GBA and BioNet

GBA Objectives

- develop, promote, and support non-commercial biofoundries
- intensify collaboration and communication
- collectively develop responses to challenges
- enhance visibility, impact and sustainability
- explore globally relevant and impactful collaborative projects

BioNet platform

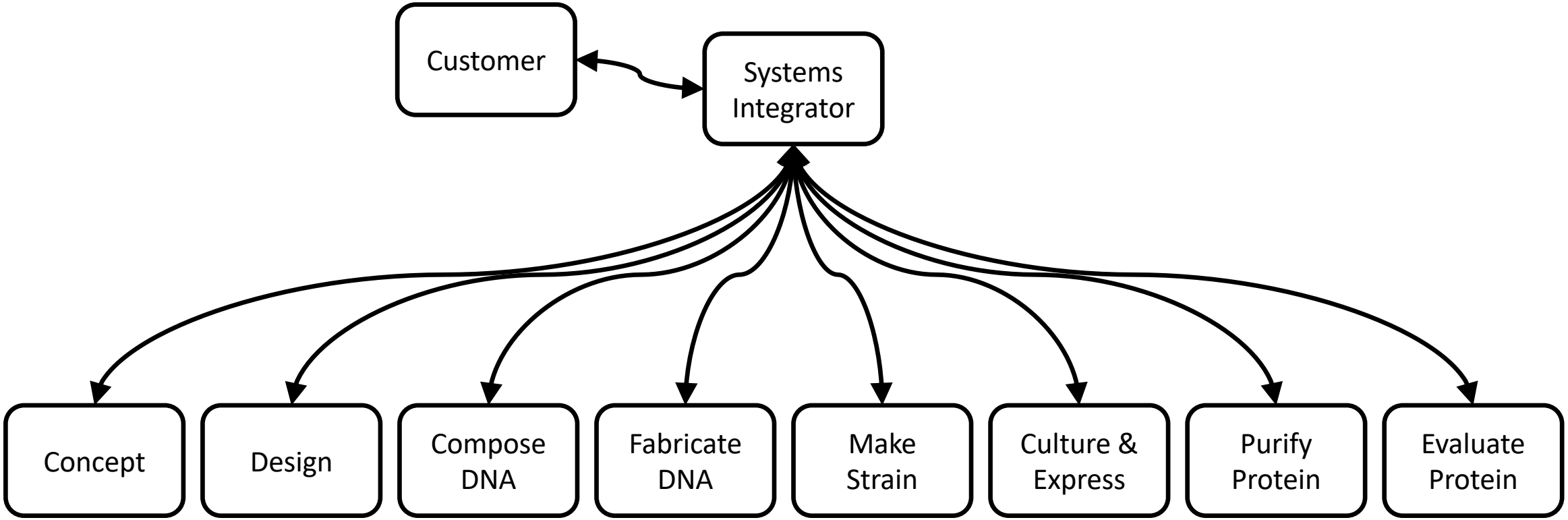
- harmonize programmable access to capabilities
- controlled vocabularies for capabilities, parameters, operations
- standardize capacities

“Tabletop” exercise as a BioNet Alpha Test

- Correspondence role-playing game
  - simulate a disaggregated Synthetic Biology project
- MITRE as Systems Integrator
  - send requirements & specifications weekly as ***Requests***
- Participants ”perform” operations
  - 2 operations per weekly cycle
  - operation results returned in stylized ***Returns***
- Sample workflow ***Requests*** & ***Returns*** provided at each cycle

What this Tabletop IS, and ISN'T

- Alpha test of BioNet concept
  - practice a disaggregated workflow
- Simulation of BioNet transactions
  - using a model project
- Engage community of practitioners (GBA) to design and build BioNet
  - what are the handoffs?
  - interfaces?
  - architecture?
- It is NOT...
  - an assessment of anyone's ability, experience, knowledge, or mastery
  - to provide "correct" or optimal results



Protein Synthesis Workflow, Call-and-Response

Systems integrator orchestrates operations that are spread across multiple organizations

Flexibly configured according to what makes sense

What you get and what you return every week

Week	Request	Return
1	• [**Concept**] description • [**Design**] criteria	• Participant's amino acid sequences [**Design**]
2	• [**Compose_DNA**] request • [**DNA_Fabrication**] request	• DNA sequences [**Compose_DNA**] • Results of [**DNA_Fabrication**]
3	• [**Strain_Construction**] request • [**Culturing and Expression**] request	• Results of [**Strain_Construction**] • Results of [**Culturing and Expression**]
4	• [**Performance_Evaluation**] request	• Results of [**Performance_Evaluation**]
5	• Debrief	• Debrief remarks

Weekly Feedback Elicited

I like... I wish... What if..?

What made sense to you?

What was hard to understand?

What was easy to do? hard?

Any suggestions?

Tabletop Timing

August 2023

Sun	Mon	Tues	Wed	Thu	Fri	Sat
			2	3	4	5
6	Week 1: Concept & Design 7	8	9	10	11	12
13	Week 2: Compose DNA & DNA Fabrication 14	15	16	17	18	19
20	Week 3: Strain Construction & Culturing and Expression 21	22	23	24	25	26
27	Week 4: Sample Preparation & Performance Evaluation 28	29	30	31	September 1	2
3	Week 5: Debrief 4	5	6	GBA Copenhagen 7	8	9

Our Tabletop Experiment: Make a Tooth-whitening Enzyme

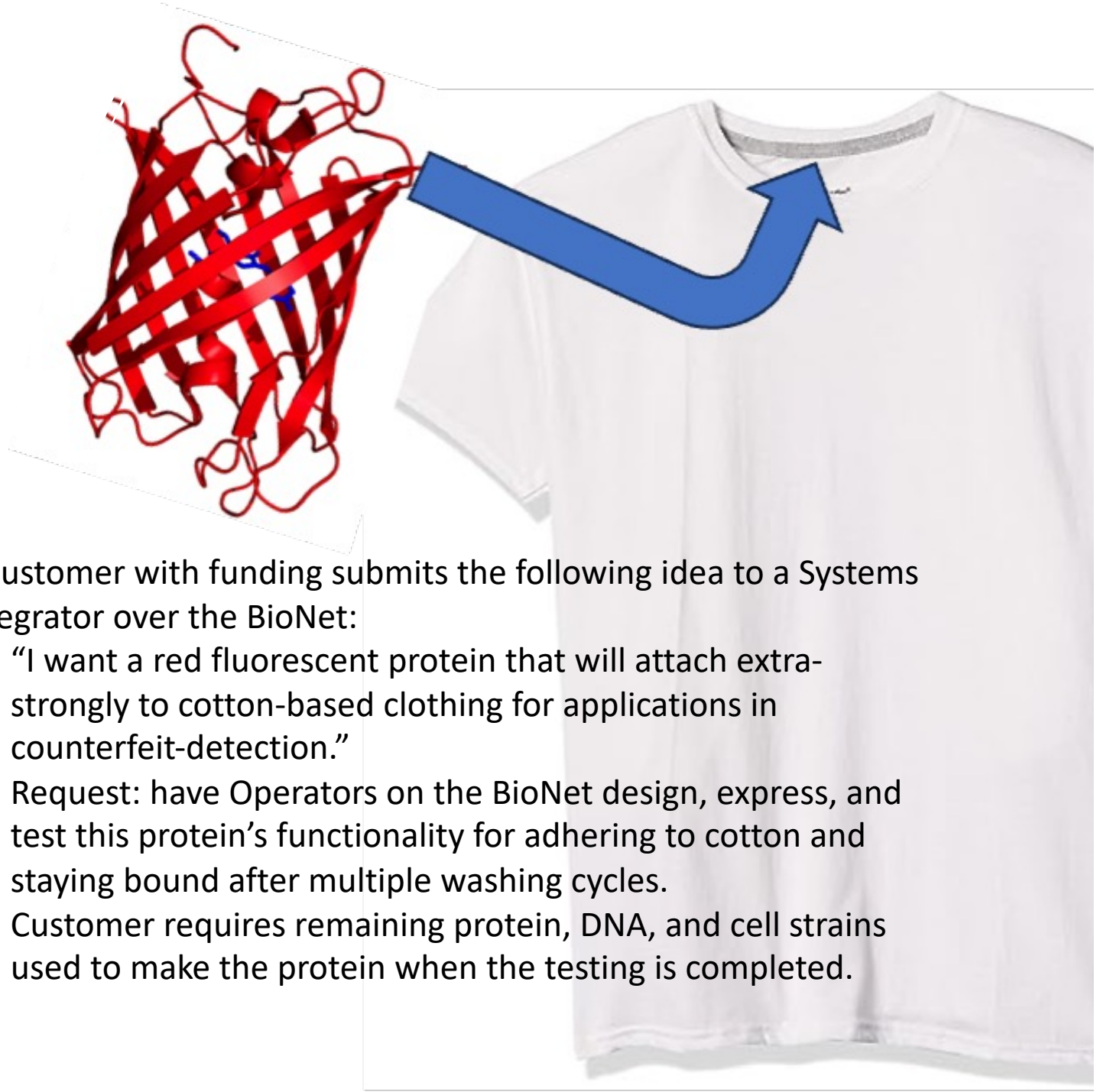
- Fuse enamel-binding peptide with perhydrolase for a tooth whitening product
- Customer requires design and production of 50 mg each of the fusion protein and the perhydrolase-only control for evaluation
- Workflow includes contract lab testing of enzyme activity retention after toothbrushing
- No affinity purification tags

Example of a BioNet Tabletop

An example of the Week 1-4 handoffs between a Systems Integrator and Foundries

Sample of a Completed Workflow

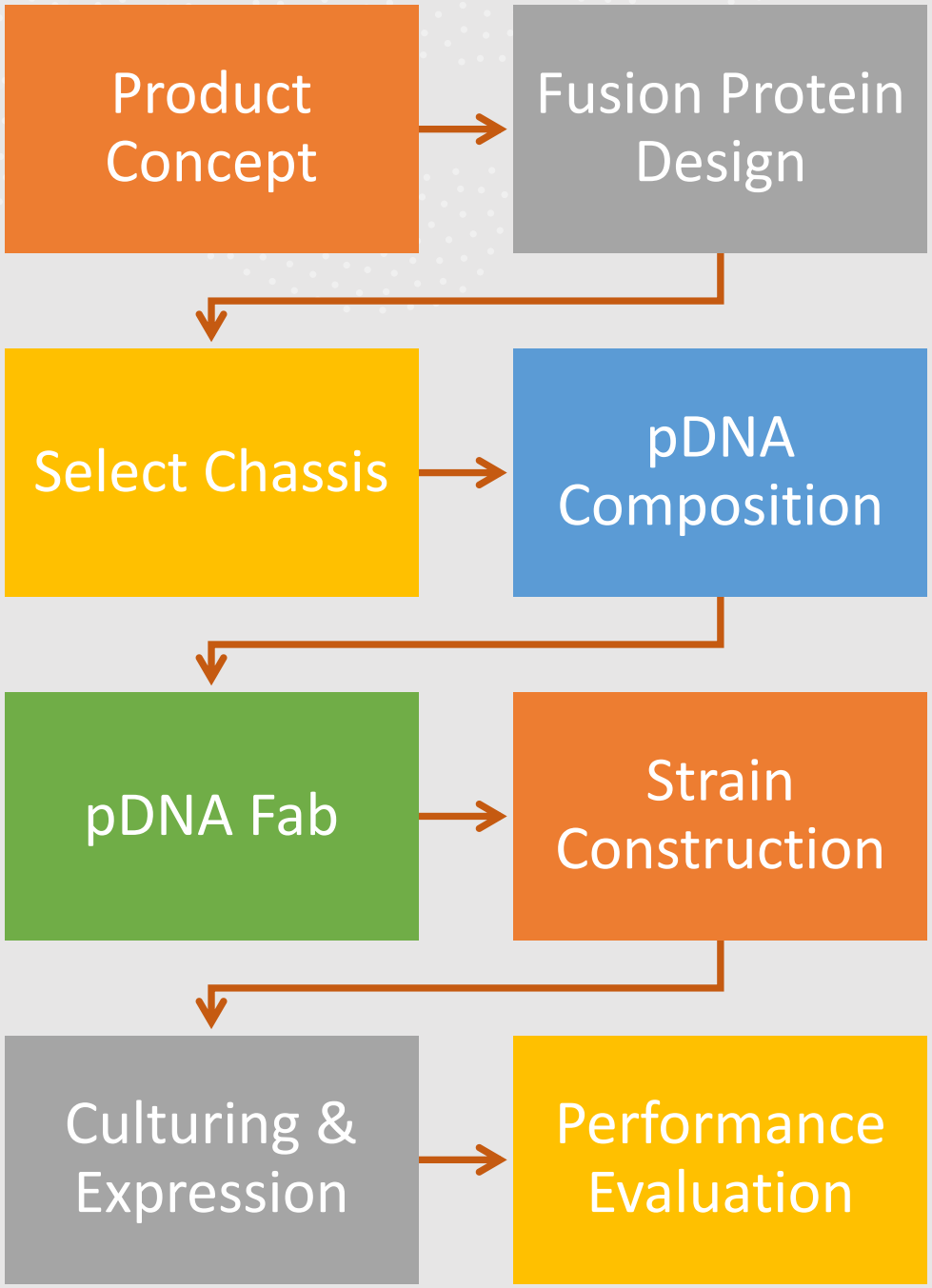
- Worked Example: Cellulose-binding RFP
- “Cotton Tattoo”
  - Design fluorescent protein that will stick to cotton
  - Produce protein
  - Perform lab tests to evaluate persistence after washing



A customer with funding submits the following idea to a Systems Integrator over the BioNet:

- “I want a red fluorescent protein that will attach extra-strongly to cotton-based clothing for applications in counterfeit-detection.”
- Request: have Operators on the BioNet design, express, and test this protein’s functionality for adhering to cotton and staying bound after multiple washing cycles.
- Customer requires remaining protein, DNA, and cell strains used to make the protein when the testing is completed.

“Cotton Tattoo” Workflow



Sample Workflow Week 1: Concept & Design

OPERATION (FUNCTION) CALLED	CALLER	REQUEST SUMMARY	BIOFOUNDRY RESPONDER	RESPONSE SUMMARY
Concept	Customer	• Design and produce modified red fluorescent protein that will stick to cotton even after washing	Systems Integrator	• Accepts project integration job from Customer
Design	Systems Integrator	• Design a fusion protein of monomeric Red Fluorescent Protein (mRFP), linker, cellulose-binding domain • Provides references useful to Designer • Decides that expression in *E. coli* will be sufficient	Designer	• Chooses specific genetic parts: CBM9 cellulose-binding domain, $(PT)_4P$ linker, mRFP1, and their order • Designs amino acid sequence of fusion protein "CBM9-RFP_fusion" that satisfies Design request

Sample Workflow Week 2: Compose & Fabricate DNA

OPERATION (FUNCTION) CALLED	CALLER	REQUEST SUMMARY	BIOFOUNDRY RESPONDER	RESPONSE SUMMARY
Compose DNA	Systems Integrator	• Shares designed protein sequence • Requests codon optimization for *E. coli* • Specifies plasmid backbone characteristics	DNA Composer	• Sequence of composed plasmid pFAB3993 • With CBM9-RFP_fusion codon-optimized for *E. coli* • Annotated plasmid map
DNA Fabrication	Systems Integrator	• Submits order for clonal plasmid pFAB3993 • Requests purity QC, quantitation, sequencing	DNA Fabricator	• Fabricates plasmid • Provides requested data & sequencing results • Confirms shipment of fabricated DNA to Strain Constructor

Sample Workflow Week 3:
Strain Construction & Culturing and Expression

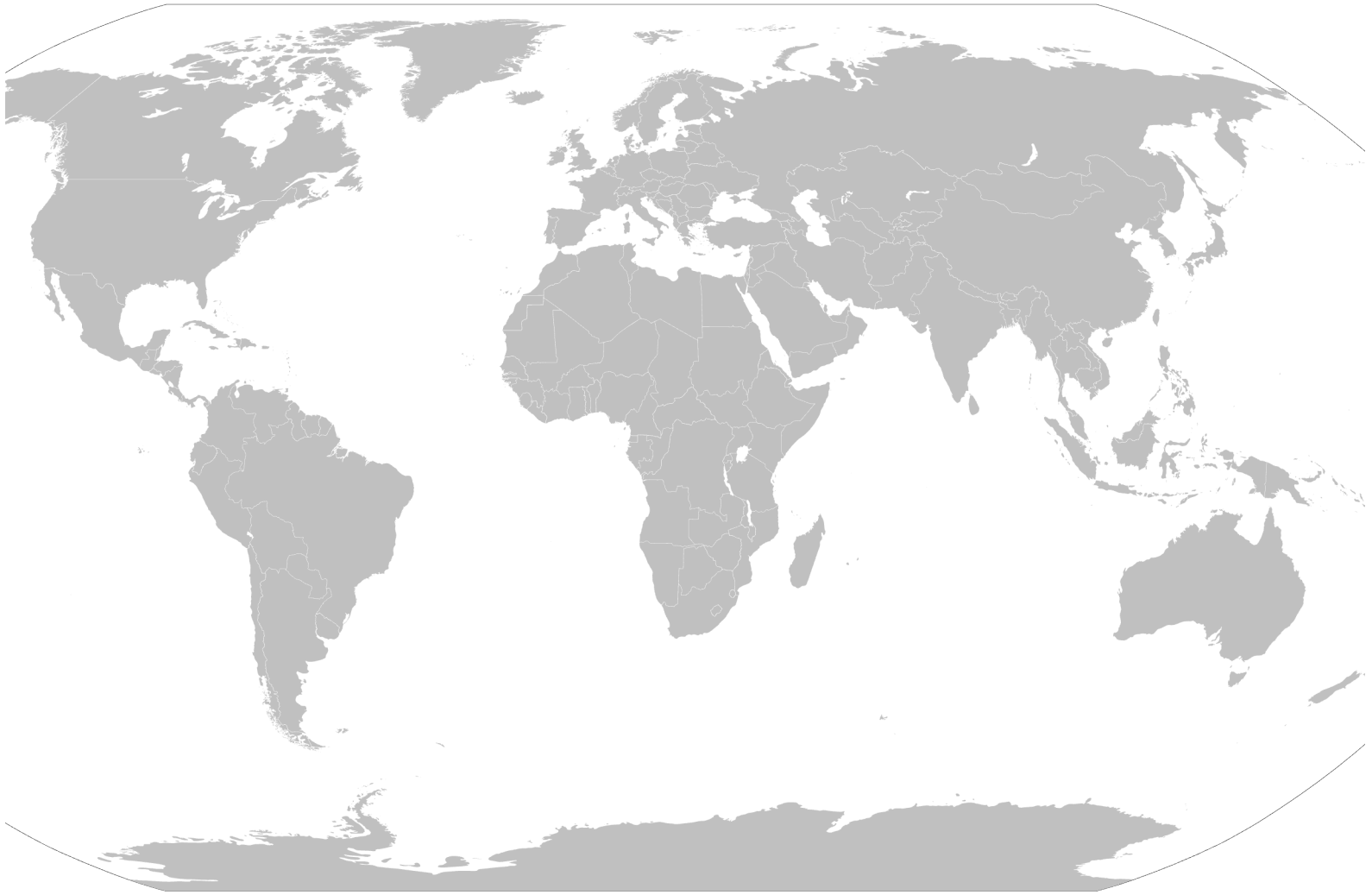
OPERATION (FUNCTION) CALLED	CALLER	REQUEST SUMMARY	BIOFOUNDRY RESPONDER	RESPONSE SUMMARY
Strain Construction	Systems Integrator	• Selects *E. coli* BL21(DE3) as chassis organism. • Requests additional control plasmid (Addgene) • Issues requirements for plasmid transformation and clone validation	Strain Constructor	• Performs transformation and validation as requested • Report of procedures followed and results • Confirms transfer of clones to next performer
Culturing And Expression	Systems Integrator	• Requests fermentations of CBM9-RFP_fusion and RFP control strains • Specifies data collection & reporting: growth curves, fluorescence • Specifies biomass transfer to Sample Prep dept.	Strain Engineer	• Raw data and report as requested • Confirms transfer of biomass to Sample Preparation performer

Sample Workflow Week 4:
Sample Preparation & Performance Evaluation

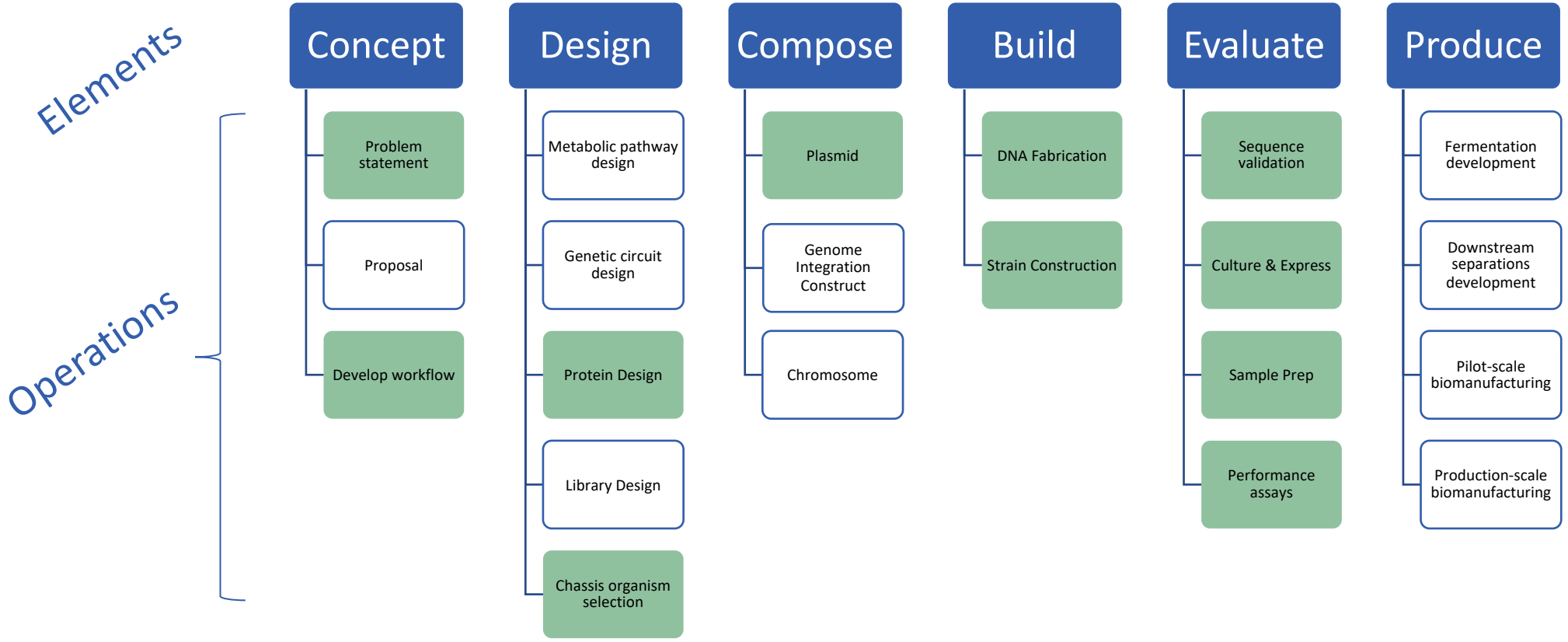
OPERATION (FUNCTION) CALLED	CALLER	REQUEST SUMMARY	BIOFOUNDRY RESPONDER	RESPONSE SUMMARY
Performance Evaluation	Systems Integrator	• Provides protein info to assist with Sample Prep method selection • Data to return: PAGE images, protein quant results, fluorescence measurements • Provides references useful to assay developer & guidance to adapt assays • Outlines testing to perform & results to return	Performance Evaluator	• Description of protocols run • Report of procedures followed and results • Raw data • Report of protein deposition and wash resistance results

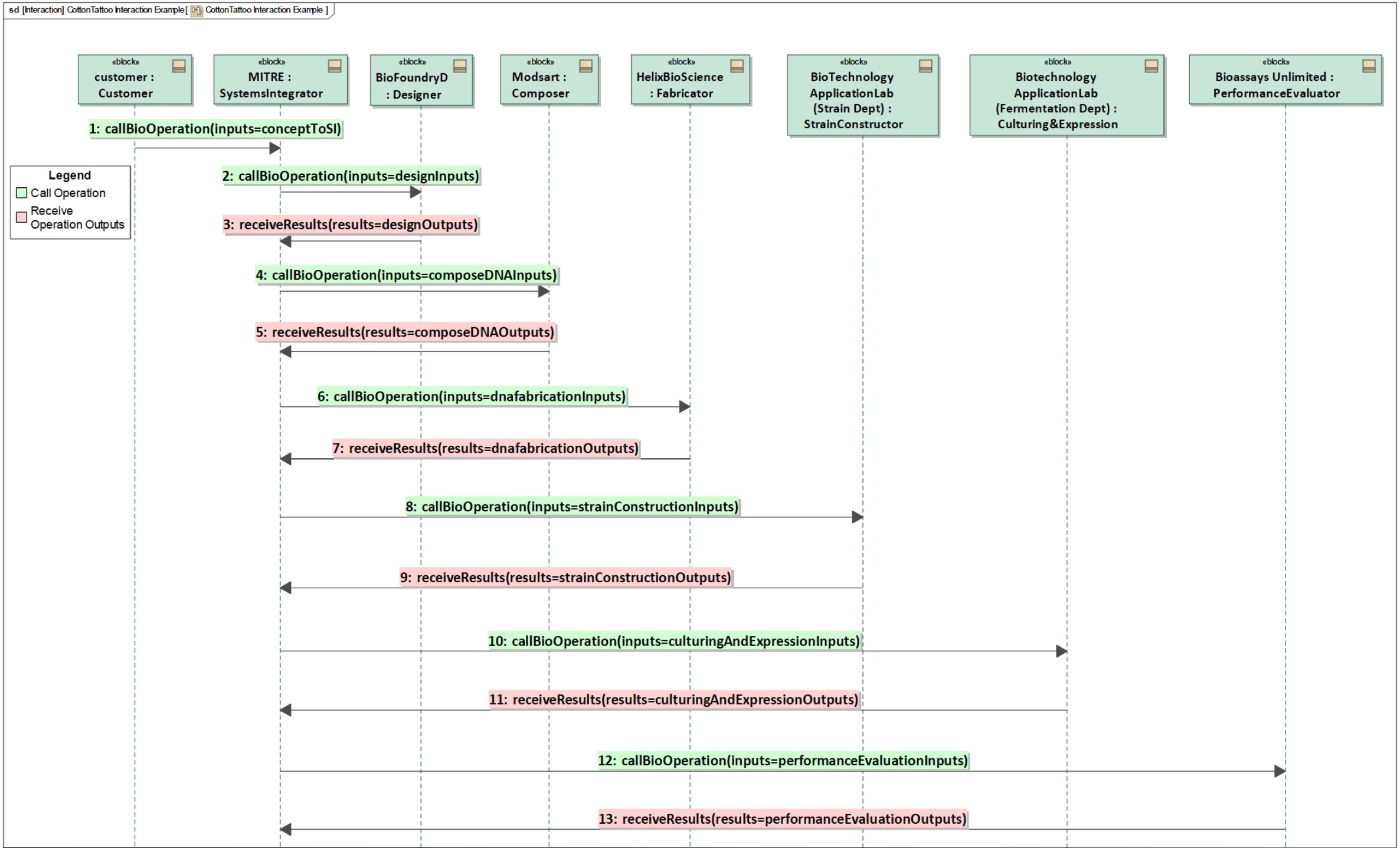
7 Labs participated in this Tabletop Run

- Australian Genome Foundry
- CSIRO BioFoundry, Australia
- DAMP Lab, Boston, USA
- SyNBiOSyS, Norway
- VTT Finland
- A*STAR SIFBI Singapore
- Global Institute for Food Security, Canada



Tabletop Workflow Elements and Operations

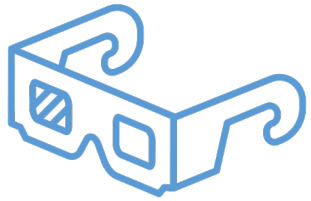



sd [Interaction] CottonTattoo Interaction Example [CottonTattoo Interaction Example]
«block» customer : Customer
«block» MITRE : SystemsIntegrator
«block» BioFoundryD : Designer
«block» Modsart : Composer
«block» HelixBioScience : Fabricator
«block» BioTechnology ApplicationLab (Strain Dept) : StrainConstructor
«block» Biotechnology ApplicationLab (Fermentation Dept) : Culturing&Expression
«block» Bioassays Unlimited : PerformanceEvaluator
Legend
Call Operation
Receive Operation Outputs
1: callBioOperation(inputs=conceptToSI)
2: callBioOperation(inputs=designInputs)
3: receiveResults(results=designOutputs)
4: callBioOperation(inputs=composeDNAInputs)
5: receiveResults(results=composeDNAOutputs)
6: callBioOperation(inputs=dnafabricationInputs)
7: receiveResults(results=dnafabricationOutputs)
8: callBioOperation(inputs=strainConstructionInputs)
9: receiveResults(results=strainConstructionOutputs)
10: callBioOperation(inputs=culturingAndExpressionInputs)
11: receiveResults(results=culturingAndExpressionOutputs)
12: callBioOperation(inputs=performanceEvaluationInputs)
13: receiveResults(results=performanceEvaluationOutputs)

Some Feedback from participants

- I like...
  - chance to perform rational design
  - clear & specific instructions
  - worked example for every week
  - standard templates
- I wish...
  - more design guidance, feedback
  - specified DNA assembly method
  - how much protocol detail do I need?
- What if...
  - we had a catalog of GBA capabilities...

Cool stuff and questions



Cool stuff

used AlphaFold

detailed DNA compositions

compositions submitted in Benchling

Questions

data sharing

- privacy – data, sequence, protocols
- want secure platform
- how to share large datasets?

how to share labile materials?

how to pick performers from a network?

- especially for novel assays

VERY preliminary debrief

- recognition that workflow can be systematic, abstract across different performers
- “understand better how different biofoundries could and should be connected”
- insight – collective strengths greater than any individual foundry brings
  - “wish I could see & discuss other people’s answers...”
- all participants interested in doing this again
- interest in doing a “real” project

Tabletop Takeaways

- Socializing BioNet Concept
- Validate Disaggregation Model
- Abstraction
- Parameterization

- Next Steps
  - cross-lab workflows
  - more complex workflows
  - psuedocode interaction
  - Python Operations Library