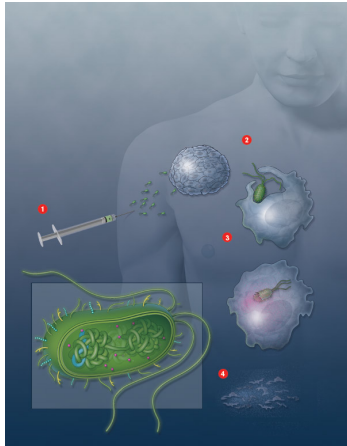


Interactive Experimentally- Driven Algorithms for Optimized DNA Assembly

Evan Appleton, Jenhan Tao & Douglas Densmore
*5th International Workshop on Bio-Design Automation,
Imperial College London
London, England, UK
July 13th, 2013*

Synthetic Biology



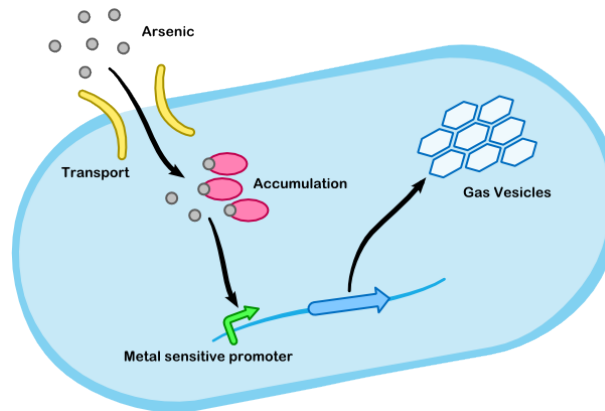
Cancer Killing Bacteria

[Anderson *et al.* 2006]



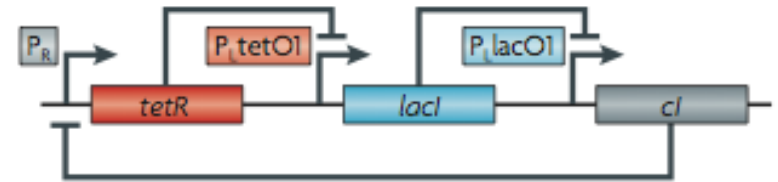
Artemisinin Production

[Ro *et al.* 2006]



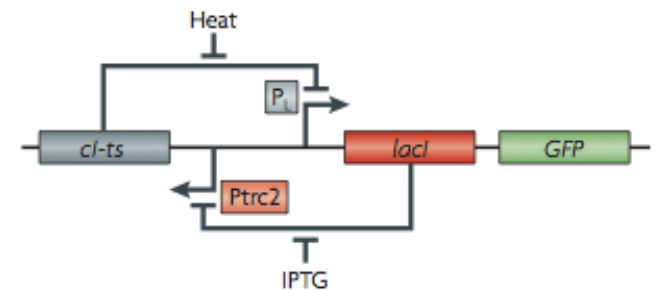
Arsenic Filtering System

Groningen – iGEM 2009



Repressilator

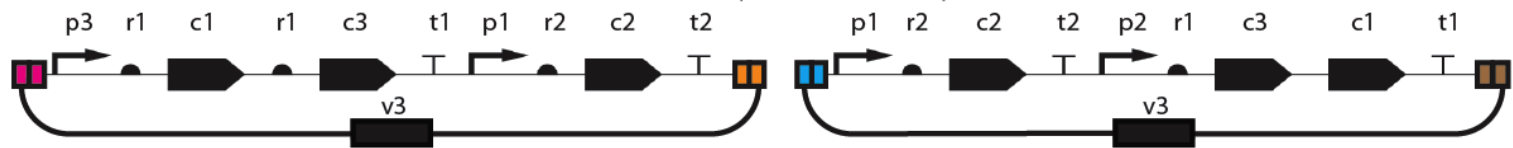
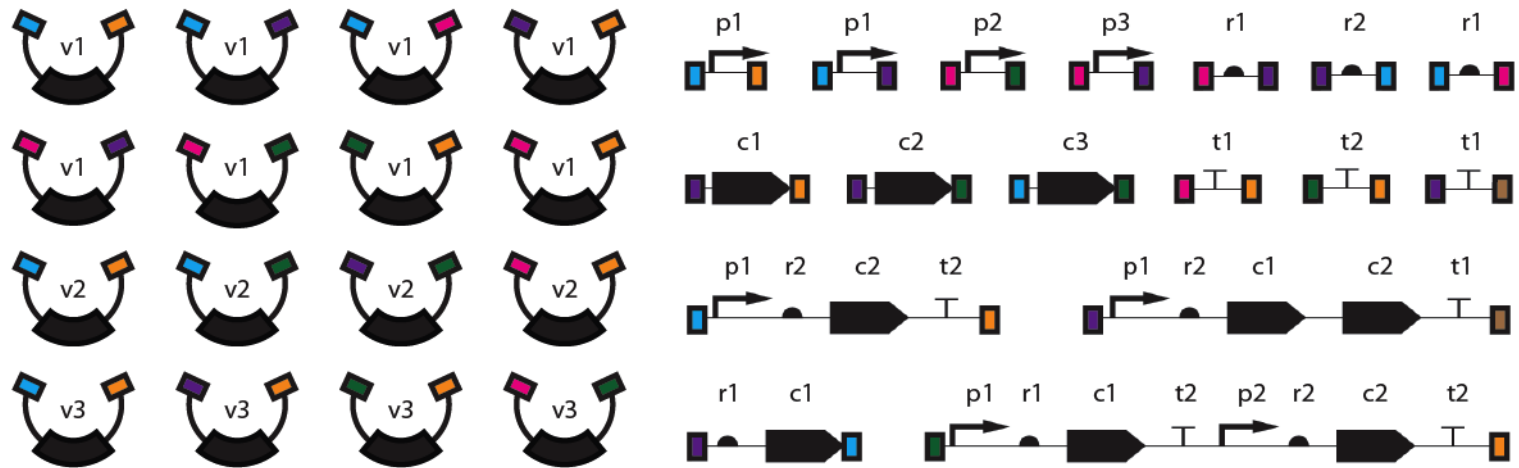
[Elowitz *et al.* 2000]



Toggle Switch

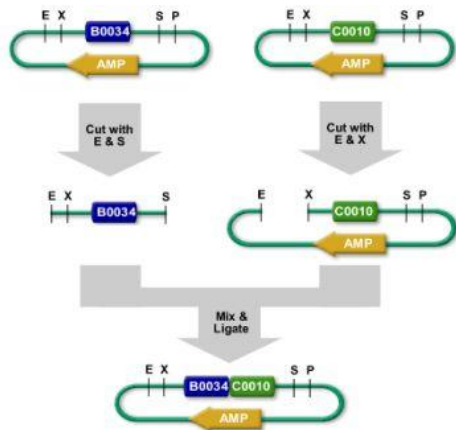
[Gardner *et al.* 2000]

DNA Assembly Planning

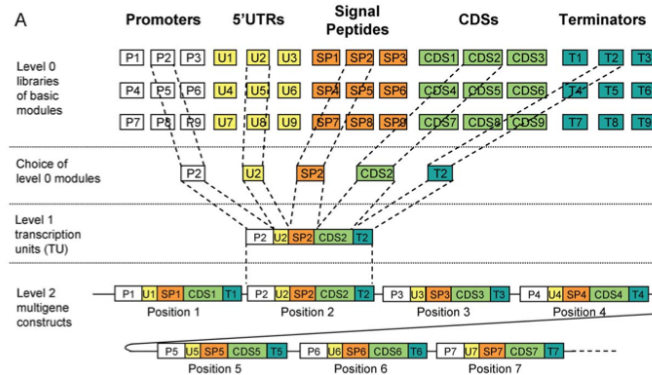


DNA Assembly Methods

BioBricks™ Assembly

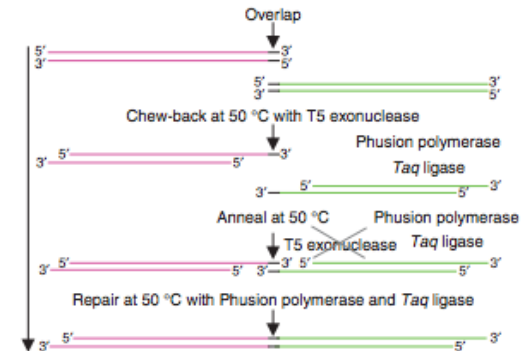


Modular Cloning Assembly



Weber *et al.* 2011

Gibson Assembly



Gibson *et al.* 2009

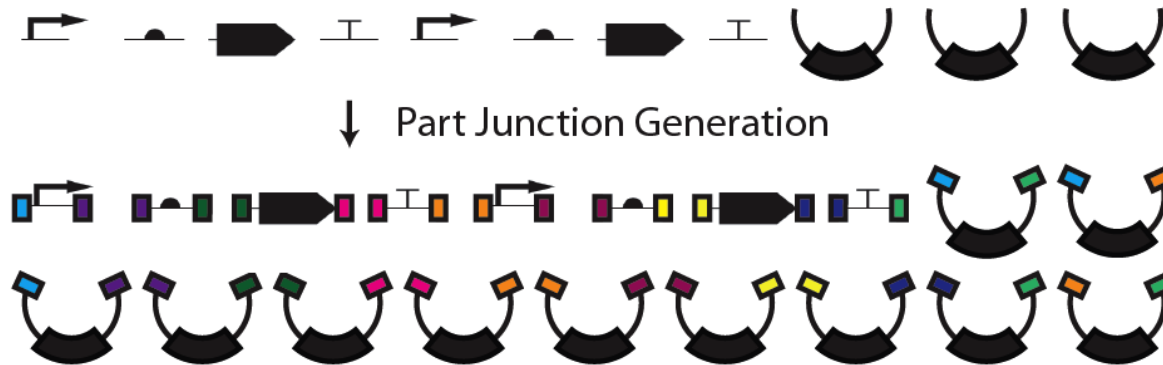
- Binary Assembly
- Assembly site scarring (6bp)
- All part junctions are the same

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

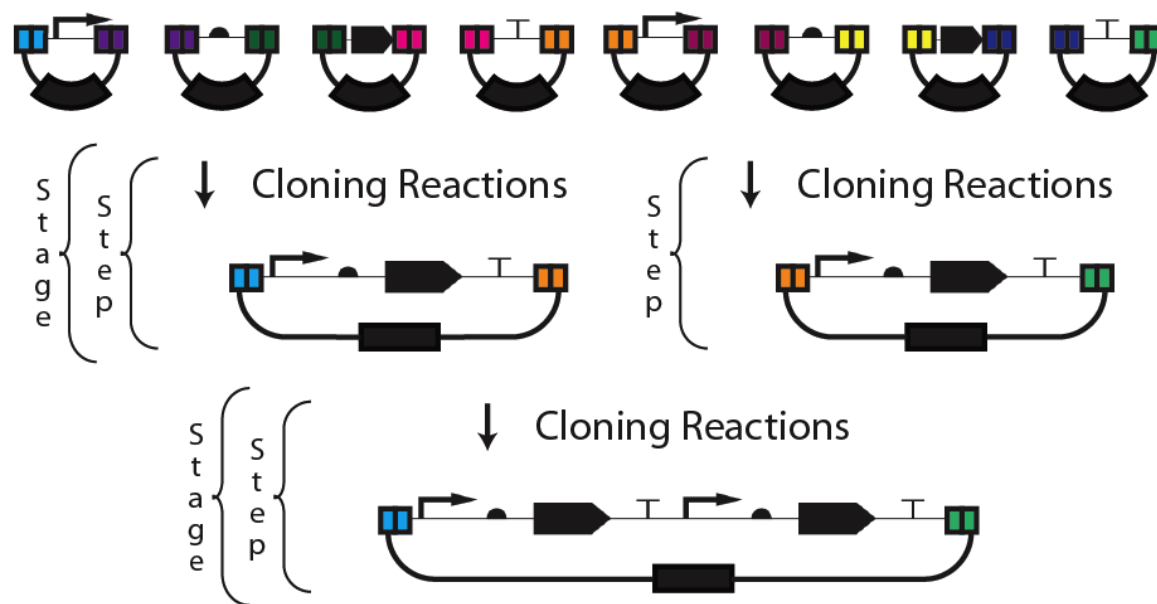
- One-pot assembly
- No assembly site scarring
- All part junctions are unique

Modeling DNA Assembly

1.



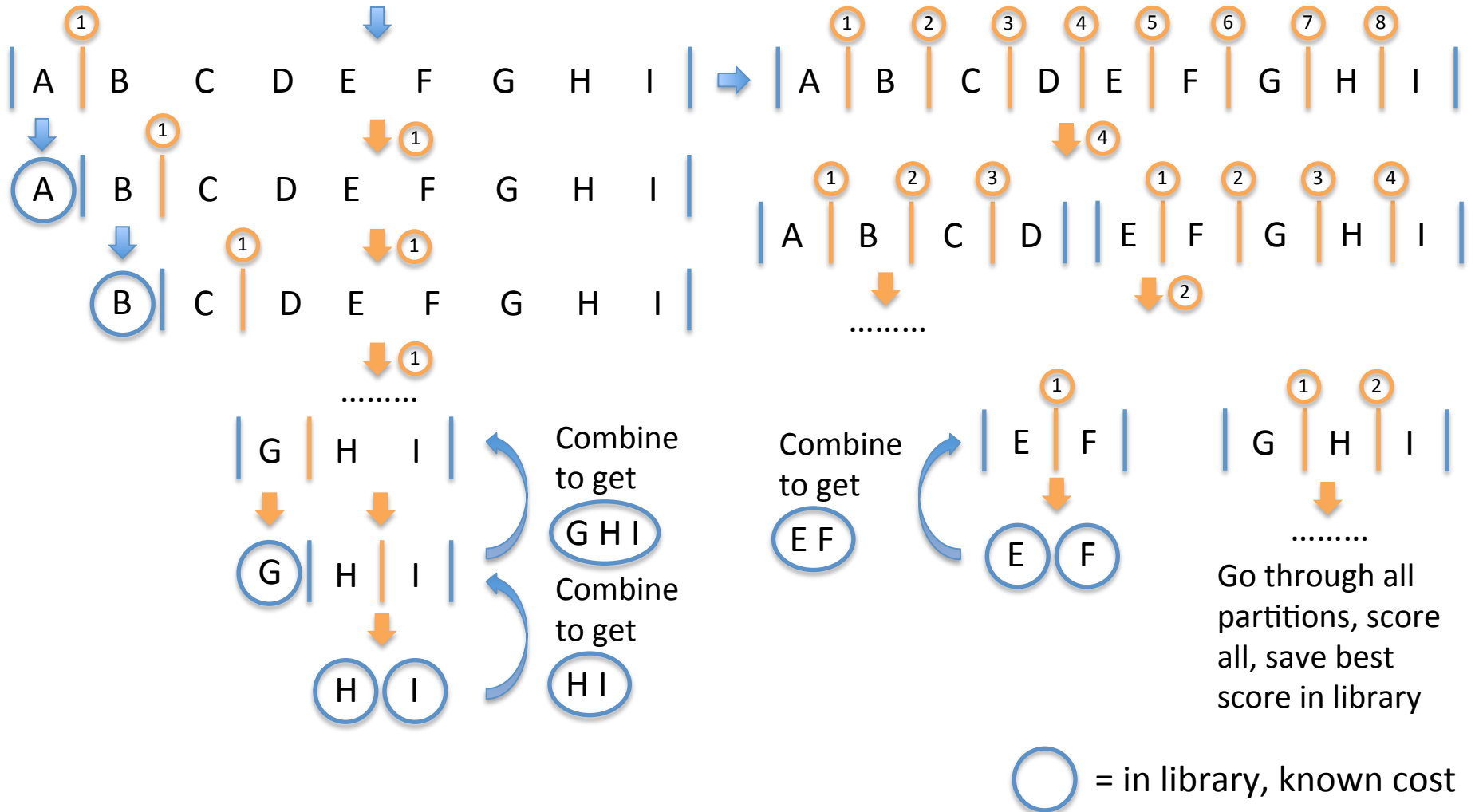
2.



Binary (2-Way) Hierarchical Algorithm

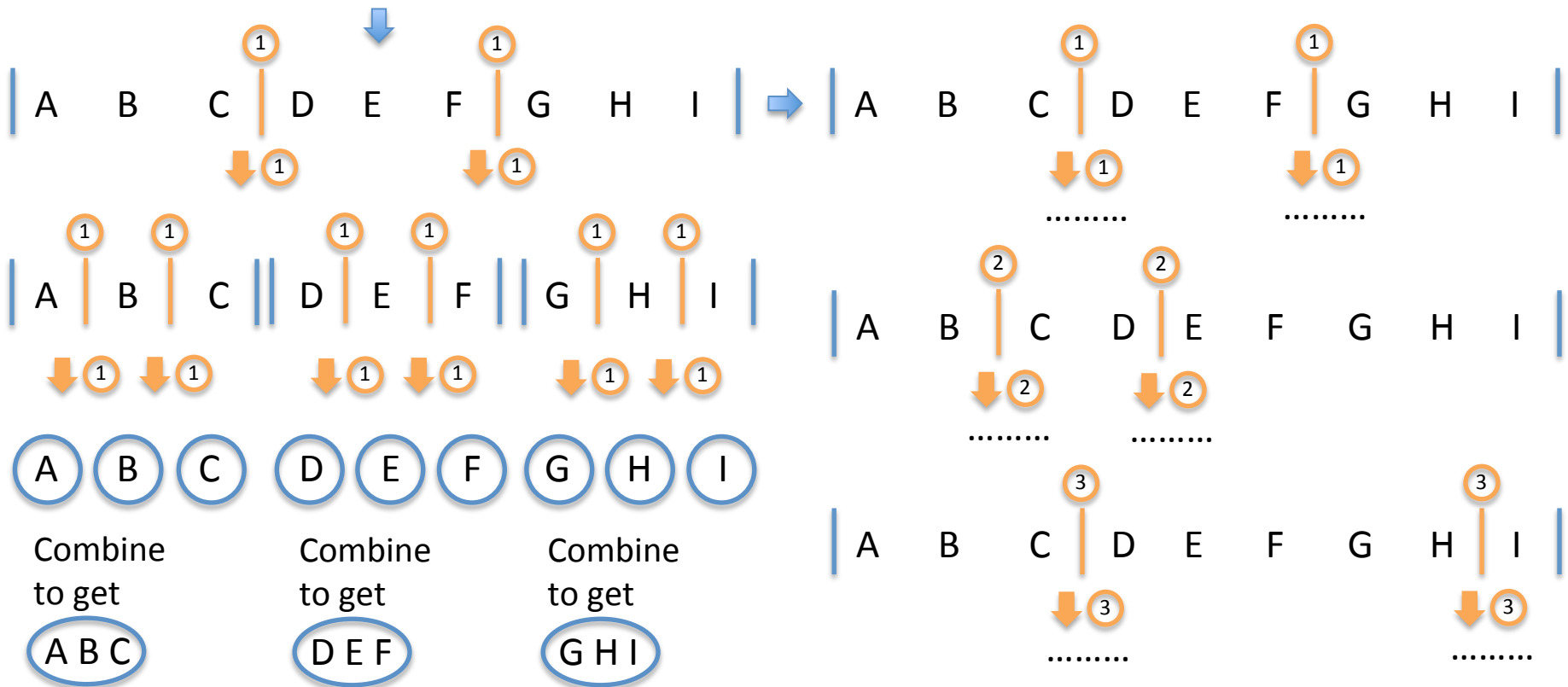
Goal Part: A B C D E F G H I

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



One Pot (n -Way) Hierarchical Algorithm

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



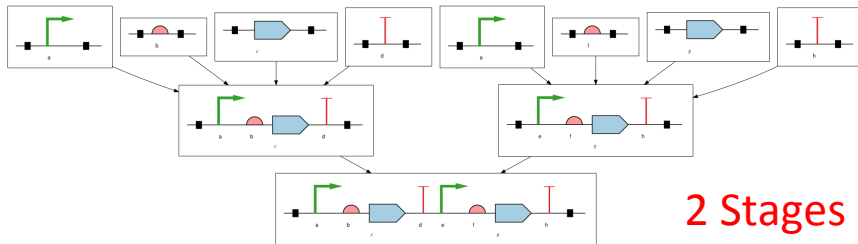
If n = number of possible breaks, k = number of selected breaks, n choose k possible break sets for each recursive call

Search all possible break sets of size 1 to upper limit

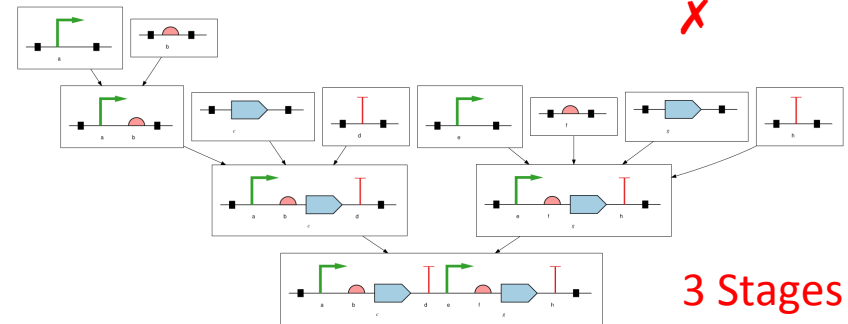
 = in library, known cost

Hierarchical Assembly Heuristics

Heuristic #1: Stages

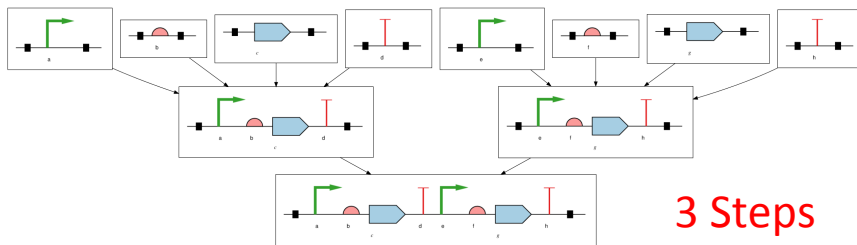


2 Stages

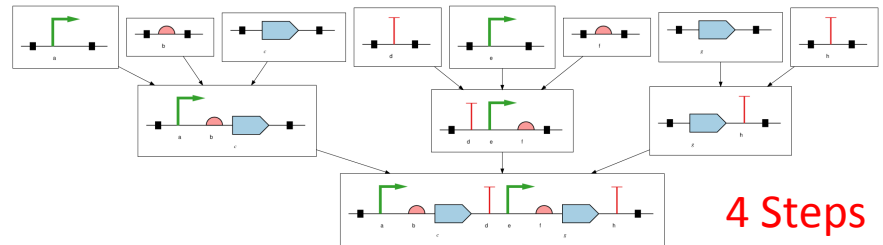


3 Stages

Heuristic #2: Steps



3 Steps



4 Steps

Heuristic #3: Efficiency

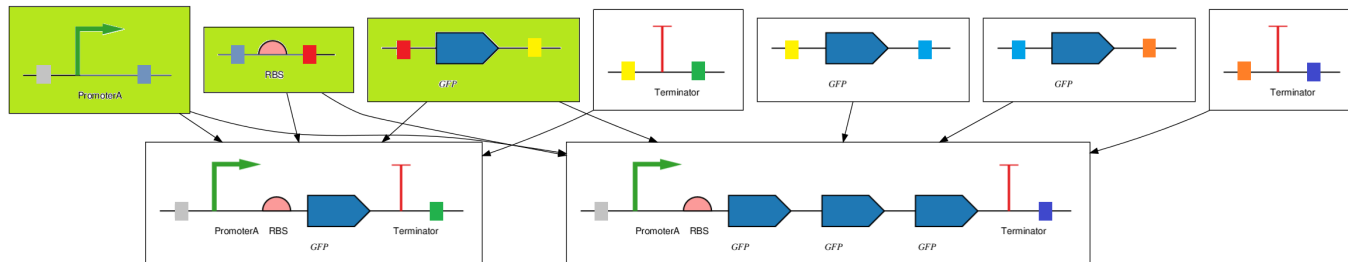
- Efficiency as a function of parts assembled per cloning reaction

Heuristic #4: Modularity and Sharing

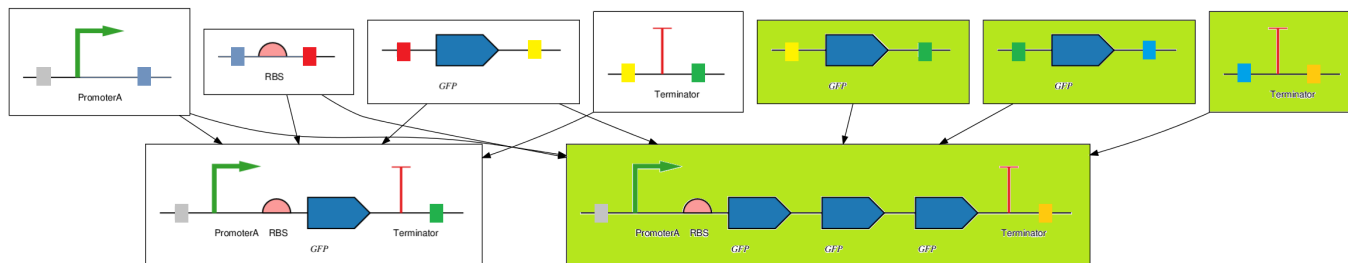
- Plans with opportunities to share intermediates are scored higher

Part Junction Modularity Optimization

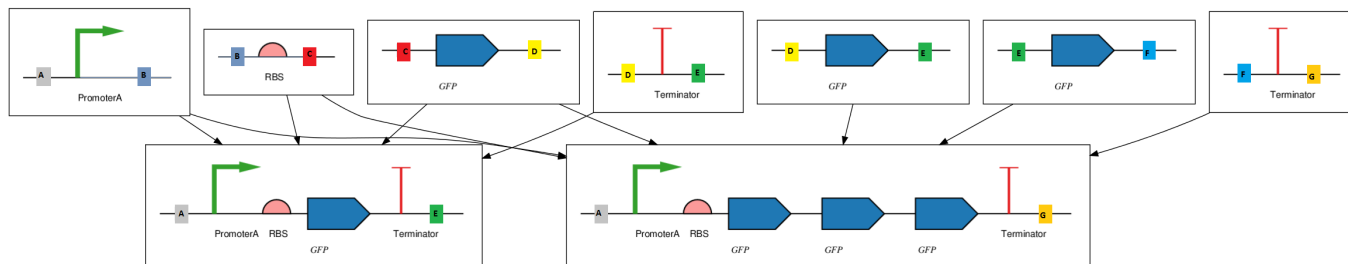
1. Determine which parts can be shared (green shows shared parts)



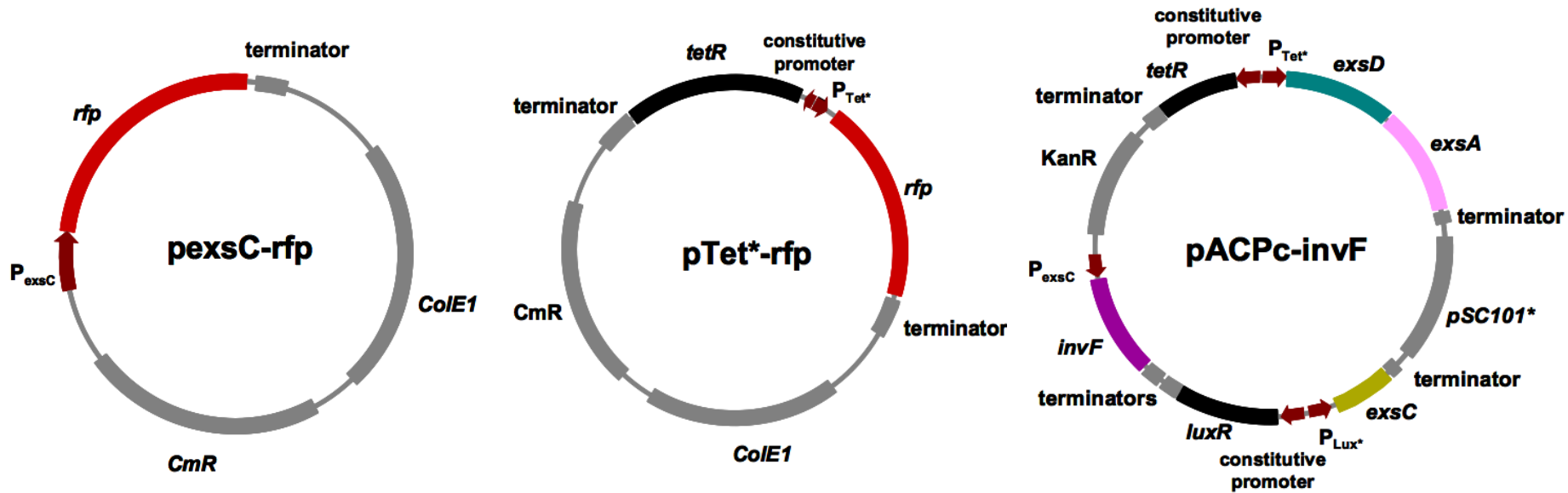
2. Minimize number of overhangs used (green shows parts affected)



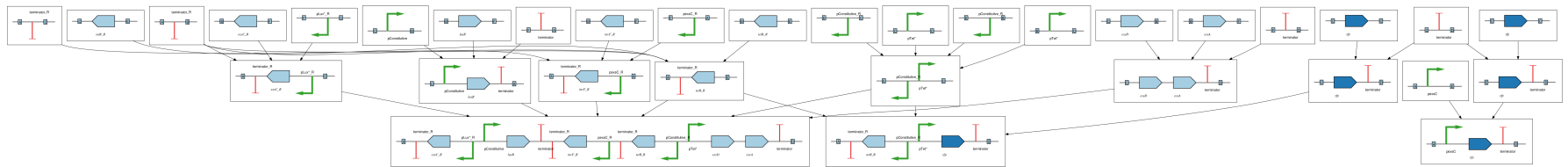
3. Use a library to finalize part junctions



Layered Genetic Logic Gates



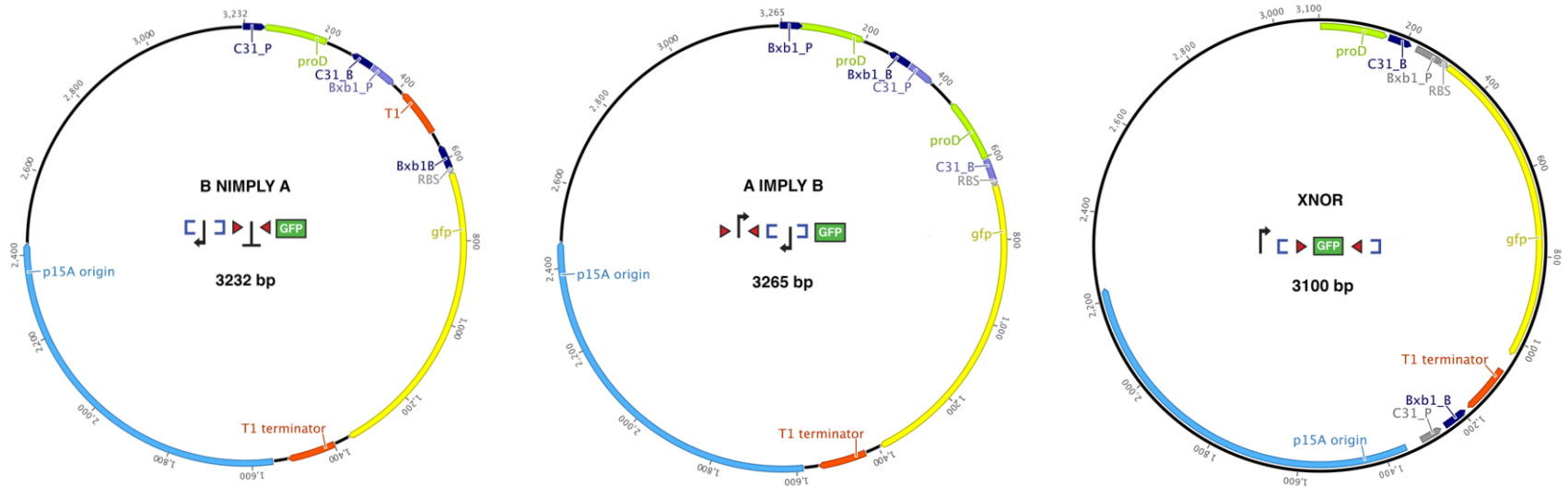
[Moon *et al.* 2012]



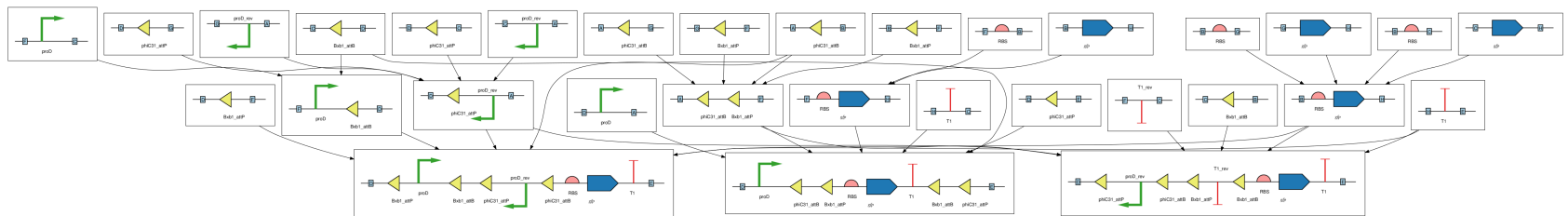
MoClo Assembly

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

Genetic Logic/Memory Circuits



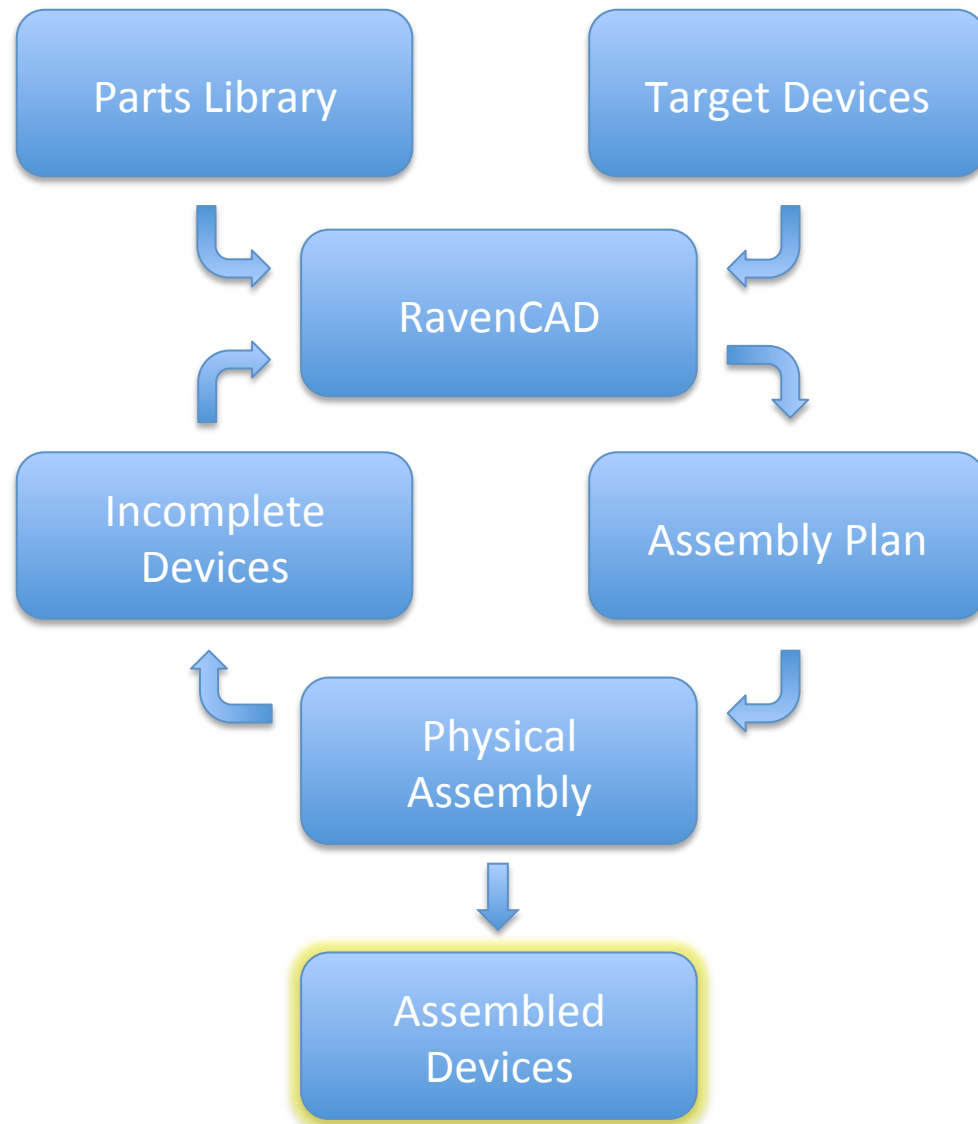
[Suiti *et al.* 2013]



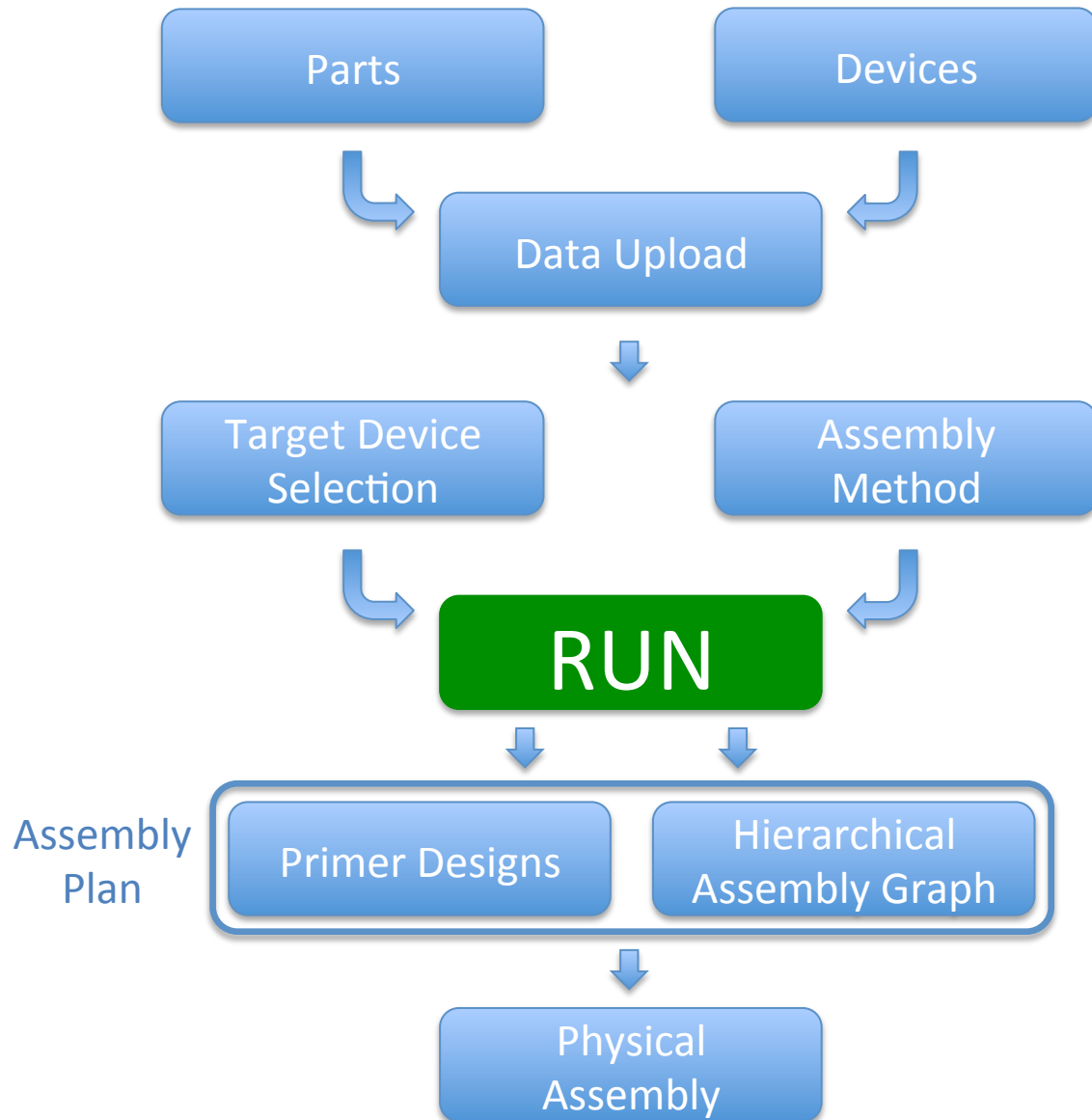
MoClo Assembly

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

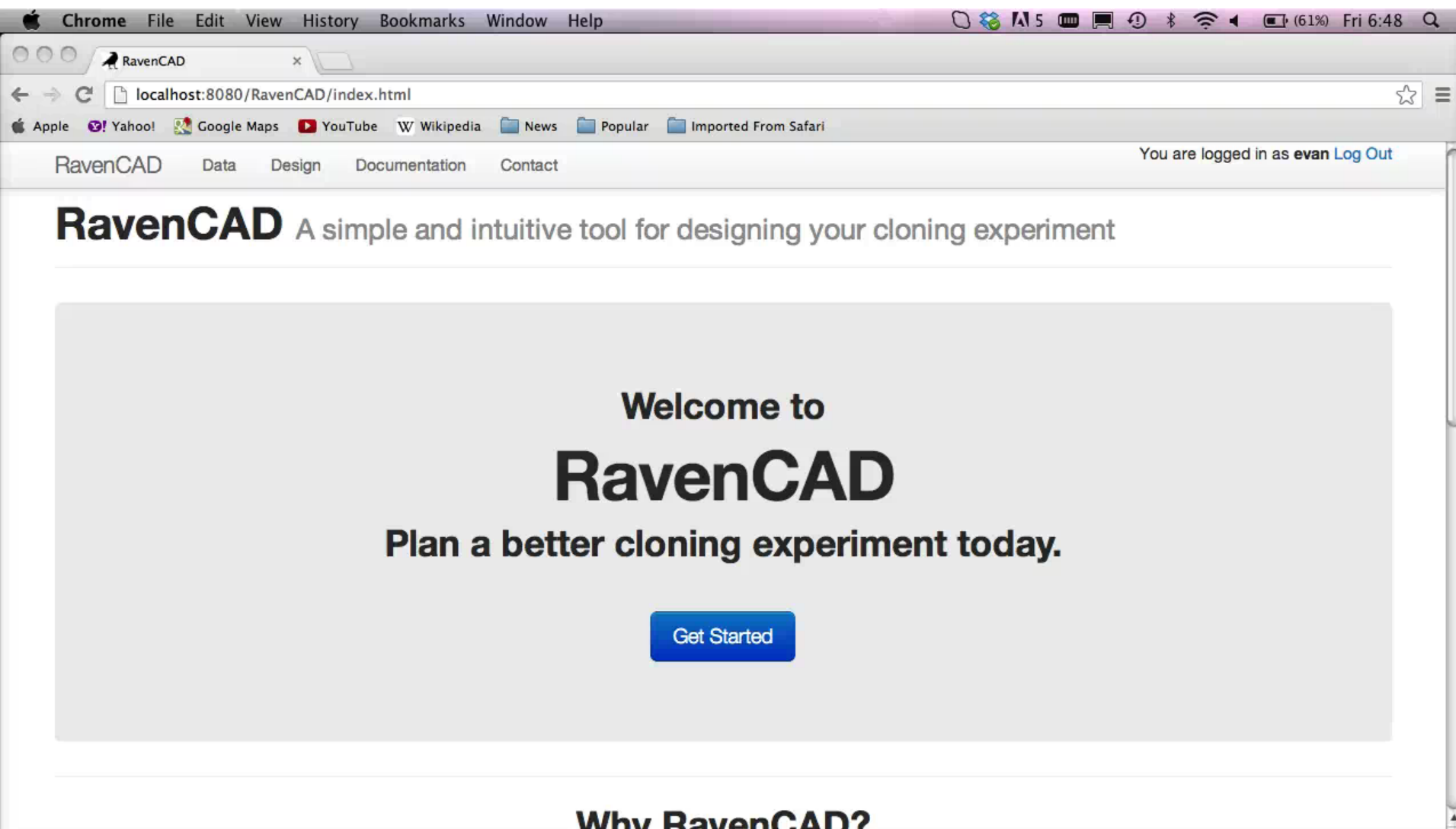
Assembly Workflow



RavenCAD Workflow



RavenCAD Introduction



Library Part Re-Use

The screenshot shows a web browser window with the address bar displaying `localhost:8080/RavenCAD/import.html`. The page title is "RavenCAD" and the navigation bar includes links for "Data", "Design", "Documentation", and "Contact". A user is logged in as "evan" with a "Log Out" link.

The main heading is "RavenCAD Add, browse, and edit your parts and vectors". Below this, there are three buttons: "Add More Data", "Design Assembly", and "Reset Data".

A file upload section contains a "Choose Files" button, an "Upload" button, and the text "No file chosen".

Below the upload section, there are tabs for "Parts", "Vectors", and "Parts and Vectors". The "Vectors" tab is currently selected.

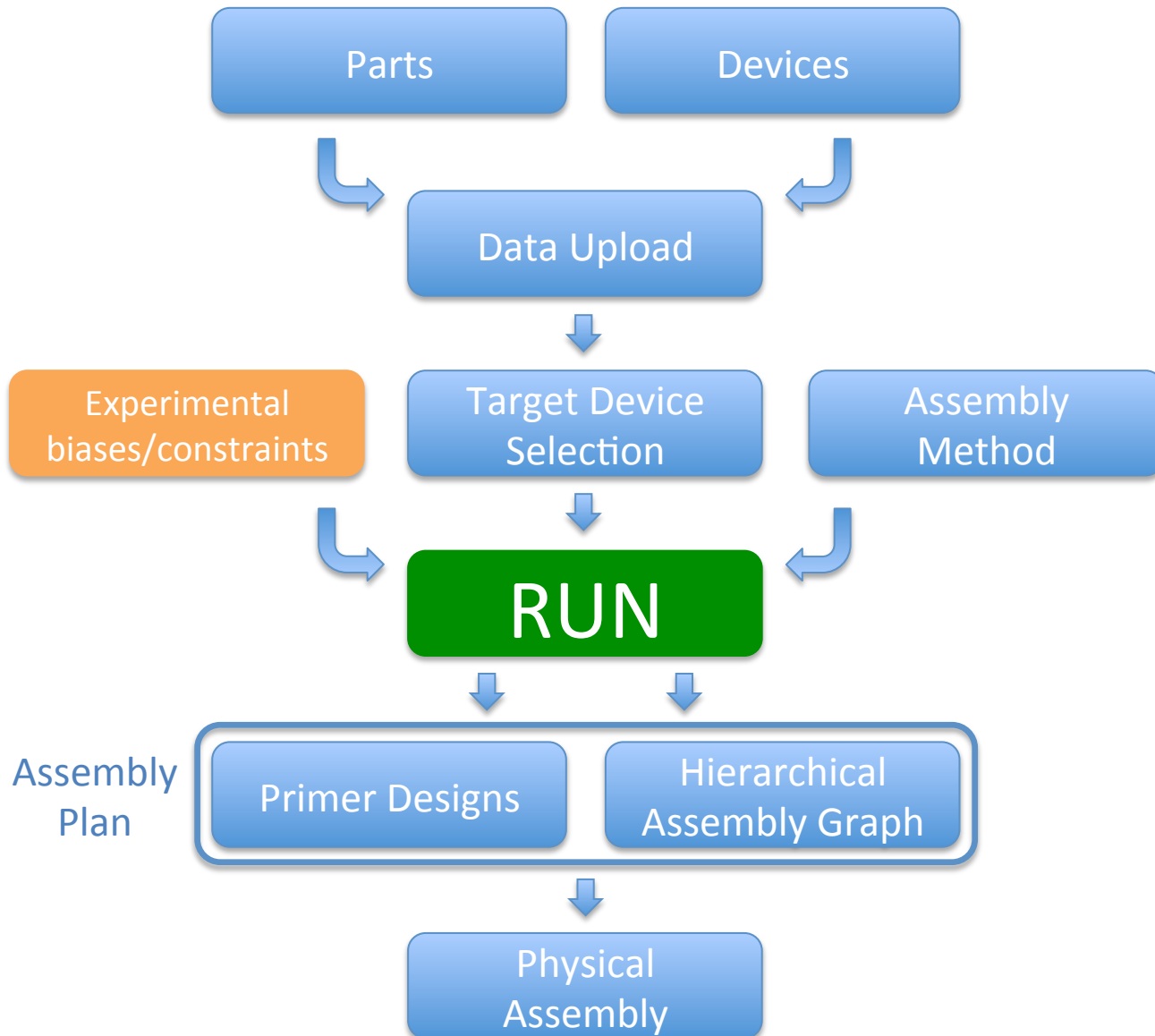
A search bar is labeled "Search:". Below it is a table with the following columns: "uuid", "Name", "Sequence", "LO", "RO", "Type", and "Composition". The table is currently empty, with the message "No data available in table" displayed below it.

At the bottom, it says "Showing 0 to 0 of 0 entries". The footer text reads: "Designed and built by [Jenhan Tao](#) and [Evan Appleton](#) at [CIDAR](#). Code licensed under [LICENSE](#)".

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

The screenshot shows a web browser window with the address bar displaying `localhost:8080/RavenCAD/import.html`. The page title is "RavenCAD" and the navigation bar includes links for "Data", "Design", "Documentation", and "Contact". The user is logged in as "evan" with a "Log Out" link.

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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
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 - Noah Davison