Differences in software performance for analyses of

High Throughput Ion Torrent DNA barcode data

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# Introduction

In recent years, Traditional Chinese Medicines (TCMs) have become increasingly popular in western countries. These TCMs make use of endangered species of plants or animals and may contain potentially dangerous components. This is a serious danger to the continued existence of certain plants and animals in the wild. In order to better protect these organisms and to prevent harm to humans, it is necessary to identify which species and components are present in TCMs. Since TCMs contain a mix of species they are currently analyzed with DNA barcoding using a high‐throughput technique: Ion Torrent sequencing. This saves a lot of time and money.

Careful information of the DNA barcoding regions present in these medicines is necessary.

However, without good software, it might prove very hard to make correct observations of the DNA barcodes found in TCM samples. To do this, three different packages were compared: GENEIOUS, CLC BIO and Life Technologies Ion Torrent software(Torrent Suite). This was done by comparing a dataset from a single TCM sample on all three packages, so that decent assumptions could be made about each package's unique set of features and methods in relation to their function of properly identifying (illegally‐used) plant species.  
 Important functions compared included the way data was imported, the workload necessary to gain proper results, how much options there were in modifying BLAST settings and how accurate the results were. Other important factors evaluated were user friendliness of the program and visual representation of the results. A program with clear, color‐coded results ranked higher than a program that only displayed names in a single list of names and scores. Additionally, this project included the creation of a pipeline, the steps of which were initially be decided upon via an ad‐hoc basis. Following review of these steps, a streamlined version was decided upon and created.

# Research questions

The following questions were formulated.

## Problem 1

Why do different software packages give different BLAST results?

## Hypothesis 1

Different programs use different settings, perform more or less accurate BLASTs, trim the reads differently or not at all, and/or do not use the same reference databases.

## Problem 2

What would be the added value of a newly designed pipeline next to already existing software?

## Hypothesis 2

The newly designed pipeline would be for free (licenses for other software are expensive), offers more flexibility for data handling, and/or produces more accurate results.

# Goals

This project had several goals. Because this is a educational project most of the goals were about educating ourselves and gaining new skills.

* Writing reports in English.
* Gain experience in a 20 weeks project.
* Examining software.
* Learn how to recommend software based on own research.
* Programming a pipeline.
* Learning to work with Microsoft Word.
* Communication with teachers and clients.
* Project management.
* Learning to write a Plan of Action showing insight in assignment.

# Products

At the end of the project the following products were handed over:

* Preliminary report consisting of our findings concerning the examined software packages and an advice on the program that performed the best during the testing.
* A pipeline that can automatically trim the Ion Torrent dataset, BLAST (P) the trimmed dataset, reliably identify organisms in the dataset and show the results in a clear and simple format.
* Manual of the pipeline, explaining how to use the program and containing further information (e.g. formats, options etc).
* Final report, this will give a detailed explanation about the methods, findings of the project and the code of the pipeline program (well documented for further use).

Furthermore, several presentations were given. For school two presentations were held and a third (final) presentation was held for Naturalis.

# Project boundaries

For the first part of the project the main focus lied on discovering why three software-packages produced different results while the input remains the same.  
 The second part of the project consisted of building a pipeline to make analyzing the data generated by the sequencer easier and more user friendly (only display less complicated options, fixed parameters, etc).  
 It was important not to stray too far from these set tasks, if such a thing occurred we planned an additional meeting to discuss this problem. The duration of the project according to the modulewijzer for each member was 224 hours distributed over a time period of 20 weeks.

# Quality

In order to maintain a high quality report the following points were monitored:

* Correct grammar.
* Substantive level of information.
* Style.
* Lay-out.
* Correct image subscripts.
* Relevance.
* The source of the information.

The final report was read by all members prior to submission and checked again for any mistakes.

# Project organization

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# Project agreements

The role of secretary was exchanged weekly between Roeben and the others. The presidency was fixed and thus was not exchanged by the members. During the meetings, the progress of the overall planning was reviewed and modified if necessary. Any irregularities and/or problems of the previous week were discussed. The action points of the meeting were mailed within 24 hours by the secretary to the project members and supervisors.  
 The project supervisor and clients were available via email and personal contact for questions and/or uncertainties. Each week there was a meeting with the project supervisor as scheduled on the school planning. The clients were kept informed via a weekly email containing results and progress made. Emails were replied as fast as possible. For this project Github was used. All members of the project group had access to the Github folder used for the project. Everyone put data, script components and parts of the Plan of Action and report in the shared Github folder when done. Every time a member made changes to a document a version number was added to the document name. Each member had his own part to work on the script so comments in the script were added containing the date and time and the changes made/work done. A new document was made containing the version number and the changes made in that version.   
 In the occasion that any member was not able to join the weekly meetings he was expected to inform the other members of his absence as soon as possible. Both the module supervisor and clients received a copy of the final products by e-mail.

# Planning

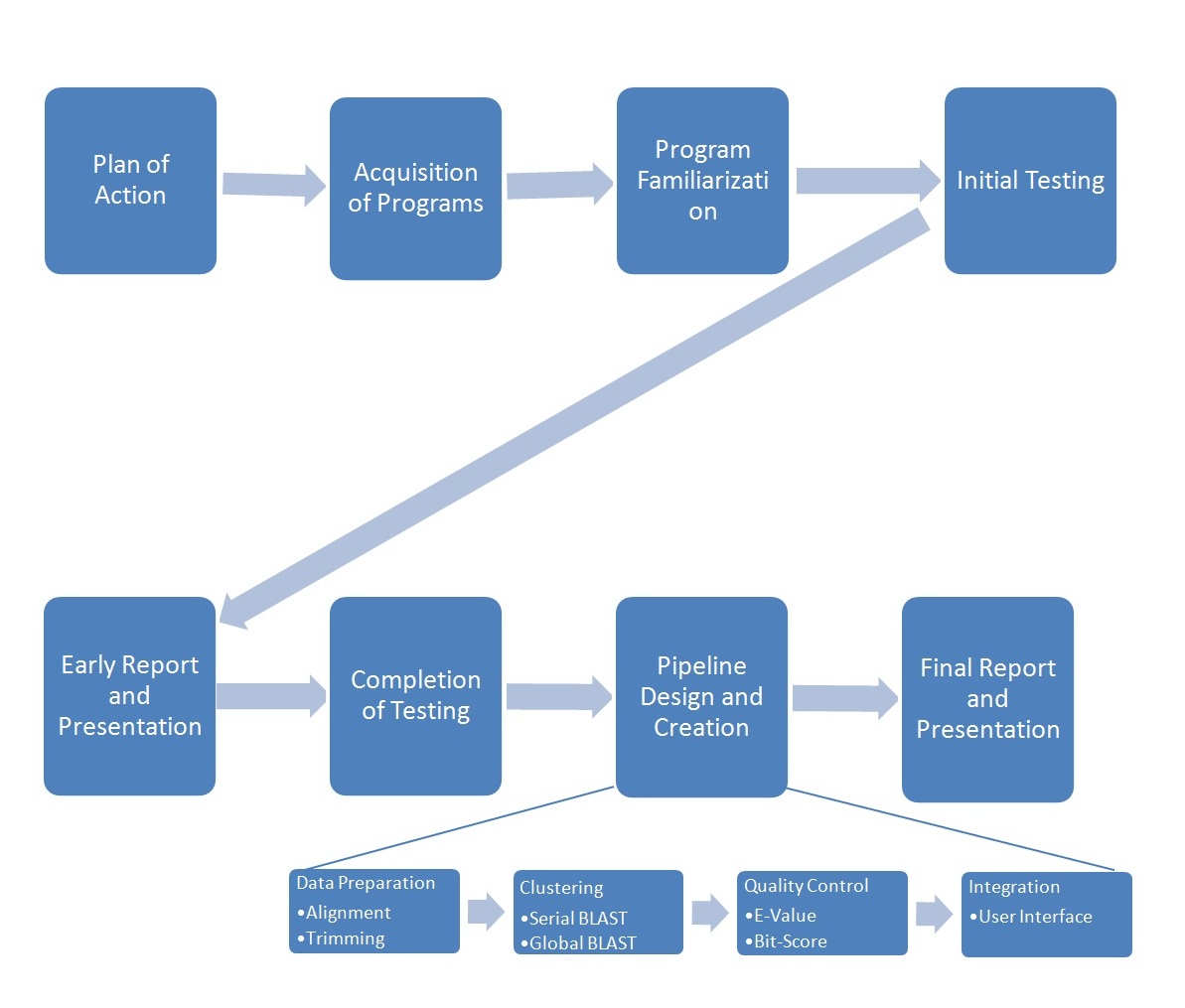
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **What** | **Person** | **Who** | **Start** | **End** | **Deliverables** |
| Plan of action | Alex | Alex,Roeben,Thomas | 24-9-2012 | 1-10-2012 | Plan of Action .docx/.pdf |
| Software Testing (data input, time, number of actions) | Roeben |  | 28-9-2012 | 9-10-2012 | Put data on Github |
| Software Testing (output, settings, validity) | Thomas |  | 9-10-2012 | 17-10-2012 | Put results on Github |
| Software Testing with incense data | Alex |  | 17-10-2012 | 30-10-2012 | Put results on Github |
| CLC BIO |  | Roeben |  |  |  |
| GENEIOUS |  | Thomas |  |  |  |
| Torrent Suite |  | Alex |  |  |  |
| Preliminary report | Thomas | Alex,Roeben,Thomas | 2-11-2012 | 9-11-2012 | Report |
| Presentation 1 | All | Alex,Roeben,Thomas | 22-11-2012 | 29-11-2012 | PowerPoint |
| Pipeline Script (research) | Thomas | Alex,Roeben,Thomas | 31-10-2012 | 7-11-2012 | Put challenge on Github |
| Pipeline Script (programming) | Roeben | Alex,Roeben,Thomas | 7-11-2012 | 27-12-2012 | Put scripts on Github |
| Pipeline Script (validation) | Alex | Alex,Roeben,Thomas | 18-12-2012 | 11-1-2013 | Script, validation report |
| Presentation 2 | All | Alex,Roeben,Thomas | 16-1-2013 | 21-1-2013 | PowerPoint |
| Final report | Alex | Alex,Roeben,Thomas | 14-1-2013 | 28-1-2013 | Report |

Table 1: Planning.

Incense data: Naturalis provided us with an Ion Torrent data set acquired from incense with fully known ingredients provided by the retailer. In this way, we could compare performances of different software packages to validate the results obtained.

## Software Testing Flowchart

## Project Flowchart



# Risk analysis

Main probable causes of project failure included the unavailability of licenses or the sudden incorporation of a large update halfway through the project, which would in theory require a complete redo of that part of the research process.  
 Smaller concerns included hardware failure (e.g. some computers do not have enough memory for some of the tasks of the software) and other mechanical failures. Personal injury during the project might also have resulted in loss of knowledge and results.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Risk | Description | Chance of appearance | Impact | Chance of discovery | Risk factor |
| 1 | Inadequate programming experience | 3 | 2 | 1 | 6 |
| 2 | Problems due to hardware failure | 3 | 3 | 1 | 9 |
| 3 | Bad communication/communication error | 3 | 3 | 2 | 18 |
| 4 | Not following up on agreements | 2 | 3 | 2 | 12 |
| 5 | Missing deadline | 3 | 5 | 4 | 60 |
| 6 | Project members quitting the project | 2 | 4 | 2 | 16 |
| 7 | Low quality products | 2 | 4 | 2 | 16 |

Table 2: Risk analysis.

For all risk factors 1 is low and 5 is high. Three factors were taken into account: chance of appearance , impact and chance of discovery. The risk factor was calculated by multiplying the three individual values . Anything under 10 constitutes a minor risk. A score between 11 and 29 is considered a risk that warrants small preventive measures. Scores above 30 are serious risks that need to be contained as much as possible (see Appendix 1).

# Materials and Methods

We compared three software packages with a first IonTorrent dataset provided by Naturalis by

running a default analysis and scoring the identifications obtained.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name Program** | **Developer** | **Version** | **Platform** |
| CLC BIO Workbench | CLC BIO. | 6.7.1 | Linux/Windows |
| Ion Torrent suite | Life Technologies. |  | Linux |
| GENEIOUS | Biomatters Ltd. | 6.0.3 | Windows |

We compared user friendliness, program functionality, integration with online applications and databases and output provided in terms of E-value and similarity.

## About Ion Torrent

The Torrent suite does not have options or functions to trim or BLAST sequences. There are plug-ins available yet none of them will add the ability to trim. Torrent suite has a different focus than the other programs and requires a different level of user skill. Its main purpose is to process the data an Ion Torrent cycle delivers. Its main features include searching for gene variations and assembly.

The software itself is at face value simple to use but can be a bit tricky at times. It comes as a virtual machine which needs to be set-up before it can be used. While someone with experience in Linux and virtual machines in general will find it easy to do, it can be daunting for someone with no experience.

## About GENEIOUS

Installing GENEIOUS is a comforting experience that does not require much interaction on the part of the user. It is easy to work with and the menu is usually clean and simple. Tutorials are readily available and are one of the first things one sees when they boot up the program. These tutorials are partitioned in segments with exercises with varying difficulty, ranging from a Beginner tutorial to ones useful to any Advanced user. It can be concluded is not hard to get used to working with GENEIOUS as long as one follows these tutorials. They are available online as well.

## About CLC Genomics Workbench

CLC Genomics Workbench, hereafter referred to as CLC BIO, is extremely easy to use and definitely seems to be designed by a team of professionals. It breezes through its tasks, and equally positive statements can be made about its stability.

Unlike GENEIOUS. CLC BIO does not grant you a tutorial when one first starts it. However, the argument can be made that CLC BIO is essentially the non-freeware version of GENEIOUS, as the two programs are strikingly similar down to the interface and options menus. If one is familiar with the one, they can nearly be guaranteed to be somewhat familiar with the other.

When using BLASTn on CLC BIO for the first time, the default settings will be equal to the ones you find on the NCBI website, with one important difference. The score for a mismatch is -3 instead of -2. This might cause differentiating results.

## About the pipeline program CITES Checker

This project included designing a pipeline that will try to provide an enhanced, customized solution for the type of problem that is generally encountered when analyzing Ion Torrent data from TCMs, i.e. finding different results when using different commercially available software, paying a lot of money for a license and still not knowing if CITES species are present in a dataset without doing additional analyses. By creating the pipeline ourselves we could eliminate the need to pay for a 10,000 dollar license and allow an untrained user to make use of the same functions and gain accurate results similar to those found in commercially available programs.

Specifically, this pipeline was built to be able to automatically trim Ion Torrent dataset(s), BLAST these and then reliably identify organisms in the dataset and identify these as CITES species. The results are shown in a clear, concise and simple format. The user gives the program the location of a fastq file. After specifying all desired parameters, the sequences are trimmed, and run through the BLAST database. After that, a quality check is done that filters hits above 97% coverage and 95% identity.

# Results

Below the presence/absence of the three packages compared is provided for different aspects.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CLC BIO** | **GENEIOUS** | **Torrent Suite** |
|  |  |  |  |
| In-Program download function | + | + | - |
| In-Program sequence searcher | + | + | + |
| De Novo Assembly | + | + | +- |
| Epigenomics | + | + | - |
| Transcriptomics | + | + | +- |
| Resquencing analysis | + | + | + |
| Tracking Tools | + | + | - |
| NGS Core Tools | + | + | - |
| BLAST | + | + | - |
| Molecular Biology tools | + | + | - |
| Trim Primers | + | + | - |
| Trim on Phred quality | + | + | - |
| Classical Sequence | + | + | + |
| Aligning to reference genome | + | + | + |
| Output in FASTQ | + | + | + |
| Output in SAM/BAM | + | + | + |

Table 3: Comparison of functionality between CLC BIO, GENEIOUS and Torrent Suite. Plus (+) means the function is present in the software, minus (-) means it is not present and plus minus (+-) means it is not present by default but can be activated by way of plug in.

For a fair comparison we first looked up the default settings for each program .

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **CLC BIO** | **GENEIOUS** | **Torrent Suite** | **NCBI BLAST** |
| Match score | 1 | 1 | N/A | 1 |
| Mismatch score | -3 | -2 | N/A | -2 |
| Read size | 11 | 28 | N/A | 28 |
| Gap existence | 5 | Linear | N/A | Linear |
| Gap extension | 2 | Linear | N/A | Linear |
| Filter low complexity | + | + | N/A | + |
| Algorithm | BLASTn | megaBLAST | N/A | megaBLAST |
| Database | NCBI Nucleotide Collection(nr) | NCBI Nucleotide Collection(nr) | N/A | NCBI Nucleotide Collection(nr) |

Table 4: Comparison of the default setting of the programs used for BLAST. Note that for Torrent Suite only N/A was scored because it lacks the ability to use BLAST.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided. Note that we are keeping to CLC BIO’s settings.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Description** | **Total score** | **Query coverage** | **E value** | **Maximum identity** | **Accession** |
| 1 | *Zymoseptoria passerinii* | 121 | 26% | 3,00E-24 | 98% | JF700879 |
| 2 | [Fungal sp.](http://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_209981600) | 97.6 | 35% | 4,00E-17 | 100% | FJ235950 |
| 3 | [*Trichosporon asahii*](http://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_402694686) | 392 | 100% | 5,00E-106 | 100% | JX174411 |
| 4 | [*Emericella similis*](http://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_184161573) | 133 | 83% | 7,00E-28 | 99% | EU448279 |
| 5 | [*Aquilaria sinensis*](http://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_238696002) | 369 | 100% | 7,00E-99 | 100% | FJ980392 |

Table 5: Selection of the first 5 contigs on which a BLAST search was performed. The search was done with the BLAST implementation on the NCBI website using our default values. For all results see Appendix 2.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with CLC BIO using the same settings as CLC BIO.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Description** | **Total score** | **Query coverage** | **E-value** | **Max identity** | **Accession** |
| 1 | *Zymoseptoria passerinii* | 121,42 | 26,97 | 2.66E-24 | 98 | JF700879 |
| 2 | Fungal sp. | 97,63 | 33,81 | 3.86E-17 | 100 | FJ235950 |
| 3 | *Trichosporon asahii* | 393 | 100 | 4.69E-106 | 100 | JX174411 |
| 4 | *Emericella similis* | 133,31 | 83,53 | 7.00E-28 | 99 | EU448279 |
| 5 | *Aquilaria sinensis* | 369,21 | 100 | 6.79E-99 | 100 | FJ980392 |

Table 6: Selection of the first 5 contigs on which a BLAST search was performed. The search was done with the BLAST implementation of CLC BIO using our default values. For all results see Appendix 2.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with GENEIOUS using the same settings as CLC BIO.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Accession** | **Description** | **Sequence Length** | **Query Coverage** | **E-value** | **Total Score** | **Pairwise**  **identity** |
| 1 | AB260871 | *Litsea glutinosa* | 204 | 83.47% | 1.35e-88 | 3,35E+02 | 97.5% |
| 2 | AY695067 | *Dactylella lysipaga* | 262 | 99.61% | 6.52e-118 | 4,32E+02 | 97.3% |
| 3 | AY970100 | Uncultured ascomycete isolate | 196 | 99.49% | 3.86e-89 | 3,37E+02 | 98.5% |
| 4 | DQ467995 | *Aspergillus nomius* | 85 | 100.00% | 8.52e-28 | 1,33E+02 | 95.3% |
| 5 | EF567419 | *Penicilliopsis clavariiformis* | 78 | 42.08% | 1.44e-18 | 1,02E+02 | 89.7% |

Table 7: Selection of the first 5 contigs on which a BLAST search was performed. The search was done with the BLAST implementation of GENEIOUS using our default values. For all results see Appendix 2.

To be absolutely sure the programs returned the same hits, we decided that using more extreme settings would be a good test of accuracy. However, outside of the basics settings we used, it proved difficult to find “extreme” settings that all three programs could handle. Ultimately we decided to modify the NCBI website to CLC BIO’s and GENEIOUS’ settings separately. This was decided after discovering that GENEIOUS did not always match NCBI BLAST settings.

We did a second comparison to NCBI BLAST with GENEIOUS. We performed a global BLAST on all 33 contigs in the Ion Torrent sample with both programs, then did it again with the settings equaling GENEIOUS. It is to be noted that GENEIOUS and NCBI BLAST do not support equal extreme settings. We have instead taken the closest possible values for the second test.

|  |  |  |  |
| --- | --- | --- | --- |
| Settings test 1 | **CLC BIO** | **BLAST** | **GENEIOUS** |
| Word length | 11 | 11 | - |
| Gap cost | 5, 5 | 5, 5 | - |
| Match/Mismatch | 4, -5 | 4, 5 | - |
| Settings test 2 | **CLC BIO** | **BLAST** | **GENEIOUS** |
| Word length | - | 11 | 11 |
| Gap cost | - | 4, 5 | 4, 4 |
| Match/Mismatch | - | 4, -5 | 4, -5 |

Table 8: Table explaining the two comparisons done between NCBI BLAST and CLC BIO and GENEIOUS (test 1). A dash means that modifying these settings in this software made the comparison between the different packages too inaccurate.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with CLC BIO using the same settings as NCBI BLAST.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Length of consensus sequence** | **Accession** | **Description** | **Total score** | **Query coverage** | **E-value** | **Max identity** |
| 1 | 157 | FJ761676 | Uncultured fungus clone Singleton | 136,55 | 70,12 | 5.91E-29 | 81 |
| 2 | 56 | FJ235950 | Fungal sp. | 82,02 | 41,73 | 1.54E-12 | 93 |
| 3 | 198 | JX174411 | *Trichosporon asahii* | 311,06 | 100 | 1.73E-81 | 100 |
| 4 | 85 | JN798502 | *Aspergillus niger* | 118,64 | 98,82 | 1.46E-23 | 96 |
| 5 | 186 | FJ980392 | *Aquilaria sinensis* | 292,36 | 100 | 7.37E-76 | 100 |

Table 9: Results after adjusting default settings of CLC BIO to a more extreme value.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with NCBI BLAST using the same settings as CLC BIO.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Total score** | **Accession** | **Description** | **Query coverage** | **E-value** | **Max identity** |
| 1 | 136 | FJ761676 | Uncultured fungus clone singleton | 68% | 6,00E-29 | 81% |
| 2 | 82.0 | FJ235950 | Fungal sp. | 43% | 2,00E-12 | 93% |
| 3 | 311 | JX174411 | *Trichosporon asahii* | 100% | 2,00E-81 | 100% |
| 4 | 118 | JQ963339 | *Aspergillus niger* | 97% | 1,00E-23 | 96% |
| 5 | 292 | FJ980392 | *Aquilaria sinensis* | 100% | 7,00E-76 | 100% |

Table 10: Results of NCBI BLAST after adjusting default settings of NCBI BLAST to the settings of CLC BIO. The similarity is notable.

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with GENEIOUS using the same settings as NCBI BLAST.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Read** | **Accession** | **Description** | **Identity** | **E-value** | **Bit Score** | **Query Coverage** |
| 1 | AB260871 | *Litsea glutinosa* | 97.5% | 7.76e-89 | 335.726 | 83.47% |
| 2 | AY695067 | *Dactylella lysipaga* | 97.7% | 2.72e-117 | 430.251 | 99.61% |
| 3 | AY970100 | Uncultured ascomycete isolate | 98.5% | 6.25e-90 | 339.362 | 99.49% |
| 4 | DQ467995 | *Aspergillus nomius* | 96.5% | 1.51e-27 | 132.133 | 100.00% |
| 5 | EF567419 | *Penicilliopsis clavariiformis* | 89.7% | 3.02e-18 | 101.23 | 42.08% |

Table 11: Results of GENEIOUS after adjusting default settings to extremer settings (test 2).

We then performed a global BLAST on all 33 contigs in the Ion Torrent sample provided with NCBI BLAST using the same settings as GENEIOUS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Contig** | **Description** | **Total score** | **Query coverage** | **E-value** | **Max identity** |
| 1 | Uncultured fungus clone singleton | 131 | 68% | 3,00E-27 | 81% |
| 2 | Fungal sp. | 76.9 | 43% | 5,00E-11 | 93% |
| 3 | *Trichosporon asahii* | 288 | 100% | 7,00E-75 | 100% |
| 4 | *Aspergillus versicolor* | 111 | 97% | 1,00E-21 | 96% |
| 5 | *Aquilaria sinensis* | 271 | 100% | 1,00E-69 | 100% |

Table 12: Results of NCBI BLAST after adjusting default settings as closely as possible to the settings of GENEIOUS. It is notable that the results revert back to the results found in the CLC BIO comparison but with slightly different scores.

Lastly we compared the Phred scores of the different programs.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CLC BIO** | **GENEIOUS** | **Torrent Suite** |
| Quality | 0.05 | 0.05 | N/A |

Table 11: Phred scores used as trimming settings.

Finally, we ran the sample through our own CITES Checker pipeline.

…

Table 12. Results obtained with CITES Checker pipeline.

# Discussion

## Settings

Several differences were found by us between the different software packages compared.

First of all not all programs supported the same features. CLC BIO and GENEIOUS were much more comprehensive as compared with Torrent Suite.

Secondly, we found CLC BIO to be deviant in default settings, leading to a significantly different result with the global BLASTs. We experimentally tested this by adjusting the default settings of CLC BIO with those of GENEIOUS, which resulted in similar, but not exactly similar results.

Thirdly, with Torrent Suite one cannot trim reads or access the internet.

## Accuracy

Interesting to note is that the results obtained with GENEIOUS differed not because the output was different but because of the way the output was listed. It is tough to sort the results in a way similar to NCBI and CLC BIO. Sometimes the results contained multiple organisms with exactly the same scores. These were then listed alphabetically on NCBI BLAST and CLC BIO but sorted on total score in GENEIOUS. Because a secondary sorting on alphabetical order could not be done, shuffling made the results obtained look different.

## Completeness

We were already aware, from information given by the producer, that *Litsea glutinosa* was included in the test set on contig 1. The only program that consistently found this organism was GENEIOUS. This suggests that GENEIOUS is the most suited to this kind of research. It also seems to be the case that GENEIOUS uses a substantially different method of BLAST compared to CLC BIO and NCBI BLAST (Which both use the exact same system).

# Conclusions

## Problem 1

Why do different software packages give different results?

## Hypothesis 1

Different programs use different settings, perform more or less accurate BLASTs, trim the reads differently or not at all, and/or do not use the same reference databases.

## Answer 1

The reason why different software gave different results is because the settings for BLAST in CLC BIO and GENEIOUS are different. Ion Torrent does not give results at all since it does not have a functionality capable of performing BLASTs and cannot trim. The programs that are capable of this however, use the same databases but with different default settings for the global BLASTs.

## Problem 2

What is the added value of a newly designed pipeline next to already existing software?

## Hypothesis 2

The newly designed pipeline is for free (licenses for other software are expensive), offers more flexibility for data handling, and produces more accurate results.

## Answer 2

The reason why ….

# References