

CFPS coopetition [draft]

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Executive Summary

We are proposing a coopetition to learn more about human-agent collaboration in the life sciences with a particular focus on experimental work (so-called “wet lab” work). We believe that, in biology, there is virtually unlimited opportunity for scientists and AI to work together for the advancement of science. We believe the reasoning models are particularly interesting to study in the context of human-agent collaboration because they necessitate an allocation of tasks within the scientific method between humans and agents. The question is less IF humans and agents should work together and more HOW humans and agents should work together. What contributions are humans best suited to make and what contributions are agents best suited to make in life sciences research? How can human scientists improve the quality and impact of AI scientists’ work and how can AI scientists improve the quality, impact and velocity of human scientists’ work? How might that change as models + harnesses + tools advance?

Building on [prior work](#), we selected a scientific objective with a quantitatively measurable outcome. The goal of the coopetition is to design cell-free protein synthesis (CFPS) reactions from an available menu of CFPS reagents to maximize the specific cost (\$/g or \$/unit of enzyme activity) of three proteins from cell-free protein synthesis:

1. sfGFP - a common fluorescent protein used in biological research labs
2. PETase - an enzyme used in the degradation of plastic (PET)
3. reteplase - a therapeutic protein with multiple disulfide bonds which must form correctly to produce active enzyme.

It is well known that different CFPS reaction compositions produce very different active protein titers. These three proteins range from relatively straightforward (sfGFP) to relatively challenging but not impossible (reteplase) to produce via cell-free protein synthesis. All three targets have high-throughput quantitative assays available and validated on Ginkgo’s autonomous lab.

We propose to compare two different approaches via the coopetition:

1. A human-led approach in which ~4 CFPS research groups design CFPS reactions for each of the three target proteins. Each group is free to use any commercially-available or open-source AI model, harness or tooling to assist with their CFPS reaction design. We ask that you refrain from using unreleased models in your work, including unreleased models from companies or university research groups.
2. An agent-led approach in which OpenAI will use a team of agents to design CFPS reactions for each of the three target proteins. OpenAI is free to use any released or unreleased AI model, harness or tooling to assist with their CFPS reaction design.

There will be 3 rounds to the competition and each round comprises 384-well plates: 16 plates per target per approach for 96 384-well plates total. Each 384-well plate contains 80 unique reaction compositions at a replication factor of 4 plus standards, positive controls and negative controls. All CFPS reaction composition plate designs will be validated using a pydantic model to

ensure that all CFPS reaction designs conform to the experimental setup and execution guidelines of the competition.

For the human-led approach, we will split the 16 384-well plates for each target amongst ~4 CFPS research groups. All CFPS reaction designs are shared amongst the CFPS research groups upon plate request submission and Ginkgo will share experimental results to all participating research groups upon successful plate execution (but not OpenAI). The CFPS research groups are welcome but not obligated to work together on their CFPS reaction composition plate designs prior to submission to Ginkgo. Similarly, OpenAI's agent-led team will receive their results after successful plate execution of their designs. Ginkgo plans to synchronize both plate design submission deadlines for and data return to the human-led teams and the agent-led teams.

Reagent selection is a critical aspect of CFPS reaction design and Ginkgo may not have all reagents available on the existing menu of options. To address this limitation, prior to each round of the competition, we will give both the human-led teams (collectively) an opportunity to add up to [20] reagents that are ONLY available for their experiments. The list of [20] reagents will be split amongst the CFPS research groups and all groups within the human-led approach will have access to the full list of [20] reagents. Again, the human groups are welcome but not obligated to coordinate their work reagent selection. The reagents selected by the human-led approach will not be shared with OpenAI's agent-led team. OpenAI's agent-led team will similarly be able to select [20] reagents and their selection will not be shared with the groups participating in the human-led approach.

Since CFPS reactions produce more protein with better oxygenation, in each round, Ginkgo will select the top-performing conditions and re-test them in 96-well plates: 4 plates per target protein per approach for 24 plates total. The 96-well plates will again have a replication factor of 4 and thus Ginkgo will re-test 80 total reaction conditions for each target protein for each approach at the 96-well plate scale. Then, Ginkgo will select the top-performing conditions and re-test them in 2mL tubes: 1 96-well plate equivalent per target protein per approach for 6 plate equivalents total. The 2mL tubes will again have a replication factor of 4 and thus Ginkgo will re-test 20 total reaction conditions for each target protein for each approach at the 2mL scale. The best performing CFPS reaction composition at 2mL scale will be considered the "winner" for that target protein.

The remainder of the document aims at providing a detailed overview of the experimental set up and experimental execution of our proposed competition.

Detailed description

Competition design

The goal of the coopetition is to maximize the specific cost (\$/g or \$/unit of enzyme) activity for three proteins from cell-free protein synthesis:

1. sfGFP - a common fluorescent protein used in biological research labs
2. PETase - an enzyme used in the degradation of plastic (PET)
3. reteplase - a therapeutic protein used in Acute Myocardial Infarction (AMI), in Acute Ischemic Stroke (AIS) and Pulmonary Embolism (PE) to initiate fibrinolysis. It has multiple disulfide bonds which must form correctly to produce active enzyme.

We are proposing three rounds to the coopetition. Each round consists of 96 plates - 48 384-well plates designed by human-led teams (collectively) and 48 384-well plates designed by OpenAI. The human-led participants will each be allocated an equal portion of plates totalling the 48 plates. The plates will be allocated evenly across the three target proteins: 16 for each target for the human-led teams and 16 for each target for the agent-led team. Each 384-well plate contains 80 unique CFPS reaction conditions at a replication factor of 4, plus standards, positive controls and negative controls.

Upon generation and return of the 384-well plate results, Ginkgo will cluster the top performing conditions, pick the best performing CFPS reaction composition from each cluster and re-test in 96-well plates (4 plates) per target. The human-led teams and OpenAI are each free to provide input on which reaction conditions from their respective 384-well plates should be re-tested at 96-well plate scale. A total of 80 unique reactions will be tested at the 96-well scale at a replication factor of 4.

Upon generation and return of the 96-well plate results, Ginkgo will cluster and pick the top performing conditions and re-test in 2mL tubes (equivalent to 1 96-well plate including controls and standards) per target. A total of 20 unique reactions will be tested at the 96-well scale at a replication factor of 4. 2mL tubes will serve as the canonical reaction conditions for declaring winning CFPS reaction compositions given that they yield the highest protein titers [Olsen et al., 2026]. CFPS reactions at all scales (384-well plates, 96-well plates and 2mL tubes) will have a total reaction volume of 20 μ L.

CFPS reaction designs and results from human-led teams will be shared amongst all participating human-led teams, but not OpenAI. Similarly, OpenAI will have access to their CFPS reaction designs and results. The human scientist-led teams are free but not obligated to work together on their CFPS reaction composition plate designs if they choose. We plan to synchronize both plate design submission and data return to the human-led teams and the agent-led teams.

In summary, there will be 3 rounds and each round will be comprised of

- 384-well plates - 16 per target per group for 96 plates total
- 96-well plates - 4 per target per group for 24 plates total
- 2-mL tubes - 1 96-well plate equivalent per target per group for 6 plate equivalents total

We plan to run batches of sixteen 384-well plates per day. Each day is a different target protein - 8 plates from human-led teams and 8 plates from OpenAI.

CFPS reaction design submission (participants)

Submit CFPS plate designs in JSON format, validated with a standardized pydantic model. The project GitHub repository (<https://github.com/ginkgobioworks/autonomous-cfps>) contains the model and example inputs. You define:

- A plate definition, including replicate structure (4 per experimental sample), reserved columns (1-4, used by Ginkgo for standard curves and controls).
- Reagent compositions for your experimental sample. These are specified in nL, must be a multiple of 25nL, and sum to 11000nL total.
- Unique sample IDs to identify each sample, using a replication factor of 4. You can arrange your samples as you want across the plate. The samples are arrayed down columns 5-24 of a 384 well plate in the order specified.

Ginkgo will create one Google Bucket for the agent-led approach and one Google Bucket for the human-led approach to upload your finalized CFPS reaction plate designs:

```
gcloud auth login --update-adc
gcloud storage cp local_dir/to_upload.json local_dir/to_upload2.json
gs://ginkgo_yourbucket/to_ginkgo/20260922/
```

The pydantic model validates the inputs to confirm that all CFPS reactions are well-specified and conform to the experimental setup guidelines and reagent availability. It also produces a human readable summary of the submission that can help identify any unintended issues.

Preparing Your Plate Submission

Submit your CFPS plate designs as JSON, validated against a standardized Pydantic model. The model lives in the project repo alongside example inputs and generator scripts; use it to confirm every reaction is well-specified and conforms to the experimental setup and reagent availability. It also prints a human-readable summary of your submission to help catch unintended issues before you send it.

1. Get the code

```
git clone https://github.com/ginkgobioworks/autonomous-cfps.git
cd autonomous-cfps
uv sync      # creates the venv and installs the package + deps in editable mode
```

The models (CFPSReagentList, Sample, Plate, PlateBatch, SampleType) and worked examples ship in the repo — start from an example JSON or generator script and adapt it.

2. Understand the plate layout (where your 80 sample IDs come from)

Designs go on a **384-well plate** (16 rows × 24 columns), `array_order="column"`:

- **Columns 1–4 are reserved** for Ginkgo (standard curves and controls) — you don't fill these, and all of Ginkgo's controls live here.
- **You fill columns 5–24:** 20 columns × 16 rows = **320 wells**.
- Every design is run with a **replicate factor of 4**, so $320 \div 4 = 80$ **unique sample IDs per plate** — all of them yours, all experimental.

Replicates must share one sample ID and identical reagents. The four wells of a design all carry the same `sample_id` and the same `reagent_list` — that's how the model groups and validates replicates.

You may arrange samples anywhere across columns 5–24; with `array_order="column"` they fill down each column in list order.

3. Define a sample's reagent composition

All reagent volumes are in **nL**, must be **multiples of 25 nL**, and the full `reagent_list` must **sum to 20,000 nL (20 µL)**. Within that:

- **Fixed — do not change:** `base_buffer = 2000`, `lysate = 5000`, and **exactly one** DNA template (`template_sfgrp / template_petase / template_reteplase`) = 2000. That's 9,000 nL locked.
- **You design the remaining ~11,000 nL** freely from the available reagents, using `nuclease_free_water` to top up to 20,000 total.
- Any reagent marked **out of stock must be 0**; the chosen DNA template must match the plate's `protein_target`.

4. Convert compositions into a plate object that runs

Build each unique design once, expand it to 4 replicates sharing its `sample_id`, assemble the Plate, then validate — the model raises on any violation (bad total, non-25 nL steps, wrong replicate count, out-of-stock reagent, template mismatch):

```
from autonomous_cfps.models import Plate, Sample, SampleType, CFPSReagentList
```

```

# One design = one sample_id + one reagent_list summing to 20000 nL
def design(sample_id: str, **reagents) -> Sample:
    return Sample(
        sample_id=sample_id,
        sample_type=SampleType.EXPERIMENTAL,
        reagent_list=CFPSReagentList(**reagents), # base_buffer/lysate/template fixed; water fills to 20000
    )

designs = [
    design("rxn_001", template_sfgfp=2000, potassium_glutamate=1500, nuclease_free_water=...),
    # ... 80 unique experimental designs total ...
]

# Expand every design to 4 replicates (same sample_id, same reagents) -> 320 wells
samples = [d for d in designs for _ in range(4)] # 80 x 4 = 320

plate = Plate(
    plate_id="my_plate_001",
    samples=samples, # must be exactly 320 in column order
    positive_controls=0,
    positive_controls_mixed=0, # controls live only in reserved cols 1-4
) # validates on construction

print(plate.get_plate_dataframe().to_string()) # human-readable summary

with open("to_upload.json", "w") as f:
    f.write(plate.model_dump_json(indent=2))

```

Multiple plates that share a bounded reagent supply can be wrapped in a `PlateBatch`, which additionally checks total demand against the reagent inventory caps.

5. Upload to Ginkgo

Ginkgo creates a Google bucket per contestant. Upload your validated JSON into the dated `to_ginkgo/` folder:

```

gcloud auth login --update-adc
gcloud storage cp to_upload.json to_upload2.json gs://ginkgo_yourbucket/to_ginkgo/20260922/

```

Experimental Setup (Ginkgo)

Inputs

Target Proteins

Protein Name	superfolder green fluorescent protein (sfGFP) - Strep tagged
Protein Molecular Weight	27,015 Da (g/mol)
Protein Amino Acid Length	240 amino acids (including the 8 amino acids-long Strep tag)
Further FP Base Information	https://www.fpbases.org/protein/8B9TX/ (plus 26 additional amino acids on C-terminus)
Ginkgo ID	m9142078 (protein)
Sequence	MSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTCLKFICTTGKLPVPWPTLVTTLTLYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFSHNVIYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEKGRDTHMVLHEYVNAAGITWSPQFEK
protein_target	sfgfp-strep-m9134733

Protein Name	PETase (<i>Thermobifida fusca</i> cutinase 2 (TfCut2))
Protein Molecular Weight	33,157 Da (g/mol)
Protein Amino Acid Length	309 amino acids
Further Uniprot Information	Q6A0I4 (with N-terminal CSL–Capper/StrepII/linker–TEV fusion and C-terminal HiBiT tag)
Ginkgo ID	m9360457 (protein)
Sequence	MEKKIWSHPQFEKGGSGENLYFQSGGASPAGEGANPYERGPNTDALLEASSGPFPSVSEENVSRLSASGFGGGTIYYPRENNYGAVALSPGYTGEASIAWLGERIASHGFFVITIDTITTLDDQPDRAEQNLNAAALNHMINRASSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHLNKNWSSVTVPITLIIGADLDTIAPVATHAKPFYNLSLPSISKAYLELDGATHFAPNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGLFGEVEEYRSTCPFGSGVSGWRLFKKIS
protein_target	TfCut2

Protein Name	reteplase
Protein Molecular Weight	44,136 Da (g/mol)
Protein Amino Acid Length	399 amino acids
Further Uniprot Information	UniProt P00750 Truncated based on: Gething, et al. (1988) <i>The EMBO Journal</i> . Yin & Swartz. (2004). <i>Wiley InterScience</i> . With N-terminal CSL tag, C-terminal HiBiT tag
Ginkgo ID	m9307103 (protein)

	taaaatgcttgatggctcggaagaggcataaattccgtcagccagtttagtctgacca tctcatctgtaacatcattggcaacgctacctttgccatggttcagaaacaactctg gcgcacggttcccatataatcgatagattgtcgacactgattgcccacattat cgcgagcccatttatacccatataaatcagcatccatggtggaatttaaatcgcggct tcgagcaagacggtttcccggtgaatatggctcataaacaccccttgattactgttta tgtaagcagacagttttattgttcagatgatataattttatcttggtgcaatgtaac atcagagattttgagacacaacgtgagatcaaaggatcttcttgagatcctttttt ctgcccgtaatctgctgcttgcaacaaaaaaaccacgcgtaccagcgggtggtttgt ttgcccgatcaagagctaccaactccttttccgaaggtaactggcttcagcagagcg cagataccaaatactgttcttctagttagccgtagttaggccaccacttcaagaac tctgtagcaccgcctacatacctcgctctgctaactcctgttaccagtggtgctgcc agtggcgataaagtctgtcttaccgggttggaactcaagacgatagttaccggataag gcgcagcggctcgggtgaacggggggttcgtgcacacagcccagcttgagcgaacg acctacaccgaactgagatacctacagcgtgagctatgagaagcgcacgcgttccc gaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgc acgagggagcttccaggggaaacgcctggtatctttatagtcctgtcgggtttcgc cacctctgacttgagcgtcgattttgtgatgctcgtcaggggggcggagcctatgg aaaaacgccagcaacgcgatcccgcaaaagtcccagcaacgcgatcccgcaaaatta atacgactcactataggagaccacaacgggttccctctagaataattttgtttaa ctttaagaaggagatatacatatgagcaaaagtgaaagactgtttaccggcgttggtg cagattctggtggaactggatggcgatgtgaacgggtcacaaattcagcgtgctggtg gaaggtgaaggcgatgccacgatggcaaaactgacgctgaaatttatctgcaccacc ggcaaaactgccggtgccgtggccgacgctggtgaccaccctgacctatggcgttcag tgttttagtcgctatccggatcacatgaaacgtcacgatttctttaaactctgcaatg ccggaaggctatgtgcaggaacgtacgattagctttaagatgatggcaaatataaa acgcgcgccgttggtgaaattgaaggcgataccctgggtgaaccgcattgaaactgaaa ggcacggatttttaagaagatggcaatatcctggggcataaaactggaatacaacttt aatagccataatgtttatattacggcggataaacagaaaaatggcatcaaagcgaat tttaccgttcgccataacggtgaagatggcagtggtgcagctggcagatcattatcag cagaataccccgatgtgtgatggctccggtgctgctgccggataatcattatctgagc acgcagaccggttctgtctaaagatccgaacgaaaaaggcacgcgggaccacatgggt ctgcacgaatatgtgaatgcggcaggtattacgtggagccatccgcagttcgaaaaa taagtcgaccggtgctaaacaaagcccgaaggaagctgagttggctgctgccaccg ctgagcaataactagcataacccttggggcctctaaacgggtcttgaggggttttt tgc
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DNA Template Type	circular plasmid DNA
DNA Template Molecular Weight	1,660,535 Da
DNA Template Base Pair Length	2696 bp
Initial DNA Concentration	50 nM
Final DNA Concentration	5 nM
Ginkgo ID	m9307404 (TFcut2)
Sequence	tgaagccaattctgattagaaaaactcatcgagcatcaaataaaactgcaatttat tcatatcaggattatcaataccatatttttgaaaaagccggtttctgtaatgaaggag aaaactcaccgagggcagttccataggatggcaagatcctggtatcggctcgcgattc cgactcgtccaacatcaatacaacctattaatttccctcgtcaaaaaataaggttat caagtgagaaatcaccatgagtgacgactgaatccggtgagaatggcaaaagtttat gcatttcttccagacttgttcaacaggccagccattacgctcgtcatcaaaatcac tcgcatcaaccaaacggtatttcattcgtgattgcccgtgagcgcagacgaaatacgc gatcgctgttaaaaggacaattacaacagggaatcgaaatgcacccggcgcaggaaca ctgccagcgcacatcaacaattttcacctgaatcaggatattcttctaataactgga atgctgttttccggggatcgagtggtgagtaacctgcatcatcaggagtacgga taaaatgcttgatggctcggaagaggcataaattccgtcagccagtttagtctgacca tctcatctgtaacatcattggcaacgctacctttgccatggttcagaaacaactctg gcgcacggttcccatataatcgatagattgtcgacactgattgcccagacattat cgcgagcccatttatacccatataaatcagcatccatggtggaatttaaatcgcggct

	tcgagcaagacggtttcccggtgaatatggctcataaacaccccttgattactgttta tgtaagcagacaggttttattgttcatgatgatataattttatcttgccaatgtaac atcagagattttgagacacaacgtgagatcaaaggatcttcttgagatcctttttt ctgcgcgtaaatctgctgcttgcaaacaaaaaaccacgctaccagcggtggtttgt ttgcccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcg cagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaac tctgtagcaccgcctacataacctcgctctgctaatacctgttaccagtggtctgcc agtggcgataagtctgtcttaccgggttggtactcaagacgatagtaccggataag gcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttgagcgaacg acctacaccgaactgagataacctacagcgtgagctatgagaaagcgccacgcttccc gaaggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgc acgagggagcttccaggggaaacgcctggtatctttatagtcctctcggtttcgc cacctctgacttgagcgtcgatttttgtgatgctcgtcagggggcgaggcctatgg aaaaacgccagcaacgcgatcccgcgaaagtcttaatacgaactcactataggagac cacaacggtttccctctagaaataatttgtttaactttaagaaggagatatacata tggaagaagaagataggtcgcatccacaattcgagaaggcggtcaggagaaaaact tatactttcaaagtggcggcgagcggcgaggcggtgccaacccctacgagc gtggcccaaaccacccagcgcgttgctcgaagccagttcaggtcctttctctgct ctgaggagaacgtctcaagattgtcagctagcgggtttgggtggtgggaccatatact acccccgcgagaacaataacttacggcgcggtggccatatctccaggctacacaggt cagaagcctcgattgcttggctgggtgaacgcattgccagccacggattcgtagtca tcacgatcgataccataacaacgctggaccagcccgactcacgcgctgagcaattga atgccgcacttaatacatgatcaaccgagcgtcttccacggttcgctctcggatcg actcctctcgcttagcagtgatgggacatagcatgggtggcgggcgaccctgagac tggcaagccagcgccccggacgtgaaagccgctataccgctcacgccttggcacttaa acaagaactggtcttccgtgacggttcccacattgataataggagcagatttagaca ccatcgccccggtagccacgcacgctaagcctttctacaatagcctgccgtctagta tttcaaaggcctaccttgagctcgacggcgccacgcactttgctcctaataatccga acaaaatcattggcaaatactctgtcgctggttaaagcgcttcgtagacaatgata cccgataacaccaatctctgtgccccggccccgctgacggtttattcggagaagtgc aagaataccgatccacgtgccggttcgggtggttctggtgtgagcggttggcggtgt tcaagaagattagctaagtcgaccggtgctaacaaagccgaaaggaaagctgagtt ggctgctgccaccgctgagcaataactagcataaaccccttggggcctctaaccgggt cttgaggggttttttgc
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DNA Template Type	circular plasmid DNA
DNA Template Molecular Weight	1,826,806 Da
DNA Template Base Pair Length	2966 bp
Initial DNA Concentration	50 nM
Final DNA Concentration	5 nM
Ginkgo ID	m9307400 (vTPA)
Sequence	tgaagccaattctgattagaaaaactcatcgagcatcaaatgaaactgcaatttat tcatatcaggattatcaataccatatttttgaaaaagccggttctgtaatgaaggag aaaactcaccgaggcagttccataggatggcaagatcctggtatcggtctgcgattc cgactcgtccaacatcaatacaacctattaatttccctcgtcaaaaataagggttat caagtgagaaatcaccatgagtgcgactgaatccggtgagaatggcaaaagtttat gcatttctttccagacttggtcaacaggccagccattacgctcgtcatcaaaatcac tcgcatcaaccaaaccgttattcattcgtgatcgccctgagcgagacgaaatacgc gatcgctgtttaaaggacaattacaaacaggaatcgaatgcaacggcgcgaggaaca ctgccagcgcatcaacaataattttcacctgaatcaggatattcttctaatactgga atgctgttttccggggatcgagtggtgagtaaccatgcatcatcaggagtacgga taaaatgcttgatggtcggaagaggcataaattccgtcagccagtttagtctgacca tctcatctgtaacatcattggcaacgctacctttgccatggttcagaaacaactctg gcgcatcggttcccatacaatcgatagattgtcgacactgattgcccgcacattat cgcgagcccatttatacccatataaatcagcatccatgttggaaatttaactcgcggt tcgagcaagacggtttcccggtgaatatggctcataaacaccccttgattactgttta

	tgtaagcagacagtttttattgttcatgatgatataatttttatcttgtgcaatgtaac atcagagattttgagacacaacgtgagatcaaaggatcttcttgagatcctttttt ctgcgcgtaatctgctgcttgcaacaaaaaaaccaccgctaccagcgggtgttgt ttgccggatcaagagctaccaactcttttccgaaggtaactggcttcagcagagcg cagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaac tctgtagcaccgcctacatacctcgctctgctaatacctgttaccagtggctgctgcc agtggcgataagtcgtgtcttaccgggtggactcaagacgatatgttaccggataag ggcgagcggctcgggctgaacggggggttcgtgcacacagcccagcttgagcgaacg acctacaccgaactgagatacctacagcgtgagctatgagaaagcggccacgcttccc gaagggagaaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgc acgagggagcttccaggggaaacgcctgggtatctttatagtcctgtcgggttccgc cactctgacttgagcgtcgatttttgtgatgctcgtcagggggcgagcctatgg aaaaacgccagcaacgcgatcccgcgaaagtcttaatacgcactcactataggagac cacaacggtttccctctagaaataattttgtttaactttaagaaggagatatacata tggagaagaagatatggtcgcatccacaattcgagaaggcggtcaggagaaaact tatactttcaaagtggcggcgagccggcaggcggcggttaacagtgattgttact tcggtaacgggtcagcttatcgtggcactcactctttgactgaatcggtgctagct gtctcccctggaattcaatgatcttgatcggtaaagtttacacagctcaaaatccga gcgctcaggctctcgggctcgggaagcacaaactactgccgtaacccagacggcgatg ccaagccttggtgccacgttttaaagaaccggcggttgacatgggagtagctcgatg ttccgtcctgttcaacctgtggtttacgtcaatacagtcaccccaatttcgcatta agggcgactgttcgcccagacattgcgtcacatccctggcaagcggcaattttcgcta agcaccgtcgatctcccggtagcgattcttatgtggcggtatactgatctcgtcgt gtggatacttagtgccgcgcactgtttccaagagcgtttcccgctcaccacttga cgggtatatgggtcgaacatatcgtgtgtgcccgggaggaagaacaaaagtccg aggtagagaagtatatagttcataaagaattcgacgcgatacttatgacaacgaca tcgcggttatgcaactcaaaagcgatagtagcagatgcgcccaagaaagctctgtgg ttcgtacggtctgcctgccaccagcggacttacaactgccagactggactgagtggtg aactttcagggtagcgaagcacgaggcattgagccattctatagtgagcgtttga aggaagctcacgttagactgtacccgagttcacgctgtacatcgcaacacttgctga acagaacggtgacagataatatgctctgcgcgggtgatacacgctcaggcggacctc aagccaaccttcacgatgcttgtcaaggagacagtggcggccactgggtgtgtctca acgacggtcggatgaccttagtaggaattatttccctggggccttgggtgtggtcaaa aggacgtgcctggtgtttatacaaaggtcaccaattaccttgactggatacgcgata acatgcgtccgggtggttctggtgtgagcggctggcggtgttcaagaagattagct aagtcgaccggctgctaacaaagccgaaaggaagctgagttggctgctgccaccgc tgagcaataaactagcataaaccccttggggcctctaaccgggtcttgaggggttttt gc
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Cell Lysate

We are producing the CFPS cell lysate internally, via large-scale fermentation of *E. coli* and downstream cell lysis. Lysate is generated from a fermented *E. coli* (BL21 Star (DE3)) strain. We grew cells overnight at 30°C in LB media to prepare starter culture. The following day, we combined all starter cultures and confirmed OD. We aseptically charged inoculum to Ginkgo's FM39 production medium supplemented with isopropyl-β-D- thiogalactopyranoside (IPTG) to induce T7 RNA polymerase expression in 5L Bioflo 320 vessels (Eppendorf) for fed-batch style fermentation with temperature, oxygen, pH, glucose and antifoam control. Once fermentation was complete, we harvested and pelleted cultures. We discarded the supernatant and washed the pellet three times in the harvest/lysis buffer. After the third wash the pelleted material was flash frozen in liquid nitrogen and stored at -80°C.

We removed the washed fermentation pellet from the freezer and thawed at room temperature. We supplemented the harvest/lysis buffer with DTT to the thawing pellet and agitated it to ensure a homogenous mixture. Once fully thawed and homogenous, we lysed the material with a PandaPLUS 2000 homogenizer (GEA). We clarified the resulting lysate twice via centrifugation.

We then flash froze and stored the final clarified lysate at -80°C. The lysate was later thawed, aliquoted into 384 well PCR plates, 10 uL per well, and frozen at -80°C for future use.

For reteplase screening, we produced batches of DSB CFPS cell lysate for reactions requiring oxidizing conditions. To make DSB lysate, DTT was omitted from the harvest/lysis buffer and an additional alkylation reaction was performed on the lysate before aliquoting to 384-well plates. Specifically, the lysate was prepared as described above with DTT-free harvest/lysate buffer throughout and flash frozen and stored at -80°C. 500 uM iodoacetamide was then added to thawed DTT-free lysate and the lysate was incubated at room temperature and then quenched with 500 uM cysteine. The DSB lysate was then aliquoted into 384 well PCR plates, 10 uL per well, and frozen at -80°C for future use in reteplase CFPS reactions.

Note that Ginkgo CFPS and CFPS Disulfide kits may also be purchased via our [Ginkgo Reagents store](#), though purchased CFPS kits may use different lysate lots than those used in the coopetition. Bulk lysate may be purchased as a custom order by contacting reagents@ginkgo.bio.

CFPS Reagents

We make a variety of reagents available to all parties participating in the coopetition. These reagents are generally those reported to have been used in the CFPS literature. We acknowledge it is not a comprehensive list of all reagents ever used in CFPS. Reagent costs are based on those reported by Olsen *et al.* (2025), where possible, for comparison of reaction costs obtained in this work to prior state of the art (SOTA).

Reagent	Vendor & Catalog #	Stock Concentration	Units	Components	Cost per mL
Core Components					
Cell lysate	N/A	1	X	<i>E. coli</i> (BL21 STAR (DE3)) strain	\$4.000
DSB Cell lysate	N/A	1	X	<i>E. coli</i> (BL21 STAR (DE3)) strain	\$4.000
DNA template	N/A	0.050	uM	Plasmid m9134733 encoding sfGFP	\$1.933
DNA template	N/A	0.050	uM	Plasmid m9307404 encoding PETase	\$2.078
DNA template	N/A	0.050	uM	Plasmid m9307400 encoding reteplase	\$2.284
Diluent					
Nuclease-free water**	Invitrogen 10-977-023	N/A	N/A	Nuclease-free water	\$0.000
Salt & Buffer Components					
Potassium glutamate K(Glu)	Sigma G1501	0.875	M	L-Glutamic acid potassium salt monohydrate	\$0.070
Magnesium glutamate	Sigma 49605	0.500	M	L-Glutamic acid hemimagnesium salt tetrahydrate	\$0.065

Reagent	Vendor & Catalog #	Stock Concentration	Units	Components	Cost per mL
Mg(Glu) ₂					
Ammonium glutamate	Biosynth FG28929	1	M	L-glutamic acid ammonium salt	\$0.72
HEPES-KOH pH 7.5	Boston Bioproducts BBH-75-K	1	M	HEPES, potassium hydroxide	\$0.130
Potassium formate	Alfa Aesar A14551	0.5	M	Potassium formate, acetic acid, potassium hydroxide	\$0.004
Transcription Components					
Adenosine 5'-triphosphate (ATP)	Sigma A2383	0.100	M	Adenosine 5'-triphosphate disodium salt hydrate, potassium hydroxide	\$1.539
Cytidine 5'-triphosphate (CTP)	Sigma C1506	0.100	M	Cytidine 5'-triphosphate disodium salt, potassium hydroxide	\$27.937
Guanosine 5'-triphosphate (GTP)	Sigma G8877	0.100	M	Guanosine 5'-triphosphate sodium salt hydrate, potassium hydroxide	\$39.343
Uridine 5'-triphosphate (UTP)	Sigma U6625	0.100	M	Uridine 5'-triphosphate trisodium salt hydrate, potassium hydroxide	\$37.846
Adenosine 5'-monophosphate (AMP)	Sigma O1930	0.100	M	Adenosine 5'-monophosphate disodium salt, acetic acid	\$0.699
Cytidine 5'-monophosphate (CMP)	Sigma C1006	0.100	M	Cytidine 5'-monophosphate disodium salt, acetic acid	\$1.270
Guanosine 5'-monophosphate (GMP)	Sigma G8377	0.100	M	Guanosine 5'-monophosphate disodium salt, acetic acid	\$0.533
Uridine 5'-monophosphate (UMP)	Sigma U6375	0.100	M	Uridine 5'-monophosphate disodium salt, acetic acid	\$0.839
Adenosine	Sigma A9251	0.025	M	Adenosine, Potassium hydroxide	\$0.002
Guanosine	Sigma G6264	0.025	M	Guanosine, Potassium hydroxide	\$0.012
Cytidine	Sigma C4654	0.025	M	Cytidine	\$0.047
Uridine	Sigma U3750	0.025	M	Uridine, Potassium hydroxide	\$0.003
Thymidine	Sigma T9250	0.025	M	Thymidine, Potassium hydroxide	\$0.108
Adenine	Sigma A8626	0.025	M	Adenine, Potassium hydroxide	\$0.010
Guanine	Sigma G11950	0.025	M	Guanine, Potassium hydroxide	\$0.003
Cytosine	Sigma C3506	0.025	M	Cytosine, Acetic acid	\$0.034

Reagent	Vendor & Catalog #	Stock Concentration	Units	Components	Cost per mL
Uracil	Sigma U1128	0.025	M	Uracil, Potassium hydroxide	\$0.002
Thymine	Sigma T0376	0.025	M	Thymine, Potassium hydroxide	\$0.007
Translation Components					
17 amino acid mix	See below	0.050	M	see below*	\$0.083
Tyrosine pH 12	Sigma T8566	0.050	M	L-Tyrosine, potassium hydroxide	\$0.011
Cysteine	Sigma C7352	0.200	M	L-Cysteine	\$0.024
Putrescine	Sigma P5780	0.500	M	Putrescine dihydrochloride	\$0.435
Core ATP Regeneration Components					
Sodium pyruvate	Sigma P2256	100	g/L	Sodium pyruvate	\$0.164
Maltose	Sigma M5895	50	g/L	D-(+)-Maltose monohydrate	\$0.020
Ribose	Sigma R7500	100	g/L	D-(-)-Ribose	\$0.180
Maltodextrin 17	Sigma 419699	300	g/L	Maltodextrin , dextrose equivalent 16.5 - 19.5	\$0.122
Glucose	Teknova G0520	200	g/L	Glucose	\$0.00222 *Sigma G8270 price to match Olsen <i>et al.</i> (2025)
Phosphoenolpyruvic acid cyclohexylammonium salt	Fisher P07581G	0.1	M	Phosphoenolpyruvic acid, cyclohexylammonium salt, potassium hydroxide	\$2.202
Oxaloacetic acid	Sigma O7753	0.5	M	Oxaloacetic acid, potassium hydroxide, acetic acid	\$0.925
Succinic acid	TCI S0100	0.5	M	Succinic acid, potassium hydroxide	\$0.004
Co-factors					
Folinic acid	Sigma TA9H98774066	10	g/L	Folinic acid, potassium hydroxide	\$8.978
NAD	Sigma N6522	0.100	M	β -Nicotinamide adenine dinucleotide, potassium hydroxide	\$33.348
cAMP	Sigma A9501	0.200	M	Adenosine 3',5'-cyclic monophosphate, potassium hydroxide	\$7.480
Spermidine	Sigma S0266	0.250	M	Spermidine	\$1.045
Nicotinamide	Sigma N7559	0.1	M	nicotinamide	\$0.002

Reagent	Vendor & Catalog #	Stock Concentration	Units	Components	Cost per mL
CoA	Sigma C3144	0.05	M	Coenzyme A sodium salt hydrate	\$104.0
Detergents					
Brij-35	Ricca Chemical 1145-32	10	% v/v	Brij® 35	\$0.027
Solvents					
Dimethyl sulfoxide (DMSO)	Sigma 472301	60	% v/v	Dimethyl sulfoxide	\$0.203
Enzymes					
Pyruvate oxidase (1.5U/mg)	Ag Scientific P-1600-5KU	10	g/L	Pyruvate oxidase, Potassium phosphate monobasic, Potassium phosphate dibasic	\$48.150
Catalase (>50000U/ml)	Sigma 02071	50000	U/ml	Catalase, Potassium phosphate monobasic, Potassium phosphate dibasic	\$5.000
Phosphates					
Sodium hexametaphosphate	Sigma 305553	0.150	M	Sodium hexametaphosphate	\$0.004
Dilithium Acetyl Phosphate	Sigma A1349332	0.050	M	Dilithium acetyl phosphate, potassium hydroxide	\$0.273
Potassium phosphate dibasic	Sigma P3786	0.500	M	Potassium phosphate dibasic	\$0.035
Potassium phosphate monobasic	Sigma P0662	0.500	M	Potassium phosphate monobasic	\$0.010
Potassium phosphate 1.6:1 dibasic:monobasic (molar ratio)	Sigma P3786 & P0662	0.5	M	Potassium phosphate dibasic, Potassium phosphate monobasic, Potassium hydroxide	\$0.024
Potassium phosphate 1.6 :1 monobasic:dibasic (molar ratio)	Sigma P3786 & P0662	0.5	M	Potassium phosphate dibasic, Potassium phosphate monobasic, Potassium hydroxide	\$0.018
Other					
Base buffer	Individual components listed in table	10	X	HEPES, Potassium hydroxide, Arginine, Isoleucine, Aspartic Acid, Alanine, Asparagine, Histidine, Proline, Serine, Threonine, Valine, Leucine, Phenylalanine, Tryptophan, Methionine, Lysine, Glycine, Glutamine, Glacial acetic acid, tyrosine, cysteine, L-Glutamic acid potassium salt monohydrate, L-Glutamic acid hemimagnesium salt tetrahydrate	\$0.220
DsbC	N/A	0.0001	M	Disulfide bond isomerase (DsbC) E. coli 25.5 kDa	\$5632
Glutathione (reduced)	Sigma G4251	0.1	M	Reduced glutathione (GSH)	\$0.17
Glutathione (oxidized)	Sigma G4376	0.17	M	Oxidized glutathione (GSH)	\$0.73

*17 amino acid mix is composed of Arginine (A8094), Isoleucine (I2752), Aspartic Acid (A7219), Alanine (A7627), Asparagine (A4159), Histidine (H8000), Proline (P0380), Serine (S4500), Threonine (T8625), Valine (V0513), Leucine (L8000), Phenylalanine (P5482), Tryptophan (T0254), Methionine (M9625), Lysine (Santa Cruz sc-207804), Glycine (G7126), Glutamine (G3126) and

glacial acetic acid (A6283). All amino acids sourced from Sigma, except where otherwise noted. Catalog numbers in parentheses.

**Nuclease-free water volume needs to be matched with the total volume of other CFPS reagents, to maintain consistent final CFPS reaction volume (20 μ L).

Additional reagents

We recognize that some participants may wish to use reagents not included in the above list and that identification and inclusion of new reagents is a contributor to advancements in cell-free protein synthesis research. Prior to each round of the coopetition, we expect to give both the human-led teams (collectively) an opportunity to add up to [10 or 20] reagents that are ONLY available for their experiments. OpenAI's agent-led team will receive the same. All requested reagents must be commercially available. We note that some reagents, particularly DNA- and protein-based reagents, may have longer lead times for sourcing. Thus, reagents requested prior to one round may not be available until a subsequent round. If we are unable to source a particular reagent in time for a round, Ginkgo will notify you and you may swap it for another selection.

Outputs

Destination (CFPS Reaction) Plate Layouts

The follow plate layout is followed for every output destination CFPS reaction plate. Standards and negative controls (3 technical replicate wells / each) are located in plate columns 1-3. Experimental samples are located in plate columns 5-24 (N=4 technical replicates), and are randomly distributed, to derisk against any “plate effects” - together with the Ginkgo positive control (N=8 technical replicates). Additional Ginkgo positive control technical replicates (N=16) are also located at a fixed position in column 4. The composition of this positive control is not disclosed and is simply used to confirm correct experimental execution via the autonomous lab.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	s1	s1	s1	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
B	s2	s2	s2	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
C	s3	s3	s3	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
D	s3	s3	s3	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
E	s4	s4	s4	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
F	s6	s6	s6	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
G	s7	s7	s7	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
H	s8	s8	s8	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
I	s9	s9	s9	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
J	s10	s10	s10	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
K	s11	s11	s11	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
L	s12	s12	s12	PC	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
M	s13	s13	s13	C	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
N	s14	s14	s14	C	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E

O	s15	s15	s15	C	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
P	NC	NC	NC	C	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E

Legend:

S - standard

NC - negative control

PC - positive control

C - control. Going forward, these wells will be negative controls containing lysate, CFPS reagent and no DNA encoding the target protein. PETase and reteplase assay have higher background and benefit from additional negative control samples to establish background levels. For the initial test plates, please refer to the JSON file to confirm whether this well was used as a positive or negative control in each plate.

E - experiment

Standards

Note that we make use of so-called “reverse standard curves” - standards in which the total concentration of product plus substrate is fixed at the substrate concentration used in the assay but the product concentration varies from high to low across standards-containing wells - to better correct for background absorbance / fluorescence from the substrate.

	sfGFP (purified sfGFP)	PETase (p-nitrophenol / p-nitrophenol hexanoate)		Reteplase (AMC / IPR-AMC)	
		p-nitrophenol product	p-nitrophenol hexanoate substrate	AMC product	IPR-AMC Substrate
Standard 1	118.9 μ M	400 μ M	0 μ M	25 μ M	0 μ M
Standard 2	90 μ M	400 μ M	0 μ M	25 μ M	0 μ M
Standard 3	59.45 μ M	200 μ M	200 μ M	12.5 μ M	12.5 μ M
Standard 4	45 μ M	200 μ M	200 μ M	12.5 μ M	12.5 μ M
Standard 5	29.73 μ M	100 μ M	300 μ M	6.25 μ M	18.75 μ M
Standard 6	22.5 μ M	100 μ M	300 μ M	6.25 μ M	18.75 μ M
Standard 7	14.86 μ M	50 μ M	350 μ M	3.13 μ M	21.88 μ M
Standard 8	11.25 μ M	50 μ M	350 μ M	3.13 μ M	21.88 μ M
Standard 9	7.43 μ M	25 μ M	375 μ M	1.56 μ M	23.44 μ M
Standard 10	5.63 μ M	25 μ M	375 μ M	1.56 μ M	23.44 μ M
Standard 11	3.72 μ M	12.5 μ M	387.5 μ M	0.78 μ M	24.22 μ M
Standard 12	2.81 μ M	12.5 μ M	387.5 μ M	0.78 μ M	24.22 μ M
Standard 13	1.86 μ M	6.25 μ M	393.75 μ M	0.39 μ M	24.61 μ M
Standard 14	1.41 μ M	6.25 μ M	393.75 μ M	0.39 μ M	24.61 μ M

Standard 15 / Negative Control	0.93 μ M	0 μ M	400 μ M	0 μ M	25 μ M
Negative Control	0 μ M	0 μ M	400 μ M	0 μ M	25 μ M

Final Reaction Composition (Experiment / Positive Control Wells)

Reagent	Initial Concentration	Final Concentration	Volume (μ L)
CFPS reagents (including NF water)	variable	variable	11
DNA template	50 nM	5 nM	2
Base buffer (HEPES, Potassium hydroxide, Arginine, Isoleucine, Aspartic Acid, Alanine, Asparagine, Histidine, Proline, Serine, Threonine, Valine, Leucine, Phenylalanine, Tryptophan, Methionine, Lysine, Glycine, Glutamine, Glacial acetic acid, tyrosine, cysteine, L-Glutamic acid potassium salt monohydrate, L-Glutamic acid hemimagnesium salt tetrahydrate)	300 mM HEPES-KOH pH 7.5 2000 mM Potassium glutamate 26 mM Magnesium glutamate 10 mM 18 amino acid mix 10 mM L-cysteine	30 mM HEPES-KOH pH 7.5 200 mM Potassium glutamate 2.6 mM Magnesium glutamate 1 mM 18 amino acid mix 1 mM L-cysteine	2
Cell lysate	100% v/v	25% v/v	5
TOTAL Volume			20 μL

Final Reaction Composition (Standard / Negative Control Wells)

Reagent	Initial Concentration	Final Concentration	Volume (μ L)
Protein target standard (sfGFP) or Water (PETase and reteplase)	4X	1X	2 μ L
Water (backfill)	1X	0.6X	11 μ L
Base buffer	10X	1X	2 μ L
Cell lysate	100% v/v	25% v/v	5 μ L
TOTAL Volume			20 μL

Data analysis

sfGFP

The raw readout is endpoint fluorescence read on the CFPS reaction plate itself. Each plate has a standard curve made up of a purified sfGFP dilution series. To calculate titer, we convert fluorescence to molarity with the standard curve, then to mass concentration using the per-well

protein molecular weight ($\mu\text{M} \times \text{MW} / 1\text{e6} = \text{g/L}$). The result is corrected back to the concentration in the reaction by the sample dilution.

PETase

The raw readout is absorbance. PETase cleaves a substrate releasing p-nitrophenol, which absorbs at 405 nm. The reported concentration is PNP product, an activity proxy, converted via the standard curve, a p-nitrophenol product series in columns 1-3. OD405 (about 0 to 4) sits below the curve-fitting code's working range, so OD is multiplied by 1000 before fitting. A constant factor is absorbed into the slope and intercept, so predicted PNP is unaffected. The raw endpoint carried in the fluorescence field is $\text{OD}_{405} \times 1000$.

The endpoint is the latest timepoint at which the column-4 A-L target_control is still below OD 3.5, since it saturates near OD 4. If every timepoint saturates, the earliest is used; if none do, the latest.

Each read gets quantified on its own standard curve, giving a PNP progress curve. We calculate an initial-rate slope fit over the linear rise before the curve bows. The window is derived once from the top-expressing experimental wells and applied plate-wide. `slope\_results` reports the raw rate in OD_{405}/h with the fit R2 and `n\_reads\_used` is the reads inside that window.

Reteplase

The raw readout is fluorescence: reteplase drives cleavage of a fluorogenic peptide substrate, releasing AMC (7-amino-4-methylcoumarin), read at 345 nm excitation / 445 nm emission. The reported concentration is an activity proxy of AMC product using the standard curve in columns 1-3.

Rate is calculated as the slope fit on raw fluorescence over an adaptive window. The initial rise of the column-4 A-L target_control is used to calculate the window over the linear range of the curve. Real elapsed time is taken from each read's wall-clock timestamp, with the origin at substrate addition. This is the axis in `kinetic\_results.elapsed\_h`. `slope\_results` reports RFU/h with the fit R2 and `n\_reads\_used`.

Experimental Execution (at Ginkgo)

Manual reagent prep: Make CFPS Reagent Plates

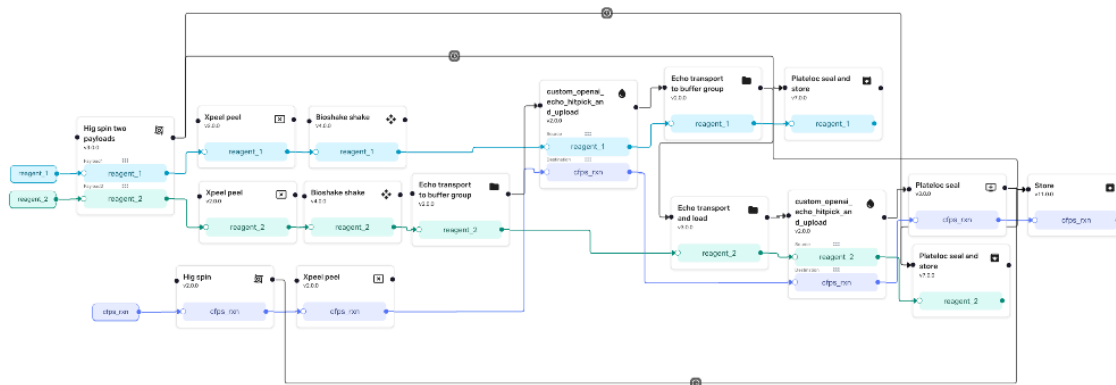
Process Steps:

#	Step	Execution
1	For requested reagents and overflow reagents, retrieve CFPS reagent Micronic tube racks from -80 C storage	Manual

2	Retrieve information on the CFPS reagent tubes needed per each CFPS reaction plate from Servicyly	Manual
3	Rearray CFPS reagent Micronic tubes into intermediate CFPS reagent tube racks. The correct number of needed tubes for each reagent and reaction plate are placed arbitrarily in a Micronic tube rack except the first 3 columns are left empty for water. Ideally keep reagent Micronic tubes on ice during this step.	Manual
4	Decap intermediate CFPS reagent Micronic tube racks	Semi-automated - IntelliXCap
5	Scan the Micronic tube rack into LIMS and use our software to confirm the right number of tubes for each reagent is in the rack.	Semi-automated - Thermo VisionMate
6	Pipette stamp 50 uL CFPS reagents from each intermediate CFPS reagent Micronic tube rack (96-tubes), to 4 quadrants of the corresponding CFPS reagent plates (384-wells)	Semi-automated - Hamilton Microlab STARplus
7	Add 50 uL nuclease-free water to designated columns of CFPS reagent plates (384-wells) using a 16-channel pipettor.	Manual
8	Seal and centrifuge CFPS reagent plates (at 1000rpm for 1min). Load CFPS reagent plates into Tundrastore at 4C.	Manual / Semi-automated - PlateLoc
9	Discard empty intermediate CFPS reagent tube racks and store the source CFPS reagent Micronic tube racks back in the -80 C storage.	Manual
10	For reagent plate 1 , CFPS reaction plate with 2 uL base buffer, and cell lysate source plate, retrieve pre-aliquoted plates from -80C and load into Tundrastore at -20C	Manual
11	Add 11 uL water to first 3 columns of CFPS reaction plate	Manual

Autonomous Lab: Part 1 Echo Cherry Pick Reagents into CFPS Destination Plates

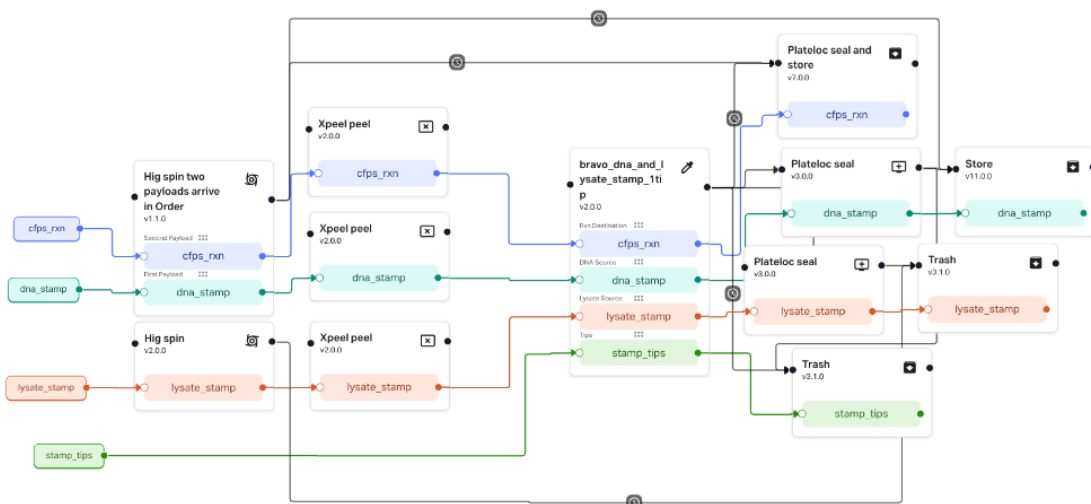
ACS Protocol: [openai_cfps_hitpicks](#)



Process Steps:

#	Step	Inputs / Outputs	Execution
1	Load	CFPS Reagent Plates	RAC system - Ambistore
2	Load	CFPS Reaction Plates	RAC system - Ambistore
3	Spin (4000 xg, 60 s, accelerate 20%, deceleration 20%)	2 CFPS Reagent Plates	RAC system - HiG3
4	Peel	2 CFPS Reagent Plates	RAC system - Xpeel
5	Shake	2 CFPS Reagent Plates	RAC system - Bioshake
6	Spin (1500 xg, 30 s, accelerate 100%, deceleration 100%)	CFPS Reaction Plate	RAC system - HiG3
7	Peel	CFPS Reaction Plate	RAC system - Xpeel
8	Hitpick	2 CFPS Reagent Plate to CFPS Reaction Plate	RAC system - Echo 525
9	Seal and store (4C)	2 CFPS Reagent Plates	RAC system - Plateloc RAC system - Steristore
10	Seal	CFPS Reaction Plate	RAC system - Plateloc
11	Store (4 C)	CFPS Reaction Plate	RAC system - Steristore

Autonomous Lab: Part 2 Lysate and DNA stamp**ACS Protocol:** [openai_cfps_bravo_stamps](#)



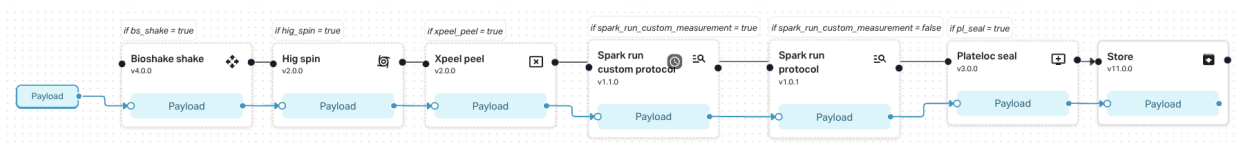
Process Steps:

#	Step	Inputs / Outputs	Execution
1	Load	DNA Plate	RAC system - Steristore
2	Load	Lysate Plate	RAC system - Tundrastore
3	Spin (1500 xg, 60 s, accelerate 100%, deceleration 100%)	CFPS Reaction Plate and DNA Plate	RAC system - HiG3
4	Spin (1500 xg, 60 s, accelerate 100%, deceleration 100%)	Lysate Plate	RAC system - HiG3
5	Peel	CFPS Reaction Plate DNA Plate Lysate Plate	RAC system - Xpeel
6	Liquid Transfer DNA template (2uL)	DNA (& optionally Standards) Plate / CFPS Reaction Plate	RAC System - Bravo 384
7	Liquid Transfer Lysate (5uL)	Lysate Plate / CFPS Reaction Plate	RAC System - Bravo 384
8	Seal and Store (4 C)	DNA (& optionally Standards) Plate	RAC System - Plateloc RAC System - Steristore
9	Seal	Lysate Plate	RAC System - Plateloc
10	Trash	Lysate Plate Tips	RAC System - TrashRAC
11	Seal and Store (30C)	CFPS Reaction Plate	RAC System - Steristore
12a	Wait (20 hours at 30C)	sfGFP and PETase CFPS Reaction Plate	RAC System - Steristore

#	Step	Inputs / Outputs	Execution
12b	Wait (10 hours at 30C)	Reteplase CFPS Reaction Plate	RAC System - Steristore
13b	Store (4C)	Reteplase CFPS Reaction Plate	RAC System - Steristore
14b	Wait (2 hours at 4C)	Reteplase CFPS Reaction Plate	RAC System - Steristore
15b	Store (Ambient)	Reteplase CFPS Reaction Plate	RAC System - Ambistore
16b	Wait (8 hours at ambient)	Reteplase CFPS Reaction Plate	RAC System - Ambistore

Autonomous Lab: Part 3a Read Fluorescence (for sfGFP)

ACS Protocol: **generic_spark_read**



Process Steps:

#	Step	Inputs / Outputs	Execution
1	Spin (1000 xg, 60 s)	CFPS Reaction Plate	RAC System - HiG3
2	Peel	CFPS Reaction Plate	RAC System - Xpeel
3	Plate Read (final read)	CFPS Reaction Plate	RAC System - Spark
4	Seal	CFPS Reaction Plate	RAC System - Plateloc
5	Store (4 C)	CFPS Reaction Plate	RAC System - Steristore

Autonomous Lab: Part 3b Dilute and run p-nitrophenol assay (for PETase)

This assay has been developed to quantify esterase activity from Cell Free Protein Synthesis (CFPS) reactions.

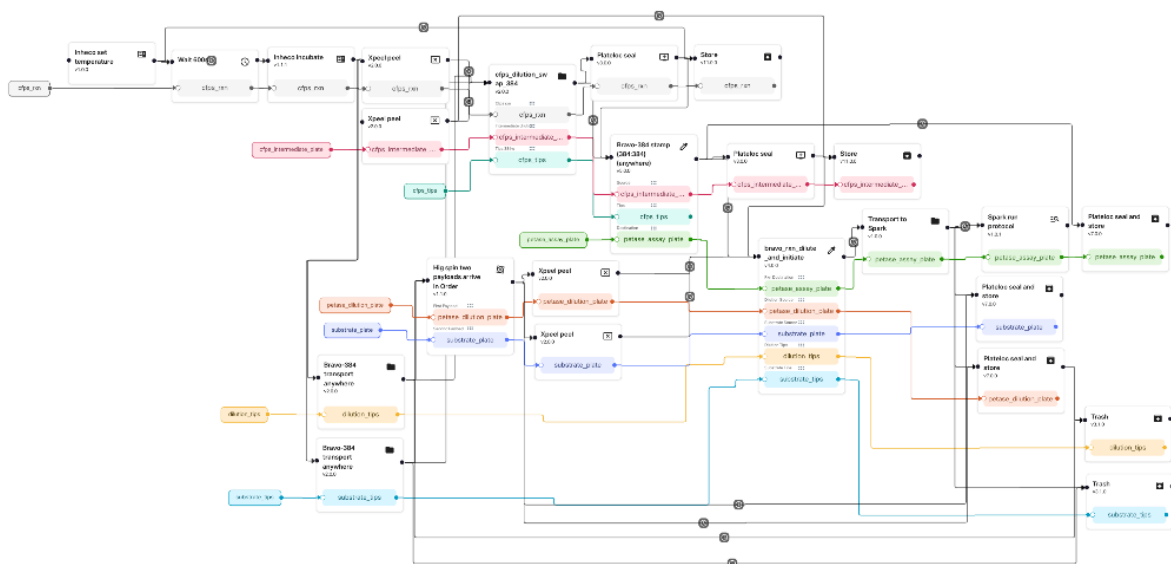
Final reaction composition:

Reagent	Initial Concentration	Final Concentration	Volume (μL)
CFPS reaction	1% (diluted 1/100 from CFPS reaction)	0.167% (600-fold dilution from CFPS reaction)	10
PBS pH 8			46
p-nitrophenol hexanoate	6mM	0.4 mM	4
TOTAL Volume			60 μL

Process Steps:

#	Step	Execution
1	Dissolve p-nitrophenol stock in 100% DMSO (100mM stock to 6mM initial concentration)	Manual
2	Array substrate/product curve into substrate plate	Manual
3	Array 175 uL PBS pH8 into CFPS intermediate plate	Semi-automated - Hamilton Microlab STARplus

ACS Protocol: **openai_cfps_petase_assay**



#	Step	Inputs / Outputs	Execution
1	Incubate (60C, 10 minutes)	CFPS Reaction Plate	RAC System - Inheco
2	Spin	Substrate plate and PETase dilution plate	RAC System - HiG3
3	Peel	CFPS Reaction Plate CFPS Intermediate Plate Substrate Plate	RAC System - Xpeel
4	Liquid transfer PBS (80 uL)	CFPS Intermediate plate / CFPS Reaction Plate	RAC System - Bravo384
5	Liquid transfer diluted CFPS reaction (5 uL)	CFPS Reaction Plate / CFPS Intermediate plate	RAC System - Bravo384
6	Seal and store (4C)	CFPS Reaction Plate	RAC System - Plateloc and RAC System - Steristore

7	Liquid transfer diluted CFPS (10 uL)	CFPS Intermediate plate / PETase Assay Plate	RAC System – Bravo384
8	Seal and store (4C)	CFPS Intermediate Plate	RAC System - Plateloc and RAC System - Steristore
9	Liquid transfer PBS pH 8 (46 uL)	PETase Dilution Plate / PETase Assay Plate	RAC System - Bravo384
10	Liquid transfer p-nitrophenol substrate (4 uL)	Substrate Plate / PETase Assay Plate	RAC System - Bravo384
11	Incubate on Bravo deck (2 mins)	PETase Assay Plate	RAC System – Bravo384
12	Plate read 405 nm (15 reads over 30 mins)	PETase Assay Plate	RAC System - Spark
13	Seal and Store (-20C)	Substrate Plate	RAC System – Plateloc and RAC System – Tundrastore
14	Seal and Store (4C)	PETase Assay Plate	RAC System - Plateloc and RAC System – Steristore
15	Seal and Store (4C)	PETase Dilution Plate	RAC System – Steristore

Autonomous Lab: Part 3c IPR-AMC peptide cleavage assay (for reteplase)

This protocol has been developed to quantify reteplase activity directly in cell-free lysate. Reteplase activity is used as a stand-in for proper disulfide bond formation. An AMC reference standard can be measured at the same time to generate a concentration curve to standardize measurements. Be sure to run an additional negative control (a reaction with no reteplase plasmid) to use with each AMC reference measurement.

Final reaction composition:

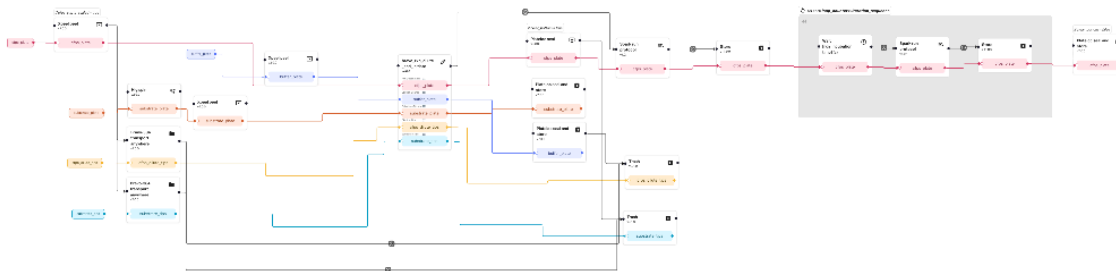
Reagent	Initial Concentration	Final Concentration	Volume (μL)
CFPS reaction	100%	50%	20
Assay buffer			18
IPR-AMC substrate	0.5 mM	25 μM	2
TOTAL Volume			40 μL

Process Steps:

#	Step	Execution
1	Dissolve AMC stock in 100% DMSO (0.5 mM initial concentration)	Manual

2	Dissolve IPR-AMC stock in 100% DMSO (0.5 mM initial concentration)	Manual
3	Array 10 uL substrate/product curve into substrate plate	Manual
4	Array 50 uL assay buffer into each well of assay buffer plate	Semi-automated - Hamilton Microlab STARplus

ACS Protocol: **openai_cfps_bravo_stamps_reteplase**



#	Step	Inputs / Outputs	Execution
1	Peel	CFPS Reaction Plate Assay Buffer Plate	RAC System - Xpeel
2	Spin	Substrate Plate	RAC System - HiG3
3	Peel	Substrate Plate	RAC System - Xpeel
4	Liquid transfer assay buffer (18 uL)	Assay Buffer plate / CFPS Reaction Plate	RAC System – Bravo384
5	Liquid transfer IPR-AMC substrate and AMC standard (2 uL)	Substrate Plate / CFPS Reaction Plate	RAC System - Bravo384
6	Seal	CFPS Reaction Plate	RAC System - Plateloc
7	Looping plate read fluorescence measurement (excitation of 345 nm and emission of 445 nm, bottom read), read 15 times over ~8 hours, storage at ambient temperature between reads	CFPS Reaction Plate	RAC System - Spark
8	Seal and Store (ambient)	Assay Buffer Plate	RAC System – Plateloc RAC System – Ambistore

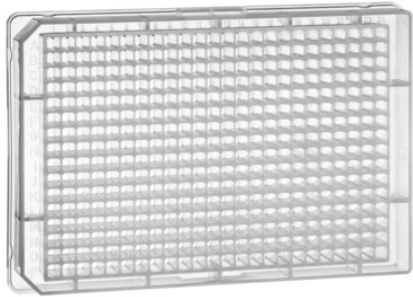
9	Seal and Store (-20C)	Substrate Plate	RAC System – Plateloc RAC System - Tundrastore
10	Seal and Store (4 C)	CFPS reaction plate	RAC System – Plateloc and RAC System – Steristore

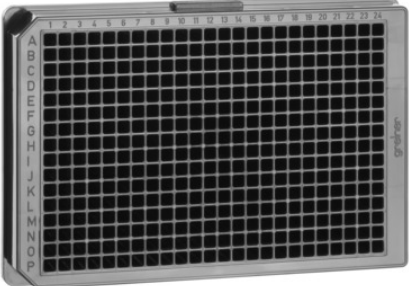
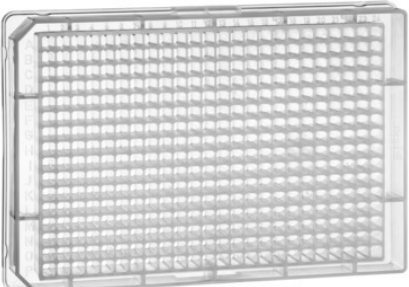
Appendix

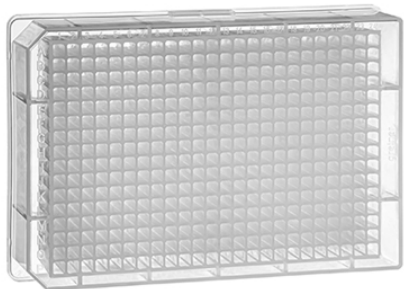
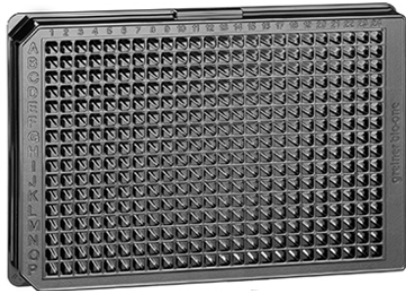
Assay reagents used during experimental execution

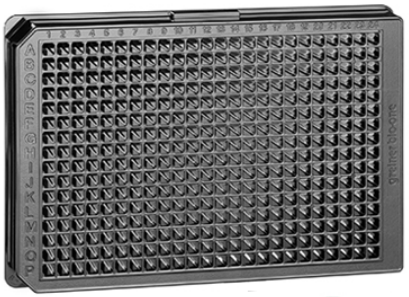
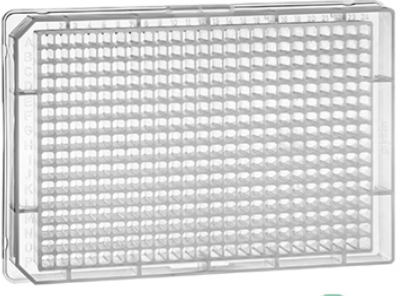
Reagent	Vendor & Catalog #	Stock Concentration	Units	Components
Core Components				
PBS pH 8 Ginkgo ID e12435	Teknova P5275	1	X	137.0 mM Sodium Chloride, 2.7 mM Potassium Chloride, 4.3 mM Disodium Phosphate, 1.4 mM Monopotassium Phosphate, NaOH to pH to 8
p-nitrophenol hexanoate Ginkgo ID m9344278	Sigma AMBH3155FE71	100	mM	p-nitrophenol hexanoate, DMSO
p-nitrophenol standard Ginkgo ID m2433310	Sigma 241326	100	mM	p-nitrophenol, DMSO
Assay buffer Ginkgo ID e12461	Ginkgo prepared	1	X	50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% Tween 20
IPR-AMC substrate Ginkgo ID e12460	Echelon Biosciences 855-18	0.5	mM	IPR-AMC, DMSO *note that this is light sensitive and must be kept out of direct light whenever possible
7-Amino-4-methylcoumarin (AMC) / tissue plasminogen-activator (tPA) standard Ginkgo ID e8967	Sigma 257370	0.5	mM	AMC, DMSO *note that this is light sensitive and must be kept out of direct light whenever possible

Consumables used during experimental execution

Generic Name: Reagent Stock Storage Tube Rack Purpose: long-term (mostly -80 C) reagent (including pDNA) storage Vendor: Micronic (cat #: MP52702-Y20) Ginkgo Container Type: Micronic M96-2 0.30 ml	
Generic Name: Reagent Source Plate Purpose: reagent acoustic cherry picking Vendor: Beckman Coulter (cat #: 001-14555) Ginkgo Container Type: 384-well Plate Echo PP Cost:	
Generic Name: DNA & Standards Source Plate Purpose: DNA template (and sometimes standards) storage for stamping Vendor: Greiner (cat #: 781280) Ginkgo Container Type: 384-v-greiner-781280-pp Cost: \$6.68 per plate	

Generic Name: Lysate Source Plate Purpose: Lysate storage for stamping Vendor Name: Eppendorf (cat. # 951020711) Ginkgo Container Type: 384-pcr-eppendorf-9510207XX Cost: \$12.23 per plate	
Generic Name: sfGFP CFPS Reaction Plate Purpose: CFPS reaction incubation and fluorescence readout Vendor Name: Greiner (cat #: 781209) flat black-bottom, black plate Ginkgo Container Type: 384 Greiner black-well clear-bottom Cost: \$6.45 per plate	
Generic Name: PETase CFPS Reaction Plate Purpose: CFPS reaction for PETase enzyme Vendor: Greiner (cat #: 781280) Ginkgo Container Type: 384-v-greiner-781281-pp Cost: \$6.68 per plate	

Generic Name: CFPS Dilution Plate Purpose: Dilution plate for PETase CFPS reaction Vendor: Greiner (cat #: 781271) Ginkgo Container Type: 384-v-greiner-781271-deep Cost: \$9.94 per plate	 
Generic Name: PETase Substrate Source Plate Purpose: Substrate storage for PETase assay Vendor Name: Eppendorf (cat. # 951020711) Ginkgo Container Type: 384-pcr-eppendorf-9510207XX Cost: \$12.23 per plate	
Generic Name: PETase Assay Plate Purpose: Absorbance readout Vendor Name: Greiner (cat #: 781092) Ginkgo Container Type: 384 Greiner black-well clear-bottom Cost: \$12.60 per plate	 

Generic Name: Reteplase CFPS Reaction Plate and Reteplase Assay Plate Purpose: Reteplase CFPS reaction and Fluorescence readout from reteplase assay Vendor Name: Greiner (cat #: 781092) Ginkgo Container Type: 384 Greiner black-well clear-bottom Cost: \$12.60 per plate	 
Generic Name: Assay Buffer Plate Purpose: Assay buffer for dilution of reteplase CFPS reaction for the reteplase assay Vendor: Greiner (cat #: 781280) Ginkgo Container Type: 384-v-greiner-781281-pp Cost: \$6.68 per plate	 
Generic Name: Reteplase Substrate Source Plate Purpose: Substrate storage for reteplase assay Vendor Name: Eppendorf (cat. # 951020711) Ginkgo Container Type: 384-pcr-eppendorf-9510207XX Cost: \$12.23 per plate	

Ginkgo's RAC-based Autonomous Lab



Device / Device Vendor	Purpose	Device Copy
Beckman Coulter Echo 525	Re-arraying (nL), stamping (nL)	5
Agilent Bravo (384 Head)	Plate stamping (uL, 384w)	4
Agilent Bravo (96 Head)	Plate stamping (uL, 96w)	6
Formulatrix Floi8	Cherry picking, Liquid handling	1
Agilent (BioTek) Multiflo FX	Bulk dispensing (uL)	8
Formulatrix Tempest	Bulk dispensing (uL)	2
Thermo Cytomat	Shaking incubation with humidity control	1
Inheco Shaking Incubator	Shaking incubation	12
QInstruments Bioshake (Model 3000)	Plate shaking (orbital)	19
QInstruments Bioshake (Model 5000)	Plate shaking (orbital)	1
ThermoFisher Automated Thermal Cycler	Thermal cycling	24
BioRad CFX Opus 384	Quantitative thermal cycling (qPCR)	4

Device / Device Vendor	Purpose	Device Copy
Agilent 406fx	Plate washing	2
Bluewasher	Plate washing	1
Azenta (Brooks) a4S	Plate sealing (breathable seals)	3
Agilent Plateloc	Plate sealing (clear seals)	1
Agilent Plateloc	Plate sealing (aluminum seals)	8
Azenta (Brooks) XPeel	Plate peeling	8
BioNex HiG3	Centrifugation	8
HighRes Biosolutions Microserve	Ambient storage (tips)	5
HighRes Biosolutions Ambistore	Ambient storage (plates)	5
HighRes Biosolutions Steristore	4C - 37C Storage (plates), static incubation	7
HighRes Biosolutions Tundrastore	-20C - 4C Storage (plates)	2
Agilent VCode	Plate barcoding	4
Ginkgo TrashRAC	Waste disposal	5
BMG Labtech Pherastar	Plate reading (spectrophotometry, luminescence, fluorescence)	3
Tecan Spark	Plate reading (spectrophotometry, luminescence, fluorescence)	2
Magnemotion	Plate transport	1

*In green, devices used in this project

Ginkgo Team

Person	Responsibility
Reshma Shetty	Project Lead
Rob Dahl	Operations (Supply Chain)
Brad Chapman	Software Dev (Data)
Ryan Harry	Software Dev (Request Processing)
Alexus Smith	Assay Dev, Operations
Ronan Donovan	Assay Dev, Operations

* other staff involved: Ginkgo's media team (CFPS reagent stocking / prep), Reagents team (lysate, DNA template and purified protein standards).