	16S Only ¹	16S & 18S ¹	Genome Copy ²	Cell Number ³
<i>Pseudomonas aeruginosa</i>	14	5.1	4.6	7.8	7.9
<i>Escherichia coli</i>	14	12.4	11.2	10.9	11.0
<i>Salmonella enterica</i>	14	12.7	11.4	11.2	11.2
<i>Enterococcus faecalis</i>	14	12.1	10.9	18.8	18.8
<i>Staphylococcus aureus</i>	14	19	17.1	19.6	19.6
<i>Listeria monocytogenes</i>	14	17.3	15.6	17.8	17.9
<i>Bacillus subtilis</i>	14	21.4	19.2	13.2	13.2
<i>Saccharomyces cerevisiae</i>	2	NA	10	0.63	0.32

Dataset 2: Kelp decomposition

LIMNOLOGY
and
OCEANOGRAPHY



Limnol. Oceanogr. 65, 2020, 3066–3084

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doi: 10.1002/limo.11574

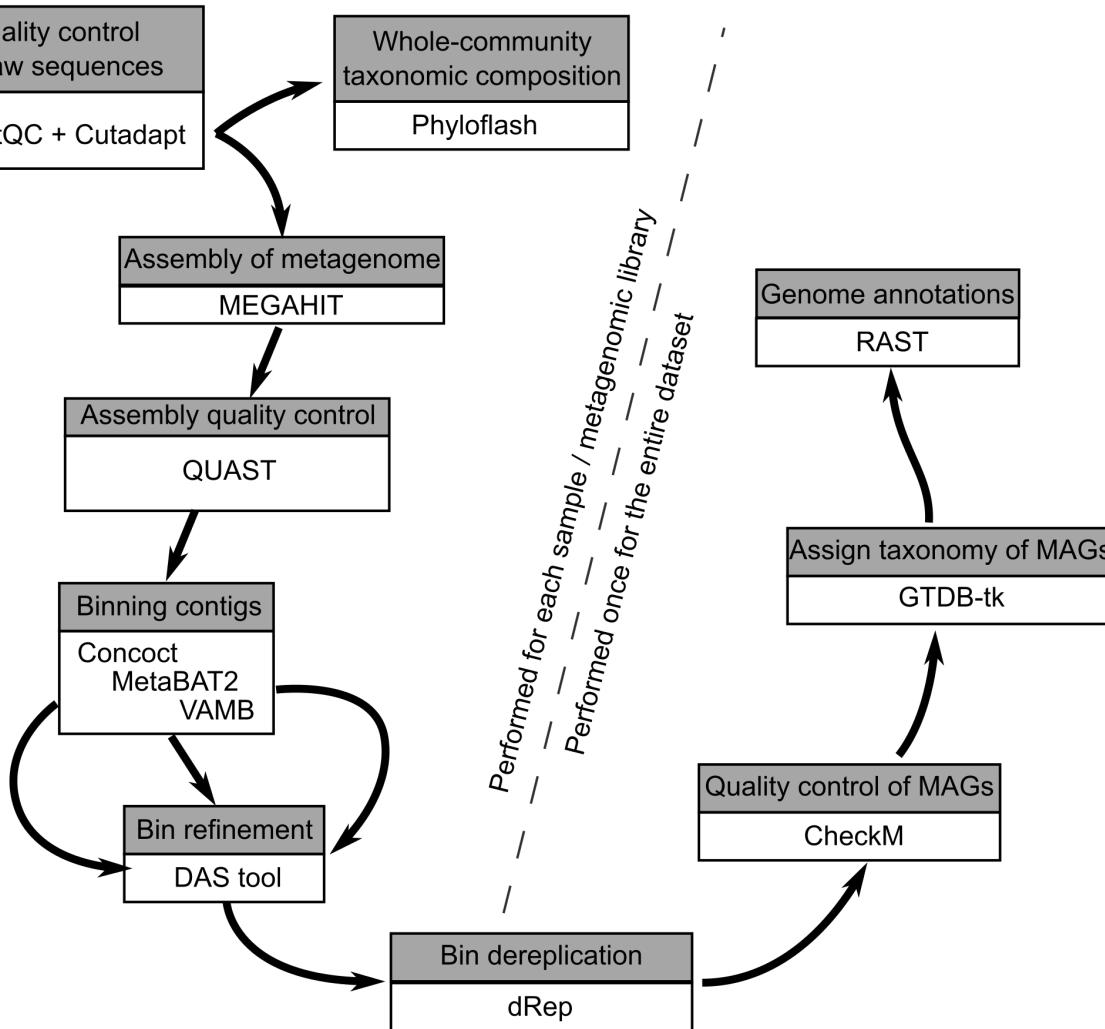
Kelp deposition changes mineralization pathways and microbial communities in a sandy beach

Marit R. van Erk ^{1,*} Dimitri V. Meier,^{1,a} Timothy Ferdelman,¹ Jens Harder,¹ Ingeborg Bussmann,² Dirk de Beer¹

¹Max Planck Institute for Marine Microbiology, Bremen, Germany

²Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Helgoland, Germany





We will use various scientific software packages to assemble Metagenome-Assembled-Genomes (MAGs) from these sequence data.

Quality control
of raw sequences

= FastQC + Cutadapt

Whole-community
taxonomic composition

Phyloflash

Assembly of metagenome

MEGAHIT

Assembly quality control

QUAST

Binning contigs

Concoct
MetaBAT2
VAMB

Bin refinement
DAS tool

Bin derePLICATION

dRep

Performed for each sample / metagenomic library

Genome annotations

RAST

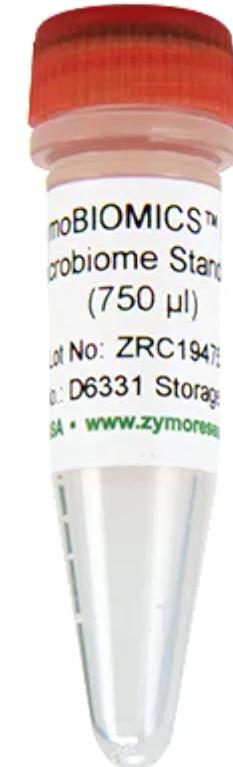
Assign taxonomy of MAGs

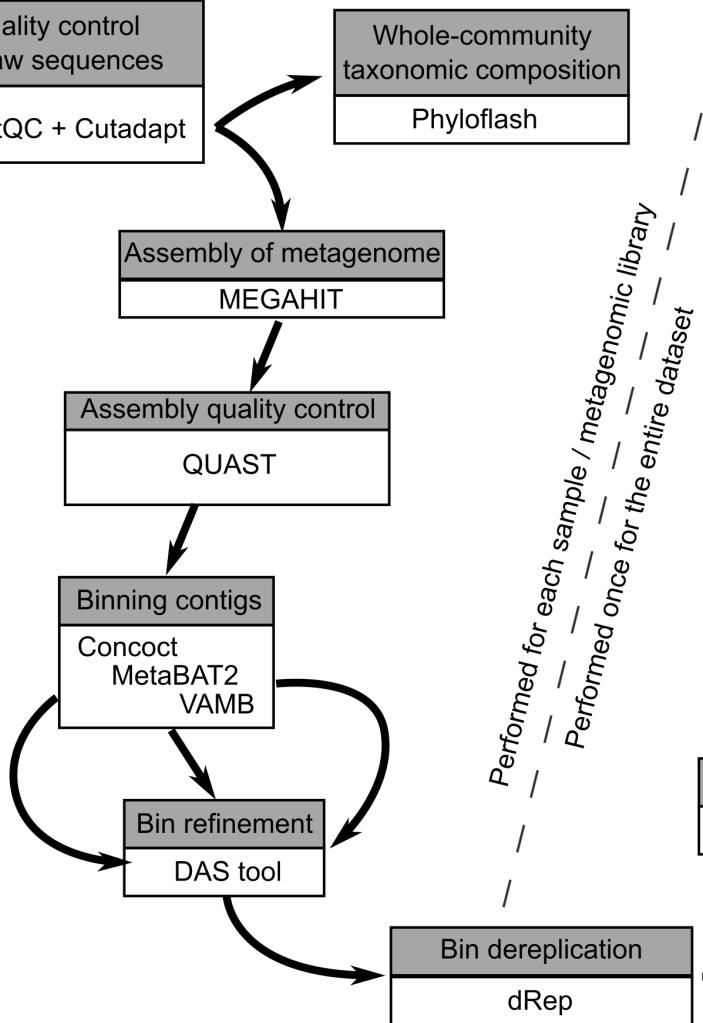
GTDB-tk

Quality control of MAGs

CheckM

First, we will do this with a simple mock community.





Then you will apply what you have learned to discover MAGs from the Kelp decomposition dataset.

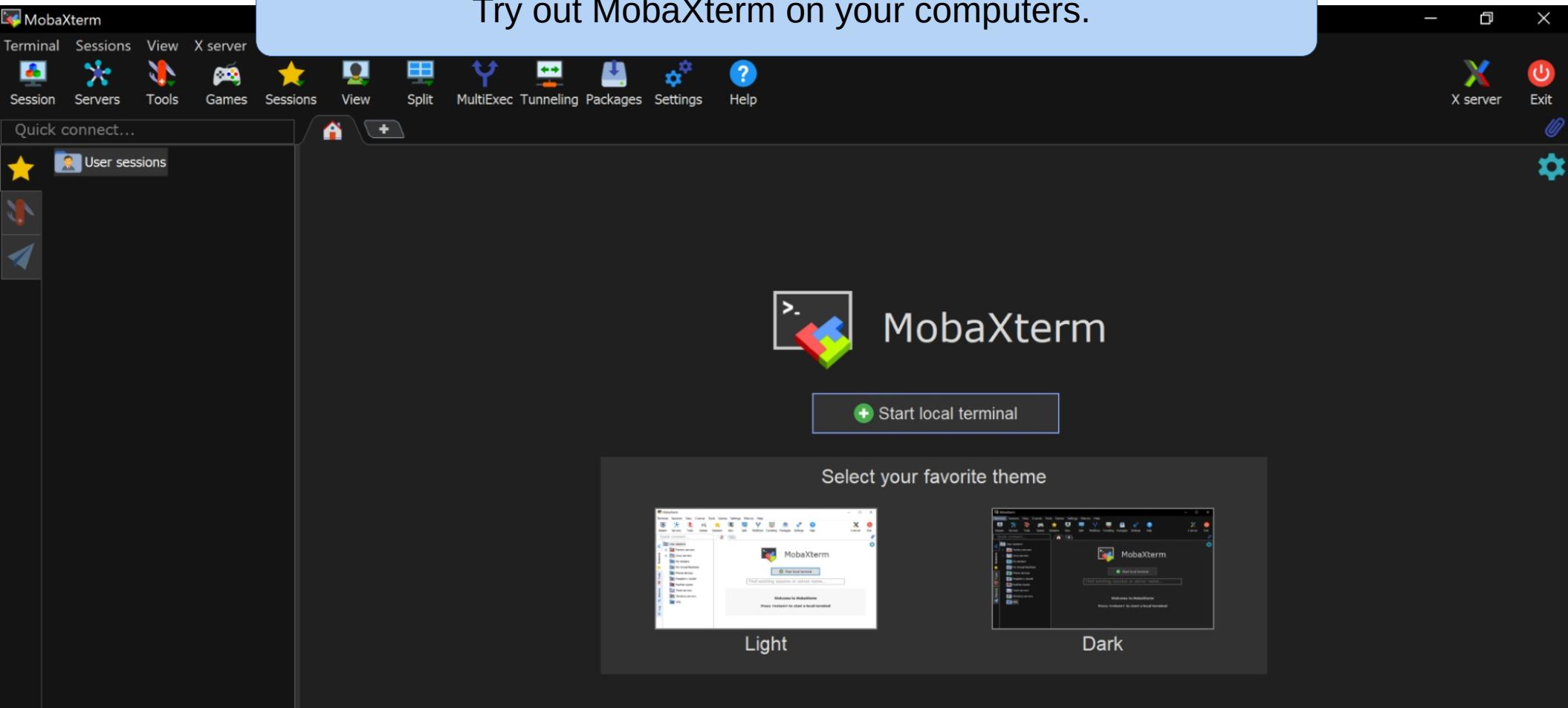


We'll keep track of what we do in scripts that will be kept in an online github repository.

1. Scientific computing and Linux (today!)
2. Metagenomic methods
3. Metabarcoding methods

Download using these links. Or use the links in the skript PDF on page 21.

Try out MobaXterm on your computers.



Let's check everyone's installation. Try starting a local terminal.

Now let's try to connect to our de.NBI virtual machines.

129.70.51.6 (ubuntu) (3)

Session

Session settings

SSH

Choose a session type...

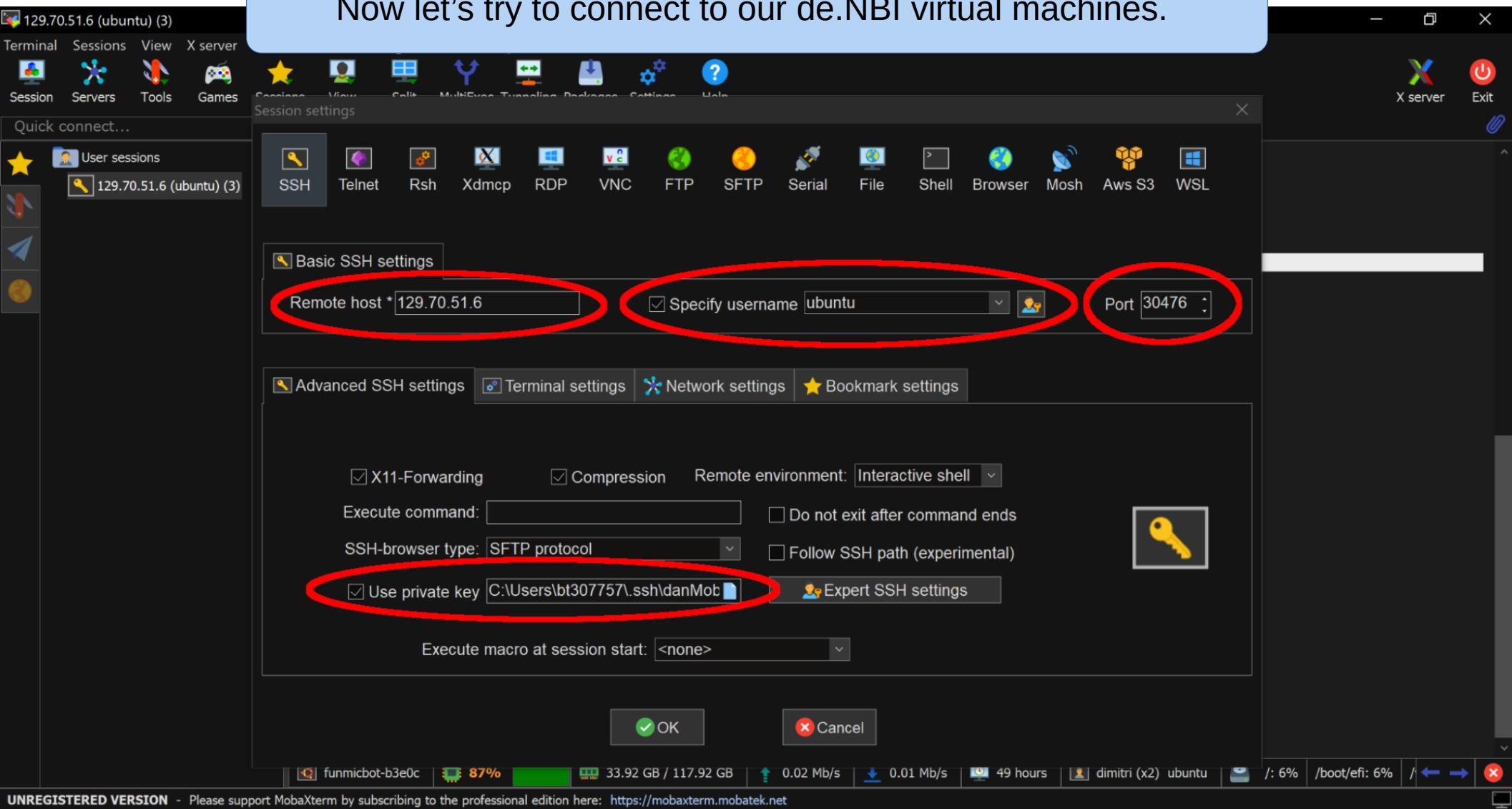
OK Cancel

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X server Exit

The screenshot shows the MobaXterm interface. In the top left, there's a session titled "129.70.51.6 (ubuntu) (3)". Below it is a toolbar with icons for Terminal, Sessions, View, X server, Session (which is highlighted with a red circle), Servers, Tools, Games, Sessions, View, Split, MultiExec, Tunneling, Packages, Settings, and Help. To the right of the toolbar is a "Session settings" window. This window has a "Quick connect..." section with a dropdown menu showing "/home/ubuntu/" and a "Name" field. Below this is a list of session types: SSH (highlighted with a red circle), Telnet, Rsh, Xdmcp, RDP, VNC, FTP, SFTP, Serial, File, Shell, Browser, Mosh, Aws S3, and WSL. A red arrow points from the "Session" icon in the toolbar to the "Session settings" window. Another red arrow points from the "SSH" icon in the session list to the "Choose a session type..." dialog, which is displayed in the center of the screen. The dialog has a small icon of a computer monitor with three colored cubes and the text "Choose a session type...". At the bottom of the dialog are "OK" and "Cancel" buttons. The status bar at the bottom shows "/boot/efi: 6%" and navigation icons. The footer indicates "UNREGISTERED VERSION" and provides a link to the professional edition.

Now let's try to connect to our de.NBI virtual machines.

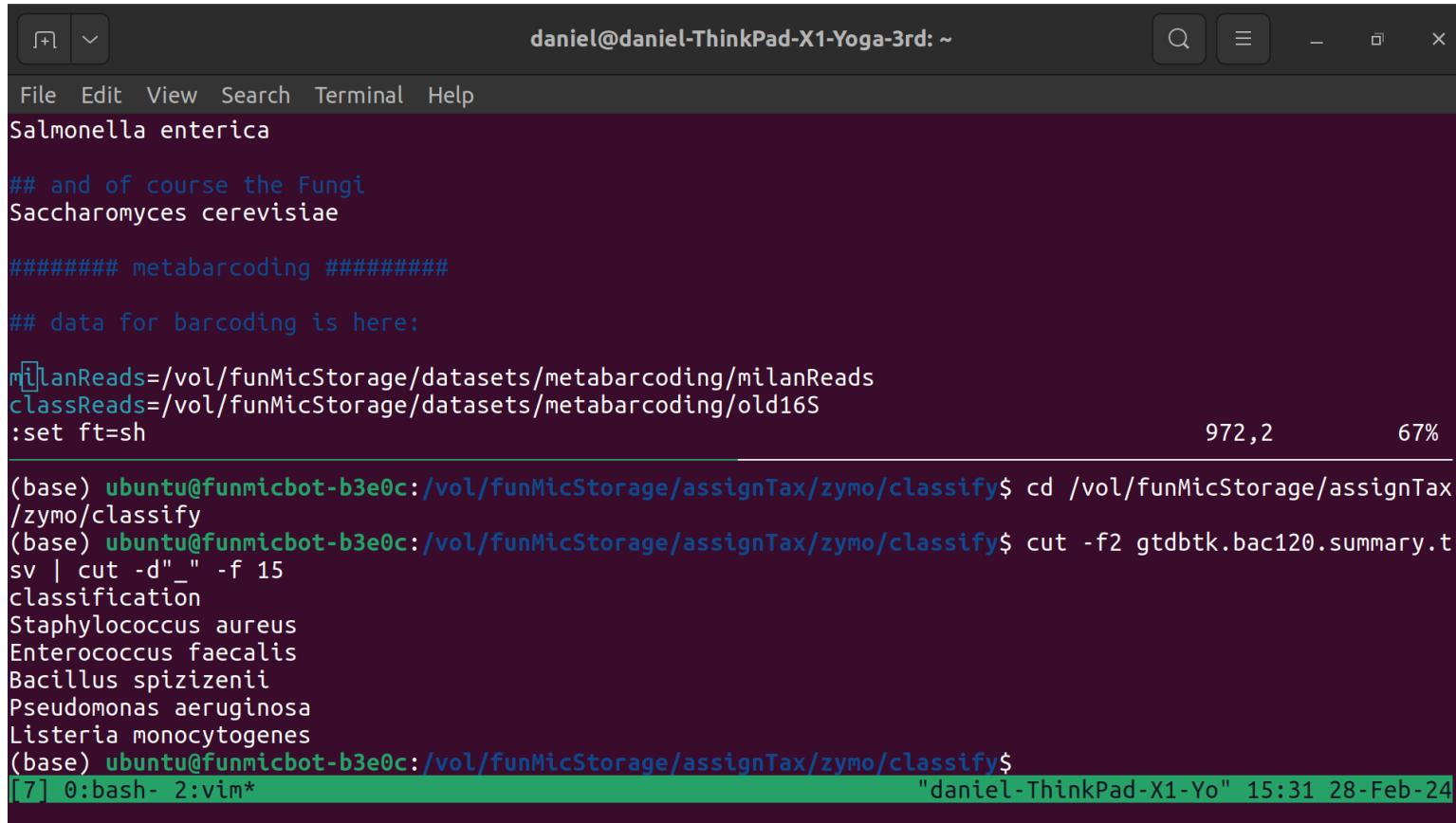


First task: connect to VMs

Milan	ssh -p 30284 -i /path/to/your/privateKey ubuntu@129.70.51.6
Julia	ssh -p 30381 -i /path/to/your/privateKey ubuntu@129.70.51.6
Maria	ssh -p 30355 -i /path/to/your/privateKey ubuntu@129.70.51.6
Anja	ssh -p 30452 -i /path/to/your/privateKey ubuntu@129.70.51.6
Susanne	ssh -p 30461 -i /path/to/your/privateKey ubuntu@129.70.51.6
Rebekka	ssh -p 30372 -i /path/to/your/privateKey ubuntu@129.70.51.6

Second task: learn about linux terminals

Once you are in, open up the script for today, and let's learn about linux.



The screenshot shows a terminal window titled "daniel@daniel-ThinkPad-X1-Yoga-3rd: ~". The window includes standard OS X-style controls (minimize, maximize, close) and a menu bar with File, Edit, View, Search, Terminal, and Help. The main pane displays a script for metabarcoding analysis:

```
File Edit View Search Terminal Help
Salmonella enterica
## and of course the Fungi
Saccharomyces cerevisiae
##### metabarcoding #####
## data for barcoding is here:
milanReads=/vol/funMicStorage/datasets/metabarcoding/milanReads
classReads=/vol/funMicStorage/datasets/metabarcoding/old16S
:set ft=sh
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/assignTax/zymo/classify$ cd /vol/funMicStorage/assignTax/zymo/classify
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/assignTax/zymo/classify$ cut -f2 gtdbtk.bac120.summary.tsv | cut -d"_" -f 15
classification
Staphylococcus aureus
Enterococcus faecalis
Bacillus spizizenii
Pseudomonas aeruginosa
Listeria monocytogenes
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/assignTax/zymo/classify$ [7] 0:bash- 2:vim*                                     "daniel-ThinkPad-X1-Yo" 15:31 28-Feb-24
```

(cut to terminal)

Second task: get a file from your VM to your computer

MiB Mem : 4.2/120747.0 [|||||]
MiB Swap: 0.2/1024.0 [

PID	USER	PR	NI	VIRT	RES	SHR	S	%CPU	%MEM	TIME+	COMMAND
310297	dimitri	20	0	55.1g	3.4g	23084	S	1252	2.9	4:22.26	java
293349	ubuntu	20	0	7764	3444	3172	S	0.7	0.0	0:02.88	bash
14	root	20	0	0	0	0	I	0.3	0.0	0:45.25	rcu_sched
287908	ubuntu	20	0	17444	8644	6008	S	0.3	0.0	0:02.17	sshd
297392	ubuntu	20	0	11032	4020	3172	R	0.3	0.0	0:02.78	top
308737	dimitri	20	0	7764	3468	3192	S	0.3	0.0	0:00.25	bash

```
(base) ubuntu@funmicbot-b3e0c:~$ vol
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage$ ls
Kelp_logs assemblies assignTax databases lost+found mapping phyloFlashOut ref
anaconda3 assemblyQC binning datasets magQC metabarcoding rawReadQC refiningBins
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage$ cd binning/
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning$ ls
MetaBAT zymo
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning$ cd zymo/
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo$ ls
concoct metabat vamb
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo$ cd concoct
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo/concoct$ ls
PCA_components_data_gt1000.csv clustering_gt1000.csv concoctContigs_10K.bed fasta_bins
```

You will need to transfer files from your de.NBI virtual machine to your local computer often.

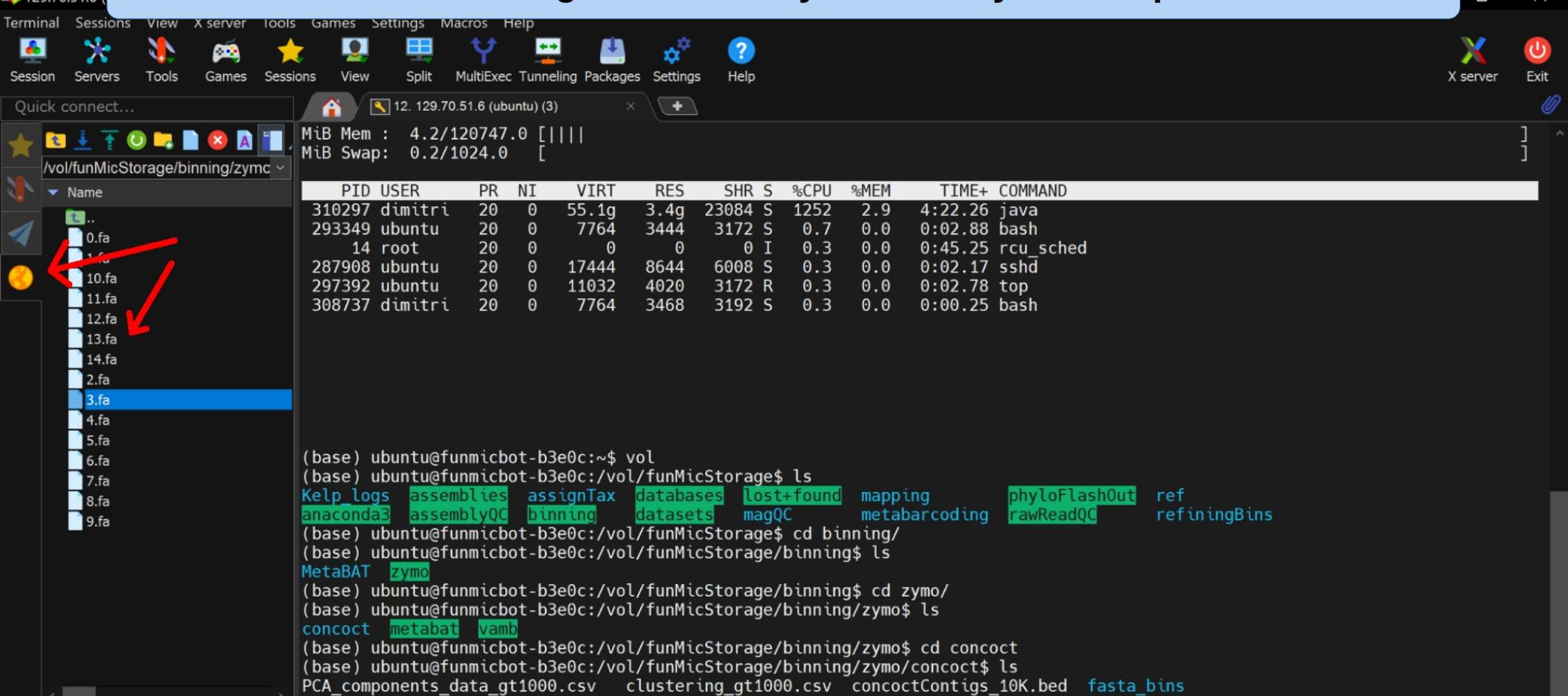
Terminal Sessions View X server Tools Games Settings Macros Help

Session Servers Tools Games Sessions View Split MultiExec Tunneling Packages Settings Help

X server Exit

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Second task: get a file from your VM to your computer



MiB Mem : 4.2/120747.0 [||||]
MiB Swap: 0.2/1024.0 [

PID	USER	PR	NI	VIRT	RES	SHR	S	%CPU	%MEM	TIME+	COMMAND
310297	dimitri	20	0	55.1g	3.4g	23084	S	1252	2.9	4:22.26	java
293349	ubuntu	20	0	7764	3444	3172	S	0.7	0.0	0:02.88	bash
14	root	20	0	0	0	0	I	0.3	0.0	0:45.25	rcu_sched
287908	ubuntu	20	0	17444	8644	6008	S	0.3	0.0	0:02.17	sshd
297392	ubuntu	20	0	11032	4020	3172	R	0.3	0.0	0:02.78	top
308737	dimitri	20	0	7764	3468	3192	S	0.3	0.0	0:00.25	bash

```
(base) ubuntu@funmicbot-b3e0c:~$ vol
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage$ ls
Kelp_logs assemblies assignTax databases lost+found mapping phyloFlashOut ref
anaconda3 assemblyQC binning datasets magQC metabarcoding rawReadQC refiningBins
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage$ cd binning/
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning$ ls
MetaBAT zymo
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning$ cd zymo/
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo$ ls
concoct metabat vamb
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo$ cd concoct
(base) ubuntu@funmicbot-b3e0c:/vol/funMicStorage/binning/zymo/concoct$ ls
PCA_components_data_gt1000.csv clustering_gt1000.csv concoctContigs_10K.bed fasta_bins
```

There are many ways to do this, but we'll try MobaXterm's builtin abilities.

Terminal Sessions View X server Tools Games Settings Macros Help

Session Servers Tools Games Sessions View Split MultiExec Tunneling Packages Settings Help

X server Exit

UNREGISTERED VERSION - Please support MobaXterm by subscribing to the professional edition here: <https://mobaxterm.mobatek.net>

And final note: keep a script of what you do!!!

```
#include <Arduino.h>
#define LED 13
void setup()
{
    pinMode(LED, OUTPUT);
}
void loop()
{
    digitalWrite(LED, HIGH);
    delay(250);
    digitalWrite(LED, LOW);
    delay(250);
}
```

```
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_digital.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_digital.c
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_digital.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_pulse.c
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_shift.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_shift.c
avr-gcc -o .pioenvs/uno/libFrameworkArduino.a pioenvs/uno/FrameworkArduino/CDC.o pioenvs/uno/FrameworkArduino/HardwareSerial0.o pioenvs/uno/FrameworkArduino/HardwareSerial1.o pioenvs/uno/FrameworkArduino/HardwareSerial2.o pioenvs/uno/FrameworkArduino/HardwareSerial3.o pioenvs/uno/FrameworkArduino/I2cAddress.o pioenvs/uno/FrameworkArduino/I2cRegisters.o pioenvs/uno/FrameworkArduino/I2cTransmitter.o pioenvs/uno/FrameworkArduino/I2cTransmitterDriver.o pioenvs/uno/FrameworkArduino/Interrupts.o pioenvs/uno/FrameworkArduino/USBCore.o pioenvs/uno/FrameworkArduino/StringUtils.o pioenvs/uno/FrameworkArduino/Math.o pioenvs/uno/FrameworkArduino/String.o pioenvs/uno/FrameworkArduino_wiring.o pioenvs/uno/FrameworkArduino_abl.o pioenvs/uno/FrameworkArduino_main.o pioenvs/uno/FrameworkArduino_new.o pioenvs/uno/FrameworkArduino_wiring.o pioenvs/uno/FrameworkArduino_wiring_analog.o pioenvs/uno/FrameworkArduino_wiring_pulse.o pioenvs/uno/FrameworkArduino_wiring_shift.o
avr-ranlib .pioenvs/uno/libFrameworkArduino.a
avr-g++ -o .pioenvs/uno/firmware.elf -Os -mmcu=atmega328p -Wl,--gc-sections,--relax,.pioenvs/uno/src/blink.o -L/Users/zach/.platformio/packages/lscripts -L.pioenvs/uno -Wl,--start-group -L.pioenvs/uno/libFrameworkArduinoVariant.a .pioenvs/uno/libFrameworkArduino.a -Wl,--end-group
avr-g++ -o .pioenvs/uno/firmware.hex -R .eprom .pioenvs/uno/firmware.elf .pioenvs/uno/firmware.hex
avr-size --mcu=atmega328p -c -d .pioenvs/uno/firmware.elf
AVR Memory Usage
Device: atmega328p
Program:   998 bytes (3.0% Full)
(.text + .data + .bootLoader)
Data:      9 bytes (0.4% Full)
(.data + .bss + .noinit)

=====
[BUILD SUCCESS] Took 1.89 seconds
=====

Built target platformio_build
Compilation finished at Fri Dec 25 23:08:44
```

13:12 U-[blink]src/blink.cpp All C++/PlatformIO pair ws yes + 55: 0 R=compilation 0x Compilation exit [0] Project

With scientific computing, it can sometimes take hours to build, debug, and successfully execute a single program, sometimes just one line of code.

And final note: keep a script of what you do!!!

```
#include <Arduino.h>
#define LED 13
void setup()
{
    pinMode(LED, OUTPUT);
}
void loop()
{
    digitalWrite(LED, HIGH);
    delay(250);
    digitalWrite(LED, LOW);
    delay(250);
}
```

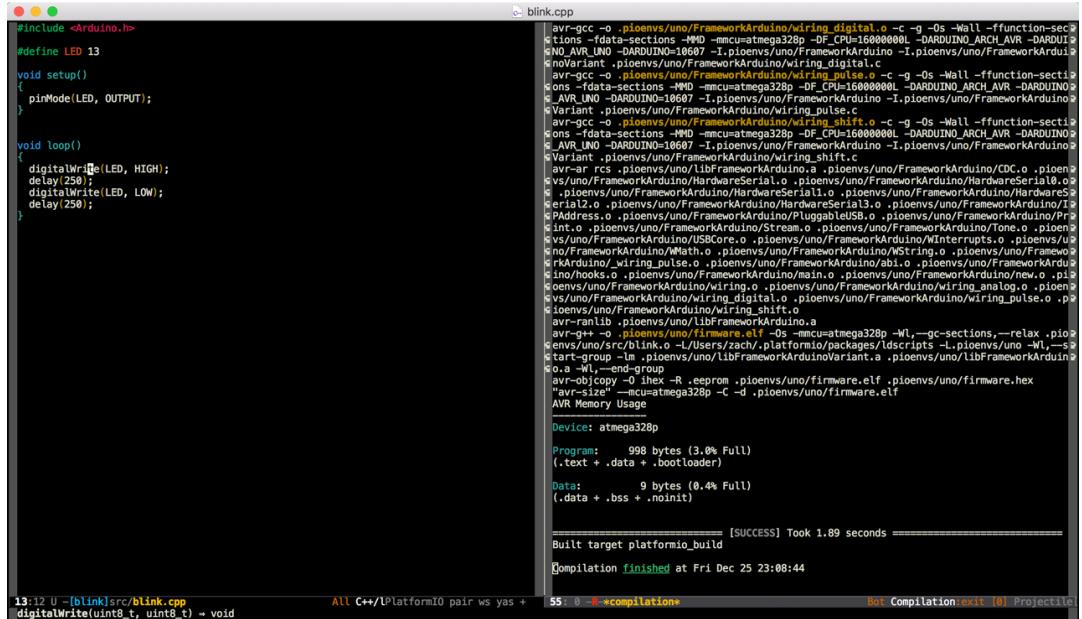
```
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_digital.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_digital.c
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_digital.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_pulse.c
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avr-gcc -o .pioenvs/uno/libFrameworkArduino.a pioenvs/uno/FrameworkArduino/CDC.o pioenvs/uno/FrameworkArduino/HardwareSerial0.o pioenvs/uno/FrameworkArduino/HardwareSerial1.o pioenvs/uno/FrameworkArduino/HardwareSerial2.o pioenvs/uno/FrameworkArduino/HardwareSerial3.o pioenvs/uno/FrameworkArduino/I2cAddress.o pioenvs/uno/FrameworkArduino/I2cRegisters.o pioenvs/uno/FrameworkArduino/I2cTransmitter.o pioenvs/uno/FrameworkArduino/I2cTransmitterDriver.o pioenvs/uno/FrameworkArduino/Interrupts.o pioenvs/uno/FrameworkArduino/USBCore.o pioenvs/uno/FrameworkArduino/StringUtils.o pioenvs/uno/FrameworkArduino/Math.o pioenvs/uno/FrameworkArduino/String.o pioenvs/uno/FrameworkArduino_wiring.o pioenvs/uno/FrameworkArduino_wiring_pulse.o pioenvs/uno/FrameworkArduino_abl.o pioenvs/uno/FrameworkArduino_hooks.o pioenvs/uno/FrameworkArduino_main.o pioenvs/uno/FrameworkArduino_new.o pioenvs/uno/FrameworkArduino_wiring.o pioenvs/uno/FrameworkArduino_wiring_analog.o pioenvs/uno/FrameworkArduino_wiring_pulse.o pioenvs/uno/FrameworkArduino_wiring_shift.o
avr-ranlib .pioenvs/uno/libFrameworkArduino.a
avr-g++ -o .pioenvs/uno/firmware.elf -Os -mmcu=atmega328p -Wl,--gc-sections,--relax,.pioenvs/uno/src/blink.o -L/Users/zach/.platformio/packages/lscripts -L.pioenvs/uno -Wl,--start-group -L.pioenvs/uno/libFrameworkArduinoVariant.a .pioenvs/uno/libFrameworkArduino.a -Wl,--end-group
avr-g++ -o .pioenvs/uno/firmware.hex -R .eprom .pioenvs/uno/firmware.elf .pioenvs/uno/firmware.hex
avr-size -A .pioenvs/uno/libFrameworkArduinoVariant.a -d .pioenvs/uno/firmware.elf
AVR Memory Usage
Device: atmega328p
Program:   998 bytes (3.0% Full)
(.text + .data + .bootLoader)
Data:      9 bytes (0.4% Full)
(.data + .bss + .noinit)

=====
[BUILT] Took 1.89 seconds =====
Built target platformio_build
Compilation finished at Fri Dec 25 23:08:44
```

13:12 U-[blink]src/blink.cpp All C++/PlatformIO pair ws yes + 55: 0 R=compilation 0x Compilation:exit [0] Project

You will often want to return to your commands days or months later. Other scientists will want to know precisely what commands and settings you used.

And final note: keep a script of what you do!!!



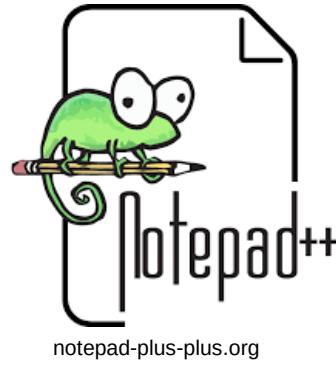
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  delay(250);
  digitalWrite(LED, LOW);
  delay(250);
}
```

```
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_digital.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_digital.c -I.pioenvs/uno/FrameworkArduino/Wiring_digital.h -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_pulse.c -avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_shift.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduinoVariant -I.pioenvs/uno/FrameworkArduino/Wiring_shift.c -avr-ar rcs .pioenvs/uno/libFrameworkArduino.a .pioenvs/uno/FrameworkArduino/CDC.o .pioenvs/uno/FrameworkArduino/HardwareSerial.o .pioenvs/uno/FrameworkArduino/HardwareSerial1.o .pioenvs/uno/FrameworkArduino/HardwareSerial2.o .pioenvs/uno/FrameworkArduino/HardwareSerial3.o .pioenvs/uno/FrameworkArduino/I2CAddress.o .pioenvs/uno/FrameworkArduino/I2CRegisters.o .pioenvs/uno/FrameworkArduino/Interrupts.o .pioenvs/uno/FrameworkArduino/USBCore.o .pioenvs/uno/FrameworkArduino/WInterrupts.o .pioenvs/uno/FrameworkArduino/Math.o .pioenvs/uno/FrameworkArduino/StringUtils.o .pioenvs/uno/FrameworkArduino_wiring_pulse.o .pioenvs/uno/FrameworkArduino/abi.o .pioenvs/uno/FrameworkArduino/main.o .pioenvs/uno/FrameworkArduino/new.o .pioenvs/uno/FrameworkArduino/wiring.o .pioenvs/uno/FrameworkArduino/wiring_analog.o .pioenvs/uno/FrameworkArduino/wiring_digital.o .pioenvs/uno/FrameworkArduino/wiring_shift.o .pioenvs/uno/FrameworkArduino/wiring_usart.o .pioenvs/uno/firmware.elf -L/Users/zach/.platformio/packages/dscripts -Lpioenvs/uno -Wl,-T -Wl,firmware.lds -R .eprom -R .hex -R .hex -R .firmware.hex -avr-size -v -mcu=atmega328p -c -d .pioenvs/uno/firmware.elf
AVR Memory Usage
Device: atmega328p
Program:   998 bytes (3.0% Full)
(.text + .data + .bootLoader)
Data:      9 bytes (0.4% Full)
(.data + .bss + .noinit)

[SUCCESS] Took 1.89 seconds
Built target platformio_build
Compilation finished at Fri Dec 25 23:08:44

13:12 U-[blink]src/blink.cpp    55: 0 ~R~compilation*       56: Compilation:exit [0] Projectile:
```



www.vim.org



www.gnu.org/software/emacs/tour/

Keep a running text-file script of all that you do. Comment it with your own mental notes so you can remember what you did and why in human language.
Pick a good **text editor** (not word processor!!) and learn to use it.