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An integrated custom design tool for PCR resequencing

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Short Abstract: Increasing attention has been devoted to SNP discovery and genotyping in an effort to associate disease/phenotypes with gene variations and mutations, but reliable primer design and data analysis remain two of the major challenges to overcome. We developed a flexible design tool that allows selection of different genomic targets.

Long Abstract:
Increasing attention has been devoted to SNP discovery and genotyping in an effort to associate disease/phenotypes with gene variations and mutations and to determine evolutionary relationships, but reliable primer design and data analysis remain two of the major challenges to overcome. Development of a flexible design tool that allows researchers to select dependable PCR-sequencing primers for different genomic targets with user-defined parameters would greatly facilitate resequencing.

In order to help the scientific community to better use PCR and CE-sequencing for SNP discovery, we recently released the VariantSEQr™ primer designs of 15K+ human genes to the public via NCBI's ProbeDB.
To improve upon this one-size-fits-all approach, we have developed an integrated web-based tool which incorporates target sequence selection/submission, primer design, and data analysis into a connected workflow. Users can choose genes, transcripts and other identifiers, select any region in the genome, or upload their own sequence as well as specify design parameters, e.g. amplicon length, primer Tm etc. The web interface then submits the job to a backend pipeline, which takes advantage of proprietary primer picking and predictive quality assurance processes that generated Applied Biosystem's VariantSEQr™ primers. All currently known SNP/MNP sites in the genomic sequence are avoided during primer design. The resulting primers are then checked for genomic redundancy and the probability of success in PCR. An exhaustive search is performed to produce the optimal tiling of the amplicons covering the target region. The generated primer information is formatted as a tab-delimited file so it can be easily uploaded in oligo vendors' ordering sites. Template files for automated discovery of variants compatible with Seqscape® software are also generated. Utilization of this primer design tool can substantially reduce the effort required to design and optimize robust resequencing primers.