Interactive Experimentally-Driven Algorithms for Optimized DNA Assembly

Evan Appleton, Jenhan Tao & Douglas Densmore

*5th International Workshop on Bio-Design Automation, Imperial College London, London, England, UK*

*July 13th, 2013*
Synthetic Biology

Cancer Killing Bacteria
[Anderson et al. 2006]

Artemisinin Production
[Ro et al. 2006]

Arsenic Filtering System
Groningen – iGEM 2009

Repressilator
[Elowitz et al. 2000]

Toggle Switch
[Gardner et al. 2000]
DNA Assembly Planning
DNA Assembly Methods

**BioBricks™ Assembly**
- Binary Assembly
- Assembly site scarring (6bp)
- All part junctions are the same

**Modular Cloning Assembly**
- One-pot assembly
- Assembly site scarring (4bp)
- Some part junctions are the same

**Gibson Assembly**
- One-pot assembly
- No assembly site scarring
- All part junctions are unique

Gibson et al. 2009

Weber et al. 2011
Modeling DNA Assembly

1. Part Junction Generation

2. Cloning Reactions
Binary (2-Way) Hierarchical Algorithm

Goal Part: A B C D E F G H I

Library: A, B, C, D, E, F, G, H, I

Combine to get: G H I
Combine to get: E F
Combine to get: H I

Go through all partitions, score all, save best score in library

☐ = in library, known cost
One Pot (n-Way) Hierarchical Algorithm

Goal Part:  A B C D E F G H I

Combine to get
A B C

Combine to get
D E F

Combine to get
G H I

If n = number of possible breaks, k = number of selected breaks, n choose k possible break sets for each recursive call

Search all possible break sets of size 1 to upper limit

= in library, known cost
Hierarchical Assembly Heuristics

Heuristic #1: Stages

2 Stages ✓

3 Stages ✗

Heuristic #2: Steps

3 Steps ✓

4 Steps ✗

Heuristic #3: Efficiency

- Efficiency as a function of parts assembled per cloning reaction

Heuristic #4: Modularity and Sharing

- Plans with opportunities to share intermediates are scored higher
Part Junction Modularity Optimization

1. Determine which parts can be shared (green shows shared parts)

2. Minimize number of overhangs used (green shows parts affected)

3. Use a library to finalize part junctions
Layered Genetic Logic Gates

MoClo Assembly
3 Goal parts, 2 Stages, 10 Steps (3 Shared), 28 PCRs

[Moore et al. 2012]
Genetic Logic/Memory Circuits

MoClo Assembly
3 Goal parts, 2 Stages, 7 Steps (3 Shared), 49 PCRs
Assembly Workflow

Parts Library

RavenCAD

Incomplete Devices

Assembly Plan

Physical Assembly

Assembled Devices
RavenCAD Workflow

- Parts
- Devices
- Data Upload
- Target Device Selection
- Assembly Method
- RUN
- Assembly Plan
  - Primer Designs
  - Hierarchical Assembly Graph
  - Physical Assembly
Welcome to

RavenCAD

Plan a better cloning experiment today.

Get Started

Why RavenCAD?
Library Part Re-Use
Experimental Input/Feedback

• What if the output assembly plan doesn’t work?
  – The user gets stuck on a specific assembly step
  – The user desires to functionally test certain intermediate constructs
  – The output doesn’t fit well with a currently implemented system of assembly

• It’s OK!
  – Force or Bias intermediates or part junctions
    • Manual entry of forced/biased steps for intermediates
    • Manual entry of forced part junctions
    • List-based entry for intermediates and part junctions

• Efficiency
  – Efficiency associated with number of parts/rxn. for a given assembly method
RavenCAD Workflow

- Parts
- Devices
- Data Upload
- Experimental biases/constraints
- Target Device Selection
- Assembly Method
- RUN
- Assembly Plan
- Primer Designs
- Hierarchical Assembly Graph
- Physical Assembly
Efficiency Input Toggling
Future Work

• Primer design optimizations

• *De Novo* assembly planning
  – List of characterized restriction enzymes
  – Knowledge of complex DNA structures
  – Outputs plans with combinations of synthesis, restriction-ligation and homologous recombination

• Sequence-only input
  – Determines which sequences in a set of target devices and library of parts can be shared the most
Acknowledgements

- **CIDAR Lab**
  - Douglas Densmore
  - Jenhan Tao
  - Swapnil Bhatia
  - Ernst Oberortner
  - Traci Haddock
  - Stephanie Paige
  - Cassie Huang
  - Rishi Ganguly
  - Swati Banerjee-Carr
  - Sonya Iverson
  - Janoo Fernandes
  - Alejandro Lechuga

- **Collaborators**
  - **BBN Technologies**
    - Jake Beal
    - Aaron Adler
    - Fusun Yaman
  - **Belta Lab (BU)**
    - Calin Belta
    - Ebru Aydin-Gol
  - **Weiss Lab (MIT)**
    - Ron Weiss
    - Noah Davison