

A field guide to automated cloning

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... nothing to be scared off?



The Team



Raik Grünberg



Jim Xu



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Tyers lab, IRIC, University of Montreal

Fast Prototyping of Synthetic Protein Systems

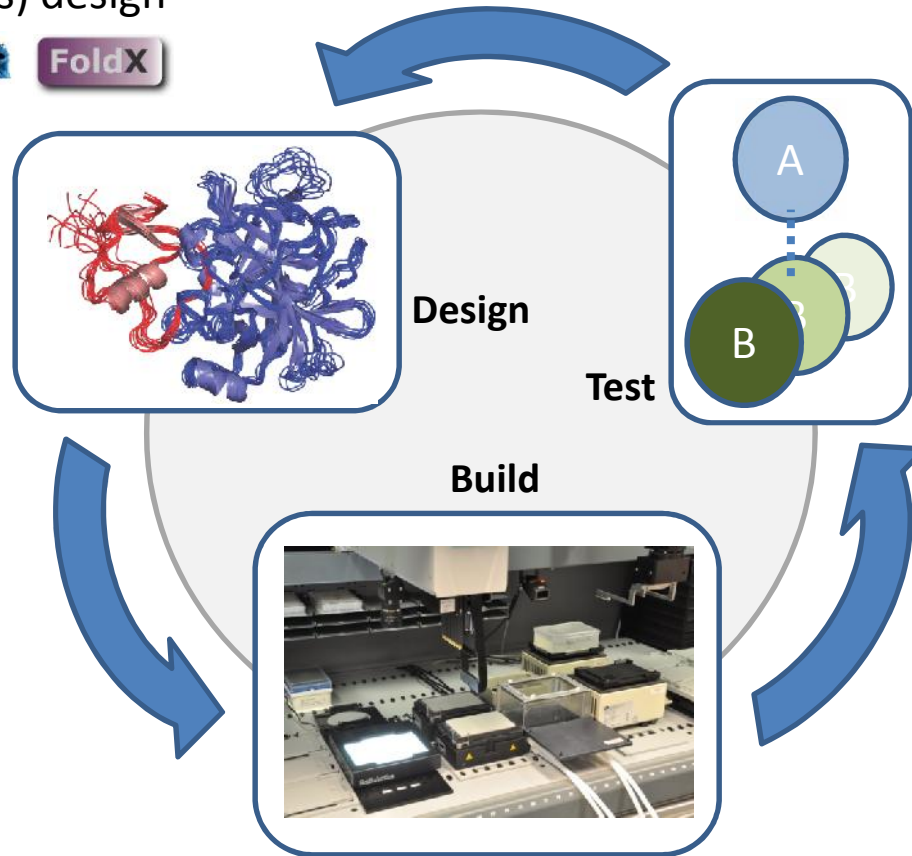
computational protein
(systems) design



cell-free expression

*

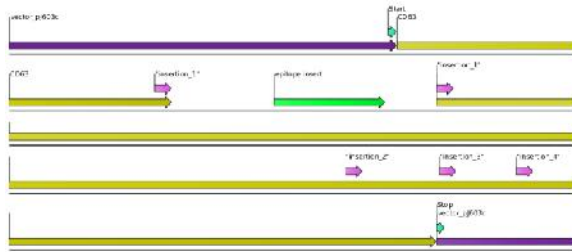
purification-free
interaction measurements



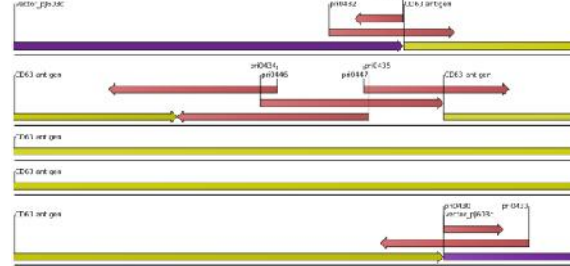
automated DNA assembly

DNA Assembly Workflow

1 construct design



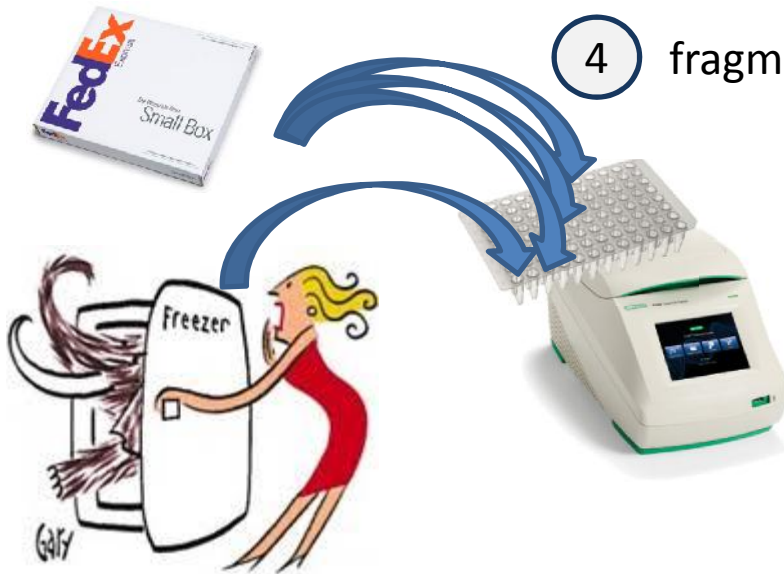
2 assembly design



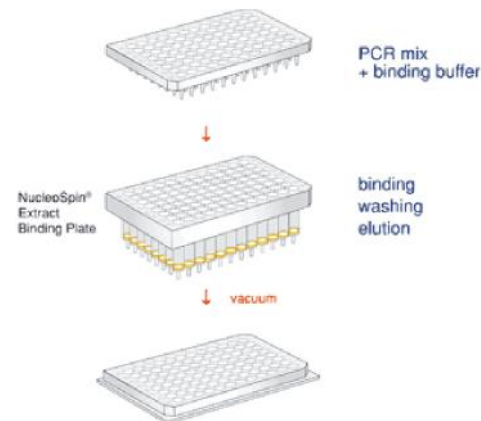
3 primer & gene synthesis



4 fragment PCR



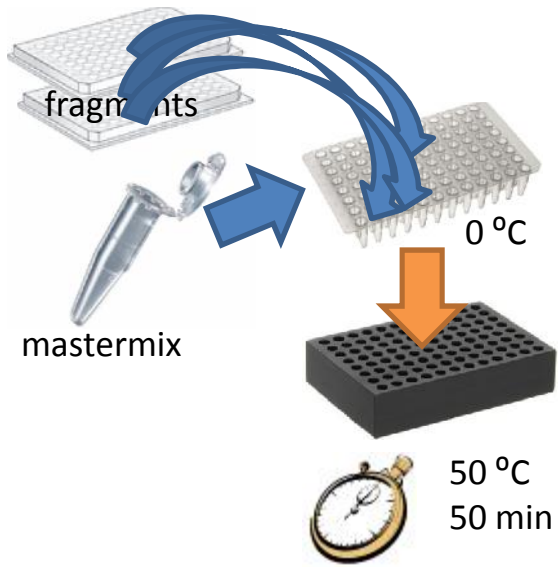
5 PCR cleanup



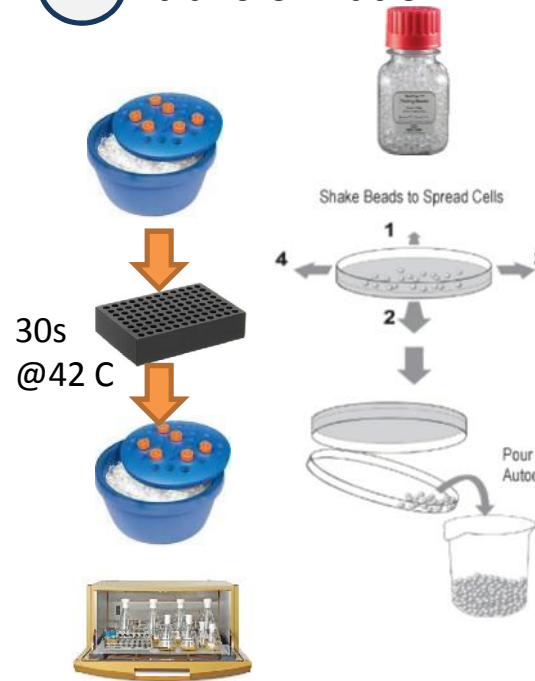
6 concentration/normalization



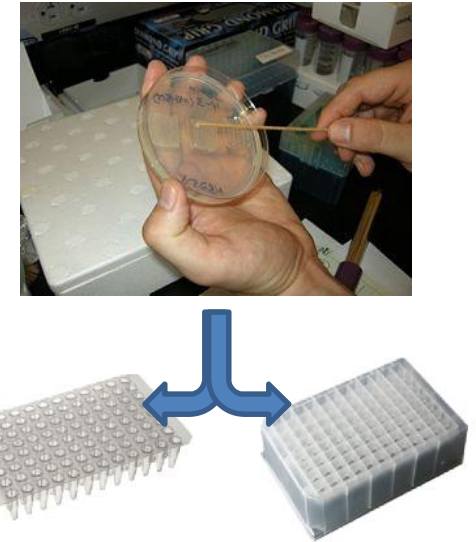
7 isothermal assembly



8 transformation



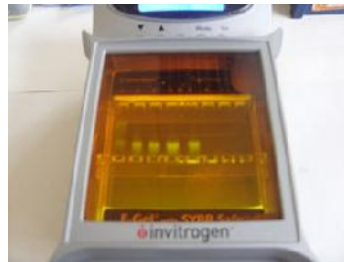
9 colony picking



10 colony PCR



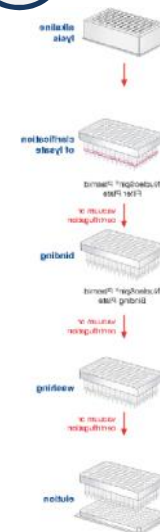
11 gel electrophoresis



12 incubation



13 DNA miniprep



What robots cannot (easily) do...



...money can solve.



Shuttling samples to
various incubators & storage



Automated Storage Systems

transformation



QPix 400

colony picking

gel electrophoresis



Fragment Analyzer

...lots of money...



Shuttling samples to
various incubators & storage



Automated Storage Systems

transformation



QPix 400

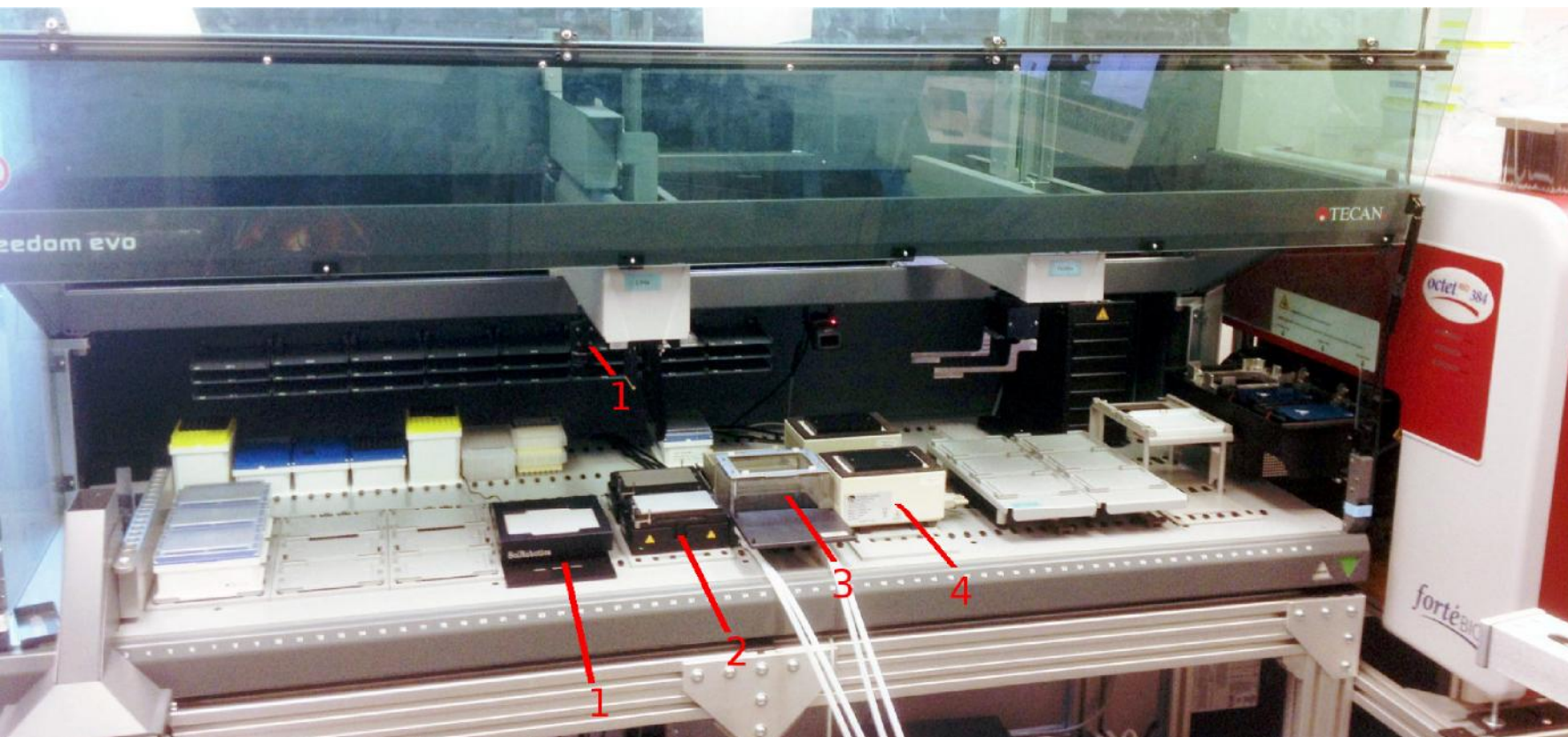
colony picking

gel electrophoresis



Fragment Analyzer

Robotic Setup



1
on-deck
Colony picking

2
Thermoshakers

3
regulated
vacuum

4
-20 to 110 C
heating/cooling

5
Dockable worktables for
ForteBio and plate reader



1

Colony picking

2

Thermoshakers

3

regulated
dual vacuum

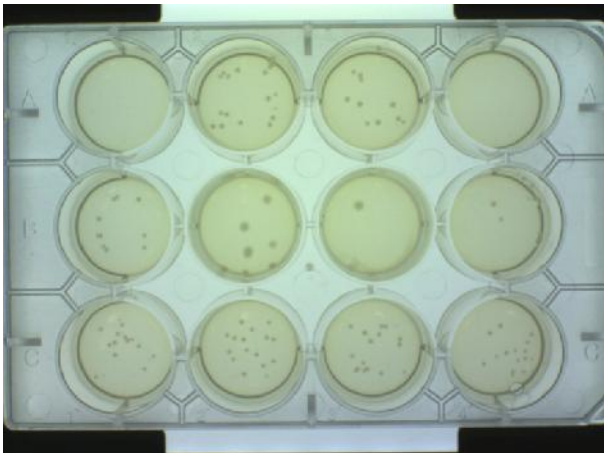
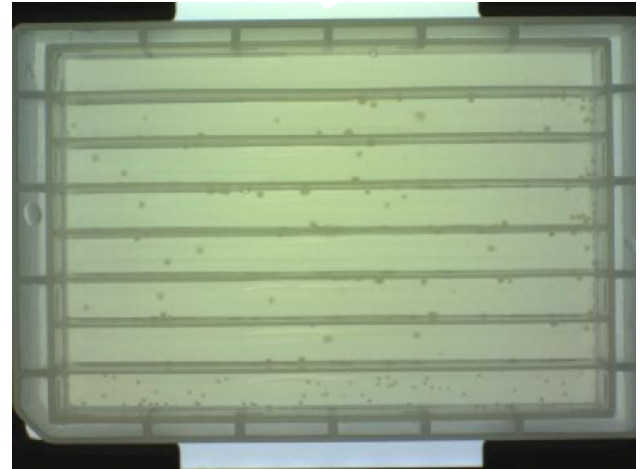
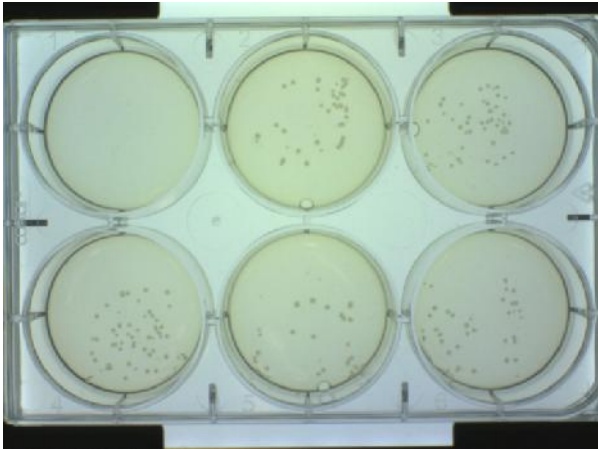
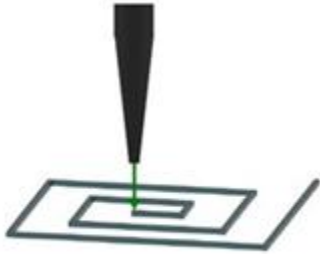
4

-20 to 110 C
heating/cooling

colony picking

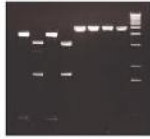


Transformation





Robotic Workflow



1

Fragment PCR-setup

2

PCR cleanup

3

DNA normalization

4

Gibson assembly

5

Transformation

6

Colony picking setup

7

Colony picking

8

Colony PCR setup

9

Cherry-pick / inoculation

10

Glycerol stock

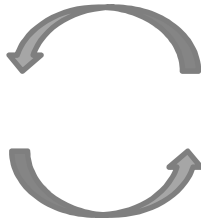
11

DNA Miniprep

PCR cherry picking

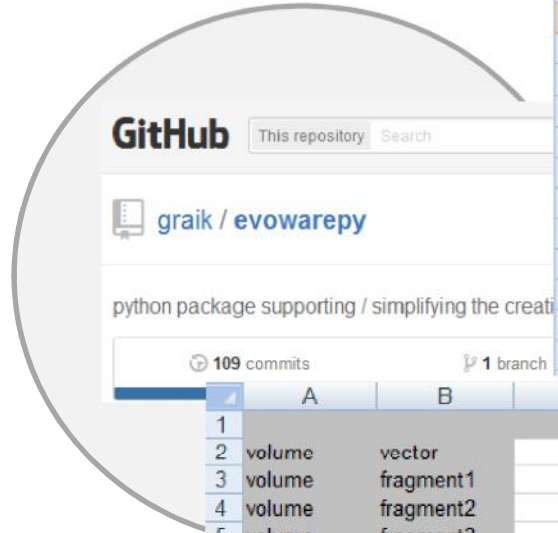
Agar filling

Kit regeneration



- 1 ☒ Fragment PCR-setup
- 2 ☒ DNA normalization
- 3 ☒ Gibson assembly
- 4 Transformation
- 5 Colony picking setup
- 6 Colony picking
- 7 Colony PCR setup
- 8 ☒ Cherry-pick / inoculation
- 9 ☒ Glycerol stock
- 10 DNA Miniprep

☒ PCR cherry picking

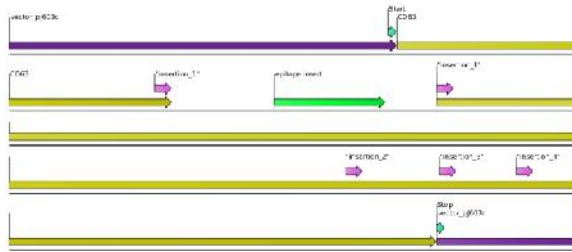


	A	B	C	D
2				
3	format	SB11	384	
4				
5				
6	Part List		source	
7	ID	sub-ID	plate	pos
8	sb0101	2	SB10	A1
9	sb0101	3	SB10	A3
10	sb0102	1	SB10	G1
11	sb0102	2	SB10	A5
12	sb0103		SB10	H12
13				
14	sb0104	1	SB11	1
15	sb0104	2	SB11	8

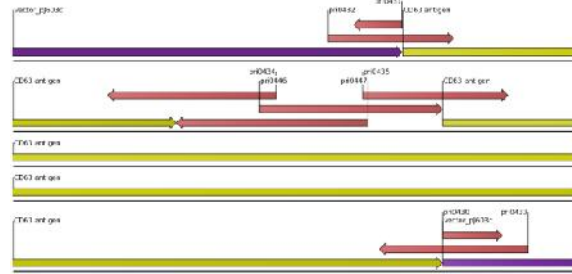
	A	B	C	D	E	F	G	H	I	J	K
1											
2	volume	vector		3 µl							
3	volume	fragment1		2 µl							
4	volume	fragment2		2 µl							
5	volume	fragment3		2 µl							
6	volume	fragment4		2 µl							
7											
8	assembly reactions										
9											
10	ID	sub-ID	target			vector		fragment1		fragment2	fragn
11	sb0501		GA-1	A1		sbv0009		sbf0100		sbf0110	
12	sb0502		GA-1	B1		sbv0009		sbf0101		sbf0111	
13	sb0503		GA-1	C1		sbv0009		sbf0102		sbf0112	
14	sb0504		GA-1	D1		sbv0009		sbf0103		sbf0113	
15	sb0505		GA-1	E1		sbv0009		sbf0104		sbf0114	
16	sb0506		GA-1	F1		sbv0009		sbf0105		sbf0115	
17	sb0507		GA-1	G1		sbv0009		sbf0106		sbf0116	
18	sb0508		GA-1	H1		sbv0009		sbf0107		sbf0117	
19											
20	sb0509		GA-1	A2		sbv0009		sbf0100		sbf0118	
21	sb0510		GA-1	B2		sbv0009		sbf0101		sbf0119	

New Bottlenecks

1 construct design



2 assembly design



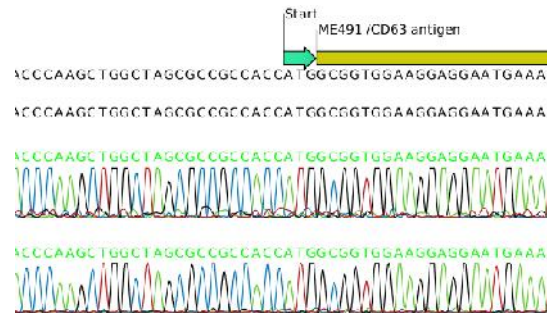
3 primer & gene synthesis



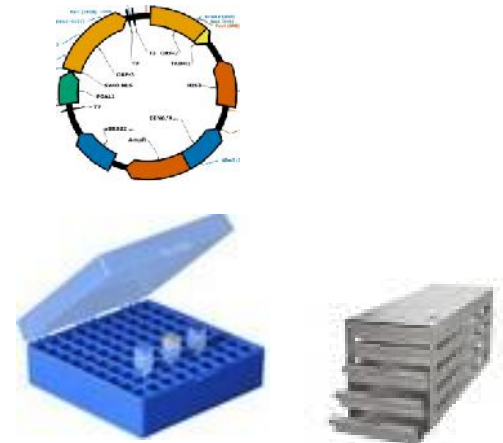
4 DNA assembly



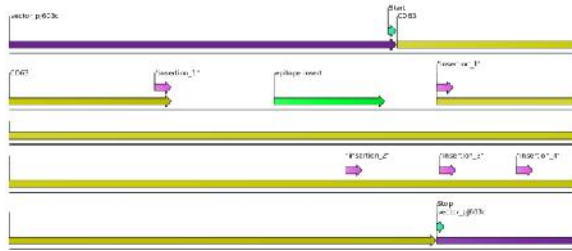
5 Quality Control



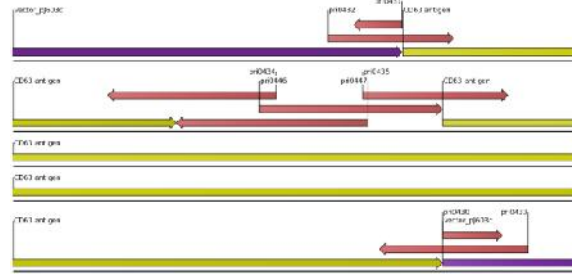
6 Sample & Construct Management



1 construct design



2 assembly design



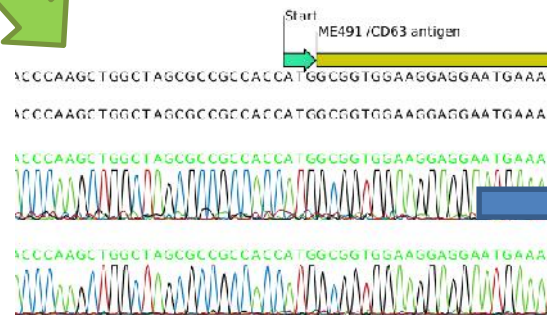
3 primer & gene synthesis



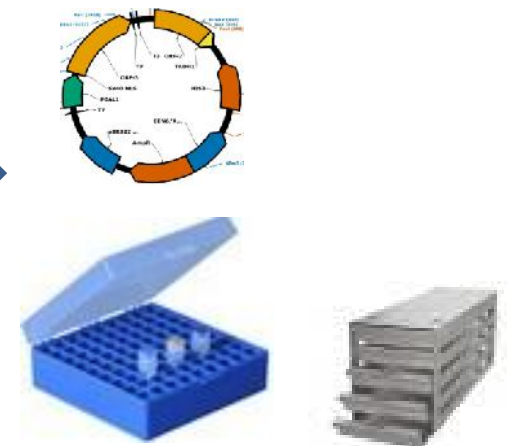
4 DNA assembly



5 Quality Control




6 Sample & Construct Management



Rotten Microbes
<http://rotmic.org>

Overview

Data Sheets (Constructs)		
	DNA constructs	+Add
	Modified Cells	+Add
	Oligonucleotides	+Add
	Proteins	+Add
	Chemicals & Reagents	+Add

Actual Samples		
	DNA Samples	+Add
	Cell stock Samples	+Add
	Oligonucleotide Samples	+Add
	Protein Samples	+Add
	Chemical & Reagent Samples	+Add
	All Samples	

Storage Locations & Containers		
	Locations (freezers or rows)	+Add
	Racks (or shelves)	+Add
	Containers (boxes)	+Add

Miscellaneous	
Projects	+Add
Sequencing Results	+Add
My personal settings	



DNA construct

rg2041 (FR-20-Che_pJEx411a)

[History](#)[Edit](#)

Description

by Raik Gruenberg (raik), Projects: hiFRET;

FRB-mCherry reference construct with long linker.

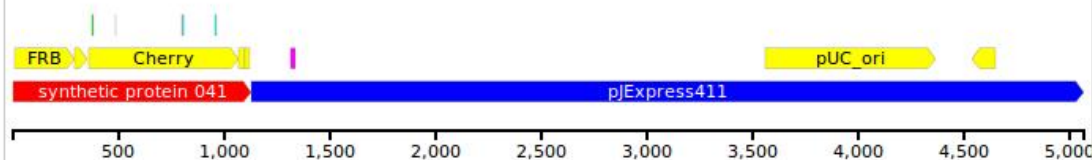
Note: Expression in BL21 typically produces an additional, truncated protein product. This is due to a cryptic RBS within the mCherry N-terminal. This lighter protein can make up more than 50% of total and needs to be removed by gel filtration.

Sequence links and composition

Insert	Vector Backbone	Vector Markers
rg2041ec FR-20-Che	sbv0001 pJEx411a	mrk0002 (KanR)
[Fragment / CDS]	[bacterial expression]	

Attachments

No attachments found.

Sequence ([Text](#) | [Graphic](#))

Related Entries: 2 (Hide/Show)

rg2041 is used within the following **Cells**:

used as	in	Species	Description	Samples
plasmid	sbc0134 (FR-20-Che_pJEx411a@Top10)	E. coli / Top10		1
plasmid	sbc0144 (FR-20-Che_pJEx411a@BL21DE3)	E. coli / BL21DE3		1

Samples (Hide/Show)

[Add DNA Sample](#)[Add Cell Sample](#)

There are **3 samples** (DNA or cell stock) registered for this DNA construct.

location	ID/ Pos.	Status	prepared	by	Type	Description	Sequencing
F1 / Synbio3A / sbD02	01	ok	Aug. 21, 2012	raik	DNA	miniprep from BCN plate	
MT80 / #03 / sbC02	01	ok	Sept. 13, 2012	raik	Cell	single col. restreaked from...	

Feedback

Status: **available**



0 comments

Classification

Plasmid /
[generic plasmid](#)

DNA properties

- **Length:** 5070 bp
- **GC content:** 50.0

Record

- **registered:**
June 19, 2014, 6:48 p.m.
by Raik Gruenberg (raik)
- **last modified:**
June 1, 2015, 8:36 a.m.
by Raik Gruenberg (raik)

Export

[Download GenBank file](#)



Content

DNA construct sb0330 (CD63wt_pJ603c)

control construct expressing wt CD63

Sample Description

Sequenced OK

Sequencing

Add Sequencing

	date	ordered By	traces	comments	result	
DJX01-12_20140813_7	2014-08-12	jim	1	(Created through trace fil...	confirmed	Delete

Help: Click first column to view / edit sequencing entries.

Source and derived samples

DL-JX : 57
DNA sb0330
(CD63wt_pJ603c)

miniprep

**This Sample**

Related Samples: 2

The following samples have the same content as DJX01 : 12:

location	ID/ Pos.	prepared	by	Type	Content	Description	Status
F1 / SynBio2C / DJX01	13	July 3, 2014	jim	DNA	sb0330 (CD63wt_pJ603c)		ok
F1 / SynBio2C / DL-JX	57	May 30, 2014	jim	DNA	sb0330 (CD63wt_pJ603c)	Sequenced ok.	ok

Help: Move mouse over content link to see content description. Move mouse over sample description to see full length description.

Feedback

Status: **ok****fresh!** score: **100%**

1 comment:

fresh: 1 | rotten: 0 | no rating: 0

Specifications

- **Concentration:** 103.0 ng/μl
- **Amount:** 50.0 μl
- **Medium/Buffer:** EB
- **Experiment #:**

Record

- **prepared:**
2014-07-03
by Jim Xu (jim)
- **registered:**
July 8, 2014, 10:42 a.m.
by Jim Xu (jim)
- **last modified:**
July 8, 2014, 10:42 a.m.
by Jim Xu (jim)

**fresh! Raik Gruenberg - June 1, 2015, 8:52 a.m.**

good colony count in robotic transformation.

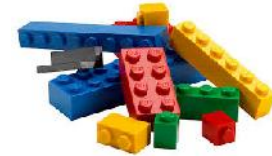
--delete this comment--

Key Lessons

- ▶ Useful platform for \$250k (or less)



- ▶ Be modular & improve iteratively



- ▶ Embrace Excel 

- ▶ Have two + x independent users



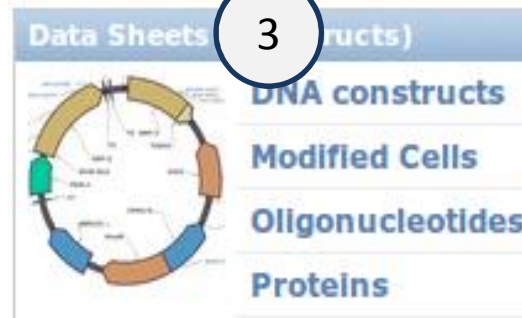
Vision / Outlook

2



Optimized Workflows

3



LIMS Integration

1



Standardized Synthetic Biology Workstation(s)

4

**SynBio / MolBio
Automation App Store**



Conclusion

- 1 Automated cloning is getting within reach of “normal” labs.
- 2 This creates new needs for the tracking of large numbers of samples, constructs, quality control data, protocol versioning, logs ...
- 3 A key challenge is the transfer of workflows / protocols between different robotic setups.

