**UNIVERSITY OF CAPE TOWN COMPUTATIONAL BIOLOGY DIVISION**

**Bioinformatics Support Request**

Please provide us with more information on your request for support. Complete the form as comprehensively as possible, and please indicate where there is still uncertainty.

**Please note, the earlier we are involved the better – for example, it would be better for us to be involved during the study design and even grant application stage**.

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| **CONTACT DETAILS** |
| Date of request | **19/02/2019** |
| Name | **Pieter J de Waal** |
| Email address | **pieterdewaal@telkomsa.net** |
| Research Group/Department | **Department Allergology, RXH, Cape Town** |
| Faculty | **Pediatrics and Child Health, UCT** |
| IF student, name & email of supervisor | **Prof Mike Levin.** **Email: michael.levin@uct.ac.za** |

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| **PROJECT DETAILS** |
| 1. What is the scientific question? |
| Identification and description of the microbiomic biodiversity found in traditionally fermented milk products versus fresh, unpasteurized cow’s milk from rural South Africa. Three (3) milk samples will be collected from farms in rural Eastern Cape: unpasteurized cow’s milk, traditionally homemade fermented milk and commercially bought fermented milk. The three sample types will be compared with each other.Do the three sample types differ from each other?How can this knowledge be translated into the Clinical Allergology? |
| 2. Who are the partners on the project? |
| Prof Shane Murray (CPGR, Cape Town), responsible for 16SrRNA extraction an production of a taxonomic report.  |
| 3. What type of collaboration with CBIO is expected? For a project that is done as collaboration or for a fee, we will put the agreement in writing. |
| Project towards an MPhil study (Allergology) |
| 4. Are there any ethical issues we should be aware of? |
| NO. All ethical issues have been thoroughly dealt with by the Animal Ethics Committee and Department of Agriculture (Eastern Cape). |
| 5. How much work is expected from CBIO and when? |
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| 6. What type of data will be generated (e.g. sequencing, genotyping, expression, etc.) and what technology platform will be used? |
| The Illumina® 16S metagenomics workflow will be used to analyze the three differently sourced milk types. Each sample will be analyzed in duplicate, producing a total number of six extracted libraries. A positive control will also be included in the analyses. The hypervariable V3 and V4 regions of the 16S ribosomal RNA gene (16S rRNA) will be amplified, using an Illumina® pre-designed primer pair.  Barcoding for multiplexing of the samples will entail the use of the Nextera XT®-Index Kit.The Illumina® software will analyze the sequencing reads and produce a taxonomic report of each sample, which will include calculating and reporting of the total reads of organisms, as well as the identification of total reads of the top Kingdom, Phylum, Class, Order, Family, Genus and in some cases, the Species. |
| 7. When do you expect the data? Does it need to be transferred from somewhere else? |
| Data collection will take place from 01/03/2019 to 03/03/2019. The samples will be transported to CPGR on Monday, 04/03/2019. Results of the data analysis will be available within two weeks from then. |
| 8. How large will the data be? How long does it need to stored for, and have you made arrangements for storage?  |
| This will dependent on the report generated from CPGR. All the information will be stored on a flash disk, obtained from Prof Shane Murray, after analysis took place. |
| 9. What bioinformatics analysis needs to be done? Which tools are required? |
| Alpha and beta diversity (Shannon diversity (H′) and Bray-Curtis dissimilarity index). Calculation of the relative abundance of each OTU per specimen. Fisher’s exact test for two-way tables. Construction of Log ratio biplots, dendograms and other illustrative (graphical) representation of data analysis. |
| 10. If a collaborative model is being used, what papers are envisaged and who will the authors be? |
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**PLEASE FORWARD THE COMPLETED FORM TO:**

Nicola.mulder@uct.ac.za