# DNA extraction and amplification of faecal samples from the White Fronted Terns (*Sternula nereis*).

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Report

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APPLIED MOLECULAR SOLUTIONS

## Executive Summary

This report describes the success of DNA extraction and amplification of fresh faecal samples collected from White Fronted Tern *Sterna striata* in the field. Sample handling involved immediate placement in ethanol field followed by freezing at -20$^{o}$C a few hours later on the day of collection.. DNA was extracted from 10 faecal samples collected from birds (*Sternula nereis*). PCR using primers targeting Chordata was then used to selectively amplify short sections of the 16S gene which can be used for prey identification . PCR products were run on an agarose gel, as a success/fail test for amplification. DNA was successfully amplified in four of the ten samples. Attempts to amplify DNA using primers targeting Malacostraca and a second pair of PCR primers, targeting a 180-270bp section of the 16S gene of multiple taxa, were unsuccessful.

## Introduction

Our laboratory has successfully obtained amplifiable DNA from regurgitate and faecal samples collected from adult Australasian gannets (*Morus serrator*) in which the samples had been initially chilled and then frozen on the day of collection. Attempts to extract and amplify DNA recovered from faecal samples (preserved in ethanol) from Buller's Shearwaters *Puffinus bulleri* and Fairy Prions *Pachyptila turtur* have been unsuccessful.

Many variables affect the outcomes of faecal DNA analysis, particularly sample degradation and contamination. Modifications made to sample collection methods, the extraction techniques used, and changes to the PCR protocol used can help to mitigate some of the issues which arise and affect the outcome of DNA amplification. Here, we report on the success of DNA extraction and amplification of faecal samples collected from White Fronted Terns *(Sterna striata)*. In contrast to samples from Buller's Shearwater and Fairy Prions immediate ethanol preservation of faecal samples was followed by freezing of the samples at -20$^{o}$C later the same day.

## Method

Samples were collected fresh and stored in 70% ethanol followed by freezing and storage at -20°C until DNA extractions. DNA was extracted from a total of ten faecal samples, sourced from White Fronted Terns, using two different methods, an isopropanol extraction technique and the Qiagen® DNA Stool kit.

A 155bp section of the 16S gene in Chordata was PCR amplified using the primers Chord_16S_F_TagA (5'- ATG CGA GAA GAC CCT RTG GAG CT) and Chord_16S_R_Short (5'- CCT NGG TCG CCC CAA C) (Deagle et al., 2009) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 0.2 µL MgCl (25mM), 3µL of DNA template, and 5.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). A second PCR reaction was carried out using the primers Mala_16S1F (5'-TGA CGA TAA GAC CCT) and Mala_16S2R (5'- CGC TGT TAT CCC TAA AGT AAC T) (Deagle et al., 2005), which target a 200bp section of the 16S gene in Malacostraca. These PCR reactions had the same composition as those using Chordata primers, and incorporated three positive controls using 3µL each of shrimp (*Decapoda*), squid (*Teuthida*), and muscle (*Bivalvia*) DNA, in addition to a negative control using 3µL of MQ water.

The DNA samples which were extracted using the Qiagen® DNA Stool kit were amplified in a second round of PCR reactions, using a modified protocol in an effort to reduce the presence of primer dimers in the PCR product. Samples were amplified in 20µL reactions containing 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 2µL of DNA template, and 6.4µl of MQ water.

In all reactions, thermal cycling was conducted in a Surecycler 8800 (Agilent Technologies). An initial denaturation period of 15 minutes at 94 °C was followed by 33 cycles of denaturation for 20 seconds at 94 °C, annealing for 90 seconds at 48.7 °C, and extension for 45 seconds at 72 °C, followed by a final extension for 2 minutes at 72 °C. PCR products were run on a 1% agarose gel and visualised in an UVIDOC HD6 Touch (UVITEC Cambridge), to determine the amplification success of each of the samples.

A second pair of PCR primers, targeting a 180-270bp section of the 16S gene of multiple taxa, was also trailed. DNA samples were PCR amplified using the primers 16S1F (GACGAKAAGACCCTA) and 16S2R (CGCTGTTATCCCTADRGTAACT) (Deagle, 2007) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 1.0 µL BSA, 0.2 µL MgCl (25mM), 3µL of DNA template, and 4.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). Testing of this primer pairs efficacy prior to this trial showed that it would amplify DNA from fish, but not from the other taxa of interest, therefore only whitebait DNA was included as a positive control.

**Results**

Of the samples extracted using the isopropanol technique, no DNA amplification was detected seen in the 10 samples using the Malacostraca primers. One sample amplified using the Chordata primers. With both primers, electrophoresis examination of the PCR products showed the presence of significant amounts of primer dimers in the reactions.

One the same set of samples we then used the Qiagen® DNA Stool kit to extract DNA followed by amplification in a PCR reaction that excluded additional MgCl. The samples in which we used used the Malacostraca primers did not amplify successfully, however the success rate of the samples amplified using the Chordata primers increased from one in ten (isopropanol extraction), to four in ten (Qiagen® DNA Stool kit extraction). A significant reduction in the presence of primer dimers in the electrophoresis gel was observed. The 16S1F/16S2R primers, while successful in amplifying fish DNA from a test sample of fish tissue, were not effective when used on the faecal samples, giving no positive results.

## Discussion

While the optimal method to use for DNA extraction may vary with the oil content of each individual sample, in this study the use of the Qiagen® DNA Stool kit improved the PCR amplification success rate from 10% to 40%. This suggests this approach is the better option for use with faecal samples collected from the White Fronted Terns,

An attempt was made to improve amplification success by trialling new PCR primers and modifying the protocol from what work had been done previously. While the use of the new 16S1F/16SR primers failed to produce the desired result, the removal of additional MgCl in the reaction did eliminate the presence of primer dimers, which would be expected to led to cleaner results were the samples to be sequenced. It should be noted, that the GoTaq® PCR Master Mix used in the PCR contains MgCl, thus the change made represented a reduction in total MgCl, not the complete removal of it from the reaction.

The successful amplification of 4 of 10 samples using the Chordata primers, but noneusing the Malacostraca primers, is in line with expectations of the largely fish based diet of White Fronted Terns Tern (Mills 2013).

## References


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