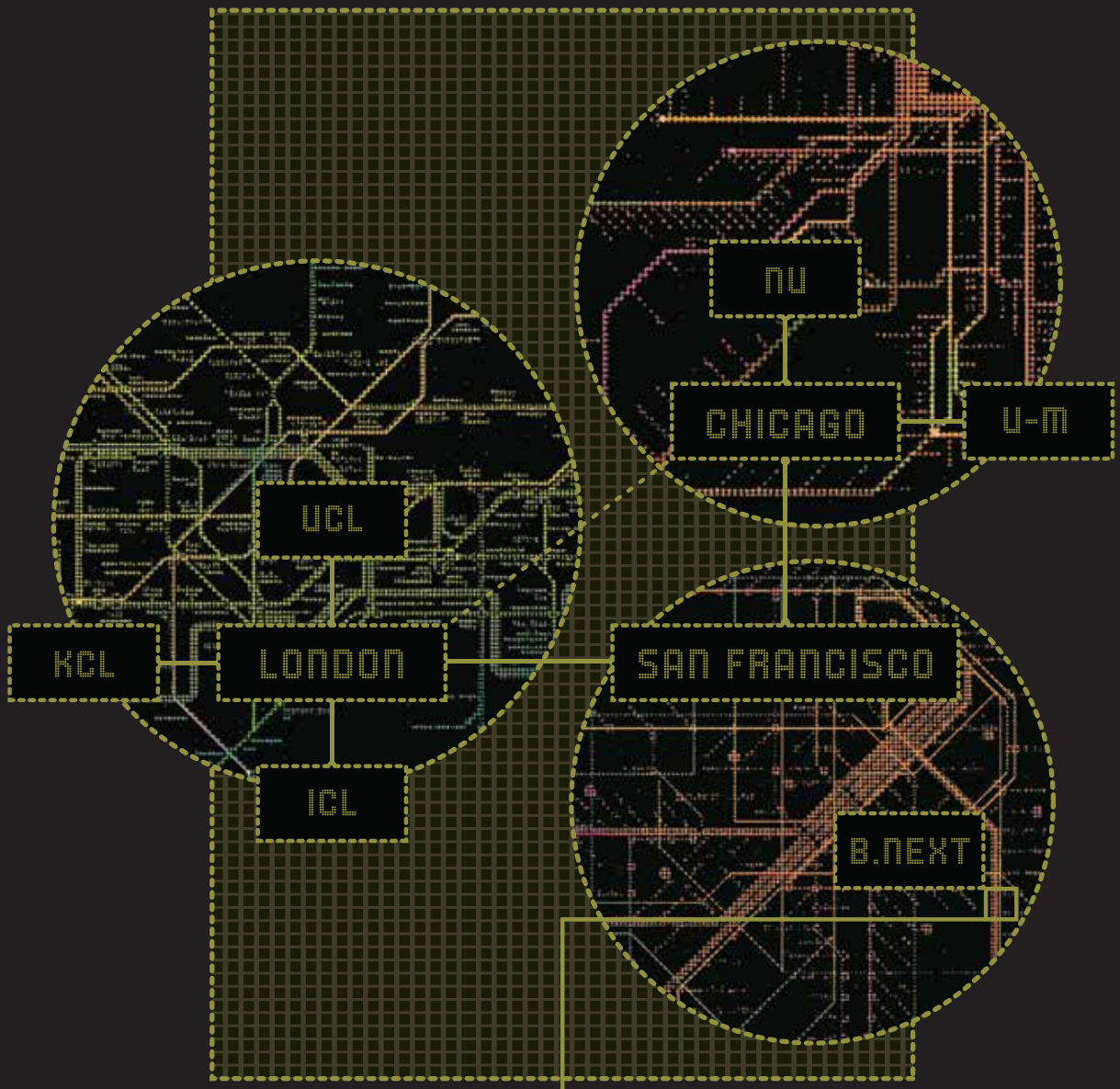
DEUCELS



KICKOFF WORKSHOP	
DATE	DEC 8-12 2025
LOCATION	NUCLEUS LABS
NODE	SAN FRANCISCO

BOOK OF PROTOCOLS

DevCells Kickoff Workshop Protocols

Overview

This booklet contains modified versions of the open source Nucleus protocols that are needed to implement and validate the PPK Energy Module in Cytosols and Cells. Complete versions of these protocols are available at https://nucleus.bnext.bio/. These protocols are living documents and are updated and expanded upon approximately every three months.

In this workshop, we will implement, validate, and optimize the PPK Energy Module in Cytosols and Cells, and use Nucleus Hub to analyze and Developer Notes to share the results. These techniques and processes will leave you prepared to make Developer Cells (DevCells).

Contents

Credits

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- Build A Cell

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Cover image by Rebecca Konte

Notes

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Day 1: Cytosol

Overview

The goal of this experiment is to compare the productivity of Cytosol using two distinct energy modules: 1) creatine kinase and creatine phosphate (CP/CK) and 2) polyphosphate kinase and polyphosphate (PPK/PolyP). Cytosol productivity will be assessed by measuring the fluorescence of expressed deGFP.

Cytosol reaction setup

Component	CP/CK [µL]	PPK/PolyP [µL]	-PPK Negative Control [µL]	-DNA Negative Control [µL]
SMix	10.5			10.5
SMixΔCP		10.5	10.5	
tRNA	3.5	3.5	3.5	3.5
PMix	4.2	4.2	4.2	4.2
Ribosomes	6.3	6.3	6.3	6.3
RNAse Inhibitor	1.75	1.75	1.75	1.75
pOpen-deGFP DNA template	0.85	0.85	0.85	0
Magnesium acetate	0	1.4	1.4	0
PPK2		1.22		
PolyP		2.10	2.10	
Water	7.9	3.18	4.4	8.75
Total master mix volume [µL]	35	35	35	35

Protocol

- [ ] **Assemble Cytosol reactions**
  - [ ] Remove all components listed in the Reaction Setup table above from appropriate cold storage.
  - [ ] Thaw reagents on ice.
  - [ ] Prepare a PCR tube, on ice, to assemble reactions into.

> 👉 **Note:** Prepare the reaction on ice or a cold block to prevent protein expression from starting during assembly. This ensures the plate reader captures the complete fluorescence kinetics for deGFP expression.

  - [ ] For a given reaction, assemble by adding the volume of reagents from the table in the order listed. Pay special attention to the handling of the Cytosol components:
    - [ ] Vortex SMix: Ensure thorough mixing; 10s vortex / 10s rest on ice; should be transparent with no visible precipitate; and add to the reaction tubes.

- [ ] Vortex or pipette mix tRNA, and add to the reaction tubes.
- [ ] Vortex or pipette mix PMix, and add to the reaction tubes.
- [ ] **Do NOT vortex** ribosomes: *gently* pipette mix or flick the tube, and add to the reaction tubes.
- [ ] Add remaining reactions in the order they appear.

- [ ] Mix the master mix thoroughly by pipetting up and down 10–15 times until it appears homogeneous and clear.
- [ ] Close lids on the PCR tubes and briefly spin down to eliminate bubbles.
- [ ] Hold assembled reactions on ice until ready for measurement.
- [ ] Array 10 µL into each of three assigned wells of a black 384-well optical plate and take note of your plate map.

> 👉 **Tip:** Set the P20 pipette to 10.1 µL to draw the master mix, then dispense into the plate well by pushing the plunger to the first stop only—this prevents bubble generation in the reaction.

- [ ] Measure deGFP fluorescence in the plate reader while incubating at 37°C.

- [ ] **Return reagents to their appropriate storage locations.**
  - [ ] Add a black dot to the lid of each of Cytosol component. The number of dots indicates freeze–thaw cycles.

Notes

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Day 2: Cells

Overview

The goal of this experiment is to compare the productivity of Cells composed of Cytosols using two distinct energy modules: 1) creatine kinase and creatine phosphate (CP/CK) and 2) polyphosphate kinase and polyphosphate (PPK/PolyP). Cytosol productivity will be assessed by measuring the fluorescence of expressed deGFP.

Cytosol reaction setup

Component	CP/CK [µL]	PPK/PolyP [µL]	-PPK Negative Control [µL]	-DNA Negative Control [µL]
SMix	12			12
SMixΔCP		12	12	
tRNA	4	4	4	4
PMix	4.8	4.8	4.8	4.8
Ribosomes	7.2	7.2	7.2	7.2
RNase Inhibitor	2	2	2	2
pOpen-deGFP DNA	0.95	0.95	0.95	0
Magnesium acetate	0	1.6	1.6	0
PPK2		1.4	0	
PolyP		2.4	2.4	
Optiprep	1.33	1.33	1.33	1.33
Water	7.72	2.32	3.72	8.67
Total master mix volume [µL]	40	40	40	40

Protocol

- [ ] **Assemble outer solutions**
  - [ ] Prepare 1.5 mL microcentrifuge tubes labelled with the appropriate reaction.
  - [ ] Mix glucose stock solution and water according to the following table:

Component	CP/CK [µL]	PPK/PolyP [µL]	-PPK Negative Control [µL]	-DNA Negative Control [µL]
Glucose (2 M)	570	637	580	570
Water	430	363	420	430
Total	1000	1000	1000	1000

📌 **Note:** The outer solution concentration may vary and should be based on the inner solution's osmolarity.

- [ ] **Assemble Cytosol reactions**
  - [ ] Remove all components listed in the Reaction Setup tableabove from appropriate cold storage.
  - [ ] Thaw reagents on ice.
  - [ ] Prepare 1.5 mL microcentrifuge tubes, on ice, to assemble reactions into.

👉 **Tip:** Prepare the reaction on ice or a cold block to prevent protein expression from starting during assembly. This ensures the plate reader captures the complete fluorescence kinetics for deGFP expression.

  - [ ] For a given reaction, assemble by adding the volume of reagents from the table in the order listed. Pay special attention to the handling of the Cytosol components:
    - [ ] Vortex SMix: Ensure thorough mixing; 10s vortex / 10s rest on ice; should be transparent with no visible precipitate; and add to the reaction tubes.
    - [ ] Vortex or pipette mix tRNA, and add to the reaction tubes.
    - [ ] Vortex or pipette mix PMix, and add to the reaction tubes.
    - [ ] **Do NOT vortex** ribosomes: *gently* pipette mix or flick the tube, and add to the reaction tubes.
    - [ ] Add remaining reactions in the order they appear
  - [ ] Mix the master mix thoroughly by pipetting up and down 10–15 times until it appears homogeneous and clear.
  - [ ] Close lids on the microcentrifuge tubes and briefly spin down to eliminate bubbles.
  - [ ] Pipette out 10 µL of the reaction for osmolarity check using a Vapor Pressure Osmometer before starting encapsulation.

🚨 **Critical:** Adjust the outer solution concentration so its osmolarity is 100–120 units lower than the inner solution when measured on a Wescor EliTech Vapro 5600 Vapor Pressure Osmometer.

  - [ ] Hold assembled reactions on ice until ready for encapsulation.
- [ ] **Encapsulate Cytosols into Liposomes**
  - [ ] Set up a 1.5 mL tube rack with two 1.5 mL microcentrifuge tubes for each liposome encapsulation. Number the tubes according to the number of reactions assembled. Label the two tubes for each reaction:
    - [ ] **T**—transfer
    - [ ] **L**—liposomes
- [ ] Add 300 uL of the appropriate glucose outer solution to each of the tubes labelled **T**.
- [ ] Add 150 uL of the lipid-oil mixture (at room temperature) on top of each assembled Cytosol reaction.

☐ Emulsify the lipid-oil and Cytosol reaction by running the tube along a row of empty slots on the 1.5 mL tube rack. Run it down 20–30 times until the solution forms a stable emulsion with an even milky color.

> 👉 **Important:** While running the tubes on the tube rack, hold the cap firmly to prevent it from coming off during vigorous mixing.

☐ Immediately layer each emulsion over the outer solution. Slowly pipette the entire emulsion down the side of the corresponding **T** tube.

☐ Centrifuge **T** tubes at 9000 g for 10 min at room temperature to pellet the liposomes.

> 👉 **Tip:** Align the hinges of each **T** tube towards the outside of the centrifuge rotor, so that the final pellet location will be indicated by the tube hinge since the liposome pellet may not always be visible.

☐ Extract the liposomes from each **T** tube:

- ☐ Remove the oil layer and lipid debris from the top of each **T** tube by gently pipetting with a 1000 uL pipette set to 800 uL.
- ☐ Gently pipette mix the pellet 10-15 times with the outer solution.
- ☐ Extract liposomes by pipetting 50-100 uL of pellet and outer solution from **T** and transfer liposome sample to the respective liposome tube **L**.

> 🚨 **Critical:** Do not transfer the entire solution. It is important to avoid transferring the top of the solution which may contain a residual oil layer.

☐ Hold liposomes on ice until you are prepared to begin measurement.

☐ Pipette the liposomes into a well on a 384-well glass bottom plate. If the density appears too high under the microscope, dilute the liposomes with the outer solution for better data analysis.

☐ **Return reagents to their appropriate storage locations.**

- ☐ Add a black dot to the lid of each of Cytosol component. The number of dots indicates freeze–thaw cycles.

Notes

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Day 3: Integration

Overview

The goal of this experiment is to integrate the two energy modules 1) creatine kinase and creatine phosphate (CP/CK) and 2) polyphosphate kinase and polyphosphate (PPK/PolyP) into Cytosol. We will assess the productivity of the cytosol by varying the concentration of four key reactants: 1) magnesium acetate, 2) PPK, 3) PolyP, and 4) CP. The components have been prepared at the stock concentrations given in the table below:

Component	Stock Concentration
Magnesium acetate	200 mM
PolyP	500 mM
PPK	57 uM
CP	1000 mM

Protocol

- [ ] **Plan your experiment**
  - [ ] Work with your group to develop a strategy for maximizing productivity of the Cytosol containing integrated energy modules.
  - [ ] Fill in the table below with the details of the reactions you will prepare.

Component	Condition 1 [µL]	Condition 2 [µL]	Condition 3 [µL]	Positive Control [µL]	-DNA Negative Control [µL]
SMixΔCP	10.5	10.5	10.5	10.5	10.5
tRNA	3.5	3.5	3.5	3.5	3.5
PMix	4.2	4.2	4.2	4.2	4.2
Ribosomes	6.3	6.3	6.3	6.3	6.3
RNAse Inhibitor	1.75	1.75	1.75	1.75	1.75
pOpen-deGFP DNA template	0.85	0.85	0.85	0.85	0
Magnesium acetate					
PPK2					
PolyP					
CP				0.7	0.7
Water				7.2	8.05

Component	Condition 1 [µL]	Condition 2 [µL]	Condition 3 [µL]	Positive Control [µL]	-DNA Negative Control [µL]
Total master mix volume [µL]	35	35		35	35

- [ ] Fill out the provided CDK-compatible platemap template with the details of your group's experiment

- [ ] **Assemble Cytosol reactions**
  - [ ] Remove all components listed in the Reaction Setup table above from appropriate cold storage.
  - [ ] Thaw reagents on ice.
  - [ ] Prepare a PCR tube, on ice, to assemble reactions into.

> 👉 **Note:** Prepare the reaction on ice or a cold block to prevent protein expression from starting during assembly. This ensures the plate reader captures the complete fluorescence kinetics for deGFP expression.

  - [ ] For a given reaction, assemble by adding the volume of reagents from the table in the order listed. Pay special attention to the handling of the Cytosol components:
    - [ ] Vortex SMix: Ensure thorough mixing; 10s vortex / 10s rest on ice; should be transparent with no visible precipitate; and add to the reaction tubes.
    - [ ] Vortex or pipette mix tRNA, and add to the reaction tubes.
    - [ ] Vortex or pipette mix PMix, and add to the reaction tubes.
    - [ ] **Do NOT vortex** ribosomes: *gently* pipette mix or flick the tube, and add to the reaction tubes.
    - [ ] Add remaining reactions in the order they appear.
  - [ ] Mix the master mix thoroughly by pipetting up and down 10–15 times until it appears homogeneous and clear.
  - [ ] Close lids on the PCR tubes and briefly spin down to eliminate bubbles.
  - [ ] Hold assembled reactions on ice until ready for measurement.
  - [ ] Array 10 µL into three assigned wells of a black 384-well optical plate and take note of your plate map.

> 👉 **Tip:** Set the P20 pipette to 10.1 µL to draw the master mix, then dispense into the plate well by pushing the plunger to the first stop only—this prevents bubble generation in the reaction.

  - [ ] Measure deGFP fluorescence in the plate reader while incubating at 37°C.

- [ ] **Return reagents to their appropriate storage locations.**
  - [ ] Add a black dot to the lid of each of Cytosol component. The number of dots indicates freeze–thaw cycles.

Notes

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Appendix: Preparation of Lipids

Overview

This protocol describe the preparation of POPC:cholesterol lipid-in-oil mixtures used on Day 2. Lipids can also be prepared in advance and stored at 4°C for up to 48 hours.

Protocol

- [ ] **Prepare lipids in mineral oil**
  - [ ] Add 1 mL of mineral oil to the 1.8 mL glass vial using a 1 mL pipette.

> ⚠️ Work inside of a fume hood when handling chloroform and lipids

  - [ ] Add the lipids to the glass vial on top of the mineral oil using the appropriate glass syringe.

Component	Target Percentage (%)	Molecular Weight (g/mol)	Stock concentration (mg/mL)	Volume to add (uL)
POPC	70	760.076	25	162.17
Cholesterol	29.95	386.654	50	17.65
Liss-Rhod PE	0.05	1301.71	1	4.96

  - [ ] Briefly vortex the lipid-oil mixture for 5 seconds to mix.
  - [ ] Evaporate the chloroform from the lipid–oil mixture:
    - [ ] Place glass vial in a 55°C dry bath in a fume hood.
    - [ ] Shield with aluminum foil to protect from light.
    - [ ] Evaporate uncovered for 4 hours.
  - [ ] In a glass bottle, add 4 mL of chloroform using a glass 10 mL serological pipette.
  - [ ] Clean syringes by rinsing with chloroform 5 times into an empty glass bottle. Store the syringes with the plunger removed inside the fume hood for 3–4 hours to allow remaining chloroform to evaporate.
  - [ ] After 4 hours of incubation at 55°C in a dry bath in a fume hood, allow the lipid-oil mixture to cool to room temperature for 10-15 minutes.

> 💡 The lipid–oil mixture can be used immediately after cooling to room temperature or stored at 4°C for up to one week. Protect from light by storing in an opaque container or wrapping the vial with aluminum foil.

  - [ ] Return the plungers to the syringes and store them in their designated location.
  - [ ] Dispose of chloroform waste following applicable chemical safety guidelines.

Appendix: Stock Concentrations

Overview

This section contains details about the stock solutions for all components used during this workshop.

Base Cytosol

Component	Stock Concentration	Unit	Final concentration	Unit	Volume for master mix [µL]
SMix	3.33	×	1	×	10.5
tRNA	35	mg/ml	3.5	mg/ml	3.5
PMix	15	mg/mL	1.80	mg/mL	4.2
Ribosomes	10	µM	1.8	µM	6.3
pOpen-deGFP DNA template	124	nM	3	nM	0.85
RNAse Inhibitor	40000	U/mL	2000	U/mL	1.75
Water					6.5
Total master mix volume [µL]					35

Additional Supplements

Component	Stock Concentration
Magnesium acetate	200 mM
PolyP	500 mM
PPK	57 uM
CP	1000 mM
Optiprep	1.32 mg/uL

Lipid-in-Oil Mixtures

Component	Target Percentage (%)	Molecular Weight (g/mol)	Stock concentration (mg/mL)	Volume to add (uL)
POPC	70	760.076	25	162.17
Cholesterol	29.95	386.654	50	17.65
Liss-Rhod PE	0.05	1301.71	1	4.96

Reference Standard

Component	Concentration
NIST-traceable Fluorescein	1 uM

Appendix: Bill of Materials

Overview

This section contains product information for all materials used during this workshop.

Materials Introduced on Day 1

Name	Product	Manufacturer	Storage Conditions
Energy Solution	SMix	b.next	-85C to -75C
Cytosol Protein Mix	PMix	b.next	-85C to -75C
E. coli Ribosomes	Ribosomes	b.next	-85C to -75C
E. coli tRNAs	tRNAs	b.next	-85C to -75C
Magnesium acetate	Magnesium acetate	Sigma-Aldrich (M5661)	-85C to -15C
DNA template	pOpen-deGFP	b.next	-85C to -15C
Polyphosphate (polyP)	Polyphosphate, Medium Chain (p100)	Kerafast (EUI005)	-85C to -15C
polyphosphate kinase (PPK) enzyme	PPK2	b.next	-85C to -75C
RNAse Inhibitor, Murine	RNAse Inhibitor, Murine	NEB (M0314S)	-85C to -15C
Nuclease-free water	Nuclease-free water	ThermoFisher Scientific (AM9916)	4C to 30C
PCR tubes	Thin-walled, RNase-free PCR tubes	ThermoFisher Scientific (AM12225)	4C to 30C
Optical Adhesive Film	MicroAmp Optical Adhesive Film	ThermoFisher Scientific (4311971)	15C to 30C
384-well plate	384 SV NoBind	Greiner Bio-One (784900)	15C to 30C
Fluorescein	Fluorescein - NIST-Traceable Standard.	Invitrogen (F36915)	4C

Materials Introduced on Day 2

Name	Product	Manufacturer	Storage
Glucose	D-(+)-Glucose, 99.5%	Sigma-Aldrich (G8270-1KG)	Room temperature
POPC	16:0-18:1 PC (POPC)	Avanti Research (A80557)	-20C
Liss-Rhod-PE	18:1 Liss Rhod PE	Avanti Research (A81150)	-20C

Name	Product	Manufacturer	Storage
Cholesterol	Cholesterol (plant)	Avanti Research (A80100)	-20C
Mineral Oil	Mineral oil, pure	Thermo Scientific (415080010)	Room temperature
Glass Syringe	Glass Syringe 250 uL and 50 uL	Hamilton (14-815-238, 14-815-216)	Room temperature
Glass vials	1.8 mL glass vials	Fisher Scientific (03-339-22A)	Room temperature
Chloroform	Chloroform, 99+%	Thermo Scientific (158210010)	Flammable storage cabinet - Room temperature
Glass serological pipette	10 mL glass serological pipettes	Corning (7077-10N)	Room temperature
384-well glass bottom plates	384-well glass bottom plates	Cellvis (P384-1.5H-N)	Room temperature
Osmometer	Vapor Pressure Osmometer	Wescor EliTech Vapro 5600 Vapor Pressure Osmometer	Room temperature
Optiprep	Optiprep	Serumwerk bernburg (1893)	Room temperature
Polyphosphate kinase (PPK) enzyme	PPK2	b.next	-85 to -75C
Polyphosphate (polyP)	Polyphosphate, Medium Chain (p100)	Kerafast (EUI005)	-85C to -15C

Materials Introduced on Day 3

Name	Product	Manufacturer	Storage
Creatine Phosphate	Sodium creatine phosphate dibasic tetrahydrate	Sigma-Aldrich (27920)	4C