Novel Chassis and Yeast States: The Automated Time Series Experimental Request

**Primary point of contact: Enoch and Steve**

**Date of preparation: 1/30/2019**

**arabinose**

**Dna roteomics arabinose proteom**

MG1655\_LPV3\_LacI\_Sensor\_pTac\_AmeR

SC\_Sorbitol Kan rs1bga86uq52tz

## Goal:

\*Develop an experimental workflow for verifying the functionalityof engineered biological function across short and long-term temporaldynamics.

\*Generate data to study host-circuit interactionsin model vs. non-model hosts using a fusion of flow, [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1), plate reader, and absolute proteomicsdata.

## Rationale:

*Provide a short rationale for the goal (or subgoals). Include references to related experiments/data/literature that are relevant.*

engineered

The operational envelope of most biologicalcircuits are characterized at static, or fixed, timepoints, without accounting for the natural temporalprocesses, cell cycle dynamics, or transition-phase dynamics (from log to stationary) that occur as a function of host state and environment.

This experimental request will facilitatecollection of time-series data, to address these questions.

## Experimental and Analytical Approach:

timepoints

**Expected data and analysis:**

What’s the data going to look like? What comparisons are going to be made?

*-n timepoints, 1 temperatures, comparison of all samples and all replicates.*

*Where might it be performed? By whom?*

Transcriptic (or UW BioFab) will perform all culturing steps and sampling steps for an experiment protocol using their fully automated workcells. Transcriptic (or UWBioFab) will perform flow and plate reader assays on samples.

Ginkgo will perform [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)and proteomic proteomics assays.

*What’s the experimental design?*

Recovery from overnight [glycerol](https://hub.sd2e.org/user/sd2e/design/CAT_G33_500/1)stocks from a 96-well plate, passaging for a period of time, then sampling, dilution (optional) to prevent overgrowth, and measurement in plate reader/[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)/flow/proteomics.

*What data are you expecting to collect/receive? Is there an ETL process in place and who is responsible?*

We need TACC/Netrias/TwoSix/Raytheon BBN involvement for plate reader, flow, [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1), and relative proteomics ETL.

Absolute [proteomics](https://hub.sd2e.org/user/sd2e/intent_parser/proteomics/1) returns the fraction of a sample’s proteome made up of a particular protein[,](https://hub-staging.sd2e.org/user/sd2e/intent_parser/protein/1) limited to a set of requested proteins. Relative Proteomics returns an amount, relative to a control sample, of each detected protein in a sample.

*Are there expected results, and if so what are they? State any assertions about the dependence/independence of measurements. If possible, estimate how much data expected.  
  
How will the data be analyzed? By what groups/stakeholders?*

TA1 performers, TA2 performers in the yeast states and novel-chassis challenge problem group.

*How was the number of replicates chosen?*

DARPA suggested at least 8 biological replicates per lab; the number of replicates will be constrained by the number of samples that can fit within a pipeline.

*Are there subsequent planned experiments based on outcomes and will they (re)use existing TA3 protocols? What’s their expected throughput?*

Yes, there will be an ask for identical experiment from multiple challenge problem groups, including yeast states and novel-chassis.

*Are there analysis that can be placed in ETL which would automate the sending of future experiments?*

Yes, perhaps not strictly in ETL, but within TA1 there are performers including Dan, Alex, and Enoch working on automated experiment planning.

*What are expectations on timeline?*

Depends on Transcriptic development process.

***Strain Definitions:***

***Yeast States:***

*XNOR gate strains 1-4,* [*NOR00*](https://hub-staging.sd2e.org/user/sd2e/design/UWBF_73000x3A0x20static0x20XNOR_00/1) *control, UW BioFab w.t. yeast strain*

Strain Antibiotics:

NA for yeast.

***Novel Chassis:***

Strains Definitions:

1- a control strain (Wild-type MG1655)

2- a strain with a genomically integrated [PhlF](https://hub.sd2e.org/user/sd2e/design/PhlF/1) gate ([PhlF](https://hub.sd2e.org/user/sd2e/design/PhlF/1) is transcribed from a minimal [pTac](https://hub.sd2e.org/user/sd2e/design/pTac/1) promoter, and YFP from p[PhlF](https://hub.sd2e.org/user/sd2e/design/PhlF/1) promoter)

3- a strain with a genomically integrated IcaR gate (IcaR is transcribed from a minimal [pTac](https://hub.sd2e.org/user/sd2e/design/pTac/1) promoter, and YFP from pIcaR promoter)

4- a strain with a genomically integrated NAND circuit composed of the above [PhlF](https://hub.sd2e.org/user/sd2e/design/PhlF/1) and IcaR gates (note that here [PhlF](https://hub.sd2e.org/user/sd2e/design/PhlF/1) is transcribed from a minimal pBAD promoter)

Strain Antibiotics -- for overnight growth:

[Kan](https://hub-staging.sd2e.org/user/sd2e/design/pAN1717/annotation15/1): 50 µg/ml

Chlor: 35 µg/ml

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 |
| [Kan](https://hub-staging.sd2e.org/user/sd2e/design/pAN1717/annotation15/1) |  | x | x | x |
| Chlor |  | x | x | x |
| None | x |  |  |  |

Strain Antibiotics -- for growth after dilution from overnight (both pre- and post-induction, starting from step 4A):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 |
| [Kan](https://hub-staging.sd2e.org/user/sd2e/design/pAN1717/annotation15/1) |  | x | x | x |
| Chlor |  |  |  |  |
| None | x |  |  |  |

***Conditions:***

Temperature Conditions (apply to all strains in all induction conditions):

30 C for yeast states

Chemical Conditions (*defined* for each strain):

Coloring indicates grouping for relative proteomics

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain ID | 0 mM IPTG,  0 mM arabinose | 0.25 mM IPTG,  0 mM arabinose | 0 mM IPTG,  25 mM arabinose | 0.25 mM IPTG,  25 mM arabinose |
| 1 | x | x | x | x |
| 2 | x | x |  |  |
| 3 | x | x |  |  |
| 4 | X | x | x | X |

\* Strain 8 with the inducer is being used as the relative proteomics comparison master sample.

***Reagents:***

***Yeast States Media:***

Yeast Extract Peptone 2% Dextrose media

Synthetic Complete 2% Dextrose media

***Novel Chassis Media:***

M9 + glucose + CAA:

* [M9 media salts](https://hub.sd2e.org/user/sd2e/design/teknova_M1902/1) (6.78 g/L Na2HPO4, 3 g/L KH2PO4, 1 g/L NH4Cl, 0.5 g/L NaCl; Sigma- Aldrich, MO, M6030)
* 0.34 g/L thiamine hydrochloride (Sigma-Aldrich, MO, T4625)
* 0.4% D- glucose (Sigma-Aldrich, MO, G8270)
* 0.2% Casamino acids (Acros, NJ, AC61204-5000)
* 2 mM MgSO4 (Sigma-Aldrich, MO, 230391)
* 0.1 mM CaCl2 (Sigma-Aldrich, MO, 449709)

***Experimental protocol:***

The protocol is a time-series automated deep well plate adaptation of Adam Lehman, Steve Haase’s time-series [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1) protocol, with the distinction of an additional fork of sampling into 1) a 96 well plate for plate reader run and 2) the original transfer to 24 well plates for ‘omics/flow analysis.

*Explanation / rationale for each step. What’s very important and what’s less important to do exactly as described and why.*

Must Be Done As Specified, Up to Consensus with TA3 performers, Up to individual TA3 performer

Protocol Steps (*apply* to all strains in all chemical/induction and temperature conditions):

Step 0: Inoculate 96 deep v-bottom plate from glycerol stock to liquid media **M**

Step 1A: Recover for **N** hrs in liquid media **M**

Step 1B: Passage volume of **V µL** into 96 deep v-bottom plate (Stamp),

Step 1C: Incubate for **N\_P** hours

Step 1D: Go to Step 1B with variables for **X** loops (maintain variables)

#SH&EY: Eventually, we want OD growth rate specific dilution, postpone for initial standup.

Step 2: Induce/Media Transfer (starts timepoints t0) in a 96 deep v-bottom plate

[Fast Time-Series Sampling, Low Throughput Mode]:

For **Well\_Index in range(0,Source\_Plate.Total\_Wells,1)**:

Step 2.A: Take off cover (evap lid or breathable seal via x-peel)

Step 2.B: Remove **Plate\_{PRFlow}, Plate\_{**[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)**}, Plate\_{Proteomics}**

Step 2.C: Transfer Source\_Plate from (warm) incubator to liquid handler - 1.5 mL

Step 2.D: Transfer **V\_{plate}** µL from Source\_Plate.Well\_Index to **Plate\_{PRFlow}** for plate reader

Step 2.E: Pull **V\_{flow}** µL from Source\_Plate.Well\_Index to **Plate\_{PRFlow}** for flow cytometer

Step 2.F: Pull **V\_{**[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)**}** µL from Source\_Plate.Well\_Index to **Plate\_{**[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)**}** for [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)

Step 2.G: Pull **V\_{Proteomics}** µL from Source\_Plate.Well\_Index to **Plate\_{Proteomics}** for Proteomics (optional for yeast-states, required for novel-chassis)

Step 2.H: Incubate Source\_Plate at (warm) incubator at **T\_{incub}** for **T\_{interval} min**

Step 2.I : Incubate **Plate\_{PRFlow}, Plate\_{**[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)**}, Plate\_{Proteomics}** at (cold) incubator

[Slow Time-Series Sampling, High Throughput Mode]:

Every 2 dbling times = 2\***T\_{dbl}**

Step 2.A: Take off cover (evap lid or breathable seal via x-peel)

Step 2.B. Transfer **Plate\_{Source}** from incubator to liquid handler (assuming a starting volume of 1.5 mL)

Step 2.C: [Plate & Flow Sampling] Subsample at **N\_PF** (default = 100 µL) of culture volume from **Plate\_{Source} to Plate\_{PRFlow}.** Seal and stack **Plate\_{PRFlow}** from step 2.C at 4C (>8 hrs)

Step 2.D: [Dilution Step] Subsample at **N\_V (default =** *100 µL*) of culture volume from **Plate\_{Source}**(96-deep v-bottom plate) into **N\_V** (**default** = *300 µL, 4 fold dilution*) of media next **M\_{Next}** (default = **M\_{Next] == M\_{Prev}**) into a separate clear-bottom round well 96 well microtiter plate - 300 uL

Step 2.E: [[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1) sampling] Add RNA Protect to source 96-deep v-bottom plate, route for 4C storage (up to 4 weeks at most!) --> [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1) sample prep - 1.1 mL to

# e.g. 1.5 mLs:

Step 2.F: Define source 96-deep v-bottom plate as new 96-deep v-bottom plate

Step 2.G: Incubate at temperature **T** C

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For each plate in stack:

Step 3.A: Take plate from 4C incubator, transfer with robotic arm to plate reader for static read.

Step 3.B: Bring out plate from workcell, place in stack for flow cytometry analysis

Step 4 : Human walks plate stacks over to flow cytometry workcell.

For each plate in flow cytometry stack:

Step 4.A: Read plate in flow cytometer.

Step 4.B: Run controls, calibrations, etc.

Step 5: Human ships **Plate\_{Proteomics}** and **Plate\_{**[RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1)**}** to appropriate venue.

*Is their expected reuse of this protocol beyond this experimental request?*

*Many times over, via Novel Chassis, Riboswitches, and YeastStates challenge problems.*

*Are there controls and success parameters we can use when developing the protocol to ensure it's been developed faithfully (Are there known runs with data we can use to validate protocol development against?)? Ideally there would be points within the protocol that could be checked along the way to better define any issues in protocol translation that may arise.*

## Automation of experimental requests and data return:

*Can the protocol be represented with current XPlan operators? Yes. If not who/timeline for extension if needed.*

*What metadata needs to be provided where and by who for analysis?*

Sequence information for all strains is needed for [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1) analysis. Strains have been sequenced at ginkgo but initial reference sequences need to be put into synbiohub.

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| Lab: Ginkgo |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **IPTG** | **Kanamycin Sulfate** | [**L-arabinose**](https://hub.sd2e.org/user/sd2e/design/Larabinose/1) | **measurement-type** | **file-type** | **replicate** | **strains** | **timepoint** | **samples** |
| 0.0 mM,7e-5 mM | 0.0019 mM | 0 mM, 0.0125 mM | FLOW | FCS | 4 | MG1655, MG1655\_LPV3,MG1655\_RPU\_Standard | 5.0 hour, 18 hour | 96 |
| 0 mM,7e-5 mM |  | 0 mM, 0.0125 mM | RNA\_SEQ | FASTQ, FASTQ | 4 | MG1655, MG1655\_LPV3 | 5.0 hour, 18 hour | 128 |
| 0 mM,7e-5 mM | 4.98e-8 mM | 0 mM, 0.0125 mM | RNA\_SEQ | FASTQ, FASTQ | 4 | MG1655\_RPU\_Standard | 5.0 hour, 18 hour | 64 |
| 0 mM,7e-5 mM | 4.98e-8 mM | 0 mM, 0.0125 mM | PLATE\_READER | PLAIN,CSV | 4 | MG1655, MG1655\_LPV3 | 5.0 hour, 18 hour | 128 |

*Old Samples: [1088, 1056, 64, 2112]*

*Does an ETL pipeline exist for all analyses? If not, what is the workaround and by who/on what timeline should it be implemented?.*

Yes.

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| Lab: UW\_BIOFAB |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| [**IPTG**](https://hub.sd2e.org/user/sd2e/design/IPTG/1) | **measurement-type** | **file-type** | **replicate** | **strains** | **timepoint** | **samples** |
| 0.0 mM | FLOW | FCS | 4 | AND\_00 | 3.0, 6.0, 12.0, 24.0, 48.0 hour | 20 |
| 5.0 mM | FLOW | FCS | 4 | AND\_00 | 3.0, 6.0, 12.0, 24.0, 48.0 hour | 20 |
| 100.0 mM | FLOW | FCS | 4 | AND\_00 | 3.0, 6.0, 12.0, 24.0, 48.0 hour | 20 |
| 2.0 uM | FLOW | FCS | 4 | AND\_00 | 3.0, 6.0, 12.0, 24.0, 48.0 hour | 20 |

Old Samples = [20, 20, 20, 20]

## 

## Potential / expected challenges / prioritization : *What are some potential ways the experiment or analysis could fail or fail to provide answers to the goal? Are their other routes? Are there some parts / subgoals that should be prioritized over others? Related -- to what degree are some of these subgoals relevant across multiple challenge problems?*

## Identified stakeholders:

CP WG: Novel chassis

TA1: UCSB, Duke, PNNL, Wyss Institute, Netrias

TA2: MIT

TA3: Transcriptic, Ginkgo

TA2 ingest: SIFT

TA3: Transcriptic, Ginkgo

TA4: TACC

DARPA: Susan, Jen, Conrad

OTHER:

## Data Upload Progress:

Please use the table below to track progress of data that was uploaded. We are not going to be computer parsing from here, so keep things simple and unstructured. It’s just a touch point for folks like me so that we don’t lose it in Slack.

|  |  |  |  |
| --- | --- | --- | --- |
| **Measurement type** | **Ginkgo** | **UW Biofab** | **Transcriptic** |
| [RNAseq](https://hub-staging.sd2e.org/user/sd2e/transcriptic_rule_30_q0_1_09242017/A01_1_rnaSeq_10/1) | All data is uploaded and accessible on TACC through the Science Table |  | List out set of conditions (this can be unstructured) -- date -- directory |
| Plate reader time series | Not yet available |  | List out set of conditions (this can be unstructured) -- date -- directory |
| Global, relative Proteomics | All data is uploaded and accessible on TACC through the Science Table | N/A | List out set of conditions (this can be unstructured) -- date -- directory |
| Absolute Proteomics | All data is uploaded and accessible on TACC through the Science Table | N/A | List out set of conditions (this can be unstructured) -- date -- directory |
| Flow | All data is uploaded and accessible on TACC through the Science Table |  | List out set of conditions (this can be unstructured) -- date -- directory |
| Genome seq | Status unknown | N/A | List out set of conditions (this can be unstructured) -- date -- directory |

Adfadf

Asdfasdf

Adfad

test MG1655 test

## Experiment Results

exp1 run on 6/30/2019  
exp2 run on 7/30/2019  
exp3 run on 8/30/2019  
exp4 run on 9/30/2019

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| --- |
| Lab: UW\_BIOFAB |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| [**Ara**](https://hub.sd2e.org/user/sd2e/design/Larabinose/1) | [**IPTG**](https://hub.sd2e.org/user/sd2e/design/IPTG/1) | **measurement-type** | **file-type** | **replicate** | **strains** | **timepoint** | **temperature** | **samples** |
| 0, 1 mM | 0, 1, 2 mM | RNA\_SEQ | FASTQ | 4 | [AND\_00](https://hub.sd2e.org/user/sd2e/design/UWBF_7376/1) | 3, 6, 12, 24 hour | 37 celsius | 72 |
| 0, 1 mM | 0, 1, 2 mM | FLOW | FCS | 4 | [NOR\_00](https://hub.sd2e.org/user/sd2e/design/UWBF_6390/1) | 24, 48 hour | 37 celsius | 48 |

|  |
| --- |
| Lab: Transcriptic |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **measurement-type** | **file-type** | **replicate** | **strains** | **timepoint** | **Beta-estradiol** | **temperature** | **samples** |
| PLATE\_READER | CSV | 12 | UWBF\_24926, UWBF\_24952, UWBF\_24959, UWBF\_24960, UWBF\_24961, UWBF\_24962, UWBF\_24963 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hours | 0 X | 30 C | 1512 |
| PLATE\_READER | CSV | 3 | UWBF\_23970, UWBF\_6390, UWBF\_24864 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 0 X | 30 C | 162 |
| PLATE\_READER | CSV | 12 | UWBF\_24926, UWBF\_24952, UWBF\_24959, UWBF\_24960, UWBF\_24961, UWBF\_24962, UWBF\_24963 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 200 nM | 30 C | 1512 |
| PLATE\_READER | CSV | 3 | UWBF\_23970, UWBF\_6390, UWBF\_24864 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 200 nM | 30 C | 162 |
| PLATE\_READER | CSV | 12 | UWBF\_24926, UWBF\_24952, UWBF\_24959, UWBF\_24960, UWBF\_24961, UWBF\_24962, UWBF\_24963 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 0 X | 35 C | 1512 |
| PLATE\_READER | CSV | 3 | UWBF\_23970, UWBF\_6390, UWBF\_24864 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 0 | 35 C | 162 |
| PLATE\_READER | CSV | 12 | UWBF\_24926, UWBF\_24952, UWBF\_24959, UWBF\_24960, UWBF\_24961, UWBF\_24962, UWBF\_24963 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 200 nM | 35 C | 1512 |
| PLATE\_READER | CSV | 3 | UWBF\_23970, UWBF\_6390, UWBF\_24864 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 hour | 200 nM | 35 C | 162 |