



## **Rødebro 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

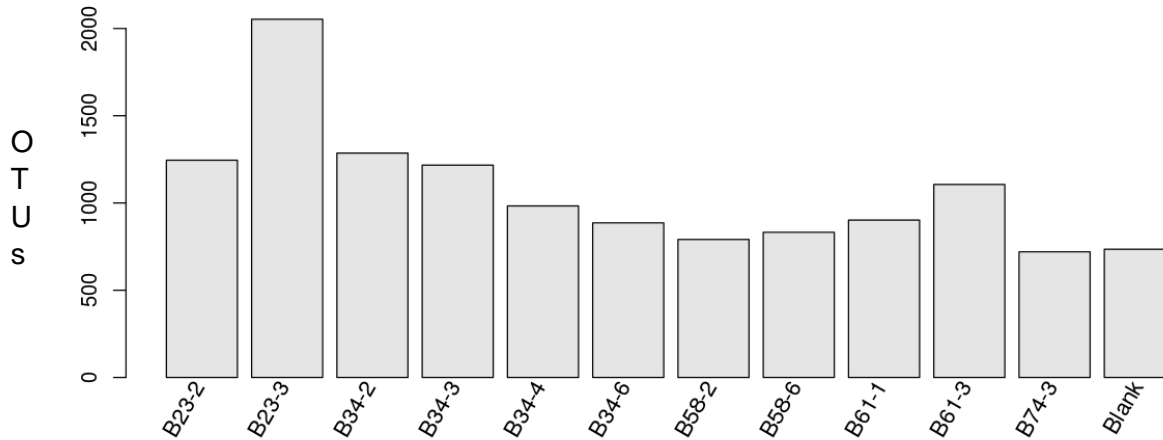
SiREM received a total of 50 Sterivex filters: 12 samples (in quadruplicate) and 1 blank (in duplicate) containing biomass from groundwater at the Røderko site on 26 May 2014 for Gene-Trac *Dehalococcoides* (Gene-Trac<sup>®</sup> Dhc) and *vcrA* analysis (Gene-Trac<sup>®</sup> VC) and 454 pyrotag next 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

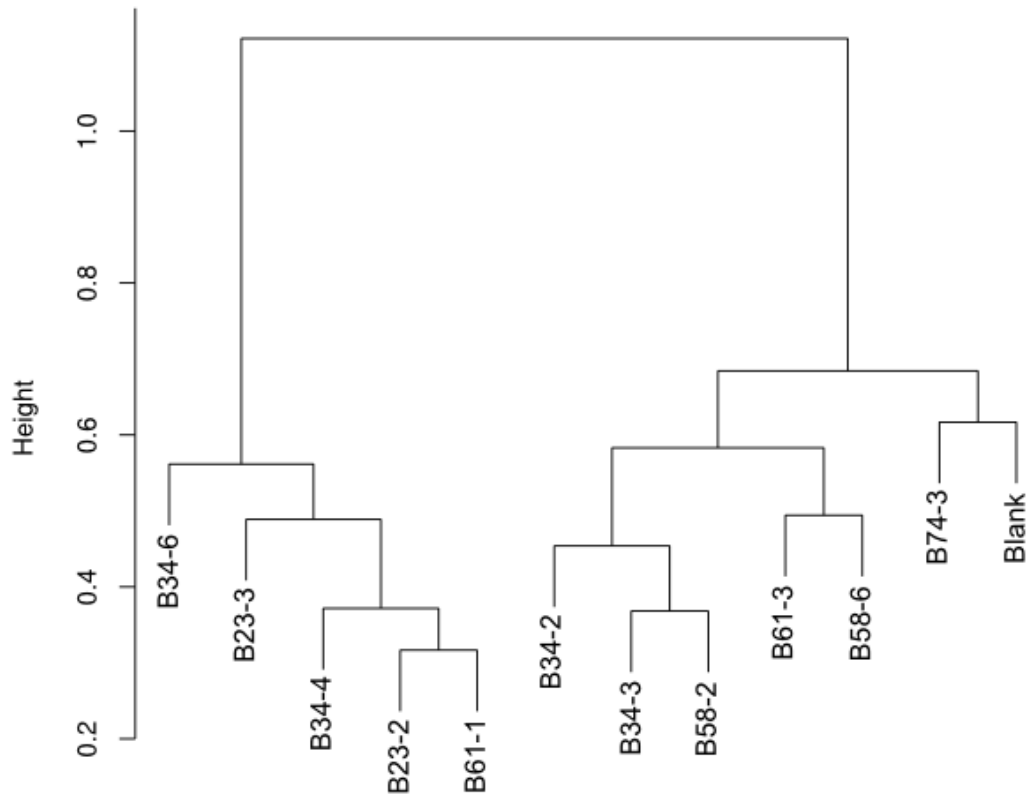
Sample ID	Groundwater Volume Filtered (mL)	DNA Concentration (ng/μL)	260:280	Total DNA Extracted (ng)	Extractable DNA Concentration in Groundwater (ng/L)
B58-6	660	16.1	1.8	402	609
B61-3	1000	9.6	1.8	239	239
B61-1	1000	9.1	1.9	228	228
B23-3	400	9.5	1.9	237	593
B23-2	400	10.4	1.9	259	647
B74-3	400	10.1	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



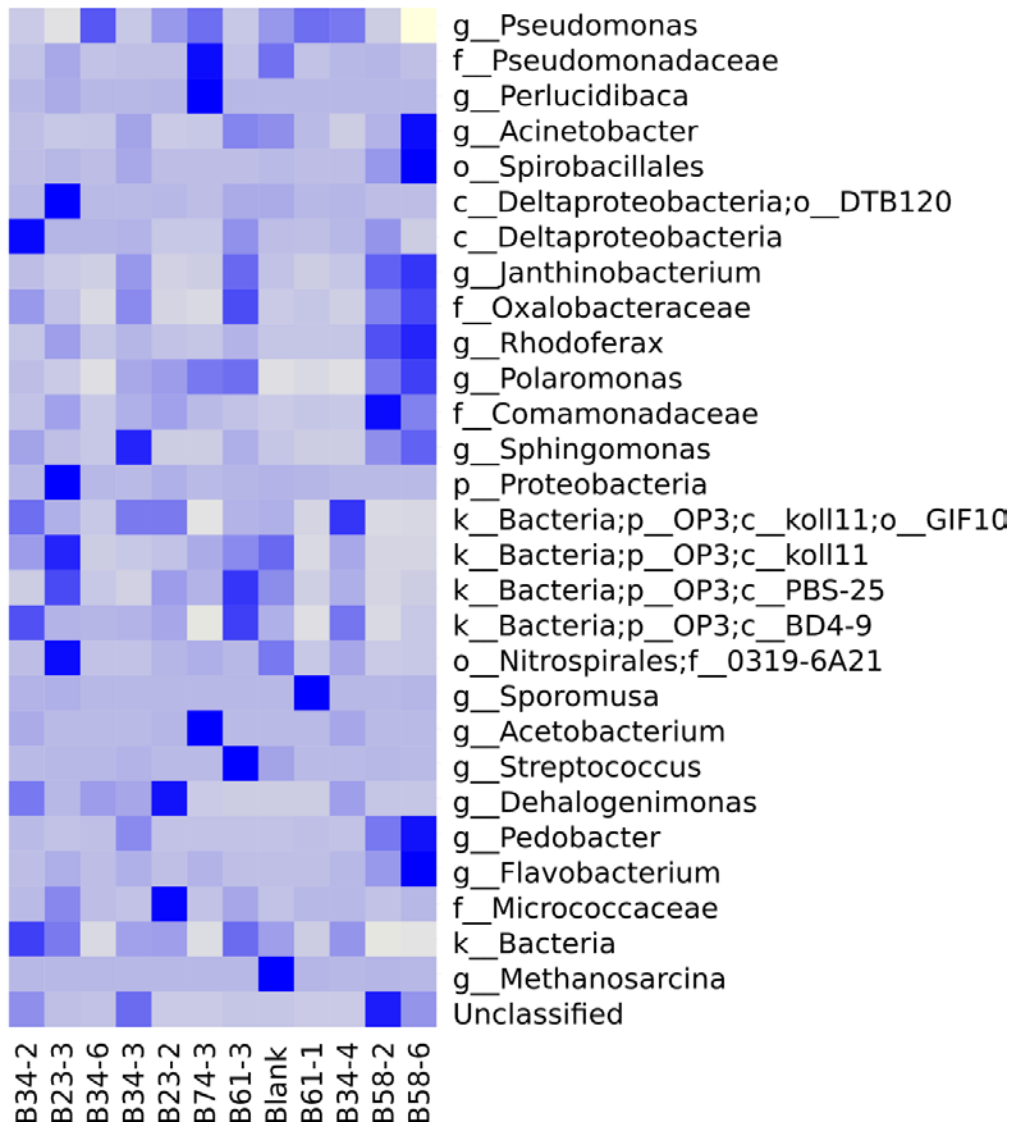
**Figure 1.** Operational taxonomic units (OTUs) obtained per sample based on sequences exhibiting a 97% 16S rRNA gene similarity. OTU number per sample is an indicator of microbial community diversity with sample B23-3 demonstrating the greatest number of OTUs (over 2,000) and B74-3 demonstrating lower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

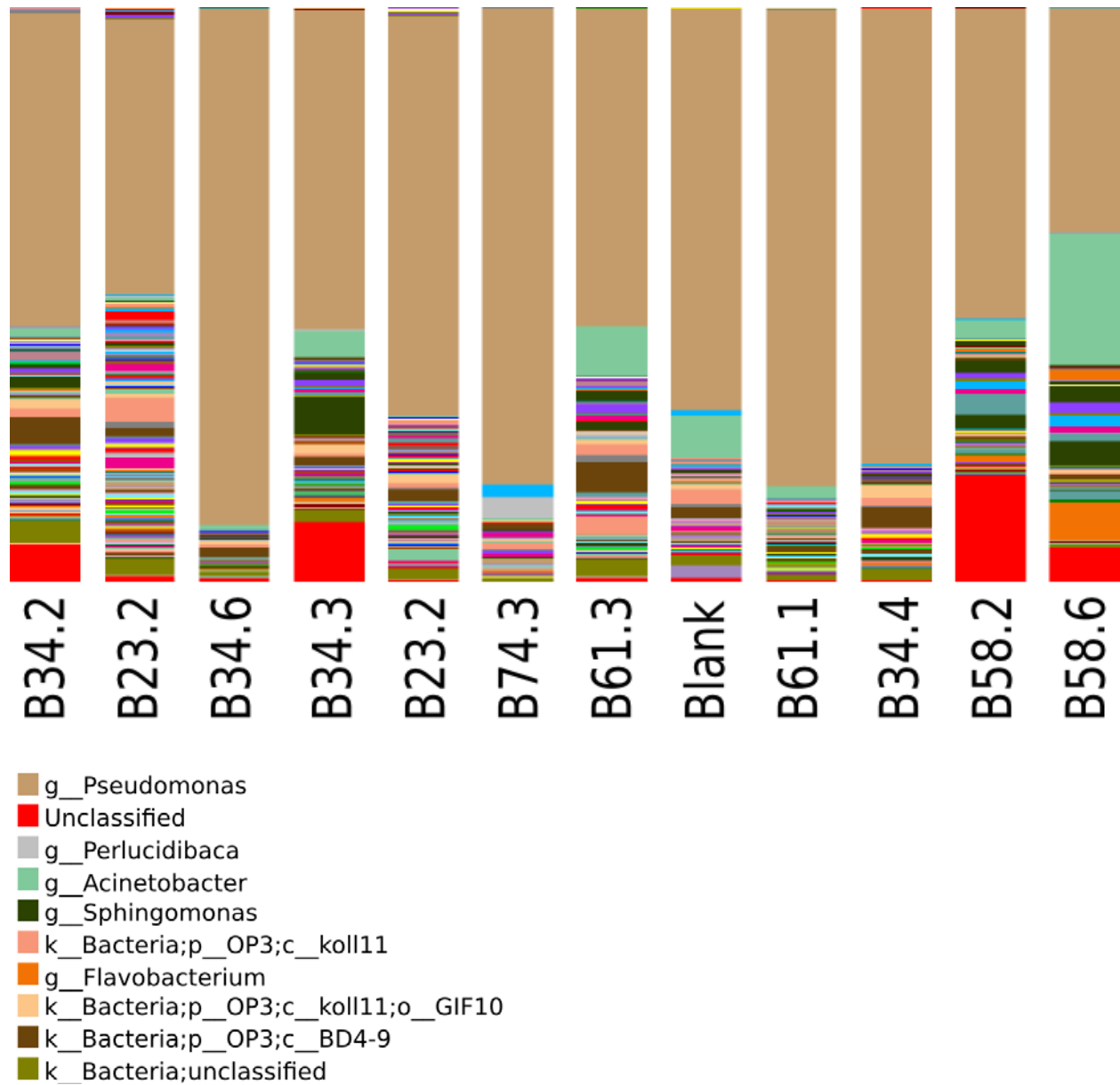
	B34-2	B23-3	B34-6	B34-3	B23-2	B74-3	B61-3	Blank	B61-1	B34-4	B58-2	B58-6
B34-2	<b>0.00</b>	0.62	0.56	0.41	0.55	0.55	0.50	0.58	0.53	0.46	0.45	0.51
B23-3	0.62	<b>0.00</b>	0.53	0.56	0.44	0.56	0.67	0.67	0.40	0.48	0.61	0.71
B34-6	0.56	0.53	<b>0.00</b>	0.59	0.52	0.61	0.66	0.72	0.45	0.49	0.65	0.68
B34-3	0.41	0.56	0.59	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>	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	<b>0.00</b>



**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 <b>Kodekro</b>		Project # <b>2188 - 032 414 (Quote)</b>		<b>Analysis</b>											
Project Manager <b>Alice BADIN / Helte BROHOLT</b>				Preservative <b>00 0 0</b>											
Email Address <b>alice.badin@unine.ch / mmbr@env.dtu.dk</b>				<div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> <p>Gene-Trac Dhc</p> <p>Gene-Trac VC</p> <p>Gene-Trac Dhb</p> <p><b>VcrA</b></p> <p><b>Pyroleg 454</b></p> </div> <div style="width: 50%; text-align: right;"> <p><b>Preservative Key</b></p> <p>0. None</p> <p>1. HCl</p> <p>2. Other _____</p> <p>3. Other _____</p> </div> </div>											
Company <b>University of Neuchâtel</b>															
Address															
Phone # <b>0041-7987 33884</b>		Fax #													
Sampler's Signature <i>[Signature]</i>		Sampler's Printed Name <b>ALICE BADIN</b>													

Customer Sample ID	Sampling		Matrix	# of Containers	Analysis												Other Information			
	Date	Time			Gene-Trac Dhc	Gene-Trac VC	Gene-Trac Dhb	VcrA	Pyroleg 454	1	2	3	4	5	6	7		8	9	10
B58-6	20/5/14	10h00		4	X	X	X	X												~ 300-400 µl
B61-3	19/5/14	14h30		4	X	X	X	X												
B61-1	19/5/14	15h45		4	X	X	X	X												
B71-3	19/5/14	11h30		4	X	X	X	X												~ 900-1000 µl
B23-3	19/5/14	18h20		4	X	X	X	X												~ 300-400 µl
B23-2	19/5/14	17h30		4	X	X	X	X												~ 300-400 µl
B74-3	19/5/14	18h45		4	X	X	X	X												~ 300-400 µl
<del>B58-6</del>				4	X	X	X	X												~ 300-400 µl
<del>B58-6</del>				4	X	X	X	X												~ 300-400 µl
B58-2	20/5/14	11h30		4	X	X	X	X												~ 300-400 µl

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

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



Project Name <u>Røddekrø</u>		Project # <u>2188-0324114</u>		Analysis																	
Project Manager <u>Alice BADIN / Mette BROHOLT</u>				Preservative <u>0</u>		<u>0</u>		<u>0</u>		<u>0</u>											
Email Address <u>alice.badin@unine.ch / mubr@env.dtu.dk</u>				Gene-Trac Dhc		Gene-Trac VC		Gene-Trac Dhib		VerA		Pyrolog 454								Preservative Key 0. None 1. HCl 2. Other _____ 3. Other _____	
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>																			
Customer Sample ID		Sampling		Matrix		# of Containers		Other Information													
		Date		Time																	
<del>B34-4</del>																					
B34-4		20/5/14		12h30		4		X		X		X		X				~300-400 uel			
B34-3		20/5/14		13h20		4		X		X		X		X				~300-400 uel			
B34-2		20/5/14		14h15		4		X		X		X		X				~300-400 uel			
B34-6		20/5/14		15h00		4		X		X		X		X				~300-400 uel			
Bk (Blank)		20/5/14		18h00		2		X		X		X		X				300 uel			

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

Relinquished By:		Received By:		Relinquished By:		Received By:		Relinquished By:		Received By:	
Signature _____		Signature <u>[Signature]</u>		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 _____	