

DNA Assembly is Not for You

Leave it to machines !

Valentin Zulkower
Edinburgh Genome Foundry
EASTBIO Dundee, May 18th, 2018

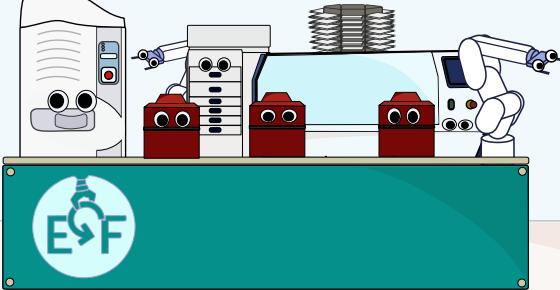
I NEED 18,000
ASSEMBLIES FOR MY
MASTERS PROJECT



GUYS ?



RIGHT AWAY !



Program

Main message: DNA Assembly is very human-unfriendly.

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Plan: We will see some common pitfalls of DNA design, assembly, and validation. And how software can help.

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... without hangover !

Sequence design

Can you polish a gene?

Parts-based design

LEGO Madness !

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Will it assemble ?

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Hi robots !

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Restriction digests
Say no to digestion problems!

Sanger sequencing
A primer on primers selection

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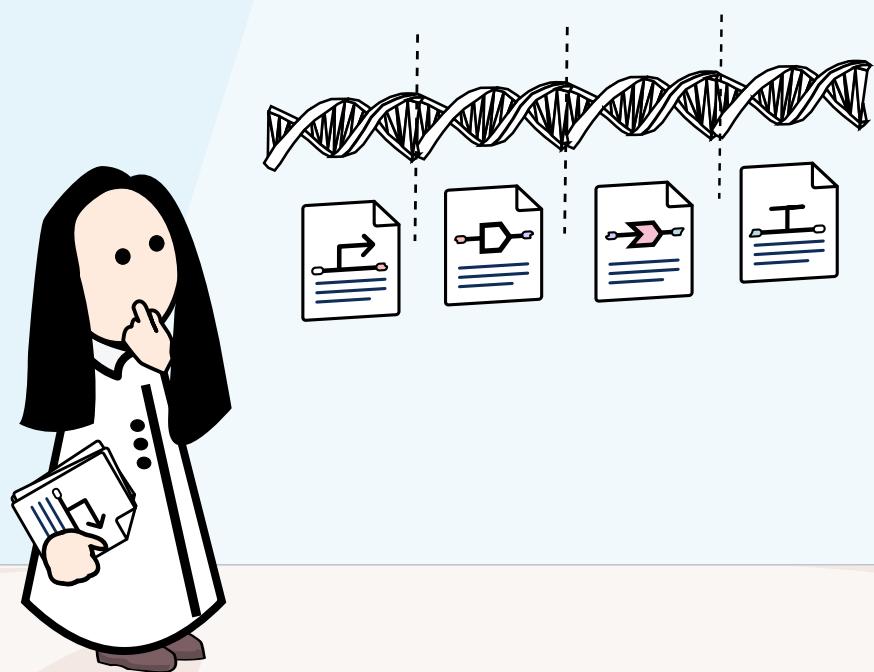
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1. Design



Overhangs design for Type 2S restriction assembly

Pitfalls of overhang design

1. Avoid similar overhangs

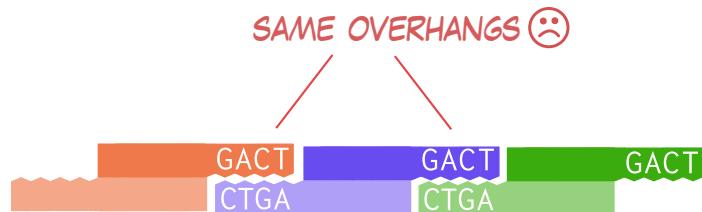
Pitfalls of overhang design

1. Avoid similar overhangs



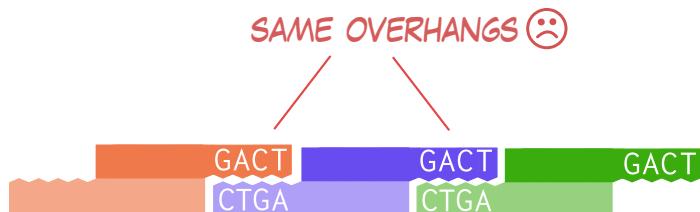
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Pitfalls of overhang design

1. Avoid similar overhangs
2. Beware of reverse-complements

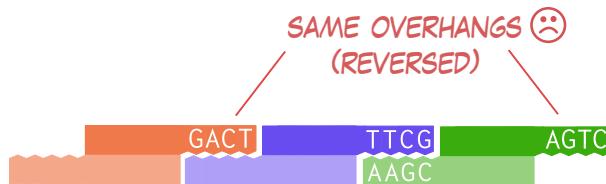
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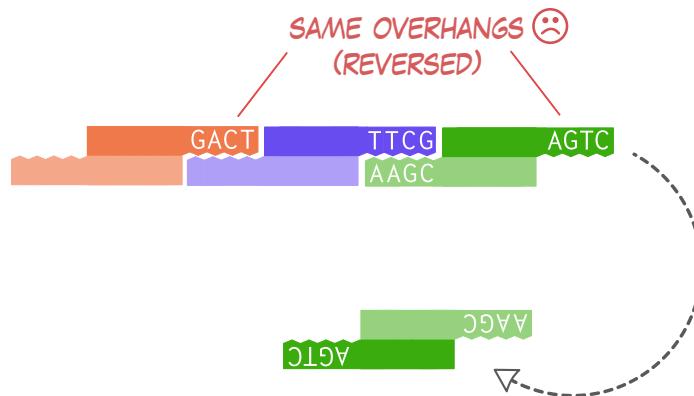
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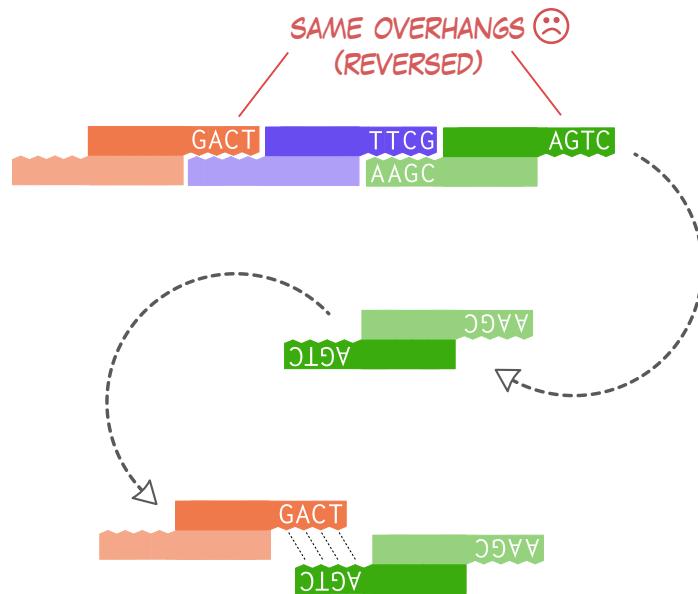
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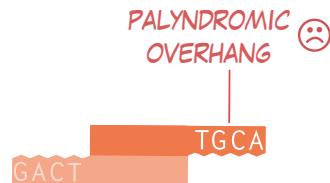


Pitfalls of overhang design

1. Avoid similar overhangs
2. Beware of reverse-complements
3. No palindromic sequences

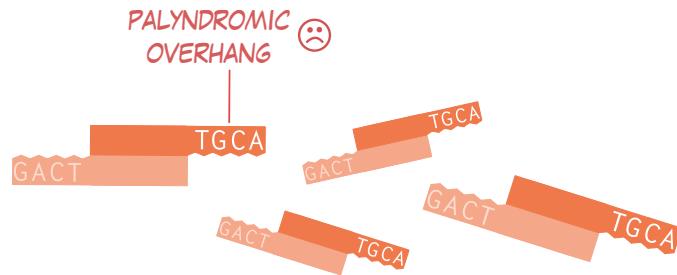
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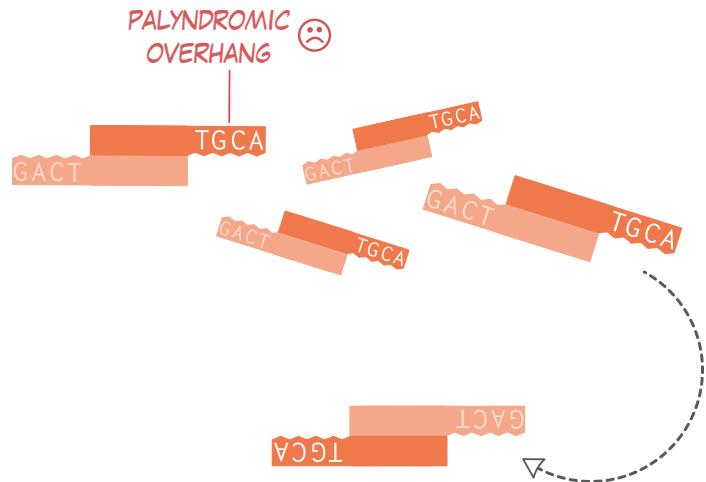
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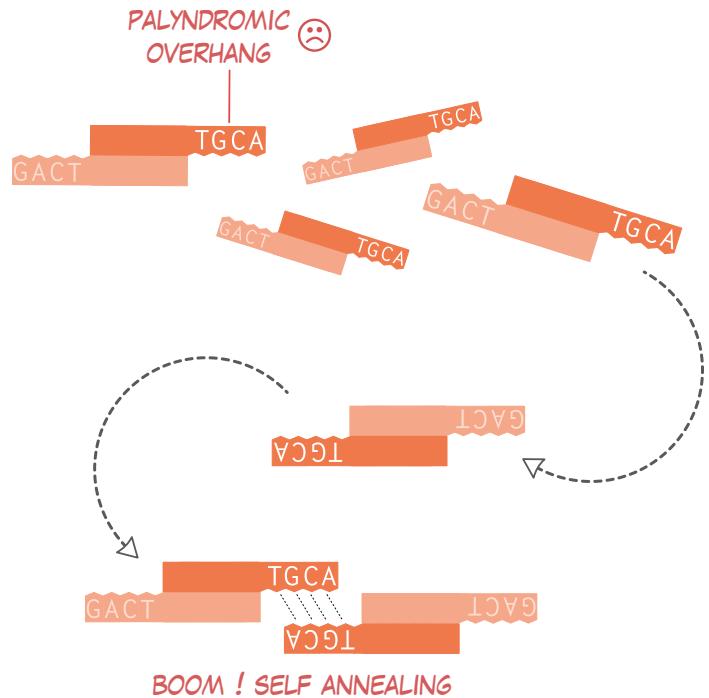
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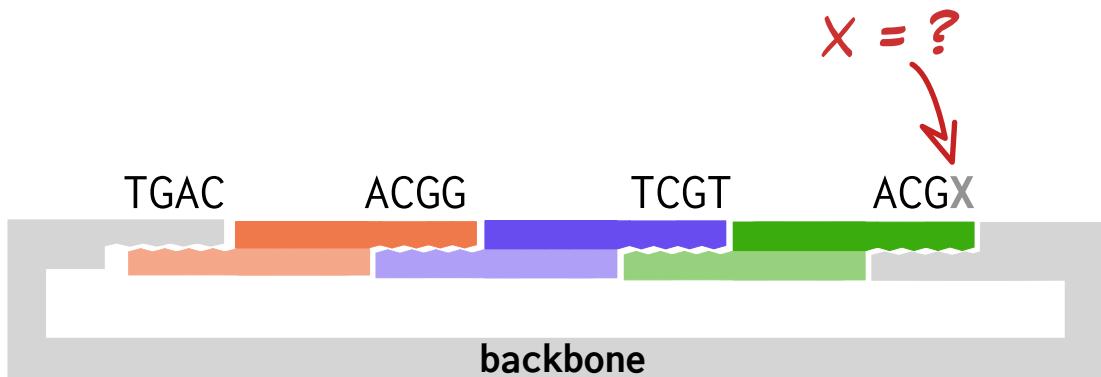


Pitfalls of overhang design

1. Avoid similar overhangs
2. Beware of reverse-complements
3. No palindromic sequences
4. Avoid 0% or 100% GC

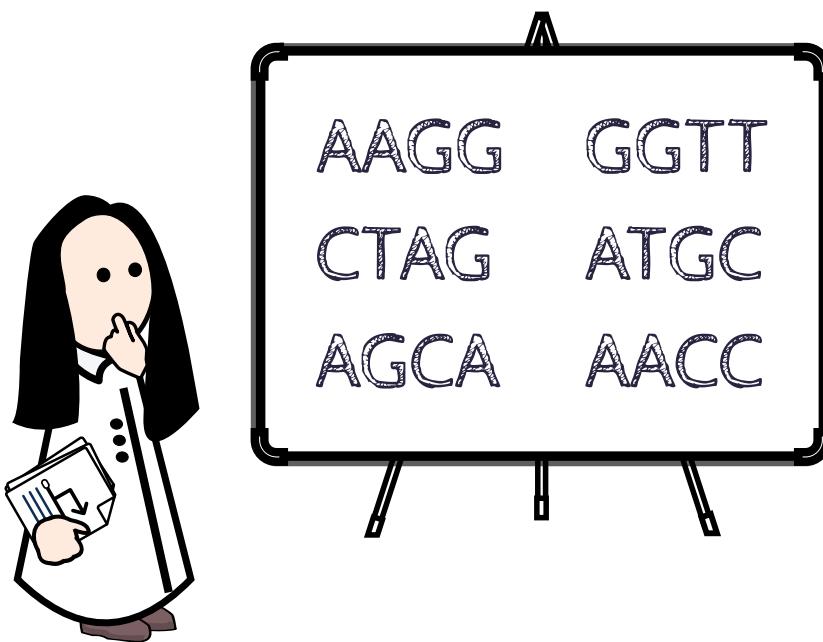
Overhangs design exercice 1

Which nucleotide will work best ?

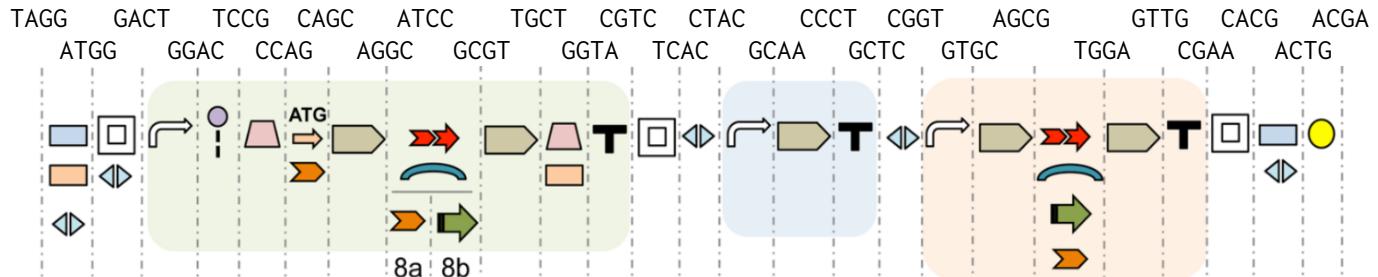


Overhangs design exercice 2

Is that a valid collection of overhangs ?



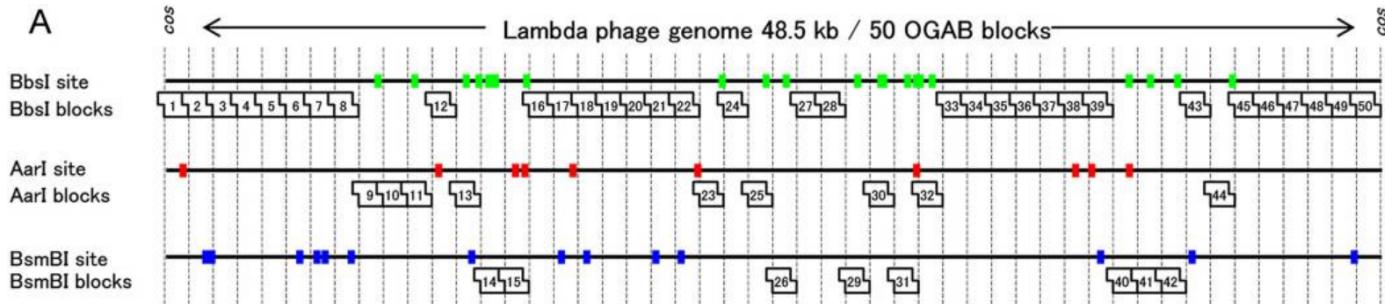
Real-world overhang design: EMMA



After Martella et al., ACS Syn. Bio. 2017

- ▶ 27 compatible overhangs
- ▶ Some mandatory overhangs (e.g. containing ATG)

Real-world overhang design: virus assembly

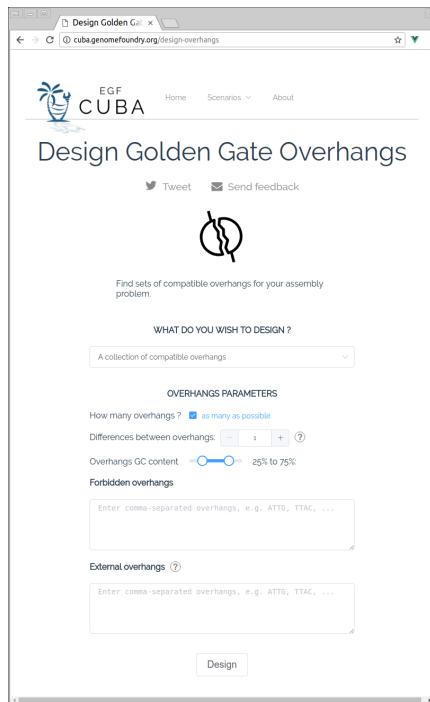


from Tsuge et al., Sci Rep. 2015

- ▶ 50 compatible overhangs
- ▶ Must be as equally spaced as possible in the sequence.

Overhang design software

cuba.genomefoundry.org/design-overhangs



Overhang design software

cuba.genomefoundry.org/design-overhangs

collection size
overhang similarity tolerance
mandatory / forbidden overhangs
Allowed GC%



The screenshot shows the 'Design Golden Gate Overhangs' page. On the left, there's a sidebar with a blue icon of a person with a brain, followed by the text 'EGF CUBA' and links for 'Home', 'Scenarios', and 'About'. Below this is the main title 'Design Golden Gate Overhangs' with social sharing buttons for Twitter and 'Send feedback'. The main area has a heading 'WHAT DO YOU WISH TO DESIGN ?' with a dropdown menu set to 'A collection of compatible overhangs'. Under 'OVERHANGS PARAMETERS', there are three sections: 'How many overhangs?' (radio button selected for 'as many as possible'), 'Differences between overhangs' (input field showing '1' with a plus sign and a help icon), and 'Overhangs GC content' (a slider set from 25% to 75%). Below these are two input fields: 'Forbidden overhangs' containing 'Enter comma-separated overhangs, e.g. ATTC, TTAC, ...' and 'External overhangs' containing 'Enter comma-separated overhangs, e.g. ATTC, TTAC, ...'. At the bottom is a large 'Design' button.

Overhang design software

cuba.genomefoundry.org/design-overhangs

collection size
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The screenshot shows the 'Design Golden Gate Overhangs' page. At the top, there's a logo for 'EGF CUBA' and navigation links for 'Home', 'Scenarios', and 'About'. Below the logo, there are social sharing buttons for 'Tweet' and 'Send feedback'. The main title is 'Design Golden Gate Overhangs' with a magnifying glass icon. A sub-instruction says 'Find sets of compatible overhangs for your assembly problem.' Under the title, there's a dropdown menu set to 'A collection of compatible overhangs'. The 'OVERHANGS PARAMETERS' section includes: 'How many overhangs?' with a radio button for 'as many as possible', 'Differences between overhangs' (set to 1), 'Overhangs GC content' (set to 25% to 75%), and a 'Forbidden overhangs' input field containing 'AAAG, AAC...'. Below that is an 'External overhangs' input field containing 'TTAG, TTCA, TTTC'. At the bottom is a 'Design' button.



AAAG, AAC...
AGTA, ATAC, ATCT, ATTG, CACG, CTGG, GACC, GCTG,
GGAG, GTCG, TAGA, TATG, TCAC, TCGG, TGCG, TGGC,
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AAAG, AAC...
AGTA, ATAC, ATCT, ATTG, CACG, CTGG, GACC, GCTG,
GGAG, GTCG, TAGA, TATG, TCAC, TCGG, TGCG, TGGC,
TTAG, TTCA, TTTC,

sequence
cutting regions
forbidden overhangs
Allowed GC%



Overhang design software

cuba.genomefoundry.org/design-overhangs

collection size
overhang similarity tolerance
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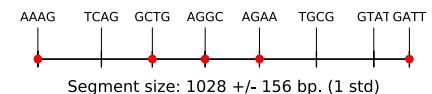


The screenshot shows the CUBA software interface for designing Golden Gate Overhangs. At the top, there's a logo for EGF CUBA, a navigation bar with Home, Scenarios, and About, and social sharing buttons for Twitter and Send feedback. The main title is "Design Golden Gate Overhangs". Below the title is a search bar with placeholder text "Find sets of compatible overhangs for your assembly problem." A large blue question mark icon is centered below the search bar. The next section is titled "WHAT DO YOU WISH TO DESIGN ?" with a dropdown menu set to "A collection of compatible overhangs". Under "OVERHANGS PARAMETERS", there are three sections: "How many overhangs?", "Differences between overhangs", and "Overhangs GC content". The "Overhangs GC content" section includes a slider from 25% to 75% and a current value of 50%. Below these are two input fields for "Forbidden overhangs" and "External overhangs", both with placeholder text "Enter comma-separated overhangs, e.g. ATTC, TTAC, ...". At the bottom is a "Design" button.

sequence
cutting regions
forbidden overhangs
Allowed GC%



AAAG, AACG, AAGT, AACG, ACCG, AGCC, AGGG,
AGTA, ATAC, ATCT, ATTG, CACG, CTGG, GACC, GCTG,
GGAG, GTCG, TAGA, TATG, TCAC, TCGG, TGCG, TGGC,
TTAG, TTCA, TTTC,



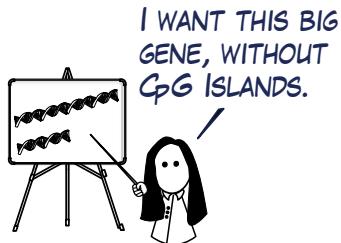
Sequence polishing: introductory exercise

Sequence specifications can depend on its purpose, assembly method, synthesis limitations, and validation method.

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CUSTOMER



remove CG
keep protein

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CUSTOMER

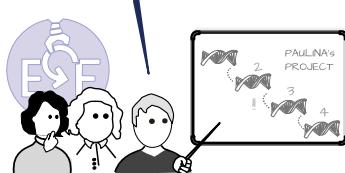
I WANT THIS BIG
GENE, WITHOUT
CpG ISLANDS.



remove CG
keep protein

EGF

LET'S REMOVE BSMBI SITES
AND INSERT A NCOI SITE



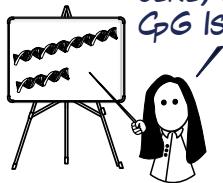
remove CGTCTC
insert CCATGG

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CUSTOMER

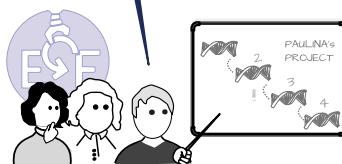
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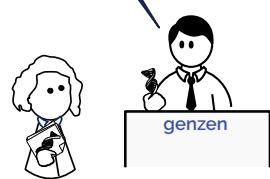
LET'S REMOVE BSMB1 SITES
AND INSERT A NCO1 SITE



remove CGTCTC
insert CCATGG

DNA PROVIDER

SORRY, WE CANNOT MAKE
PARTS WHICH CONTAIN
BSAI SITES.



remove GGTCTC

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CUSTOMER

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EGF

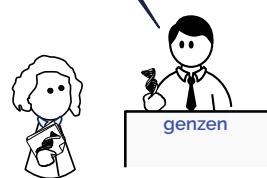
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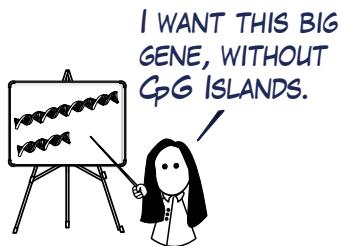
remove GGTCTC

M	S	S	G	G	S	R	L	H	G	V
A	T	G	A	G	T	C	T	C	G	A
A	G	C	A	G	T	G	G	A	G	T
T	C	A	T	C	A	G	T	A	G	T
T	C	C	T	C	C	G	G	A	G	T
T	C	G	T	C	G	T	C	C	T	T
T	C	T	T	C	T	T	C	C	G	T

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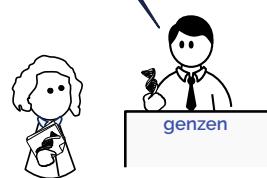
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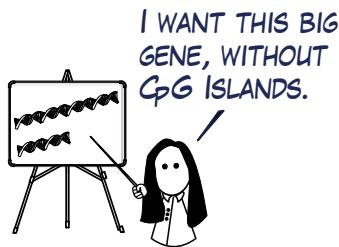
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T	C	A	T	C	A	G	G	C	G	T
T	C	C	T	C	C	G	G	T	G	T
T	C	G	T	C	G	T	C	C	T	A
T	C	T	T	C	T	C	C	G	T	T

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A	T	G	A	G	C	G	T	C	A	T
A	G	C	A	G	T	G	G	A	G	T
T	C	A	T	C	A	G	G	C	G	C
T	C	C	T	C	C	G	G	T	G	T
T	C	G	T	C	G	T	C	C	G	C
T	C	T	T	C	T	T	C	C	G	T

Real-world sequence polishing examples

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- ▶ *I want a 60,000bp sequence that is orthogonal to yeast, but same GC%*

Real-world sequence polishing examples

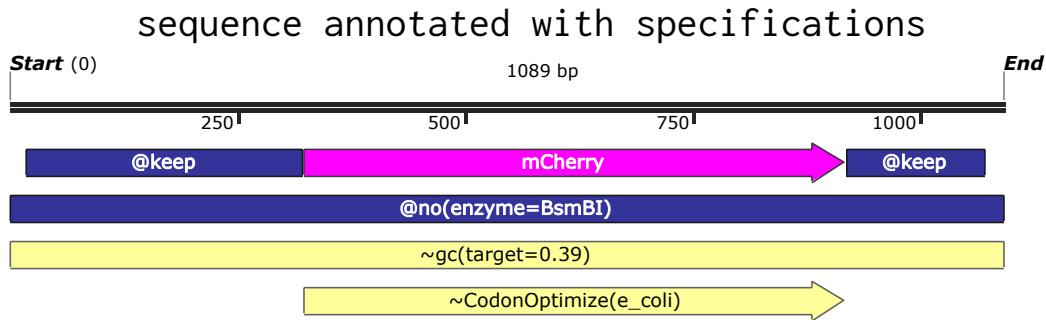
- ▶ *I want a 60,000bp sequence that is orthogonal to yeast, but same GC%*
- ▶ *I want this 50,000bp chromosome segment, but freed from as many common 4bp restriction sites as possible. Conserve ORFs if possible.*

Real-world sequence polishing examples

- ▶ I want a 60,000bp sequence that is orthogonal to yeast, but same GC%
- ▶ I want this 50,000bp chromosome segment, but freed from as many common 4bp restriction sites as possible. Conserve ORFs if possible.
- ▶ I want to domesticate these genes for E. coli. They should also pass the constraints of Synthesis Company (no homopolymers, no hairpins, no repeats)

Sequence polishing software

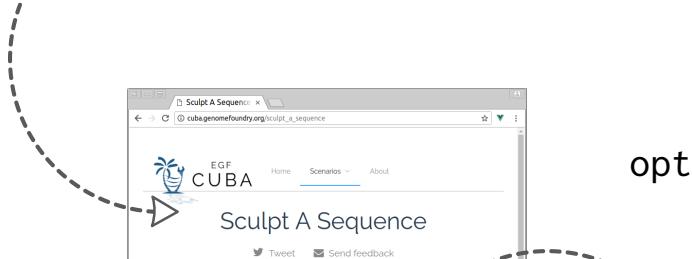
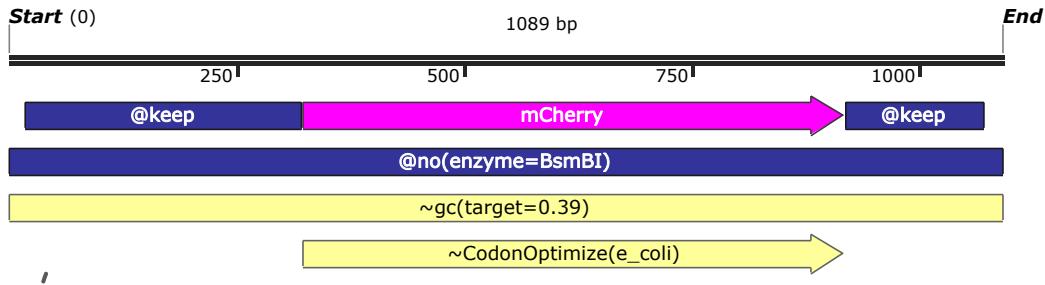
cuba.genomefoundry.org/sculpt_a_sequence



Sequence polishing software

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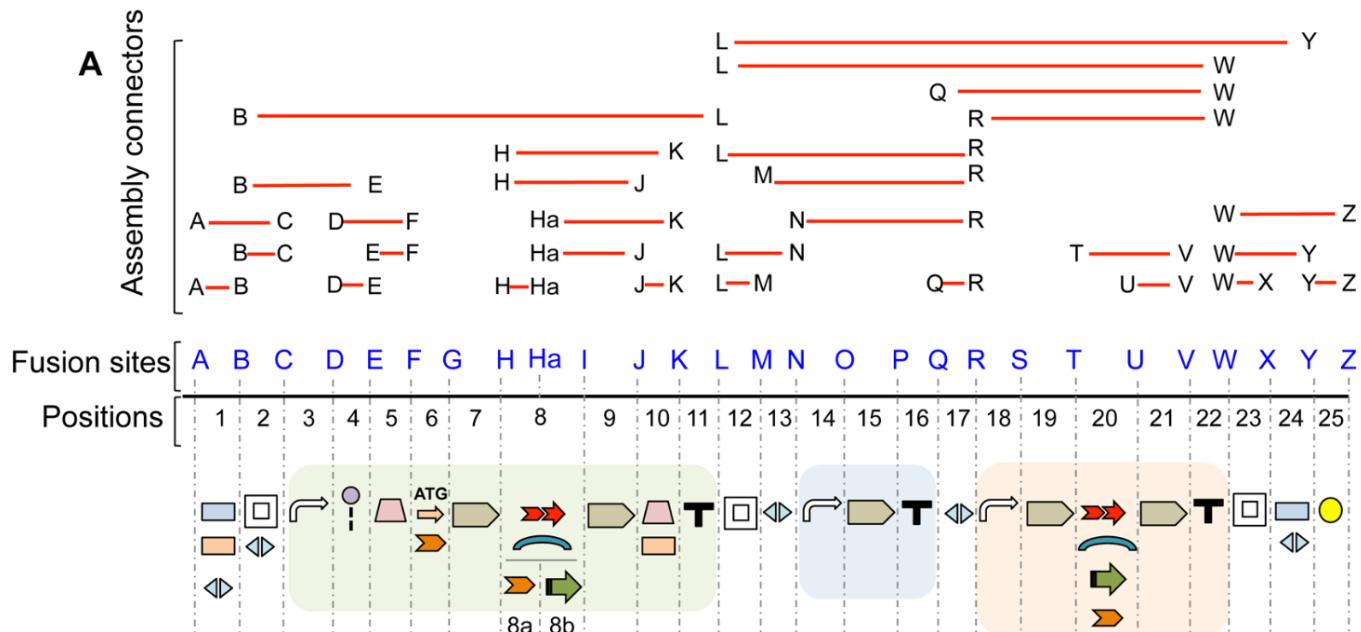
sequence annotated with specifications



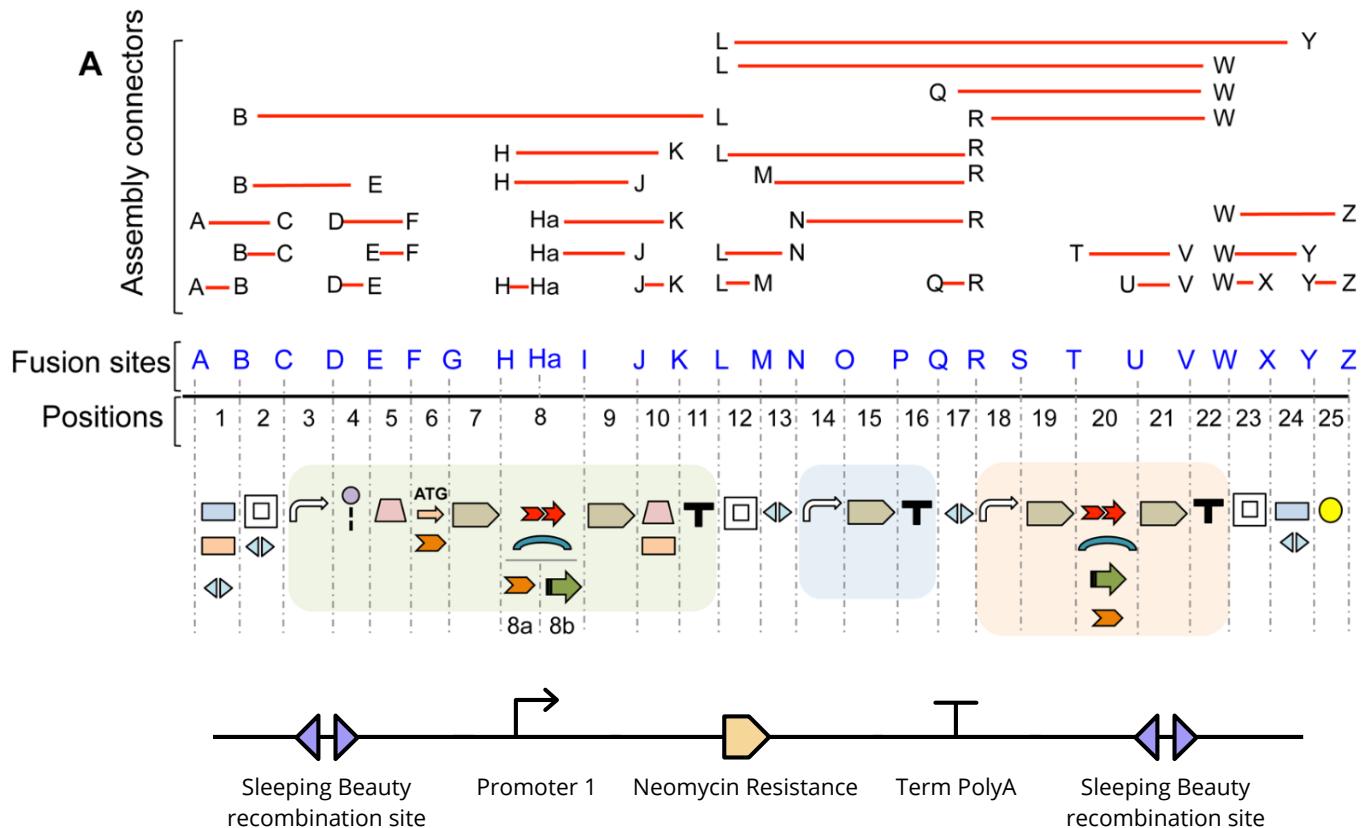
optimized sequence



Parts based assembly: easy peasy ?

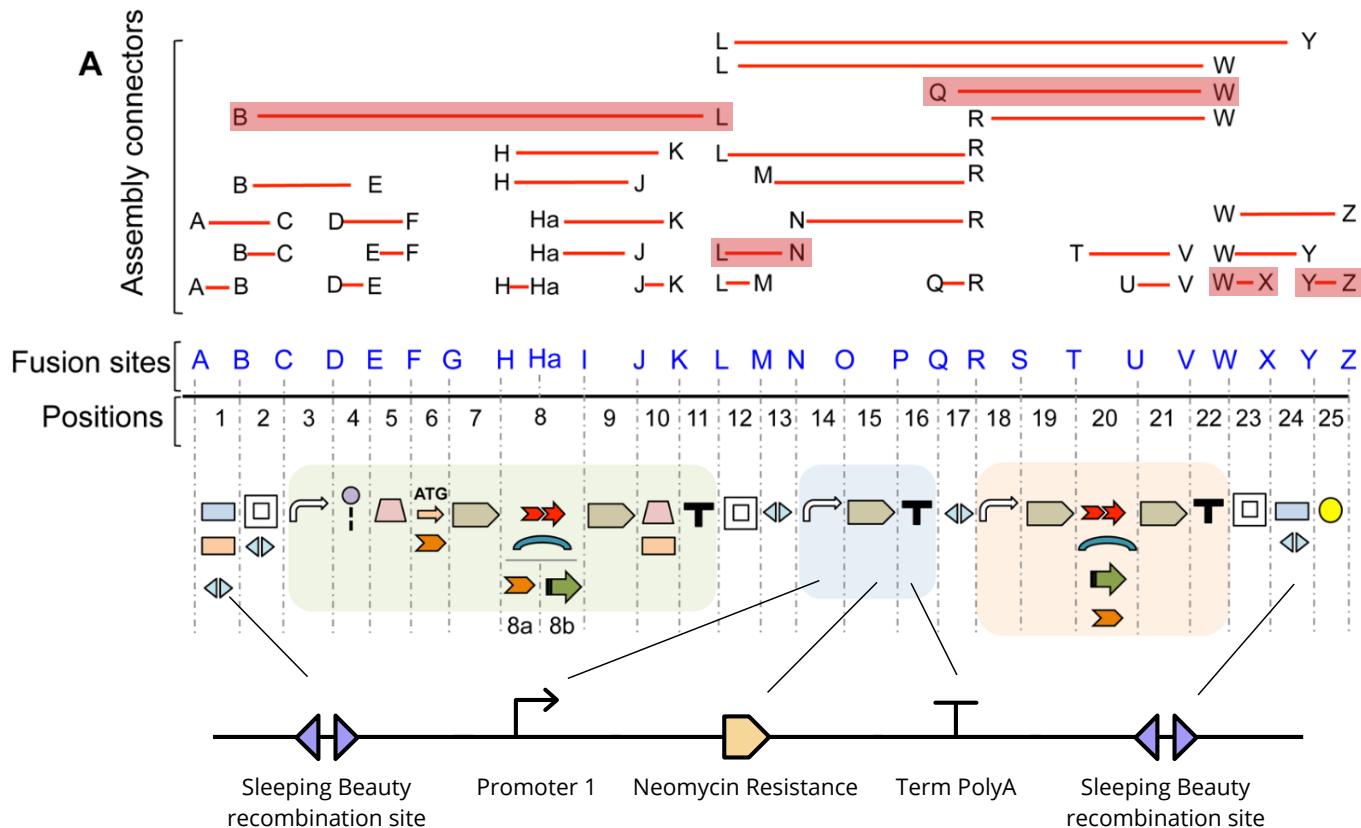


Parts based assembly: easy peasy ?



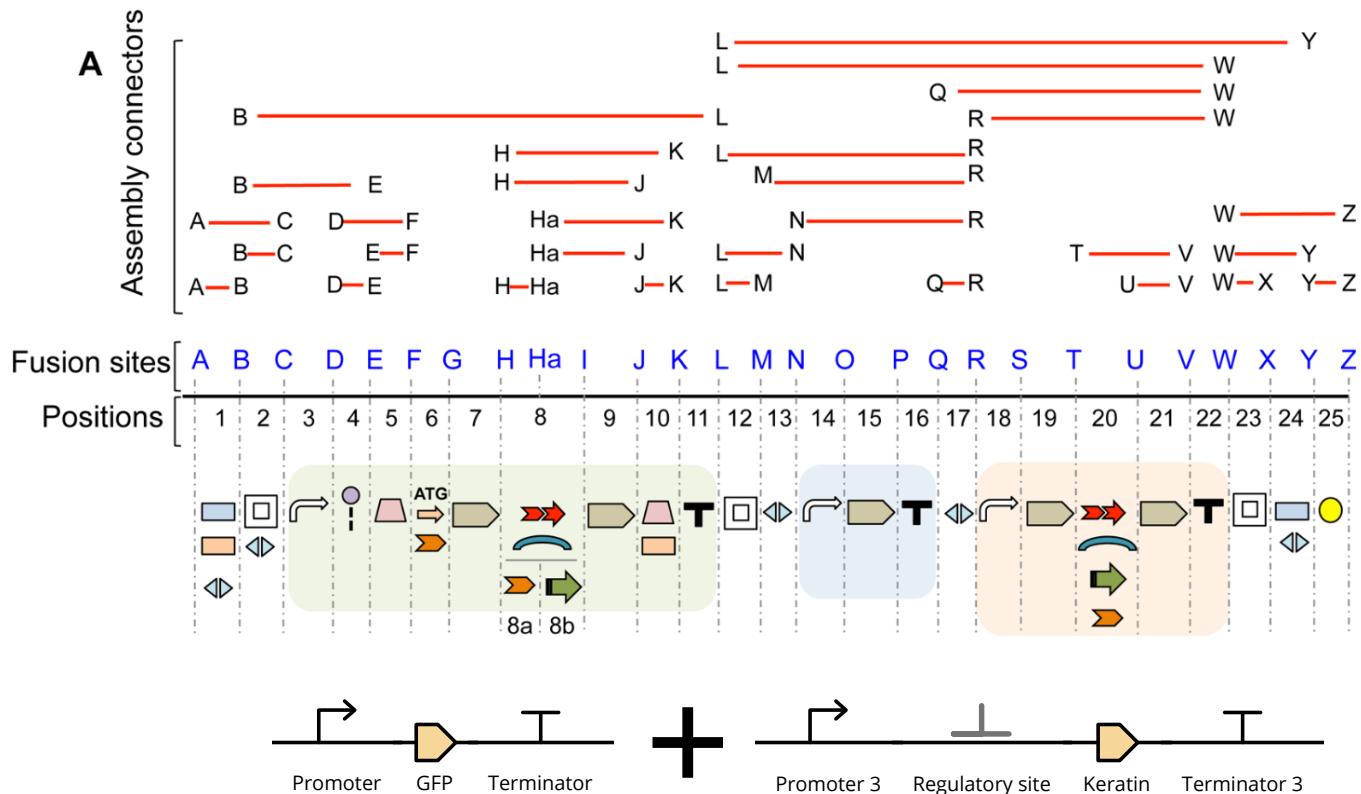
Question: Where would you place the parts ?

Parts based assembly: easy peasy ?



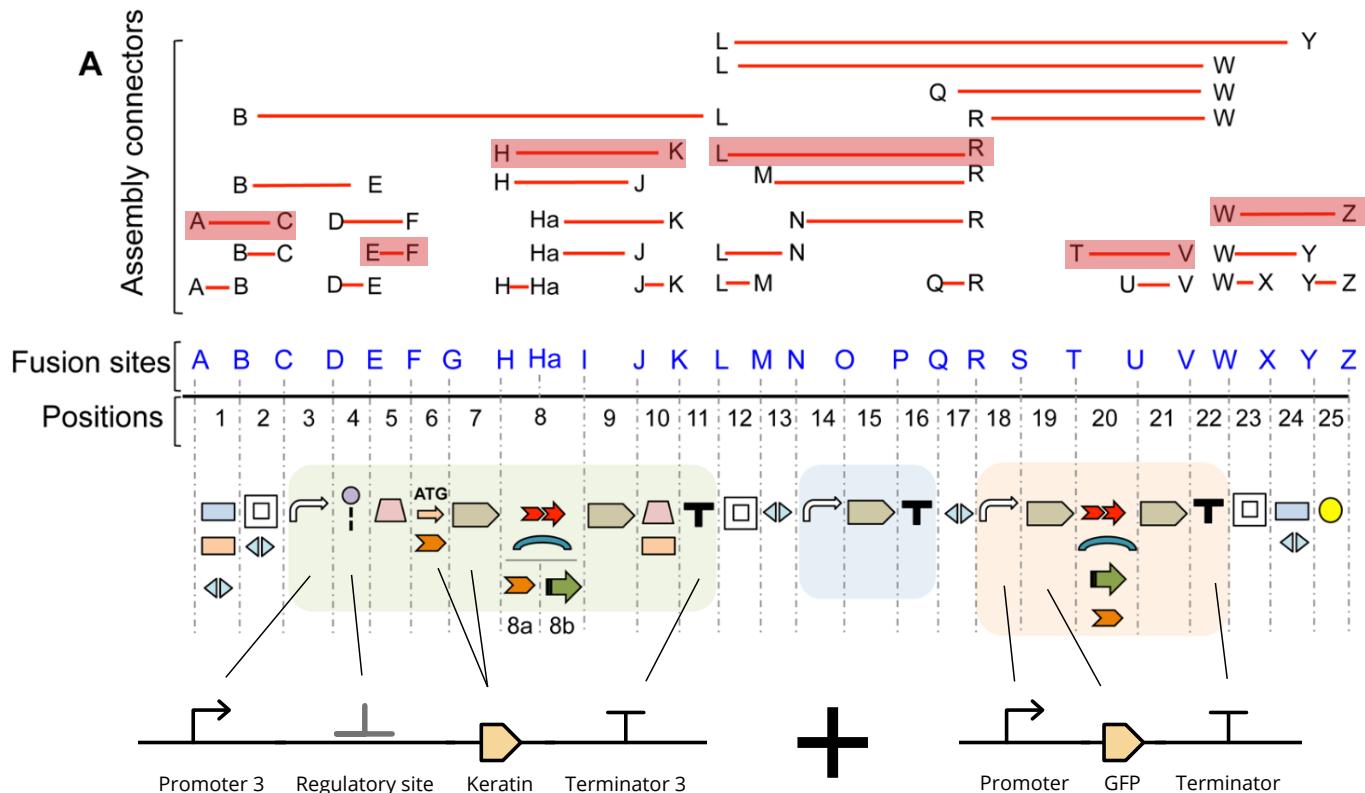
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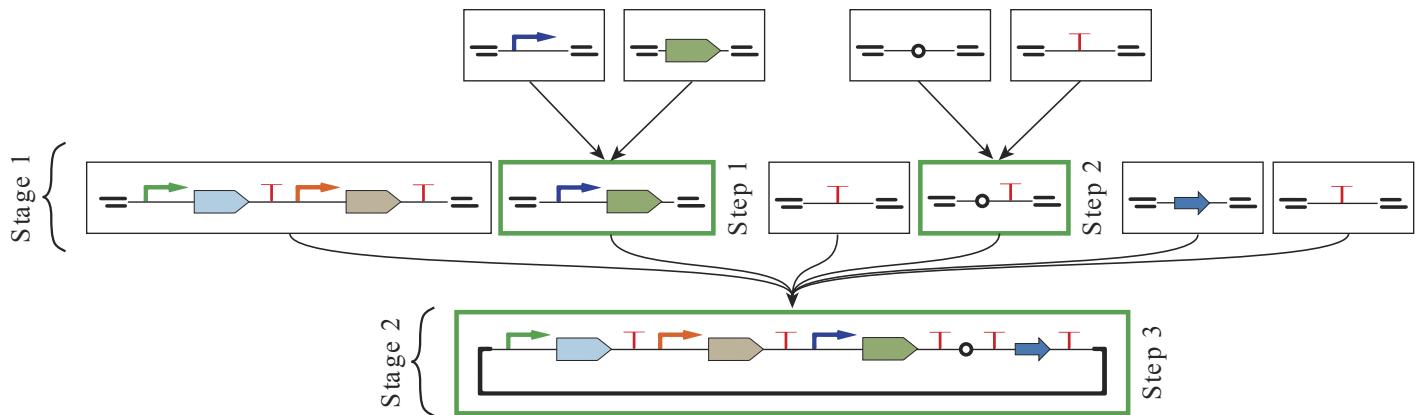
Part-based design with Autodesk Genetic Constructor

The screenshot displays the Autodesk Genetic Constructor software interface, showing four different genetic constructs:

- 5 - Episomal, single bicistronic unit:** A grid with positions 3 through 11. Positions 3, 6, 7, 8a, 9, and 11 contain CAGp. Positions 6 and 7 contain ATG-BoxC (LTae) and Ni-IgGL sequence. Position 7 also contains mTgFP2. Positions 8a and 9 contain L7Ae - Weiss, mNeonGreen, and mRuby2. Position 8b contains CI-KDEL and IRES2. Position 11 contains SV40 polyA.
- 6A - Episomal, Two bicistronic units:** A grid with positions 3 through 18. Positions 3, 6, 7, 8, 9, 11, and 18 contain CAGp. Positions 6 and 7 contain ATG-BoxC (LTae) and Ni-IgGL sequence. Position 7 also contains mTgFP2. Position 8 contains L7Ae and mNeonGreen. Position 9 contains Linker 2 and FcFy Luciferase -3XFLAG. Position 11 contains SV40 polyA. Position 18 contains CMVp.
- 2A - Episomal, single tagged fusion protein:** A grid with positions 3 through 9. Positions 3, 6, 7, 8, and 9 contain CAGp. Positions 6 and 7 contain Ni-IgGL sequence, EF1αp, TRE30p, and CMVp, Tet. Position 7 also contains Ni-SV40-NLS, Ni-myristylation signal, 3X-FLAG, ATG-BoxC (LTae), Ni-Palmitoylation sequence, Kozak, ATG, and Ni-MLS. Position 8 contains mTgBP2, L7Ae, L7Ae - Weiss, FcFy Luciferase, mRuby2, Bx81, mKate2, and mNeonGreen. Position 9 contains p2a Porcine teschovirus-1, Linker 2, Linker 1, FcFy Luciferase -3XFLAG, mNeonGreen, DmR, BSDR, NeoR, PuoR, α-Tubulin, mRuby2, and mTagBP2.
- 1B - Single tagged protein:** A grid with positions 3 through 11. Positions 3, 6, 7, 11, and 12 contain CAGp. Positions 6 and 7 contain ATG-BoxC (LTae) and Kozak, ATG.

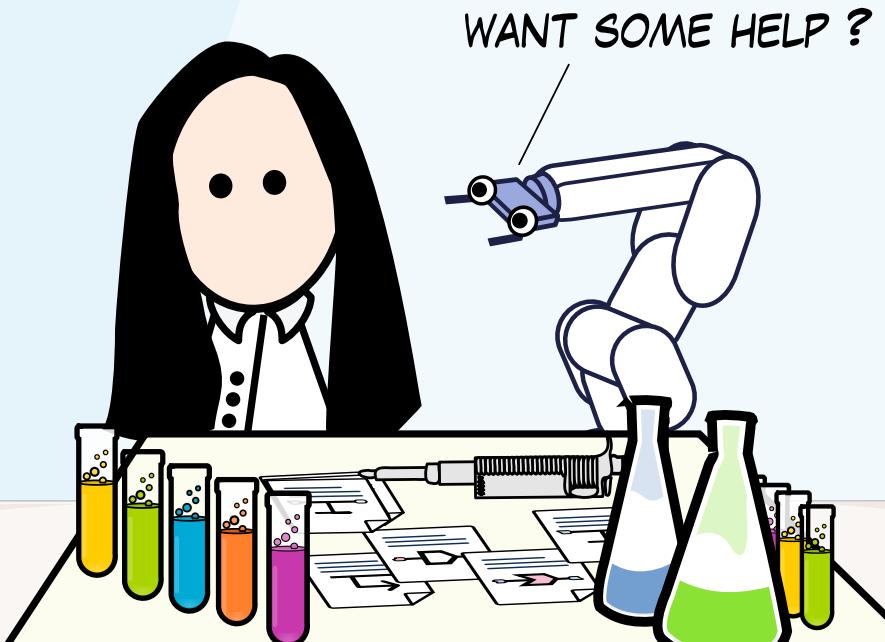
The interface includes a sidebar for "Projects" and "By Project" search, and tabs for "SEQUENCE" and "GSL EDITOR".

Automated part selection with RavenCAD

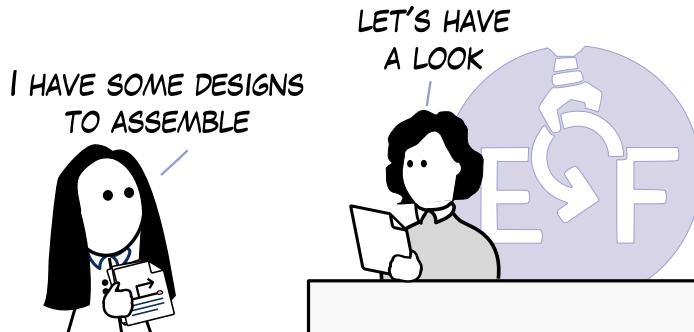


From Appleton et al., Nat Methods 2014

2. Assembly

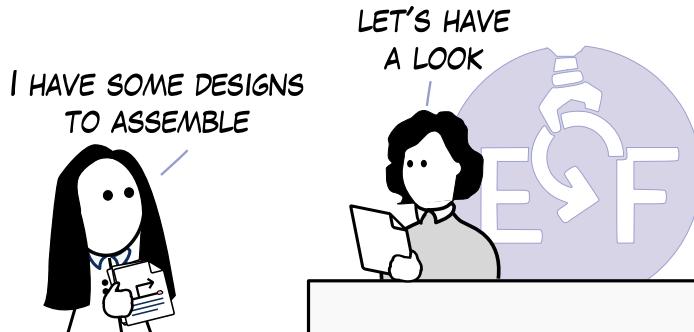


Assembly pre-validation



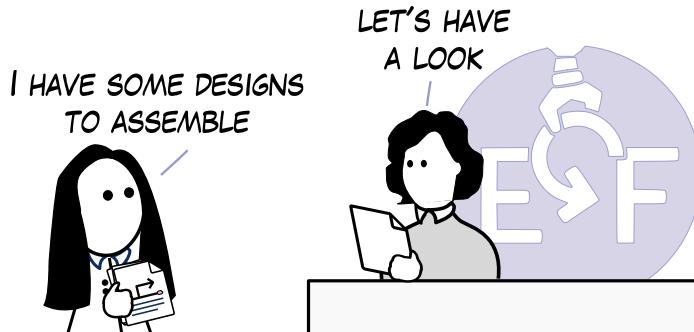
Assembly 1	promoter_PKA	neogreen	term_pkA	insulator	HA_0001	HC_amp
Assembly 2	promoter_PkA	mCherry	term_pkA	insulator	HA_1002	HC_amp
Assembly 3	promoter_PKA	YFP	term_pkA	insulator	HA_0202	HC_amp
Assembly 4	promoter_AR1	neogreen	UTR53	insulator	HA_0003	HC_amp
Assembly 5	promoter_AR1	neogreen	neogreen	term_pkA	HA_0203	HC_amp
Assembly 6	promoter_ZDF	YFP	UTR53	term_pkA	insulator	HA_0001
Assembly 7	promoter_ZDF	mCherry	term_pkA	insulator	HA_0003	HC_amp
Assembly 8	promoter_ZDF	neogreen	term_pkA	insulator	HA_0401	HC_amp

Assembly pre-validation



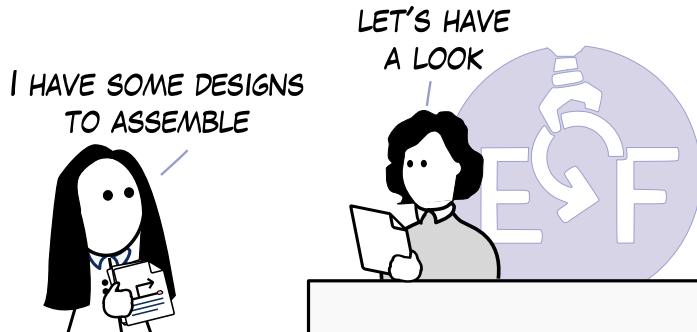
Assembly 1	promoter_PKA	neogreen	term_pkA	insulator	HA_0001	HC_amp
Assembly 2	promoter_PkA	mCherry TYPO?	term_pkA	insulator	HA_1002	HC_amp
Assembly 3	promoter_PKA	YFP	term_pkA	insulator	HA_0202	HC_amp
Assembly 4	promoter_AR1	neogreen	UTR53	insulator	HA_0003	HC_amp
Assembly 5	promoter_AR1	neogreen	neogreen	term_pkA	HA_0203	HC_amp
Assembly 6	promoter_ZDF	YFP	UTR53	term_pkA	insulator	HA_0001
Assembly 7	promoter_ZDF	mCherry	term_pkA	insulator	HA_0003	HC_amp
Assembly 8	promoter_ZDF	neogreen	term_pkA	insulator	HA_0401	HC_amp

Assembly pre-validation



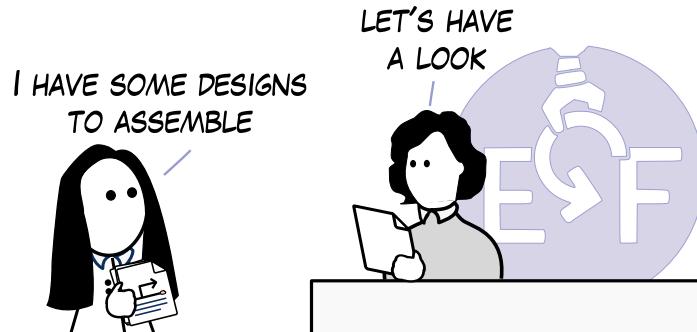
Assembly 1	promoter_PKA	neogreen	term_pkA	insulator	HA_0001	HC_amp
Assembly 2	promoter_PkA	mCherry	term_pkA	insulator	HA_1002	HC_amp
Assembly 3	promoter_PKA	YFP	term_pkA	insulator	HA_0202	HC_amp
Assembly 4	promoter_AR1	neogreen	UTR53	insulator NO TERMINATOR?	HA_0003	HC_amp
Assembly 5	promoter_AR1	neogreen	neogreen	term_pkA	HA_0203	HC_amp
Assembly 6	promoter_ZDF	YFP	UTR53	term_pkA	insulator	HA_0001
Assembly 7	promoter_ZDF	mCherry	term_pkA	insulator	HA_0003	HC_amp
Assembly 8	promoter_ZDF	neogreen	term_pkA	insulator	HA_0401	HC_amp

Assembly pre-validation



Assembly 1	promoter_PKA	neogreen	term_pkA	insulator	HA_0001	HC_amp
Assembly 2	promoter_PkA	mCherry ↖TYPO?	term_pkA	insulator	HA_1002	HC_amp
Assembly 3	promoter_PKA	YFP	term_pkA	insulator	HA_0202	HC_amp
Assembly 4	promoter_AR1	neogreen	UTR53 NO TERMINATOR?	insulator	HA_0003	HC_amp
Assembly 5	promoter_AR1	neogreen DUPLICATE?	↔ neogreen	term_pkA	HA_0203	HC_amp
Assembly 6	promoter_ZDF	YFP	UTR53	term_pkA	insulator	HA_0001
Assembly 7	promoter_ZDF	mCherry	term_pkA	insulator	HA_0003	HC_amp
Assembly 8	promoter_ZDF	neogreen	term_pkA	insulator	HA_0401	HC_amp

Assembly pre-validation



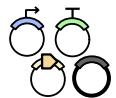
Assembly 1	promoter_PkA	neogreen	term_pkA	insulator	HA_0001	HC_amp
Assembly 2	promoter_PkA	mCherry ↖TYPO?	term_pkA	insulator	HA_1002	HC_amp
Assembly 3	promoter_PkA	YFP	term_pkA	insulator	HA_0202	HC_amp
Assembly 4	promoter_AR1	neogreen	UTR53 NO TERMINATOR?	insulator	HA_0003	HC_amp
Assembly 5	promoter_AR1	neogreen DUPLICATE?	↔ neogreen	term_pkA	HA_0203	HC_amp
Assembly 6	promoter_ZDF	YFP	UTR53	term_pkA	insulator	HA_0001 X NO BACKBONE?
Assembly 7	promoter_ZDF	mCherry	term_pkA	insulator	HA_0003	HC_amp
Assembly 8	promoter_ZDF	neogreen	term_pkA	insulator	HA_0401	HC_amp

Assembly pre-validation with simulated cloning

Assembly pre-validation with simulated cloning

SIMULATED CLONING

GENETIC PARTS



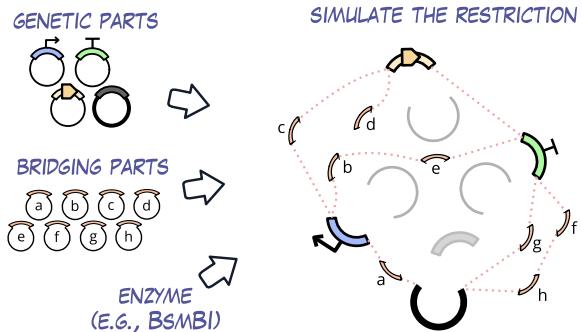
BRIDGING PARTS



ENZYME
(E.G., BSMBI)

Assembly pre-validation with simulated cloning

SIMULATED CLONING



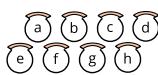
Assembly pre-validation with simulated cloning

SIMULATED CLONING

GENETIC PARTS

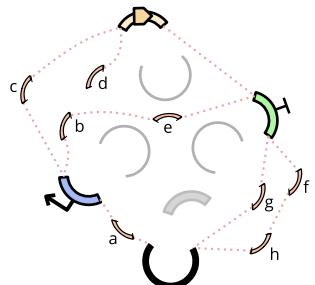


BRIDGING PARTS

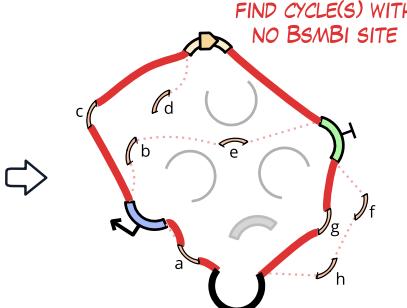


ENZYME
(E.G., BSMBI)

SIMULATE THE RESTRICTION



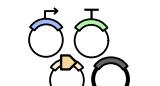
FIND CYCLE(S) WITH
NO BSMBI SITE



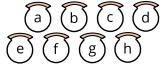
Assembly pre-validation with simulated cloning

SIMULATED CLONING

GENETIC PARTS

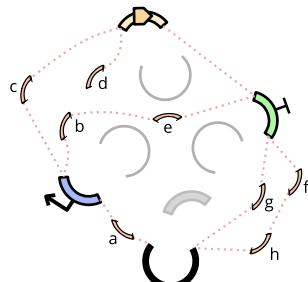


BRIDGING PARTS

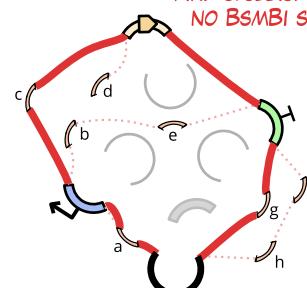


ENZYME
(E.G., BSMBI)

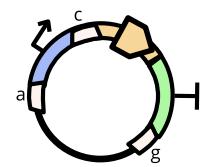
SIMULATE THE RESTRICTION



FIND CYCLE(S) WITH
NO BSMBI SITE



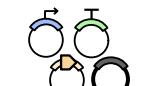
COMPUTE THE FINAL
ASSEMBLY SEQUENCES



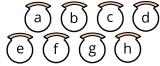
Assembly pre-validation with simulated cloning

SIMULATED CLONING

GENETIC PARTS

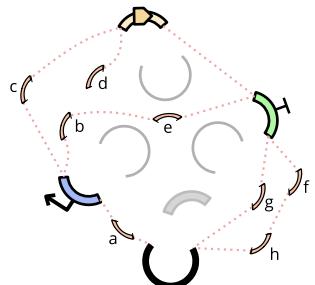


BRIDGING PARTS

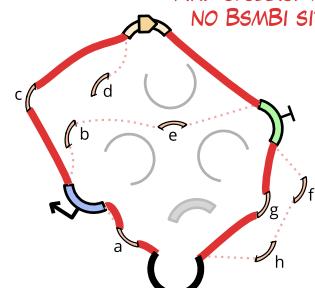


ENZYME
(E.G., BSMBI)

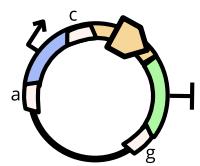
SIMULATE THE RESTRICTION



FIND CYCLE(S) WITH
NO BSMBI SITE



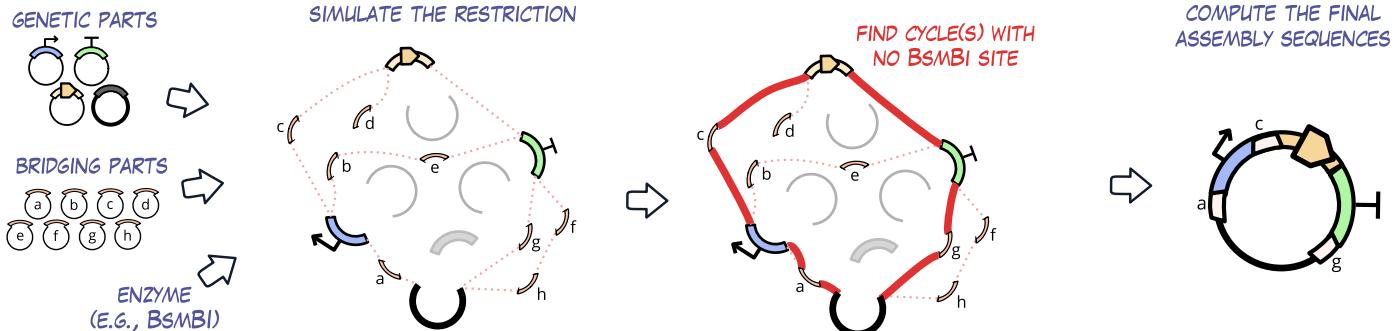
COMPUTE THE FINAL
ASSEMBLY SEQUENCES



PARTS CONNECTIONS GRAPH

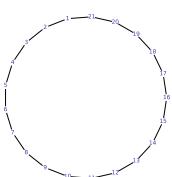
Assembly pre-validation with simulated cloning

SIMULATED CLONING



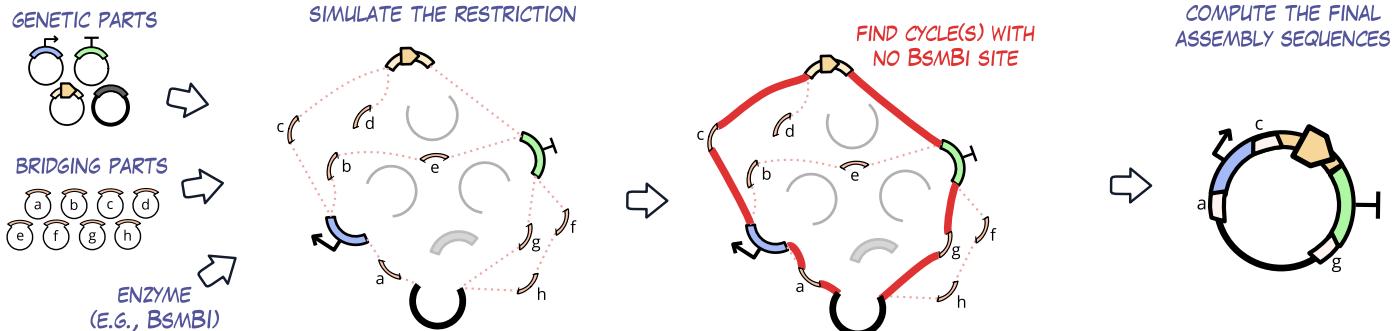
PARTS CONNECTIONS GRAPH

VALID ASSEMBLY

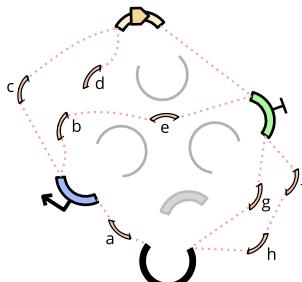


Assembly pre-validation with simulated cloning

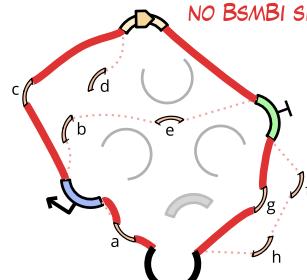
SIMULATED CLONING



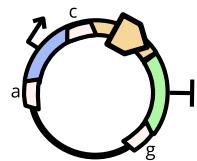
SIMULATE THE RESTRICTION



FIND CYCLE(S) WITH
NO BSMBI SITE

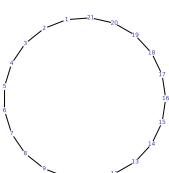


COMPUTE THE FINAL
ASSEMBLY SEQUENCES

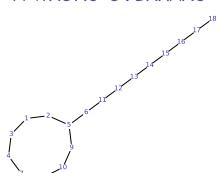


PARTS CONNECTIONS GRAPH

VALID ASSEMBLY

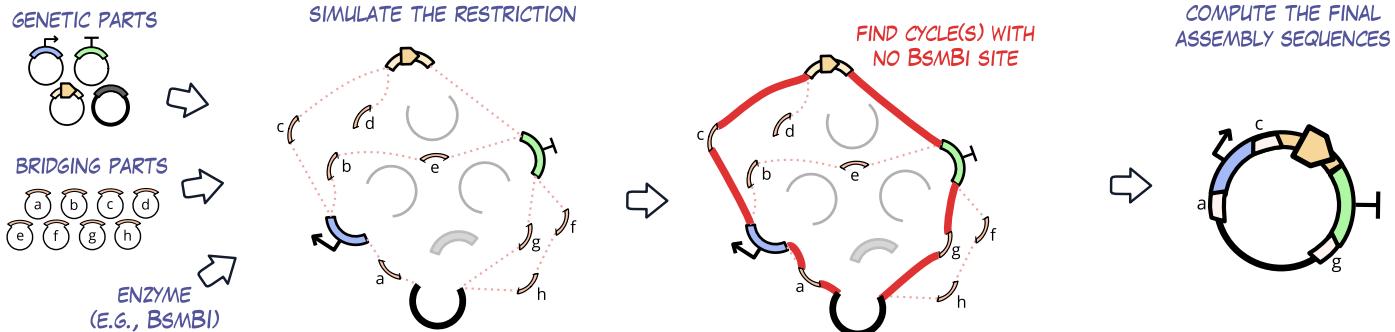


ONE PART HAS
A WRONG OVERHANG

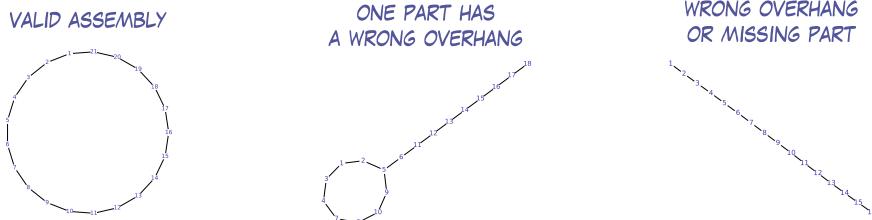


Assembly pre-validation with simulated cloning

SIMULATED CLONING

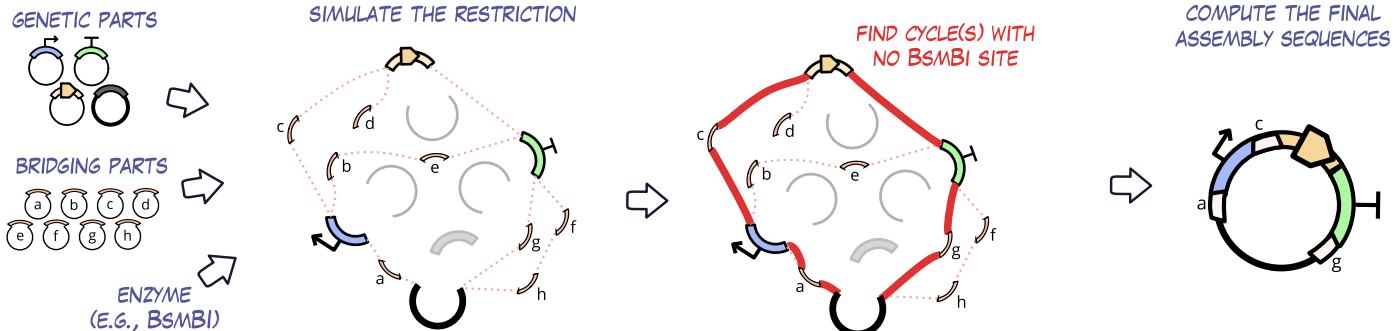


PARTS CONNECTIONS GRAPH

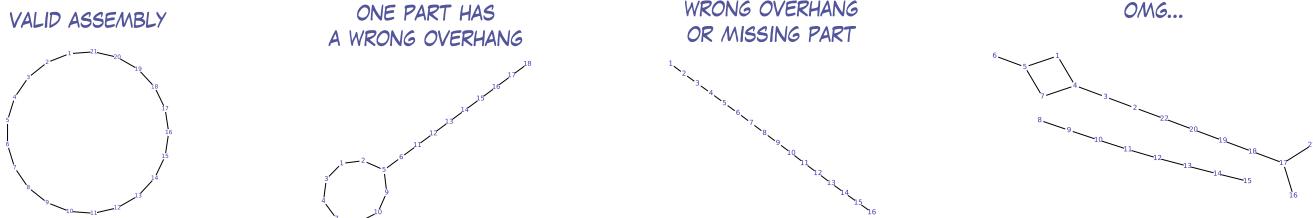


Assembly pre-validation with simulated cloning

SIMULATED CLONING

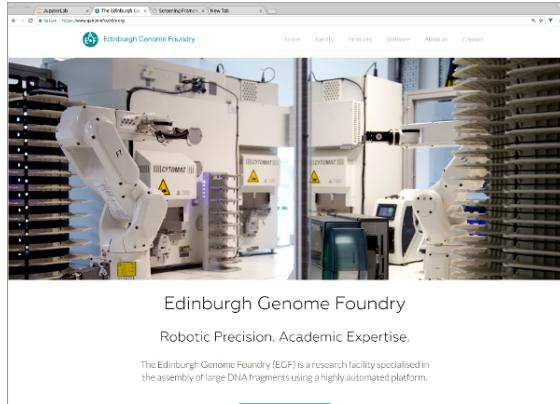


PARTS CONNECTIONS GRAPH



No hands ! DNA assembly in the cloud.

Edinburgh Genome Foundry

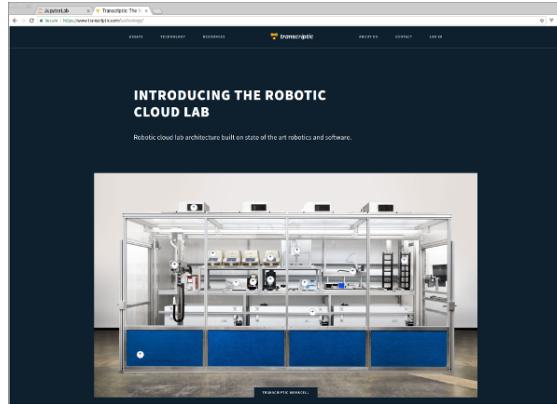


Edinburgh Genome Foundry

Robotic Precision. Academic Expertise.

The Edinburgh Genome Foundry (EGF) is a research facility specialised in the assembly of large DNA fragments using a highly automated platform.

Transcriptic

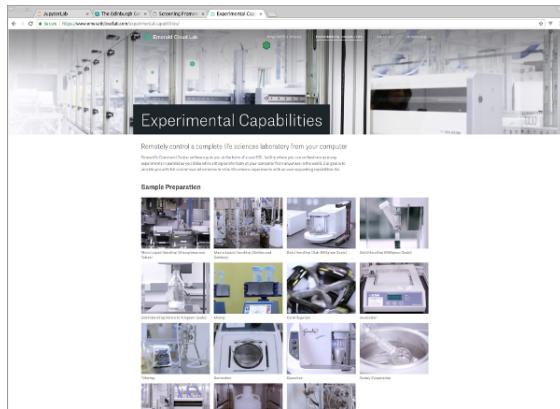


INTRODUCING THE ROBOTIC CLOUD LAB

Robotic cloud lab architecture built on state of the art robotics and software.



Emerald Therapeutics



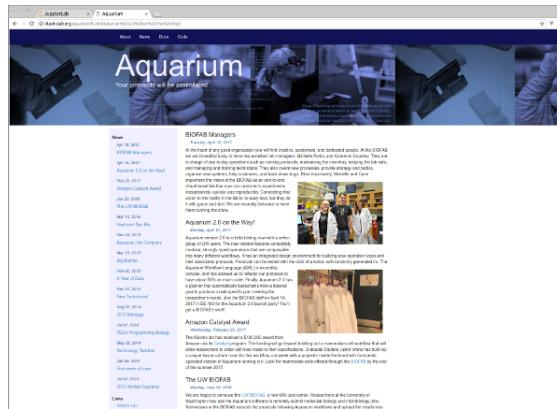
Experimental Capabilities

Remotely control a complete life sciences laboratory from your computer

Sample Preparation



Aquarium



Biorobotics Manager

As part of our R&D programme we will be creating, assessing, and optimising robots. In the Biorobotics Manager, you can upload your own designs or download existing ones. You can also upload your own images and videos, and manage your training libraries. They also have new processes, private storage and testing.

Biorobotics 2.0 on the Way!

Aquarium version 2.0 is currently being tested and refined. It will be available, already programmed so that you can quickly start using it. It will be a much more user friendly environment for training new students and researchers. Aquarium 2.0 will be up and running Q3M 2018. We are currently working on the design and development of the software, and will be releasing a beta version in June 2018. Finally, Aquarium 2.0 will produce much faster speeds than previous versions, and will be able to handle larger datasets. We are currently working on the design and development of the software, and will be releasing a beta version in June 2018. Finally, Aquarium 2.0 will produce much faster speeds than previous versions, and will be able to handle larger datasets.

Amazon Catalyst Award

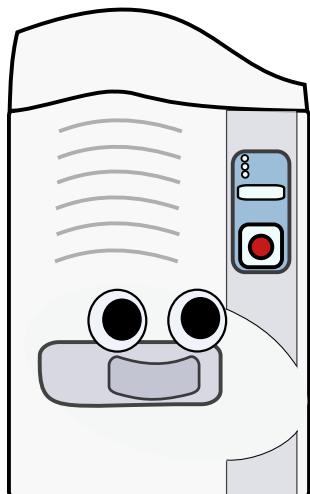
We thank the Amazon Catalyst program for the \$50,000 award that will help us to develop Aquarium. This will allow us to hire additional staff to work on the development of the software, and to purchase more hardware to test and refine the software. We are currently working on the design and development of the software, and will be releasing a beta version in June 2018. Finally, Aquarium 2.0 will produce much faster speeds than previous versions, and will be able to handle larger datasets.

The UW Biorobotics Manager

We are happy to release the UW Biorobotics Manager, a new UW core course. Researches at the University of Washington have developed a software tool called the UW Biorobotics Manager, which allows users to easily program robots in the Biorobotics lab by simply dragging and dropping commands and adding the most impor-

Assembly time !

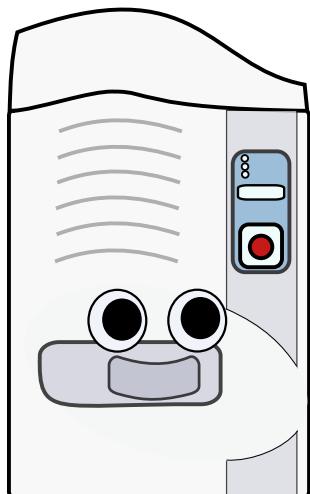
Question: What does this picklist do ?



Source Plate	Source Well	Destination Plate	Destination well	Volume (nL)
P1	A1	P2	A1	50
P1	B3	P2	A1	21
P1	B5	P2	A1	48
P1	C12	P2	A1	20
P1	A1	P2	A2	40
P1	F11	P2	A2	48
P1	G2	P2	A2	25
P1	G5	P2	A2	21
P1	A1	P2	A3	50
P1	B5	P2	A3	24
P1	B6	P2	A3	81
P1	B7	P2	A3	20

Assembly time !

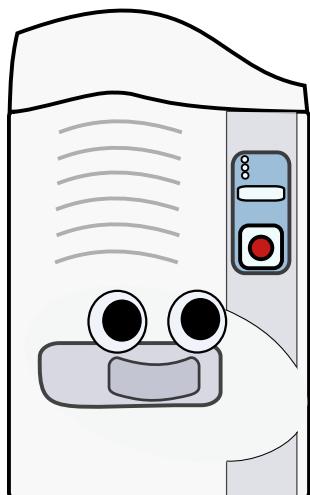
Question: What does this picklist do ?



Source Plate	Source Well	Destination Plate	Destination well	Volume (microL)
P1	A1	P2	A1	100
P1	A5	P2	B1	100
P1	B5	P2	C1	100
P1	B12	P2	D1	100
P1	C10	P2	E1	100
P1	C11	P2	F1	100
P1	C12	P2	G1	100
P1	F3	P2	H1	100
P1	G1	P2	A2	100
P1	G2	P2	B2	100
P1	H11	P2	C2	100
P1	H12	P2	D2	100

Assembly time !

Question: What does this picklist do ?



Source Plate	Source Well	Destination Plate	Destination well	Volume (microL)
P1	A1	P1	B1	25
Trough	--	P1	B1	75
P1	B1	P1	C1	25
Trough	--	P1	C1	75
P1	C1	P1	D1	25
Trough	--	P1	D1	75
P1	D1	P1	E1	25
Trough	--	P1	E1	75
P1	E1	P1	F1	25
Trough	--	P1	F1	75
P1	F1	P1	G1	25
Trough	--	P1	G1	75

Remarks on automated pipetting

Remarks on automated pipetting

- ▶ Automated pipetting improves precision, reproducibility, speed, costs.

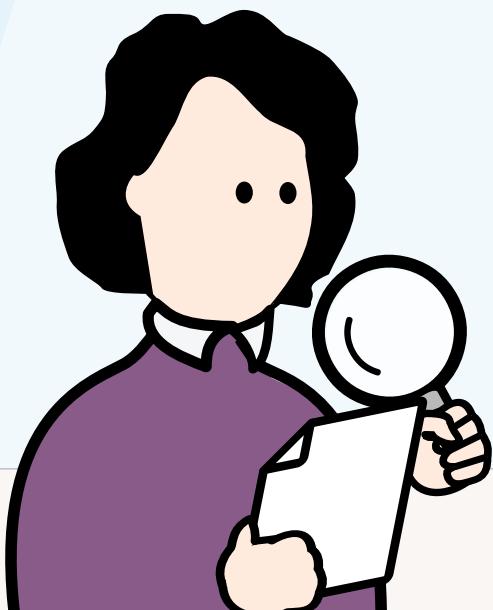
Remarks on automated pipetting

- ▶ Automated pipetting improves precision, reproducibility, speed, costs.
- ▶ It does **not** resolve the problem's complexity (you still need to write picklists), or the risk of error.

Remarks on automated pipetting

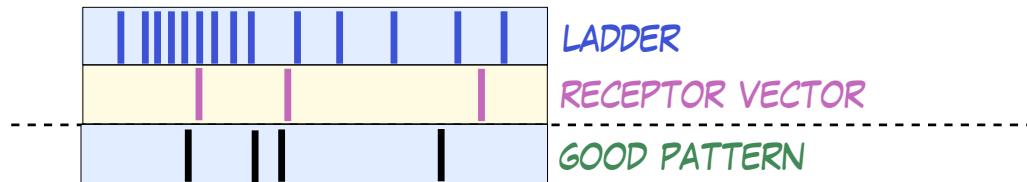
- ▶ Automated pipetting improves precision, reproducibility, speed, costs.
- ▶ It does **not** resolve the problem's complexity (you still need to write picklists), or the risk of error.
- ▶ See PR-PR, Antha, Plateo, for examples of software helping with picklist generation.

3. Validation



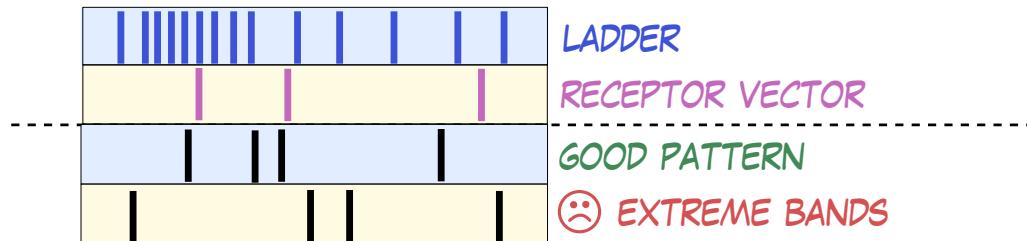
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



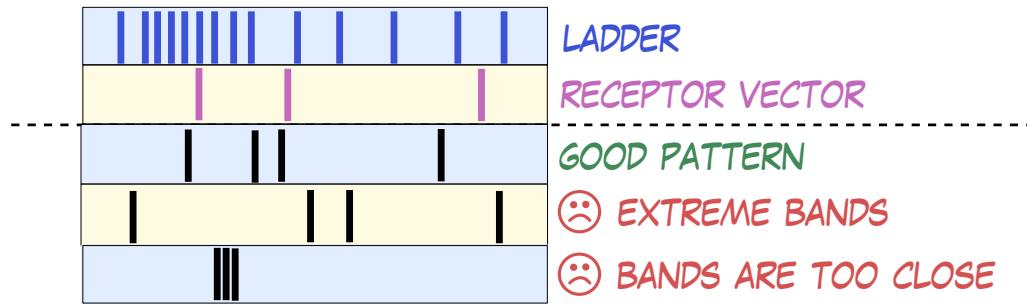
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



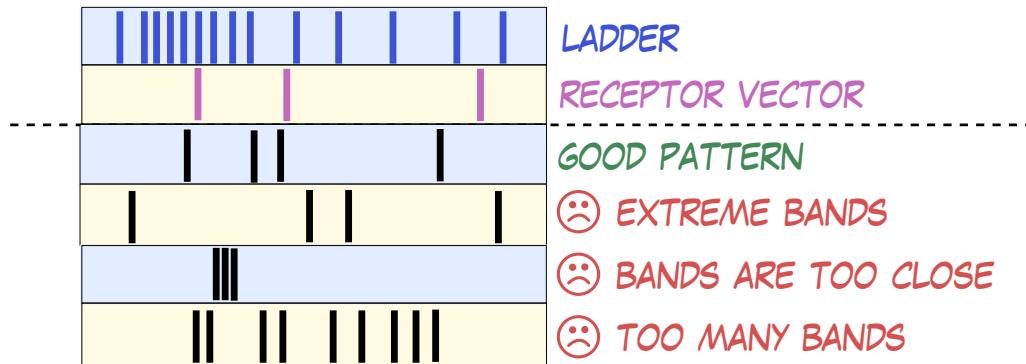
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



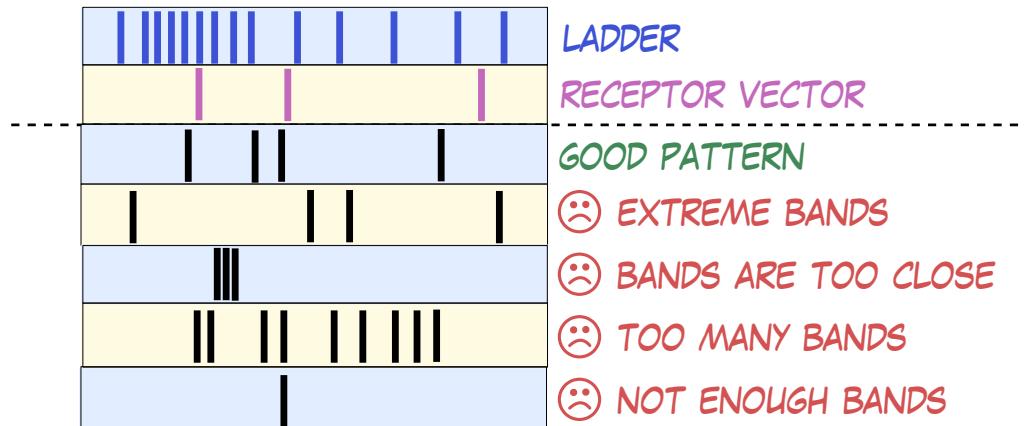
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



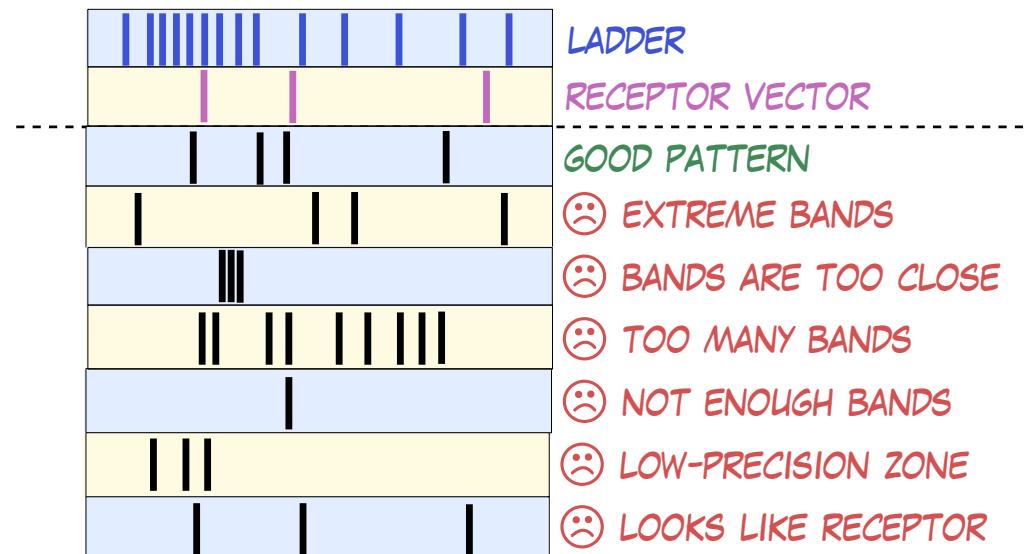
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



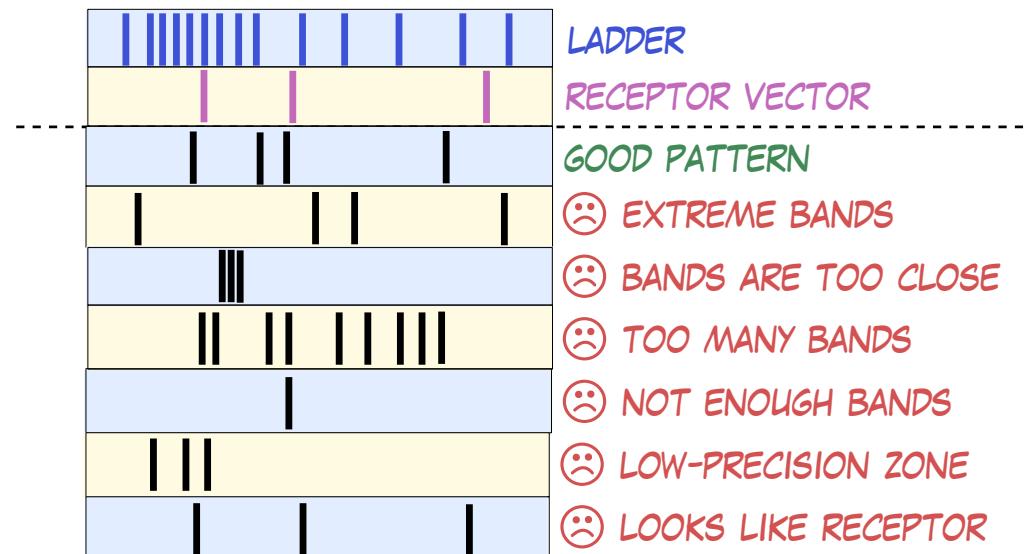
Computer-aided restriction digests

Shoot yourself in the foot with a bad choice of enzymes !



Computer-aided restriction digests

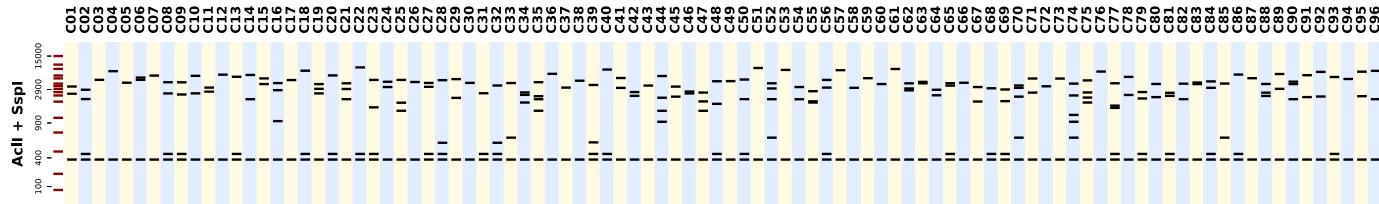
Shoot yourself in the foot with a bad choice of enzymes !



Other problems: methylation-sensitive enzymes, enzymes with incompatible activation temperatures.

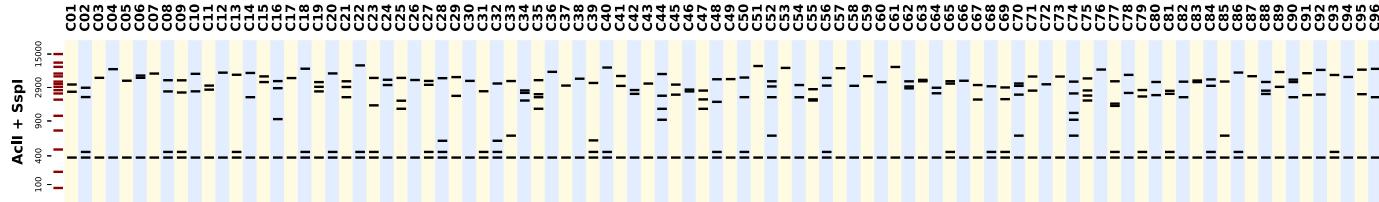
EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION

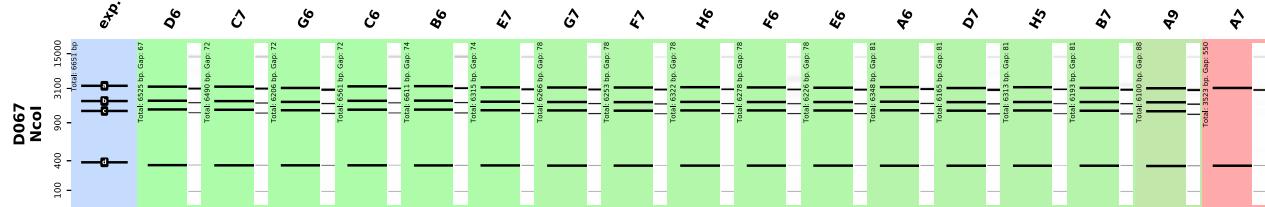


EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION

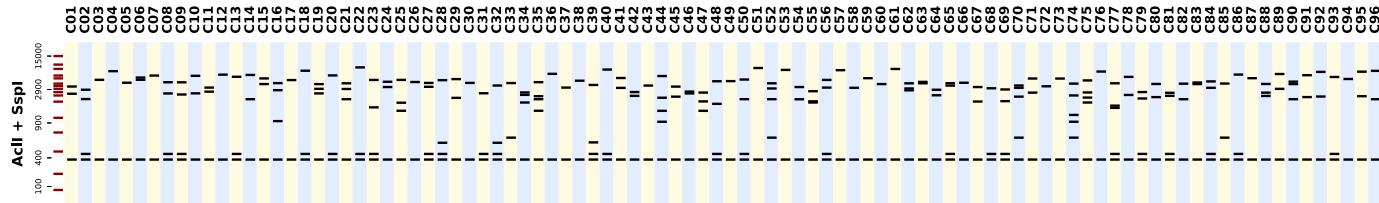


EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION

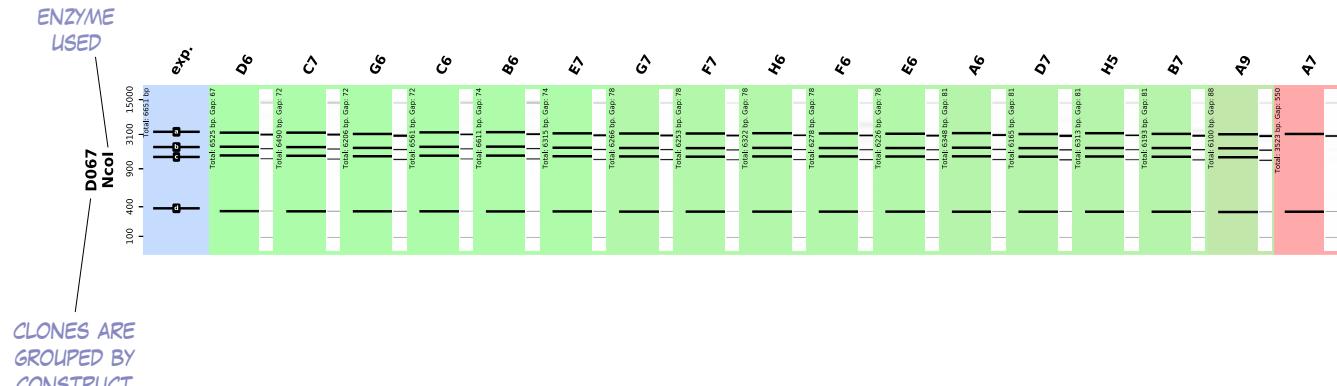


EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION

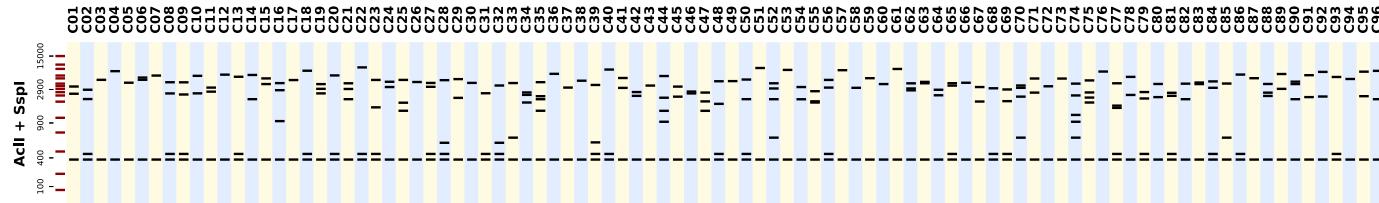


EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION

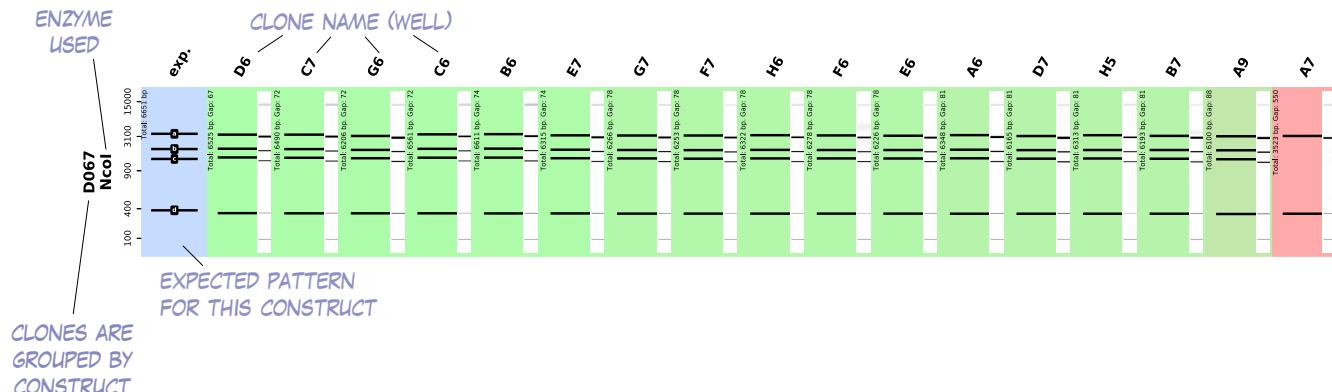


EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION

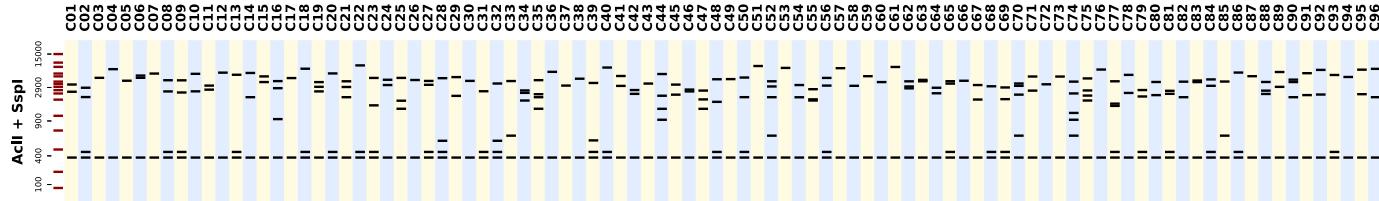


EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION

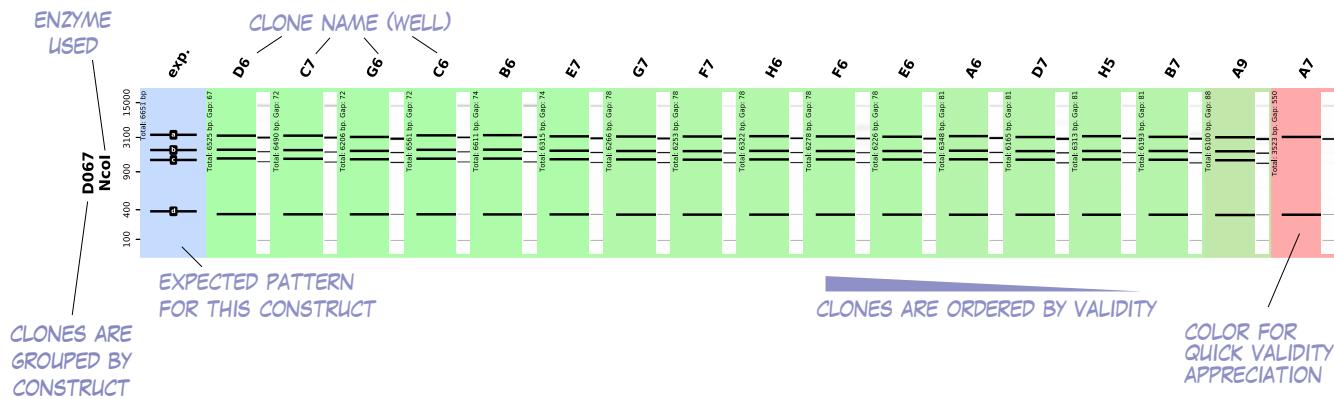


EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION



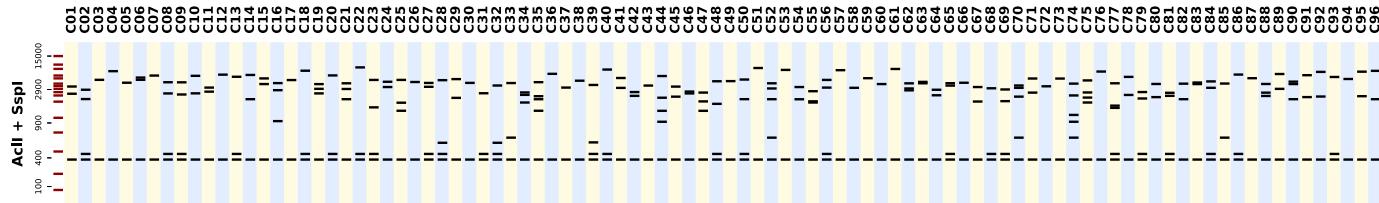
EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION



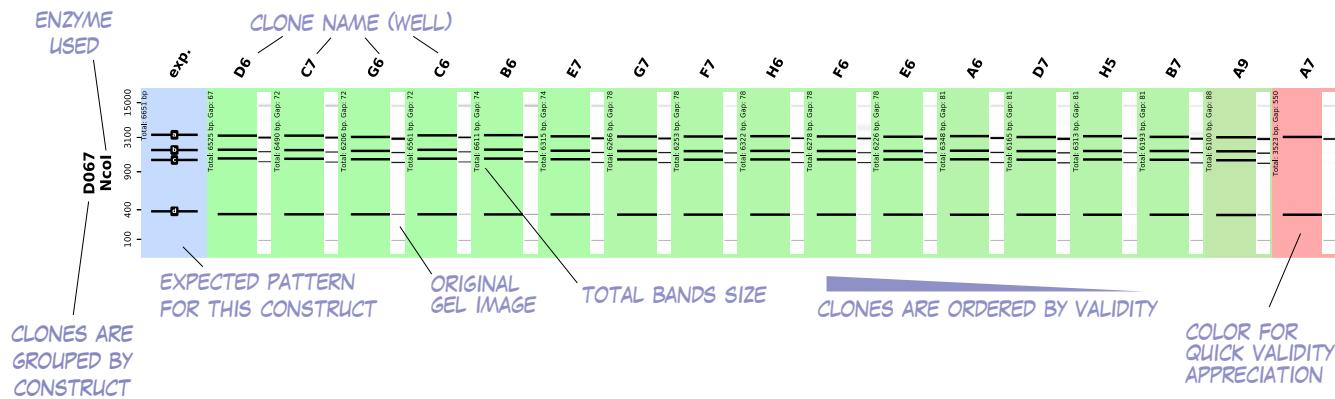
CLONES ARE GROUPED BY CONSTRUCT

EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION



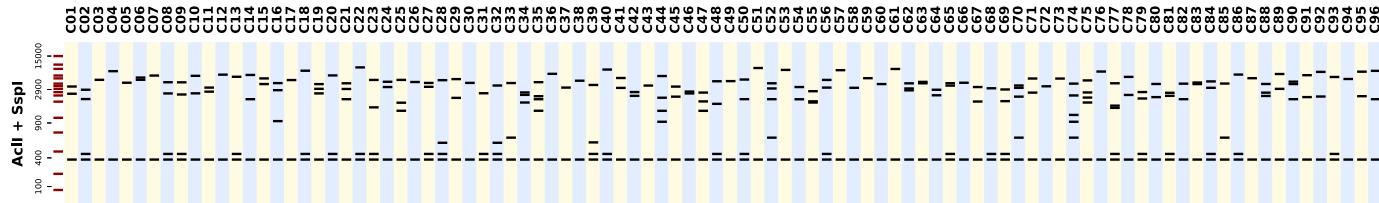
EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION



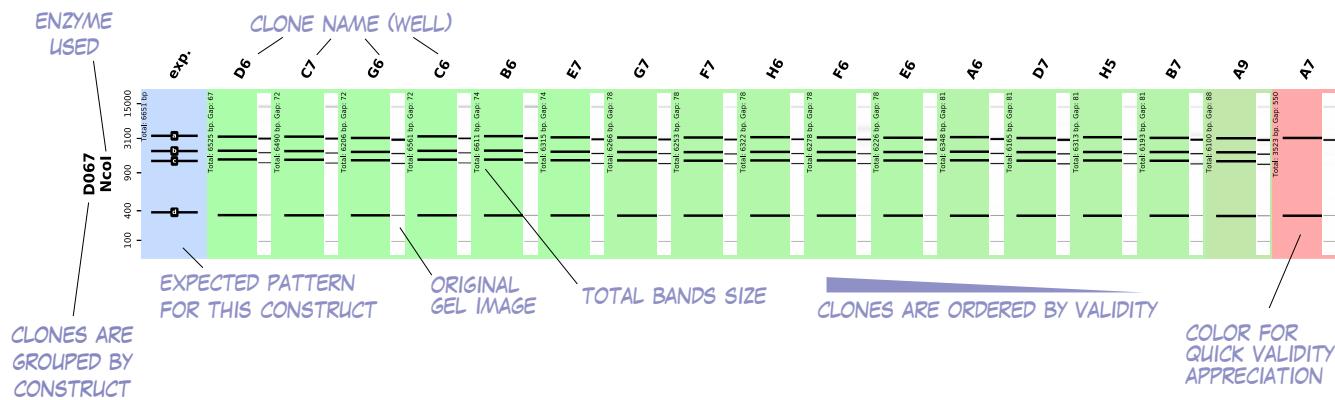
CLONES ARE GROUPED BY CONSTRUCT

EGF restriction digest software

AUTOMATIC DIGESTION SELECTION FOR WHOLE-PLATE VALIDATION

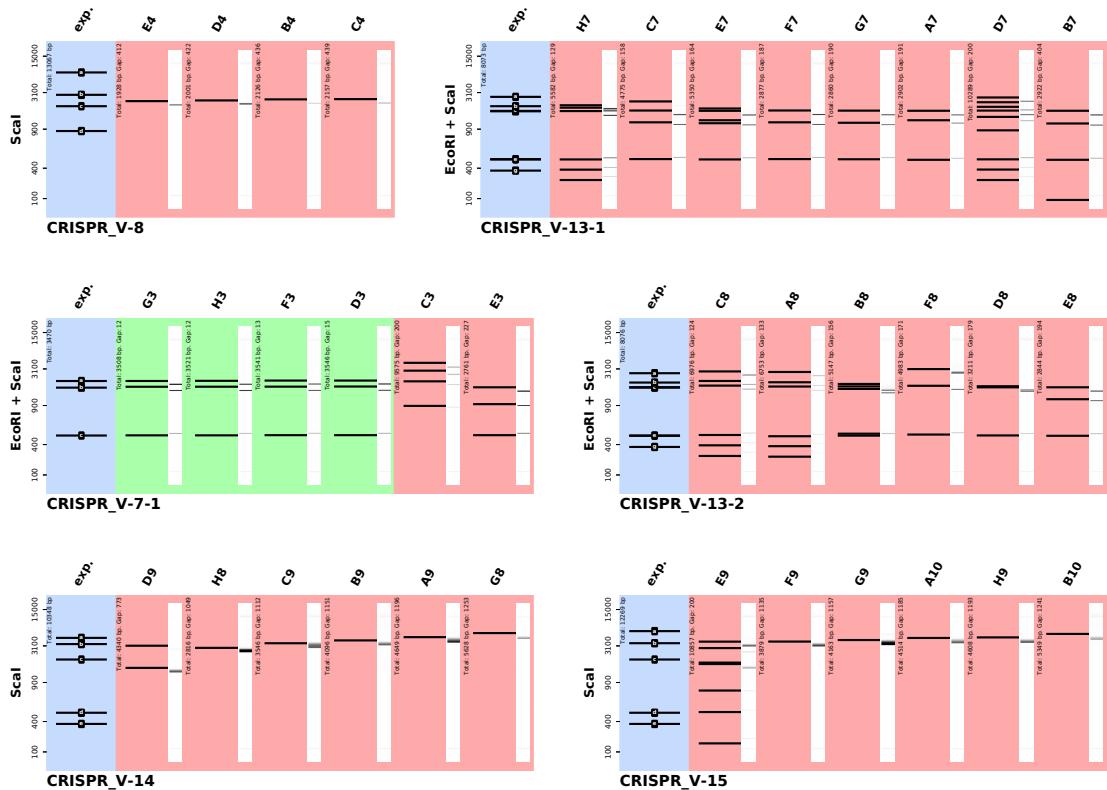


EXTENSIVE REPORT GENERATION FOR CONSTRUCT VALIDATION



CLONES ARE GROUPED BY CONSTRUCT

Aaaaaaargh ! Aaaaaaaaaaaaaaaargh !



What do you think happened ?

Find the saboteurs !

FAILED
ASSEMBLIES

SUCCESSFUL
ASSEMBLIES

PARTS

NAME	Value	max_Di	max_M	min_P	max_P	min_V	max_V								
ASSEMBLY-A	600	/	/												
ASSEMBLY-B	600	/	/												
ASSEMBLY-C	600	/													
ASSEMBLY-D	600	/													
ASSEMBLY-E	600	/													
ASSEMBLY-F	600	/													
ASSEMBLY-G	600	/													
ASSEMBLY-H	600	/													
ASSEMBLY-I	600	/													
ASSEMBLY-J	600	/													
ASSEMBLY-K	600	/													
ASSEMBLY-L	600	/													
ASSEMBLY-M	600	/													
ASSEMBLY-N	600	/													
ASSEMBLY-O	600	/													
ASSEMBLY-P	600	/													
ASSEMBLY-Q	600	/													
ASSEMBLY-R	600	/													
ASSEMBLY-S	600	/													
ASSEMBLY-T	600	/													
ASSEMBLY-U	600	/													
ASSEMBLY-V	600	/													
ASSEMBLY-W	600	/													
ASSEMBLY-X	600	/													
ASSEMBLY-Y	600	/													
ASSEMBLY-Z	600	/													

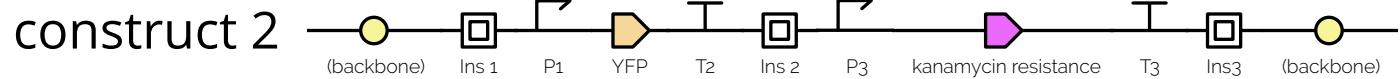
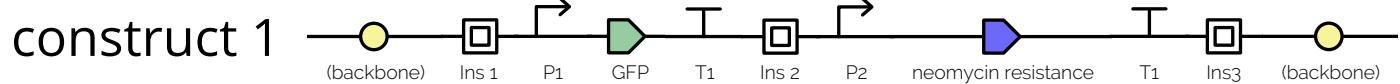
Find the saboteurs !

FAILED ASSEMBLIES

SUCCESSFUL ASSEMBLIES

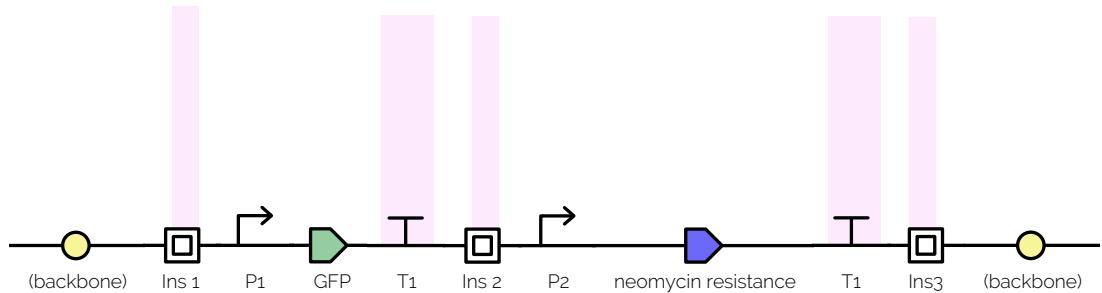
PARTS

Optimal Sanger Sequencing plan

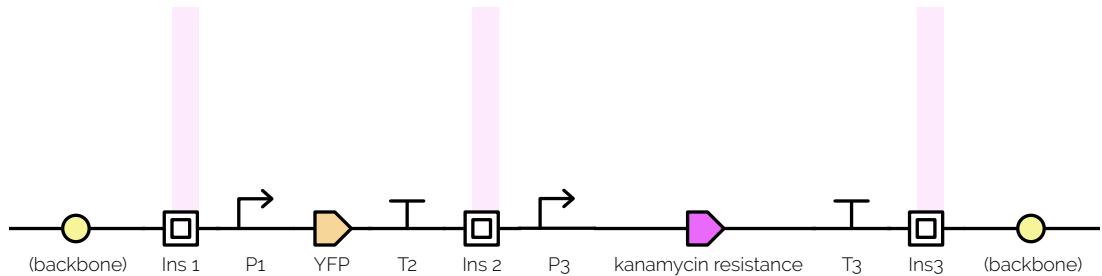


Optimal Sanger Sequencing plan

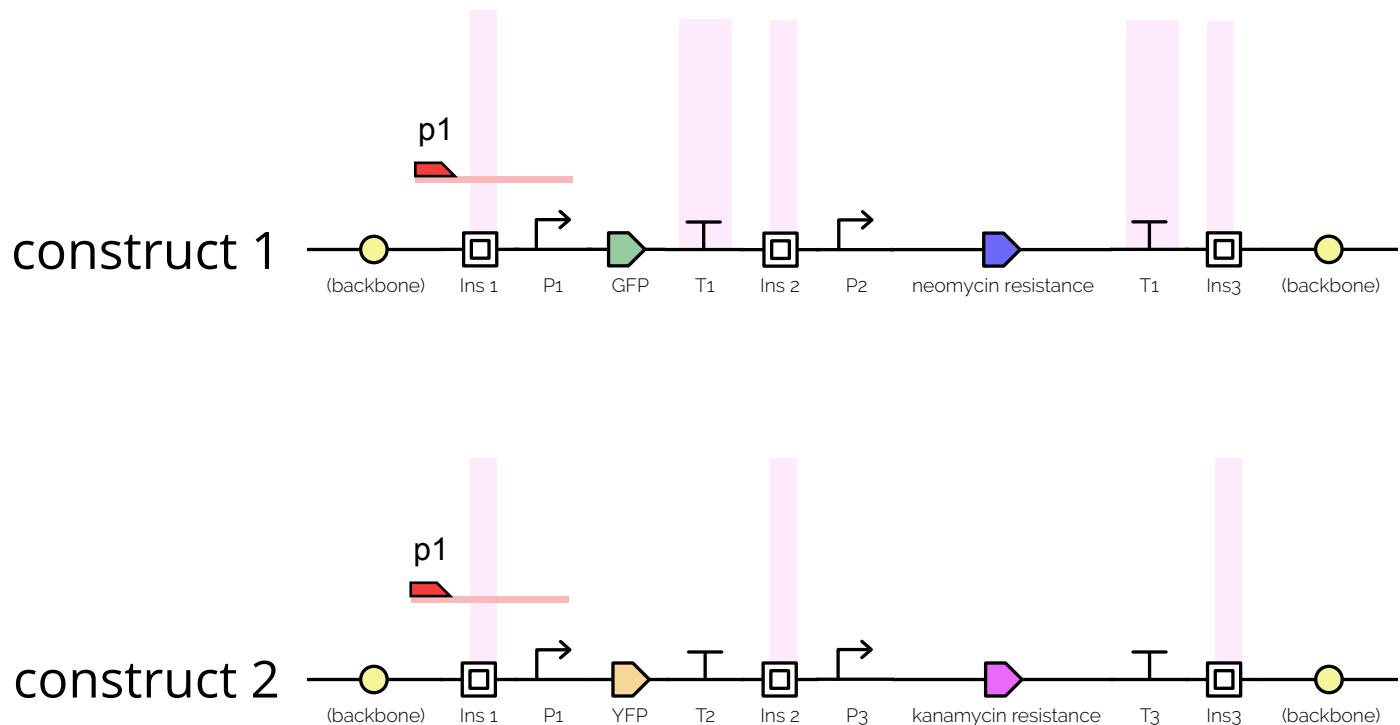
construct 1



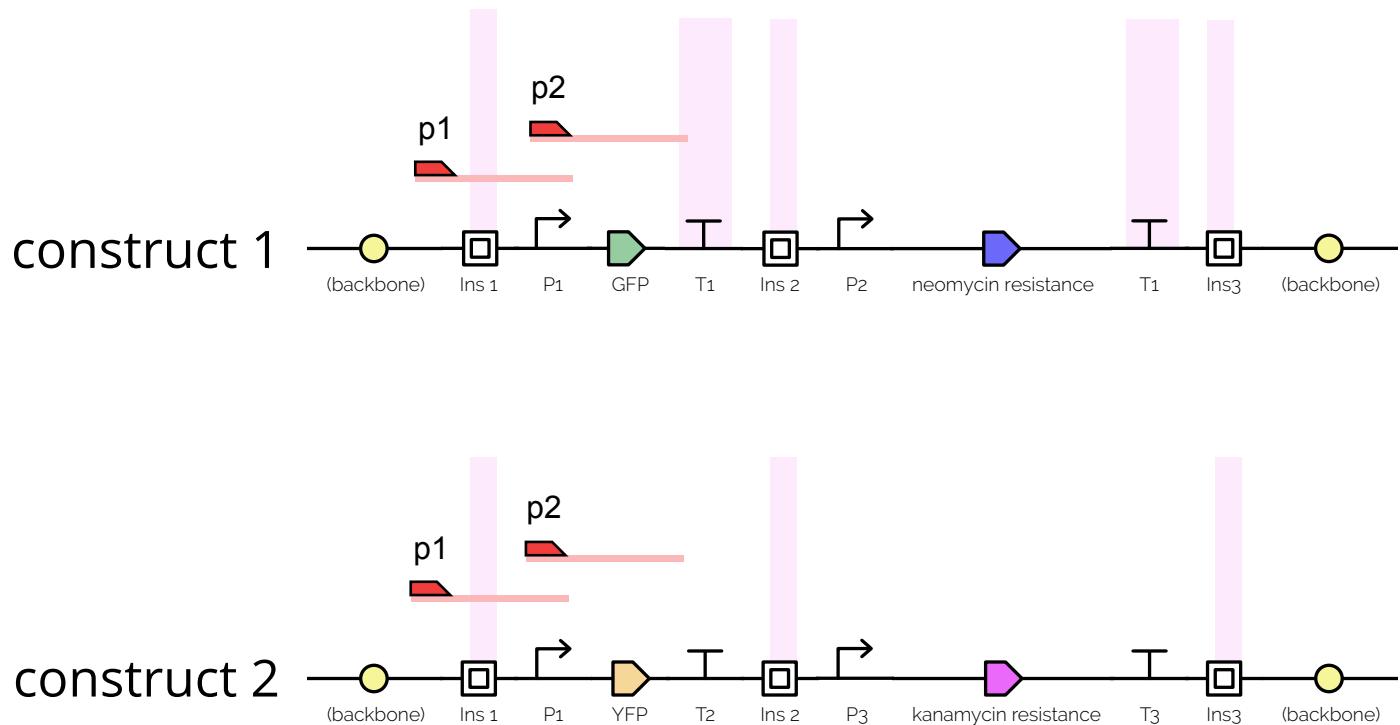
construct 2



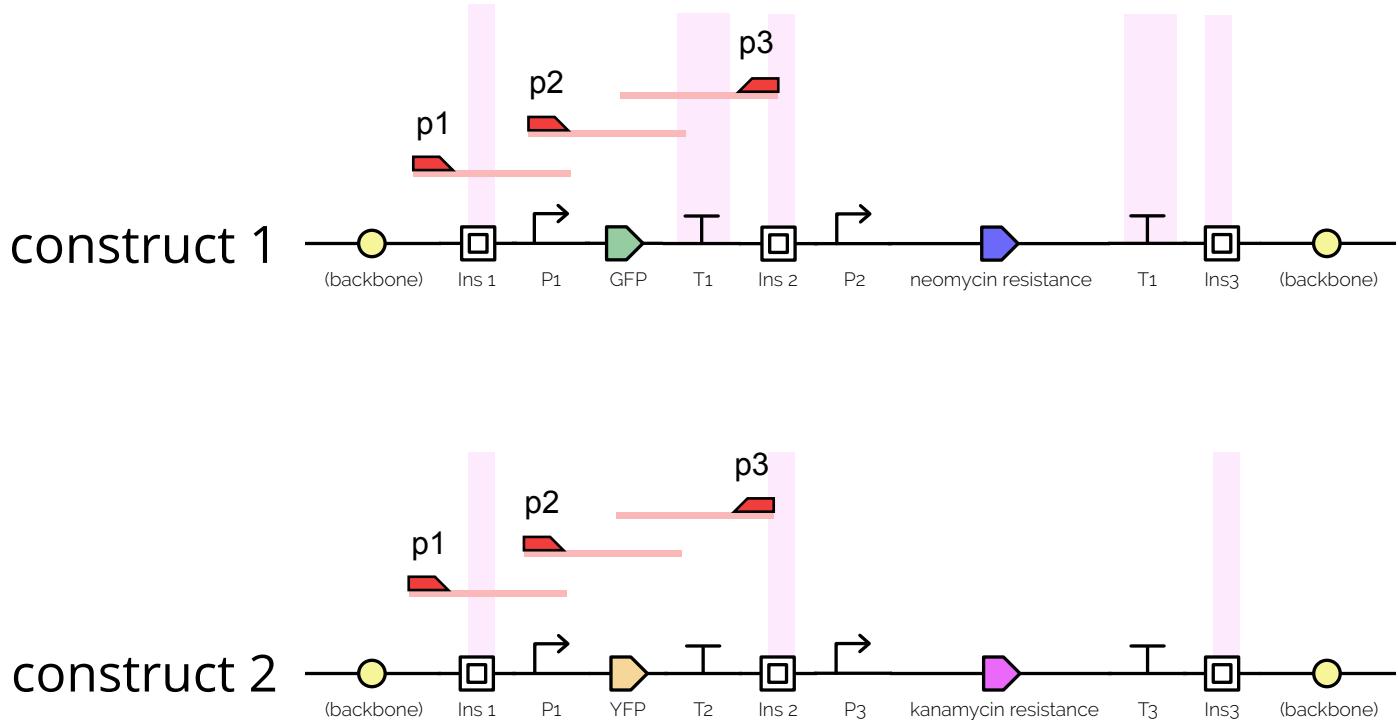
Optimal Sanger Sequencing plan



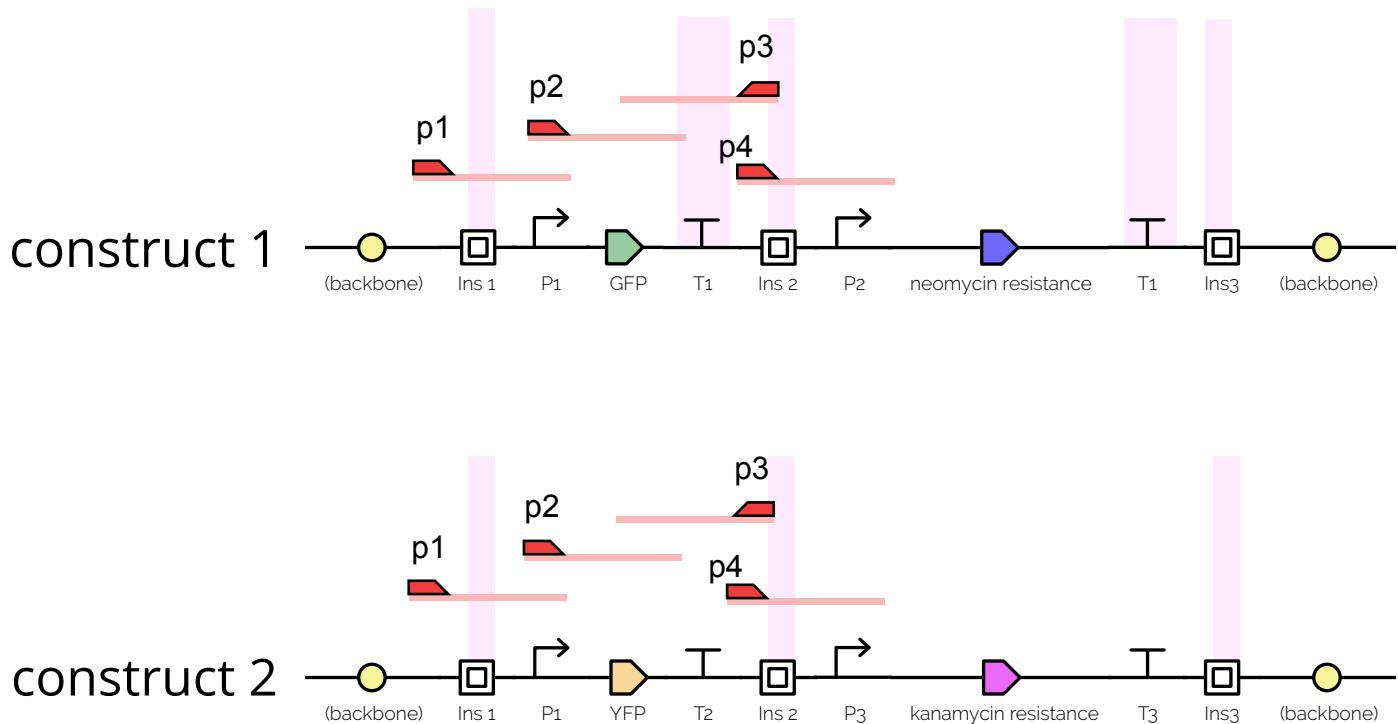
Optimal Sanger Sequencing plan



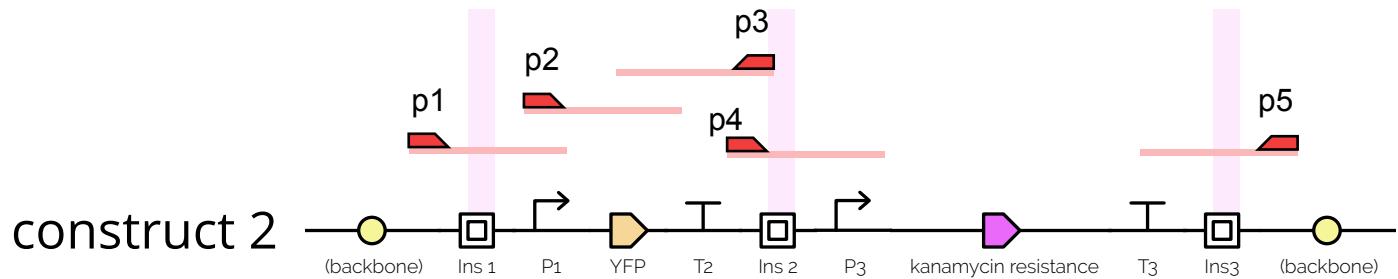
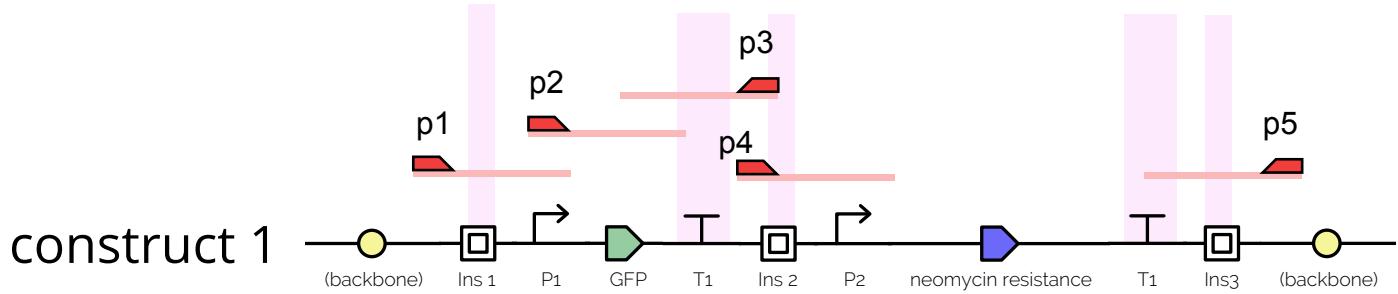
Optimal Sanger Sequencing plan



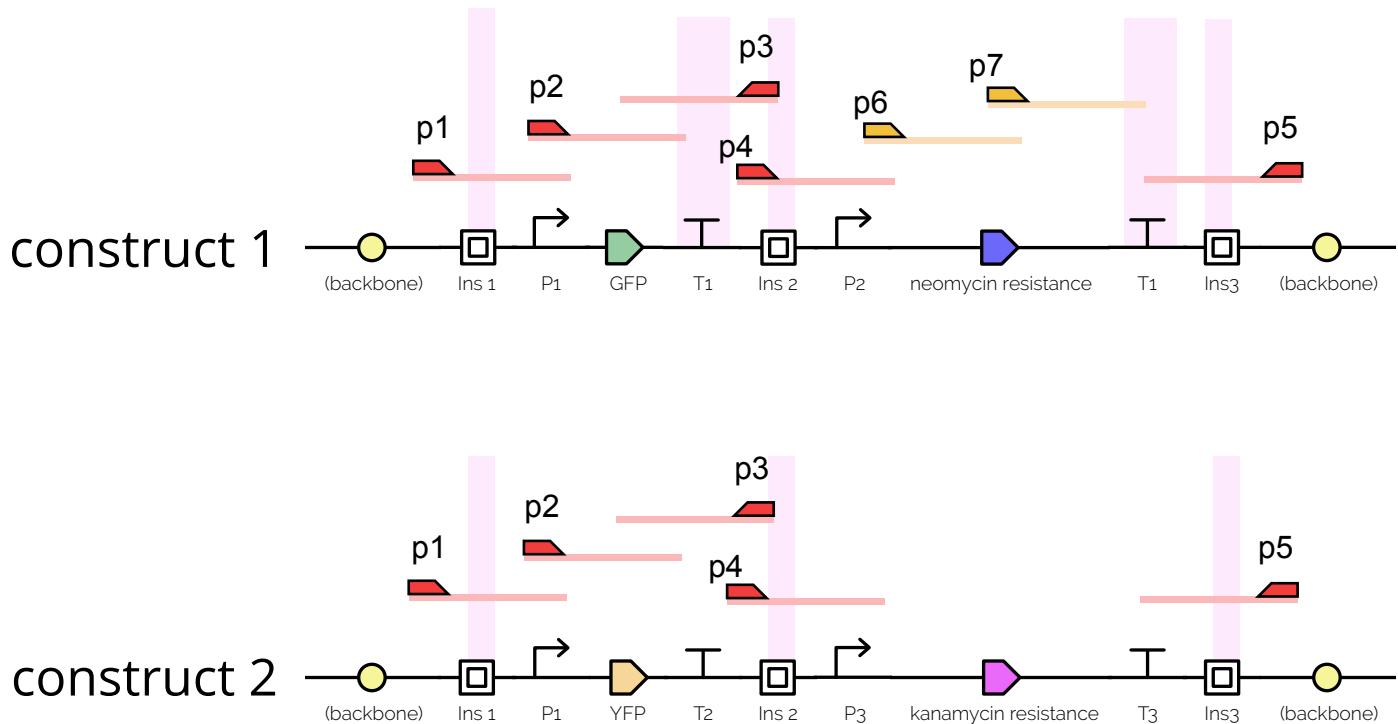
Optimal Sanger Sequencing plan



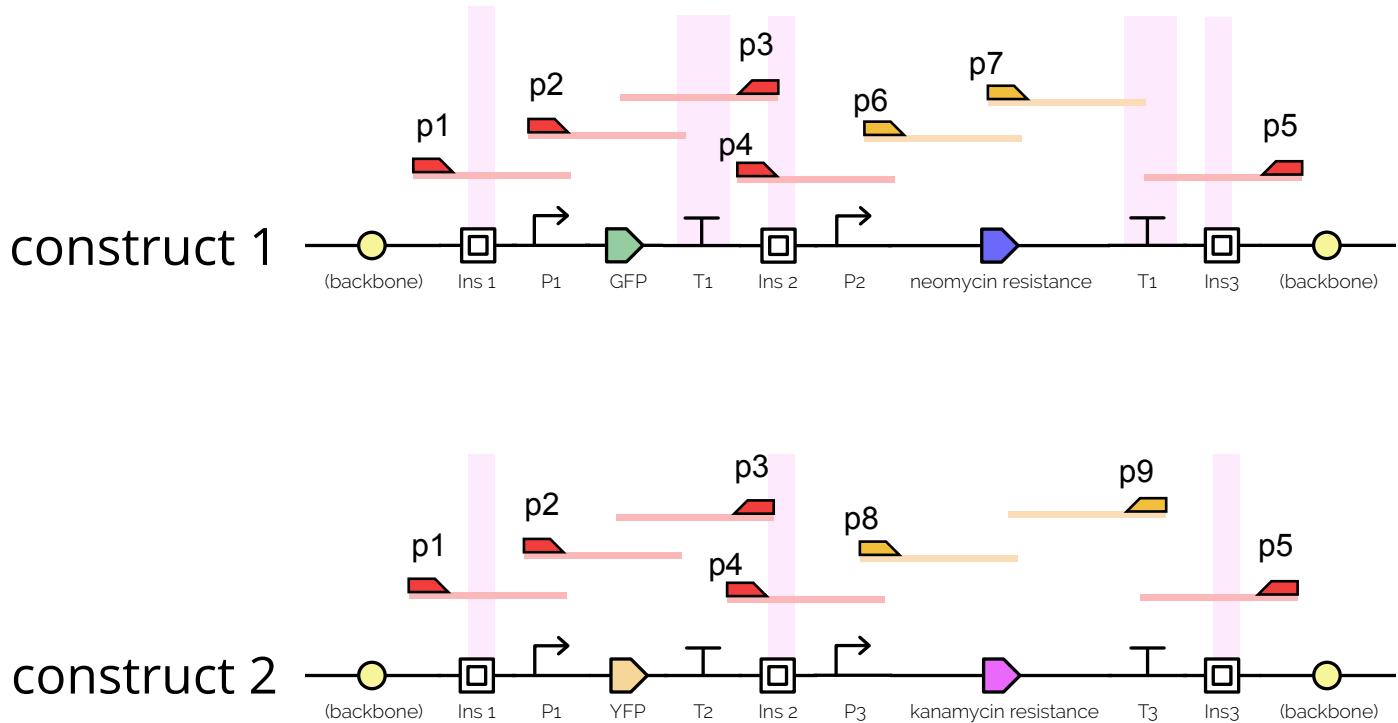
Optimal Sanger Sequencing plan



Optimal Sanger Sequencing plan



Optimal Sanger Sequencing plan



Primer selection software

constructs
sequences

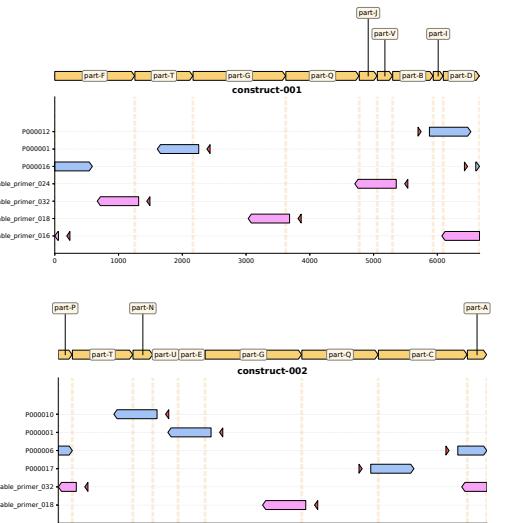


Screenshot of the "Select Primers" interface from the EGF CUBA genome browser. The page title is "Select Primers". It features a logo of a palm tree and the text "Find the best primers to your constructs, for validation or identification purposes." A dropdown menu shows "Validation Type: Sanger Sequencing". Below this are sections for "CONSTRUCTS SEQUENCES" (with a "Drop files here or click to select" box) and "AVAILABLE PRIMERS" (with a similar "Drop files here or click to select" box). A checkbox "Sequences are circular" is checked. At the bottom, a slider indicates the "ideal read range: from 150bp after primer annealing to 800bp".

available
primers



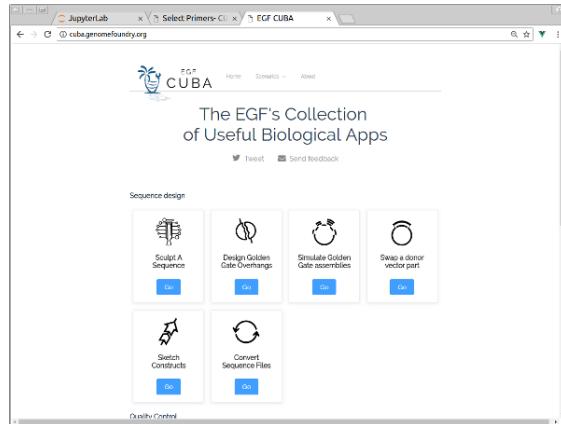
optimal sanger sequencing plan



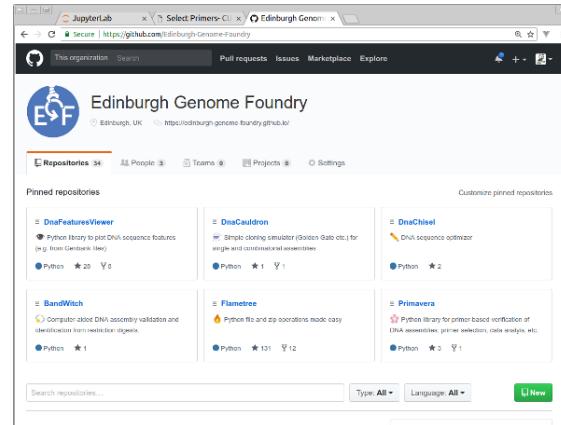
Conclusion

- ▶ The path to DNA assembly is dark and full of terrors.
One mistake can cost weeks and plenty of money.
- ▶ Advances in molecular biology and growing software investment should solve many issues in the next years.
- ▶ Next time you design or assemble DNA, have a look at the latest software... or call the Foundry !

Conclusion: links



Public web apps:
cuba.genomefoundry.org



Computational libraries on Github:
[Edinburgh-Genome-Foundry](https://github.com/Edinburgh-Genome-Foundry)

Contact:
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