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Author(s) ² : Tuval Ben Yehezkel
Participant(s) ³ : Weizmann
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² i.e. name of the person(s) responsible for the preparation of the document

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Abstract

Based on years of experience in DNA editing we have realized that the construction and editing of a complex DNA library is, to a large extent, an exercise in failure management. The reasons for this are mainly three-fold: (1) there is no one biochemical methodology that is optimal for every DNA editing and synthesis task. As a result, most large scale DNA editing projects tend to at least partially fail, often for unknown reasons. The second main reason (2) for large scale DNA editing failure is that high throughput projects require automation, which is often error prone. The third reason for editing failure is faulty computational design of DNA editing reagents, mainly of PCR primers. The subject of this deliverable is to implement CADMADs strategy for recovering from construction failures due to these main two reasons.

Keywords⁷:

Y operation, Gibson, Primer design, Automation, DNA editing

Introduction

a. Aim / Objectives

To lay a strategy, or an algorithm, for a fixed number of corrective operations that will be taken during the editing of a DNA library in order to resolve construction failures.

b. State of the Art

There is no strategy that we know of in the art that deals with the automated recovery from construction failures in an robotic DNA editing and synthesis system in which large DNA libraries are produced.

c. Innovation

The innovative aspect of this deliverable is that construction failures are not dealt with individually. Instead, there is an automated decision making process that applies a standard “error recovery battery” of operations aimed at addressing the most common construction failure reasons. These are applied automatically when a failure is detected.

2. Implementation

The most frequent reasons for failures in construction are: (1) failures in the assembly protocol (Gibson, Y or other) used, which turn out to be incompatible for the specific DNA editing task (2) pipetting (or other robotic) failures which occur in the operation of the liquid handling robots CADMAD technology uses. In order to mitigate these risks we implemented a recovery operation for each of them, as follows, which resolves the problem in most of the instances and (3) faulty design of primers for PCR.

3. Results

If a Y operation fails we apply the following set of operations to recover from the failure:

⁷ Keywords that would serve as search label for information retrieval

- (1) It is our experience that there is a certain probability of failure in every reaction and most of these failures are random. As a result, an effective strategy to recover from these random pipetting errors is to simply repeat the operation. If it still fails after this step this means that the error is likely not a pipetting error. If it succeeds it means that the error was originally a pipetting error.
- (2) Our second strategy for recovering from failures is aimed at recovering from instances in which switching from one assembly protocol to another resolved the issue. To this end we have implemented a fully automated Gibson operation and intend to further implement assembly PCR. As a result, we will be able to automatically switch from one assembly protocol to another when a construction failure occurs during the construction of CADMAD libraries.
- (3) The results of our project for improved primer design (reported in the deliverables of WP2) will be used in order to redesign new primers for Y operations that failed the first two failure recovery operations. It is our experience that replacing primers with other that were designed differently solves some of the failures.

4. Conclusions

Implementation of our error recovery strategy will enable a relatively wide array of responses in cases of construction failures in the editing of CADMAD libraries. According to experience with several past editing project in which many construction failures were encountered we estimate that a significant portion of the failures may be recovered using this strategy. During the high throughput editing of DNA libraries for CADMAD partners during the 3rd year we will evaluate the fraction of editing failures that were successfully