Step 1.1: Identify Seed Sequence(s) and Create Hit Table

Overview. The first step in the design process is to identify the Seed Sequence(s) and to create the Hit Table. The Seed Sequence(s) represent the DNA sequence(s) that the primers are designed to amplify. The Hit Table is a list of DNA sequences with various degrees of similarity to the Seed Sequence(s), from which the target and non-target sequences can be derived. The Hit Table is created by subjecting the Seed Sequence(s) to a BLAST (blastn) analysis.

> *Note*. Although Steps 1.1 and 1.2 are designed to identify and collect target and non-target DNA sequences, there are certainly other strategies for accomplishing this task, which the user may decide to use instead of or in combination with our steps. The only requirement for using the primer design module of PRISE (Step 2) is that the target and non-target sequences be available in separate FASTA-formatted text files.

Identify the Seed Sequence(s): Identify the sequence(s) that the primers are intended to amplify and save them in FASTA format as a plain text file.

Create the Hit Table: Subject the Seed Sequence(s) to a nucleotide BLAST analysis using the program on the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). In this analysis, the user needs to select the appropriate **Database** and number of **Max target sequences**, which, in our experience, will typically be a minimum of 500. The **Max target sequences** option is located in the **Algorithm parameters** section. In the current version of the program, after clicking on the **BLAST** button, click on **Formatting options**. Here, set **Show Alignment** as **Plain text** and **Alignment View** as **Hit Table**. In addition, set **Alignments** in the **Limit results** section to the value that was used for the **Max target sequences**. Click **View report** and save the output as a text file. This file is the Hit Table. Note that some web browsers do not allow output to be saved as text files.