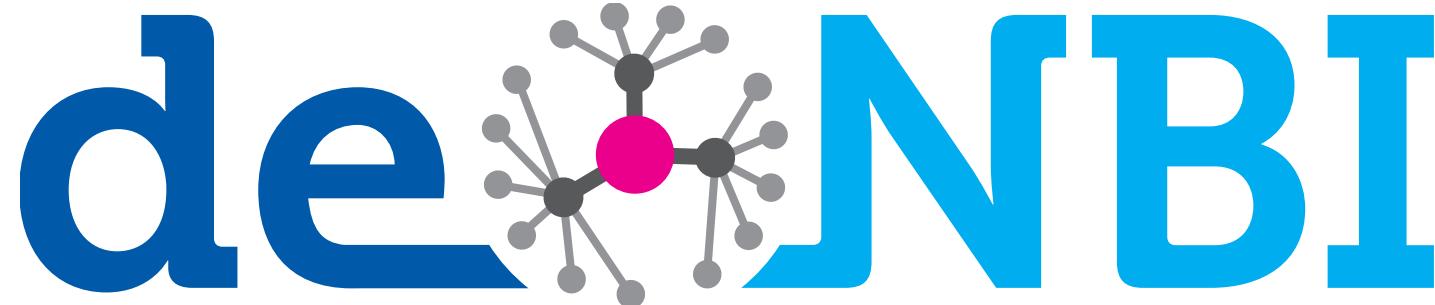


# Functional microbiome research – bioinformatics section

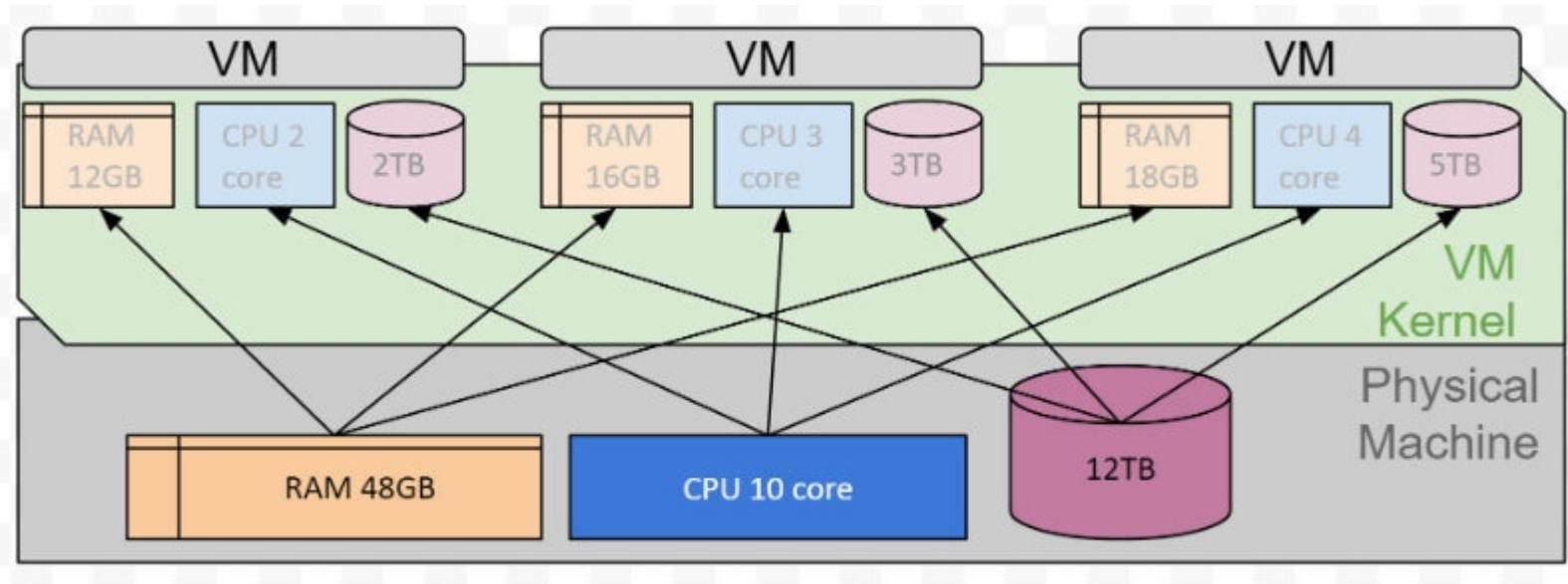
Session 1 – intro to linux



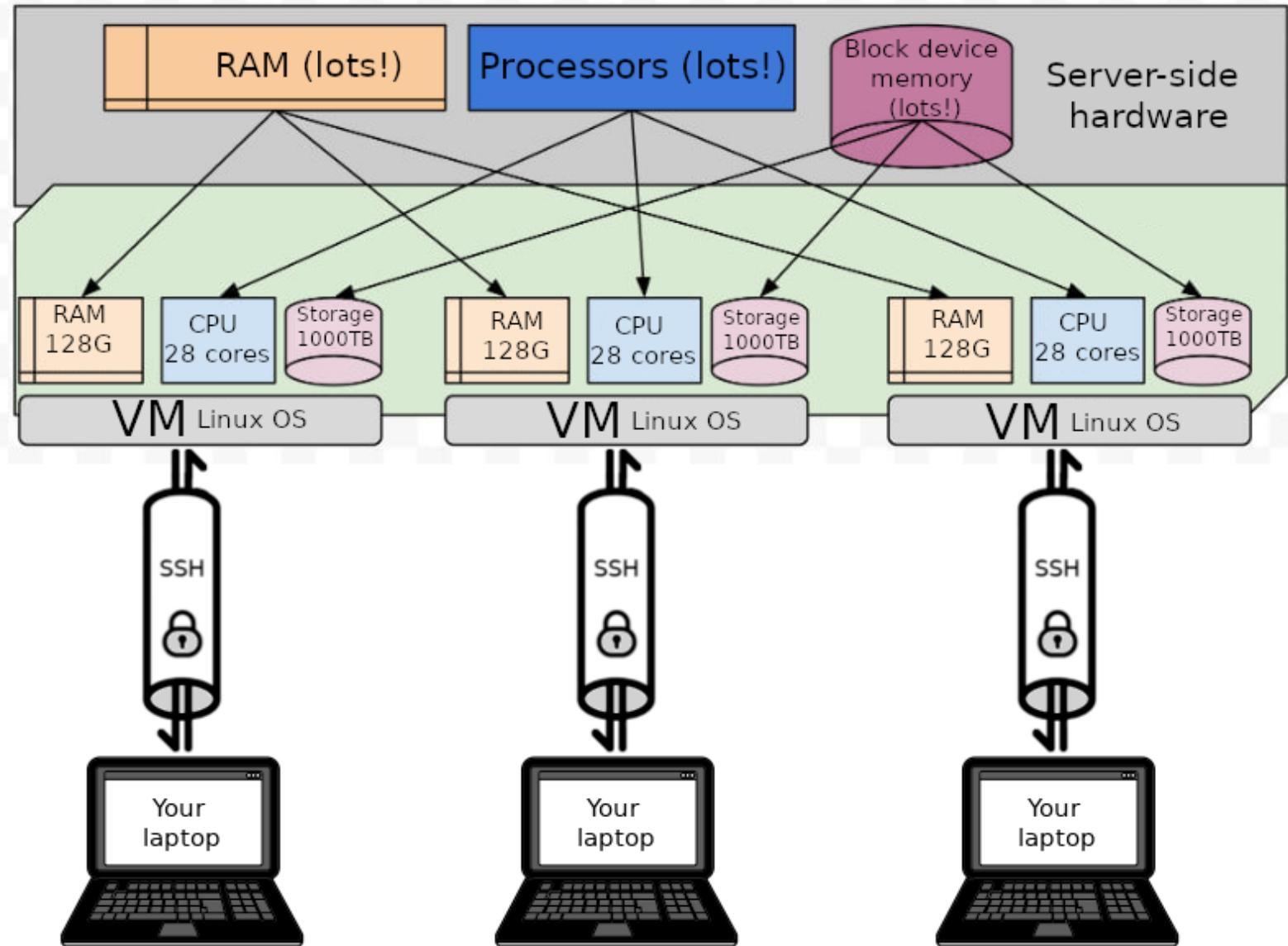
GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

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

Distributed computing and compute clusters are essential tools for scientists.



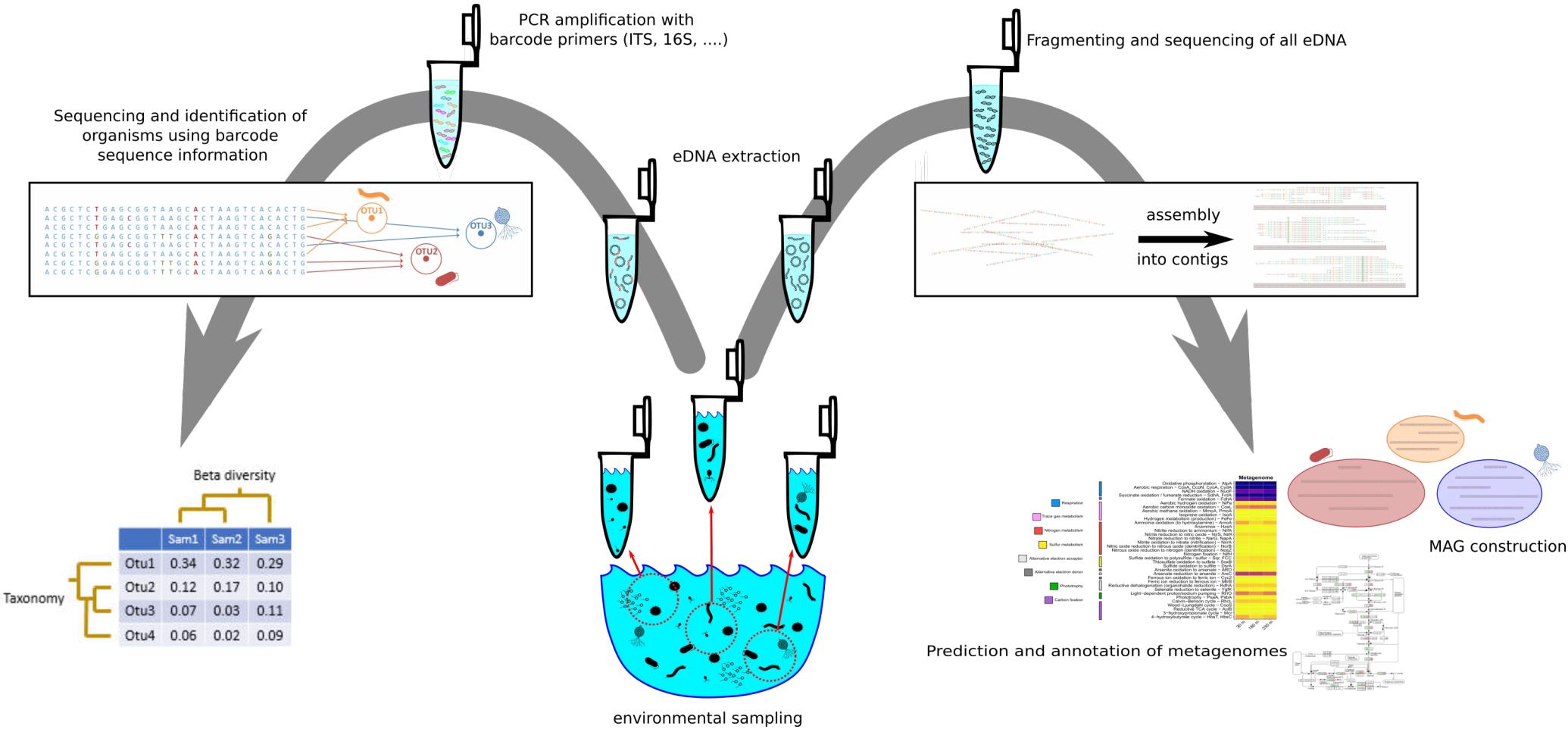
We use our local computers to talk to shared remote servers that have lots of high-powered processors, and storage and RAM.



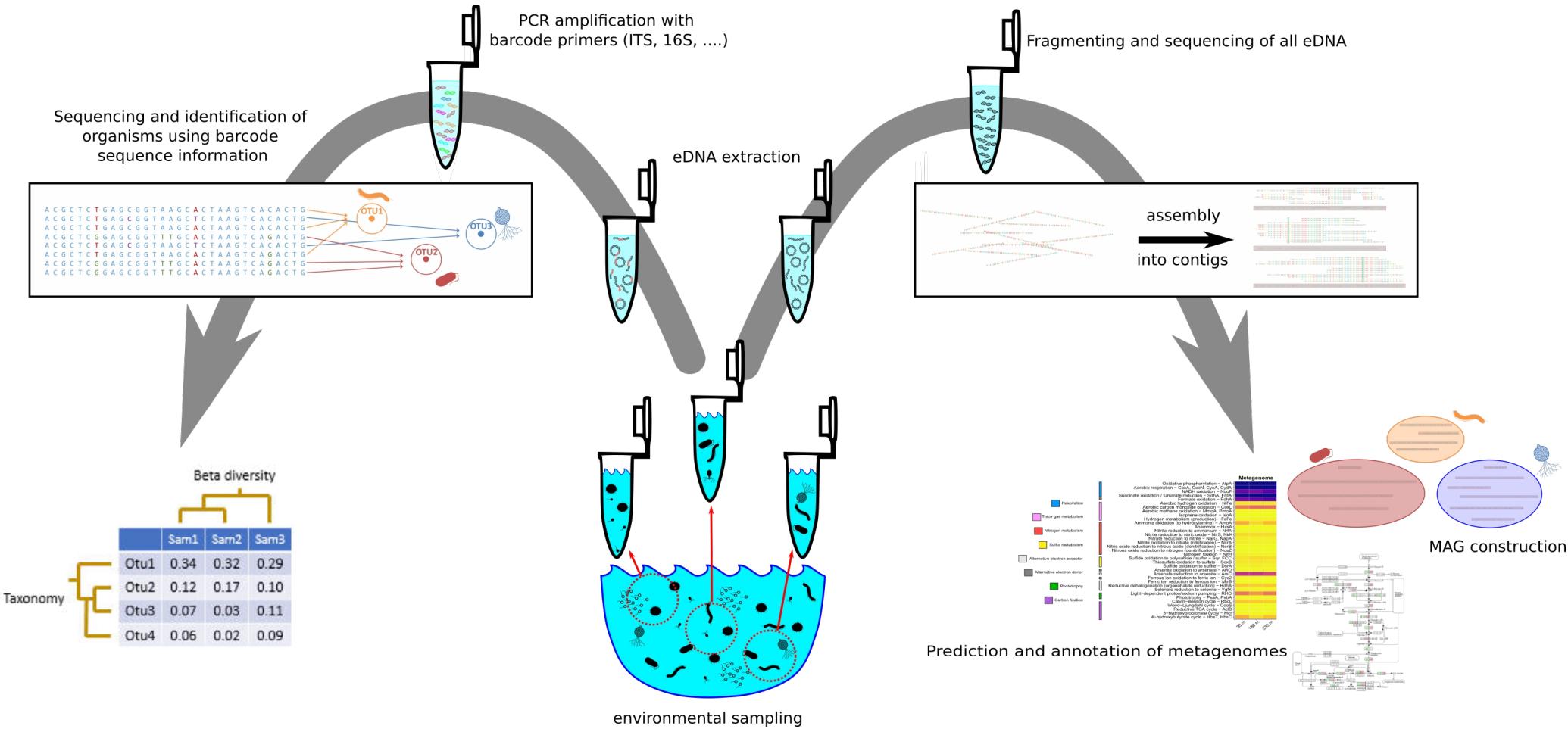
de.NBI / Universität  
Bielefeld

Us.

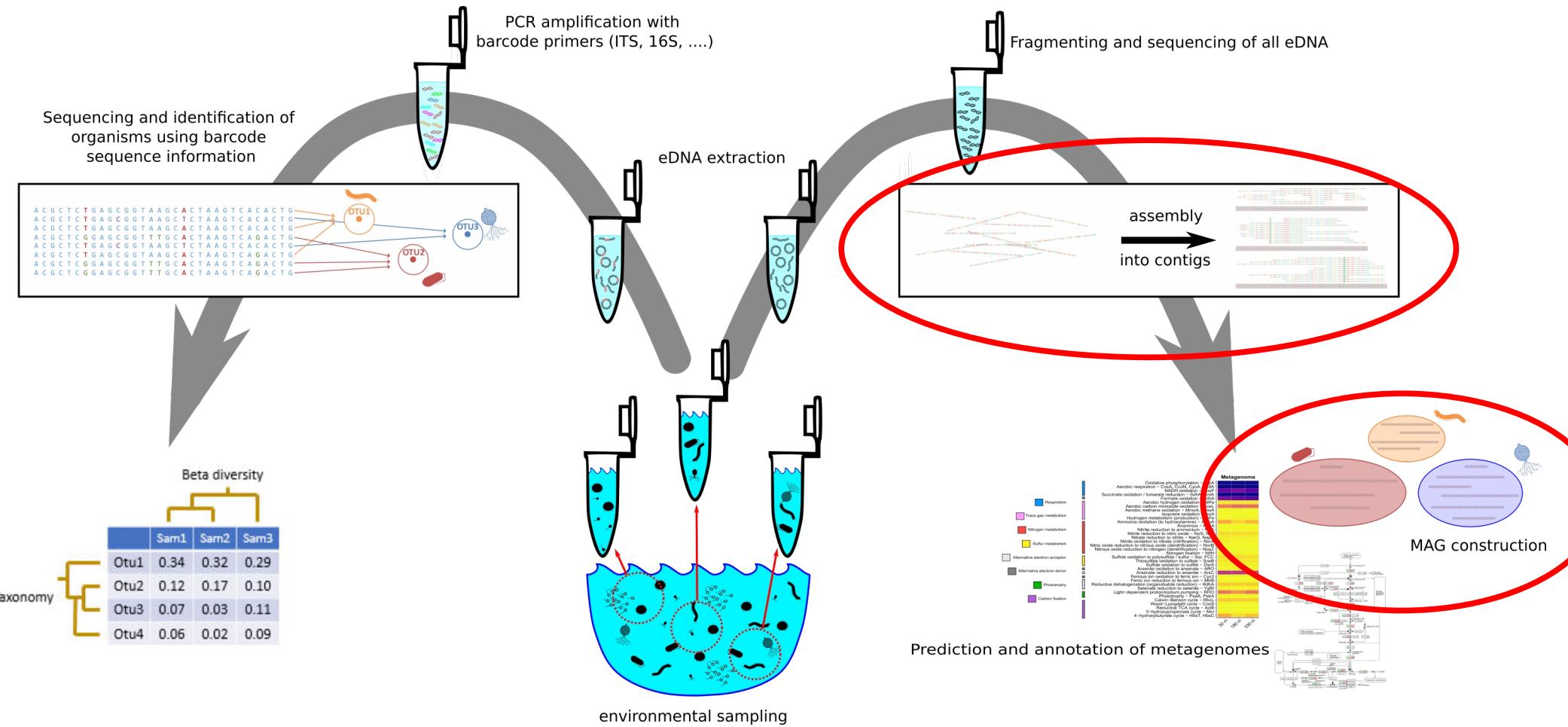
# Review – what do we do with DNA sequence data?



# Why do we need distributed computing to do this?



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



# Dataset 2: Kelp decomposition

LIMNOLOGY  
and  
OCEANOGRAPHY



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© 2020 The Authors. *Limnology and Oceanography* published by Wiley Periodicals LLC on behalf of Association for the Sciences of Limnology and Oceanography.  
doi: 10.1002/limo.11574

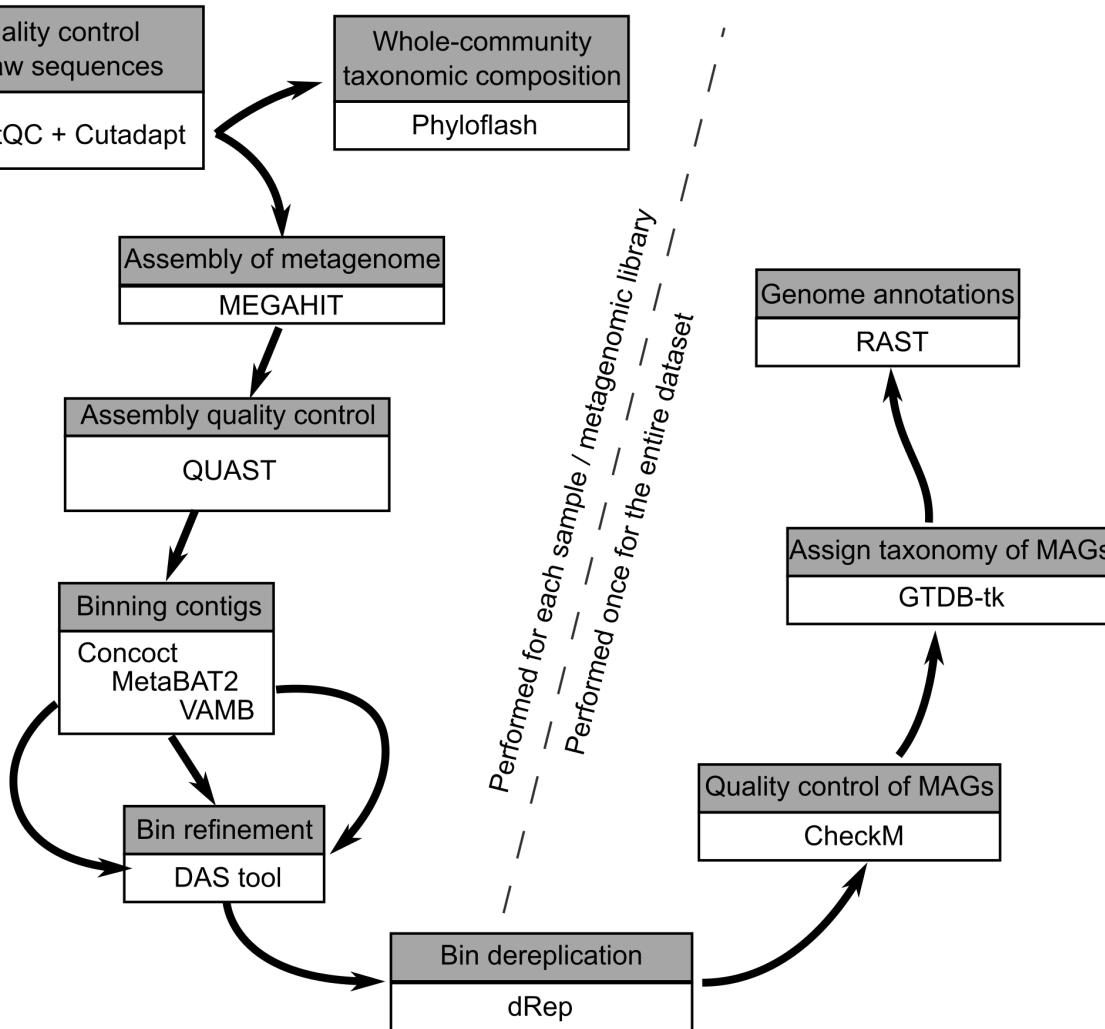
## Kelp deposition changes mineralization pathways and microbial communities in a sandy beach

Marit R. van Erk <sup>1,\*</sup> Dimitri V. Meier,<sup>1,a</sup> Timothy Ferdelman,<sup>1</sup> Jens Harder,<sup>1</sup> Ingeborg Bussmann,<sup>2</sup> Dirk de Beer<sup>1</sup>

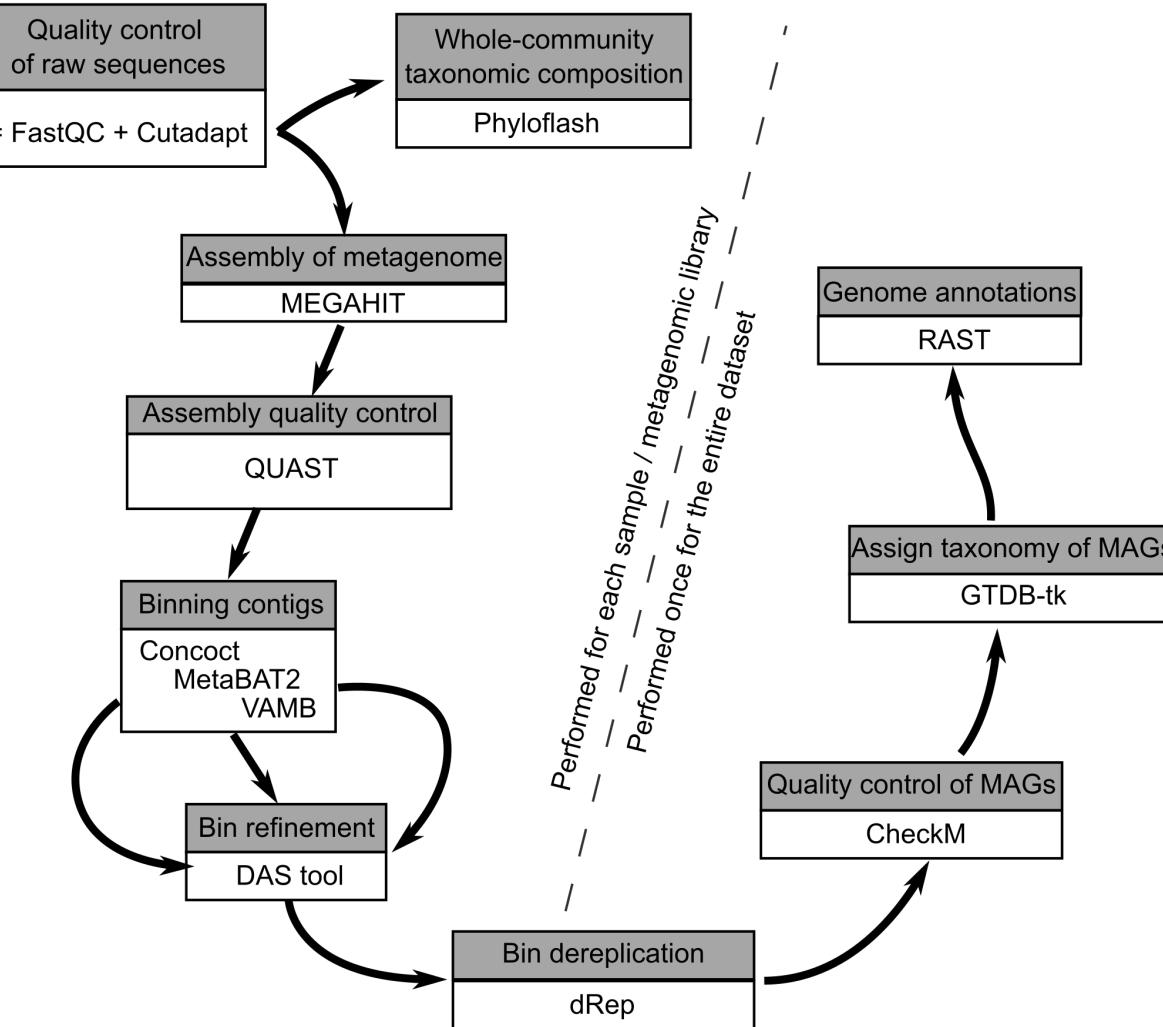
<sup>1</sup>Max Planck Institute for Marine Microbiology, Bremen, Germany

<sup>2</sup>Alfred 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.



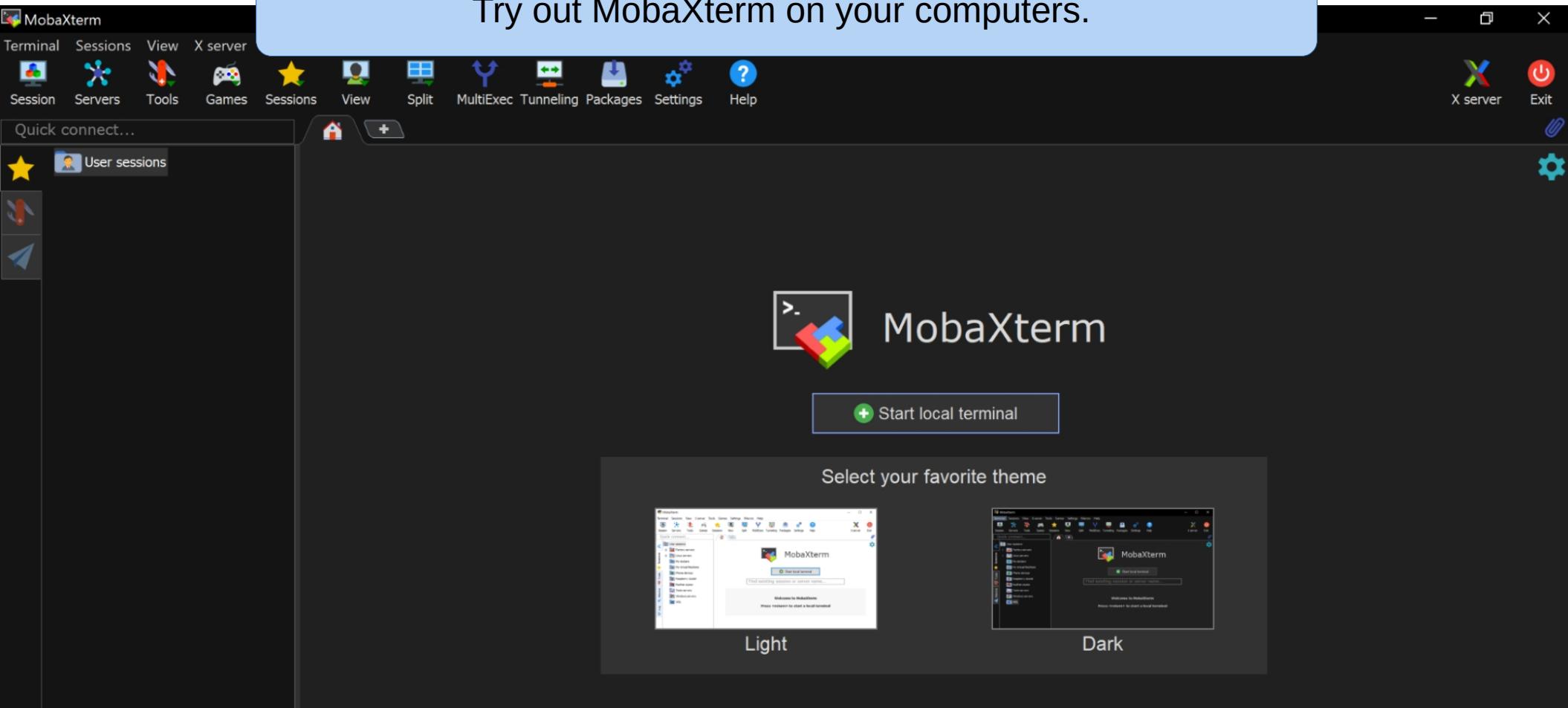
You will use these tools to discover the ecological story in the Kelp decomposition dataset.



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
4. Multivariate SIP analysis

# 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.

Sessions View X server

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

X server Exit

Quick connect... Session settings

SSH Telnet Rsh Xdmcp RDP VNC SFTP Serial File Shell Browser Mosh Aws S3 WSL

Choose a session type...

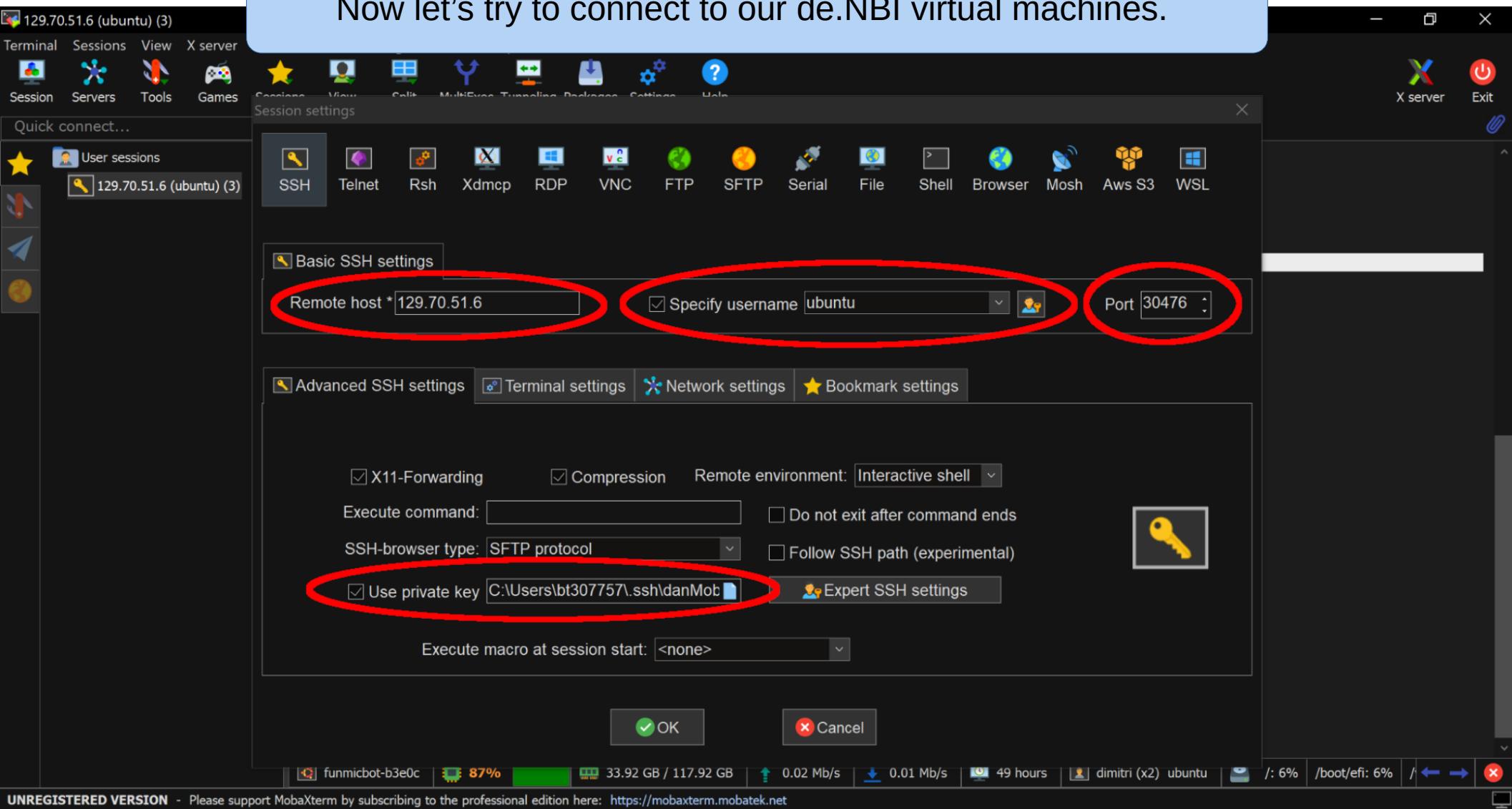
OK Cancel

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/boot/efi: 6% / ← → ×

The screenshot shows the MobaXterm interface. At the top, there's a menu bar with tabs like Terminal, Sessions, View, and X server. Below the menu is a toolbar with icons for Session, Servers, Tools, Games, Sessions, View, Split, MultiExec, Tunneling, Packages, Settings, and Help. On the left, there's a sidebar titled 'Quick connect...' containing a list of session names and a 'Remote monitoring' section. In the center, a 'Session settings' dialog is open, showing various connection types: SSH (which is highlighted with a red circle), Telnet, Rsh, Xdmcp, RDP, VNC, SFTP, Serial, File, Shell, Browser, Mosh, Aws S3, and WSL. At the bottom of the dialog are 'OK' and 'Cancel' buttons. Red arrows point from the 'Session' button in the top menu to the 'Session settings' dialog, and from the 'SSH' icon in the 'Session settings' dialog to the 'SSH' icon in the 'Quick connect...' sidebar.

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

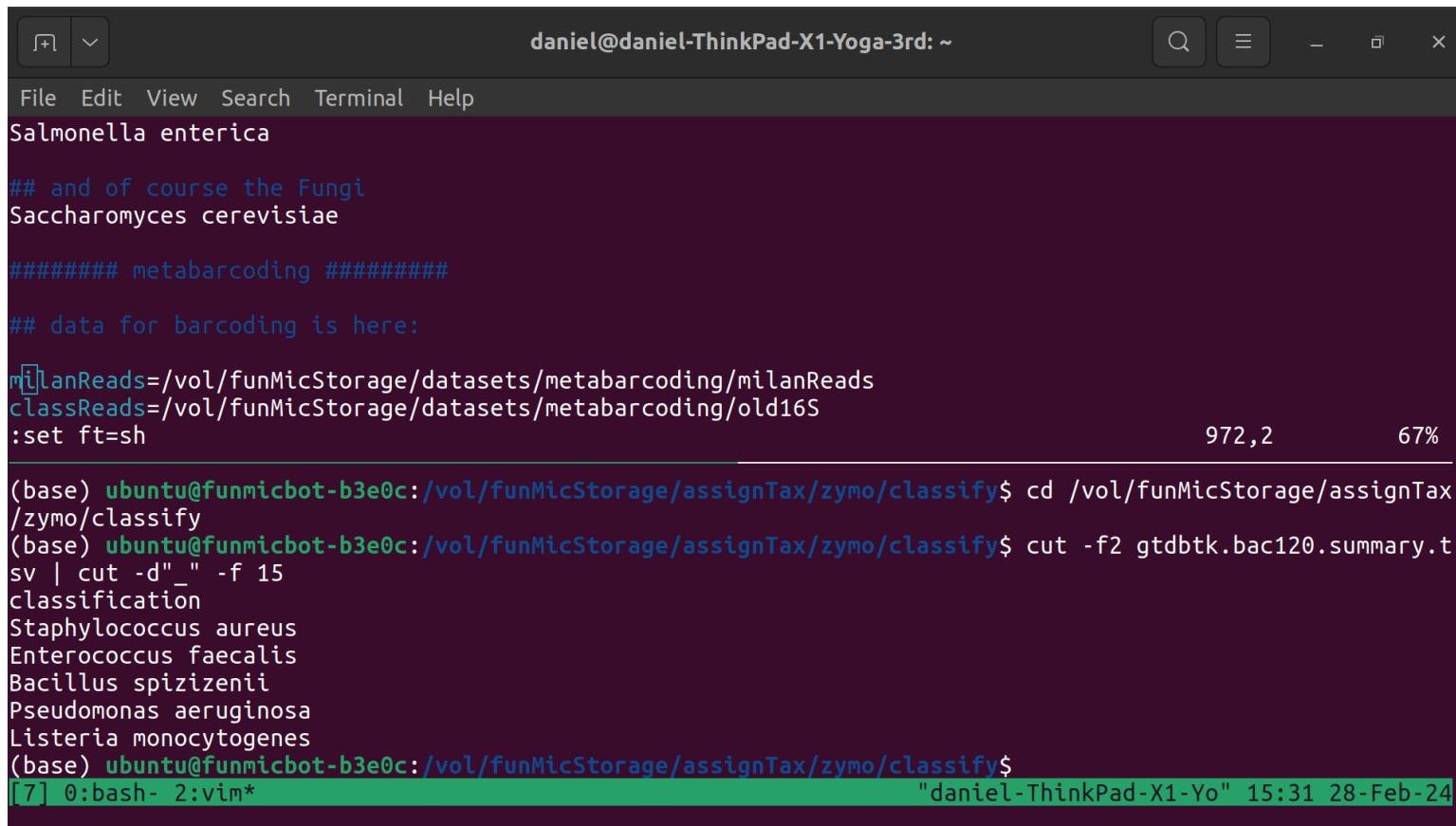


## First task: connect to VMs

Dennis Büchner	ssh -X ubuntu@129.70.51.6 -p 30323
Federico Campo	ssh -X ubuntu@129.70.51.6 -p 30281
Zoi Hock	ssh -X ubuntu@129.70.51.6 -p 30365
Dongun Kim	ssh -X ubuntu@129.70.51.6 -p 30500
Fiona Peters	ssh -X ubuntu@129.70.51.6 -p 30272
Hannes Reichel	ssh -X ubuntu@129.70.51.6 -p 30453
Romina Retsch	ssh -X ubuntu@129.70.51.6 -p 30306
Isabelle Storm	ssh -X ubuntu@129.70.51.6 -p 30460
backup	ssh -X ubuntu@129.70.51.6 -p 30414

## Second task: learn about linux terminals

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

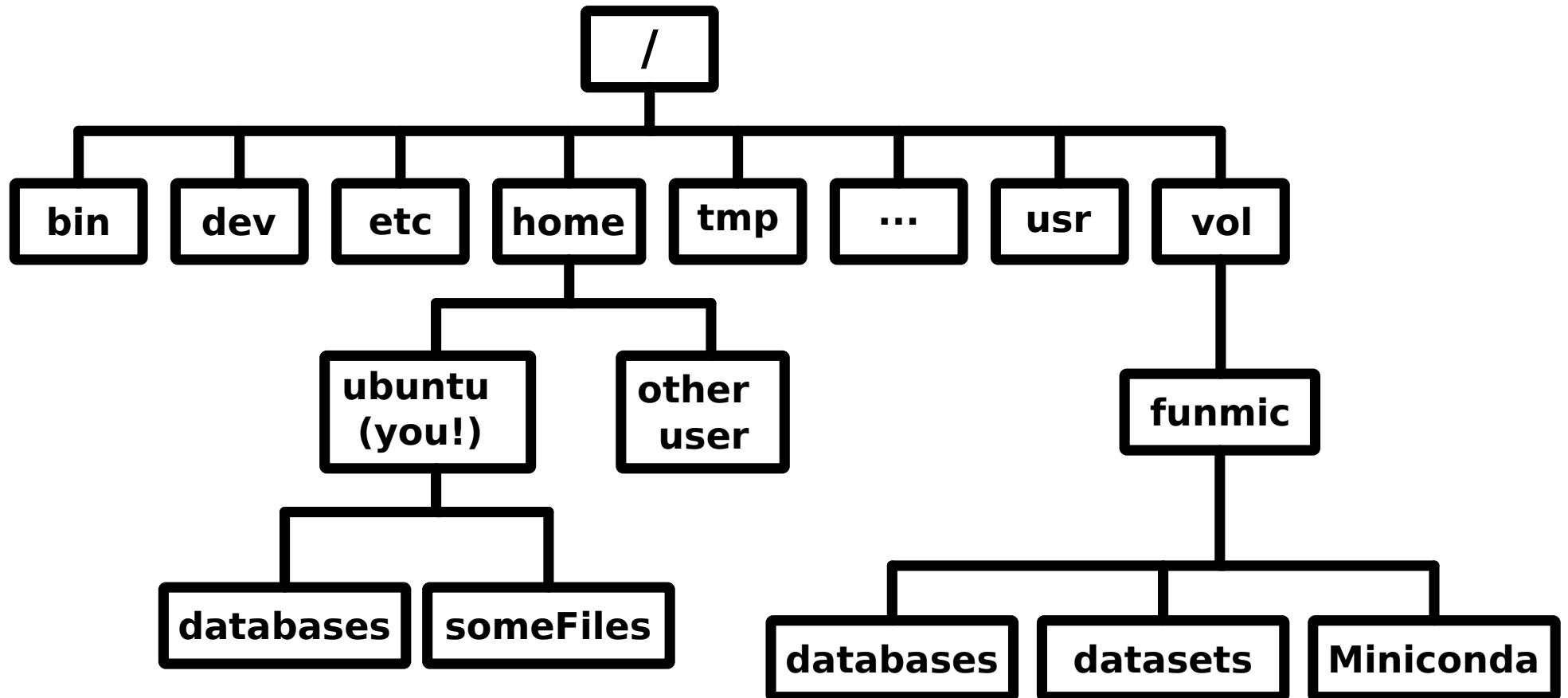


The screenshot shows a terminal window titled "daniel@daniel-ThinkPad-X1-Yoga-3rd: ~". The window includes standard OS X-style controls (minimize, maximize, close) and a menu bar with File, Edit, View, Search, Terminal, and Help. The main pane displays a script for metabarcoding analysis:

```
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)

## Image of a file tree in Linux:



## 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.

129.70.51.6 (3) 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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# 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 -MMD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_NO_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduino_noVariant .pioenvs/uno/FrameworkArduino/wiring_digital.c
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_pulse.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MMD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduino_Variant .pioenvs/uno/FrameworkArduino/wiring_pulse.c
avr-gcc -o .pioenvs/uno/FrameworkArduino/wiring_shift.o -c -g -Os -Wall -ffunction-sections -fdata-sections -MMD -mmcu=atmega328p -DF_CPU=16000000L -DARDUINO_ARCH_AVR -DARDUINO_AVR_UNO -DARDUINO=10607 -I.pioenvs/uno/FrameworkArduino -I.pioenvs/uno/FrameworkArduino_Variant .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/HardwareSerial0.o .pioenvs/uno/FrameworkArduino/HardwareSerial1.o .pioenvs/uno/FrameworkArduino/HardwareSerial2.o .pioenvs/uno/FrameworkArduino/HardwareSerial3.o .pioenvs/uno/FrameworkArduino/IPAddress.o .pioenvs/uno/FrameworkArduino/PluggableUSB.o .pioenvs/uno/FrameworkArduino/Print.o .pioenvs/uno/FrameworkArduino/Stream.o .pioenvs/uno/FrameworkArduino/Tone.o .pioenvs/uno/FrameworkArduino/USBCore.o .pioenvs/uno/FrameworkArduino/WInterrupts.o .pioenvs/uno/FrameworkArduino/WMath.o .pioenvs/uno/FrameworkArduino/WString.o .pioenvs/uno/FrameworkArduino_wiring_pulse.o .pioenvs/uno/FrameworkArduino_abi.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_digital.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/ldscripts -L.pioenvs/uno -Wl,--start-group -Lm .pioenvs/uno/libFrameworkArduinoVariant.a .pioenvs/uno/libFrameworkArduino.a -Wl,--end-group
avr-objcopy -O ihex -R .eeprom .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++/lPlatformIO pair ws yes +
digitalWrite(uint8_t, uint8_t) -> void
55: 0 -R-*compilation*
Bot Compilation:exit [0] Projectile
```