Funding Scheme: THEME [ICT-2007.8.0] [FET Open]

Paving the Way for Future Emerging DNA-based Technologies: Computer-Aided Design and Manufacturing of DNA libraries

Grant Agreement number: 265505

Project acronym: CADMAD

Deliverable number: D4.4

Deliverable name: Report on strategy and methods for recovering from construction failures

Contractual Date[1] of Delivery to the CEC: M18 postponed to M24
Actual Date of Delivery to the CEC: M25
Author(s)[2]: Tuval Ben Yehezkel
Participant(s)[3]: Weizmann
Work Package: WP4
Security[4]: Pub
Nature[5]: R
Version[6]: 0.0
Total number of pages:

1 As specified in Annex I

2 i.e. name of the person(s) responsible for the preparation of the document

3 Short name of partner(s) responsible for the deliverable

4 The Technical Annex of the project provides a list of deliverables to be submitted, with the following classification level:
Pub - Public document; No restrictions on access; may be given freely to any interested party or published openly on the web, provided the author and source are mentioned and the content is not altered.
Rest - Restricted circulation list (including Commission Project Officer). This circulation list will be designated in agreement with the source project. May not be given to persons or bodies not listed.
Int - Internal circulation within project (and Commission Project Officer). The deliverable cannot be disclosed to any third party outside the project.

5 **R (Report):** the deliverables consists in a document reporting the results of interest.
P (Prototype): the deliverable is actually consisting in a physical prototype, whose location and functionalities are described in the submitted document (however, the actual deliverable must be available for inspection and/or audit in the indicated place)
D (Demonstrator): the deliverable is a software program, a device or a physical set-up aimed to demonstrate a concept and described in the submitted document (however, the actual deliverable must be available for inspection and/or audit in the indicated place)
O (Other): the deliverable described in the submitted document can not be classified as one of the above (e.g. specification, tools, tests, etc.)

6 Two digits separated by a dot:
The first digit is 0 for draft, 1 for project approved document, 2 or more for further revisions (e.g. in case of non acceptance by the Commission) requiring explicit approval by the project itself;
The second digit is a number indicating minor changes to the document not requiring an explicit approval by the project.

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.

Keywords[7]:

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 throuput editing of DNA libraries for CADMAD partners during the 3rd year we will evaluate the fraction of editing failures that were successfully