# 8th of December - 4th meeting Debaryomyces hansenii

## Important points of previous meeting:

- Try kraken with custom database done
- ITS regions started
- SNP calling not done yet
- Know assemblers done
- Things of previous meetings that were left behind for now:
- Annotation using Maker
- BLAST of the assemblies

## New results and information:

Everything located on GitHub:

https://github.com/The-Bioinformatics-Group/Debaryomyces\_hansenii

## Kraken customDB study:

Assemblies:

https://github.com/The-Bioinformatics-

 $Group/Debaryomyces\_hansenii/tree/master/Work\_files/mahesha\_assemblies\_work folder/contamination\_check$ 

#### Raw reads:

https://github.com/The-Bioinformatics-

Group/Debaryomyces\_hansenii/tree/master/Work\_files/rawdata\_workfolder/contamination\_check

Tables with results included in this file.

Also resume of the results.

<u>ITS</u> sequences: Found in almost all strains, better conditions depending of the assembler used.

Resume at the end of this document.

## **Information about strains**

Alternative names for strains and its origin:

| Strain | Alternative strain names   | Origin   | Year |
|--------|--|--|------|
| 1001   | NCYC2572, CBS767,<br>ATCC36239, CCRC21394,<br>DBVPG6050, IFO0083,<br>JCM1990, JCM2102,<br>KCTC7645, MUCL30242, | Carlsberg laboratories, habitat associated with fermentation | 1994 |

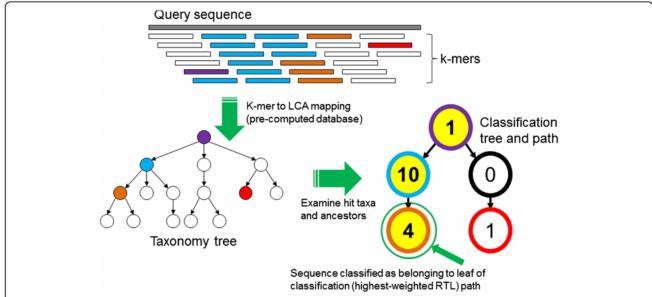
|      | NRRLY-7426, NRRLY-10976,<br>UCD74-86              |  |      |
|------|---|--|------|
| 1002 | NCYC8, NCTC2059                                   | Throat of pacient with angina                                | 1925 |
| 1003 | NCYC9, NCTC2048                                   | Dutch cheese prepared in Russia                              | 1924 |
| 1004 | NCYC10, NCTC2056                                  | Unknown  | 1925 |
| 1005 | NCYC103, NCTC1681                                 | Carlsberg laboratories, habitat associated with fermentation | 1923 |
| 1006 | NCYC459   | Soil, New Zealand  | 1955 |
| 1007 | NCYC475, CBS811, JCM1439,<br>NRRLY-1454, UCD75-11 | Fermenting Kentucky tobacco                                  | 1956 |
| 1008 | NCYC792, NCMB1230 43                              | Sea Water  | 1974 |
| 1009 | NCYC3045  | Dried salted black olives from Thassos, Greece               | 2002 |
| 1010 | NCYC3364  | Capping machine of soft drinks factory in Brazil             | 2006 |
| 1011 | CBS117  | Rennet from New Zealand                                      | 1985 |
| 1012 | CBS5140   | Skin of a man, Hungary                                       |      |
| 1013 | CBS1101, IFO0027, IFO0093                         | Salt pork  | 1946 |
| 1014 | CBS1792   | Chilled beef from Brisbane, Australia                        |      |
| 1015 | J63   | Seawater, Sweden   |      |
| 1016 | J26   | Seawater, Sweden   |      |
| 1017 | J16   | -  | -    |
| 1018 | J52   | -  | -    |
| 1019 | DBH9  | CBS767 carrying a mutation in DhHIS4 gene                    |      |

### Kraken

Kraken is an ultrafast and highly accurate program for assigning taxonomic labels to metagenomics DNA sequences. Previous programs designed for this task have been relatively slow and computationally expensive, forcing researchers to use faster abundance estimation programs, which only classify small subsets of metagenomic data. Using exact alignment of k-mers, Kraken achieves classification accuracy comparable to the fastest BLAST program. In its fastest mode, Kraken classifies 100 base pair reads at a rate of over 4.1 million reads per minute, 909 times faster than Megablast and 11 times faster than the abundance estimation program MetaPhlAn.

#### k-mer to lowest common ancestor database

At the core of Kraken is a database that contains records consisting of a k-mer and the LCA of all organisms whose genomes contain that k-mer. This database, built using a user-specified library of genomes, allows a quick lookup of the most specific node in the taxonomic tree that is associated with a given k-mer. Sequences are classified by querying the database for each k-mer in a sequence, and then using the resulting set of LCA taxa to determine an appropriate label for the sequence (Figure 1). Sequences that have no kmers in the database are left unclassified by Kraken. By default, Kraken builds the database with k=31, but this value is user-modifiable.



**Figure 1** The Kraken sequence classification algorithm. To classify a sequence, each *k*-mer in the sequence is mapped to the lowest common ancestor (LCA) of the genomes that contain that *k*-mer in a database. The taxa associated with the sequence's *k*-mers, as well as the taxa's ancestors, form a pruned subtree of the general taxonomy tree, which is used for classification. In the classification tree, each node has a weight equal to the number of *k*-mers in the sequence associated with the node's taxon. Each root-to-leaf (RTL) path in the classification tree is scored by adding all weights in the path, and the maximal RTL path in the classification tree is the classification path (nodes highlighted in yellow). The leaf of this classification path (the orange, leftmost leaf in the classification tree) is the classification used for the query sequence.

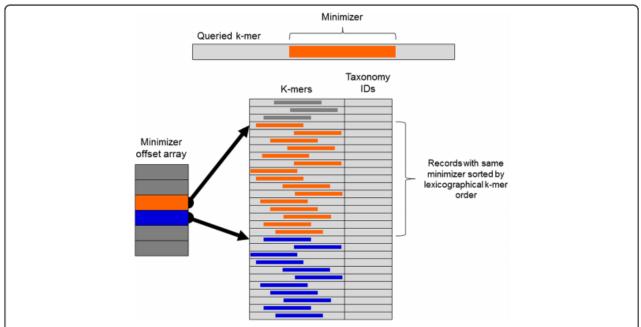
## Database structure and search algorithm

Because Kraken very frequently uses a k-mer as a database query immediately after querying an adjacent kmer, and because adjacent k-mers share a substantial amount of sequence, we utilize the minimizer concept to group similar k-mers together. To explain our application of this concept, we here define the canonical representation of a DNA sequence S as the lexicographically smaller of S and the reverse complement of S. To determine a k-mer's minimizer of length M, we consider the canonical representation of all M-mers in the k-mer, and select the lexicographically smallest of those M-mers as the k-mer's minimizer. In practice, adjacent k-mers will often have the same minimizer.

In Kraken's database, all k-mers with the same minimizer are stored consecutively, and are sorted in lexicographical order of their canonical representations. A query for a k-mer R can then be

processed by looking up in an index the positions in the database where the k-mers with R's minimizer would be stored, and then performing a binary search within that region (Figure 5). Because adjacent k-mers often have the same minimizer, the search range is often the same between two consecutive queries, and the search in the first query can often bring data into the CPU cache that will be used in the second query. By allowing memory accesses in subsequent queries to access data in the CPU cache instead of RAM, this strategy makes subsequent queries much faster than they would otherwise be.

The index containing the offsets of each group of k-mers in the database requires  $8 \times 4M$  bytes. By default Kraken uses 15-bp minimizers, but the user can modify this value; for example, in creating MiniKraken, we used 13-bp minimizers to ensure the total database size stayed under 4 GB.



**Figure 5 Kraken database structure.** Each *k*-mer to be queried against the database has a specific substring that is its minimizer. To search for a *k*-mer in the database, the positions in the database that contain *k*-mers with the same minimizer are examined. These positions are quickly found by examining the minimizer offset array for the start positions of records with the *k*-mer's minimizer (orange) and the next possible minimizer (blue). Within a range of records associated with a given minimizer, records are sorted by lexicographical ordering of their *k*-mers, allowing a query to be completed by using a binary search over this range.

#### Raw reads results

- ❖ Classified sequences: Found in the kraken custom database, sequences matching bacterial, archaeal, plasmids, viral, human or fungi domains.
- ♦ **Unclassified sequences:** Not found in the custom database.

| fastq     | Total sequences | Classified sequences | Unclassified sequences |
|-----------|-----------------|----------------------|------------------------|
| 1001 AH 1 | 2462393         | 2029147 (82.41%)     | 433246 (17.59%)        |
| 1001 AH 2 | 2462393         | 2000937 (81.26%)     | 461456 (18.74%)        |
| 1001 BC 1 | 3480996         | 2858026 (82.10%)     | 622970 (17.90%)        |
| 1001 BC 2 | 3480996         | 2832253 (81.36%)     | 648743 (18.64%)        |
| 1002 AH 1 | 1160378         | 1140993 (98.33%)     | 19385 (1.67%)          |
| 1002 AH 2 | 1160378         | 1134927 (97.81%)     | 25451 (2.19%)          |
| 1002 BC 1 | 1853630         | 1822492 (98.32%)     | 31138 (1.68%)          |
| 1002 BC 2 | 1853630         | 1805406 (97.40%)     | 48224 (2.60%)          |
| 1003 AH 1 | 2193546         | 1817189 (82.84%)     | 376357 (17.16%)        |
| 1003 AH 2 | 2193546         | 1776305 (80.98%)     | 417241 (19.02%)        |
| 1003 BC 1 | 3349842         | 2766520 (82.59%)     | 583322 (17.41%)        |
| 1003 BC 2 | 3349842         | 2709763 (80.89%)     | 640079 (19.11%)        |

| 1004 ATT 1              | 1550601            | 1422265 (01 060/)                    | 125226 (0.040/)                 |
|-------------------------|--------------------|--------------------------------------|---------------------------------|
| 1004 AH 1               | 1558691            | 1433365 (91.96%)                     | 125326 (8.04%)                  |
| 1004 AH 2               | 1558691            | 1374655 (88.19%)                     | 184036 (11.81%)                 |
| 1004 BC 1               | 2449087            | 2248776 (91.82%)                     | 200311 (8.18%)                  |
| 1004 BC 2               | 2449087            | 2189404 (89.40%)                     | 259683 (10.60%)                 |
| 1005 AH 1               | 2088288            | 2057955 (98.55%)                     | 30333 (1.45%)                   |
| 1005 AH 2               | 2088288            | 2027995 (97.11%)                     | 60293 (2.89%)                   |
| 1005 BC 1               | 2922955            | 2879153 (98.50%)                     | 43802 (1.50%)                   |
| 1005 BC 2               | 2922955            | 2835228 (97.00%)                     | 87727 (3.00%)                   |
| 1006 AH 1               | 1929277            | 335761 (17.40%)                      | 1593516 (82.60%)                |
| 1006 AH 2               | 1929277            | 324501 (16.82%)                      | 1604776 (83.18%)                |
| 1006 BC 1               | 2847843            | 492928 (17.31%)                      | 2354915 (82.69%)                |
| 1006 BC 2<br>1007 AH 1  | 2847843            | 479112 (16.82%)                      | 2368731 (83.18%)                |
| 1007 AH 1<br>1007 AH 2  | 2305984            | 2267226 (98.32%)                     | 38758 (1.68%)                   |
| 1007 AH 2<br>1007 BC 1  | 2305984<br>3473851 | 2239638 (97.12%)                     | 66346 (2.88%)                   |
| 1007 BC 1<br>1007 BC 2  |                    | 3413498 (98.26%)                     | 60353 (1.74%)                   |
| 1007 BC 2<br>1008 AH 1  | 3473851<br>1312616 | 3372019 (97.07%)                     | 101832 (2.93%)<br>31237 (2.38%) |
| 1008 AH 1<br>1008 AH 2  | 1312616            | 1281379 (97.62%)<br>1268340 (96.63%) | 44276 (3.37%)                   |
| 1008 BC 1               | 1940489            | 1893091 (97.56%)                     | 47398 (2.44%)                   |
| 1008 BC 1<br>1008 BC 2  | 1940489            | 1873108 (96.53%)                     | 67381 (3.47%)                   |
| 1008 BC 2<br>1009 AH 1  | 1944249            | 1419587 (73.01%)                     | 524662 (26.99%)                 |
| 1009 AH 1<br>1009 AH 2  | 1944249            | 1394897 (71.74%)                     | 549352 (28.26%)                 |
| 1009 ATI 2<br>1009 BC 1 | 2894462            | 2107051 (72.80%)                     | 787411 (27.20%)                 |
| 1009 BC 1<br>1009 BC 2  | 2894462            | 2077020 (71.76%)                     | 817442 (28.24%)                 |
| 1009 BC 2<br>1010 AH 1  | 1319601            | 189904 (14.39%)                      | 1129697 (85.61%)                |
| 1010 AH 2               | 1319601            | 185313 (14.04%)                      | 1134288 (85.96%)                |
| 1010 AH 2               | 1909206            | 270625 (14.17%)                      | 1638581 (85.83%)                |
| 1010 BC 1               | 1909206            | 264627 (13.86%)                      | 1644579 (86.14%)                |
| 1010 BC 2               | 768699             | 450133 (58.56%)                      | 318566 (41.44%)                 |
| 1011 AH 2               | 768699             | 446288 (58.06%)                      | 322411 (41.94%)                 |
| 1011 BC 1               | 1178421            | 687776 (58.36%)                      | 490645 (41.64%)                 |
| 1011 BC 2               | 1178421            | 680994 (57.79%)                      | 497427 (42.21%)                 |
| 1012 AH 1               | 2073205            | 359992 (17.36%)                      | 1713213 (82.64%)                |
| 1012 AH 2               | 2073205            | 350775 (16.92%)                      | 1722430 (83.08%)                |
| 1012 BC 1               | 3224847            | 556618 (17.26%)                      | 2668229 (82.74%)                |
| 1012 BC 2               | 3224847            | 544512 (16.88%)                      | 2680335 (83.12%)                |
| 1013 AH 1               | 1828482            | 1765439 (96.55%)                     | 63043 (3.45%)                   |
| 1013 AH 2               | 1828482            | 1740777 (95.20%)                     | 87705 (4.80%)                   |
| 1013 BC 1               | 2736996            | 2640904 (96.49%)                     | 96092 (3.51%)                   |
| 1013 BC 2               | 2736996            | 2604369 (95.15%)                     | 132627 (4.85%)                  |
| 1014 AH 1               | 2206902            | 1806751 (81.87%)                     | 400151 (18.13%)                 |
| 1014 AH 2               | 2206902            | 1780380 (80.67%)                     | 426522 (19.33%)                 |
| 1014 BC 1               | 3528456            | 2882136 (81.68%)                     | 646320 (18.32%)                 |
| 1014 BC 2               | 3528456            | 2837996 (80.43%)                     | 690460 (19.57%)                 |
| 1015 AH 1               | 1923464            | 1859416 (96.67%)                     | 64048 (3.33%)                   |
| 1015 AH 2               | 1923464            | 1844644 (95.90%)                     | 78820 (4.10%)                   |
| 1015 BC 1               | 3181425            | 3074019 (96.62%)                     | 107406 (3.38%)                  |
| 1015 BC 2               | 3181425            | 3044236 (95.69%)                     | 137189 (4.31%)                  |
| 1016 AH 1               | 2646260            | 2151919 (81.32%)                     | 494341 (18.68%)                 |
| 1016 AH 2               | 2646260            | 2117272 (80.01%)                     | 528988 (19.99%)                 |
| 1016 BC 1               | 3919163            | 3177692 (81.08%)                     | 741471 (18.92%)                 |
| 1016 BC 2               | 3919163            | 3130716 (79.88%)                     | 788447 (20.12%)                 |

| 1017 AH 1 | 1889165 | 1853424 (98.11%) | 35741 (1.89%)  |
|-----------|---------|------------------|----------------|
| 1017 AH 2 | 1889165 | 1828433 (96.79%) | 60732 (3.21%)  |
| 1017 BC 1 | 2738771 | 2685950 (98.07%) | 52821 (1.93%)  |
| 1017 BC 2 | 2738771 | 2659430 (97.10%) | 79341 (2.90%)  |
| 1018 AH 1 | 2628541 | 2580100 (98.16%) | 48441 (1.84%)  |
| 1018 AH 2 | 2628541 | 2552444 (97.10%) | 76097 (2.90%)  |
| 1018 BC 1 | 3662396 | 3593609 (98.12%) | 68787 (1.88%)  |
| 1018 BC 2 | 3662396 | 3555962 (97.09%) | 106434 (2.91%) |
| 1019 AH 1 | 2000344 | 1964810 (98.22%) | 35534 (1.78%)  |
| 1019 AH 2 | 2000344 | 1915216 (95.74%) | 85128 (4.26%)  |
| 1019 BC 1 | 2664496 | 2615956 (98.18%) | 48540 (1.82%)  |
| 1019 BC 2 | 2664496 | 2576230 (96.69%) | 88266 (3.31%)  |

## Table resume of the reports

| Strain       | Fungi  | Bacteria | Viruses | Archaea | Others | Unclassif ied | Deha   | Sc    | Huma<br>n |
|--------------|--------|----------|---------|---------|--------|---------------|--------|-------|-----------|
| 1001<br>AH 1 | 82.38% | 0.01%    | 0.00%   | 0.00%   | 0.01%  | 17.59%        | 78.50% | 0.01% | 0.01%     |
| 1001<br>AH 2 | 81.22% | 0.01%    | 0.00%   | 0.00%   | 0.01%  | 18.74%        | 77.38% | 0.01% | 0.01%     |
| 1001<br>BC 1 | 82.08% | 0.01%    | 0.00%   | 0.00%   | 0.01%  | 17.90%        | 78.25% | 0.01% | 0.01%     |
| 1001<br>BC 2 | 81.33% | 0.01%    | 0.00%   | 0.00%   | 0.02%  | 18.64%        | 77.53% | 0.01% | 0.02%     |
| 1002<br>AH 1 | 98.31% | 0.01%    | -       | -       | 0.01%  | 1.97%         | 93.71% | 0.01% | 0.01%     |
| 1002<br>AH 2 | 97.78% | 0.00%    | -       | -       | 0.01%  | 2.19%         | 93.20% | 0.01% | 0.01%     |
| 1002<br>BC 1 | 98.30% | 0.01%    | -       | -       | 0.01%  | 1.68%         | 93.71% | 0.01% | 0.01%     |
| 1002<br>BC 2 | 97.37% | 0.00%    | -       | -       | 0.02%  | 2.60%         | 92.82% | 0.01% | 0.02%     |
| 1003<br>AH 1 | 82.81% | 0.02%    | 0.00%   | 0.00%   | 0.01%  | 17.16%        | 78.91% | 0.00% | 0.01%     |
| 1003<br>AH 2 | 80.93% | 0.02%    | 0.00%   | 0.00%   | 0.01%  | 19.02%        | 77.08% | 0.01% | 0.01%     |
| 1003<br>BC 1 | 82.55% | 0.02%    | 0.00%   | 0.00%   | 0.01%  | 17.41%        | 78.71% | 0.00% | 0.01      |
| 1003<br>BC 2 | 80.84% | 0.02%    | 0.00%   | 0.00%   | 0.02%  | 19.11%        | 77.05% | 0.01% | 0.02%     |
| 1004<br>AH 1 | 91.91% | 0.04%    | 0.00%   | 0.00%   | 0.00%  | 8.04%         | 89.12% | 0.00% | 0.00%     |
| 1004<br>AH 2 | 88.13% | 0.04%    | 0.00%   | 0.00%   | 0.01%  | 11.81%        | 85.43% | 0.00% | 0.01%     |
| 1004<br>BC 1 | 91.77% | 0.05%    | 0.00%   | 0.00%   | 0.01%  | 8.18%         | 89.03% | 0.00% | 0.01%     |
| 1004<br>BC 2 | 89.33% | 0.04%    | 0.00%   | 0.00%   | 0.02%  | 10.60%        | 86.66% | 0.00% | 0.02%     |
| 1005<br>AH 1 | 98.48% | 0.06%    | 0.00%   | -       | 0.00%  | 1.45%         | 93.36% | 0.00% | 0.00%     |
| 1005         | 97.04% | 0.06%    | 0.00%   | -       | 0.01%  | 2.89%         | 91.97% | 0.00% | 0.01%     |

| AH 2<br>1005<br>BC 1 | 98.43% | 0.06% | 0.00% | -     | 0.00% | 1.50%  | 93.46% | 0.00% | 0.00% |
|----------------------|--------|-------|-------|-------|-------|--------|--------|-------|-------|
| 1005<br>BC 2         | 96.92% | 0.06% | 0.00% | -     | 0.01% | 3.00%  | 92.09% | 0.00% | 0.01% |
| 1006<br>AH 1         | 17.32% | 0.05% | 0.00% | -     | 0.02% | 82.60% | 12.74% | 0.02% | 0.02% |
| 1006<br>AH 2         | 16.73% | 0.04% | 0.00% | -     | 0.03% | 83.18% | 12.21% | 0.02% | 0.03% |
| 1006<br>BC 1         | 17.22% | 0.05% | 0.00% | -     | 0.02% | 82.69% | 12.69% | 0.02% | 0.02% |
| 1006<br>BC 2         | 16.73% | 0.04% | 0.00% | -     | 0.03% | 83.18% | 12.30% | 0.02% | 0.03% |
| 1007<br>AH 1         | 98.29% | 0.02% | 0.00% | -     | 0.00% | 1.68%  | 91.13% | 0.00% | 0.00% |
| 1007<br>AH 2         | 97.08% | 0.02% | 0.00% | 0.00% | 0.01% | 2.88%  | 89.97% | 0.00% | 0.01% |
| 1007<br>BC 1         | 98.23% | 0.02% | 0.00% | 0.00% | 0.00% | 1.74%  | 91.22% | 0.00% | 0.00% |
| 1007<br>BC 2         | 97.03% | 0.02% | 0.00% | 0.00% | 0.01% | 2.93%  | 90.12% | 0.00% | 0.01% |
| 1008<br>AH 1         | 97.60% | 0.01% | -     | -     | 0.01% | 2.38%  | 91.44% | 0.00% | 0.01% |
| 1008<br>AH 2         | 96.60% | 0.00% | 0.00% | 0.00% | 0.01% | 3.37%  | 90.49% | 0.00% | 0.01% |
| 1008<br>BC 1         | 97.54% | 0.01% | -     | 0.00% | 0.01% | 2.44%  | 91.52% | 0.00% | 0.01% |
| 1008<br>BC 2         | 96.50% | 0.00% | -     | -     | 0.02% | 3.47%  | 90.59% | 0.00% | 0.02% |
| 1009<br>AH 1         | 72.97% | 0.01% | 0.00% | 0.00% | 0.02% | 26.99% | 69.45% | 0.01% | 0.02% |
| 1009<br>AH 2         | 71.69% | 0.01% | 0.00% | 0.00% | 0.03% | 28.26% | 68.21% | 0.01% | 0.03% |
| 1009<br>BC 1         | 72.75% | 0.02% | 0.00% | 0.00% | 0.02% | 27.20% | 69.22% | 0.01% | 0.02% |
| 1009<br>BC 2         | 71.70% | 0.01% | 0.00% | 0.00% | 0.03% | 28.24% | 68.23% | 0.01% | 0.03% |
| 1010<br>AH 1         | 14.33% | 0.03% | 0.00% | 0.00% | 0.02% | 85.61% | 9.20%  | 0.03% | 0.02% |
| 1010<br>AH 2         | 13.97% | 0.02% | 0.00% | 0.00% | 0.02% | 85.96% | 8.94%  | 0.03% | 0.02% |
| 1010<br>BC 1         | 14.12% | 0.03% | 0.00% | 0.00% | 0.02% | 85.83% | 9.15%  | 0.03% | 0.02% |
| 1010<br>BC 2         | 13.79% | 0.02% | 0.00% | 0.00% | 0.03% | 86.14% | 8.91%  | 0.02% | 0.03% |
| 1011<br>AH 1         | 58.50% | 0.02% | 0.00% | 0.00% | 0.02% | 41.44% | 53.28% | 0.01% | 0.02% |
| 1011<br>AH 2         | 58.00% | 0.01% | 0.00% | 0.00% | 0.03% | 41.94% | 52.80% | 0.02% | 0.03% |
| 1011<br>BC 1         | 58.30% | 0.02% | 0.00% | 0.00% | 0.03% | 41.64% | 53.10% | 0.02% | 0.03% |
| 1011                 | 57.72% | 0.01% | 0.00% | 0.00% | 0.04% | 42.21% | 52.57% | 0.02% | 0.04% |

| DC 4                 |        |       |       |       |       |        |        |        |       |
|----------------------|--------|-------|-------|-------|-------|--------|--------|--------|-------|
| BC 2<br>1012<br>AH 1 | 17.31% | 0.02% | 0.00% | -     | 0.02% | 82.64% | 12.17% | 0.02%  | 0.02% |
| 1012<br>AH 2         | 16.85% | 0.02% | 0.00% | 0.00% | 0.02% | 83.08% | 11.82% | 0.02%  | 0.02% |
| 1012<br>BC 1         | 17.20% | 0.02% | 0.00% | -     | 0.02% | 82.74% | 12.13% | 0.02%  | 0.02% |
| 1012<br>BC 2         | 16.82% | 0.02% | 0.00% | 0.00% | 0.03% | 83.12% | 11.83% | 0.02%  | 0.03% |
| 1013<br>AH 1         | 96.50% | 0.04% | 0.00% | 0.00% | 0.00% | 3.45%  | 90.88% | 0.00%  | 0.00% |
| 1013<br>AH 2         | 95.14% | 0.04% | 0.00% | 0.00% | 0.01% | 4.80%  | 89.58% | 0.00%  | 0.01% |
| 1013<br>BC 1         | 96.44% | 0.05% | 0.00% | 0.00% | 0.00% | 3.51%  | 90.90% | 0.00%  | 0.00% |
| 1013<br>BC 2         | 95.09% | 0.04% | 0.00% | 0.00% | 0.01% | 4.85%  | 89.65% | 0.00%  | 0.01% |
| 1014<br>AH 1         | 81.84% | 0.01% | 0.00% | 0.00% | 0.01% | 18.13% | 78.14% | 0.00%  | 0.01% |
| 1014<br>AH 2         | 80.64% | 0.01% | 0.00% | -     | 0.01% | 19.33% | 76.97% | 0.00%  | 0.01% |
| 1014<br>BC 1         | 81.65% | 0.01% | 0.00% | 0.00% | 0.01% | 18.32% | 77.98% | 0.00%  | 0.01% |
| 1014<br>BC 2         | 80.39% | 0.01% | 0.00% | -     | 0.02% | 19.57% | 76.75% | 0.00%  | 0.02% |
| 1015<br>AH 1         | 96.65% | 0.01% | -     | -     | 0.01% | 3.33%  | 90.17% | 0.00%  | 0.01% |
| 1015<br>AH 2         | 95.87% | 0.01% | 0.00% | 0.00% | 0.02% | 4.10%  | 89.43% | 0.00%  | 0.02% |
| 1015<br>BC 1         | 96.60% | 0.01% | 0.00% | 0.00% | 0.01% | 3.38%  | 90.32% | 0.00%  | 0.01% |
| 1015<br>BC 2         | 95.65% | 0.01% | 0.00% | 0.00% | 0.02% | 4.31%  | 89.48% | 0.00%  | 0.02% |
| 1016<br>AH 1         | 81.29% | 0.01% | 0.00% | 0.00% | 0.01% | 18.68% | 76.99% | 0.01%  | 0.01% |
| 1016<br>AH 2         | 79.98% | 0.01% | 0.00% | -     | 0.01% | 19.99% | 75.72% | 0.00%  | 0.01% |
| 1016<br>BC 1         | 81.05% | 0.01% | 0.00% | 0.00% | 0.01% | 18.92% | 76.80% | 0.01%  | 0.01% |
| 1016<br>BC 2         | 79.84% | 0.01% | 0.00% | 0.00% | 0.02% | 20.12% | 75.64% | 0.01%  | 0.02% |
| 1017<br>AH 1         | 98.04% | 0.03% | 0.00% | -     | 0.02% | 1.89%  | 0.05%  | 93.14% | 0.02% |
| 1017<br>AH 2         | 96.71% | 0.03% | 0.00% | 0.00% | 0.03% | 3.21%  | 0.04%  | 91.83% | 0.03% |
| 1017<br>BC 1         | 97.99% | 0.05% | 0.00% | -     | 0.02% | 1.93%  | 0.05%  | 93.03% | 0.02% |
| 1017<br>BC 2         | 97.02% | 0.02% | 0.00% | 0.00% | 0.04% | 2.90%  | 0.04%  | 92.09% | 0.04% |
| 1018<br>AH 1         | 98.12% | 0.01% | -     | -     | 0.01% | 1.84%  | 0.04%  | 94.68% | 0.01% |
| 1018                 | 97.06% | 0.01% | 0.00% | -     | 0.02% | 2.90%  | 0.03%  | 93.62% | 0.02% |

| <b>AH 2</b> |        |       |       |       |       |       |        |        |       |
|-------------|--------|-------|-------|-------|-------|-------|--------|--------|-------|
| 1018        | 98.08% | 0.01% | 0.00% | -     | 0.02% | 1.88% | 0.04%  | 94.65% | 0.02% |
| <b>BC</b> 1 |        |       |       |       |       |       |        |        |       |
| 1018        | 97.04% | 0.01% | 0.00% | -     | 0.03% | 2.91% | 0.03%  | 93.61% | 0.03% |
| BC 2        |        |       |       |       |       |       |        |        |       |
| 1019        | 98.19% | 0.03% | 0.00% | -     | 0.00% | 1.78% | 95.54% | 0.00%  | 0.00% |
| AH 1        |        |       |       |       |       |       |        |        |       |
| 1019        | 95.70% | 0.03% | 0.00% | 0.00% | 0.01% | 4.26% | 93.10% | 0.00%  | 0.01% |
| <b>AH 2</b> |        |       |       |       |       |       |        |        |       |
| 1019        | 98.14% | 0.04% | 0.00% | 0.00% | 0.00% | 1.82% | 95.51% | 0.00%  | 0.00% |
| <b>BC</b> 1 |        |       |       |       |       |       |        |        |       |
| 1019        | 96.64% | 0.03% | 0.00% | -     | 0.01% | 3.31% | 94.04% | 0.00%  | 0.01% |
| <b>BC 2</b> |        |       |       |       |       |       |        |        |       |

1001 CBS767 reference

1006, 1010 and 1012 Probably not Debaryomyces hansenii

1009, 1011 Genome ~double size

1017, 1018 Saccharomyces cerevisiae

## Mahesh assemblies results

- ❖ Classified sequences: Found in the kraken custom database, sequences matching bacterial, archaeal, plasmids, viral, human or fungi domains.
- ♦ **Unclassified sequences:** Not found in the custom database.

| fasta | Total     | Classified sequences | Unclassified |
|-------|-----------|----------------------|--------------|
|       | sequences |                      | sequence     |
| 1001  | 840       | 684 (81.43%)         | 156 (18.57%) |
| 1002  | 804       | 735 (91.42%)         | 69 (8.58%)   |
| 1003  | 714       | 517 (72.41%)         | 197 (27.59%) |
| 1004  | 15474     | 14975 (96.78%)       | 499 (3.22%)  |
| 1005  | 2070      | 1090 (52.66%)        | 980 (47.34%) |
| 1006  | 593       | 218 (36.76%)         | 375 (63.24%) |
| 1007  | 1421      | 1162 (81.77%)        | 259 (18.23%) |
| 1008  | 1024      | 929 (90.72%)         | 95 (9.28%)   |
| 1009  | 1029      | 994 (96.60%)         | 35 (3.40%)   |
| 1010  | 396       | 297 (75.00%)         | 99 (25.00%)  |
| 1011  | 5889      | 5641 (95.79%)        | 248 (4.21%)  |
| 1012  | 454       | 200 (44.05%)         | 254 (55.95%) |
| 1013  | 1483      | 996 (67.16%)         | 487 (32.84%) |
| 1014  | 906       | 670 (73.95%)         | 236 (26.05%) |
| 1015  | 1140      | 1029 (90.26%)        | 111 (9.74%)  |
| 1016  | 723       | 545 (75.38%)         | 178 (24.62%) |
| 1017  | 1008      | 998 (99.01%)         | 10 (0.99%)   |
| 1018  | 1326      | 1298 (97.89%)        | 28 (2.11%)   |
| 1019  | 1023      | 715 (69.89%)         | 308 (30.11%) |

## Table resume of the reports

| Strain | Fungi  | Bacteria | Viruses | Archaea | Others | Unclassified | Deha   | Sc |
|--------|--------|----------|---------|---------|--------|--------------|--------|----|
| 1001   | 80.36% | 0.48%    | -       | -       | -      | 18.57%       | 74.29% | -  |
| 1002   | 89.18% | 0.25%    | -       | -       | 0.37%  | 8.58%        | 81.59% | -  |

| 1003 | 70.17% | 1.26% | 0.14% | -     | -     | 27.59% | 64.85% | -      |
|------|--------|-------|-------|-------|-------|--------|--------|--------|
| 1004 | 96.37% | 0.25% | 0.03% | -     | 0.01% | 3.22%  | 95.83% | -      |
| 1005 | 45.51% | 6.14% | 0.43% | -     | 0.05% | 47.34% | 41.59% | -      |
| 1006 | 30.19% | 4.22% | 0.51% | -     | -     | 63.24% | 25.80% | -      |
| 1007 | 79.24% | 1.69% | -     | -     | 0.21% | 18.23% | 73.61% | -      |
| 1008 | 88.96% | 0.39% | -     | -     | 0.10% | 9.28%  | 79.20% | -      |
| 1009 | 96.60% | -     | -     | -     | -     | 3.40%  | 95.43% | -      |
| 1010 | 73.48% | 0.51% | -     | -     | 0.76% | 25.00% | 66.41% | -      |
| 1011 | 95.72% | -     | -     | 0.02% | 0.07% | 4.19%  | 95.19% | 00.02% |
| 1012 | 40.53% | 0.88% | -     | -     | 0.22% | 55.95% | 33.48% | 00.22% |
| 1013 | 61.90% | 3.78% | 0.40% | -     | 0.07% | 32.84% | 55.02% | -      |
| 1014 | 72.41% | 0.33% | -     | -     | 0.11% | 26.05% | 66.67% | -      |
| 1015 | 88.51% | 0.26% | -     | -     | 0.35% | 9.74%  | 77.82% | -      |
| 1016 | 73.44% | 0.69% | -     | -     | 0.28% | 24.62% | 65.15% | -      |
| 1017 | 98.91% | 0.10% | -     | -     | -     | 0.99%  | 00.00% | 98.12% |
| 1018 | 97.89% | -     | -     | -     | -     | 2.11%  | 00.00% | 97.51% |
| 1019 | 65.59% | 3.03% | 0.20% | -     | -     | 30.11% | 61.00% | 00.20% |

1001 CBS767 reference

1006, 1010 and 1012 Probably not Debaryomyces hansenii

1009, 1011 Genome ~double size

1017, 1018 Saccharomyces cerevisiae

Results more resumed explained in the meeting.

## ITS sequences.

I have been able to get the ITS regions of all the strains except from 1018, one of the *Saccharomyces cerevisiae* strains. Another problem that I had is that all of them had the region quite extended except from some strains that casually corresponds to the two different assemblers used: Spades or MaSuRCA. The ones assembled with Spades have better regions normally containing 18s and 28s at the extremes, and the ones with MaSuRCA I was only able to find some really short sequences (1009, 1010, 1011, 1017, 1018).

When trying to get this region on the reference genome CBS767 I discovered that it doesn't exist! So there is no way to align them to a reference. I will try to find it for the other reference genome. I did some alignments that I will show you, but it is quite impossible to make some phylogeny from that to be reliable because of the MaSuRCA assemblies.