Screening for influenza A H5N1 haemagglutinin and neuramidase mutations using the new Alignment & Mutation Analysis tool in the BioNumerics® software

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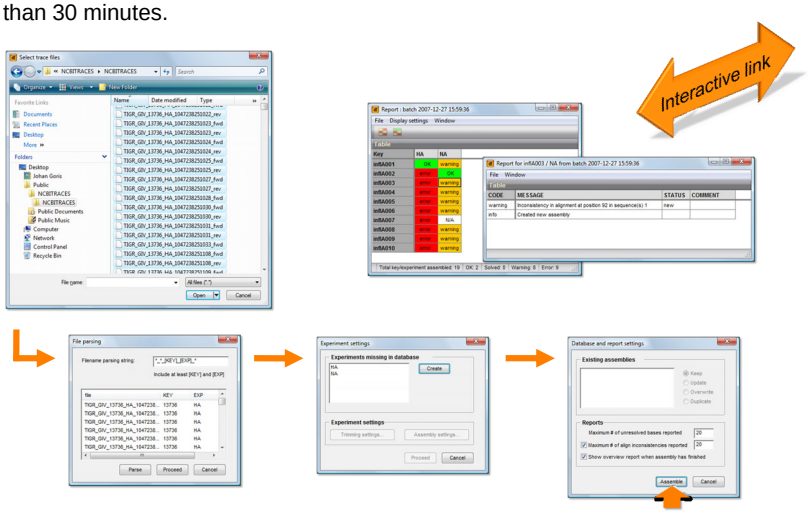
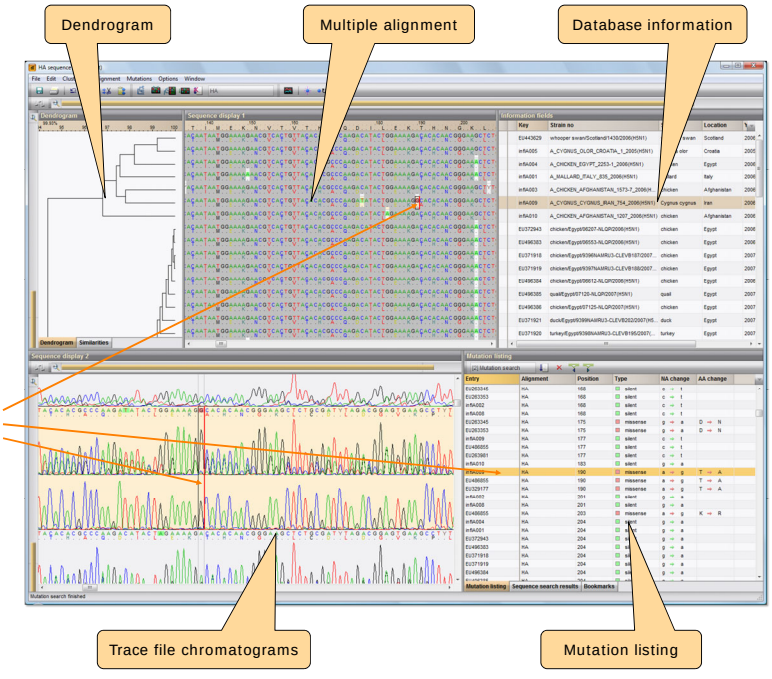
INTRODUCTION: The haemagglutinin (HA) and neuramidase (NA) genes are considered to be responsible for the major part of the antigenic variability of influenza A viruses. Reportedly, specific amino acid changes might even serve as molecular markers for the pandemic potential of avian influenza isolates. We recently developed an Alignment & Mutation Analysis tool as an extension library for the BioNumerics® software. Here, we illustrate the use of this versatile and powerful tool with publicly available sequences and trace files.

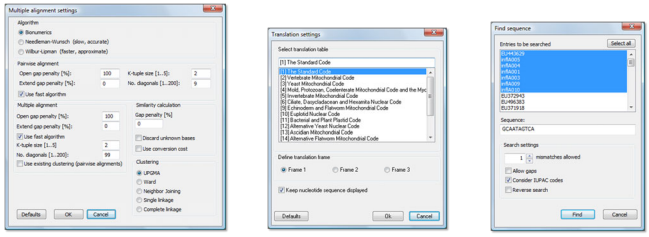
AUTOMATED ASSEMBLY OF TRACE FILES: Using the Batch Sequence Assembly plugin in BioNumerics®, trace files can be assembled automatically into a large number of sequences. Names are parsed from the trace file names or from an external template file. Optimized trimming and assembly settings can be saved for each experiment. Interactive assembly reports facilitate the manual cleanup of the sequences. Using this plugin, we were able to assemble 22,000 influenza A genome sequence trace files from the NCBI Trace Archive (http://0-www.ncbi.nlm.nih.gov.catalog.llu.edu/Traces/trace.cgi) in less than 30 minutes.



IMPORT OF SEQUENCES FROM PUBLIC DATABASES: A convenient import routine in BioNumerics® allows import of GenBank, EMBL or FASTA-formatted sequences. For example, in this study 1,800 HA and 1,500 NA sequences from H5N1 strains were imported from NCBI GenBank and the corresponding strain information was parsed into a relational database in only a few steps.

A VERSATILE ANALYSIS TOOL: In the new Alignment & Mutation Analysis tool, multiple alignments according to Needleman-Wunch, Wilbur-Lipman or BioNumerics® own proprietary algorithm, can be calculated in a single step. For the HA and NA gene sequences, a multiple alignment was created and the nucleic acid sequences translated into amino acids. Mutations were searched relative to a consensus sequence, which made mutation “hot spots” to become immediately apparent. For each mutation, its position, type (silent, missense, indel), nucleotide change and amino acid change was listed. When browsing through the mutation list, the cursor jumps automatically to the corresponding position on the alignment and curves, allowing the researcher to visually evaluate the mutation in its context.





CONCLUSION: The Alignment & Mutation Analysis tool is a powerful new addition to the BioNumerics® software. Overall, performing the complete mutation analysis in a single package offers much added flexibility, in comparison with online analysis tools.

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