

Chapter 12

Synthetic Approaches to Biology: Engineering Gene Control Circuits, Synthesizing, and Editing Genomes

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ABSTRACT

Nanobiotechnology and synthetic biology are emerging as novel fields that integrate research from science and technology to create novel organisms with new desired properties. We present here the new revolutionary methods of synthetic biology that enable us to engineer gene control circuits, edit genomes, and create de novo whole genomes. The creation of new genomes that function in the cell means that we can create new organisms that are different from those observed in nature. The synthetic genomes can contain novel combinations of genes that offer the opportunities to create novel biological species that possess predefined combination of properties. Therefore, the synthetic genomes can be regarded as a new kind of materials. The methods for whole genome assemble applied so far combined several in vitro and in vivo steps that possess certain technical limitations and shortcomings. In this chapter, we discuss all technical aspects of assembling novel genomes and their current limitations. The genome editing technologies that have been developed over the last several years based on the CRISPR-Cas system is also discussed. In addition, we present major RNA-based methods for design of gene control circuits both in prokaryotes and eukaryotes, including humans.

1. INTRODUCTION

The possibilities to engineer exogenous gene control circuits, to edit genomes, and to synthesize whole new genomes that work *in vivo* open new avenues in modern biology (Li & Borodina, 2014; Wang, Kim, & Kim, 2014), biotechnology (Bereza-Malcolm, Mann, & Franks, 2014), and biomedical research (Chien,

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Zangi, & Lui, 2015), which were unthinkable just a decade ago. These developments make synthetic biology really exciting and promising research field. Synthetic biology promises to make not less than a scientific revolution in our abilities to design and engineer living cells with predefined properties with far reaching applications. At the same time these novel technologies have their own limitations that need to be overcome to achieve practical feasibility, which is beyond the proof-of-principal (Collins & Jones, 2014; Gibson & Venter, 2014; Kalluri, Yin, Yang, & Davison, 2014; Koch, 2014; Tyagi et al., 2014). In addition, certain ethical concerns over this research should be addressed to ensure that ethical norms are preserved (“NEW DIRECTIONS: the Ethics of Synthetic Biology and Emerging Technologies,” 2010).

In fact, the development of synthetic genome technology depends heavily on the application of many other existing technologies that have been already developed, advanced and also scrutinized in terms of ethical issues. Such technologies include various DNA recombinant and cloning methods, chemical DNA synthesis, automated DNA sequencing (Penchovsky, 2013), particular next generation DNA sequencers (Rogers & Venter, 2005), somatic cell nuclear transfer (Karas et al., 2013), and others.

Therefore, it is obvious that the creation of a functional synthetic genome is mainly based on the applications of many existing methods rather on the development of brand new technologies. However, the ability to make a whole synthetic genomes opens new avenues for many useful biotechnology applications. The reading and writing genetic sequences among living cells, computers, and DNA synthesizers are transforming natural sciences by creating new life forms based on designed synthetic DNA. The present and possible future applications of these emerging technologies are also discussed.

In addition, the recently developed genome editing tools have given us the opportunity to change any sequence in prokaryotes or eukaryotes, including human cells and bacterial synthetic genomes. Moreover, this chapter discusses the design and application of various RNA-based strategies for exogenous control of gene expression (Thodey & Smolke, 2011). These new bio-molecular techniques further enhance the tool box of synthetic biology and make this new research field even more feasible.

The main conceptual point of synthetic biology is to tread genetic and cellular engineering more like a programming language i.e. like a software engineering. To reach this goal, researchers are using DNA and RNA to produce simple functional components that are called BioBricks (Altegoer, Schuhmacher, Pausch, & Bange, 2014; Lewens, 2013; Madec, Gendrault, Lallement, & Haiech, 2012; Smolke, 2009). These components should be standardized so that they can be used to make various complex structural and functional entities from whole synthetic genome to exogenous circuits for control of gene expression in the presence or absence of a predefined effector molecule. In fact, there is already a catalog that contains more than 5000 standardized molecular components (BioBricks). They include various promoters, terminators, enhancers, protein encoding sequences, and many others. This is a kind of an open source catalog. The molecular BioBricks are available for the synthetic biology community and their number is growing. They include molecular components with unified restriction cloning sites so that they can be easily cloned into a vector. There are different BioBricks that can work in bacterial or eukaryotic cells such as yeast and mammalian cell lines.

In this way, synthetic biology is creating new biomaterials that are inspired by nature but are not naturally occurring. At the same time, synthetic biology is making genetic engineering much more a real engineering technology (R. Penchovsky, 2013b) by collecting, redesigning, and re-assembling of components of living nature. The main difference with the software engineering is that the reprogrammed cell, can create its own hardware in terms of cellular compounds or entirely new cells.

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