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From Primer Generation to Chromosome Identification: A Primer Generation Genotyping Method for Bacterial Identification and Typing

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Abstract : A challenge for laboratories is to provide bacterial identification and antibiotic sensitivity results within a short time. Hence, advancement in the required technology is desirable to improve timing, accuracy and quality. Even with the current advances in methods used for both phenotypic and genotypic identification of bacteria the need is there to develop method(s) that enhance the outcome of bacteriology laboratories in accuracy and time. The hypothesis introduced here is based on the assumption that the chromosome of any bacteria contains unique sequences that can be used for its identification and typing. The outcome of a pilot study designed to test this hypothesis is reported in this manuscript. Methods: The complete chromosome sequences of several bacterial species were downloaded to use as search targets for unique sequences. Visual basic and SQL server (2014) were used to generate a complete set of 18-base long primers, a process started with reverse translation of randomly chosen 6 amino acids to limit the number of the generated primers. In addition, the software used to scan the downloaded chromosomes using the generated primers for similarities was designed, and the resulting hits were classified according to the number of similar chromosomal sequences, i.e., unique or otherwise. Results: All primers that had identical/similar sequences in the selected genome sequence(s) were classified according to the number of hits in the chromosomes search. Those that were identical to a single site on a single bacterial chromosome were referred to as unique. On the other hand, most generated primers sequences were identical to multiple sites on a single or multiple chromosomes. Following scanning, the generated primers were classified based on ability to differentiate between medically important bacterial and the initial results looks promising. Conclusion: A simple strategy that started by generating primers was introduced; the primers were used to screen bacterial genomes for match. Primer(s) that were uniquely identical to specific DNA sequence on a specific bacterial chromosome were selected. The identified unique sequence can be used in different molecular diagnostic techniques, possibly to identify bacteria. In addition, a single primer that can identify multiple sites in a single chromosome can be exploited for region or genome identification. Although genomes sequences draft of isolates of organism DNA enable high throughput primer design using alignment strategy, and this enhances diagnostic performance in comparison to traditional molecular assays. In this method the generated primers can be used to identify an organism before the draft sequence is completed. In addition, the generated primers can be used to build a bank for easy access of the primers that can be used to identify bacteria.

Keywords : bacteria chromosome, bacterial identification, sequence, primer generation
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