

## Sequence Analysis

**ReSequencer: an automated primer picking application for gene resequencing efforts.**Maulik Shah<sup>1,\*</sup>, Jeff Touchman<sup>1</sup><sup>1</sup>Translational Genomics Research Institute, Phoenix, USA

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**ABSTRACT**

**Summary:** ReSequencer is a stream-lined, cross-platform, stand-alone application that is capable of retrieving the DNA sequence of a gene's exons and then designing primers to sequence each exon. This application remotely accesses Ensembl to search retrieve the DNA sequence for any known transcript for a gene. Once the sequence for each exon is retrieved, ReSequencer runs a client-side binary of Primer3 to design primers based on the user's parameters. During the course of resequencing over 100 genes related to lung cancer, we have found ReSequencer to significantly speed up this project by successfully automating primer selection for XX % of the XXX exons involved in the project.

**Availability:** <http://www.tgen.org/software/resequencer>**1 INTRODUCTION**

As sequencing technologies continue to improve, the cost of sequencing an individual patient's set of possible disease genes becomes an affordable method of analysis. However, until the creation of ReSequencer, there were few options for sequencing genes in a batch process. The first method is to use funds to access proprietary databases of primers known to allow successful sequencing of an exon. To accomplish such a task without the use of funds, involved manually downloading the DNA sequence of each exon of each gene of interest and then using a free primer design tool, such as Primer3, to design primers. Because this process may require the user to surf web-pages and then use command line prompts to reach the end, the process becomes tedious and time-consuming.

**2 GOALS**

We present ReSequencer; a tool that makes possible automatic sequence retrieval as well as automatic per-exon primer design. The goals for ReSequencer were determined by analyzing the needs of a lung cancer study that required the resequencing of 100+ genes (XXXX+ exons). We designing ReSequencer, we aimed to provide solutions the following problems: repetitive use of slow web-interfaces and user-unfriendly command-line interface. We also aimed to create an application that would accomplish the following goals: download all exons for a given gene, design both 5' and 3' primers for each exon, generate useable output, provide this service to multiple computing platforms, and ease of use. Beyond the technical goals, our original objective was to save man hours in the lab and aid resequencing projects reach results

faster. We have shown that ReSequencer helps in these kinds of projects. More features will be added as scientists' needs become apparent.

**3 USAGE CASE**

Usage of ReSequencer is straightforward and does not require any major configuration. To start ReSequencer in Windows and Macintosh, simply double-click on the ReSequencer.jar file; use the command 'java -jar ReSequencer.jar' in Linux. Input, through a graphical user interface (GUI), is simply the name of the gene that one desires to sequence. The search is submitted to Ensembl and all known transcripts for the gene are returned for the users to browse and the select. Browsing involves double clicking on a single gene transcript. Doing so opens a browser window showing Ensembl's description of the transcript. If the user is satisfied, s/he may return to ReSequencer and click the 'Make Primers' button. At this point, ReSequencer downloads each exon's sequence and locally runs Primer3 to design primers. If desired, the user can choose which sequencing primers s/he will be using and attach them to each set of primers. Now the user can save these results to a comma-separate-value file and use it to order primers from their chosen provider.

**4 IMPLEMENTATION**

ReSequencer is mainly written in Java/Swing and is compatible with Java 1.4 and above. Three compiled binaries of Primer3 are bundled with ReSequencer. Because Primer3 is written in the C language, a version for each supported platform must be included in the ReSequencer package. Currently, Windows, Linux and Mac OS X are supported. Other library routines that are used in ReSequencer are documented in its 'About' dialog.

Although ReSequencer supplies an easy way to design primers, it is not always successful. In such cases, a different set of parameters must be provided to Primer3. The 'Advanced Options' dialog offers a variety of parameters that the user can modify to achieve optimal results.

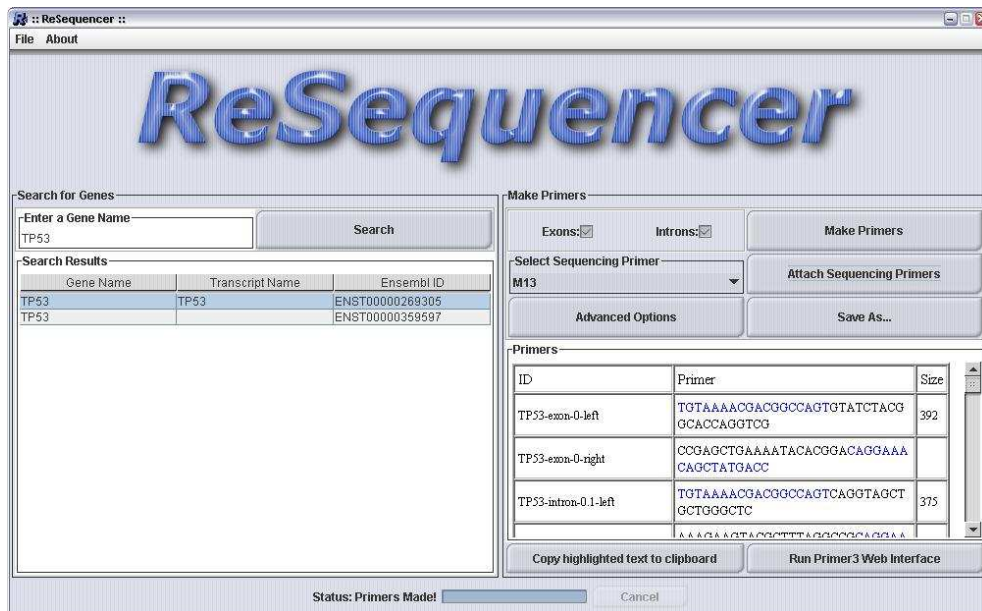
**5 RESULTS**

We used ReSequencer in the following practical situation. We aimed to design primers and sequence over 100 genes in XX patient samples. In total, there were XXXX exons; and exon was counted as two if it exceeded 400 base pairs in length. ReSequencer automatically divides exons too long for PCR into appropriately sized segments, and then designs the appropriate primers for each such segment. Primers failing to generate sufficient PCR product, were then redesigned using a manual process.

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**Fig. 1.** A Screenshot of ReSequencer. The left panel displays the various transcripts available for the TP53 gene in the Ensembl database. The right half presents the primers that have been generated with M13 Sequencing primers attached. Generating Primers and changing primer design parameters are both available.

## REFERENCES

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