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Report on methods of monitoring Y operation biochemistry

Abstract

Real time quality control is a crucial factor in multi-step construction processes.
The DNA editing technology developed by the CADMAD consortium is based on iterative execution of a basic construction step, namely the Y operation. Moreover, the basic construction is itself in fact a multi-step process which includes several enzymatic, purification and mechanical steps.
Therefore, quality controlling for the results of each of these steps is critical in our early stage of development. This deliverable aims at defining the requirements and specifications of the actions we will take in order to monitor the biochemistry of the Y operation.

Keywords[7]:

Automation, quality control, liquid handling,

1. Implementation

The implementation of our Y operation monitoring strategy takes the form of standard and well-studied methods for evaluating the length, quality and sequence of DNA molecules. All of these methods will be integrated into our production pipeline in a manner that best exploits the information they may provide us on the construction process.

2. Results

Biochemical surveillance:

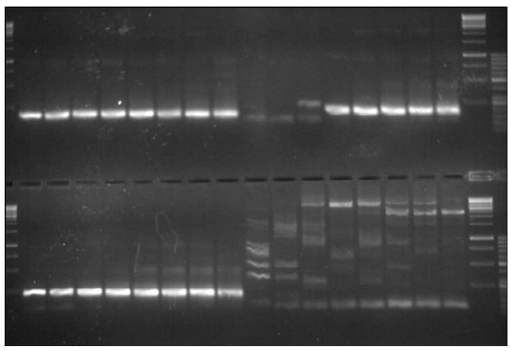
1) Standard biochemical assays that monitor the activity of the DNA processing enzymes used in DNA synthesis:
   •Monitoring of Polymerase activity
   •Monitoring of Exonuclease activity
   •Monitoring of Ligase activity

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

2) A Standard battery of different control Y operations (the basic biochemical DNA composition step).
3) Depth controls – controlling for the fact that DNA synthesis quality tends decline with the number of serial Y operations. This will be done by performing a control construction of a complete DNA editing tree that includes several consecutive Y operations.

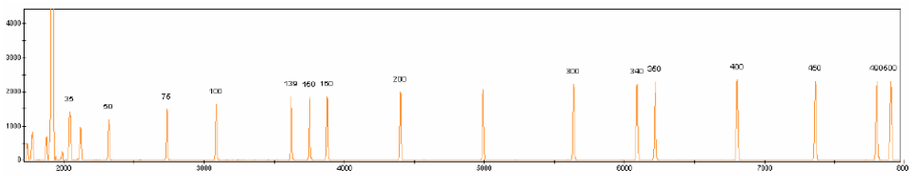
The surveillance and QC operations that will be integrated into our automated production pipeline will include:

1) Gel electrophoresis – for evaluating construction using fragment analysis

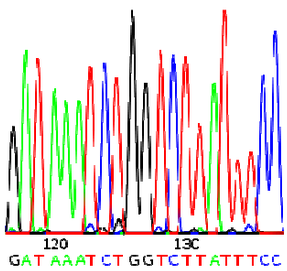


We will develop automated tools for analyzing the results of GE and CE (below) and using them to push the DNA editing task forward. These will take into account the expected lengths of fragments, their actual length and the existence of non-specific by product on top of the correct lengths.

2) Capillary electrophoresis - for evaluating construction using fragment analysis

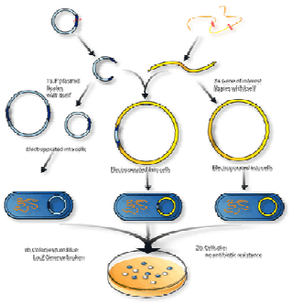


3) DNA sequencing - for evaluating error rate



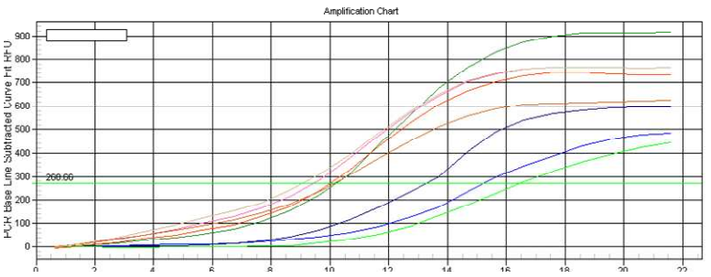
DNA sequencing will be used both to computationally dentify completely error free fragments and also for performing error correction if required using our concept of the minimal cut.

4) DNA Cloning – to isolate single construct and analyze them individually

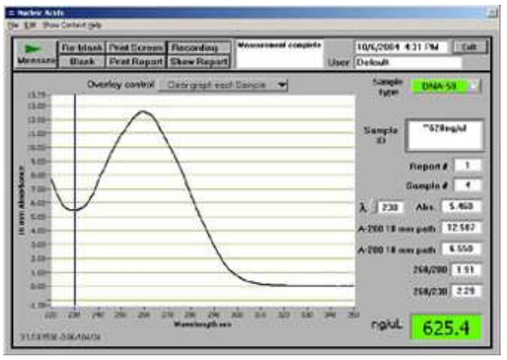


Optionally, we will integrate into our production pipeline a method we are in the process of developing which automates much of the cloning process and enables it to be streamlined in a robotic setup.

5) Real-time PCR – for monitoring PCR-based gene synthesis in real-time



6) Measurements of DNA quantity and purity



3. Conclusions

The requirements and specifications listed above for monitoring DNA resulting from DNA editing operations represents an extensive and thorough monitoring and QC strategy.
We intend to integrate these methods into our production pipeline in an automated manner and to attempt to develop computational methods so that that their analysis will also be as automated as possible.

4. References

1) Linshiz G., Ben Yehezkel T, Kaplan S., Gronau I., Ravid S., Adar R., Shapiro E. , Recursive construction of perfect DNA molecules and libraries from imperfect oligonucleotides Mol Syst Biol. 2008;4:191.
2) T. Ben Yehezkel, Linshiz G., Buaron H, Kaplan S., Shabi U., Shapiro E., De novo DNA synthesis using single molecule PCR E Nucleic Acids Res. 2008 Oct;36(17) 2 Uri Shabi , et al., & Ehud Shapiro Processing DNA Molecules as Text Systems and synthetic biology, 2010.
3) Ben Yehezkel T, Nagar S, Mackrants D, Marx Z, Linshiz G, Shabi U, Shapiro E Computer-aided high-throughput cloning of bacteria in liquid medium Biotechniques. 2011 Feb;50(2):124-7

List all abbreviations used in the document arranged alphabetically.
