Software for Automated Somatic Mutation Detection in DNA Sequence Traces

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Introduction

Mutation detection has become increasingly important in the study of cancer. Mutation Surveyor identifies mutations from a physical trace comparison using an anti-correlation algorithm with the results shown in a mutation electropherogram. The software automatically detects DNA variants directly from sequence trace data through comparison of sample to a known reference trace using multiple factors to identify mutations. The anti-correlation method calculates the differences between the reference and sample traces, showing any physical variation in a mutation electropherogram. Traditional base calling software is not capable of detecting the small percentage of somatic mutations within cells. The trace comparison method, however, does not rely on base calling and successfully identifies somatic mutations in cellular levels as low as 20 percent.

Mutation Surveyor is beneficial in the study of cancer, because the increased sensitivity of the software allows detection of somatic mutations often embedded within normal cells. Accuracy of the software in the bi-directional analysis mode is over 99%, with sensitivity to greater than 5% of the primary peak. The anti-correlation method also proves beneficial in the direct comparison of normal to cancer cells for the detection of somatic mutations. Somatic mutations occur at the rate of about 1-2ppm (part per million) in cancer samples[1,2].
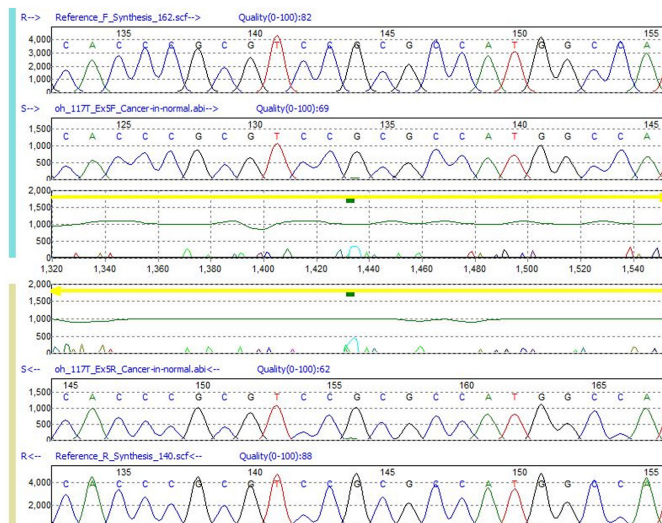
Procedure

Data Entry

1. Add 2 directional sample traces into the third panel titled "Sample Files."
2. Add GenBank sequence into the first panel titled "GenBank Sequence File."

Software Options

1. After loading the data, go to Process: Settings: Display.
2. Check the box titled "Check 2D Small Peaks (Mosaic)." This option enabled the software to display potential somatic mutations which oftentimes have low intensity.

Data Analysis

1. After running the data, potential somatic mutations will be represented by green dots in the mutation electropherogram.
2. If the peak is in fact a true somatic mutation, add the mutation by holding Ctrl and left click at the top of the peak in the mutation electropherogram.

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Results

In the example shown above, a somatic mutation has been detected within the tumor protein p53. The sample traces consisted of cancerous cells within normal patients and the reference used was a synthetic trace of tp53 exon five. The small peak detected at position 167 is in fact a somatic mutation G>GA. The best way to distinguish true somatic mutations from false positives is through examination of the peak shape. In both forward and reverse directions, the peak shape in the sample electropherograms is parabolic, which is characteristic of true mutations.

Discussion

The detection of somatic mutations using Mutation Surveyor requires high quality traces. Confirmation is recommended through other techniques including mutation enrichment by dHPLC (denaturing high pressure liquid chromatography) and TOPO cloning.

Another verification method involves cell culture, which allows selection and amplification of cancer cells. Mutations play an important role in transforming normal cells into cancerous cells. The study of cancer progression therefore requires the study of how point mutations and chromosomal mutations develop in cellular lineages. Mutations that occur in germ cells are present in all cells in resulting offspring; those that occur in any other cells are called somatic mutations and are often less apparent because they affect only portions of the body. Clinically, however, somatic mutations probably contribute to a greater number of medical problems.

Somatic mutations, which occur post-zygotically, produce a genetic mosaic, meaning that the mutation occurs in some but not all cells. They are important because they affect tissues of all ages and seem to cause most of the variation in cancer occurrence. Somatic mutations can be difficult to detect, however, due to their ambiguous nature. Mutation Surveyor is an accurate, user friendly tool which can improve the detection capacity of somatic mutations. Somatic mutation detection is a crucial step in the analysis of human cancers, and Mutation Surveyor is available to aid this process.

References

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2. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, van der Heijden MS, Parmigiani G, Yan H, Wang TL, Riggins G, Powell SM, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. SCIENCE. 2004 304(5674).

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