

Mite COI_1

- Dabert, J etal 2008. Glaucoalgae tytonis sp. n. (Analgoidea, Xozlgidae) from the barn owl Tyto alba (Strigeiformes, Tytonidae) : compiling morphology with DNA barcode data for taxon descriptions in mites (Acari). *Zootaxa* 1719: 41–52 (2008)

Extraction Date(s): 4/6/2017 – end date

Using Qiagen: DNeasy Blood & Tissue Kit

PCR Date: 4/7/3017**Technician:** CLR**Electrophoresis Date:** 4/7/2017**Submitted for SEQ Date:** 4/11/2017**Exo SAP Date:** 4/11/2017**Date Seq Results Received:** 4/12/2017

Seq #	PCR Amplific (PA #)	Ext Sample #	Gel Lane	Non Case/ Case	Acc	Host	Tissue	Ext END Date	
	pa 816	NA	2 no band	NTC - Negative Template Control (water)					
	pa 817	px 689	3 no band	NEC				4/6/2017	
1,2	pa 818	px 687	4 band	26939	1	PGroesebeak	feet	4/6/2017	
3,4	pa 819	px 688	5 band	26939	1	PGroesebeak	feet	4/6/2017	
5,6	pa 820	px 694	6 band	26939	1	PGroesebeak	feet	4/6/2017	
7,8	pa 821	px 695	7 band	26939	1	PGroesebeak	feet	4/6/2017	

PCR & SEQ Primers bcdF05 and bcdR04 **Cycling Conditions: BioRad :**MiteCOI_1 **This is :** a 644-bp region near the 5' terminus of the COI gene

Gel Prep: 1% Agarose Gel with Gel Red, Run at 110V for 45 min 250mA (1%-100ml gel = 1g agarose to 100ml 1xTAE, with 10µl Gel Red) 100 bp ladder (6µl water +4µl ladder) Load: 1µl loading dye +3µl water+ 5µl product

SEE Page 2 for Gel Image

Cycling Conditions		
	Temp (°C)	Time
Denaturation	96	3 min
35 cycles	95	10 sec
	50	30sec
	72	1 min
Final Extension	72	5 min
End	4	forever
Expected Run time: 2h 20min		

ExoSAP 2 rxns per sample for PreSequence clean up
(one rxn for each primer)

Master Mix Prep Table	1 Rxn	Total Rxns	[Final]
PCR Reaction	(µl)	9	
DDwater	9.75	87.75	
10x ExTaq Buffer	2.5	22.5	1x
Dntp mix (2.5mM each)	2.5	22.5	0.1mM
Forward Primer (10uM)	2.5	22.5	1uM
Reverse Primer (10uM)	2.5	22.5	1uM
EX Taq (5U/µl)	0.25	2.25	1.25 U
Master Mix	20		
DNA Template	5		
Total Rxn Volume	25		

Record PCR Info on:

- x Case Bench sheet
- x DNA Excel table
- x PCR Log Book
- x ParaDB if applicable

A= add when closing case**X= recorded.**

	1 Rxn (µl)
ExoSapIT	1
PCR Product	5
Cycle through "Pre-Seq" (37°C for 15min and 80°C for 20min)	
Primer (10uM)	1
DD Water	17
Final Volume	24

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NOTE: UW Biotech Center Login: Rebecca Cole PW: parasitology

PCR Notes:

- See ParaDB for Lysis/Extraction notes, power outage 1st set and modified from Dabert for 2nd set
- NEC and NTC no band
- Appears the PCR worked despite issues w/ Lysis and Extration, also some mites were in xylene prior to extraction, see extraction notes in paradb.

