JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

The SBOL.assembly Language

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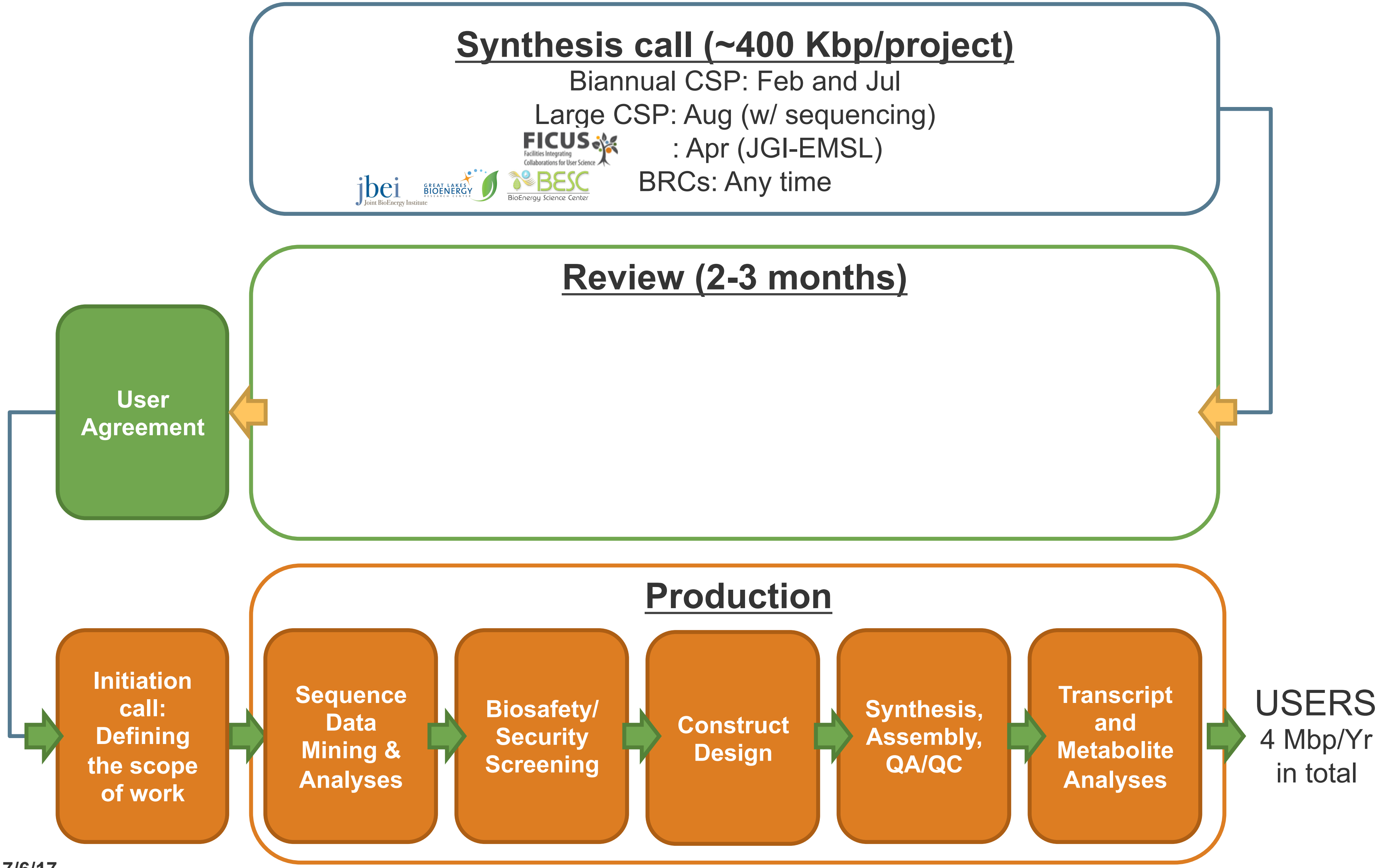
June 26th, 2017

DNA Synthesis
Community Science Program (CSP)



Synthesis call (~400 Kbp/project)

Biannual CSP: Feb and Jul

Large CSP: Aug (w/ sequencing)

FICUS: Apr (JGI-EMSL)

jbei, Great Lakes Bioenergy, BESC BRCs: Any time

Review (2-3 months)

User Agreement

Production

Initiation call: Defining the scope of work

Sequence Data Mining & Analyses

Biosafety/ Security Screening

Construct Design

Synthesis, Assembly, QA/QC

Transcript and Metabolite Analyses

USERS
4 Mbp/Yr in total

DNA Synthesis Production Pipeline

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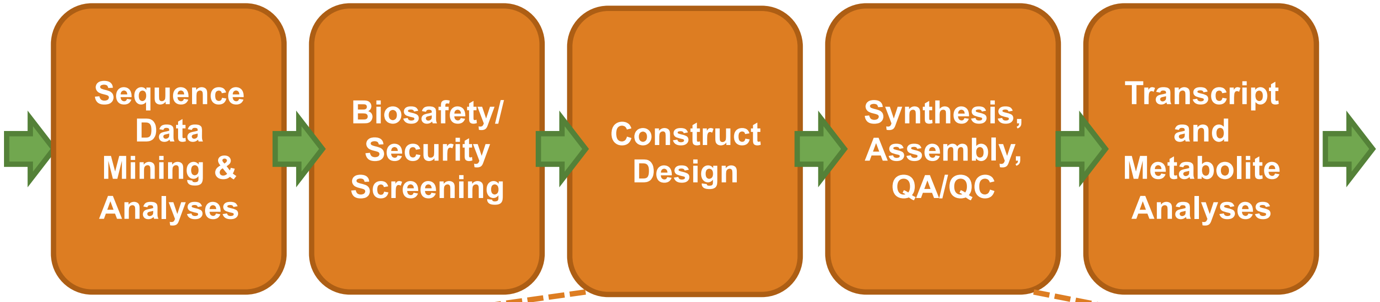
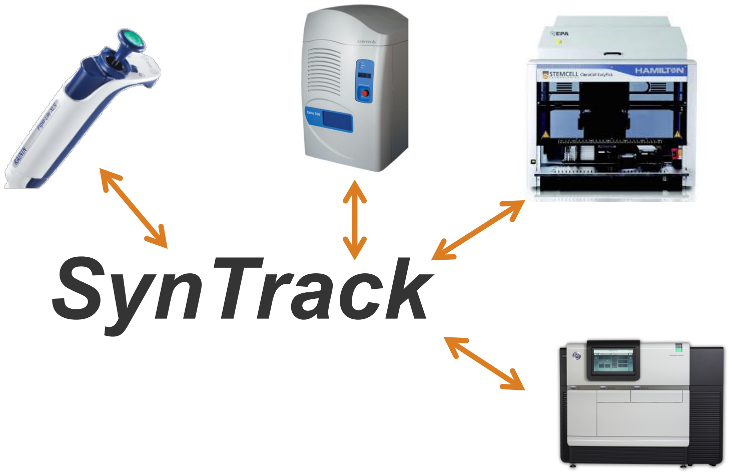

Sequence Data Mining & Analyses
Biosafety/ Security Screening
Construct Design
Synthesis, Assembly, QA/QC
Transcript and Metabolite Analyses

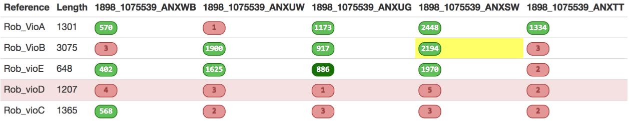
Construct Design

https://boost.jgi.doe.gov

Reverse-Translation, Codon-Juggling, Polishing, and **Partitioning**

Synthesis, Assembly, QA/QC





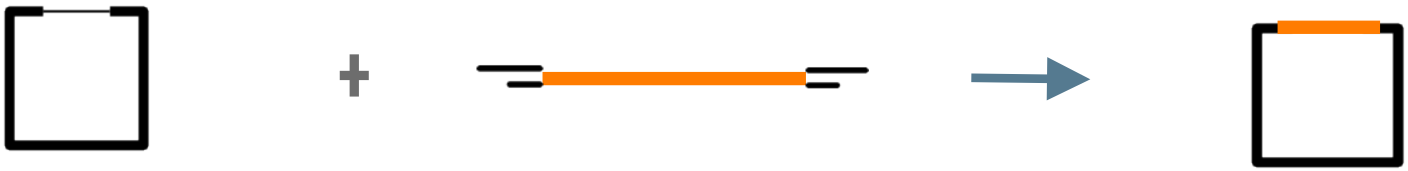
Reference	Length	1898_1075539_ANXWB	1898_1075539_ANXUW	1898_1075539_ANXUG	1898_1075539_ANXSW	1898_1075539_ANXTT
Rob_VioA	1301	570	1	1173	2448	1334
Rob_VioB	3075	3	1900	917	2194	3
Rob_vioE	648	402	1625	886	1970	2
Rob_vioD	1207	4	3	1	5	2
Rob_vioC	1365	568	2	3	3	2

QA/QC using **DNA Sequencing**

JGI's DNA Assembly Operations

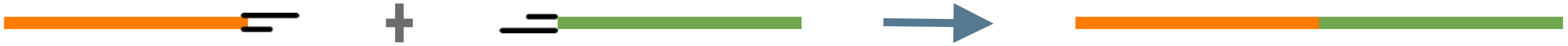
Scenario I

Insert linear DNA construct into vector



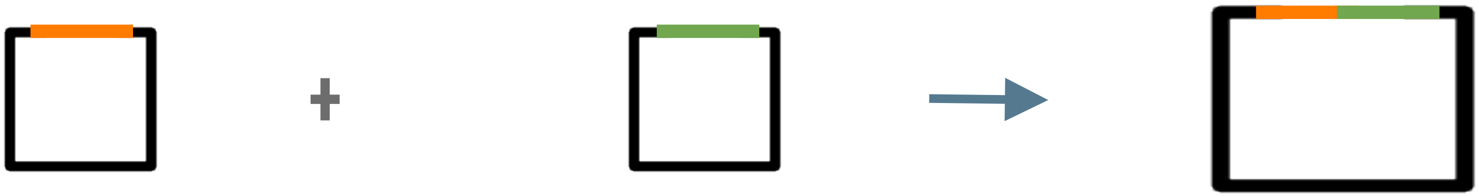
Scenario II

Assembly of linear DNA constructs



Scenario III

"Combinatorial Assembly"



Proposal

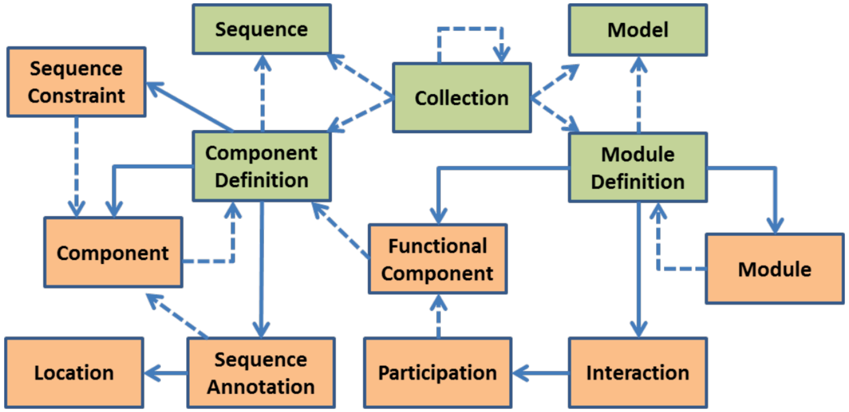
SBOL.Assembly

Operands

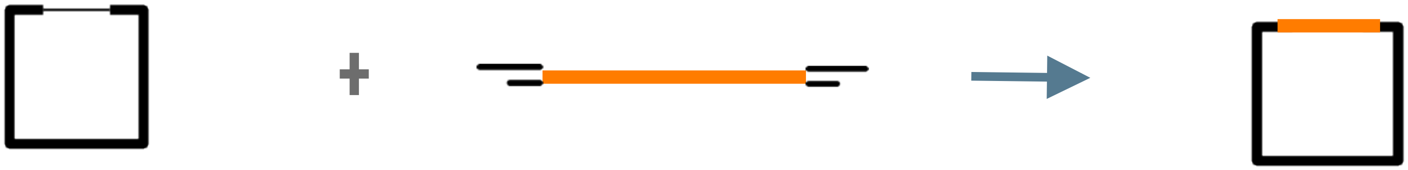
- ▬ Linear construct
- □ Circular construct
- ⇀ oligo/primer
- ◔ restriction enzymes

Operators

- *cut*
- *amplify*
- *join*

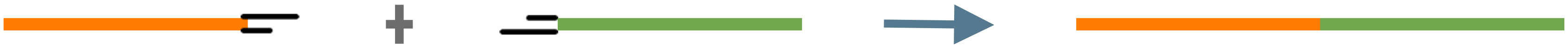


Scenario I



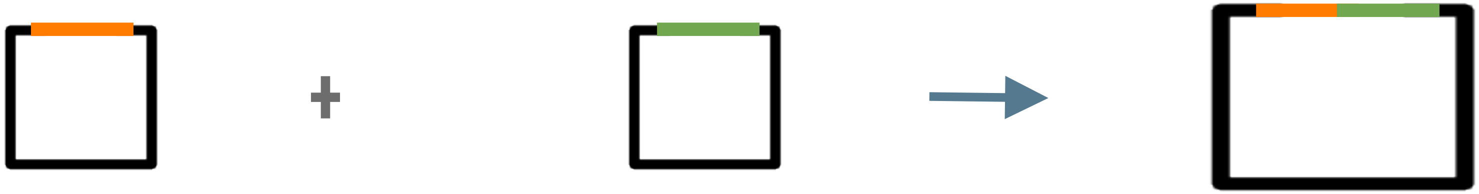
```
// cut the vector
linearized_vector = cut(vector, enzyme)
// amplify the construct
amplified_construct = amplify(construct, 5'_primer, 3'_primer)
// join the two resulting constructs
assembled_construct = join(linearized_vector, amplified_construct)
```

Scenario II



```
// amplify the construct
amplified_construct$_1$ = amplify(
    construct$_1$, 5'_primer_construct$_1$, 3'_primer_construct$_1$)
amplified_construct$_2$ = amplify(
    construct$_2$, 5'_primer_construct$_2$, 3'_primer_construct$_2$)
// join the amplified constructs
assembled_construct = join(
    amplified_construct$_1$, amplified_construct$_2$)
```

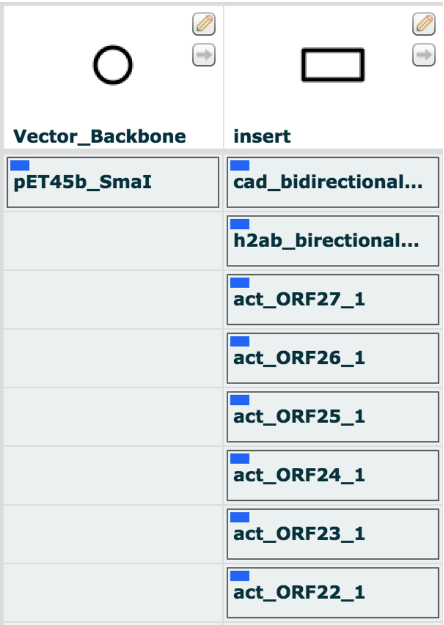
Scenario III



```
// amplify the construct
amplified_construct$_1$ = amplify(
    construct$_1$, 5'_primer_construct$_1$, 3'_primer_construct$_1$)
amplified_construct$_2$ = amplify(
    construct$_2$, 5'_primer_construct$_2$, 3'_primer_construct$_2$)
// join the amplified constructs
assembled_construct = join(
    amplified_construct$_1$, amplified_construct$_2$)
// cut the vector
linearized_vector2 = cut(vector2, enzyme)
// join the two resulting constructs
final_construct = join(linearized_vector, assembled_construct)
```

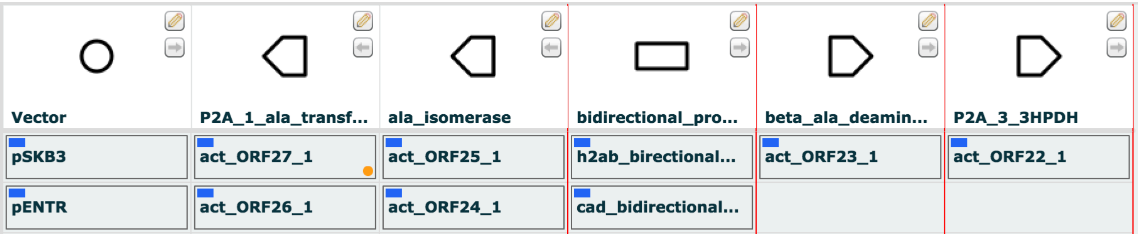
Use Case:
Multi-Level Combinatorial Assembly

Level-0



Vector_Backbone	insert
pET45b_SmaI	cad_bidirectional...
	h2ab_birectional...
	act_ORF27_1
	act_ORF26_1
	act_ORF25_1
	act_ORF24_1
	act_ORF23_1
	act_ORF22_1

Level-1



Vector	P2A_1_ala_transf...	ala_isomerase	bidirectional_pro...	beta_ala_deamin...	P2A_3_3HPDH
pSKB3	act_ORF27_1	act_ORF25_1	h2ab_birectional...	act_ORF23_1	act_ORF22_1
pENTR	act_ORF26_1	act_ORF24_1	cad_bidirectional...		

- Software Prototype
  https://github.com/eoberortner/SBOL.assembly
- Poster:
  "SBOL.assembly: Using SBOL 2.1.0 for representing DNA Assemblies"

Future of SBOL.assembly

What is the minimum set of operands and operators for the representation of DNA assembly protocols?

- Establishment of a working group
- Collaboratively define the requirements for representing DNA assemblies
- Reach consensus on the representation scheme using SBOL
- Real-world scenarios based on common DNA assembly protocols
- Development of software libraries (build atop libSBOL)
- Release of specification documents and publications

Interested?
Please contact: eoberortner@lbl.gov