

## PRIMER #1

### Coralliophila galea voucher MNHN-IM-2013-9565 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

ACCESSION PP094199

---

tactttgtacattttatattgggtatgtggtccggctcttggtggaacgggactaagtttact  
aattcgagctgaactcgggtcaaccaggagccttactcgggtgacgatcaactatacaatgt  
gattgttacggcacacgcttttggtataattttttcctagtaatgccattataattgg  
tggctttggtaattgggtggtacctcttatattaggtgctcccgacatagcttttcctcg  
actaataatatgagttttgacttcttccccctgcactattgctgcttctttcttctg  
tgctgtagaaaggggtggtgggactggctggacagtgtatccacctctctctggtaatgt  
tgcacatgccggtggctctgtagatctggctatctttagccttcatctggctggtgtatc  
ctctattttaggtgctgtaaactttattacaactgttaccaatatacgatgacagggcat  
acaatttgagcggcttcctttatattggttggctctgtaaagattactgctattttattact  
cctatctttaccggtggtggctggggctattactatggttgtaacagatcgtaactttaa  
tacaagctttttgatcccgccagaggaggagatcctatcttataaccagcacttattt

Primer

---

Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self comp	Self 3' comp	Self 3' comp
<b>Forward primer</b>	GTGCTCCCGACA TAGCTTTT	Plus	20	216	235	58.26	50.00	4.00	2.00
<b>Reverse primer</b>	AGTCCCAACACC CCTTTCTA	Minus	20	325	306	57.87	50.00	2.00	2.00
<b>Product length</b>	110								

-----  
General properties:

-----  
Primer name: forward

Primer sequence: GTGCTCCCGACATAGCTTTT

Sequence length: 20

Base counts: G=4; A=3; T=7; C=6; Other=0;

GC content (%): 50.00

Molecular weight (Daltons): 6058.99

nmol/A260: 5.56

micrograms/A260: 33.68

Basic Tm (degrees C): 52

Salt adjusted Tm (degrees C): 47

Nearest neighbor Tm (degrees C): 62.93

PCR suitability tests (Pass / Warning):

-----  
Single base runs: Pass

Dinucleotide base runs: Pass

Length: Pass

Percent GC: Pass

Tm (Nearest neighbor): **Warning: Tm is greater than 58;**

GC clamp: Pass

Self-annealing: Pass

Hairpin formation: Pass  
-----

-----  
General properties:

-----  
Primer name: reverse

Primer sequence: AGTCCCAACACCCCTTTCTA

Sequence length: 20

Base counts: G=1; A=5; T=5; C=9; Other=0;

GC content (%): 50.00

Molecular weight (Daltons): 5956.92

nmol/A260: 5.49

micrograms/A260: 32.69

Basic Tm (degrees C): 52

Salt adjusted Tm (degrees C): 47

Nearest neighbor Tm (degrees C): 63.40

PCR suitability tests (Pass / Warning):

-----  
Single base runs: Pass

Dinucleotide base runs: Pass

Length: Pass

Percent GC: Pass

Tm (Nearest neighbor): **Warning: Tm is greater than 58;**  
 GC clamp: Pass  
 Self-annealing: Pass  
 Hairpin formation: Pass

-----

## PRIMER #2

### Coralliophila galea voucher MNHN-IM-2013-9565 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

ACCESSION PP094199

tactttgtacattttatttg gtatgtgggtccggctcttggtggaacgggactaagtttact  
 aattcgagctgaactcgggtcaaccaggagccttactcgggtgacgatcaactataacaatgt  
 gattgttacggcacacgcttttgttataatTTTTTCTtagtaatgccattataaattgg  
 tggctttggtaattggttggtacctcttatattaggtgctcccacatagcttttctctcg  
 acttaataatagagttttgacttcttccccctgcaactattgctgcttctttcttctgg  
 tgctgtagaaaggggtggtgggactggctggacagtgtatccacctctctctggtaatgt  
 tgcacatgccggtggctctgtagatctggctatctttagccttcatctggctggtgtatc  
 ctctatTTTtaggtgctgtaaactttattacaactgttaccaatatacgatgacagggcat  
 acaatttgagcggcttccctttatttggttggtctgtaaagattactgctatTTTtattact  
 cctatctttaccggtggtggctggggctattactatggtgtaaacagatcgtaactTTTaa  
 TacaagctTTTTTgatcccgccagaggaggagatcctatcttataaccagcacttattt

Primer

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
<b>Forward primer</b>	GTGGTCCGGTCTTGT TGGAA	Plus	20	25	44	60.18	55.00	4.00	2.00
<b>Reverse primer</b>	ACAAAAGCGTGTGCC GTAAC	Minus	20	14	12	59.97	50.00	4.00	3.00
<b>Product length</b>	120								

General properties:

-----

Primer name: forward  
Primer sequence: GTGGTCCGGTCTTGTGGAA  
Sequence length: 20  
Base counts: G=8; A=2; T=7; C=3; Other=0;  
GC content (%): 55.00  
Molecular weight (Daltons): 6195.08  
nmol/A260: 5.29  
micrograms/A260: 32.74  
Basic Tm (degrees C): 54  
Salt adjusted Tm (degrees C): 49  
Nearest neighbor Tm (degrees C): 65.53

PCR suitability tests (Pass / Warning):

-----

Single base runs: Pass  
Dinucleotide base runs: Pass  
Length: Pass  
Percent GC: Pass  
Tm (Nearest neighbor): **Warning: Tm is greater than 58;**  
GC clamp: Pass  
Self-annealing: Pass  
Hairpin formation: Pass

-----

-----

General properties:

-----

Primer name: reverse  
Primer sequence: ACAAAGCGTGTGCCGTAAC  
Sequence length: 20  
Base counts: G=5; A=7; T=3; C=5; Other=0;  
GC content (%): 50.00  
Molecular weight (Daltons): 6135.06  
nmol/A260: 5.01  
micrograms/A260: 30.71  
Basic Tm (degrees C): 52  
Salt adjusted Tm (degrees C): 47  
Nearest neighbor Tm (degrees C): 64.53

PCR suitability tests (Pass / Warning):

-----  
Single base runs: Pass

Dinucleotide base runs: Pass

Length: Pass

Percent GC: Pass

Tm (Nearest neighbor): **Warning: Tm is greater than 58;**

GC clamp: Pass

Self-annealing: Pass

Hairpin formation: Pass  
-----