Interactive Experimentally-Driven Algorithms for Optimized DNA Assembly

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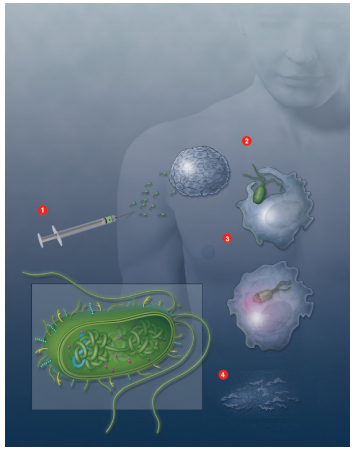
Synthetic Biology

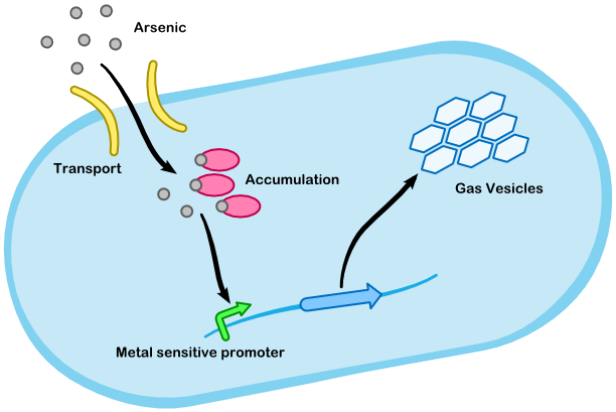


Cancer Killing Bacteria

[Anderson *et al.* 2006]

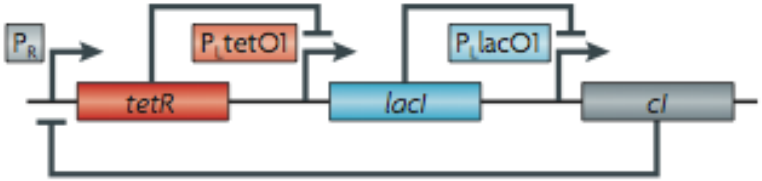
Artemisinin Production

[Ro *et al.* 2006]



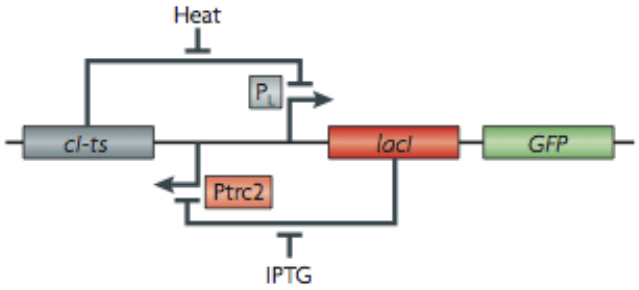
Arsenic Filtering System

Groningen – iGEM 2009



Repressilator

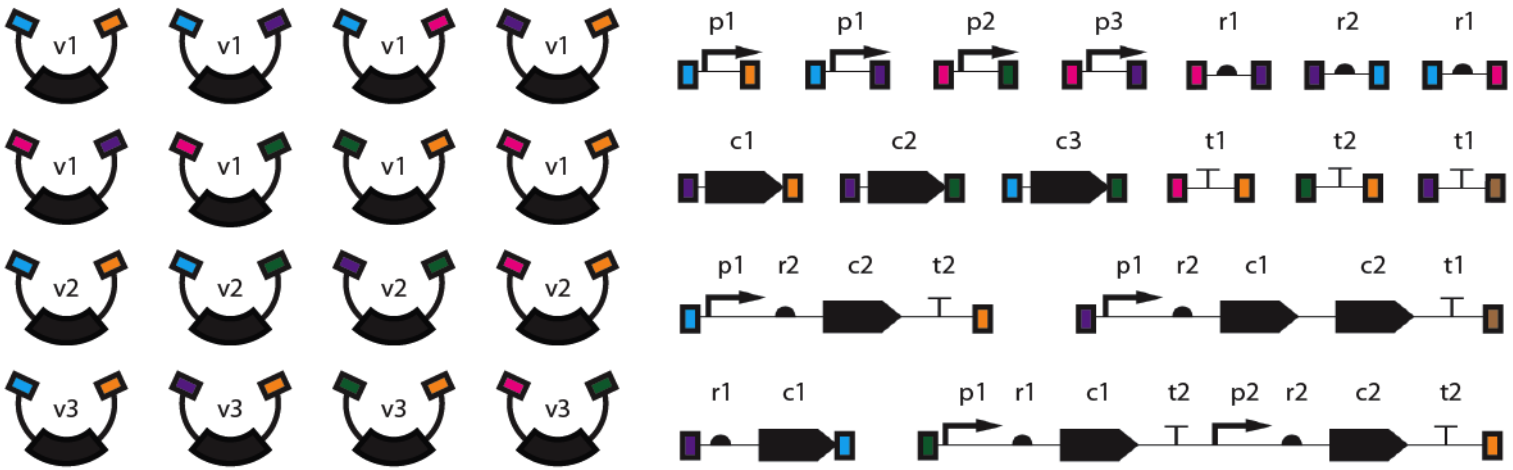
[Elowitz *et al.* 2000]



Toggle Switch

[Gardner *et al.* 2000]

DNA Assembly Planning



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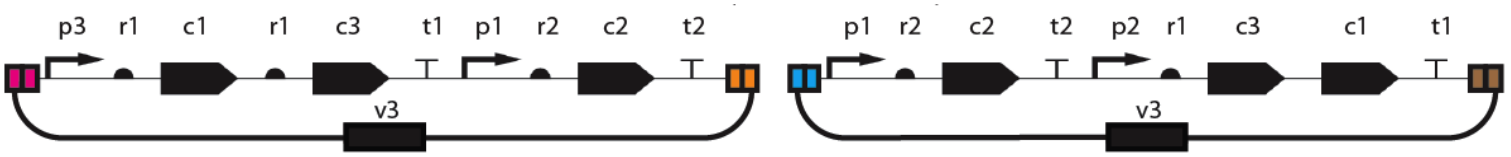
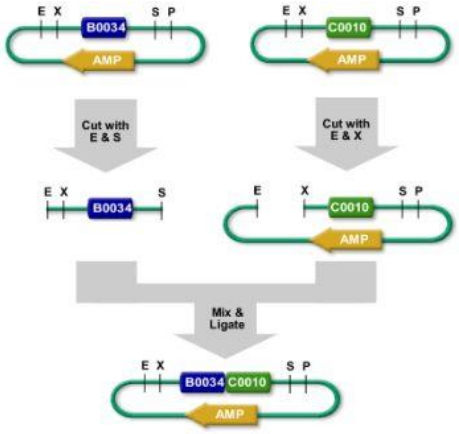


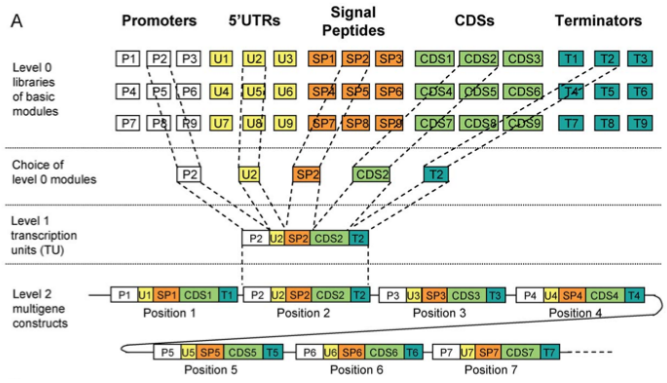
DNA Assembly Methods

BioBricks™ Assembly



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

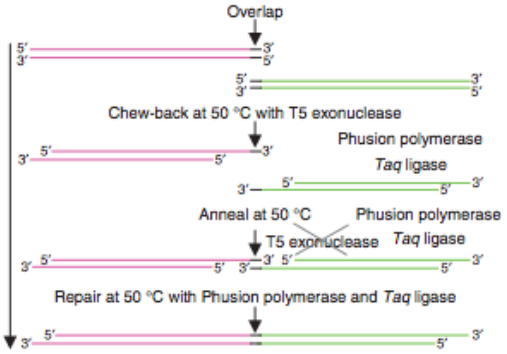
Modular Cloning Assembly



Weber *et al.* 2011

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

Gibson Assembly

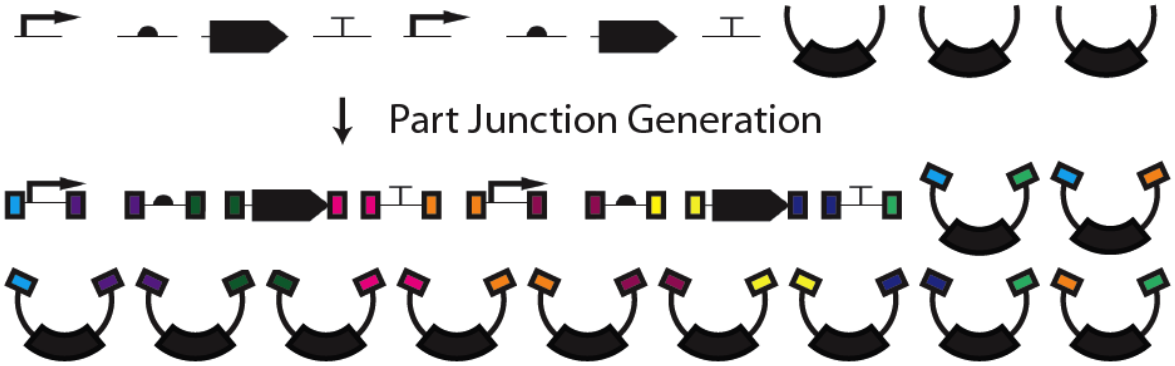


Gibson *et al.* 2009

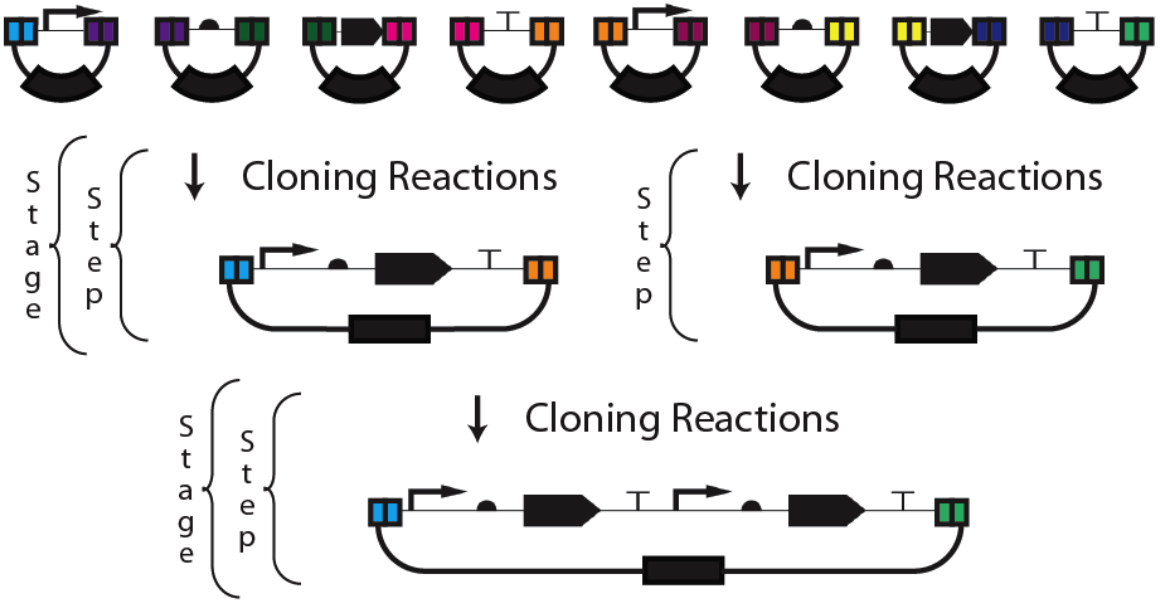
- One-pot assembly
- No assembly site scarring
- All part junctions are unique

Modeling DNA Assembly

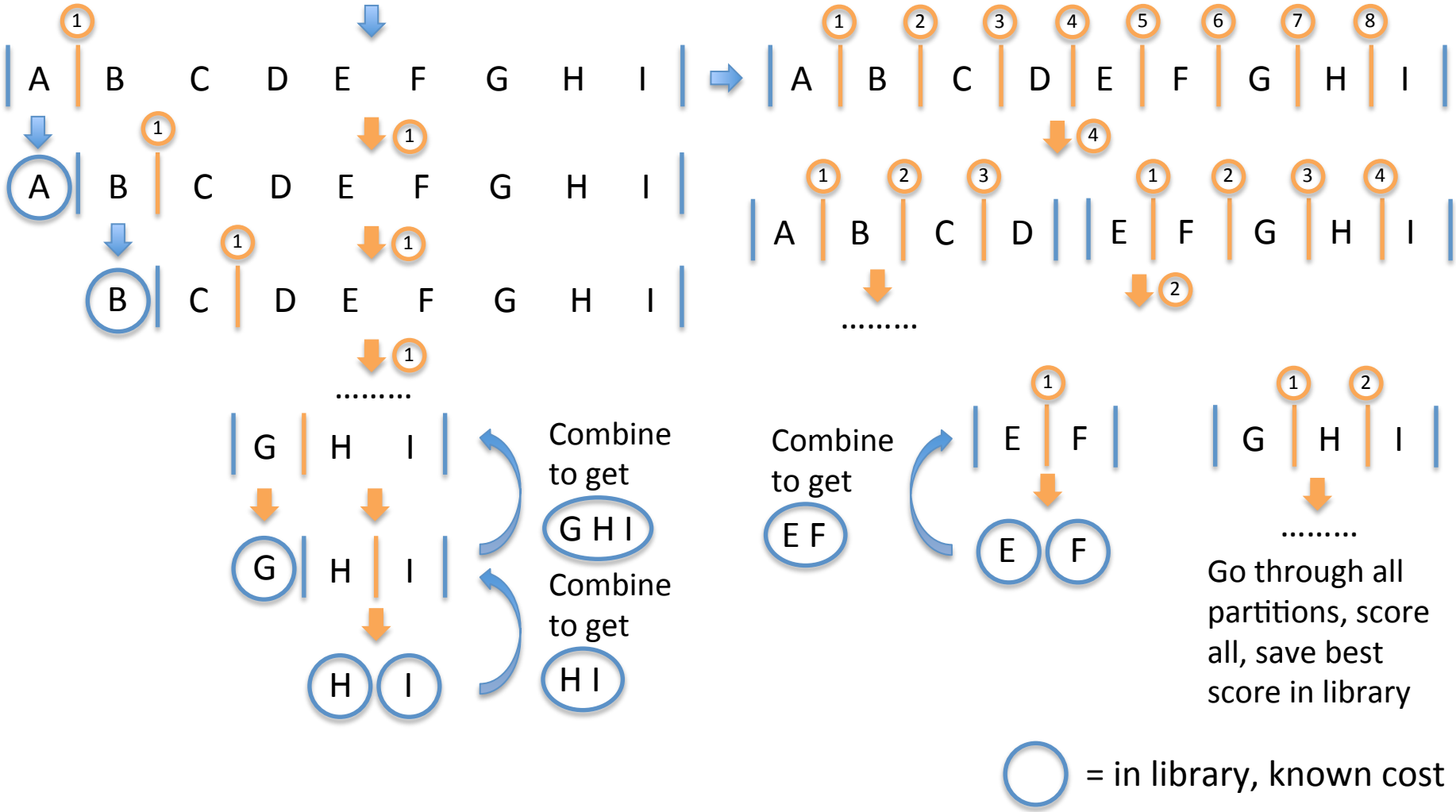
1.



2.

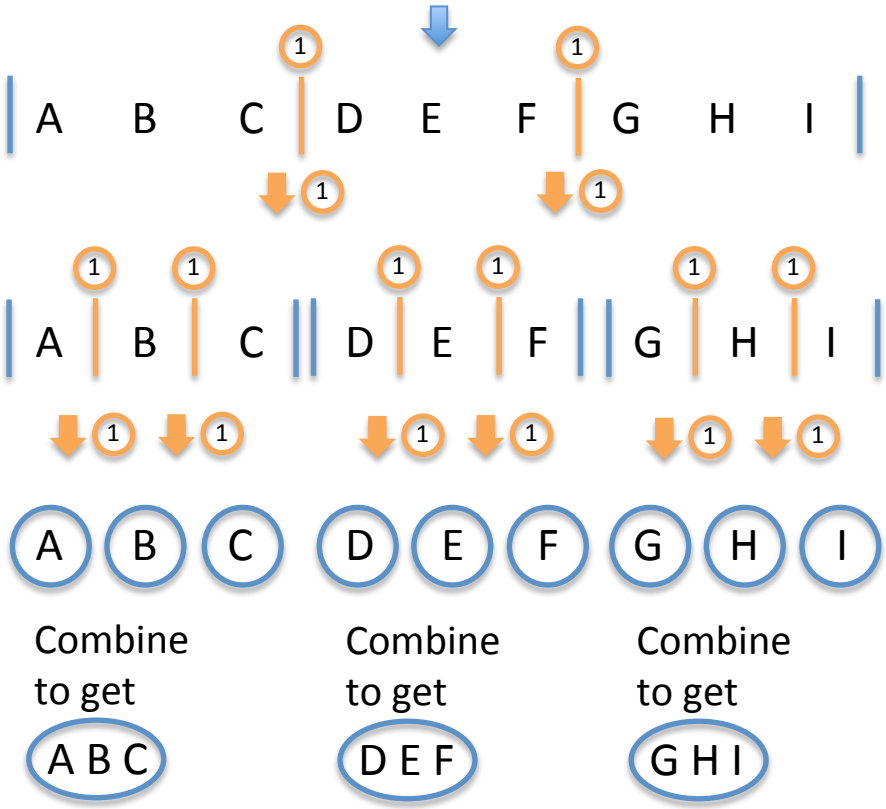


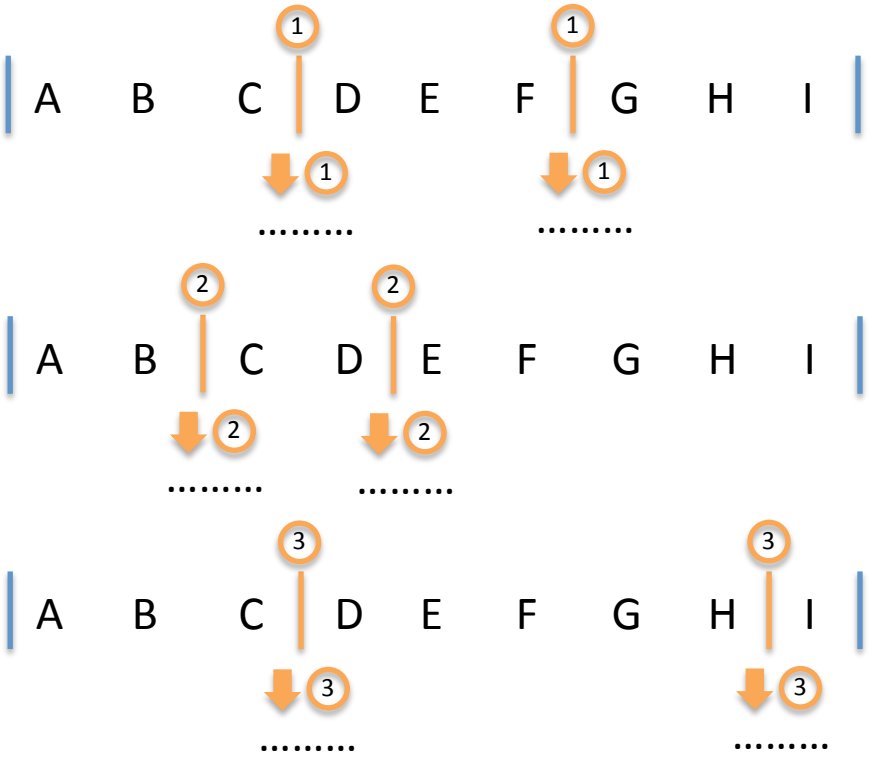
Binary (2-Way) Hierarchical Algorithm



One Pot (*n*-Way) Hierarchical Algorithm

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





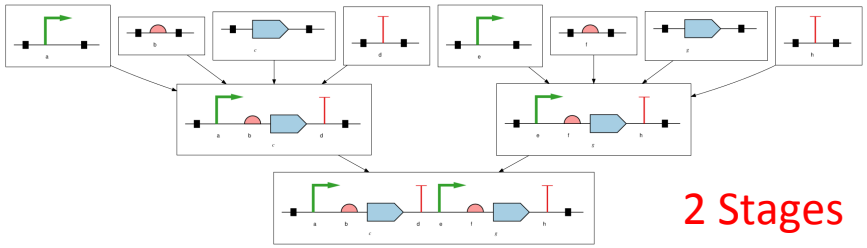
If n = number of possible breaks, k = number of selected breaks, n choose k possible break sets for each recursive call

Search all possible break sets of size 1 to upper limit

= in library, known cost

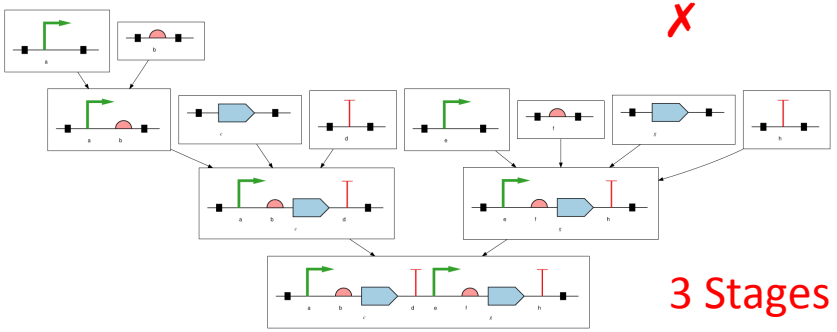
Hierarchical Assembly Heuristics

Heuristic #1: Stages ✓



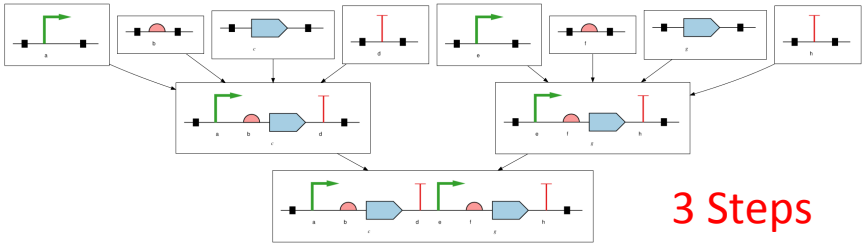
2 Stages

✗



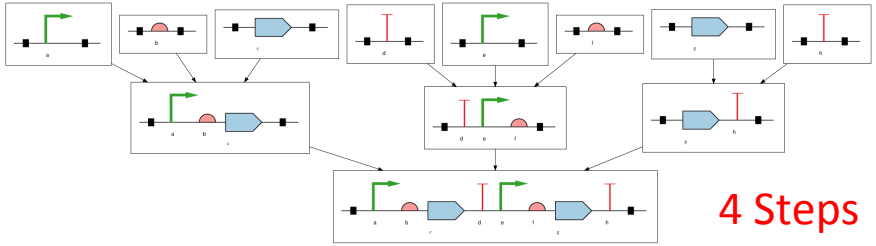
3 Stages

Heuristic #2: Steps ✓



3 Steps

✗



4 Steps

Heuristic #3: Efficiency

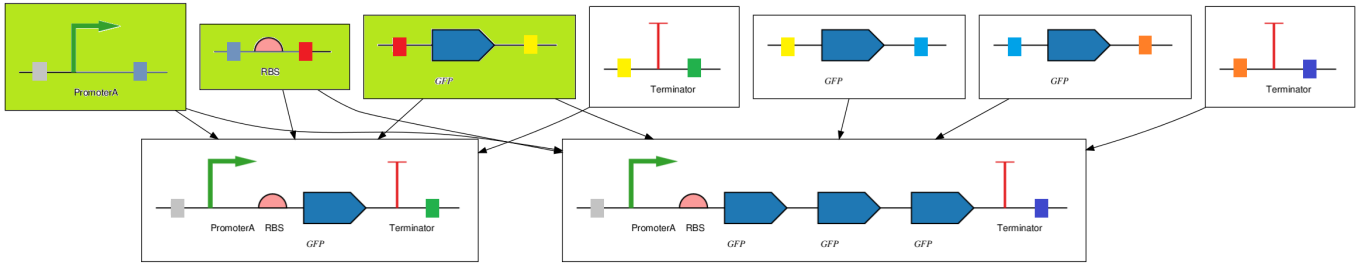
- Efficiency as a function of parts assembled per cloning reaction

Heuristic #4: Modularity and Sharing

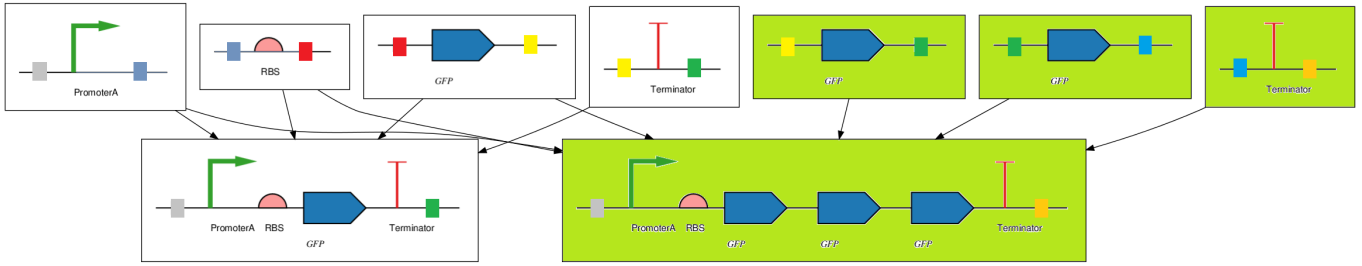
- Plans with opportunities to share intermediates are scored higher

Part Junction Modularity Optimization

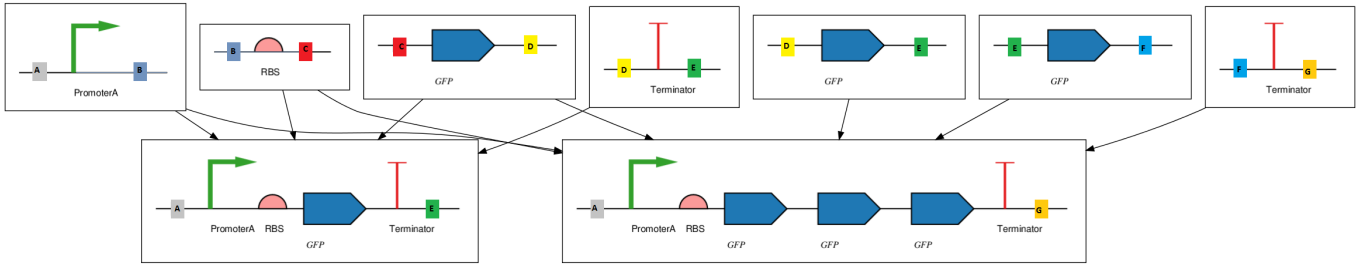
1. Determine which parts can be shared (green shows shared parts)



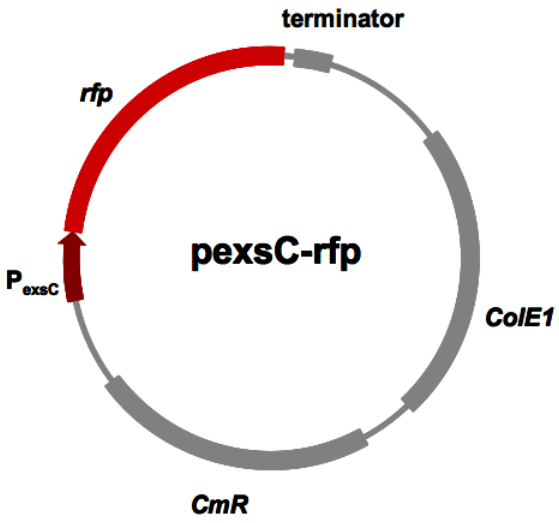
2. Minimize number of overhangs used (green shows parts affected)

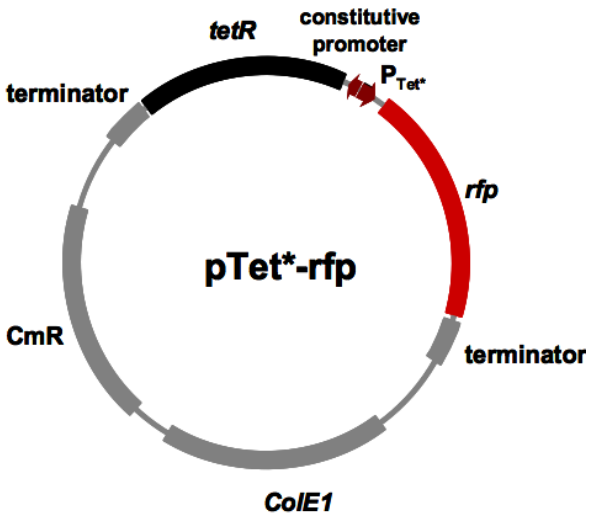


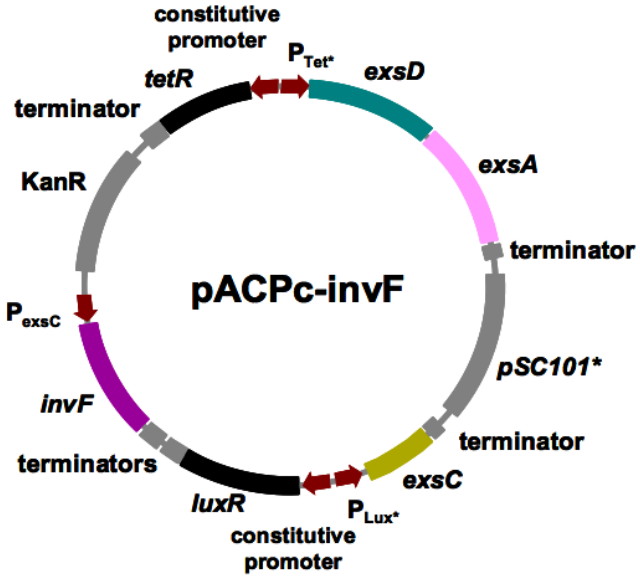
3. Use a library to finalize part junctions



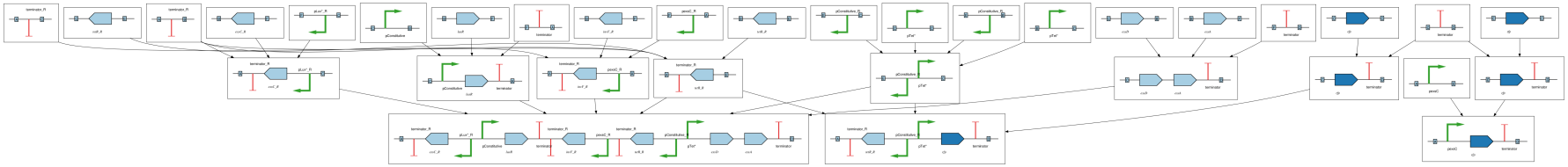
Layered Genetic Logic Gates







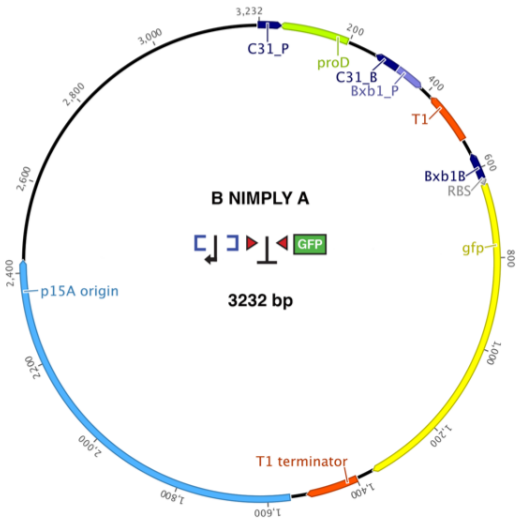
[Moon *et al.* 2012]

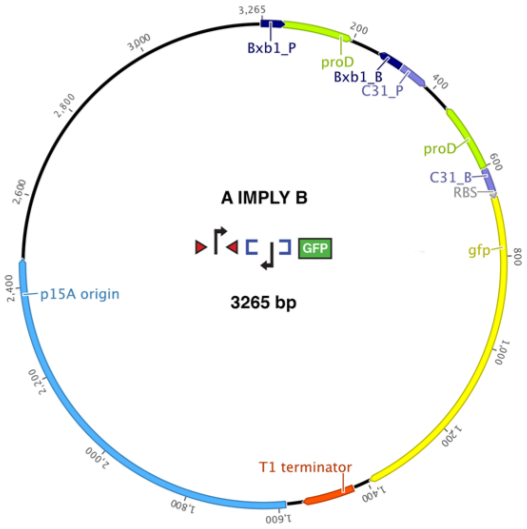


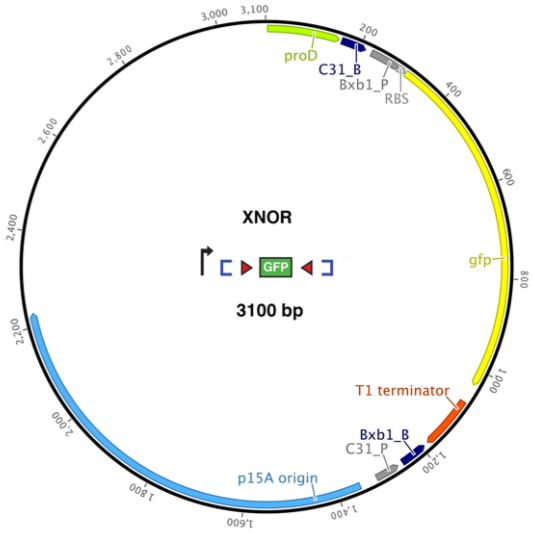
MoClo Assembly

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

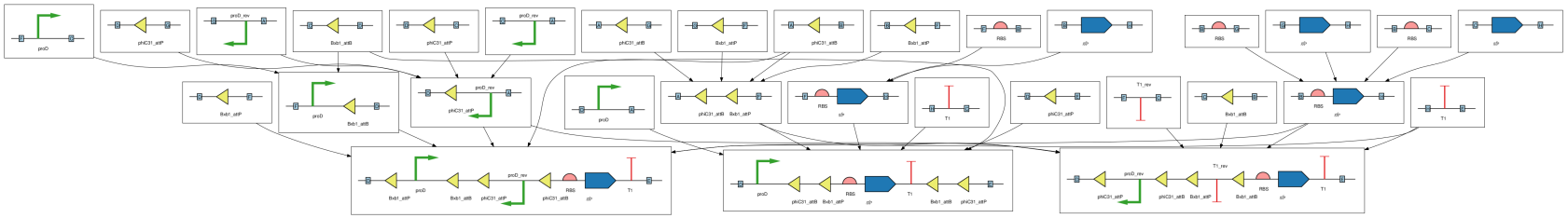
Genetic Logic/Memory Circuits







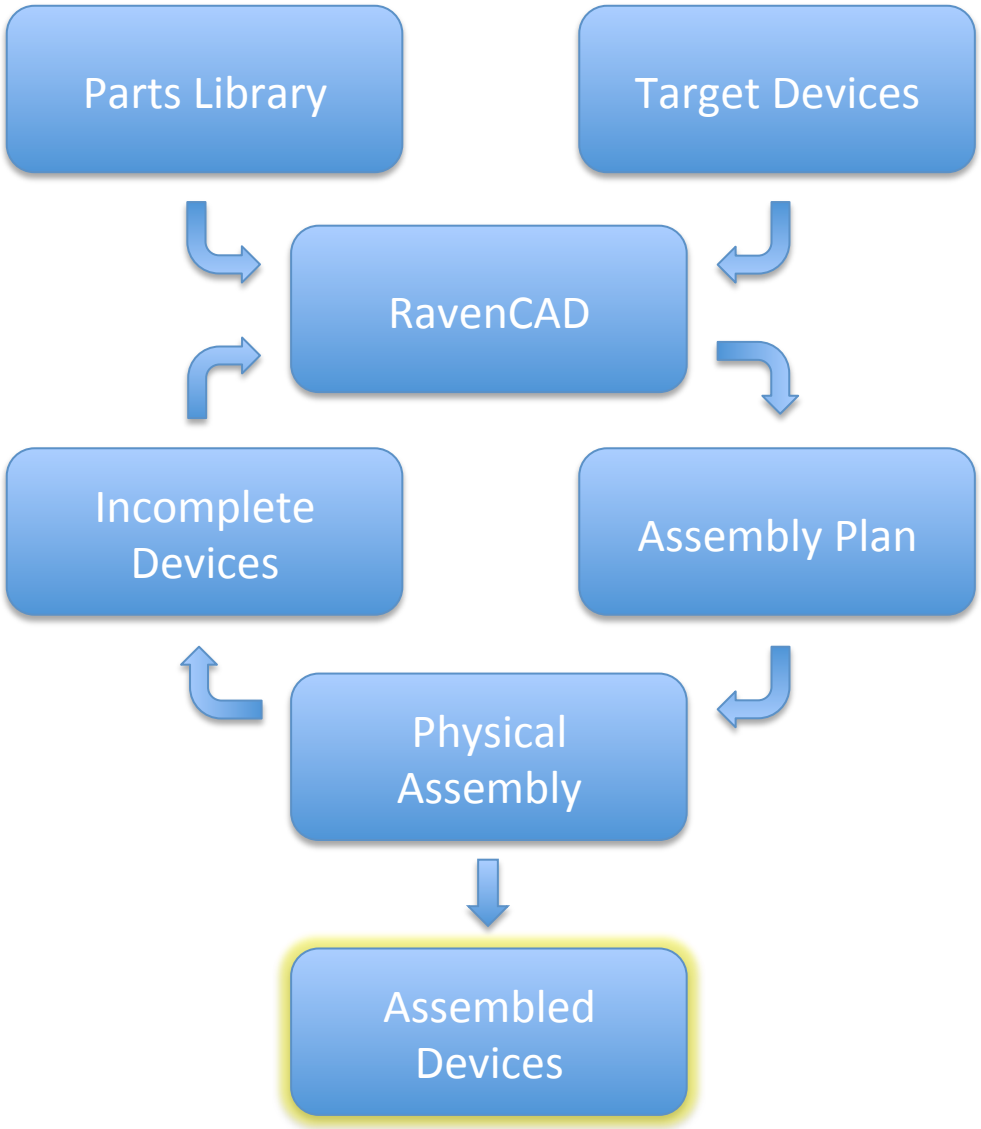
[Suiti *et al.* 2013]



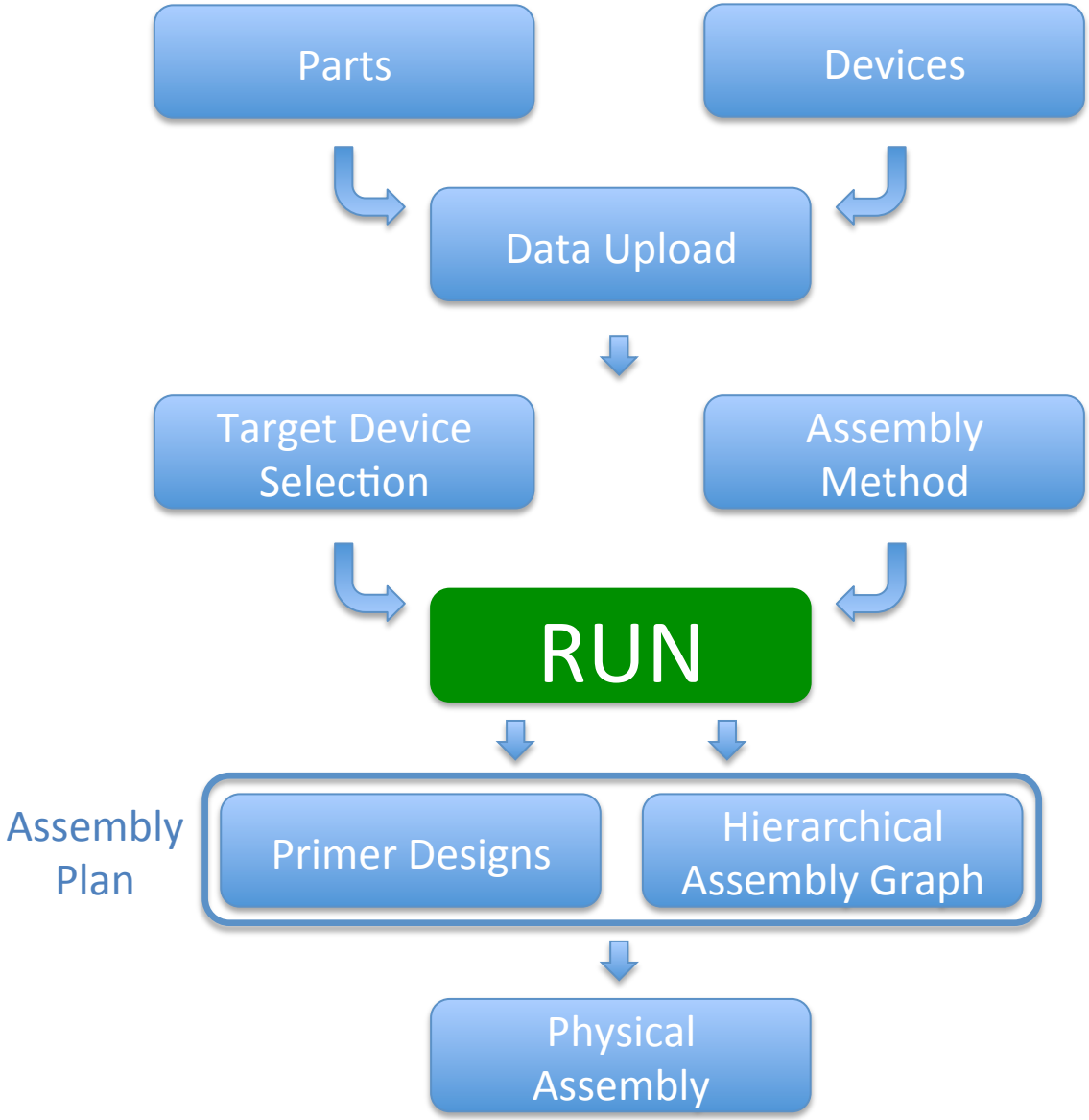
MoClo Assembly

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

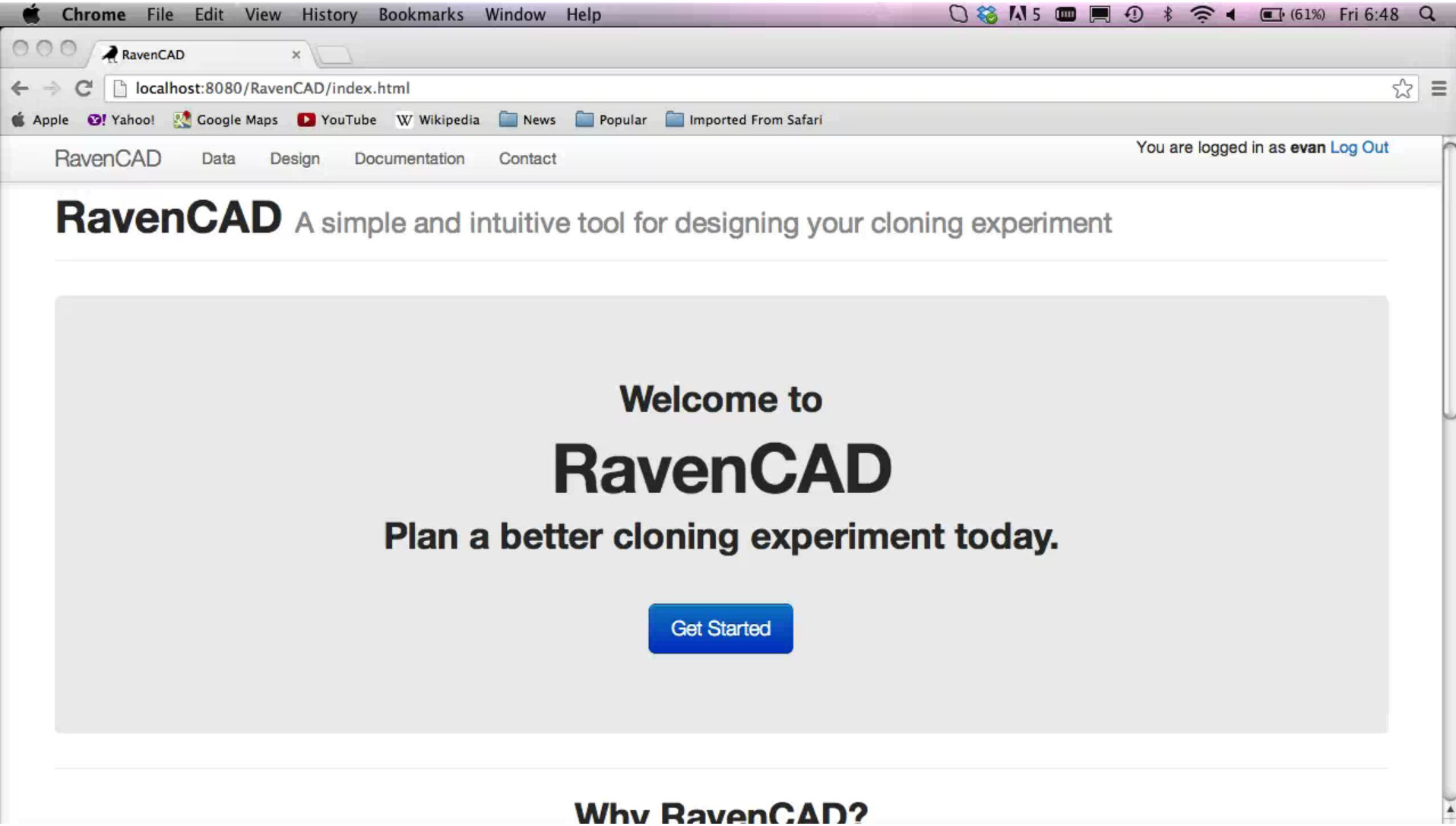
Assembly Workflow



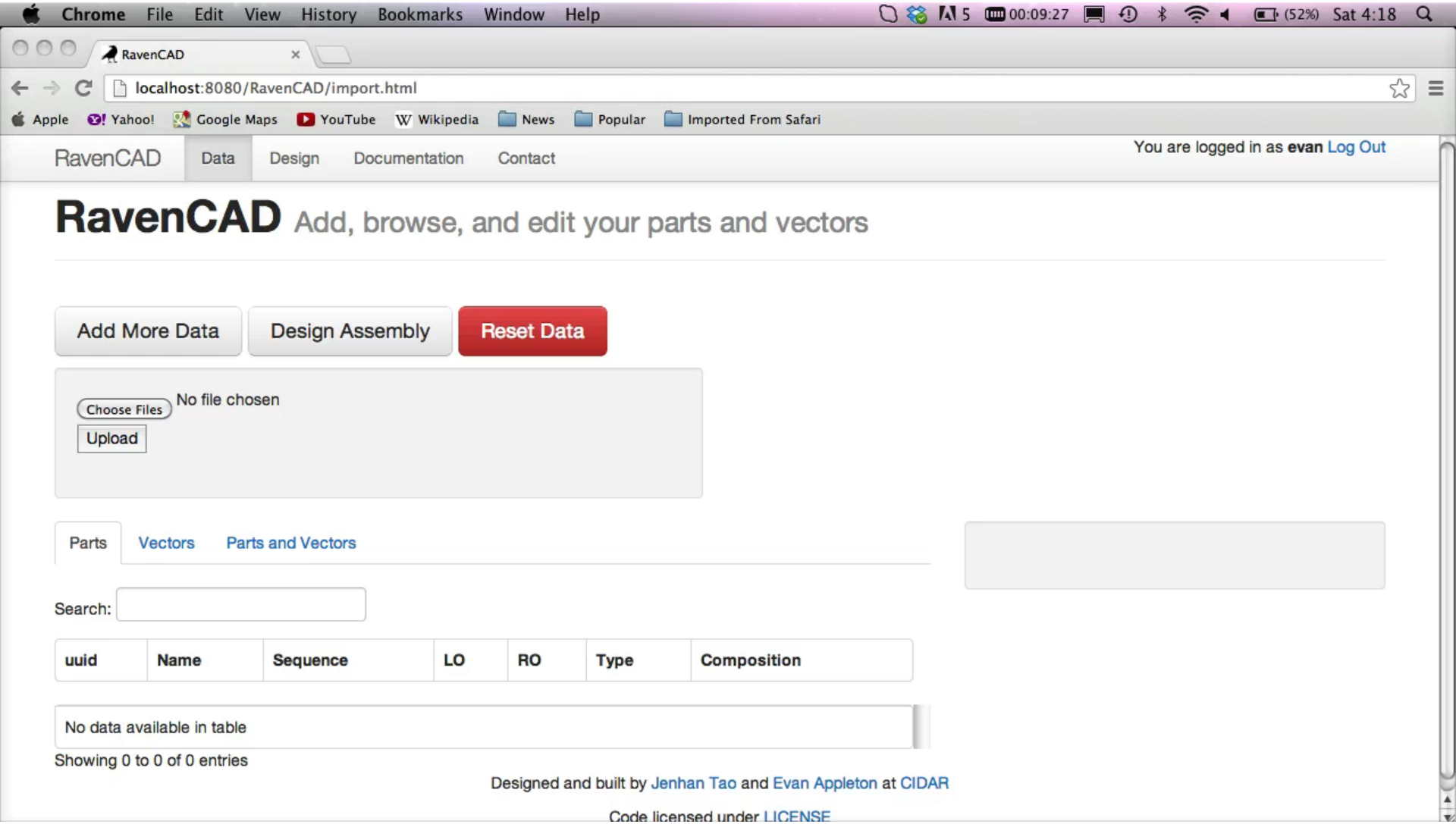
RavenCAD Workflow



RavenCAD Introduction



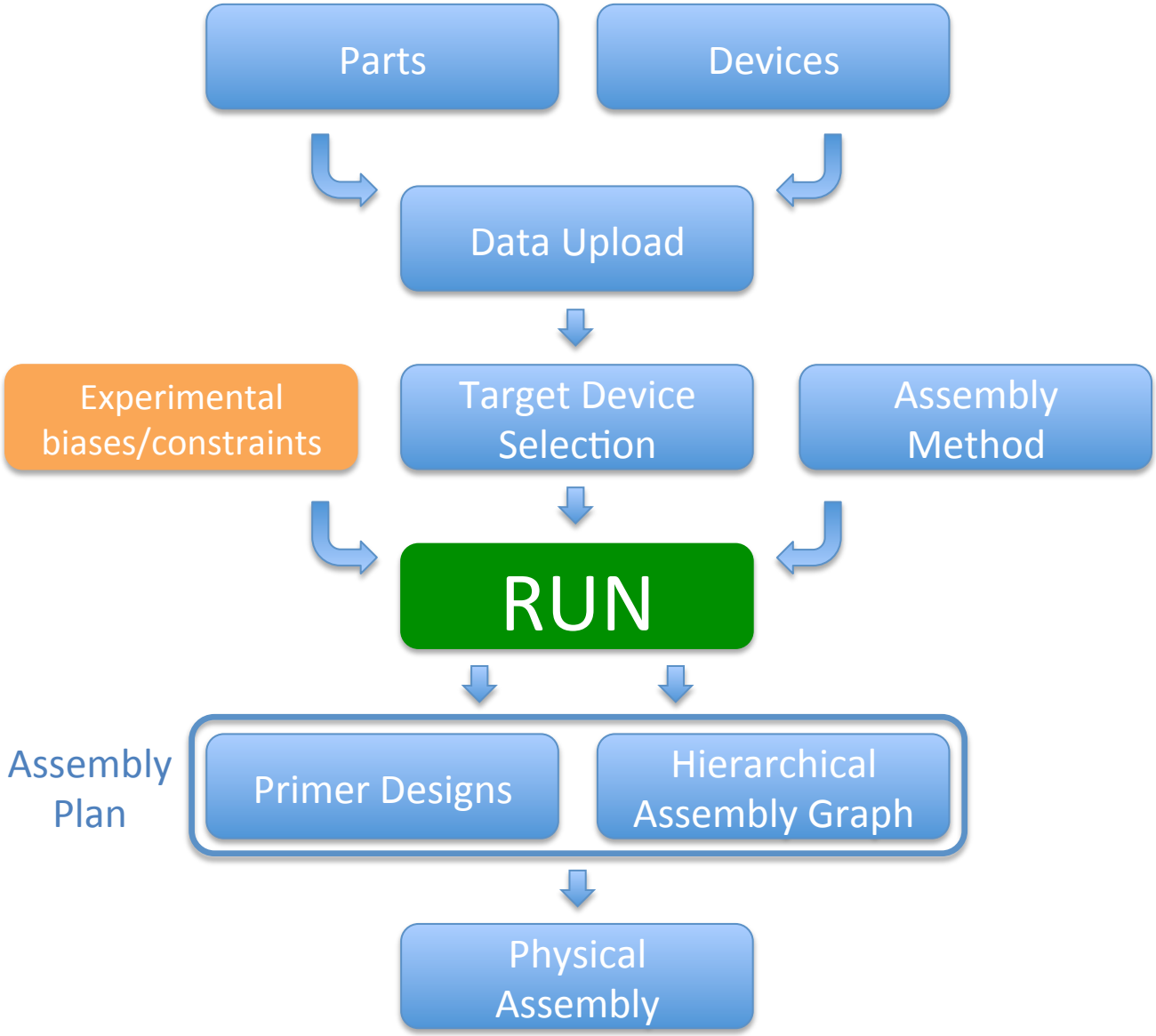
Library Part Re-Use



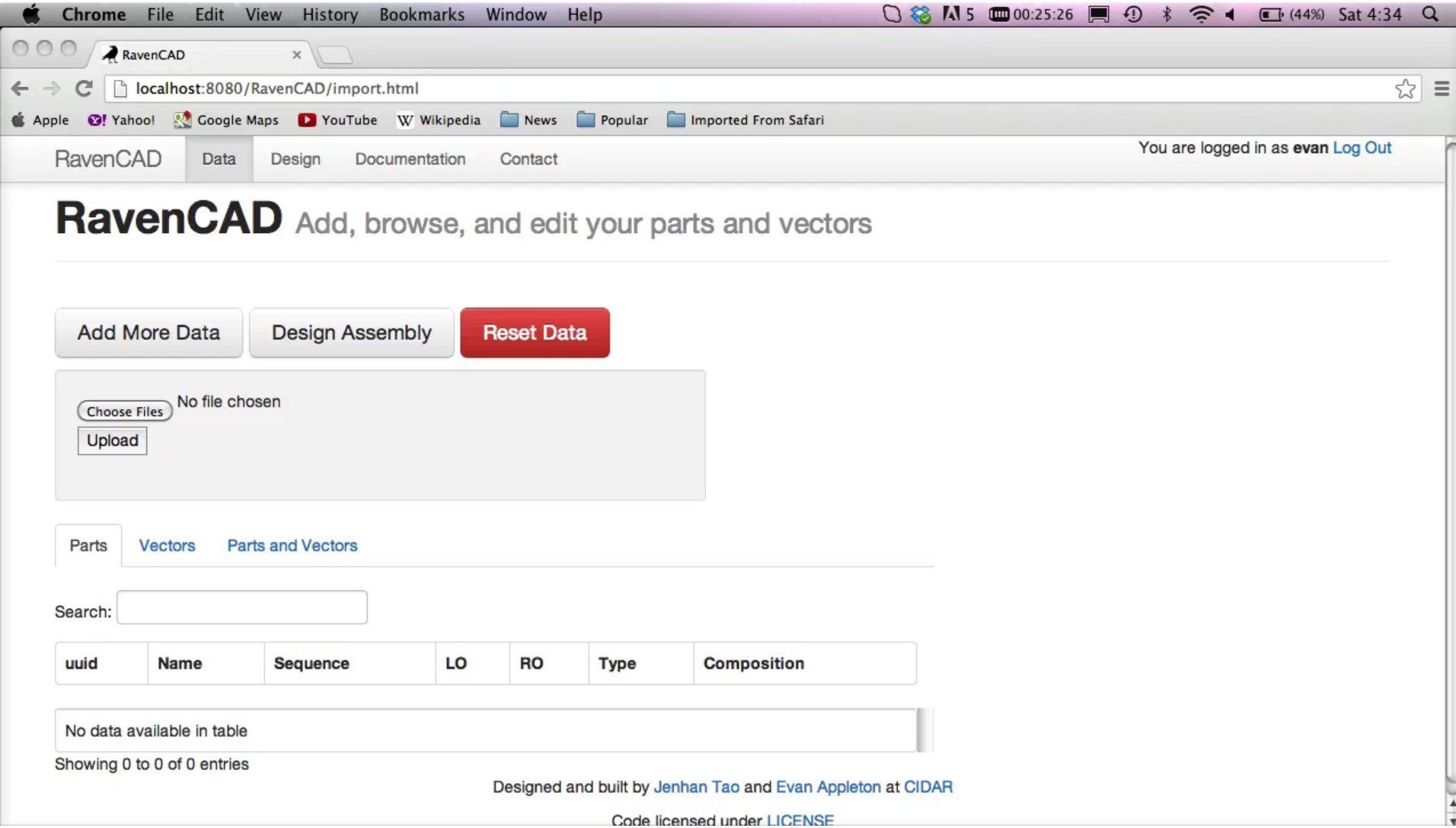
Experimental Input/Feedback

- *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



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
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  - Jenhan Tao
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  - Ernst Oberortner
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    - Aaron Adler
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    - Calin Belta
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  - Weiss Lab (MIT)
    - Ron Weiss
    - Noah Davison