Battelle
Columbus Laboratories

CHAIN OF CUSTODY RECORD

Form No. _____

Proj. No.	Project Title
G486111-T3	Source Determination Study

SAMPLERS: (Signature) Greg Headington / Dave Conner / Ben Headington

PO# 191770

DATE	TIME	SAMPLE I.D.	SAMPLE TYPE (√) FUNCTIONAL GENE TEST	Container No.	Number of Containers	Remarks
02JUN05	1340	GARFIELD	X		1	
02JUN05	1052	SUNSET	X		1	
02JUN05	1530	BANGHAM	X		1	
		~~DGH~~				

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Greg Headington	03JUN05 1620				

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)

Relinquished by: (Signature)	Date/Time	Received for Laboratory by: (Signature)	Date/Time	Remarks
				SENT TO: BioInsite

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G486111-T3	Source Determination Study

SAMPLERS: (Signature) Greg Headington / Dave Conner / Ben Headington

PO# 191770

DATE	TIME	SAMPLE I.D.	SAMPLE TYPE (√) FUNCTIONAL GENE TEST	Container No.	Number of Containers	Remarks
03JUN05	0930	LFWC-2	X		1	
03JUN05	1330	LAWC-3	X		1	

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Greg Headington	03JUN05 1620				

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)

Relinquished by: (Signature)	Date/Time	Received for Laboratory by: (Signature)	Date/Time	Remarks
				SENT TO: BioTrite

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Battelle
Columbus Laboratories

CHAIN OF CUSTODY RECORD

Form No. _____

Proj. No.	Project Title
G486111-T3	Source Determination Study

SAMPLERS: (Signature) D. Lorner

DATE	TIME	SAMPLE I.D.	SAMPLE TYPE (√) Enrichment Gene Test	Container No.	Number of Containers	Remarks
7/19/2005	15:10	MW-25-3	X			PO # 191770

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
[signature]	7/20/05 17:00				
Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Relinquished by: (Signature)	Date/Time	Received for Laboratory by: (Signature)	Date/Time	Remarks Sent to: BioInsite	

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07/22/2005 10:55 FAX 6263450698 JPL A K 002/003

Battelle
Columbus Laboratories

Form No. _____

CHAIN OF CUSTODY RECORD

Proj. No.	Project Title
G486111-T3	Source Determination Study

SAMPLERS: (Signature) D. Corner

SAMPLE TYPE (√)

DATE	TIME	SAMPLE I.D.	Functional Gene Test													Container No.	Number of Containers	Remarks
7/25/2005	13:15	MW-24-1	X															PO # 191770

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
[signature]	7/26/05 1700				
Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Relinquished by: (Signature)	Date/Time	Received for Laboratory by: (Signature)	Date/Time	Remarks	
				To: Bio Insite	

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BIOINSITE LLC, POST OFFICE BOX 1503, MURPHYSBORO, ILLINOIS 62966

Tel. (618) 549 6868, Fax (618) 687-4801, URL: WWW.BIOINSITE.COM

SUMMARY REPORT:

The genomic DNAs for samples Sunset, MW-1, and LFWC-2 were positive for the chlorite dismutase gene *cld* indicating the presence of (per)chlorate-reducing bacteria in these samples. Samples of genomic DNA from Garfield, Bangham, and LAWC-3 were negative for the chlorite dismutase gene.

Analysis of the presence of the *cld* mRNA resulted in a weak positive result for the MW-1 sample which may indicate active (per)chlorate reduction occurring at that site. The other five samples were negative for the *cld* mRNA, indicating that active (per)chlorate reduction is not occurring at these sites. However, this conclusion must be tempered by the observation that the low biomass of the samples could have biased the experimental outcome.

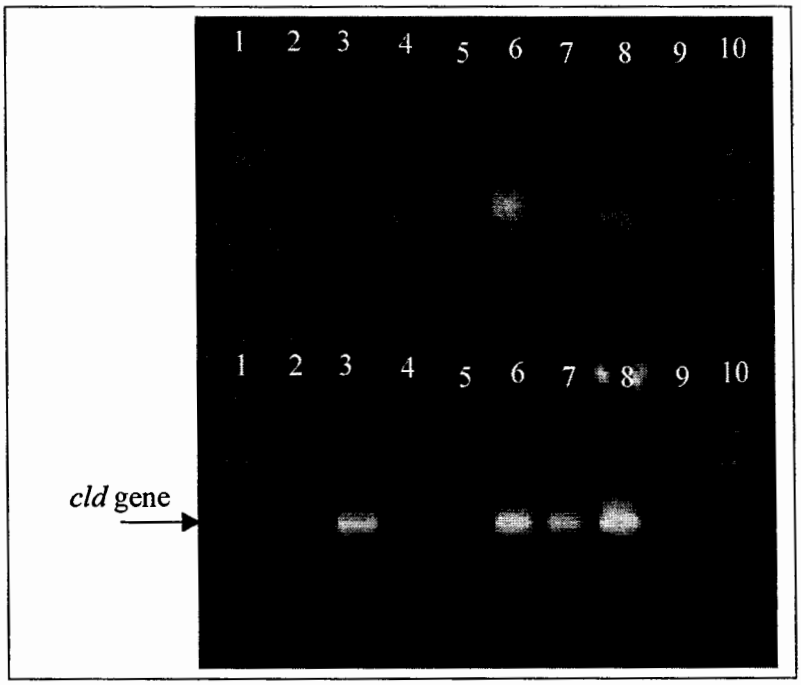
Samples received on: 4 June - 7 June, 2005
Analyzed on: 10 June - 28 June, 2005
Samples stored at: -20° C (DNA and RNA)

One-liter water samples were received from each site. The lid on the LFWC-2 sample was cracked during shipping and over half the sample leaked out, leaving ~300 ml for analysis. Each sample was filtered to concentrate the biomass and split into two equal parts to prepare genomic DNA (gDNA) and total RNA from each.

Amplifications of chlorite dismutase gene *cld* using nested primer sets on gDNA:

Top: 10 µl gDNA amplified with DCD primer set
1) 1 kb marker
2) Garfield
3) Sunset
4) Bangham
5) LAWC-3
6) MW-1
7) LFWC-2
8) Positive control
9) Negative control
10) 1 kb marker

Bottom: 5 µl DCD gDNA template amplified with UCD primer set
1) 1 kb marker
2) Garfield
3) Sunset
4) Bangham
5) LAWC-3
6) MW-1
7) LFWC-2
8) Positive control
9) Negative control
10) 1 kb marker

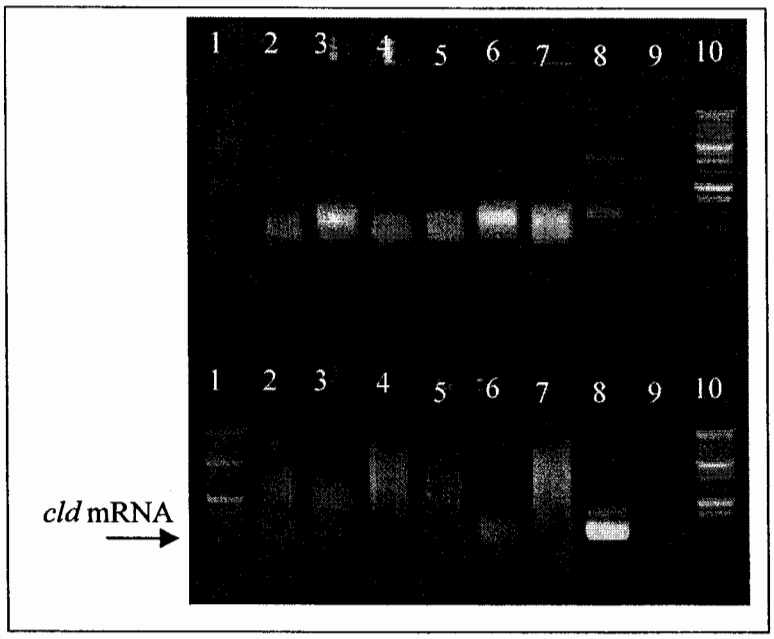


Result
Positive for chlorite dismutase gene: Sunset, MW-1, and LFWC-2.
Negative for chlorite dismutase gene: Garfield, Bangham, and LAWC-3.
Not shown: Amplifications using the standard 1 µl gDNA did not work; likely due to extremely low biomass.

Nested *cld* amplifications using cDNA prepared by reverse transcription of total RNA

Top: 10 µl cDNA amplified with DCD primer set
1) 1 kb marker
2) Garfield
3) Sunset
4) Bangham
5) LAWC-3
6) MW-1
7) LFWC-2
8) Positive control
9) Negative control
10) 1 kb marker

Bottom: 5 µl DCD template amplified with UCD primer set
1) 1 kb marker
2) Garfield
3) Sunset
4) Bangham
5) LAWC-3
6) MW-1
7) LFWC-2
8) Positive control
9) Negative control
10) 1 kb marker



Result
The presence of a faint band of the expected size is detectable in the MW-1 sample nested with the UCD primer set which may indicate the presence of *cld* mRNA in that sample (lane 6, bottom). The other five samples were all negative. (Bands present in those samples are not of the expected size and are considered negative results.)

QCQA

Sample	Type	Presence of *cld* gene	Presence of *cld* mRNA
Garfield	Experimental sample	–	–
Sunset	Experimental sample	+	–
Bangham	Experimental sample	–	–
LAWC-3	Experimental sample	–	–
MW-1	Experimental sample	+	+
LFWC-2	Experimental sample	+	–
D. aromatica	Positive control	+	+
E. coli	Negative control	–	–

[redacted]

BIOINSITE LLC, POST OFFICE BOX 1503, MURPHYSBORO, ILLINOIS 62966

Tel. (618) 549 6868, Fax (618) 687-4801, URL: WWW.BIOINSITE.COM

SUMMARY REPORT:

The genomic DNAs for samples MW-24-1 and MW-25-3 were positive for the chlorite dismutase gene *cld* indicating the presence of (per)chlorate-reducing bacteria in these samples.

Analysis of the presence of the *cld* mRNA resulted in a positive result for the MW-24-1 sample which indicates that active (per)chlorate reduction occurs at that site. Sample MW-25-3 was negative for the *cld* mRNA, indicating that active (per)chlorate reduction is not occurring at this site.

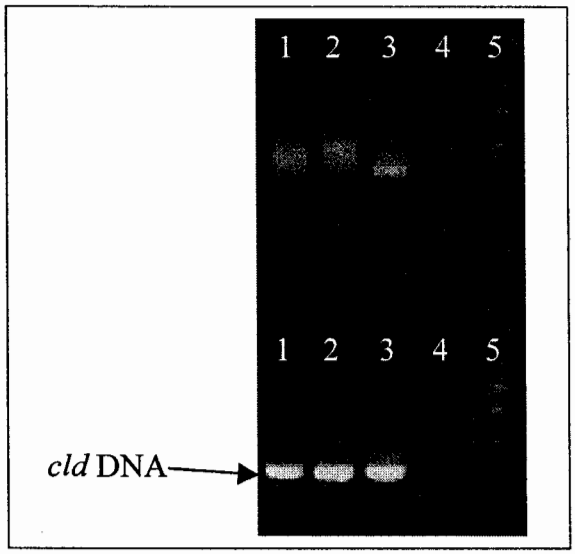
Samples received on: 21 July - 27 July, 2005
Analyzed on: 10 Aug - 12 Aug, 2005
Samples stored at: -20° C (DNA and RNA)

One-liter water samples were received from each site. Each sample was filtered to concentrate the biomass and split into two equal parts to prepare genomic DNA (gDNA) and total RNA from each.

Amplifications of chlorite dismutase gene *cld* using nested primer sets on gDNA:

Top: Amplifications with DCDF/DCDR
1) MW-24-1
2) MW-25-3
3) Positive control (RCB)
4) Negative control
5) 1 kb marker

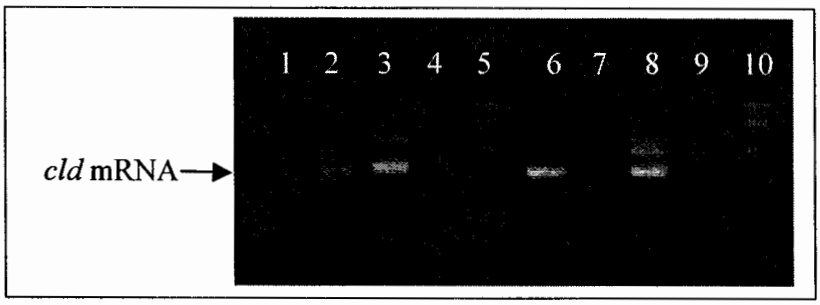
Bottom: Amplifications with UCDF/UCDR
1) MW-24-1
2) MW-25-3
3) Positive control (RCB)
4) Negative control
5) 1 kb marker



Results: Samples MW-24-1 and MW-25-3 both positive for *cld* gene (bottom lanes 1 and 2).

Nested *cld* amplifications using cDNA prepared by reverse transcription of total RNA

1) MW-24-1 cDNA touchdown
2) MW-25-3 cDNA touchdown
3) Touchdown positive control
4) Touchdown negative control
5) 1 kb marker
6) MW-24-1 cDNA nested
7) MW-25-3 cDNA nested
8) Nested positive control
9) Nested negative control
10) 1 kb marker



Results: Sample MW-24-1 positive for *cld* mRNA (lane 6). Sample MW-25-3 negative for *cld* mRNA (lane 7).

QCQA

Sample	Type	Presence of *cld* gene	Presence of *cld* mRNA
MW-24-1	Experimental sample	+	+
MW-25-3	Experimental sample	+	–
D. aromatica	Positive control	+	+
E. coli	Negative control	–	–

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Tel. (618) 549 6868, Fax (618) 687-4801, URL: WWW.BIOINSITE.COM

SUMMARY REPORT:

The genomic DNA for sample MW-17-3 was positive for the chlorite dismutase gene *cld* indicating the presence of (per)chlorate-reducing bacteria in this sample.

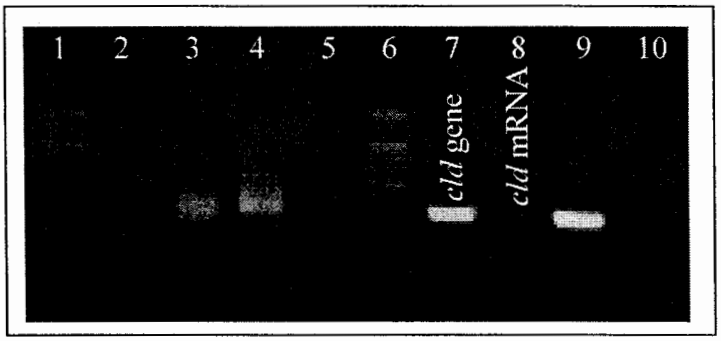
Analysis of the presence of the *cld* mRNA resulted in a negative result for the MW-17-3 sample which indicates that active (per)chlorate reduction is not occurring at this site.

Samples received on: 17 Aug, 2005
Analyzed on: 17 Aug - 26 Aug, 2005
Samples stored at: -20° C (DNA and RNA)

A one-liter water sample was received. The sample was filtered to concentrate the biomass and split into two equal parts to prepare genomic DNA (gDNA) and total RNA.

gDNA and RNA nested *cld* amplifications:

1) 1 kb marker
2) MW-17-3 gDNA with DCD primers
3) MW-17-3 cDNA with DCD primers
4) Positive control for DCD primers
5) Negative control for DCD primers
6) 1 kb marker
7) MW-17-3 gDNA with UCD primers
8) MW-17-3 cDNA with UCD primers
9) Positive control for UCD primers
10) Negative control for UCD primers



Results: Sample MW-17-3 positive for *cld* gene (lane 7). Sample MW-17-3 negative for *cld* mRNA (lane 8).

QCQA

Sample	Type	Presence of *cld* gene	Presence of *cld* mRNA
MW-17-3	Experimental sample	+	–
D. aromatica	Positive control	+	+
E. coli	Negative control	–	–