Tool for rapid annotation of microbial SNPs (TRAMS): a simple program for rapid annotation of genomic variation in prokaryotes¹

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TRAMS provides rapid and accurate SNP annotation to NGS users with varying computational experience

Next-generation sequencing (NGS) has been widely applied to a variety of microorganisms for evolutionary and epidemiological investigations. Genome wide variation (single nucleotide polymorphism, SNP; multiple nucleotide polymorphism, MNP) from NGS data analyses need functional annotation for their impact on coding sequences. We have developed a program, TRAMS, to annotate large numbers of SNPs between several strains¹.

TRAMS is especially designed for growing number of NGS users with limited computational experience who can use an executable file on WINDOWS computers without needing any installation or command lines to run. TRAMS is available as a Python² script for MacOS and Linux users and also works within Galaxy³, a highly used tool for analysing NGS data. TRAMS accurately annotated >70,000 SNPs between four *Corynebacterium diphtheriae* strains and will be very useful in analysing microbial genomic diversity.

SNPs are extracted from the genome alignment and functionally annotated using a reference genome

Four published *C. diphtheriae* genomes⁴⁻⁷ were aligned (Fig 1a) and SNPs were extracted using Mauve⁸. An input file (Fig 1b)containing the SNP positions, reference alleles and SNP alleles in a tab delimited format was generated using a script, mauve2trams.py, that is also included in the TRAMS package. SNPs were annotated against the reference genome in the GenBank format using TRAMS. WINDOWS users can use the executable version of TRAMS and enter the input file names when prompted (Fig 1c). MacOS and Linux users can download the scripts and run it by a command line (Fig. 1d).

Fig 1: genome alignment (a), extracted SNPs (b) and annotation using TRAMS (c,d)

TRAMS output: Annotation, summary and list of SNPs in overlapping features

Three output files are produced (Fig 2a-c). The annotation file contains the SNP types for each strain, the summary file contains an overview of SNP properties and the overlap file lists the SNPs that are present in overlapping features.

Fig 2: TRAMS output files. Annotation (a), run summary (b), overlapping features (c).

TRAMS can be used in Galaxy workflows

Galaxy is a bioinformatics platform that enables use of a growing number of bioinformatic tools through a single graphic user interface. Workflows can be set up that allow multiple tools to be used consecutively, enabling automated and reproducible data analysis. TRAMS was adapted to work in this environment and is available through the Galaxy toolshed for installation to local galaxy instances.

The figure below shows an example of a workflow that maps Illumina sequence data to a reference genome, extracts SNPs and annotates them using TRAMS.

Fig 3: Galaxy workflow mapping sequencing reads to a reference genome, extracting SNPs and annotating them using TRAMS

Conclusions

- We have developed a program (TRAMS) that:
- Can functionally annotate large numbers of SNPs
- Can be used using at least three platforms (Windows, Linux, MacOS)
- Is easy to use, as it requires no installation (when used on windows) and does not require complex command line arguments
- Annotates SNPs as intergenic, synonymous or nonsynonymous but subdivides nonsynonymous SNPs into nonsense, nonstart or nonstop SNPs
- Is available for Galaxy from the main Galaxy toolshed

Availability

TRAMS can be downloaded as a Python script or Windows executable from <http://sourceforge.net/projects/strathtrams/>. Instructions are included with the program.

The Python script requires Python (ver. 2.7) and the BioPython package² (ver. 1.60) to be installed. Python can be downloaded from www.python.org, Biopython is available from www.biopython.org.

References

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