Using Species-Specific and Broad Range Primers in PCR to Identify Edaphic Strains of *Chlamydomonas reinhardtii*, *C. incerta*, and Related Species

ISOLATION OF FOUR NEW STRAINS OF *CHLAMYDOMONAS REINHARDTII* (CHLOROPHYTA) FROM SOIL SAMPLES[1]

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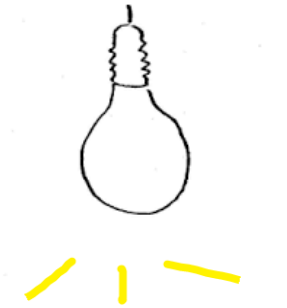
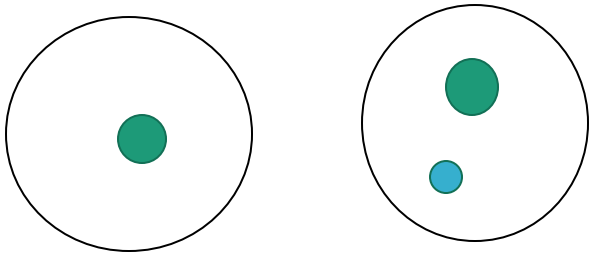
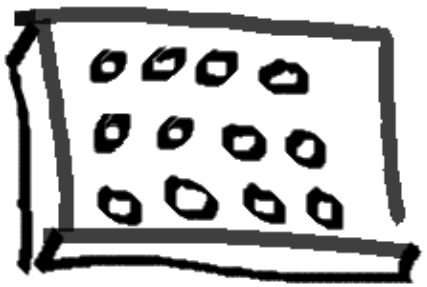
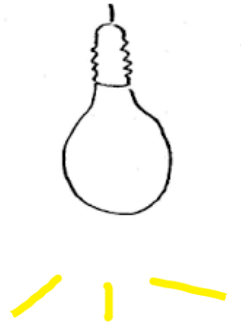








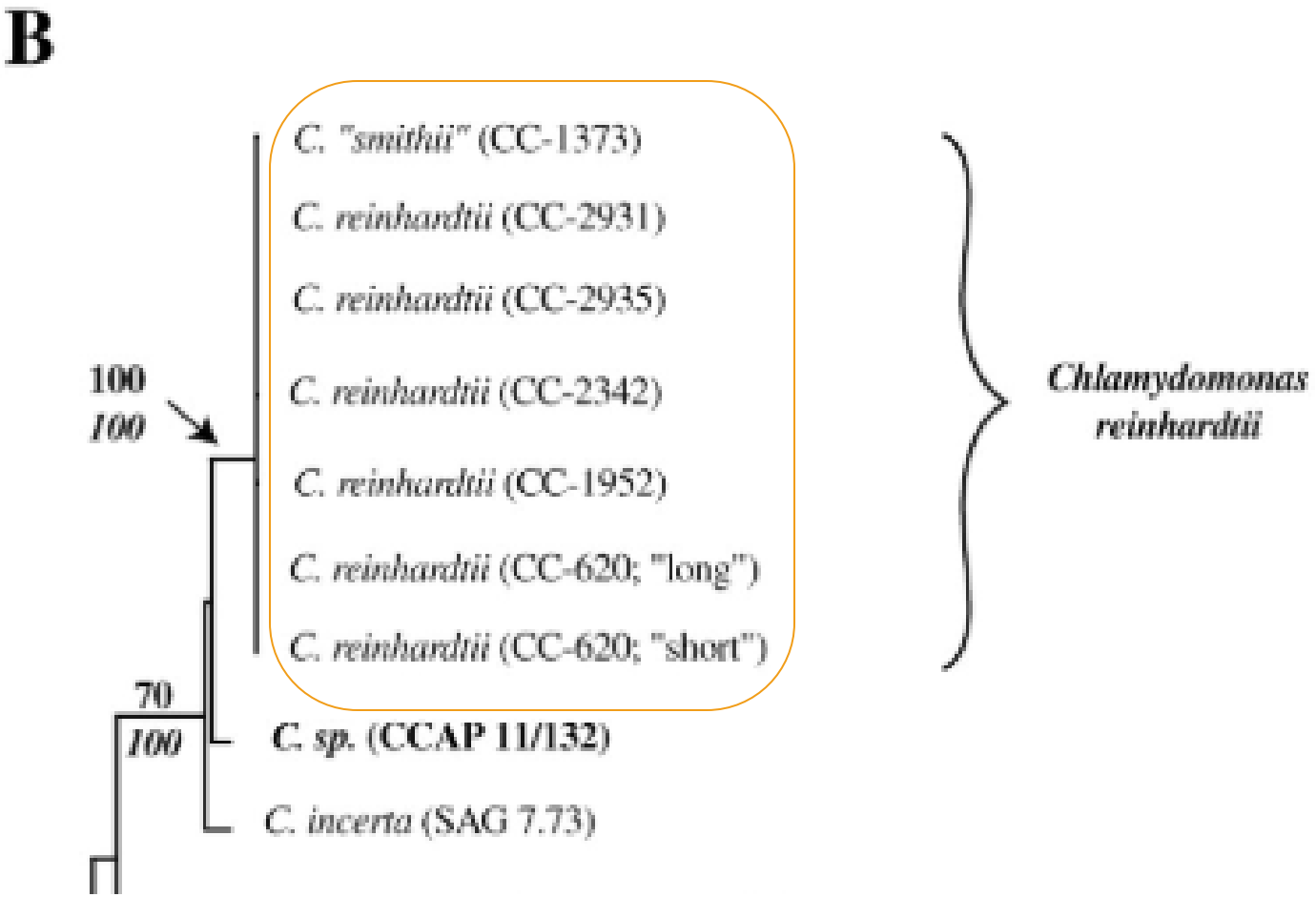
- M. Wilson, Kim & Schembri, Mark & D. Baker, Peter & Saint, Christopher. (2000). *Molecular Characterization of the Toxic Cyanobacterium Cylindrospermopsis raciborskii and Design of a Species-Specific PCR*. Applied and Environmental Microbiology. 66. 10.1128/AEM.66.1.332-338.2000.

- D Atkins, Simon & Clark, Ian & Pande, Sonal & Hirsch, Penny & R Kerry, Brian. (2005). The use of real-time PCR and species-specific primers for the identification and monitoring of Paecilomyces lilacinus. FEMS microbiology ecology. 51. 257-64. 10.1016/j.femsec.2004.09.002.

- Bérard, Annette & Dorigo, U & Humbert, Jean-François & Martin-Laurent, Fabrice. (2005). Microalgae community structure analysis based on 18S rDNA amplification from DNA extracted directly from soil as a potential soil bioindicator. Agronomy for Sustainable Development - AGRON SUSTAIN DEV. 25. 285-291. 10.1051/agro:2005004.

Primer Design

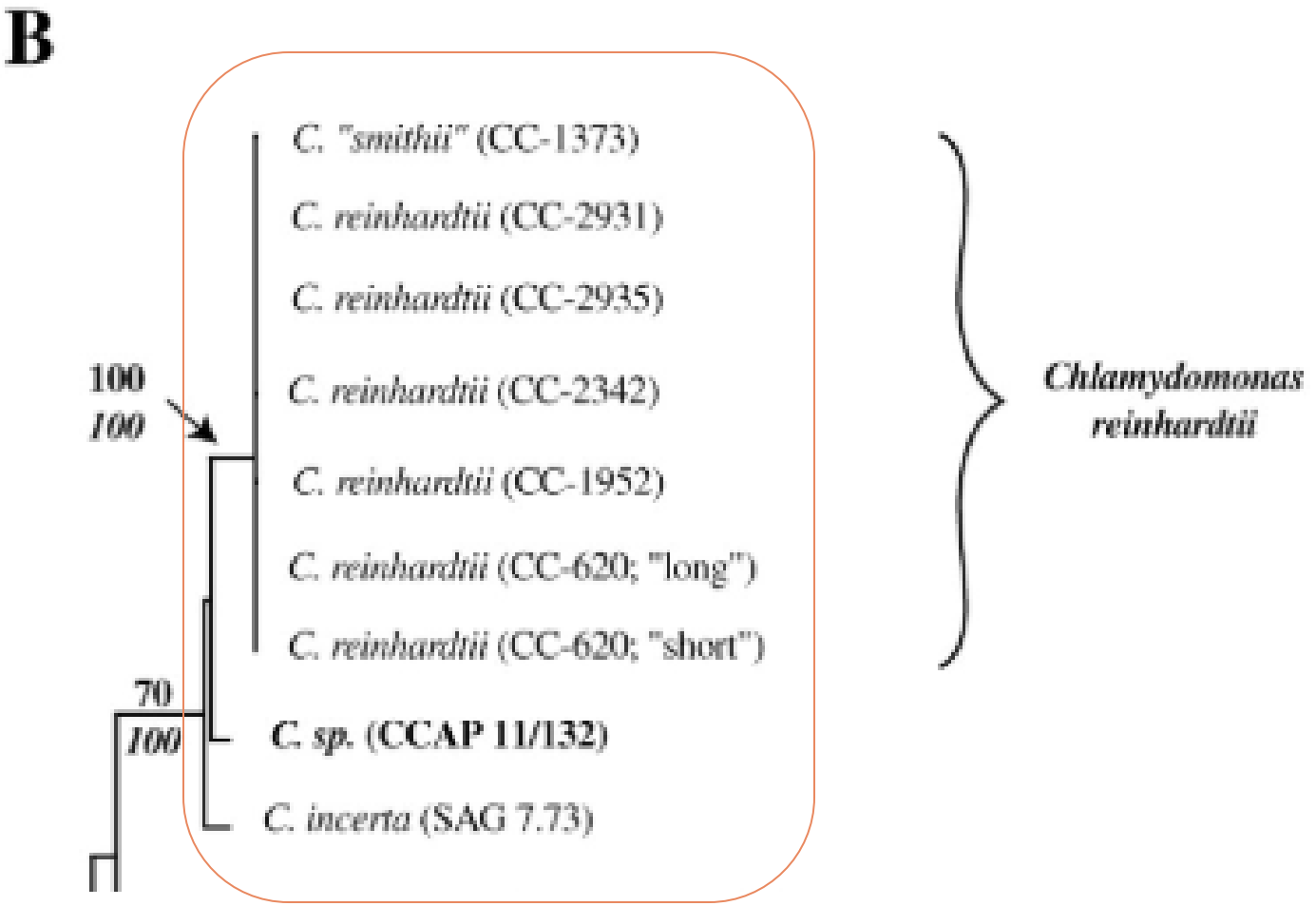
C.reinhardtii-Specific Primers



Portrait of a Species: Chlamydomonas reinhardtii,
Thomas Pröschold, Elizabeth H. Harris and Annette W. Coleman
GENETICS *August 1, 2005 vol. 170 no. 4 1601-1610;https://doi.org/10.1534/genetics.105.044503*

Broad-Range Primers

Ask rory for less prehis tric figure?



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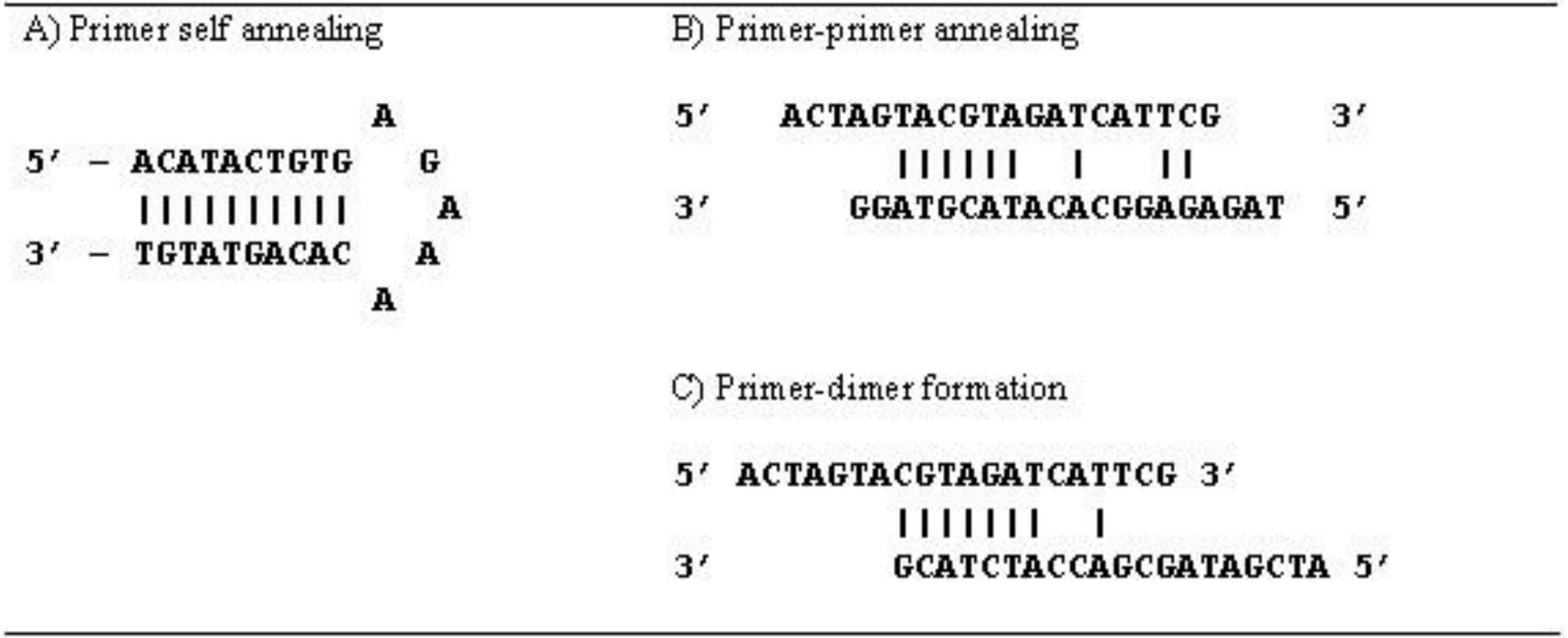
A Primer on Good Primers

- 5' A T C G C G G C C G T A A G T G G C 3' Primer Sequence
  3' T A G C G C C G G C A A T C A C C G 5' DNA

5' A T C G C G G C C G T A A G
3' T A G C G C C G G C A T T C A A A G 5' DNA

A Primer on Good Primers



Rouchka, Eric & Khalyfa, Abdelnaby & Cooper, Nigel. (2005). MPrime: Efficient large scale multiple primer and oligonucleotide design for customized gene microarrays. BMC bioinformatics. 6. 175. 10.1186/1471-2105-6-175

A Primer on Good Primers

- Tm of both primers close enough to each other
- GC content

Primer3

Primer3Plus

pick primers from a DNA sequence

More... | Source Code | Help | About

Load server settings: Default Activate Settings

Task: generic

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

Pick Primers Reset Form

Main | **General Settings** | **Advanced Settings** | **Internal Oligo** | **Penalty Weights** | **Advanced Sequence**

Sequence Id:

Paste template sequence below Or upload sequence file: Choose File No file chosen Upload File

Mark selected region: < > [ ] { } Clear Save Sequence

Excluded Regions: <

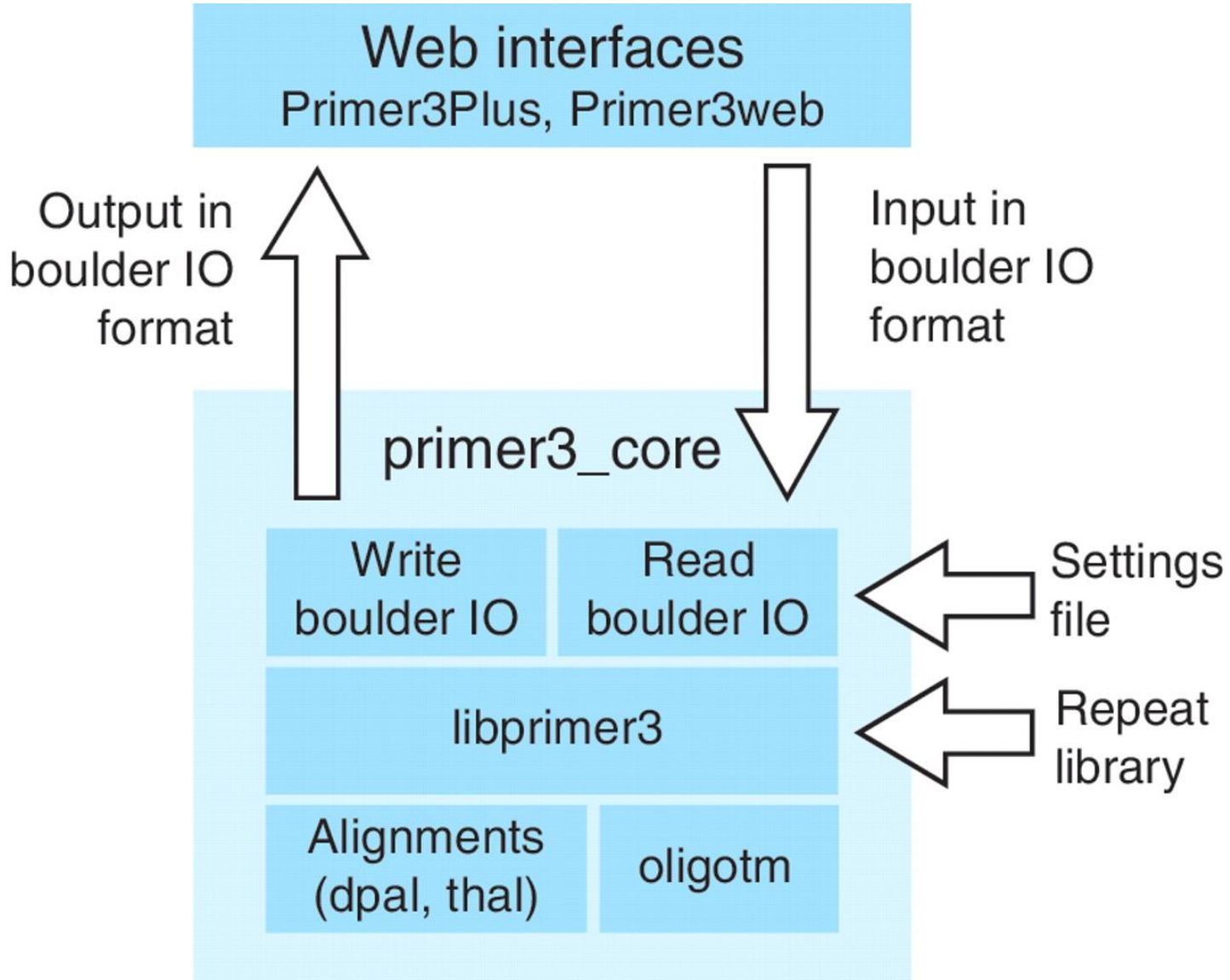
Targets: [

Included Region: {

Primer overlap positions: -

Pair OK Region List:

Pick left primer	Pick hybridization probe	Pick right primer
or use left primer below.	(internal oligo) or use oligo below.	or use right primer



- Andreas Untergasser, Ioana Cutcutache, Triinu Koressaar, Jian Ye, Brant C. Faircloth, Maido Remm, Steven G. Rozen; Primer3—new capabilities and interfaces, *Nucleic Acids Research*, Volume 40, Issue 15, 1 August 2012, Pages e115, https://doi.org/10.1093/nar/gks596

```
PRIMER_MISPRIMING_LIBRARY=incerta_mispriming_library.fasta
PRIMER_OPT_SIZE=22
PRIMER_MAX_LIBRARY_MISPRIMING=16.00
PRIMER_MAX_TEMPLATE_MISPRIMING=12
PRIMER_LIB_AMBIGUITY_CODES_CONSENSUS=0
PRIMER_INTERNAL_MIN_GC=50
PRIMER_TASK=generic
PRIMER_PICK_LEFT_PRIMER=1
PRIMER_PICK_INTERNAL_OLIGO=0
PRIMER_PICK_RIGHT_PRIMER=1
PRIMER_PRODUCT_SIZE_RANGE=250-900
PRIMER_MAX_SIZE=22
PRIMER_GC_CLAMP=3
PRIMER_MAX_END_GC=3
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SEQUENCE_TEMPLATE=ATGGCGAGTTTGCAGTCCGCGGGTGCCCCGGGCAGCGTCCCTCCCCTCCTGGATCCCAGCAAGCTAAGCATTGGACGAGTGCTTGGCCGGGGCGCCTTTGCCACAGTGTAC
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CTACACGCCAAGGCCGGCCGCCGTCCCTACAGCGACGCCACAGCGCTGTCGCTGCTGCGTGACGTGACAGAGGCGCTGGCGGCGTTGCACGCGGCGTCGCCGCCCGCCATTCACCGCGACGTGAAAGCT
GAGAACATTCTGCTCAAGCGCGAGGGACTTACGCCGGCAGCAGCAGGCGCCGGCGGTGGCTGCGCCGCGTCATCGCTGCCGGCGGTGGCATCTGGAGGTGGCGAGGGGCAGGAGCAGCCGTACCAGCAG
CTGACGGCGCGGGTGGCGGATCTGGGTCTGCACGTGCGGGTGGAGCAGCATCGCTCCGTGATGCTGCGACGCCGCGGCGCGGCCTCGCTGGACGGCTCCGCCTTTGTGGCCATCTCGCGCCACAACTCC
```

```
=
SEQUENCE_ID=g6136.t1|PACid:26892997
SEQUENCE_TEMPLATE=ATGAAGCGCCCTGACACCTTGGGTGACAGGATATGGCGGTCAGTCGATAAGAGCGTGGACCAGAGGTACCGCGGCGTGCTGCTGGTGGTGTCCATCCCGGTAATTCTAATC
CTGGCGGTCATCACGCTGGTGCCACGTTCAACGCCTTTCCACGCGGAGCGCACCCACACGCTGAAGGAGCTGCGCCATACAGGCGCGGCGCCAAAGTTCGCGATTGTGTTTGATGCGGGCAGCACAGGC
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GTGGGCAAGACCGTGGCTGCCATTGACATGGGTGGCGGCTCGATTCAGGAGGCGTTTGCGCTGGAGGACACGAATGCAAAACTGGCGCCCACGGACTACGTTGTTCAGCTGCACGGAGGCGGCAAGACC
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```

C.reinhardtii-Specific Primers

- highly specific to C. reinhardtii --> used C. incerta as mispriming library

```
PRIMER_MISPRIMING_LIBRARY=incerta_mispriming_library.fasta
PRIMER_OPT_SIZE=22
PRIMER_MAX_LIBRARY_MISPRIMING=16.00
PRIMER_MAX_TEMPLATE_MISPRIMING=12
PRIMER_LIB_AMBIGUITY_CODES_CONSENSUS=0
PRIMER_INTERNAL_MIN_GC=50
PRIMER_TASK=generic
PRIMER_PICK_LEFT_PRIMER=1
PRIMER_PICK_INTERNAL_OLIGO=0
```

```
%%bash

 #primer3 doing its work on reinhardtii input for C.reinhardtii-specific primers

/scratch/research/tmp_apps/primer3-2.3.7/src/primer3_core < reinhardtii_input2.txt > primer3_output_reinhardtii_selecti
```

```
PRIMER_LEFT_NUM_RETURNED=5
PRIMER_RIGHT_NUM_RETURNED=5
PRIMER_INTERNAL_NUM_RETURNED=0
PRIMER_PAIR_NUM_RETURNED=5
PRIMER_PAIR_0_PENALTY=5.801451
PRIMER_LEFT_0_PENALTY=0.158696
PRIMER_RIGHT_0_PENALTY=5.642755
PRIMER_LEFT_0_SEQUENCE=AGGCTGACAATATCCTGAAGGC
PRIMER_RIGHT_0_SEQUENCE=AAGAGCACTCCGTTTGCG
PRIMER_LEFT_0=475,22
PRIMER_RIGHT_0=1005,18
PRIMER_LEFT_0_TM=60.159
PRIMER_RIGHT_0_TM=58.357
PRIMER_LEFT_0_GC_PERCENT=50.000
PRIMER_RIGHT_0_GC_PERCENT=55.556
PRIMER_LEFT_0_SELF_ANY_TH=0.00
PRIMER_RIGHT_0_SELF_ANY_TH=0.00
PRIMER_LEFT_0_SELF_END_TH=0.00
PRIMER_RIGHT_0_SELF_END_TH=0.00
PRIMER_LEFT_0_HAIRPIN_TH=34.44
PRIMER_RIGHT_0_HAIRPIN_TH=45.15
PRIMER_LEFT_0_LIBRARY_MISPRIMING=14.00, startgeneg5578
PRIMER_RIGHT_0_LIBRARY_MISPRIMING=11.00, comp startgeneg20636
PRIMER_PAIR_0_LIBRARY_MISPRIMING=24.00, startgeneg5578
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PRIMER_RIGHT_0_END_STABILITY=4.8500
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PRIMER_RIGHT_0_TEMPLATE_MISPRIMING=8.0000
PRIMER_PAIR_0_COMPL_ANY_TH=0.00
PRIMER_PAIR_0_COMPL_END_TH=0.00
PRIMER_PAIR_0_PRODUCT_SIZE=531
```

.txt

Broad Range Primers

```
109
110 Query= startgeneg5578
111
112 Length=1392
113                                                                      Score     E
114 Sequences producing significant alignments:                         (Bits)  Value
115
116 g6136.t1|PACid:26892997                                             1906    0.0
117
118
119 >g6136.t1|PACid:26892997
120 Length=1392
121
122  Score = 1906 bits (1032),  Expect = 0.0
123  Identities = 1272/1392 (91%), Gaps = 0/1392 (0%)
124  Strand=Plus/Plus
125
126 Query  1     ATGAAGCGCCCTGACACCTTGGGTGACAGGATATGGCGGTCAGTCGATAAGAACGTGGAC  60
127                ||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
128 Sbjct  1     ATGAAGCGCCCTGACACCTTGGGTGACAGGATATGGCGGTCAGTCGATAAGAGCGTGGAC  60
129
130 Query  61    CAGAGGTACCGTGGCGTGCTGCTGGTGGTGTCCATCCCGGTCATTCTCATCCTGGCGGTC  120
131                ||||||||||| |||||||||||||||||||||||||||| ||||| ||||||||||||
132 Sbjct  61    CAGAGGTACCGCGGCGTGCTGCTGGTGGTGTCCATCCCGGTAATTCTAATCCTGGCGGTC  120
133
134 Query  121   ATCATGCTAGTACCCCGCTCTACGCCTTTCCATGCGGAGCGCCCGCACACCTTGAAGGAG  180
135                ||||  |||  ||  ||  ||  ||  |||||||||||  |||||||| | |||||  ||||||||
136 Sbjct  121   ATCACGCTGGTGCCACGTTCAACGCCTTTCCACGCGGAGCGCACCCACACGCTGAAGGAG  180
137
138 Query  181   CTGCGCCATACGGGCGCGGCGCCGAAGTATGCAATTGTGTTCGATGCGGGCAGCACCGGC  240
139                ||||||||||| |||||||||||  ||  ||||||||  ||||||||||||||  |||
140 Sbjct  181   CTGCGCCATACAGGCGCGGCGCCAAAGTTCGCGATTGTGTTTGATGCGGGCAGCACAGGC  240
```

Primer3Plus

pick primers from a DNA sequence

More... | Source Code | Help | About

Load server settings: Default Activate Settings

Task: generic

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

Pick Primers Reset Form

Main | **General Settings** | **Advanced Settings** | **Internal Oligo** | **Penalty Weights** | **Advanced Sequence**

Sequence Id:

Paste template sequence below Or upload sequence file: Choose File No file chosen Upload File

Mark selected region: < > [ ] { } Clear Save Sequence

Excluded Regions: <

Targets: [

Included Region: {

Primer overlap positions: -

Pair OK Region List:

Pick left primer	Pick hybridization probe	Pick right primer
or use left primer below.	(internal oligo) or use oligo below.	or use right primer

- Query= startgeneg7179
- Cre03.g161400.t1.2|PACid:26901868

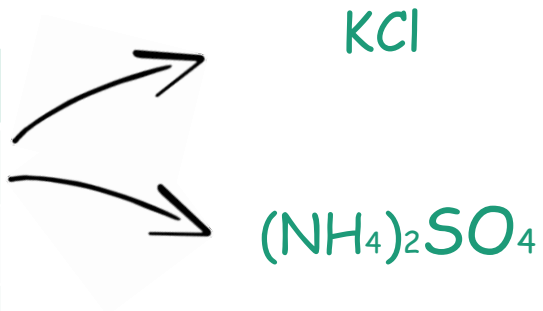
OLIGO	start	len	tm	gc%	any	3'	seq
LEFT PRIMER	573	18	59.27	66.67	5.00	2.00	GACGCCCCTGTACTACGC
RIGHT PRIMER	1026	18	59.55	61.11	7.00	0.00	CACCATCATGGGGTAGGG

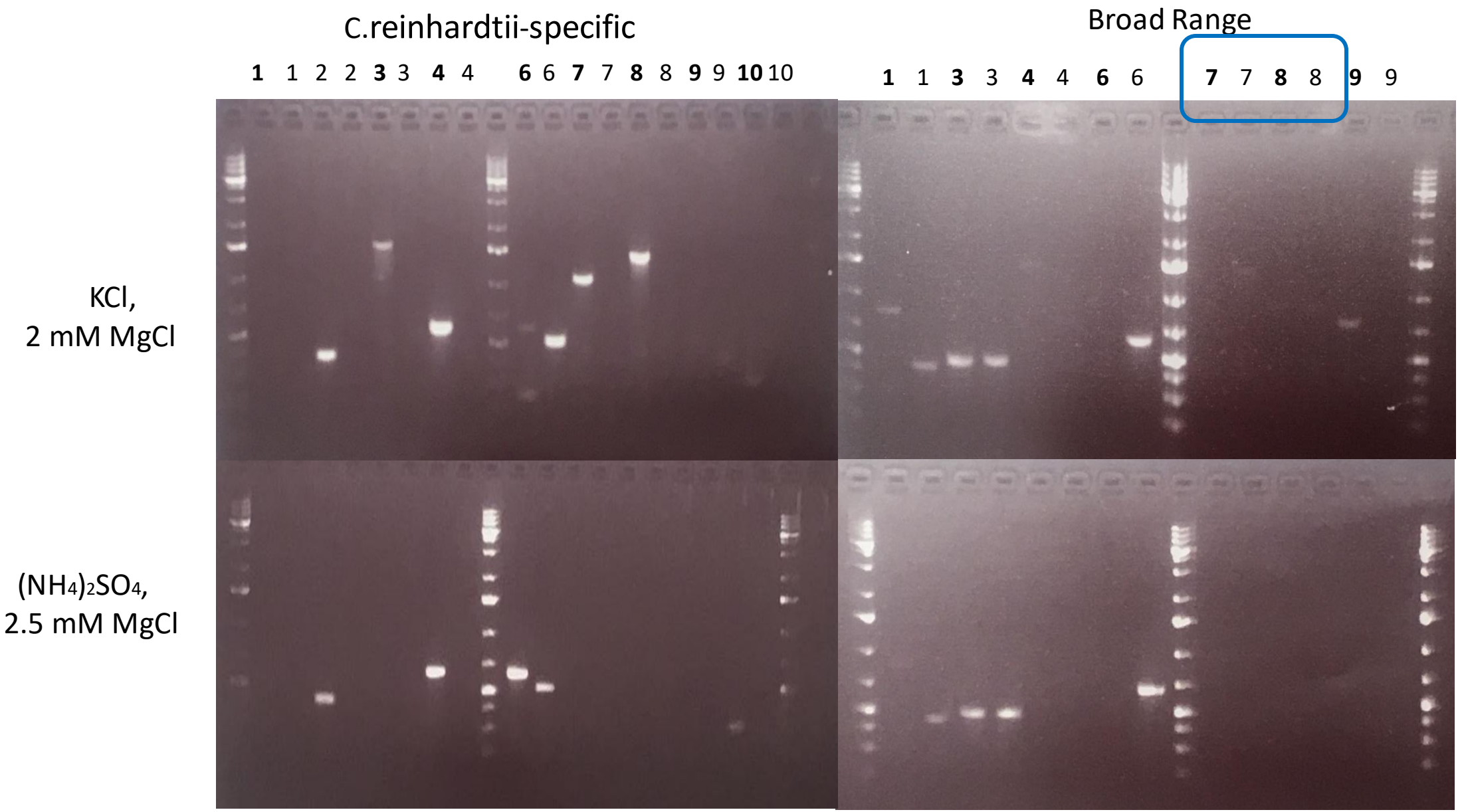
```
6616
6617  Query  555   GGACTACGTGGGCCGCGAGACGCCCCTGTACTACGCGGAGCGCCTGTCGGCGAACTACAA  614
6618                ||||||||||||| |||||||||||||| ||| |||| ||||||||||||||| |||||||
6619  Sbjct  555   GGACTACGTGGGTCGCGAGACGCCCCTTTACCACGCCGAGCGCCTGTCGGCGCACTACAA  614

6644
6645  Query  975   CCACTACATCCTGGGCTCGGCCGCCGGCCCCCACCCCTACCCCATGATGGTGCGCGAGTT  1034
6646                |||||||||||||||| |||||||| ||||||||||||||||||||||||||||||||
6647  Sbjct  975   CCACTACATCCTGGGGTCGGCCGCTGGCCCCCACCCCTACCCCATGATGGTGCGCGAGTT  1034
```

PCR
I.Primer Eval with Lab Strain DNA extract

PCR
I. Primer Eval with Lab Strain DNA extract

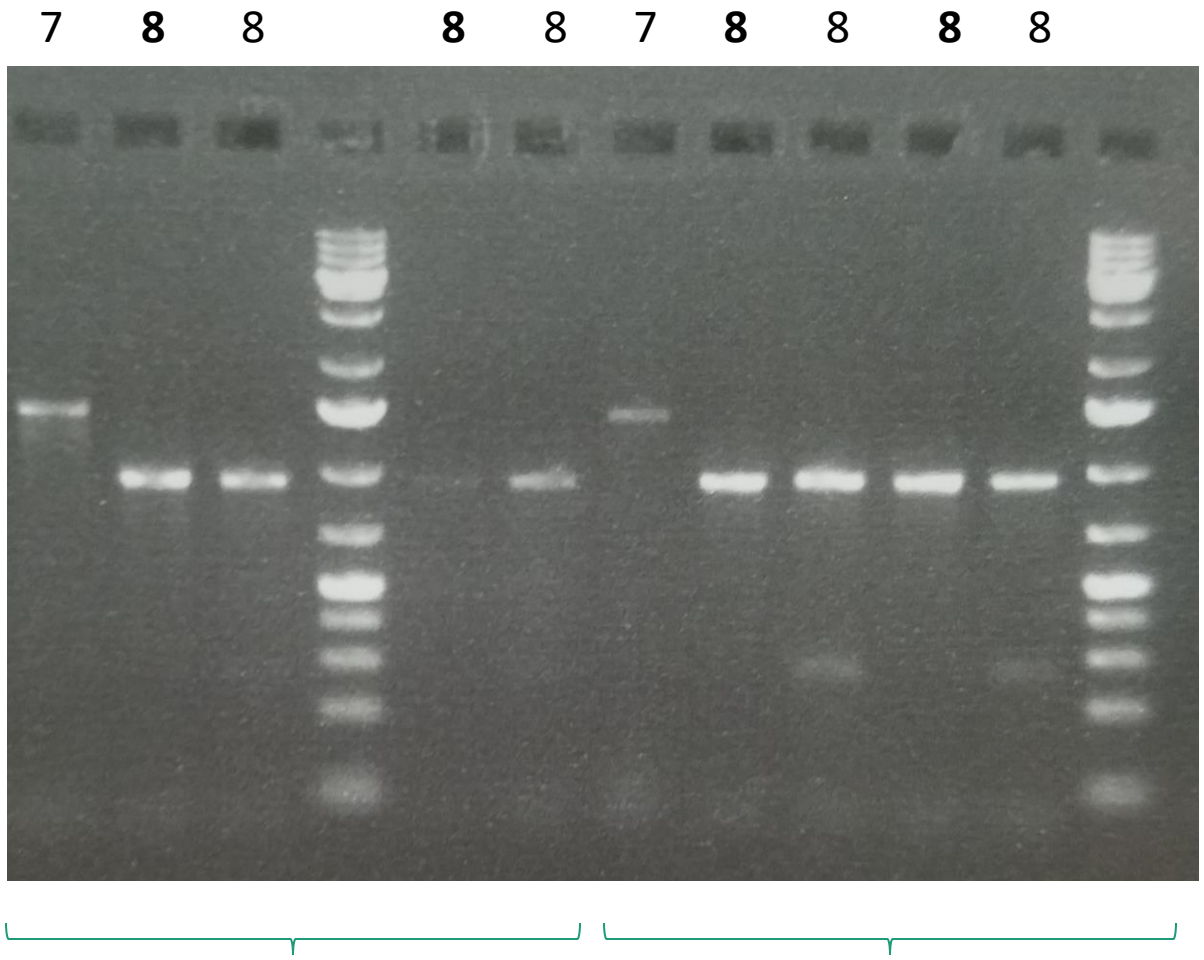
Final Concentration/ Ratio	Reagent
2.5 uL in 25 uL	Buffer
2.0 or 2.5 mM	Mg2+
0.125 uL in 25 uL	Taq Polymerase
200 mM	dNTPs
1 ng/uL	gDNA
0.5 uL in 25 uL	Forward Primer
0.5 uL in 25 uL	Reverse Primer
1 M	Betaine - Additive





Bold numerals: C. reinhardtii amplicon
Else: C. incerta amplicon


7
7
8
8

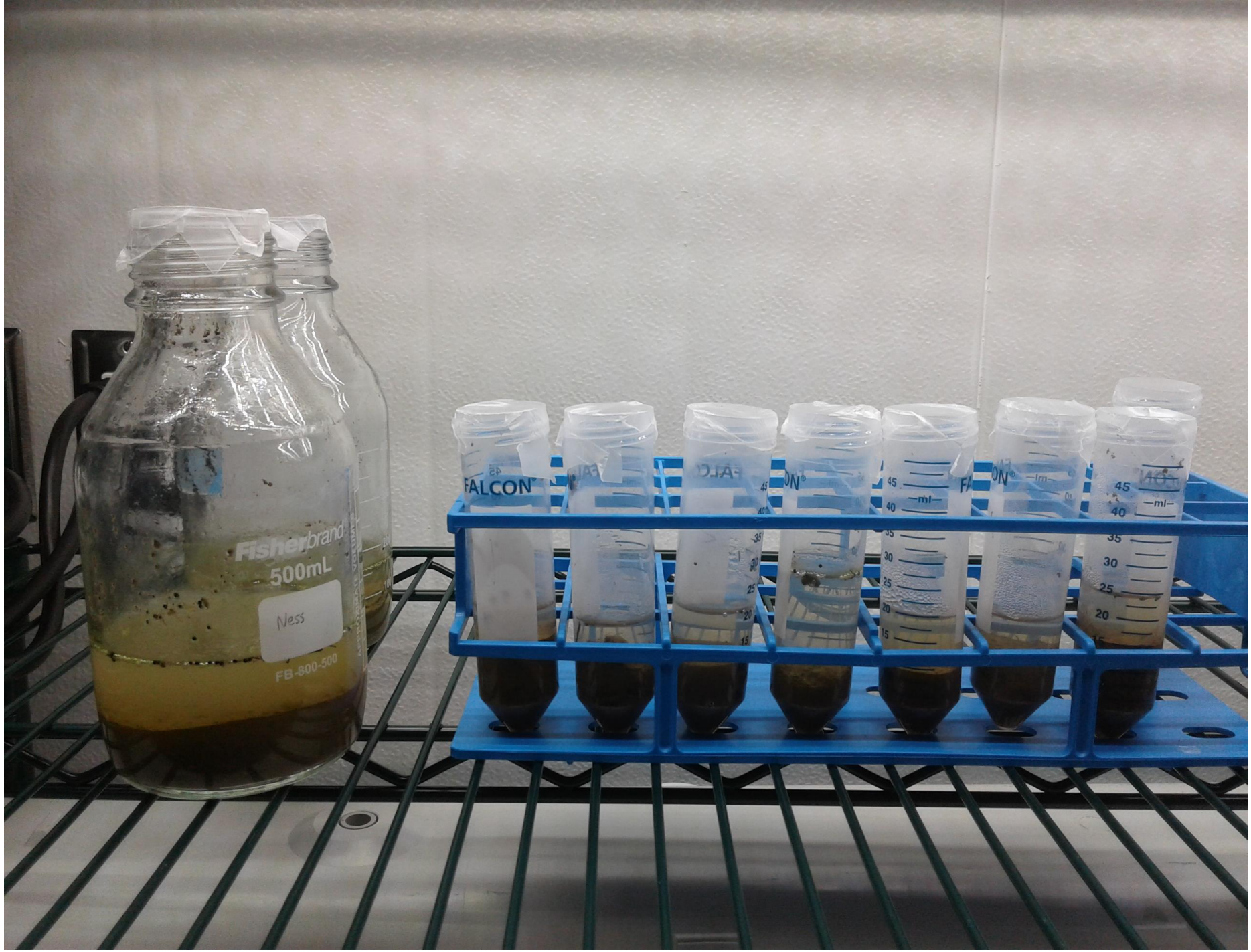

7
8
8
8
8
7
8
8
8
8
Betaine
No Betaine

Soil Sampling

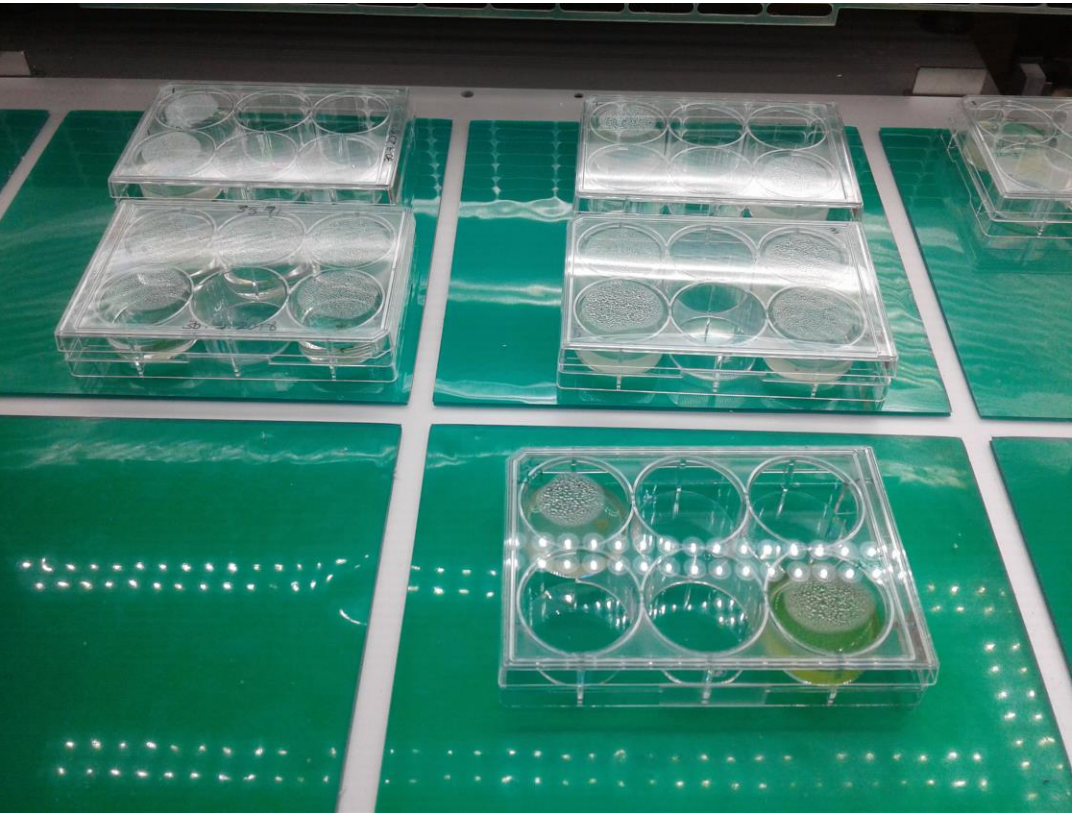
Riverwood Conservancy, Mississauga

- Culham trail
- Two samples from two very different soil types
- Upper Culham (UC) : clay/loam like
- Lower Culham (LC) : drier, shallower.

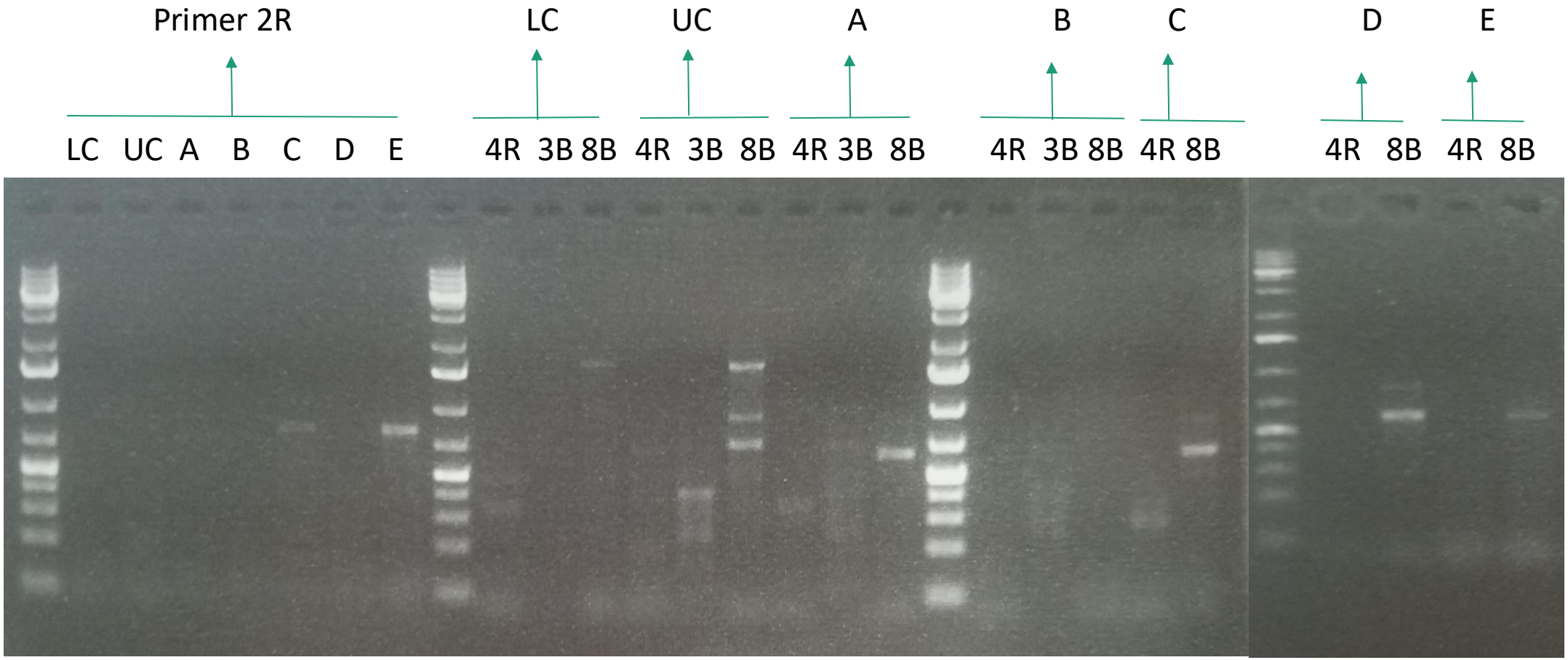
Andrew's (very) Scenic Acres, Milton


Fisherbrand
500mL
Ness
FB-800-500
FALCON
45
40
35
30
25
20
ml

To Culture or Not to Culture



Direct DNA extraction from Soil	Culture
Point of using PCR?	Will I get enough DNA?
Faster- contamination not an issue	Primer specificity requirement less stringent than it would be with indigenous soil DNA composition
Chlorella may outcompete	



Primer	Expected FS	FS-1690 &/or 3871	Fragment Size-E	Fragment Size-C	FS UC	FS A	FS D	FS LC
2R	267	4 (400 bp)	6.5 (700-	6.5				