



Rødekro 2015. Vurdering af udviklingen i den naturlige nedbrydning i nedstrømsforureningsfane efter kildeoprensning

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NEXT GENERATION SEQUENCING REPORT

Customer: University of Neuchâtel

SiREM Reference: S-3320

Project: Røderko, Denmark

Report Date: 7 October 2014

Introduction

Next generation sequencing (NGS) provides detailed characterization of microbial community structure, diversity and taxonomic identification for environmental samples by providing gene sequences from environmental DNA samples. The 454 pyrotag method used in this study generated 16S rRNA gene sequence reads of approximately 400 base pairs using PCR primers that target eubacteria and many archaea, thereby providing identity and community structure information for a significant range of prokaryotes. This report summarizes the results of NGS and data analysis performed on groundwater filter samples collected by University of Neuchâtel at the Røderko, Denmark site (the Site). This report provides data and analysis including:

- A case narrative
- Concentration and mass of extracted DNA (Table 1)
- Microbial community diversity (Figure 1)
- The relative similarity of microbial communities between different sampling locations (Table 2/Figure 2)
- The taxonomic affiliation of 16S rRNA sequences generated and the relative abundance between and within samples (Figure 3/Figure 4 and Table 3)
- Chain of custody record
- Detailed listing and sequences of OTUs in Attached Files:
Excel "Taxonomic_assignment_of_OTUs"/ FASTA: "seqs-S-3220"

Case Narrative

SiREM received a total of 50 Sterivex filters: 12 samples (in quadruplicate) and 1 blank (in duplicate) containing biomass from groundwater at the Røderko site on 26 May 2014 for Gene-Trac *Dehalococcoides* (Gene-Trac[®] Dhc) and *vcrA* analysis (Gene-Trac[®] VC) and 454 pyrotag next generation sequencing analysis. The samples arrived in good condition at a temperature of 15°C and were logged in under SiREM reference number S-3320. DNA extraction from the filters was performed on 29 May 2014. All samples were submitted for NGS, except sample B71-3, for which DNA extraction failed. The DNA extracts were submitted to the University of Toronto for PCR amplification and subsequently submitted for 454 pyrotag sequencing at Genome Quebec (Montreal, PQ) on 17 July 2014.

Results

The results obtained for DNA extraction, 454 pyrotag sequencing and data analysis including estimates of microbial diversity, information relating the similarity of microbial communities and overall microbial community composition are presented in tabular and graphical format below.

Table 1: Summary of DNA Extraction Results

Sample ID	Groundwater Volume Filtered (mL)	DNA Concentration (ng/μL)	260:280	Total DNA Extracted (ng)	Extractable DNA Concentration in Groundwater (ng/L)
B58-6	660	16.1	1.8	402	609
B61-3	1000	9.6	1.8	239	239
B61-1	1000	9.1	1.9	228	228
B23-3	400	9.5	1.9	237	593
B23-2	400	10.4	1.9	259	647
B74-3	400	10.1	1.9	253	633
B58-2	400	10.3	1.9	259	647
B34-4	400	9.4	1.7	236	590
B34-3	400	10.2	1.8	254	635
B34-2	360	10.3	1.8	257	714
B34-6	360	9.8	2.0	244	678
Blank	360	10.0	1.9	249	691

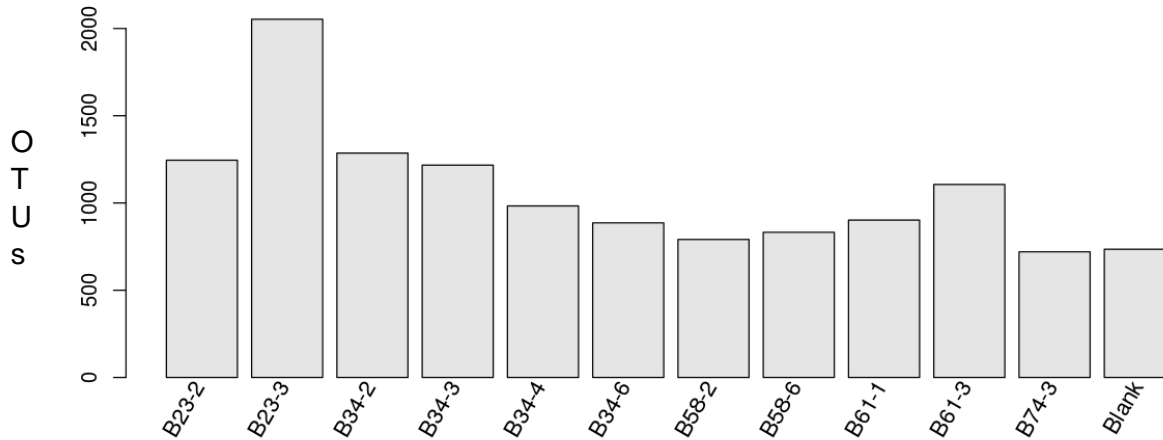


Figure 1. Operational taxonomic units (OTUs) obtained per sample based on sequences exhibiting a 97% 16S rRNA gene similarity. OTU number per sample is an indicator of microbial community diversity with sample B23-3 demonstrating the greatest number of OTUs (over 2,000) and B74-3 demonstrating lower diversity (less than 1,000). All other samples had OTU diversity intermediate between these samples.

Table 2: Dissimilarity matrix for microbial community structure of Site samples. Analysis based on Bray-Curtis dissimilarity matrix. Higher values indicate greater dissimilarity (lower similarity) between the microbial communities of paired samples.

	B34-2	B23-3	B34-6	B34-3	B23-2	B74-3	B61-3	Blank	B61-1	B34-4	B58-2	B58-6
B34-2	0.00	0.62	0.56	0.41	0.55	0.55	0.50	0.58	0.53	0.46	0.45	0.51
B23-3	0.62	0.00	0.53	0.56	0.44	0.56	0.67	0.67	0.40	0.48	0.61	0.71
B34-6	0.56	0.53	0.00	0.59	0.52	0.61	0.66	0.72	0.45	0.49	0.65	0.68
B34-3	0.41	0.56	0.59	0.00	0.52	0.51	0.54	0.63	0.49	0.47	0.37	0.48
B23-2	0.55	0.44	0.52	0.52	0.00	0.56	0.61	0.68	0.32	0.35	0.56	0.67
B74-3	0.55	0.56	0.61	0.51	0.56	0.00	0.63	0.62	0.52	0.51	0.56	0.64
B61-3	0.50	0.67	0.66	0.54	0.61	0.63	0.00	0.49	0.62	0.60	0.55	0.49
Blank	0.58	0.67	0.72	0.63	0.68	0.62	0.49	0.00	0.65	0.62	0.62	0.63
B61-1	0.53	0.40	0.45	0.49	0.32	0.52	0.62	0.65	0.00	0.36	0.51	0.64
B34-4	0.46	0.48	0.49	0.47	0.35	0.51	0.60	0.62	0.36	0.00	0.53	0.65
B58-2	0.45	0.61	0.65	0.37	0.56	0.56	0.55	0.62	0.51	0.53	0.00	0.45
B58-6	0.51	0.71	0.68	0.48	0.67	0.64	0.49	0.63	0.64	0.65	0.45	0.00

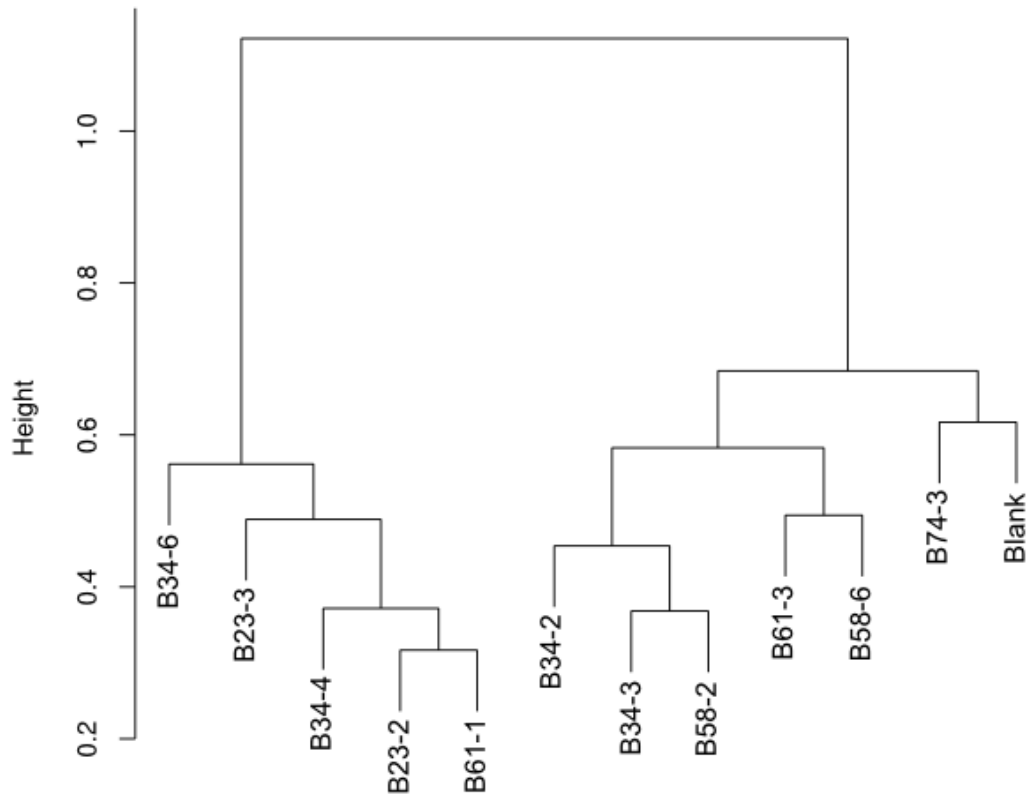


Figure 2. Cluster analyses of microbial community structure of Site samples based on Bray-Curtis dissimilarity matrix. Shorter vertical distances between samples indicate more similar microbial communities. The dissimilarity matrix (Table 2) provides numerical values for these relationships.

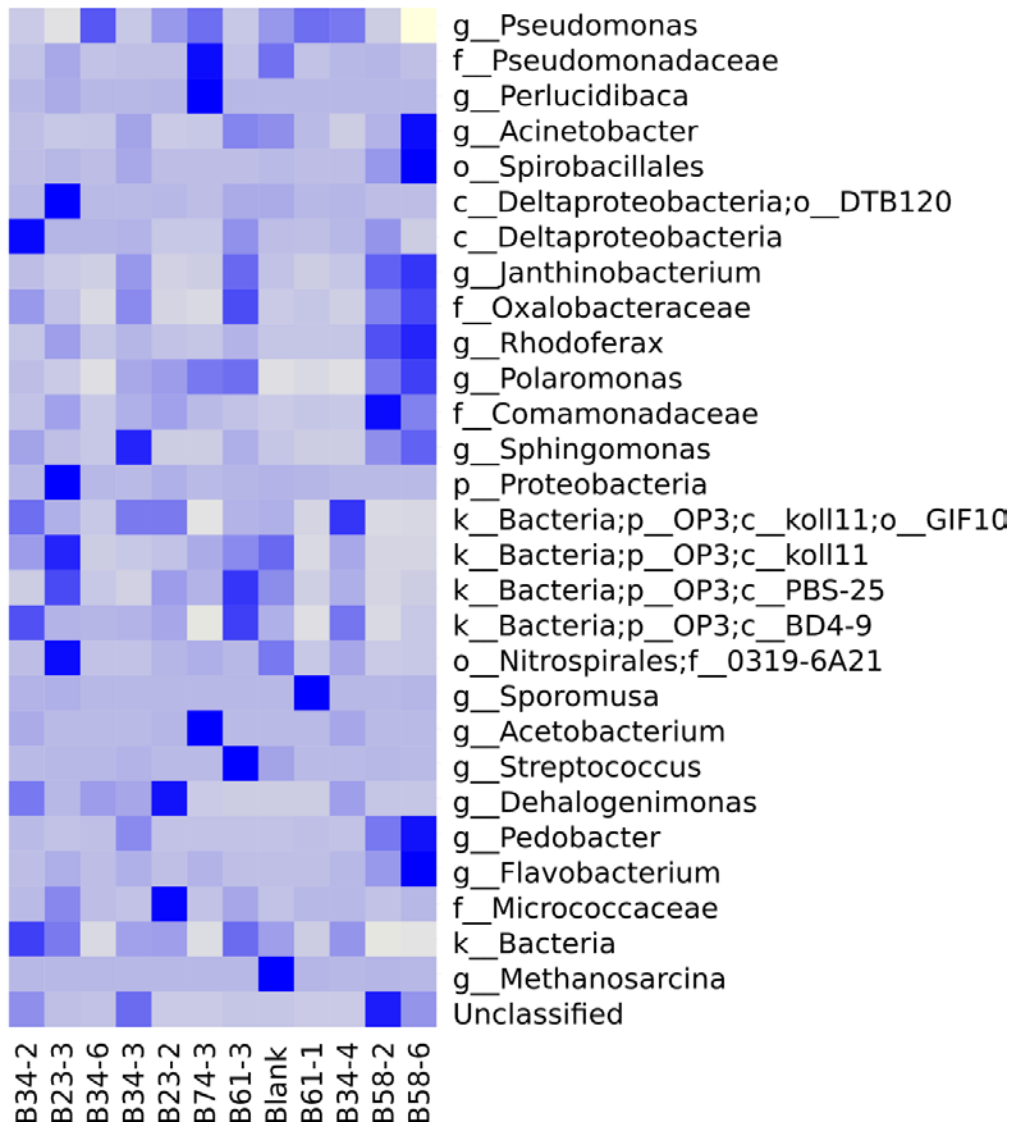


Figure 3. Heat map demonstrating taxonomic affiliation and relative abundance of key microbial groups between Site samples. Color intensity indicates relative abundance of taxonomic group in relation to other samples. Table 3 provides corresponding numerical values. k=kingdom, p=phylum, f=family, o=order, g=genus, c=class

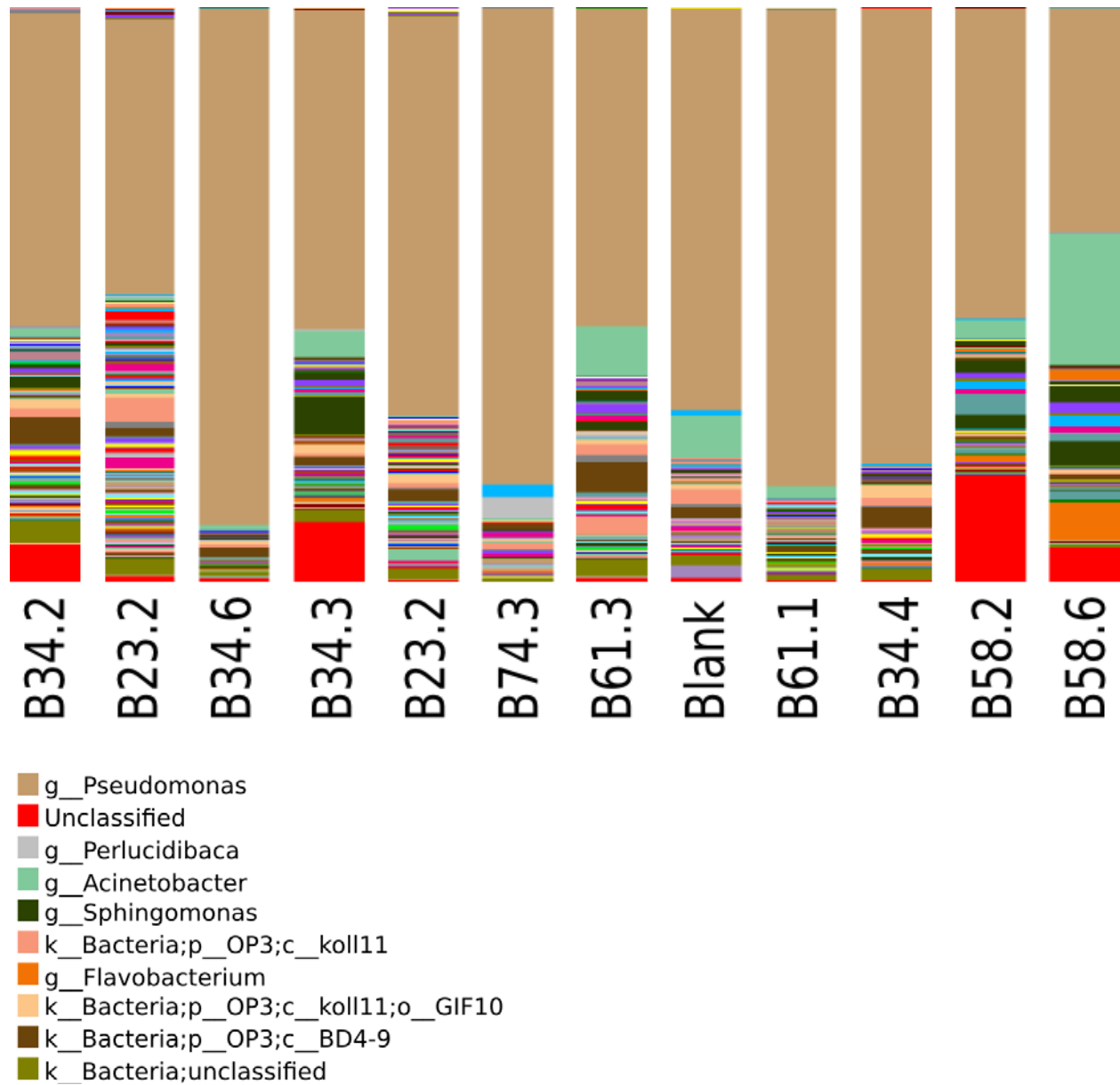


Figure 4. Stacked bar chart indicating relative abundance of OTUs identified in Site samples. OTUs representing a significant proportion of the microbial community in any sample are indicated in legend. k=kingdom, p=phylum, f=family, o=order, g=genus

Table 3. Relative abundance of taxonomic groups (within samples) representing greater than 1% of total abundance in any sample. Full Listing of all OTUs identified in the samples is provided electronically in Excel file “Taxonomic_assignment_of_OTUs”

Phylogenetic Classification	B34-2	B23-3	B34-6	B34-3	B23-2	B74-3	B61-3	Blank	B61-1	B34-4	B58-2	B58-6
Unclassified	6.54%	1.12%	0.90%	10.32%	0.25%	0.03%	0.80%	0.81%	0.25%	0.13%	18.76%	5.91%
g: <i>Methanosarcina</i>	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.95%	0.01%	0.00%	0.02%	0.00%
k__Bacteria	3.86%	2.68%	0.73%	1.89%	1.93%	0.66%	2.99%	1.93%	0.96%	2.10%	0.42%	0.51%
f__Micrococcaceae	0.09%	0.61%	0.05%	0.01%	1.88%	0.02%	0.30%	0.00%	0.11%	0.11%	0.02%	0.12%
g__ <i>Flavobacterium</i>	0.14%	0.61%	0.12%	0.53%	0.06%	0.45%	0.05%	0.05%	0.07%	0.33%	1.30%	6.45%
g__ <i>Pedobacter</i>	0.07%	0.00%	0.03%	0.48%	0.00%	0.01%	0.01%	0.00%	0.02%	0.00%	0.62%	1.44%
g__ <i>Dehalogenimonas</i>	0.46%	0.11%	0.27%	0.21%	1.02%	0.02%	0.01%	0.00%	0.01%	0.25%	0.05%	0.04%
g__ <i>Streptococcus</i>	0.01%	0.06%	0.07%	0.13%	0.00%	0.01%	3.19%	0.40%	0.00%	0.00%	0.06%	0.01%
g__ <i>Acetobacterium</i>	0.10%	0.00%	0.00%	0.00%	0.03%	1.14%	0.00%	0.00%	0.01%	0.13%	0.00%	0.00%
g__ <i>Sporomusa</i>	0.05%	0.06%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.28%	0.00%	0.00%	0.02%
o__Nitrospirales:f__0319-6A21	0.14%	1.90%	0.09%	0.07%	0.21%	0.27%	0.19%	0.81%	0.03%	0.36%	0.01%	0.03%
k__Bacteria;p__OP3;c__BD4-9	4.67%	1.58%	1.53%	1.62%	2.01%	0.00%	5.18%	1.71%	0.21%	3.57%	0.36%	0.94%
k__Bacteria;p__OP3;c__PBS-25	0.07%	1.11%	0.09%	0.02%	0.45%	0.27%	1.26%	0.57%	0.04%	0.31%	0.00%	0.06%
k__Bacteria;p__OP3;c__koll11	1.48%	4.11%	0.40%	0.53%	0.68%	1.18%	1.89%	2.65%	0.15%	1.20%	0.26%	0.27%
k__Bacteria;p__OP3;c__koll11;o__GIF10	1.63%	0.77%	0.46%	1.47%	1.47%	0.10%	0.73%	0.78%	0.30%	2.35%	0.24%	0.28%
p__Proteobacteria	0.01%	1.36%	0.01%	0.00%	0.07%	0.00%	0.03%	0.05%	0.04%	0.00%	0.00%	0.00%
g__ <i>Sphingomonas</i>	1.60%	0.53%	0.26%	6.62%	0.00%	0.08%	1.26%	0.32%	0.03%	0.08%	2.44%	4.24%
f__Comamonadaceae	0.14%	0.72%	0.02%	0.44%	0.74%	0.27%	0.14%	0.00%	0.09%	0.02%	3.45%	1.28%
g__ <i>Polaromonas</i>	0.22%	0.14%	0.00%	0.36%	0.46%	0.70%	0.77%	0.00%	0.03%	0.00%	0.69%	1.08%
g__ <i>Rhodoferax</i>	0.01%	0.48%	0.01%	0.20%	0.06%	0.00%	0.22%	0.00%	0.01%	0.00%	1.40%	1.93%
f__Oxalobacteraceae	0.78%	0.32%	0.06%	0.97%	0.13%	0.06%	1.65%	0.22%	0.30%	0.18%	1.05%	1.71%
g__ <i>Janthinobacterium</i>	0.56%	0.33%	0.25%	1.23%	0.21%	0.31%	2.02%	0.50%	0.29%	0.42%	2.15%	2.88%
c__Deltaproteobacteria	1.69%	0.24%	0.24%	0.26%	0.08%	0.09%	0.54%	0.18%	0.16%	0.22%	0.52%	0.04%
c__Deltaproteobacteria;o__DTB120	0.04%	1.47%	0.02%	0.02%	0.04%	0.01%	0.14%	0.14%	0.05%	0.06%	0.00%	0.00%
o__Spirobacliales	0.00%	0.06%	0.00%	0.21%	0.00%	0.00%	0.00%	0.02%	0.00%	0.00%	0.40%	1.95%
g__ <i>Acinetobacter</i>	1.56%	0.47%	0.75%	4.71%	0.20%	0.45%	8.51%	7.27%	2.01%	0.03%	3.14%	22.87%
g__ <i>Perluclidibaca</i>	0.00%	0.28%	0.00%	0.04%	0.08%	3.75%	0.00%	0.00%	0.00%	0.01%	0.00%	0.02%
f__Pseudomonadaceae	0.03%	0.33%	0.02%	0.07%	0.06%	2.15%	0.02%	1.01%	0.02%	0.14%	0.19%	0.04%
g__ <i>Pseudomonas</i>	54.38%	48.02%	89.89%	55.62%	69.48%	82.74%	55.29%	69.95%	82.91%	79.23%	53.97%	38.71%

k=kingdom, p=phylum, f=family, o=order, g=genus c= class

Project Name <u>Røddekrø</u>		Project # <u>2188-0324114</u>		Analysis															
Project Manager <u>Alice BADIN / Mette BROHOLT</u>				Preservative <u>0 0 0 0</u>															
Email Address <u>alice.badin@unine.ch / mubr@env.dtu.dk</u>				<div style="display: flex; justify-content: space-between;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Gene-Trac Dhc</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Gene-Trac VC</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Gene-Trac Dhib</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">VerA</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Pyrolog 454</div> </div>															
Company <u>University of Neuchâtel</u>																			
Address																			
Phone # <u>0041-79 87 38 884</u> Fax #																			
Sampler's Signature <u>[Signature]</u>		Sampler's Printed Name <u>ALICE BADIN</u>		Preservative Key 0. None 1. HCl 2. Other _____ 3. Other _____															
Customer Sample ID		Sampling														Matrix		# of Containers	
		Date Time																	
B34-4								<u>n 300-400 neel</u> <u>n 300-400 neel</u> <u>n 300-400 neel</u> <u>n 300-400 neel</u> <u>300 neel</u>											
B34-4		20/5/14 12h30		4		4													
B34-3		20/5/14 13h20		4		4													
B34-2		20/5/14 14h15		4		4													
B34-6		20/5/14 15h00		4		4													
Bk (Blank)		20/5/14 18h00		2		2													

Cooler Condition: <u>GOOD</u>		P.O. #		Turnaround Time Requested		For Lab Use Only <u>Filters</u> <u>F-01886-F-01935</u> Proposal #: _____	
Cooler Temperature: <u>15°C</u>		Billing Information		Normal <input type="checkbox"/>			
Custody Seals: Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>		Bill To:		Rush <input type="checkbox"/>			

Relinquished By:		Received By:		Relinquished By:		Received By:		Relinquished By:		Received By:	
Signature		Signature		Signature		Signature		Signature		Signature	
Printed Name		Printed Name <u>D. DiPasoli</u>		Printed Name		Printed Name		Printed Name		Printed Name	
Firm		Firm <u>SiREM</u>		Firm		Firm		Firm		Firm	
Date/Time		Date/Time <u>MAY 26 '14 12:00 pm</u>		Date/Time		Date/Time		Date/Time		Date/Time	