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Abstract

The focus of the CADMAD project is to develop a CAD/CAM system for DNA libraries. In order to realize this vision in its fullest it is critical to develop robust and efficient biochemical methodologies for DNA library construction. Specifically, we are developing and optimizing what we call the Y operation – a method for biochemically concatenating two DNA fragments into one.
During the past year we have broadened and optimized the Y operation toolkit. The reason for this is that in our experience having more than one DNA editing biochemistry significantly increases the probability of successfully completing complex DNA editing projects, such as those we will undertake during the 3rd year of CADMAD. The following deliverable is a report on our work to this end during the past year.

Keywords[7]:

Y operation, Gibson assembly, Ultramers

Introduction

a. Aim / Objectives

The aim of this deliverable is to improve the capabilities of CADMAD's main biochemistry, the Y operation.

b. State of the Art

The traditional Y operation developed and used by CADMAD has been reported before and its applicability to various DNA editing tasks has been exemplified in previous reports. Besides the Y operation there exists a plethora of biochemical DNA assembly reactions that have been published. The aim of the CADMAD consortium in this respect is to remain future-proof in the sense that we can integrate newly developed technologies as they appear into our platform.

c. Innovation

We have several innovative aspects in this deliverable. These include: (1) The development of a new DNA synthesis operation that is adapted for the use in electro-wetting based microfluidics (which we call CPA – continuous PCR assembly), (2) the full integration of the use of Ultramers, a new technology for making long synthetic oligos up to 200bp), into our standard DNA editing workflow and (3) the full integration of the Gibson assembly protocol as a new alternative to our traditional Y operation in CADMAD.

2. Implementation

Implementation of the work in this deliverable has been through (1) extensive biochemical experimentation with and optimization of the Gibson assembly protocol, primarily on a positive control system in which we construct the GFP gene (2) optimizing the use of Ultramers on our traditional Y operation and (3) developing CPA, an inside-out PCR assembly and amplification reaction that replaces the PCR primers "on-the-fly" during PCR and extends the product inside out.

[7] Keywords that would serve as search label for information retrieval

3. Results

(1) The CPA protocol was tested on ALL's microfluidic device and showed great results. The assembled products were size analysed and sequenced and negative controls indicate that assembly was indeed performed on chip.
(2) Ultramers were fully integrated into our DNA editing system as primers (up to 120 bp) and as templates (up to 200 bp) and their optimal concentrations in the assembly reactions were determined.
(3) The Gibson assembly reaction was tested for both binary assembly (of 2 fragments into one) and for multiplex assembly (up to 8 fragments). Its use has been fully integrated into the DNA editing workflow and, as a result, will be used extensively in the synthesis of CADMAD libraries.

4. Conclusions

The Y operation has been significantly broadened during the past year and as a result the CADMAD toolkit for constructing DNA libraries for end users during the 3rd year of the project has been considerably improved.

5. Abbreviations

List all abbreviations used in the document arranged alphabetically.

CPA	Continuous PCR assembly
Ultramers	Long oligos up to 200bp
Gibson assembly	A new method for combining DNA fragments