A network diagram consisting of several white nodes connected by thin white lines. Three nodes are highlighted with concentric circles, indicating a focus or specific interest. The diagram is overlaid on a background of a blue ocean with a sun flare effect.

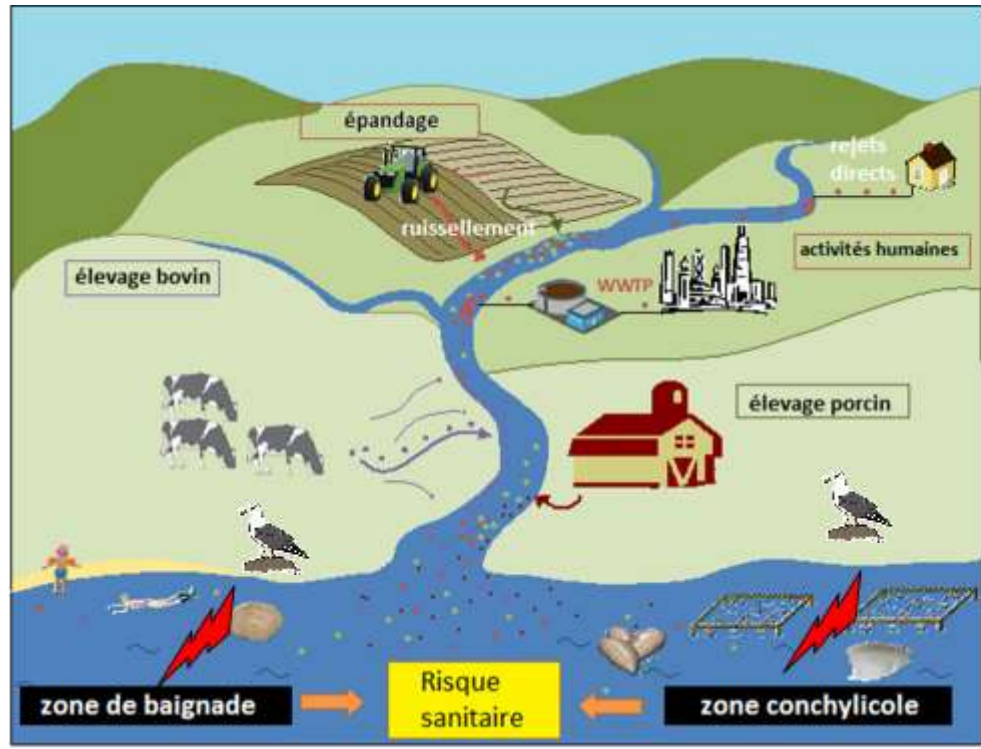
Pour une meilleure identification
des pollutions d'origine fécale du
littoral : développement d'outils
de Microbial Source Tracking et
caractérisation des *Campylobacter*
par NGS



Bactéries et virus entériques

Campylobacter

- E. coli* pathogènes
- Salmonella*
- P. aeruginosa*



Bactéries marines

Vibrio spp.



Impact sur les activités en zones côtières



Conséquences économiques : fermeture et/ou déclassification des zones conchylicoles et de baignade

Risques pour la santé : infections et toxi-infections alimentaires collectives (TIAC)

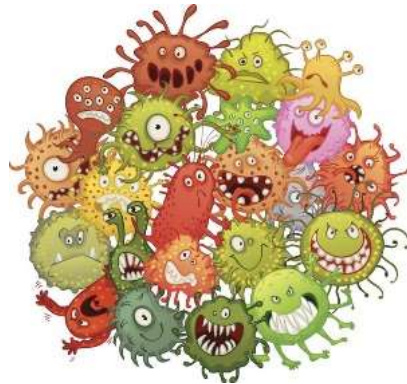
Législation Européenne

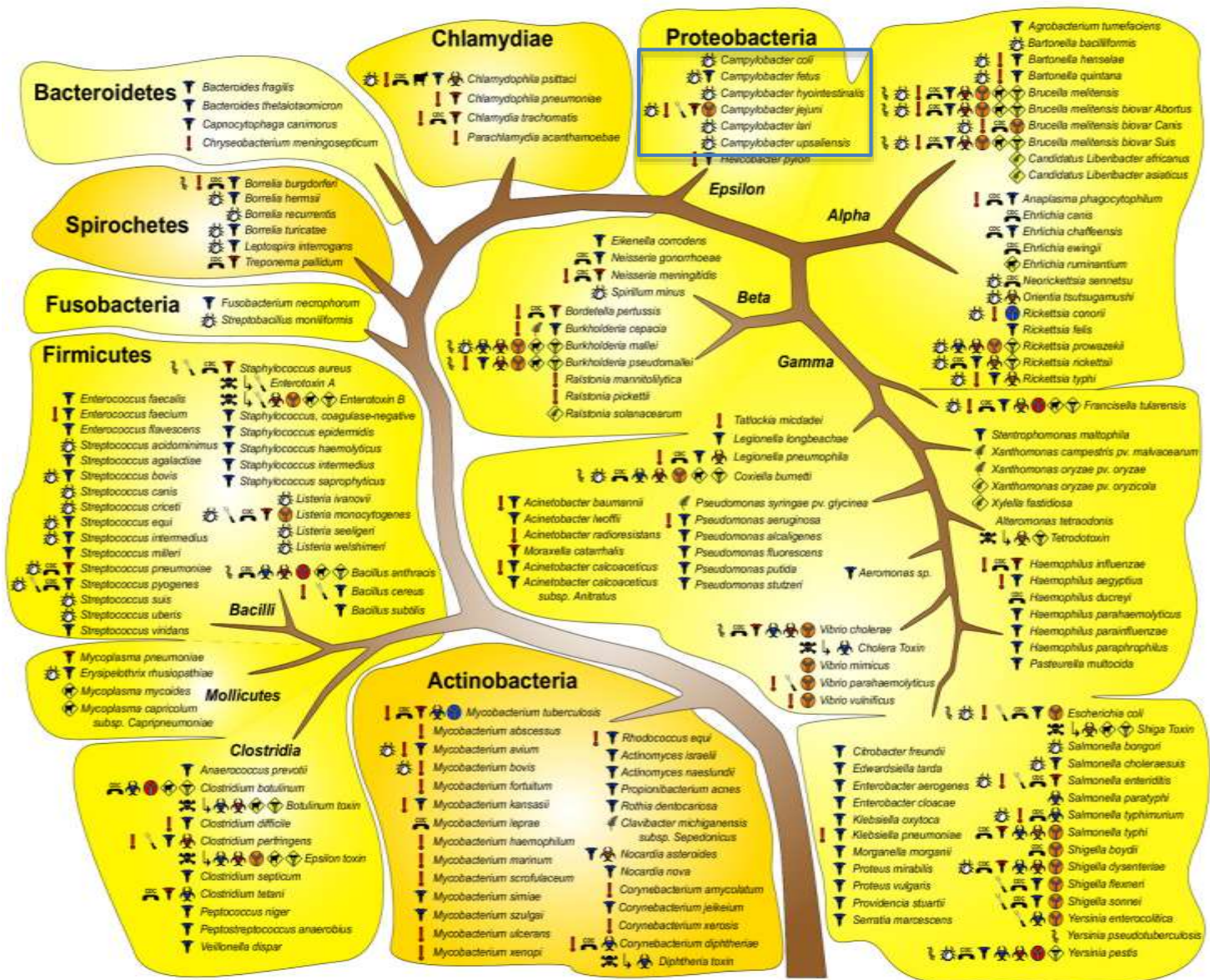
- Zones conchylicoles : règlement 854/2004/CE basée sur les [*E. coli*] dans la chair et le liquide intervalvaire
- Zones de baignade : directive 2006/7/CE basée sur les [*E. coli*] & [entérocoques intestinaux]



- Identifier les sources de pollution afin de mieux les gérer
- Caractérisation génétique des *Campylobacter spp.*

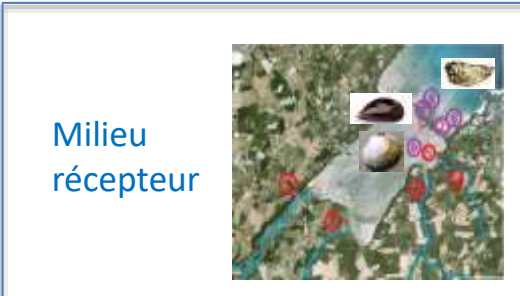
- Étudier les communautés bactériennes environnantes pour concevoir de nouvelles boîtes à outils **TSM** (Traceurs de Sources Microbiennes) afin de **différencier l'origine de la pollution**
- Étudier la **diversité** des espèces de *Campylobacter* retrouvés dans ces milieux, et cribler les gènes de **virulence** et **antibiorésistance**





- 📌 Globally Important Human Pathogen
- 📌 Medically Important Human Pathogen
- 📌 Important Animal Pathogen
- 📌 Important Plant Pathogen
- 📌 Zoonotic Agent
- 📌 Toxin
- 📌 Principal Foodborne Pathogen
- 📌 Emerging Infectious Agent

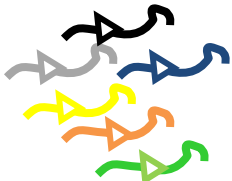




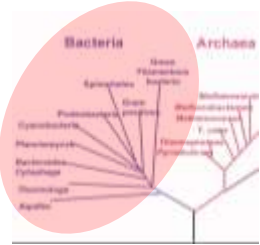
Extraction ADN



Amplification et séquençage gène ARNr 16S (V3V4)



Analyses bio-informatiques



NGS

Isolement souches pures *Campylobacter*



Extraction & séquençage du gADN



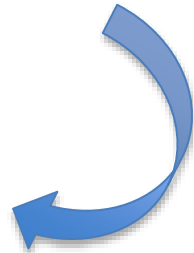
Analyses bio-informatiques



WGS

Utilisation de DATARMOR en ligne de commande Unix

Principalement des fichiers du type .fastq





Liste des outils bioinfo

	Tool name	release	cmd-line	Galaxy	Available for	Manual
3	biom-format	2.1.6	😊	😊	conversion	
14	cutadapt	1.13	😊	😊	quality-control	
19	FastQC	0.11.5	😊	😊	quality-control	
21	FLASH	1.2.11	😊	😊	data-handling	
23	FROGS	1.4.0	😊	😞	community-profiling	
24	FROGS	2.0.0	😊	😊	community-profiling	
43	mothur	1.38.1.1	😊	😞	community-profiling	
44	mothur	1.39.5	😊	😞	community-profiling	
46	MultiQC	1.2	😊	😞	quality-control	
47	NCBI Blast+	2.6.0	😊	😊	pairwise-sequence-alignment	
48	NCBI Blast+	2.2.31	😊	😞	alignment	
55	PICRUSt	1.1.0	😊	😞	phylogenetic-tree-analysis	
61	QIIME	1.9.1	😊	😞	community-profiling	
62	QIIME	2.0.0	😊	😞	community-profiling	



```
C:\Users\mboukerb\Documents\Mobaxterm\ssh\FTPRemoteFiles\312\mboukerb@datarmor\3qime_goumerl.pbs - Notepad++
Fichier Edition Recherche Affichage Encodage Langage Paramétrage Outils Macros Exécution Compléments Documents
3qime_goumerl.pbs
1 //usr/bin/env bash
2
3 #PBS -q main
4 #PBS -l walltime=24:00:00
5 #PBS -l mem=11Gb
6 #PBS -l ncpus=66
7
8 cd /home1/datasetwork/mboukerb/RisakManche/allDNA2/
9
10 ./appli/bininfo/qiime/1.9.1/env.sh
11
12 #STEPS = downstream analysis with QIIME and VSEARCH
13
14 #create a directory for the STEPS analyses
15 mkdir /home1/datasetwork/mboukerb/RisakManche/allDNA2/QIIME
16
17 ## QIIME analysis
18
19 #Make a mapfile
20 #Use the 1 make
21 #It is required
22 # $SampleID Be
23 #NOTES:
24 # <1> "fSample
25 # <2> "Treat
26 # <3> "Decon
27 # <4> If the
28 #Checke user's
29 # NOTES:
30 # <1> There
31 # error
32 # <2> If the
33 # there
34 # mkdir /home1/d
35 # validate_mapfi
36 # -p disable pr
37 # file
38
39 #OPTIONAL
40 #Usually date s
41 #demultiplex, t
42 #Repeatate $AS
43 #convert_fastqual_fastq.py -i fastq_in_fastqual -f $AS $AS2 -o $AS3/$AS4
44 #split_libraries.py -m sapping_output/mapfile_corrected.txt -f $AS5 $AS6 -b 10 -l 50 -o $AS7
45 # NOTES:
46 # <1> -m mapfile.txt: the mapfile from step 1)
47 # <2> -f $AS5 $AS6: sequence file
48 # <3> -b 10 : barcode type, 10 meaning that it's a 10 bp length of barcode
49 # <4> -l 50 : the minimum length of sequences after quality filtering. The default value (if omit this option)
50 # is 100 bp
51 # <5> -o $AS7: output directory (folder)
52 # <6> This command generates three files:
53 # histogram.txt -- histogram on sequence counts of different lengths, before and after filtering.
```

Commandes pour préparer le script

1 - Pre-process

3 - Suppression des chimères

5 - Affiliation taxonomique

7 - Obtention de la table des OTU

0 - Démultiplexage

2 - Clustering

4 - Filtrage des OTU sur l'abondance

6 - Filtres sur les résultats de BLAST

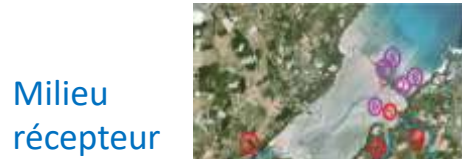
8 - Graphiques : diagramme de répartition et courbe de raréfaction

Echanges avec la cellule bioinfo pour:

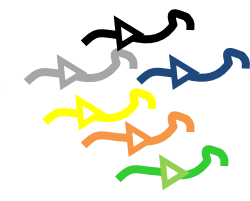
- installation des outils/bases et m2j
- solutions suite à des erreurs dans certains scripts/ codes outils
- augmentation du walltime en cas de besoin (selon queue de calcul)



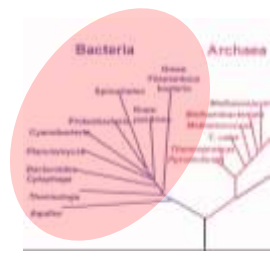
NGS: de la molécule d'ADN à la source



Extraction ADN

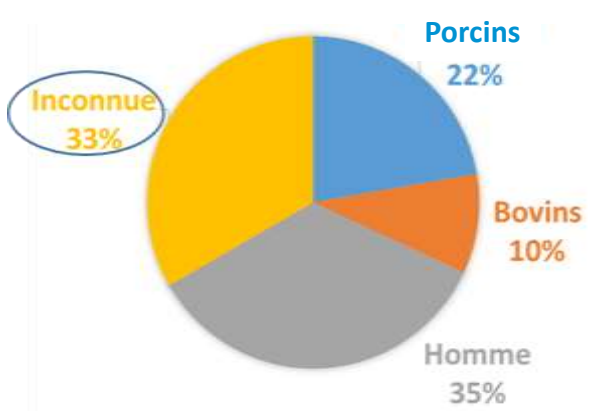


Amplification et séquençage gène ARNr 16S (V3V4)

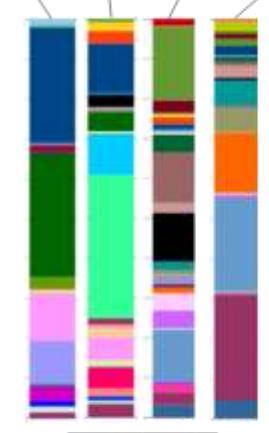


Analyses bio-informatiques

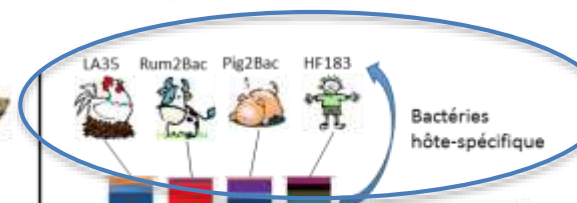
NGS



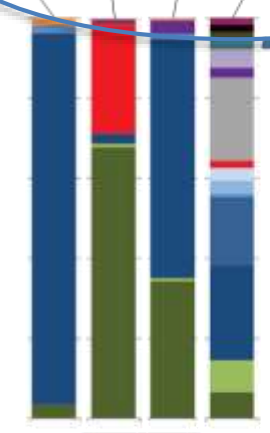
SourceTracker



Réservoirs

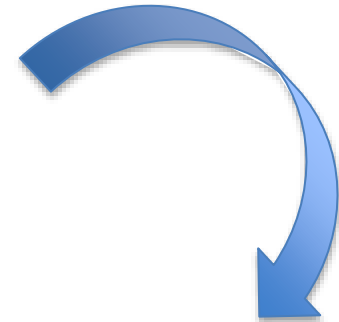


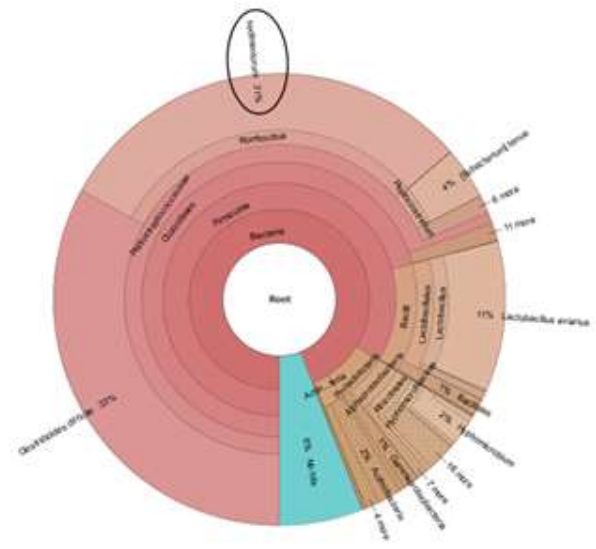
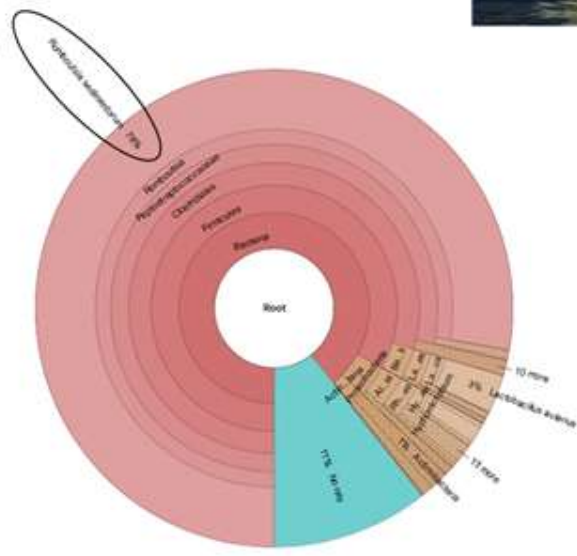
Bactéries hôte-spécifique



Sources

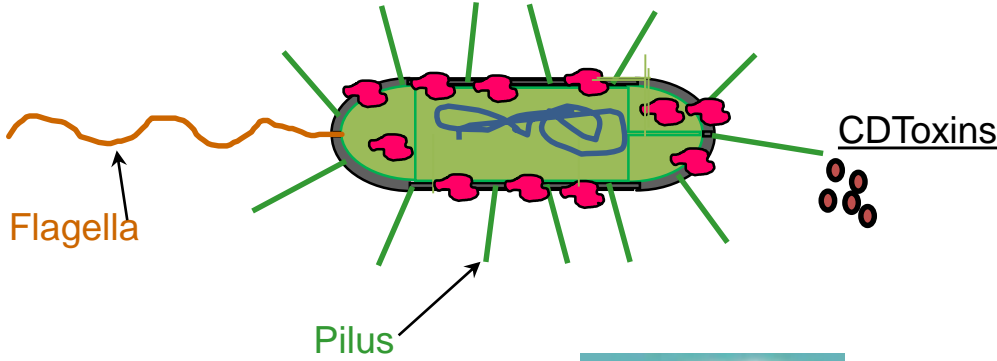
- Actinobacteria
- Bacteroidetes
- Firmicutes
- Proteobacteria
- Alphaproteobacteria
- Betaproteobacteria
- Deltaproteobacteria
- Epsilonproteobacteria
- Gammaproteobacteria
- Unclassified Proteobacteria
- Planctomycetes
- Other
- Unclassified





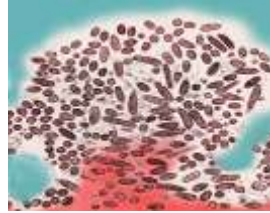
Romboutsia sp... analyses en cours pour une application en qPCR

Plusieurs espèces dont *C. jejuni*, *coli* et *lari*

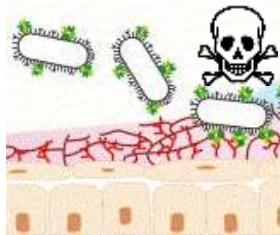


VIRULENCE

Flagella, pili...
= **adhésion et biofilm**
(hôte et environnement)



Systèmes de sécrétion (TSS)
CDToxins = *cdtA*, *B*, *C*

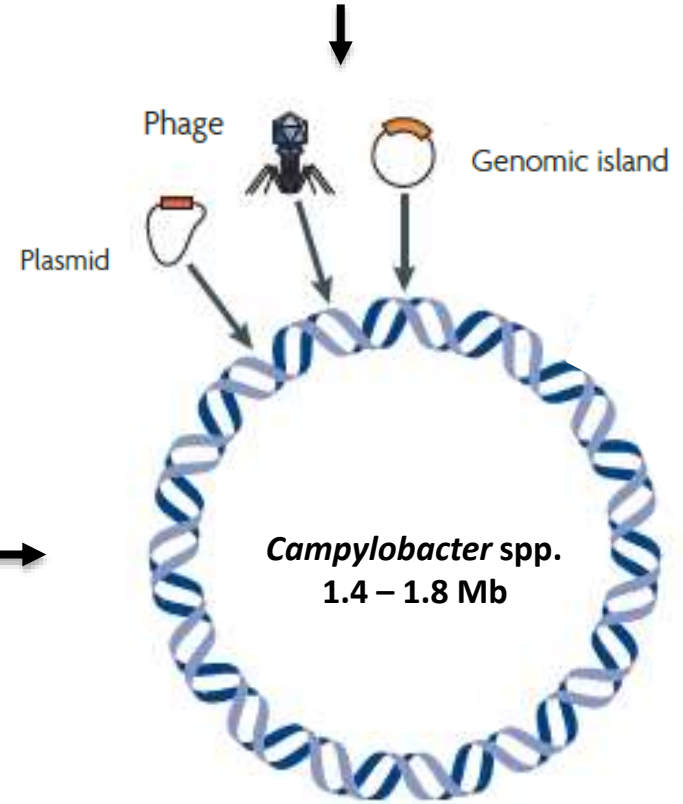


ATBr

- ... et déterminants d'ATBr
- Gènes de résistance aux antibiotiques
- CME efflux pump

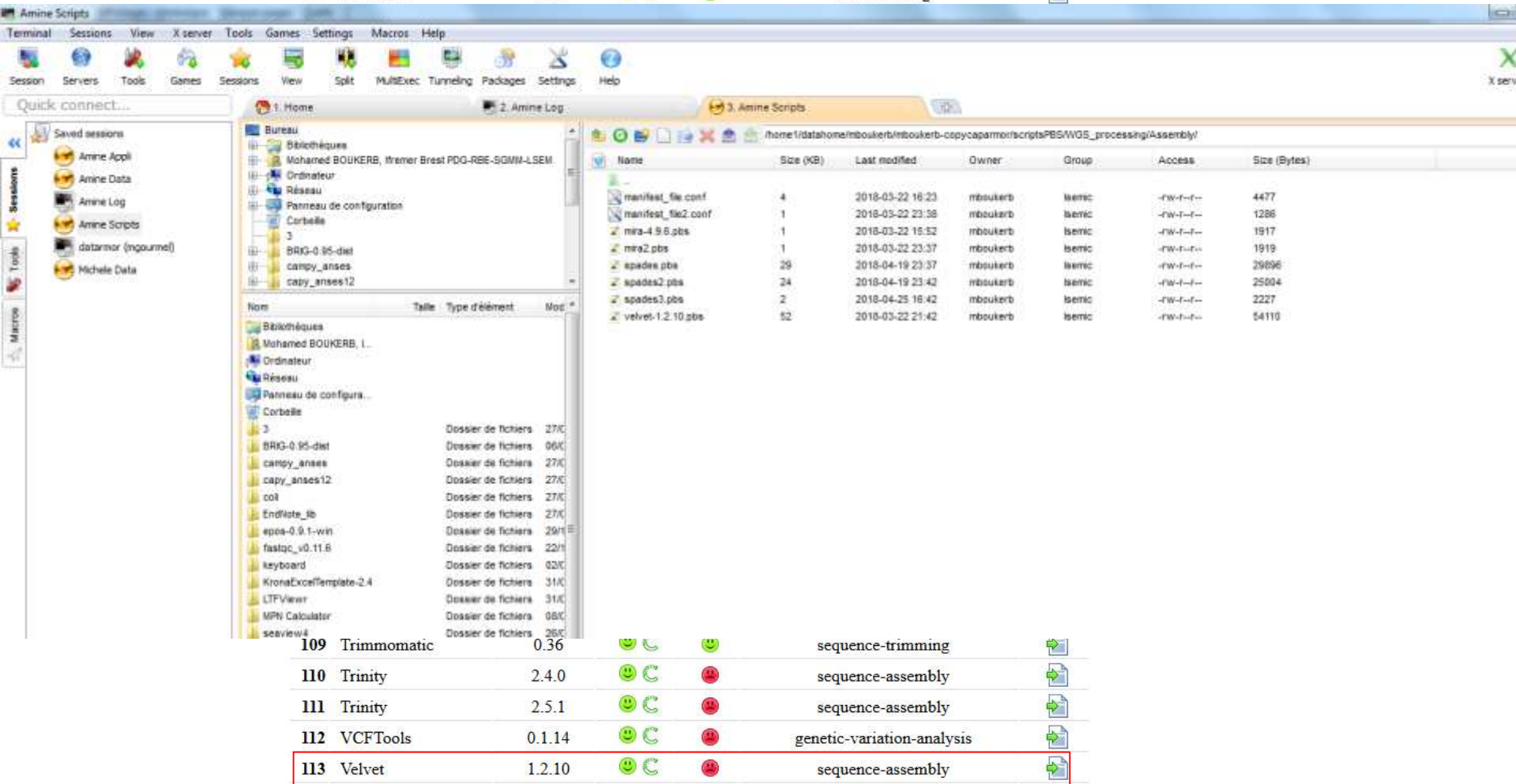


Isolement souches pures
Campylobacter



Adapted from Toft and Andersson 2010,
Freschi 2015

63	MIRA	4.9.6				sequence-assembly	
86	QuasiRecomb	1.2				recombination-detection	
87	QUAST	4.5				sequencing-quality-assessment	
88	SAMtools	1.4.1				data-handling	



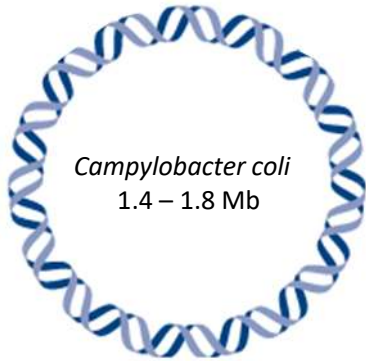
The screenshot shows the Amine Scripts interface. On the left, there is a sidebar with 'Quick connect...' and 'Saved sessions'. The main area is divided into three panes: '1. Home', '2. Amine Log', and '3. Amine Scripts'. The '3. Amine Scripts' pane displays a file explorer view of the directory `home/tdatathome/mboukerb/mboukerb-copycaparron/scripts/PBS/WGS_processing/Assembly/`. Below the file explorer, a table lists the scripts:

Name	Size (KB)	Last modified	Owner	Group	Access	Size (Bytes)
manifest_file.conf	4	2018-03-22 16:23	mboukerb	lsenic	-rw-r--r--	4477
manifest_file2.conf	1	2018-03-22 23:38	mboukerb	lsenic	-rw-r--r--	1288
mir-4.9.6.pbs	1	2018-03-22 16:52	mboukerb	lsenic	-rw-r--r--	1917
mir2.pbs	1	2018-03-22 23:37	mboukerb	lsenic	-rw-r--r--	1919
spades.pbs	29	2018-04-19 23:37	mboukerb	lsenic	-rw-r--r--	29896
spades2.pbs	24	2018-04-19 23:43	mboukerb	lsenic	-rw-r--r--	25004
spades3.pbs	2	2018-04-25 16:42	mboukerb	lsenic	-rw-r--r--	2227
velvet-1.2.10.pbs	52	2018-03-22 21:42	mboukerb	lsenic	-rw-r--r--	54110

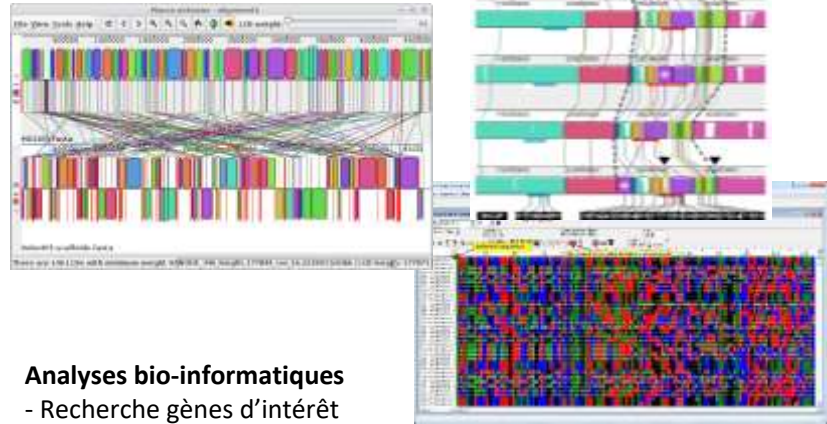
Below the file explorer, a table lists the scripts:

109	Trimmomatic	0.36				sequence-trimming	
110	Trinity	2.4.0				sequence-assembly	
111	Trinity	2.5.1				sequence-assembly	
112	VCFTools	0.1.14				genetic-variation-analysis	
113	Velvet	1.2.10				sequence-assembly	

Analyses en cours – comparaison des sorties avec QUAST



Assemblage et annotation



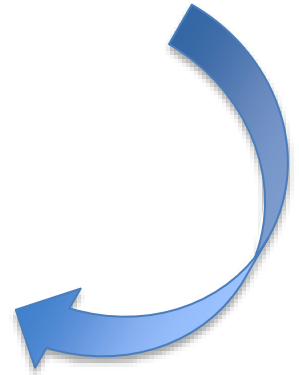
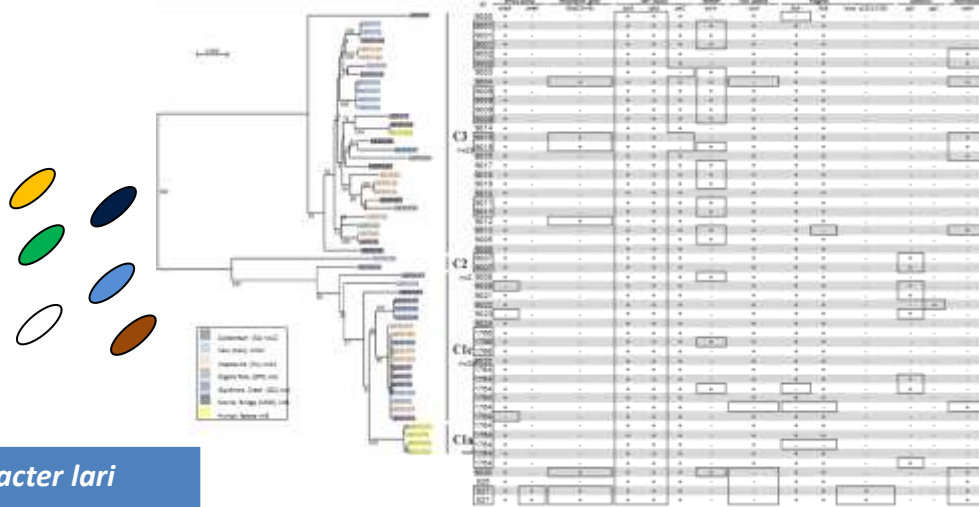
Analyses bio-informatiques

- Recherche gènes d'intérêt
- Comparaison: cliniques vs environnementales

Génotypage

ATBr

Virulence



<i>Campylobacter jejuni & coli</i>	<i>Campylobacter lari</i>
Fèces bovin, volaille, eaux...	Fientes oiseaux sauvages et coquillages



Dangerosité des souches



Ifremer, LSEM and LER, France
E. Quenot, J. Cozien, M. Gourmelon
THL Water and Health Unit, Finland
B. Jayaprakash, T. Heiskanen & T. Pitkänen
Ifremer, Cellule Bioinformatique
L. Quintric, F. Marquer, P. Durand
LGC Genomics GmbH, Berlin, Germany
Producing sequence libraries
Réserve naturelle de St-Brieuc, France
A. Sturbois
Bretagne vivante, France
B. Cadiou, Y. Jacob



Part of the Interreg IVA RiskManche project (2012-2015)
Risk management of catchments and coasts for human and health
(Coord. H. Taylor, Univ. of Brighton, UK)



Part of the FEAMP Campyshell project (2016-2019)
Campylobacter in shellfish: sources of contamination and human health risk
(Coord. M. Denis, ANSES, France)



Part of the BacTrac project (2016-2019)
Microbial source tracking in surface waters
(Coord. S. Bétat, LPL, France)



Merci pour votre attention

