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From high throughput 454 GS FLX data analysis process of 16S RNA gene sequences using barcoding to bacterial community exploration

Laurent Cauquil, Jérôme Mariette, Christine Rousseau, Asma Zened, Francis Enjalbert, Annabelle Troegeler-Meynadier, Christophe Klopp, Sylvie Combes

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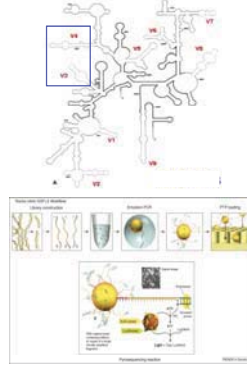
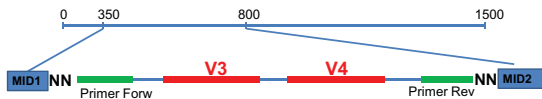
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From high throughput 454 GS FLX data analysis process of 16S RNA gene sequences using barcoding to bacterial community exploration

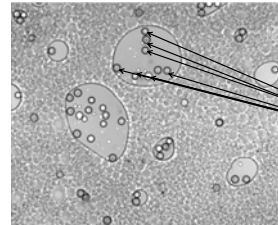
L. Cauquil¹, J. Mariette², C. Rousseau³, A. Zened¹, F. Enjalbert¹, A. Troegeler¹, C. Klopp² and S. Combes¹
¹UMR 1289 Tandem, INRA / INPT-ENSAT / ENVT, Castanet-Tolosan, France ²Plateforme bio-informatique Genotoul, Biométrie et Intelligence Artificielle, INRA, Castanet-Tolosan, France, ³INPT-ENSAT, Castanet-Tolosan, France
 Laurent.Cauquil@toulouse.inra.fr



The bacterial community of 97 cow rumen samples was explored using a 440 bp V3-V4 16S RNA amplicon. Each sample is barcoded using a couple of MIDs.



Since sequences might be randomly over represented within a run when sequencing is done using the Roche 454 pyrosequencing platform (Gomez-Alvarez et al. 2009), random nucleotide dimers were introduced in the sequence.



Eight beads instead of one

Pipeline

Rawdata : 889 029 reads



16Spyrocleaner (python)

- Length > 150 nt
- Sequence with one N are removed
- Homopolymere > 10 are removed

-19.7%

MOTHUR (<http://www.mothur.org>)

- Alignment (Silva base)
- Distance matrix
- Assigning sequences to OTU



NNpyrocleaner (python)

- Remove over represented sequences (-0.24%)

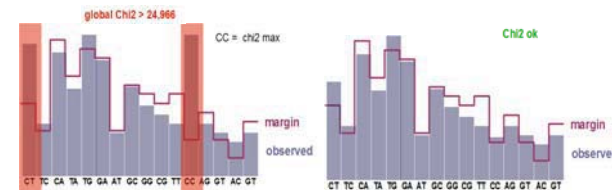
MOTHUR (<http://www.mothur.org>)

- Alignment
- Bad aligned seq were removed (-1%)
- Distance matrix
- Assigning sequences to OTU

706 155 reads
7249 reads/sample

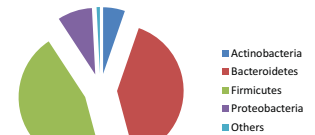
- Taxonomic assignment (bootstrap 60%)

- 868 OTU /sample (cutoff 0.1)
- Coverage of 95.9%
- Diversity indices



Taxonomy levels	Groups identified	% Affiliation
Phylum	15	100
Class	24	98
Order	51	95
Family	114	84
Genus	293	50

1.4% of reads not affiliated to any phylum were removed.

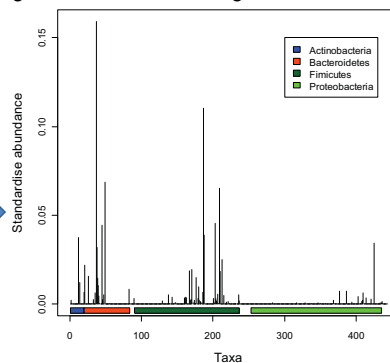


Mean distribution of major bacterial groups at the phylum level

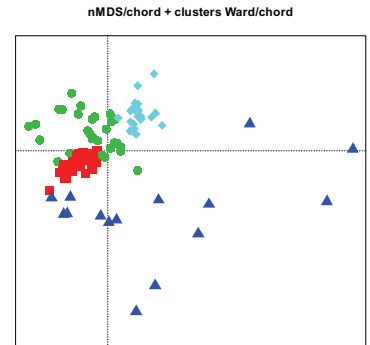
Analysis

From the 97 samples, we identified 430 different taxa leading to a taxonomic ecological matrix. The resulting data set can be used for ordination and clustering analysis (after normalisation) using R software.

X - matrix	Taxon 1	Taxon 2	Taxon 3	Taxon 7	Taxon 8	Taxon 9	...	Taxon 430
Ruminal fluid 1	0	0	0	0	57	0	...	0
Ruminal fluid 2	1	0	3	0	53	0	...	0
Ruminal fluid 3	0	0	0	0	4	0	...	0
Ruminal fluid 4	0	0	1	0	92	0	...	0
Ruminal fluid 5	0	0	1	0	25	0	...	0
Ruminal fluid 6	0	2	3	2	49	0	...	0
...
Ruminal fluid 97	0	0	4	0	75	1	...	0



Plot of the mean of the standardise abundance of each taxa upon the 97 samples



Ordination plot (nMDS) of the 97 samples and classification in 4 clusters