Clarity - Bioinformatics Market Place
DIPLOMICS
108 Albert Rd
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Analytical Study Plan

Date	14-09-2023	**Client**	Dr Carel J Oostehuizen
Project ID	Wild_dog_Microbiome		Senior Lecturer
Quote No.	MKP025	**Address**	Room 2-48.1 Zoology Building
Version	001		Department of Zoology and Entomology
Author	Etienne De Villiers		Faculty of Natural and Agricultural Sciences
	Patricia Swart		University of Pretoria, Hatfield, South Africa

Data

- N = 2
- The 12S and 16S mtDNA genes were amplified for each sample and then mixed in equal concentrations for the sequencing reaction according to Wang et al., 2022.
- Data were received from the sequencing facility in the form of .bam and .fastq files.

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Determining the prey species of wild dog using a metabarcoding approach

Project scope and objectives

Aim

The client wishes to identify species consumed by wild dogs by isolating DNA from wild dog scat and selectively PCR amplifying the mitochondrial 12S and 16S genes from these samples and sequencing on an IonTorrent sequencer (Central Analytics Facility, Stellenbosch). The amplicon sequences are then compared to a database of known 12S and 16S mtDNA sequences and the origin of the DNA deduced.

Two test samples will be used to establish the workflow and provide proof of concept. If successful, an additional 12 samples will be sequenced. The client will then use the analytical report provided upon completion of this project to conduct the analysis of the 12 samples themselves.

Methodology

A crucial step in the bioinformatic analysis of these amplicon sequences is the identification of representative sequences. This can be achieved using a clustering approach or by denoising raw sequencing reads. DADA2 (Callahan et al., 2016) is a widely adopted algorithm, released as an R library, that denoises marker-specific amplicons from next-generation sequencing and produces a set of representative sequences referred to as Amplicon Sequence Variants (ASV).

ASV sequences are compared to a reference database to verify the taxonomic composition using the “Blastn” function of the program Blast+ (Camacho et al., 2009) for the 10 best hits and an e- value of < 0.001. ASVs blasted > 98% similarity will be considered the matched species.

Outputs

- List of species identified by DADA2
- A detailed analytical report with documented code to aid the client in conducting their own future analysis.

References

- Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High- Resolution Sample Inference from Illumina Amplicon Data. Nat. Methods 2016, 13, 581–583.
- C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, T.L. Madden. BLAST+: architecture and applications. BMC Bioinformatics, 10 (2009), p. 421, 10.1186/1471-2105-10-421

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Client deliverables and responsibilities

The client is responsible for providing all the data [described above] generated by the project, the experimental design, as well as the results of analyses performed so far. Client retains ownership of the data.

CLARITY deliverables and responsibilities

1. The CLARITY team will endeavor to provide **timely help and advice** to the client throughout the duration of the project.
2. At times requested by the client, CLARITY will be responsible for delivering reports summarizing its findings. These reports will include:
    a. A statement of the scientific questions that are being asked, and a summary of the answers provided by the team's analysis (**Executive Summary**)
    b. A description of the **input data**, including any QC issues that may have been uncovered during the analysis.
    c. A description of the **analytical procedures** used by the team, including software packages and versions, and a justification of their choice of methodology.
    d. A description of the **computer hardware** or cloud environment on which the analyses were performed, and a short summary of its performance.
    e. All the **computer code** produced by the team, the code will be well commented and documented, ensuring that the entire analysis can be performed again with identical results.
    f. **Figures and tables** to illustrate the results obtained by the team.

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Milestones and Timeline

Milestone achievements will be communicated to the client.

Milestone	Timeline
• Query submission • Consultation meeting	• 1 week to schedule 30-min consultation between client and CLARITY.
• Analytical study plan • Pro-forma invoice • Terms of service • Project acceptance form	• 1 week for CLARITY to send project documentation to client. • 15 days for client to accept that project
• Create MS Teams channel for project communications. • Data transfer to consultant • Data analysis • Results report uploaded to MS Teams channel*	• Project dependent
• Results discussion	• Within 1 week from when the results report is uploaded to MS Teams channel
• Additional questions • Issue of final tax invoice • Project feedback • Project sign-off	• Within 1 week of results discussion
• Payment	• 30 days for client to pay CPGR
• Deletion of raw data • Client follow-up for reporting/tracking purposes	• Raw data will be deleted from CLARITY storage 3-months after project sign-off. • Follow up is client dependent.

** Results will not be released until a purchase order and/or payment has been received*

This project will be automatically signed off 1 week after the last component is delivered. Please note that you will be charged for any consultations after a project is signed off.

The project will be run in accordance with this analytical study plan and the standard terms and conditions of DIPLOMICS. Any deviations will be noted and reported in a timely fashion. DIPLOMICS will present an analytical report at the end of the project.

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Data transfer, usage, and access

The client is responsible for providing all the data generated by the project, the experimental design, as well as the results of analyses performed so far. The transfer of the data from the client to bioinformatician is for the purpose of obtaining bioinformatic services only. The data will be analysed by the bioinformatician as described above and a full analytical report will be returned to the client. The data will not be shared and will be handled in compliance with all applicable laws and regulations and in accordance with best practices. The raw data received from the client will be removed from secure CLARITY storage after a period of 3-months from the date of project sign-off.

Reporting

In order to improve our services, market our services, measure impact and to provide feedback to our funders we would like to request your permission to contact you in the future. This may be in the form of an email or survey. We are interested in outcomes such as degrees obtained, skills learnt, conferences attended, manuscripts published, and future work or funding received based on your results emanating from our bioinformatic services. In addition, where possible, please acknowledge CLARITY and DIPLOMICS in the above mentioned research outcomes [*This project received bioinformatics support through CLARITY, a bioinformatics service made possible by DIPLOMICS (DIstributed PLatform in OMICS), a research infrastructure programme funded by the Department of Science and Innovation through its South African Research Infrastructure Road Map Programme)*].

Your contact for this project:

Dr Patricia Swart (DIPLOMICS Bioinformatics Coordinator)
patricia.swart@diplomics.org.za
072 739 8748 (WhatsApp)

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Project and Quote Acceptance

Kindly complete all the sections below and return to Dr Patricia Swart (DIPLOMICS Bioinformatics Coordinator) patricia.swart@diplomics.org.za

By signing below you attest to having (tick the relevant boxes):

- ☐ Read and understood the requirements of the project plan and agree to the experimental design
- ☐ Accepted the quote / proforma invoice
- ☐ Agreed to the project turn-around times as stipulated in the analytical study plan
- ☐ Consent to be contacted in the future for reporting purposes

Ethics approval:

- Does the project require ethics approval? ☐ Yes ☐ No
- If yes, please provide your ethics approval number and expiry date: _____

Client details:

Title, name and surname:
Institution/affiliation:
Email address:

Date and signature:

_____ _____
Date *Signature*

Details of person responsible for payment (if different from above):

Title, name and surname:
Institution/affiliation:
Email address:

Date and signature:

_____ _____
Date *Signature*