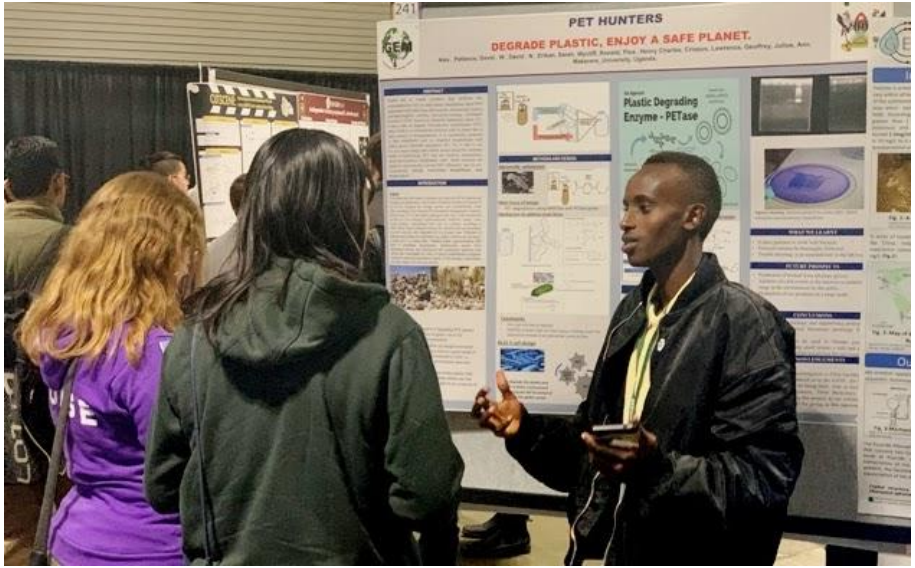
Getting started

Week 1
iGEM 2020 Summer webinars

Alexis Casas

Where do you start ? Where do you end ?

Team Marburg 2018, finalist presentation



Team Makere 2018, poster presentation

Team Pasteur Paris 2018

Team Valencia UPV 2016

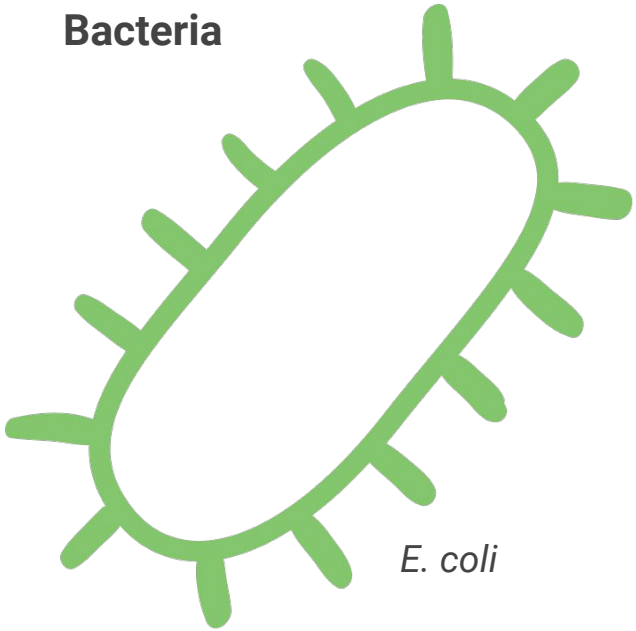
Where do you start ?

- What are you solving ? What are you engineering ?
- What should you design *in-silico* (on your computer) ?
- What are you going to be doing in the lab and why ?
- Choice of organism ?
- How does Human Practices influence your work in the lab ?

Where do you start ?

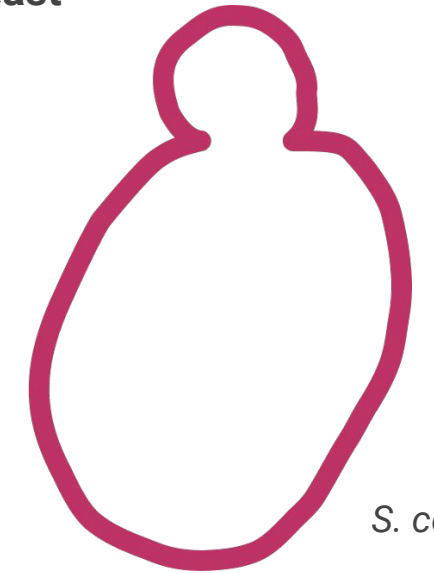
- Choice of organism ?

Bacteria



E. coli

Yeast

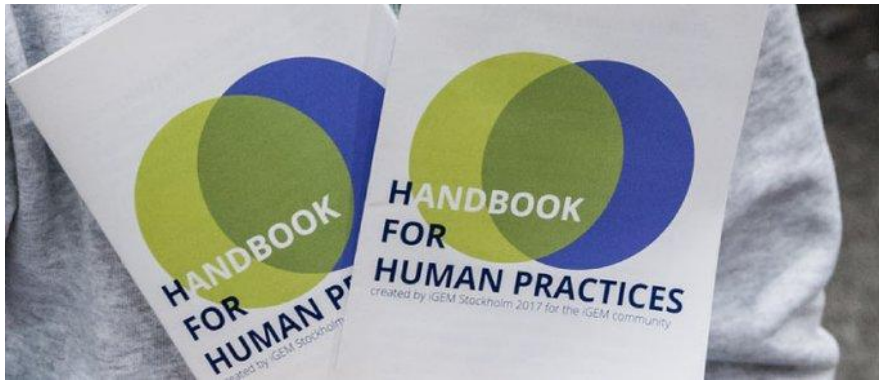


S. cervisae

Other ?

Where do you start ?

- What are you solving ? What are you engineering ?
- What should you design *in-silico* (on your computer) ?
- What are you going to be doing in the lab and why ?
- Choice of organism ?
- How does Human Practices influence your work in the lab ?
  - ➞ Integrated Human Practices



What are you engineering ?

- DNA circuit for protein expression / production
- DNA circuit as a sensor
- Biotransformation
- Data storage in DNA

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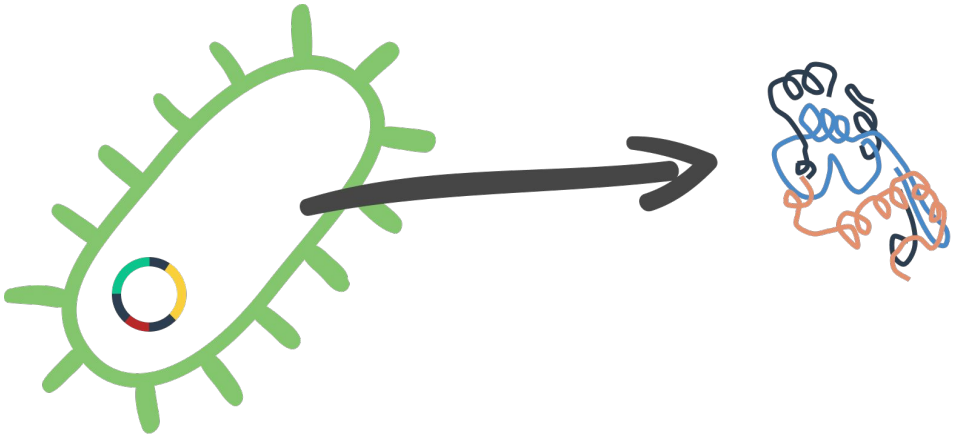

Example: Spider silk protein production
Team GreatBay SZ 2019

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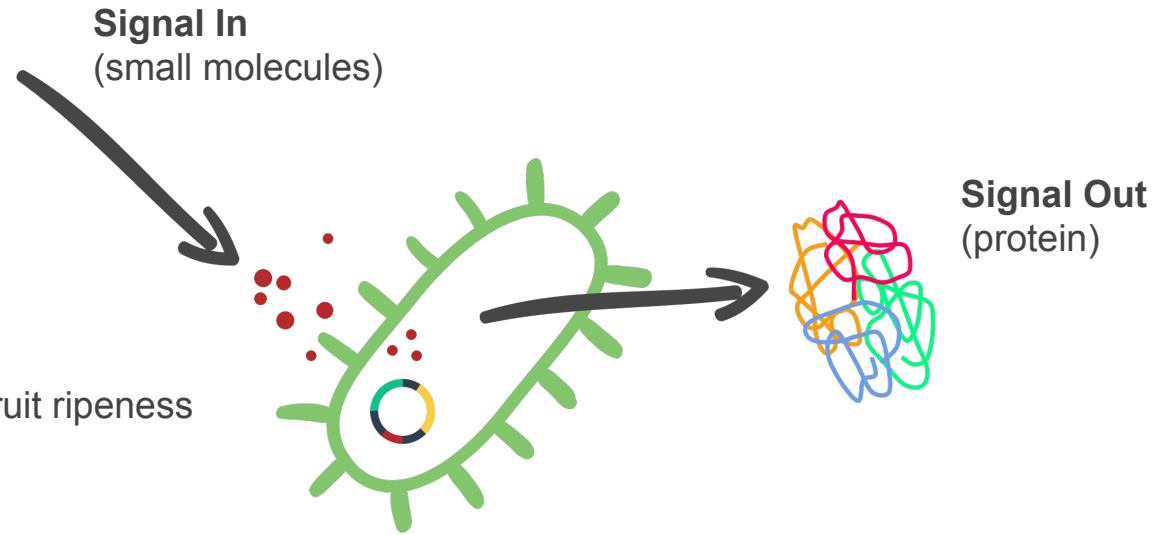

Example: Biosensor to detect fruit ripeness
Team Sydney 2016

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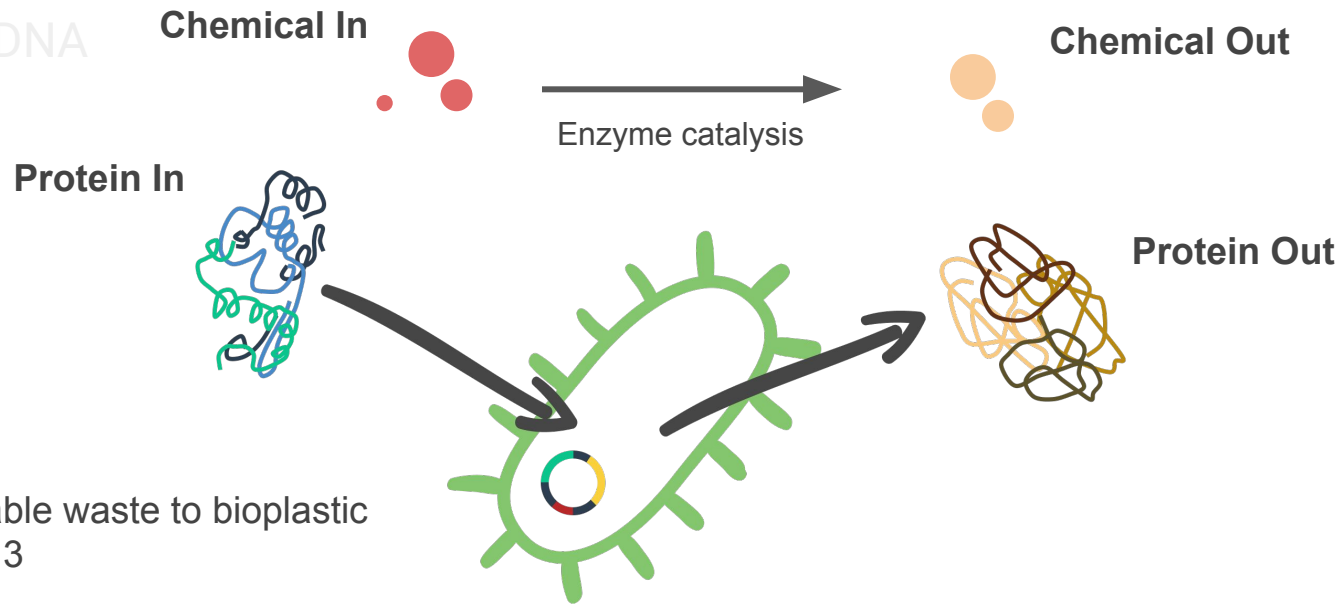

Example: Turn non-recyclable waste to bioplastic
Team Imperial College 2013

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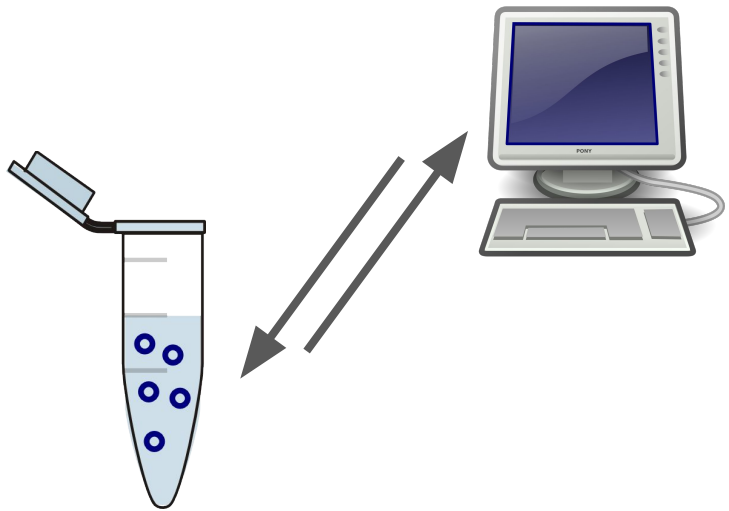


Example: DNA storage and encryption systems
Team Edinburgh 2016, Team Groningen 2016

Resources from iGEM 2020 Opening Week-end

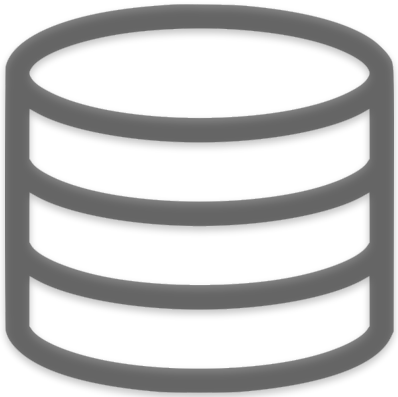
- ***Planning and Designing an iGEM Project***
  - https://youtube.com/wach?v=39nLyxYun38

- ***Cloning and Assembly Plans***
  - https://www.youtube.com/watch?v=f0Q1xeX2xzA

- ***Modelling for Synthetic Biology***
  - *https://www.youtube.com/watch?v=z7isDdt0lS0*

Tools to design your circuit

DNA design tools



Databases

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DNA design tools

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- Databases
  - Find proteins
  - Find genes
  - Find other parts (promoters, RBS etc..

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Next talk: **How to utilize databases effectively** - Ian Schlander

Designing your circuit

- Getting your DNA sequences
  - Where to search ?
  - How to use and do lookups in different databases ?
- Getting your Genes
- Designing your Transcriptional Unit (TU)
  - Choosing the promoter
  - Choosing the RBS
  - Putting the CDS together
  - Choosing the terminator
- Designing your Device (or Circuit)
  - Backbone (=Vector)
  - Resistance

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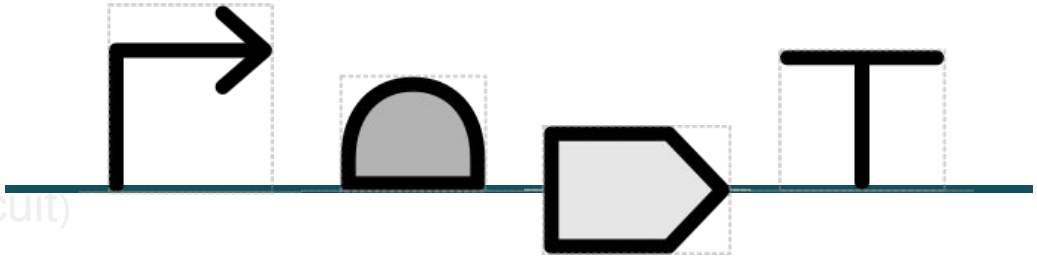

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Next talk: **Cloning Strategy** - Sonja Billerbeck

Building your circuit

- Physically getting the parts and the genes
- Steps to get your DNA from existing plasmid
- Cloning to get many copies of your gene
- Assembling the DNA together

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  Week 2 - **DNA parts and Basic Molecular Biology**

- Cloning to get many copies of your gene
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Cloning Strategy - Sonja Billerbeck

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Week 3 - **DNA assembly techniques**

Expressing / Transformation

- Expression vector
- Cloning in the host organism (=chassis)

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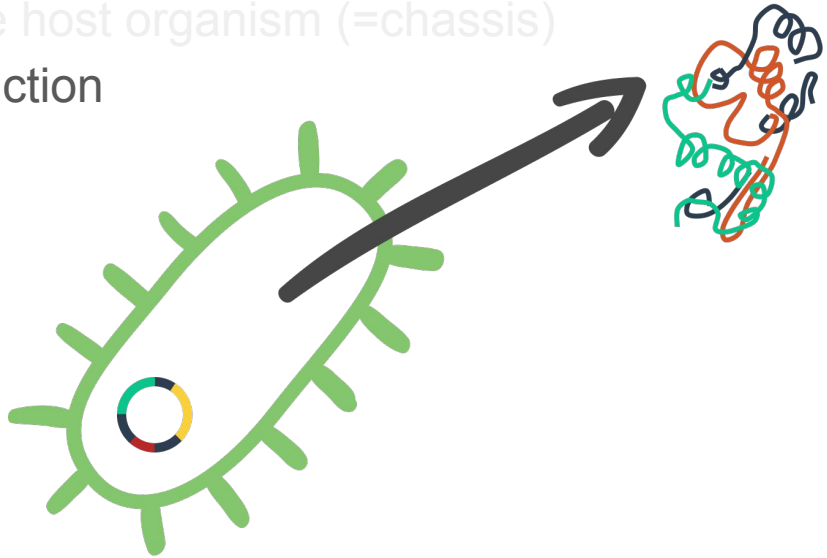
- Cloning in the host organism (=chassis)
- Protein production



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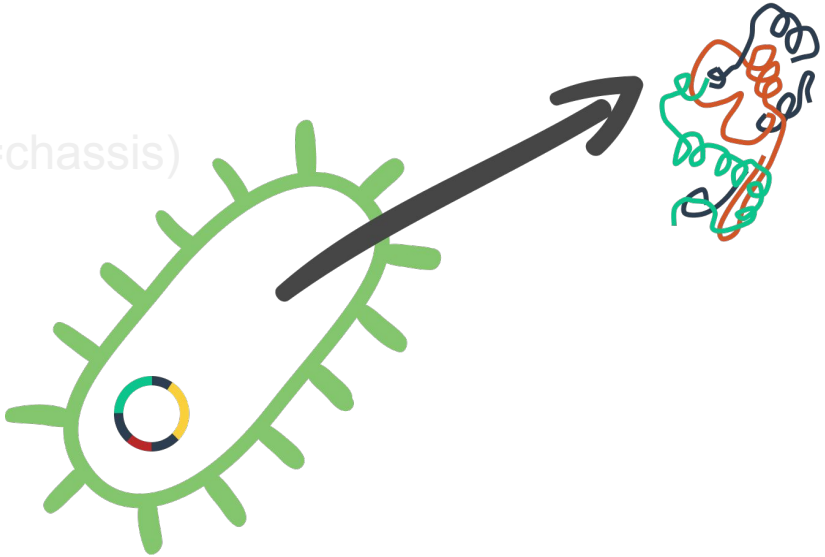


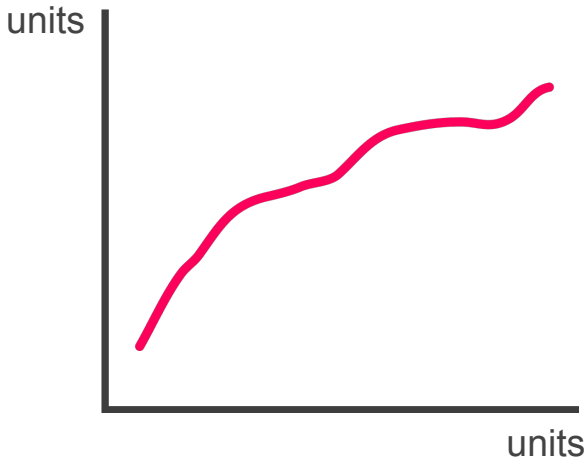
Week 2 - **DNA parts and Basic Molecular Biology**

Week 6 - **Transforming and Sequencing**

Week 8 - **Protein characterization**

Measuring

- What do you measure ?
- Designing your measurement assays



Week 5 - **Quantifying fluorescence and cell count with plate readers**

Troubleshooting

- Verify at each step
  - Gels electrophoresis
  - Sequencing
  - Demos (simulate troubleshooting steps) of software
  - Troubleshooting guides in products (ex: miniprep guide, vendor websites etc..)
  - Other assays
- Discuss with your mentor / PI
- iGEM Measurement Committee Office Hours
  - Biweekly office hours to answer questions
  - Every other Tuesday at 3am EDT and 1pm EDT starting June 9.

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Next Office Hours: **Tuesday 23rd of June 2020**

Future Webinars & Events

Thank you

Future Webinars & Events

The next two sessions in this webinar:

Getting started - Alexis Casas

How to utilize databases effectively - Ian Schlander

Cloning Strategy - Sonja Billerbeck

How to Use Biological Databases and Tools for Beginners

Measurement Committee
Ian Schlander (NREL)

Biological Databases Covered Today

1. **BioCyc** – Sequenced genomes with predicted metabolic pathways
2. **BRENDA** – Enzyme database
3. **UniProt** – Protein sequences and functional information
4. **PDB** – Protein structural data
5. **NCBI** – Biomedical and genomic information
6. **GenBank** – DNA sequences and their protein translations

Tools Demonstrated Today

1. **NCBI: BLAST** – Aligning homologous sequences for finding a protein of interest
2. **GenBank: RefSeq** – How to find a DNA sequence of your protein in the genome of an organism
3. **Clustal Omega** – Multiple sequence alignments and constructing phylogenetic trees
4. **ExPasy** – Bioinformatics resource portal

By the end you will be able to…

1. Understand what databases are available and how to navigate the database
2. Use database information to decide on sequences of proteins from different organisms that can be useful for their project
3. Use bioinformatic tools in conjunction with the database to analyze and visualize these proteins and their pathways to eventually decide which one to use.


BIOCYC
Database Collection


RCSB
PDB
PROTEIN DATA BANK


BRENDA
The Comprehensive Enzyme
Information System

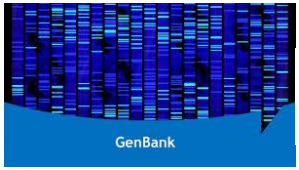

UniProt


NCBI


CLUSTAL


NCBI
BLAST


SIB
ExPASy
Bioinformatics Resource Portal


GenBank

RefSeq

Time for you to practice!

- “Database & Bioinformatic Tools for Beginners Practicum”
    - Download the document available on our webpage: https://2020.igem.org/Measurement/Webinars
- Contact measurement@igem.org and request **Ian Schlander** for further questions ☺
- Next is a presentation by Sonja Billerbeck to discuss Cloning Strategies!

Cloning Strategy

Sonja Billerbeck
Assistant Professor
University of Groningen
Netherlands

 @billerbeck_s

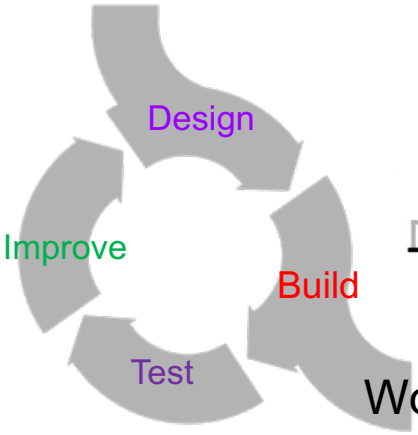
 www.BillerbeckLab.com

Cloning Strategy

Genetic information

Idea

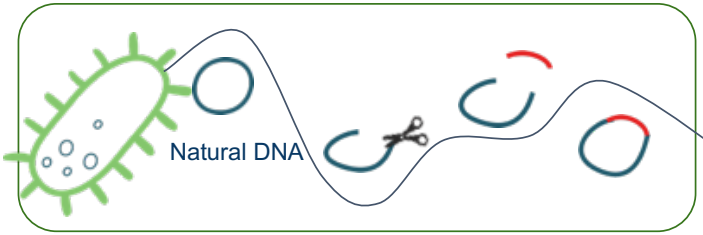
Cloning strategy

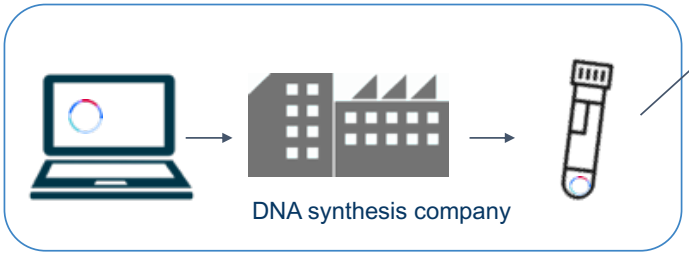


Working device

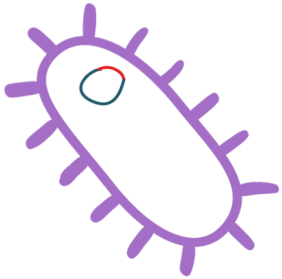
A cloning strategy needs three major considerations

Cloning





1. Host organism: Cloning *Backbone/vector*

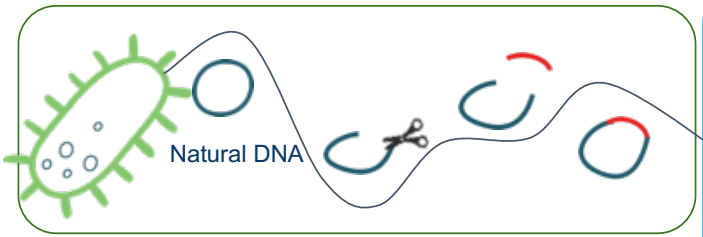


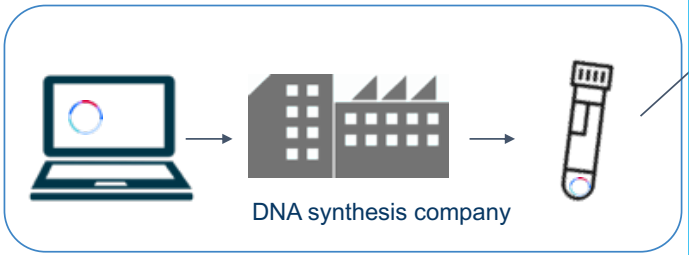
2. Assemble genes to circuits: *Assembly method*

3. Troubleshoot the circuit performance: Flexible and *modular design and assembly* strategy of the backbone and the circuits

A cloning strategy needs three major considerations

Cloning





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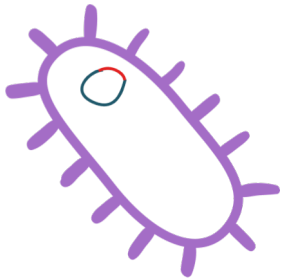


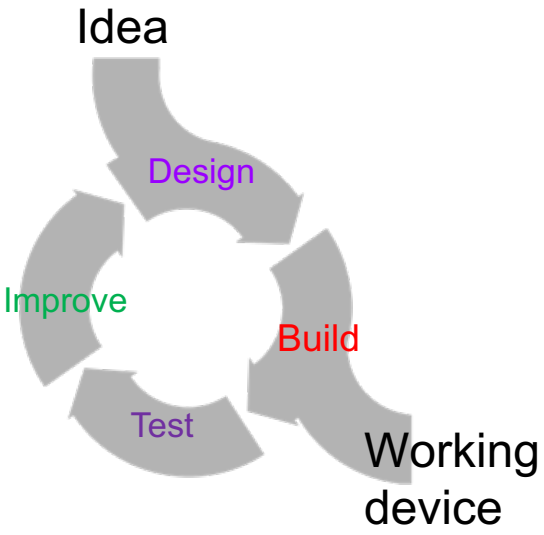
Cloning Strategy

2. Assemble genes to circuits: *Assembly method*

3. Troubleshoot the circuit performance: Flexible and *modular design and assembly* strategy of the backbone and the circuits

It is important to think of a cloning strategy during the design phase

- Cloning is an essential component of the **built** phase
- A cloning strategy enables the **improvement** phase
- The cloning strategy depends on the **design** and **test** phase



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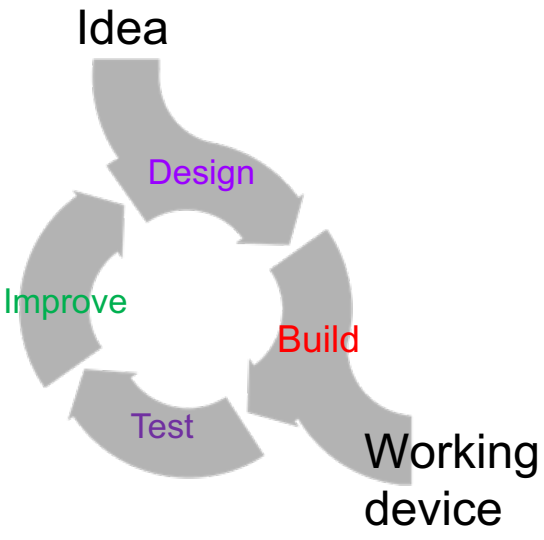


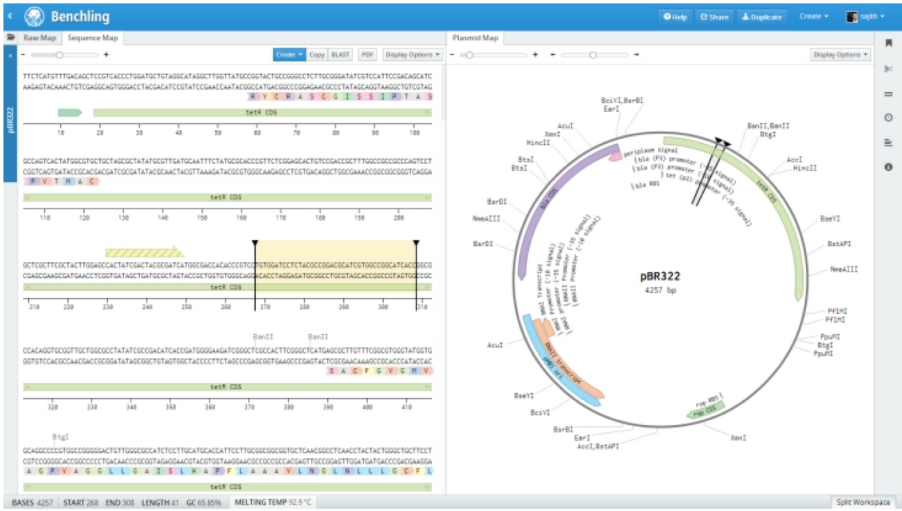
Points for consideration

The cloning strategy is developed and tested “in silico”

Plan, visualize, and document DNA cloning experiments.

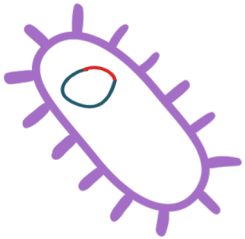
We offer Webinars on

- SnapGene
- Benchling
- geneious

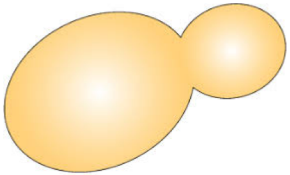


Cloning strategy question **1**: Host organism

Bacteria (*E. coli*)

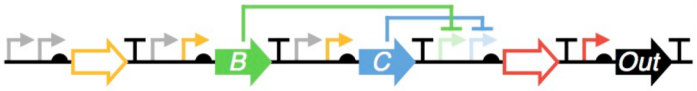


Yeast (*S. cerevisiae*)

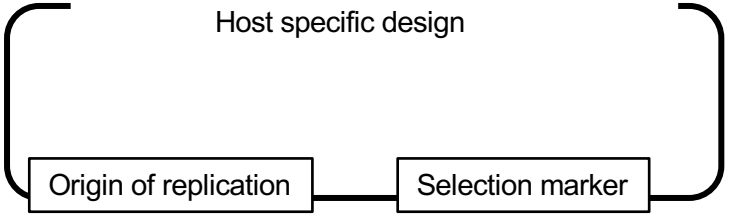


- Mammalian cells
- Plants
- Non-conventional yeast and bacteria

Circuit



Backbone/ Vector

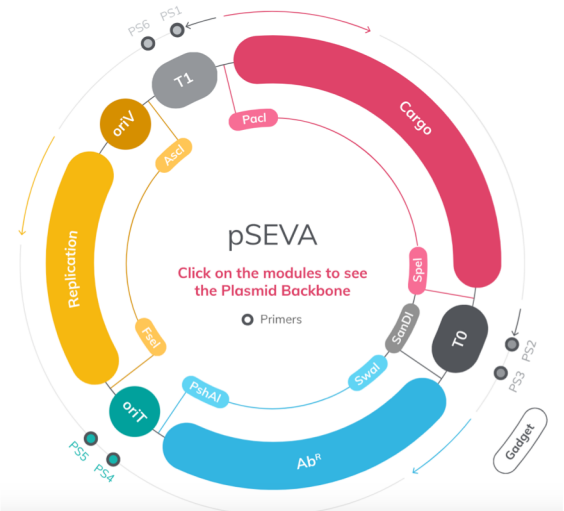


Host organism: Backbone/vector collections

Bacteria

p**SEVA** collection

Interchangeable
Selection markers
Origin of replication
Multiple cloning site for cargo



http://seva.cnb.csic.es/backbone-modules-nomenclature/

Yeast

- pRS series of plasmids

Interchangeable
Selection markers
Origin of replication
Multiple cloning site for cargo

Addgene #11258 pRS410
Addgene #11256 pRS418
Addgene #11256 pRS420

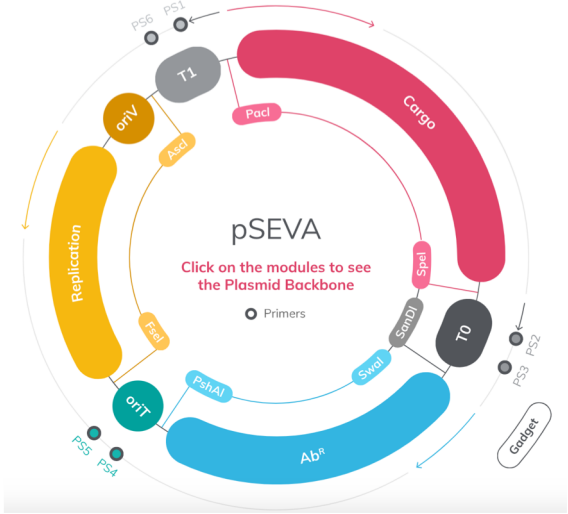
- Yeast MoClo Toolkit (Dueber Lab)

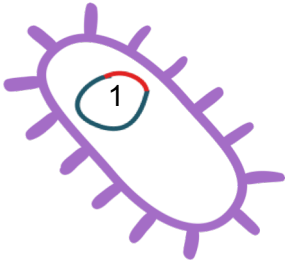
Make pRS series via golden gate assembly

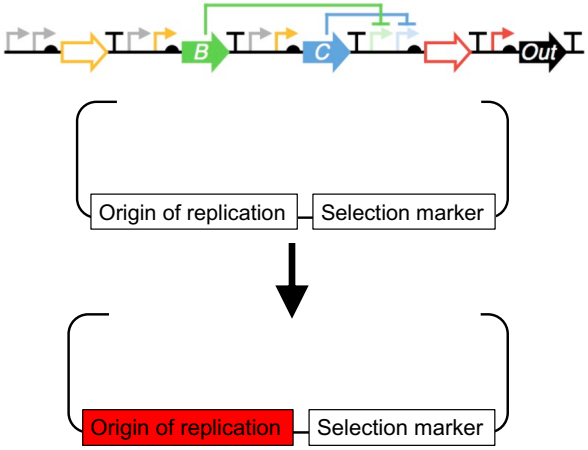
Addgene Kit #1000000061

Backbone collections: Change copy number of plasmid

Interchangeable
Selection markers
Origin of replication
Multiple cloning site for cargo

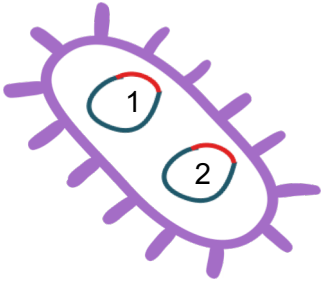


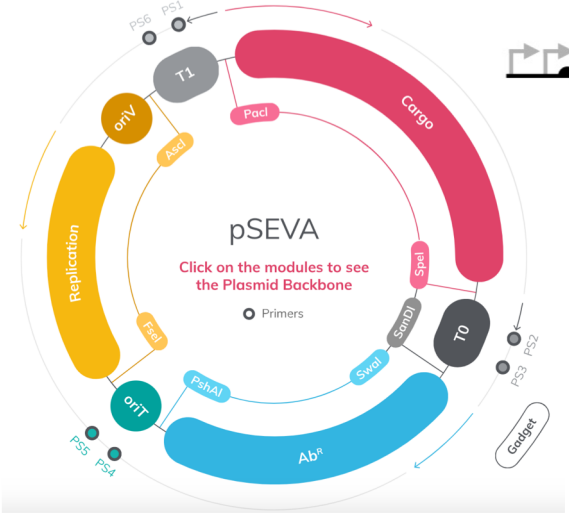


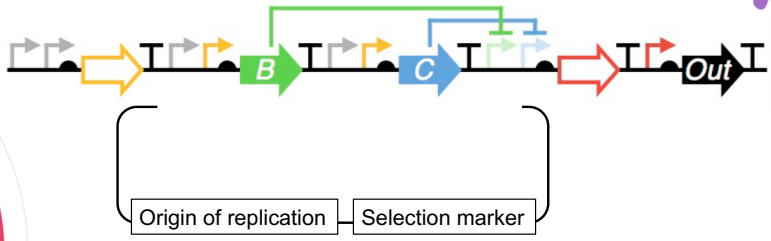


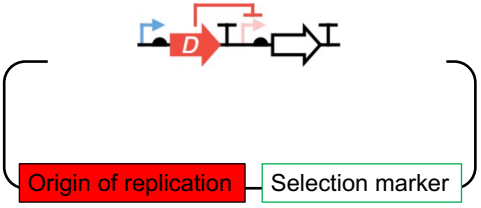
Backbone collections : Add a circuit

Interchangeable
Selection markers
Origin of replication
Multiple cloning site for cargo





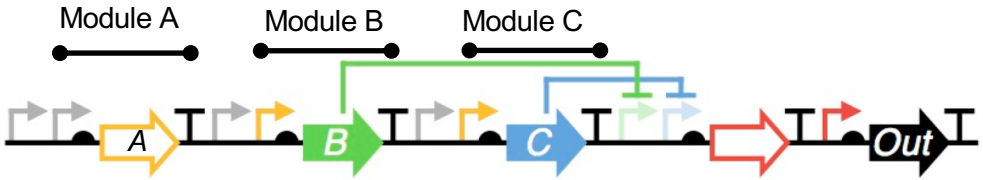




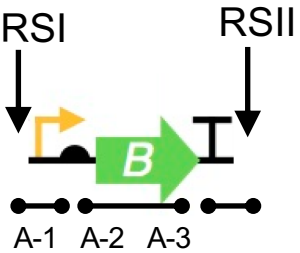
Cloning strategy question **2:** Assembly of circuits: Modularity and troubleshooting

Design Version 1

Works? ✗

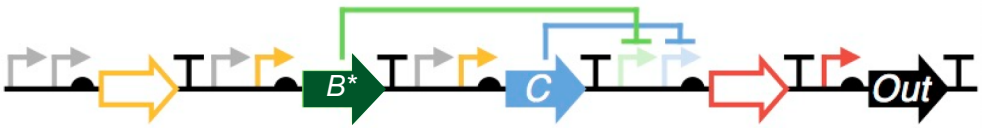


Exchange modules

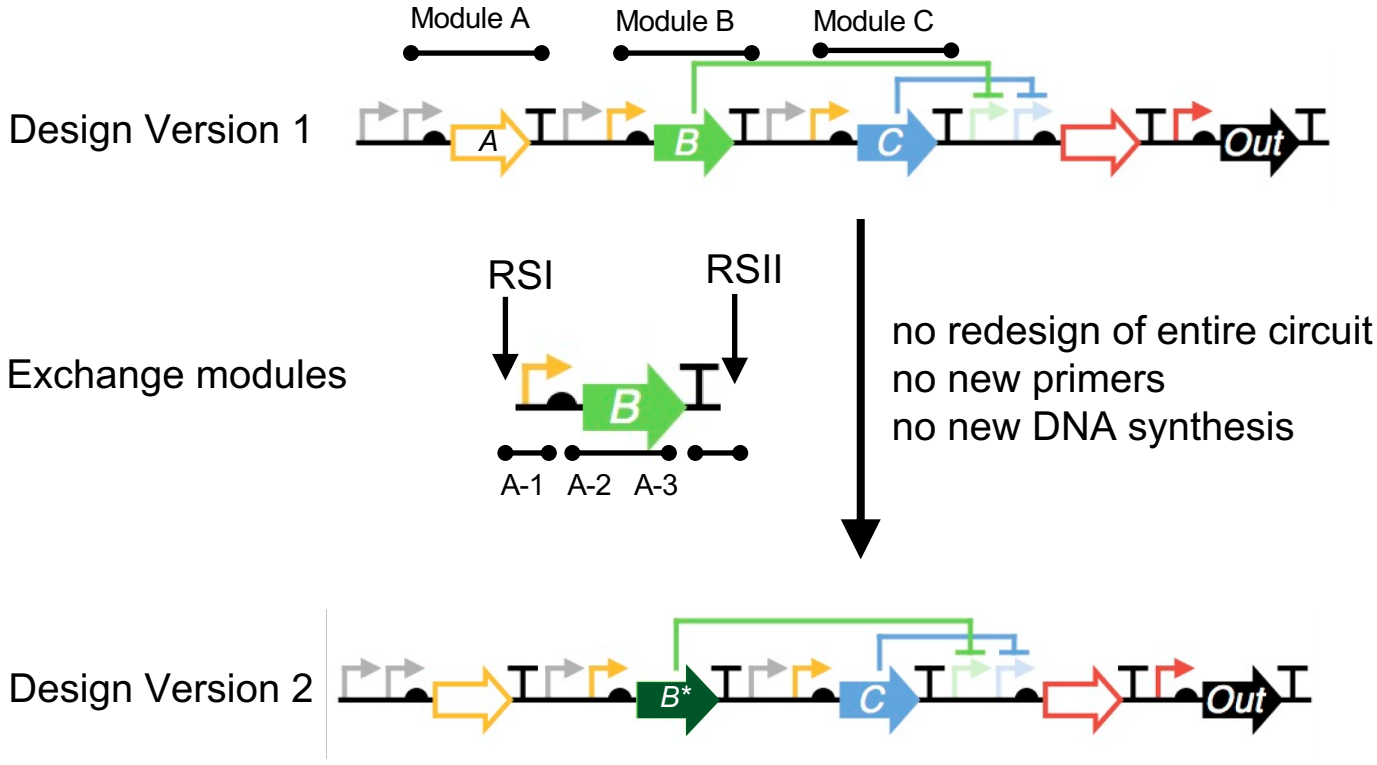


Design Version 2

Works? ✓



Modularity allows for **quick and cheap** troubleshooting

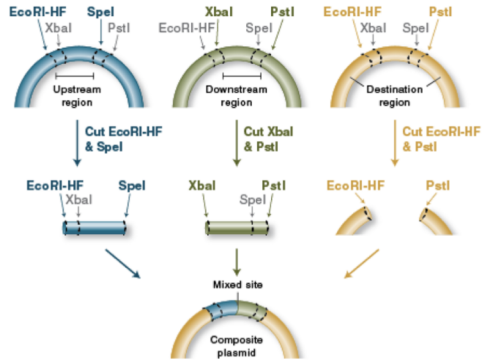


Assembly of circuits: Assembly method

Webinars

BioBrick Assembly

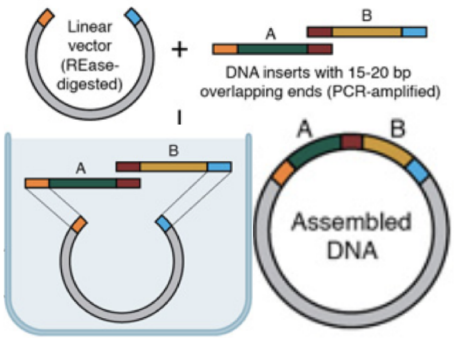
- Restriction sites flank every gene fragment, allowing “parts” to be interchanged, but introduces scar sequences



Modular ✓

Gibson Assembly

- Exonuclease creates large overhangs for annealing fragments, allowing for more accurate assemblies

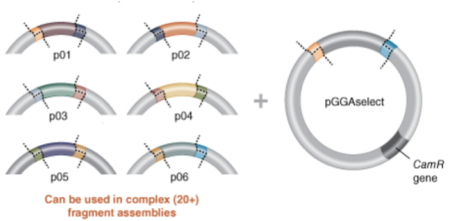


Modular ✓

Scar-less ✓

Golden Gate Assembly

- Endonuclease creates fragment-specific overhangs allowing 20+ fragments to be assembled at once in a relatively short time



Modular ✓

Scar-less ✓

Scalable ✓

Assembly of circuits: Assembly method

Webinars

BioBrick Assembly

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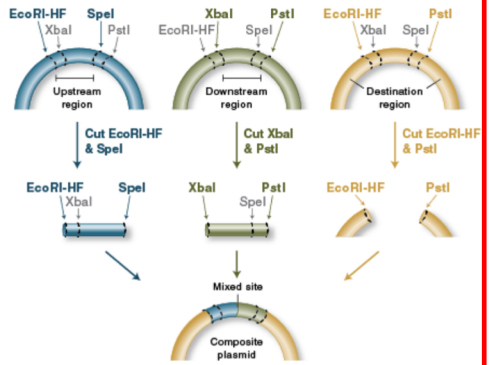

Gibson Assembly

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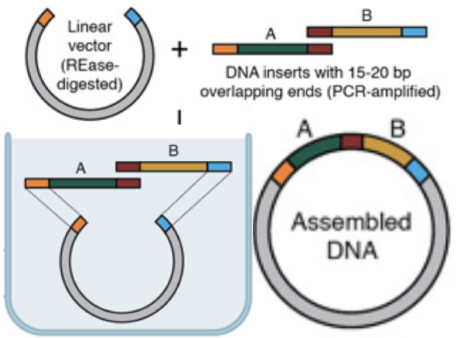

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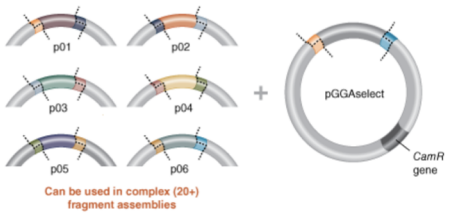
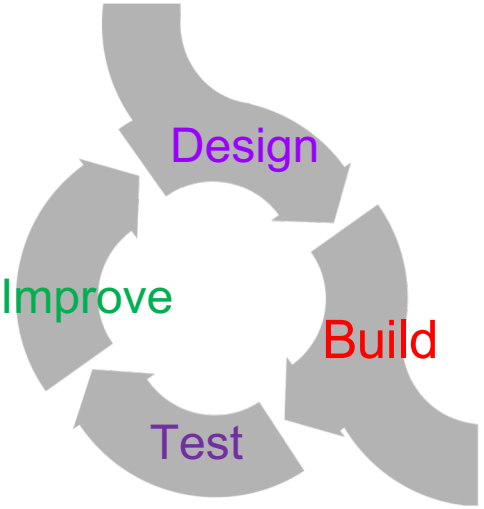


Good choice for most cloning strategies

A well-defined cloning strategy is…



…essential to run the full cycle and improve a design until it works.

Specialized webinars on:
Cloning Apps and Assembly methods