36. In silico modelling of the PCR process for detection of bacteria from clinical samples

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Our approach is based on finding species-specific bacterial repetitive sequences and designing PCR primers for those repeats. We assume that designing primers from species-specific repetitive sequences is a good approach because this assures increased sensitivity of PCR.

Detection of pathogenic bacteria from the clinical samples is continually relevant problem. One of the most exerted methods for detecting specific bacterial infections is the polymerase chain reaction (PCR). Though this method is fast, its quality of the results depends significantly on specificity and sensitivity of primers used in the reaction mixture. Motivated of afore described problem we have introduced method for more specific and sensitive detection of bacteria from different samples (clinical, environmental, veterinary etc) with PCR.

We have developed BLAST-based method for finding species-specific bacterial repetitive sequences. The copies of the unique repetitive sequence are aligned with each other by program CLUSTALW and thereafter resultant consensus sequence is used for designing primers for PCR. We assume that designing primers from species-specific repetitive sequences is a good approach because this assures increased sensitivity of PCR. For each species-specific consensus repetitive sequence we design maximally 10 primer pairs with modified version of program called PRIMER3 (1). Later this gives us enough information for choosing primers for experimental tests. For each computer-designed primerpair we calculate thermodynamic values (based on deltaG) and count non-specific annealing sites. We also find alternative products for each primer pair using BLAST and program Genome Tester (http://bioinfo.ebc.ee/genometester/) which finds PCR primers
binding sites and alternative products from a given DNA sequence. For annealing sites of primer pairs giving possible false products we calculate indels-mismatches and thermodynamic values (based on deltaG). The latter could give us the measure (after experimental tests and statistical analyzes) that characterizes the primer pairs possibility to give a false products (what is the rate of indels-mismatches in a duplex (or duplex free energy) so the duplex will not be stable enough to give us a false product or primer will not anneal to a non-target regions).

Among all candidates of the set of designed primer pairs we choose a smaller subset of statistically informative primer pairs for carrying out the experimental tests.
In the future we intend to analyze values of calculated parameters with the results of experimental tests statistically. This is for finding out the possible characteristics (and their values) of the primer pair and characteristic features for conditions of PCR experiments (the DNA polymerase, the salt and DNA concentrations etc.) which could further be used for predicting the success of PCR.

References:
1. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365-86.