



# Synthetic Biology: A Lab Manual

*Josefine Liljeruhm*

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*Synthetic Biology: A Lab Manual* is the first manual for laboratory work in the new and rapidly expanding field of synthetic biology. Aimed at non-specialists, it details protocols central to synthetic biology in both education and research. In addition, it provides all the information that teachers and students from high schools and tertiary institutions need for a colorful lab course in bacterial synthetic biology using chromoproteins and designer antisense RNAs. As a bonus, practical material is provided for students of the annual international Genetically Engineered Machine (iGEM) competition. The manual is based upon a highly successful course at Sweden's Uppsala University and is coauthored by one of the pioneers of synthetic biology and two bioengineering postgraduate students.

### An inspiring foreword is written by another pioneer in the field, Harvard's George Church:

*"Synthetic biology is to early recombinant DNA as a genome is to a gene. Is there anything that SynBio will not impact? There was no doubt that the field of SynBio needed 'A Lab Manual' such as the one that you now hold in your hands."*

### Contents:

#### • **Introduction:**

- What is Synthetic Biology, Exactly?
- The iGEM Outbreak
- A Synthetic Biology Lab Manual

#### • **Genes, Chromoproteins and Antisense RNAs:**

- *E. coli* DNA: Chromosomes, Plasmids and Copy Number
- Coupling of Transcription and Translation in Bacteria
- Promoter and Terminator for Transcription
- Ribosome Binding Site (RBS)
- Codon Bias
- Chromoproteins
- Small Regulatory RNAs (sRNAs)

#### • **Lab Rooms and Equipment:**

- The Physical Lab Spaces
- Equipment

#### • **Safety is Priority #1:**

- Fires
- Chemicals
- Biological Safety and Disposal
- Dangerous Equipment

#### • **Lab Course Projects:**

- Time and Resources
- Project Overview and Learning Objectives
- The Lab Notebook
- Lab Section 1. Preparation of Chemical Solutions and Agar Plates
- Lab Section 2. Coloring Bacteria by Adding a Promoter to a Chromoprotein Gene
- Lab Section 3: Rational Engineering of Chromoprotein Expression Level

- Lab Section 4. Other Experiments
- The “Dreaded” Exam
- **Protocols:**
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  - Protocol 1. Preparation of Solutions and Agar Plates
  - Protocol 2. Overnight Cultures with Antibiotics, and Glycerol Stocks
  - Protocol 3. BioBrick™ 3A Assembly and Gel Analysis
  - Protocol 4. Agarose Gel Electrophoresis
  - Protocol 5. Preparation of Competent *E. coli* Cells Using CaCl<sub>2</sub>
  - Protocol 6. Transformation of CaCl<sub>2</sub>-Competent *E. coli* Cells
  - Protocol 7. Bacterial Re-Streaking Techniques
  - Protocol 8. Lysis of *E. coli* Cells with Lysozyme
  - Protocol 9. Polymerase Chain Reaction (PCR)
  - Protocol 10. Inverse PCR Mutagenesis
  - Protocol 11. Colony PCR
  - Protocol 12. Gibson Assembly
- **Advanced Methods:**
  - Flow Cytometry and Cell Sorting
  - Recombination in Plasmids and the Chromosome
  - Electrocompetent Cells
- **The International Genetically Engineered Machine (iGEM) Competition:**
  - How to Start an iGEM Team
  - Uppsala iGEM 2011 — Show Color with Color
  - Uppsala iGEM 2012 — Resistance is Futile
  - Uppsala iGEM 2013 — Lactonutritious — It's Delicious
- **Appendices**

**Readership:** Students and researchers in biotechnology, cell/molecular biology and genetics.

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