Version Number:AS&M1001

Portal.Bio Products

PORTAL

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Next Gen Cell Engineering and Analytics

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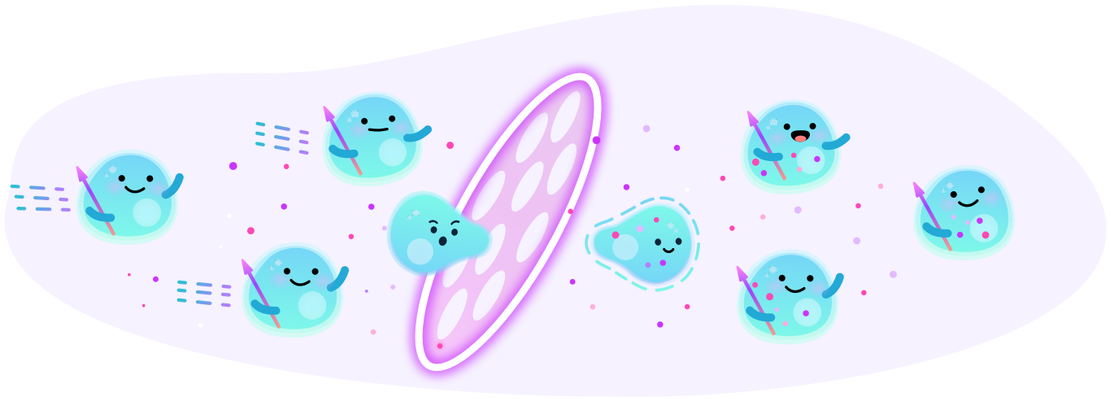
PORTAL

Contents

Technology Overview

Mechanical Delivery with Proprietary Portal Technology



Sharei, A, et al. PNAS 2013
Stewart and Sharei et al, Nature 2016

Proprietary membrane technology

Deliver >over 1B cells/min

Flexible buffer and prep requirements

Single use booster consumables

Delivery materials

- mRNA, siRNA, saRNA
- Proteins & Peptides
- CRISPR RNPs
- Small Molecules
- Polymers
- Nanoparticles
- Antibodies
- Virus

Validated cell types

- PBMCs
- T cells
- B cells
- NK cells
- iPSCs
- Monocytes
- MSCs
- HSCs

Cells exposed to **electroporation** misregulate gene expression

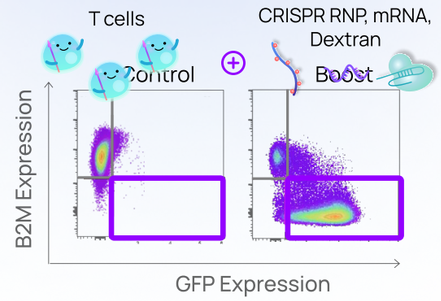
T cells: misregulated genes (>2-fold change, p<.05)

Cells exposed to **mechanoporation** are more similar to controls

T cells: misregulated genes (>2-fold change, p<.05)

DiTommaso, T et al. 2018. PNAS. PMID: 30381459.

Multiplexed delivery enables simultaneous delivery of 3 cargoes to primary human T cells



*Triple positive delivery efficiency of 85%. Dextran data not shown

- Portal's technology delivers single or multiplexed cargos (e.g. RNA, siRNA, RNP editing complexes, peptides/proteins, antibodies, small-molecules, DNA-encoded libraries (DELs) and fluorescent tracers) directly to the cytosol
- Technology demonstrated in primary immune cells (T, NK, and B cells, mixed PBMCs), stem cells (CD34+ HSCs, iPSCs, MSCs), and cell lines (HeLa, HEK-294)
- Utilizes a simple workflow applicable to high throughput screening, preclinical studies, and clinical scale-up for cell therapies

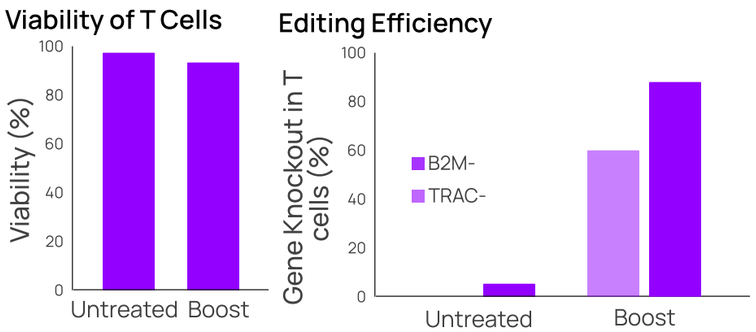
PORTAL

Case Studies: Cell Engineering

Delivery into Immune Cells

Multiplexed CRISPR Knock Out in Unstimulated T Cells

Unstimulated T cells were isolated from PBMCs and mechanoporated with a multiplexed cargo containing CRISPR RNPs targeting T Cell Receptor Alpha Constant (TRAC) and B2 microglobulin (B2M). T cells were activated and expanded for 4 days and expression of TRAC and B2M protein were assessed by flow cytometry. Viability is consistent between the untreated control and the boosted sample. The % gene knockout in T cells is >60% for B2M- and TRAC-.

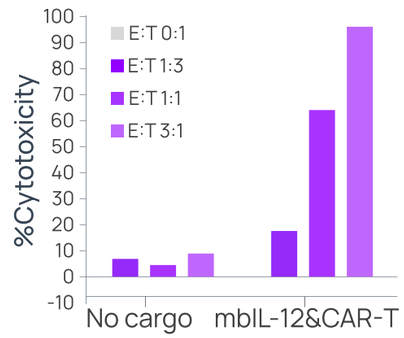


Activated T cells Engineered with Simultaneous circRNA Delivery for IL-12 and CD19 CAR

Human Pan-T cells were isolated and activated with CD3/CD28 Dynabeads for 48h and T cells were cultured an additional 4 days. Activated T cells were mechanoporated with CD19 CAR circRNA and membrane bound IL-12 (mbIL-12) circRNA. Boosted cells were rested for 2h before co-culturing with Raji cells at different ratios of effector cells (E) to target cells (T).

Flow cytometry was conducted 48h post-boost to assess expression of CD19 CAR and mbIL-12 (left) and cytotoxicity (right). Cytotoxicity was measured by quantifying reduction in CD19+ Raji cells relative to counting beads (bottom).

Cytotoxicity of mbIL-12 and CAR circRNA Boosted T cells

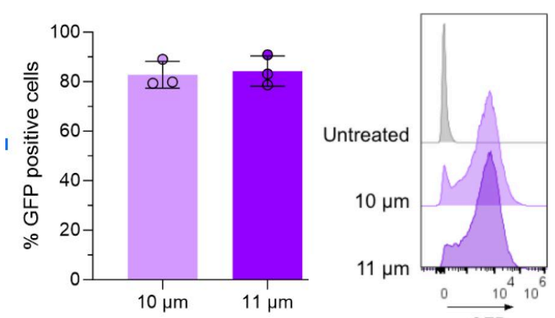


Stem Cell Delivery

Efficient mRNA Expression in Human MSCs

Mesenchymal stem cells (MSCs) were mechanoporated using the Gateway with GFP mRNA. After 20h, GFP expression in MSCs was assessed using flow cytometry and cell morphology was assessed by microscopy (right). Size (microns) refers to pore size used for mechanoporation (left).

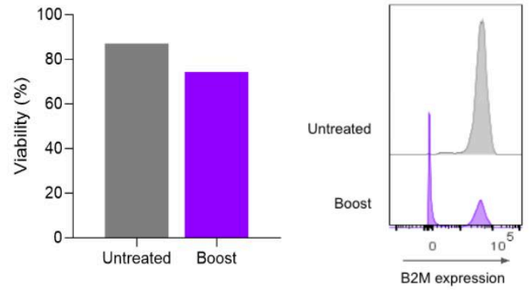
Delivery of GFP mRNA to MSCs



Hematopoietic Stem Cell (HSC) Boost with siRNA

show a high knockdown efficiency. β2 microglobulin (B2M) siRNA was mechanoporated using the Gateway into human CD34+ hematopoietic stem cells. Flow cytometry was conducted after 40 hours to assess viability and B2M expression. Viability of the CD34+ HSCs was maintained >70% (left) with a % B2M knock down of >50% (middle).

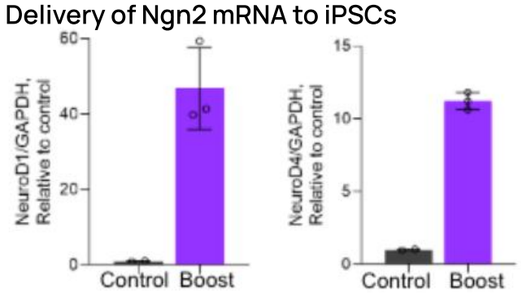
Viability of CD34+ HSCs



iPSCs Express Early Neuronal Markers 12hrs after Boosting with Ngn2 mRNA

Human iPSCs boosted with Ngn2 mRNA show expression of Ngn2 and downstream neuronal transcription factors. The induced pluripotent stem cells (iPSCs) were mechanoporated using the Gateway with neurogenin 2 (Ngn2) mRNA and dextran. Dextran delivery was assessed immediately post-boost by flow cytometry (left). Cells were harvested and total RNA was extracted 12h post boost. RT-qPCR was performed to assess expression levels of Ngn2 and neuronal transcription factors NeuroD1 and NeuroD4 (right images).

Delivery of Ngn2 mRNA to iPSCs

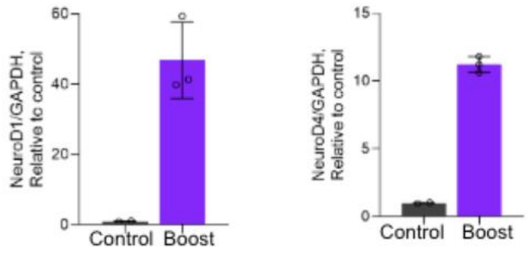


Case Studies: Cell Analytics

Peptide and DEL

Robust DNA Encoded Library Delivery in iPSCs & HeLa Cells

iPSCs (top) and HeLa's (bottom) were boosted with a fluorescently-tagged DNA encoded library (DEL) probe. Strong delivery efficiency was shown when measured using flow cytometry. Induced pluripotent stem cells (iPSCs) or HeLa cells were mechanoporated with a fluorescently-tagged DNA encoded library (DEL) probe. Delivery efficiency was measured using flow cytometry to detect the fluorescent tag immediately after boosting. Both cell types showed a >80% % probe + live cells.



Macrocycle Delivery to HeLa Cells Shows Robust Dose Response

When boosted with a FITC labelled peptide macrocycle HEK & HeLa cells showed a robust dose response inside the cell as demonstrated by the right shift of the histogram (Fig 1). The experiment was executed in a 96 well plate, supporting high throughput screening. Flow cytometry and fluorescence microscopy was conducted post-boost to assess viability and efficiency of macrocycle delivery into boosted cells (fig 2).

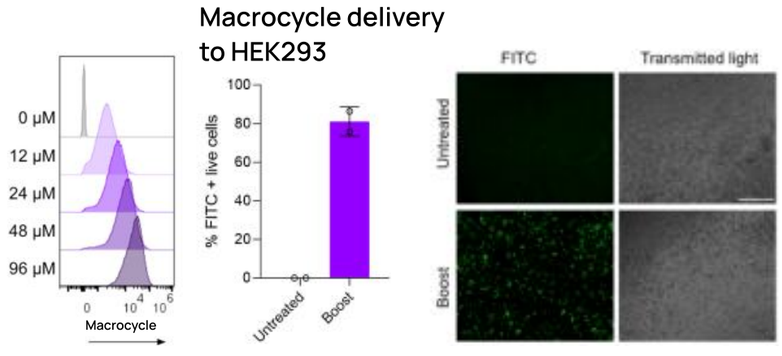
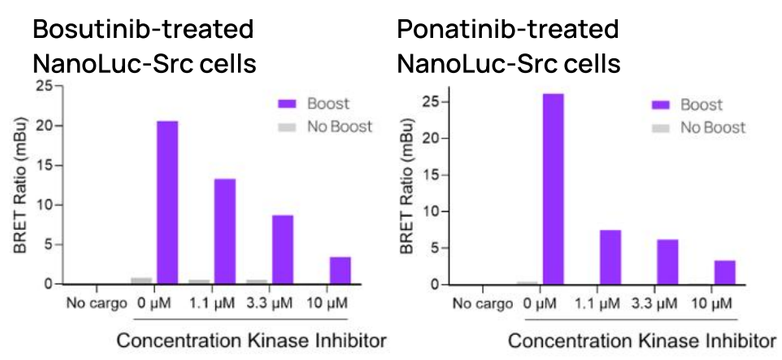


Fig 1: HeLa cells were boosted then manually added into wells of a 96-well plate pre-loaded with FITC-labeled macrocycle at 0-0.2 mg/ml. Flow cytometry was conducted post-boost to assess viability (A) and efficiency of macrocycle delivery into boosted cells (B).2: HEK293 cells were mechanoporated with a FITC-labeled macrocycle. Viability and macrocycle delivery to the untreated and boosted samples were measured by flow cytometry (A) and fluorescence microscopy (B). Scale bar = 300 µm.

Probe Delivery

Intracellular Target Engagement Assays with Impermeable Molecules

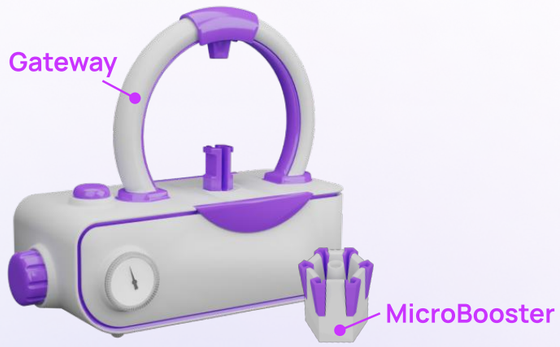
HeLa cells were transfected with SRC-NanoLuc Fusion vector then mechanoporated with varying amounts of impermeable kinase tracer. NanoGlo substrate was added and BRET (Bioluminescence resonance energy transfer) was detected on a plate reader.



HeLa cells transfected with SRC-NanoLuc Fusion vector were incubated for 2 hours with Src kinase inhibitors (Ponatinib or Bosutinib) then mechanoporated with an impermeable kinase tracer. NanoGlo substrate was added and BRET was detected on a plate reader.

Products

Gateway + MicroBooster



Gateway

R&D scale instrument for use across non-clinical applications.The Gateway is used with Booster cartridges containing a proprietary membrane optimized for delivery and cell viability that is compatible across cell concentrations and buffers.

WEIGHT	8lbs / 3.62 Kg
DIMENSIONS (HWD)	10.25" x 11.25" x 6.5" (260mm x 285mm x 165mm)
VOLUME RANGE	50-200ul
CELL NUMBER RANGE	0.5-10M/ml
TYPE	Research
COMPATIBLE WITH EQUIPMENT FROM	N/A. Standalone instrument

MicroBooster

R&D scale consumables containing Portal's proprietary silicon cores for use in cell engineering experiments **with the Gateway**. Available with pore sizes from 4-14um.

WEIGHT	24g
DIMENSIONS (HWD)	2.2" x 1.65" x 1.65" (56mm x 42mm x 42mm)
VOLUME RANGE	50-200ul
CELL NUMBER RANGE	0.5-10M/ml
TYPE	Research
COMPATIBLE WITH EQUIPMENT FROM	Gateway

Galaxy

Available For In-Line Integration

High-throughput scale instrument and consumables for screening applications with multi-well plates. The Galaxy is available as a standalone unit or as an in-line consumable integration with existing robotics.

WEIGHT	5lbs / 2.27 Kg
DIMENSIONS (HWD)	4.25" x 5" x 10.25" (110mm x 127mm x 260mm)
VOLUME RANGE	2-50ml
CELL NUMBER RANGE	1-100M/ml
TYPE	Research
COMPATIBLE WITH EQUIPMENT FROM	Eppendorf, Hamilton, Tecan, GNF, Combi, Certus Flex, PD2, and more

Millibooster

Clinical-scale GMP cartridge for engineering immune cells and stem cells. The Millibooster cartridge can be attached to existing equipment via tube-weld or luer lock connection. This setup is capable of treating >1 Billion cells/minute.

WEIGHT	118g
DIMENSIONS (HWD)	7.55" x 3.00" x 2.60" (192mm x 76mm x 66mm)
VOLUME RANGE	2-100ml
CELL NUMBER RANGE	1-100M/ml
TYPE	Research and Clinical GMP
COMPATIBLE WITH EQUIPMENT FROM	Fresenius Kabi, Thermo Fisher, Cytiva, Miltenyi

Gateway

Don’t Transfect. Just Deliver!

Multi-cargo. High viability. Better delivery.

Boost Cells with the Push of a Button

Research Scale Mechanical Delivery

Tiny Footprint

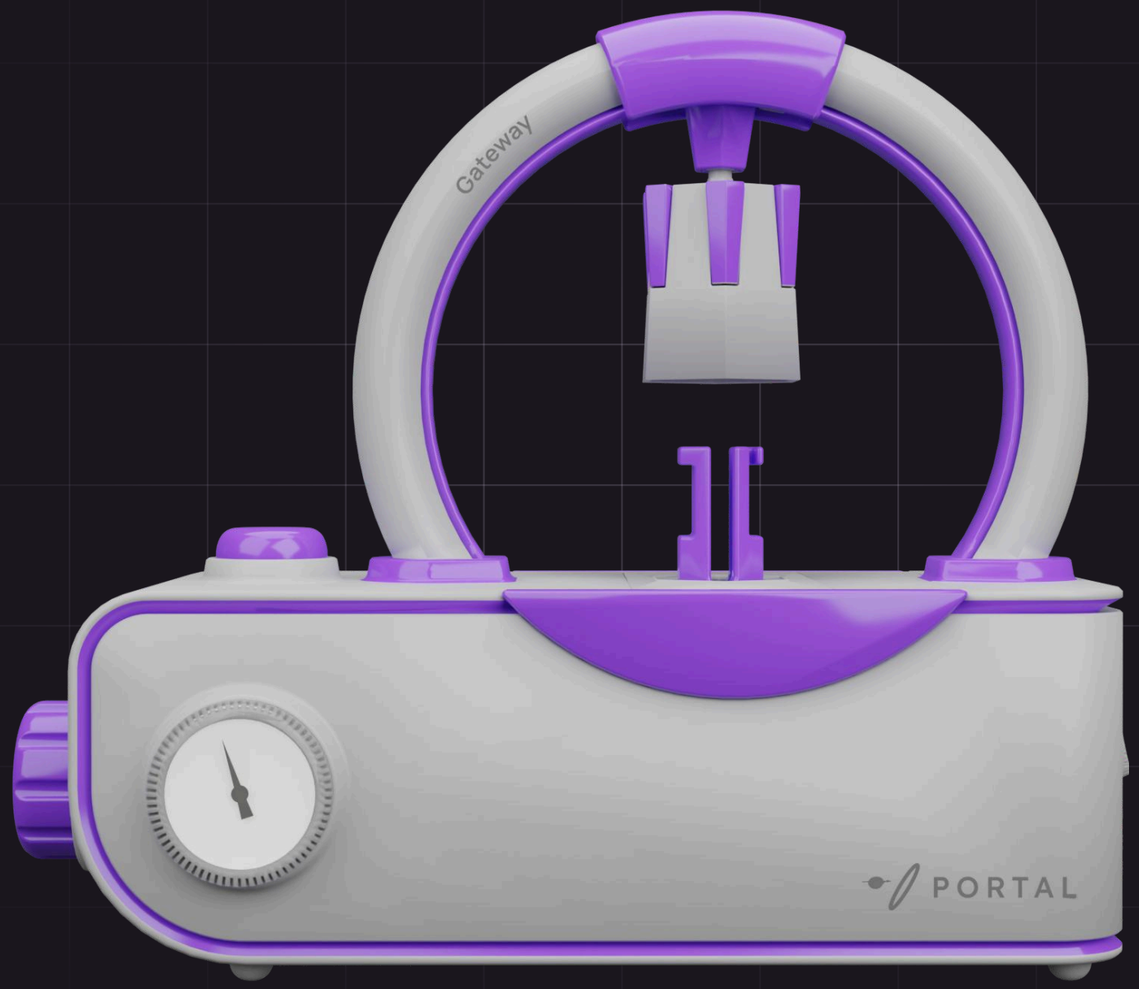
Fits in biohood with room to spare

Easy to Use

Deliver with literally the push of a button

Broad Cell & Cargo Compatibility

Excels with sensitive cell types ad traditionally hard to deliver cargoes



Galaxy

Explore New Worlds

High-throughput boosting. Compatible with manual workflows as an in-line integration and liquid handlers.

INSTRUMENT FOR LIQUID HANDLER

IN LINE INTEGRATION

Screen Multiple Cargoes at Once

High-throughput mechanical delivery

Cargo Flexibility

Screen small molecules, DELs, peptides and oligos

Easy Workflow Integration

Compatible with both manual workflows and liquid handlers

Cell and Buffer Flexibility

Compatible across cell lines, primary immune and stemcells

MicroBooster

Boosters

Optimized delivery for immune and stem cells.
More cells and larger volumes coming soon!

Proprietary Membrane

Optimized for Delivery and Cell Viability

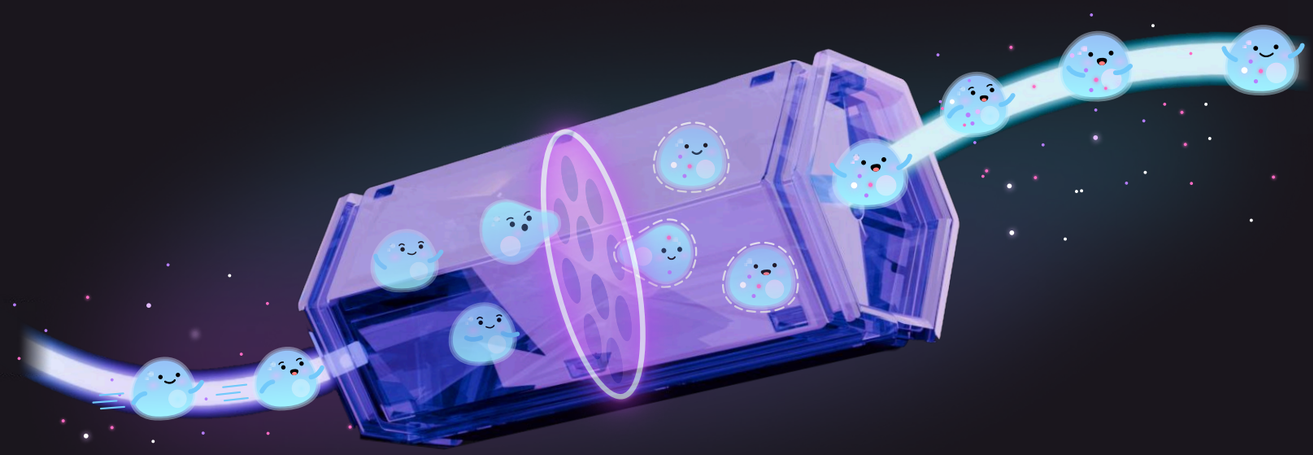
Each MicroBooster Delivers 300 uL, 15M Cells

Delivery Materials		Validated Cell Types	
mRNA, siRNA, saRNA	Polymers	PBMCs	T cells
Proteins & Peptides	Nanoparticles	B cells	NK cells
CRISPR RNPs	Antibodies	iPSCs	Monocytes
Small Molecules	Virus	MSCs	HSCs

MilliBooster

Unleashing Cell Therapy's Clinical Potential

Portal's MilliBooster integrates
with most manufacturing equipment



Delivery to

>1B cells / min

COMPATIBLE WITH

FRESENIUS KABI

Thermo SCIENTIFIC

Over 90% mRNA Expression at Scale

Delivery Materials

- mRNA, siRNA, saRNA
- Proteins & Peptides
- CRISPR RNPs
- Small Molecules
- Polymers
- Nanoparticles
- Antibodies
- Virus

Validated Cell Types

- PBMCs
- T cells
- B cells
- NK cells
- iPSCs
- Monocytes
- RBCs
- HSCs

Setup & Operating Instructions

Operating instructions

System components

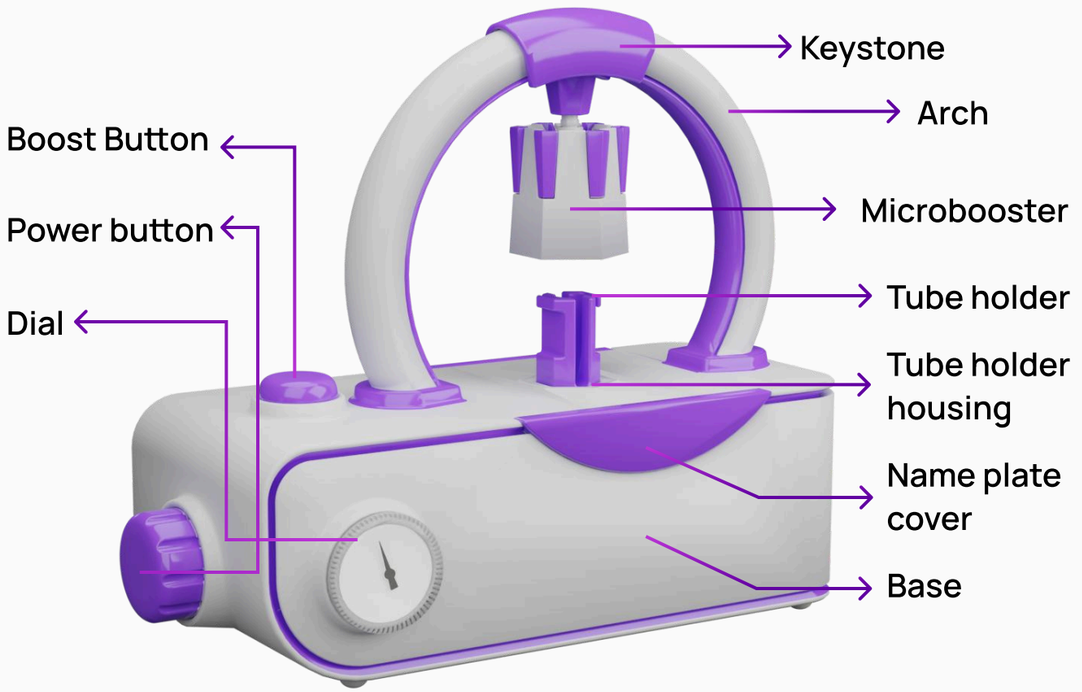


Figure 1: The Gateway. R&D scale instrument for use across non-clinical applications. The Gateway unlocks many novel applications across cell engineering and drug screening. The system is compact and designed to be used in a biosafety hood

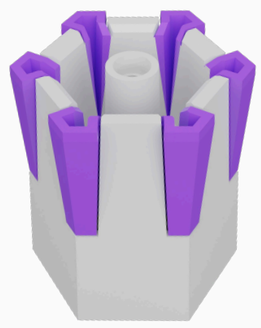


Figure 2: Microbooster. R&D scale consumables containing Portal's proprietary silicon cores for use in cell engineering experiments with the Gateway

Set up instructions

1. Carefully open the shipping box.
2. Remove the top layer of foam to expose the Gateway device and power cord.
3. Gently lift the Gateway and charger from the packaging, holding the Gateway securely at the base.
4. Inspect all components for any visible damage or missing parts. If any issues are found, contact support before proceeding.

Turning on the Gateway

1. Place the Gateway on a lab bench or inside a Biosafety Cabinet, depending on your workspace requirements.
2. To transport the device, hold the Gateway by the arch with one hand and support the base with the other
3. Plug the power cord into a standard electrical outlet.
4. Connect the other end of the power cord to the power inlet port located on the side of the Gateway.
5. Flip the power switch to the ON position.
    a. You will hear the internal pump activate as the device begins to pressurize.
    b. The Boost button will illuminate once the internal pressure reaches the pressure set on the gauge.

Handling instructions

1. Set System Pressure
    a. Turn the pressure adjustment dial located on the side of the Gateway to set the desired pressure.
    b. Monitor the gauge on the front of the device to confirm the correct pressure is reached. Allow the system to fully pressurize.
2. Prepare the Collection Area
    a. Place an open 1.5 mL collection tube onto the tube holder.
    b. Insert the tube's cap into the cap holder on the side of the tube holder. Avoid touching the interior of the cap to maintain sterility.
3. Prepare the Microbooster
    a. Verify that the Microbooster has the correct pore size for your application.
    b. Carefully open the Microbooster packaging by peeling apart the

paper and plastic from the top.
   c. Confirm the correct orientation, then gently screw the Microbooster onto the luer lock connection on the keystone.
   d. Ensure the Microbooster is secure but do not overtighten.
4. Position the Tube Holder
   a. Pull the tube holder upward until it is fully raised and the collection tube is positioned directly beneath the Microbooster's output nozzle.
5. Initiate Boosting
   a. Confirm that the gauge is still set to the desired pressure. Adjust the dial if necessary.
   b. Once the Boost button is illuminated, press and hold it to begin the boosting process.
   c. Continue holding the button until all components have passed through the Microbooster and into the 1.5 mL collection tube.
6. Complete the Process
   a. Gently press the tube holder down to return it to its original position.
   b. Carefully unscrew the Microbooster in a counterclockwise direction to remove it from the Gateway.
   c. Remove the cap from the cap holder and securely place it on the collection tube.
   d. Remove the sealed 1.5 mL tube from the holder.
7. Power Off
   a. Flip the power switch to the OFF position to shut down the device.

Experiment Quick Start Guide

Critical Boosting Parameters

Cell Health
Happy cells yield better viability and delivery performance

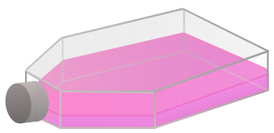
Cargo Concentration
Boosting is diffusion-based. Higher cargo concentration = higher delivery

Cell Speed + Pore Size
Smaller pore sizes and faster speeds drive cargo delivery

Cell Recovery
Membranes reseal in 30 seconds. Transfer to media and incubator

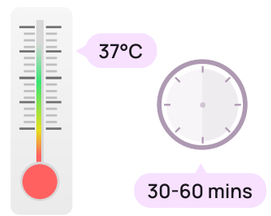
Quick Start Guide

1



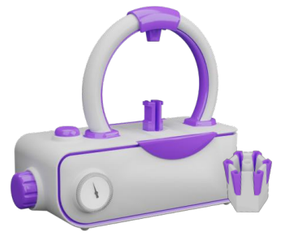
Source fresh or frozen cells and prepare in culture as necessary for specific cell type.

2



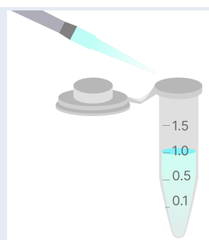
Rest any cells thawed the same day as boosting in minimal media (R10 recommended) at 37°C for 30-60 minutes.

3



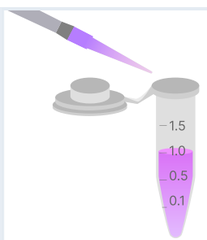
Prepare the Gateway and MicroBoosters in a Biosafety cabinet.

4



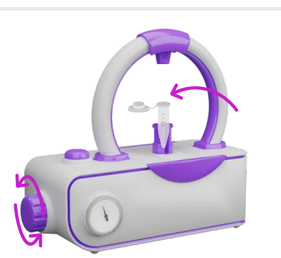
Prepare cargo mixture in delivery buffer at 2X concentration.

5



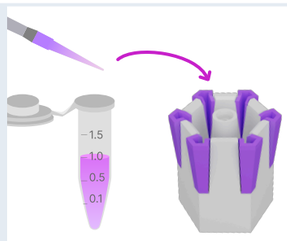
Prepare cells in a single cell suspension at 2X concentration in delivery buffer.

6



Set pressure on the Gateway and load collection tube.

7

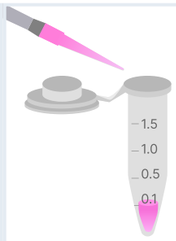


Mix cells and cargo (1:1, total volume 50-200 µl) and pipette into the top of the MicroBooster.

8

Attach MicroBooster to the top of the Gateway and press the purple button to Boost.

9



Wait at least 30 seconds, then add complete media to a collection tube.

10

Use cells immediately, or culture at appropriate seeding density for downstream applications.

Operating Parameters

Suggested Parameters

Booster Specifications

Parameter	Minimum	Maximum	Recommended
Volume	50 µl	200 µl	100 µl
Pressure	3 psi	15 psi	4-12 psi
Cell concentration	1 x 10^6 cells/mL	1 x 10^8 cells/mL	1-5 x 10^7 cells/ mL
Cargo volume			≤ 10% total volume[1]
Sample number per MicroBooster cartridge[2]	1	10	5

[1] It is not recommended to dilute the delivery buffer with the cargo buffer at a rate higher than 10% of the reaction volume, as this may alter delivery performance. If the cargo volume must exceed 10%, please adjust accordingly.
[2] Cell concentration will impact maximum sample number. Booster cartridge capacity correlates with total number of cells passed through, so samples at higher concentrations will allow fewer samples through the Booster compared to samples at lower cell concentrations which will allow for more samples to pass through the Booster.

Recommended Booster Conditions by Cell Type

Cell Type	Pore size (µm)	Recommended pressure
Human PBMCs	5- 6.5 µm	7-10 psi
Human Unstimulated T cells	4-6 µm	7-12 psi
Human Activated T cells	6 -10 µm	7-12 psi
Human NK cells	5-6.5 µm	7-12 psi
Human Monocytes	6-7 µm	7-12 psi
Human iPSCs	10-11 µm	3-6 psi
Human MSCs	10-12 µm	10-12 psi
HEK293[3]	10-12 µm	8-12 psi
HeLa[3]	9-12 µm	8-11 psi
Human B Cells	4-6 µm	9-11 psi
Dendritic Cells	4.5-6.5 µm	4-6 psi
Macrophages	11-13 µm	6-10 psi
Fibroblasts	9-12 µm	8-10 psi
Jurkat	7-9 µm	9-11 psi
A549	10-11 µm	9-11 psi
CHO	9-10 µm	9-11 psi
RPE1	9-11 µm	9-11 psi
K562	9-11 µm	6-9 psi
THP-1	9-12 µm	6-11 psi
Raji Cells	6.5-8.5 µm	8-10 psi

[3] Many human epithelial lines work well in the 100 - 12 um pore size range, but Portal recommends optimizing conditions for your specific line with several pore sizes.

Recommended Cargo Concentration[4]

Cargo type	Recommended final concentration (1X)
Dextran	0.05-0.2 mg/mL
mRNA	0.05-0.2 mg/mL
siRNA	5-15 µM
Peptides	5-10 µM
CRISPR RNP	Nuclease: 0.2 mg/mL, complex with guide at a 2.5:1 guide:nuclease molar ratio
cRNA	0.05-0.2 mg/mL
LgBit	Approximately 1:10 ratio

[4]This list represents some commonly delivered cargo. For delivery of unlisted cargo types, Portal recommends 0.1 mg/ml as a starting concentration.

General Guidelines

Required Materials Not Supplied By Portal[6]

Material	Portal's preferred product[7]
Delivery buffer	Opti-MEM[8] (Gibco 31985062) as a general buffer; Also acceptable: PBS, DMEM, RPMI, X-Vivo.
Osmolarity adjustment buffer[9]	10X PBS or 5M NaCl
Delivery tracer	Fluorescent dextran[10] (Thermo, various)
Tubes	1.5 mL, 15 mL, 50 mL
Cultureware	Culture plates or flasks in appropriate size
Cell strainer	40 µm mesh strainer (VWR 89508-342)
Cell detachment solution	Accutase (Thermo A1110501)
Media[11] Complete culturing media	Varies by cell type; please refer to individual cell type protocols for more information

[6] This is a generalized list intended for use as a quick reference or for use when optimizing new cell types. Please refer to individualized protocols by cell type for more specific information.
[7] The specifically identified reagents are used and recommended by Portal. Other reagents can be used, but the protocol should be optimized to ensure high performance.
[8] Portal recommends Opti-MEM as a buffer compatible with all types of cargo. The boost process is compatible with other minimal media, serum-free complete media, or PBS. Buffer choice may impact delivery and viability. When using an RNA-based cargo, ensure the media formulation is serum and RNase-free. It is not recommended to use complete media containing serum as a delivery buffer.
[9] Optional, as needed. Portal recommends not to dilute the delivery buffer with an aqueous cargo buffer at a rate higher than 10% of the reaction volume, as this may alter delivery performance. Instructions for adjustment can be found in Appendix 3.
[10] Portal recommends co-delivering dextran in all samples as a delivery tracer. Portal's dextran of choice is 3 kDa Cascade Blue (Thermo Fisher D7132). Portal reconstitutes dextran at 5 mg/ml in DNase/RNase free water or PBS and stores this stock at -20°C.
[11] This media formulation is used and recommended by Portal, but other complete media formulations are suitable.

Required Equipment

Equipment	Note
Cell counter	Should provide cell diameter
Mini centrifuge	For spinning 1.5 mL tubes
Benchtop centrifuge	For spinning 15 and 50 mL conical tubes
Incubator	37°C & 5% CO_2
Biosafety Cabinet	

Optional Equipment for Downstream Readouts

Equipment	Note
Flow cytometer	To analyze cargo delivery and/or cell viability
Fluorescence microscope	To analyze cargo delivery

Example Experiment Guidelines

1.1 Pre-experiment Cell Preparation

1. Prepare cells in a clean, single cell suspension and resuspend at desired concentration in the delivery buffer.
   - Recommended general range for cell concentration is 1 x 10^7 cells/mL - 5 x 10^7 cells/mL; please check specific cell recommendations.
   - Negative selection kits are preferred when isolating a pure population from PBMCs.
   - Adherent cells must first be lifted off the plate into a single cell suspension prior to cell boost.
   - Adherent cells should be passaged at least 36 hours before boosting and should be around 70-80% confluence when harvested.
   - Adherent cells from thaw should be passaged at least twice prior to cell boost.
2. Cell-specific preparations:
   - For primary suspension cells: following the isolation/preparation protocol, rest in culture media for 30-60 minutes[12] in a 37°C incubator with 5% CO_2 prior to cell boost to improve cell health.
   - For adherent cell-types: Dissociate cells into single cell suspension and boost soon after dissociation. Passing cells through a 40 µm mesh strainer may help with dissociating clumps prior to boost.

[12] Rest times greater than 60 minutes are suitable, though an overnight rest is not recommended.

1.2 Gateway and Booster Cartridge Preparation

1. Place the Gateway system into a Biosafety cabinet (BSC) after spraying with 70% ethanol, plug in the instrument, and turn the switch to the “ON” position. The purple “Boost” button will illuminate when the instrument is ready.
2. Spray the MicroBooster packaging with 70% ethanol and open the packaging in the BSC.
   - If using metal holders, please refer to Appendix 2 for instructions.

1.3 Cargo Preparation

1. Ensure cargo is at an appropriate temperature (e.g., dextran can be kept at room temperature, while RNA should be maintained on ice until ready to boost).
   - Ensure the temperature of the cells does not drop when using cargo on ice.
   - Cells and cargo may be prepared simultaneously in order to maintain a time-efficient workflow, however, the experimental flow should ensure that cells are handled out of the incubator for as short a time as possible to maintain cell health (<30 minutes).
2. Prepare a cargo master mix in the delivery buffer to be added to a cell master mix to make 1X final for both cells and cargo when combined. Note: we recommend a 1:1 V:V mixture of 2X concentration of cargo to be mixed with 2X concentration of cells to make a final concentration of 1X; however, other master mix ratios can be used (e.g. 1:100) as long as once cargo and cells are mixed the final desired 1X concentration is achieved for both cells and cargo. Other key considerations when planning a cargo sample include:
   - Wait to add the cargo to cells until immediately prior to boosting.
   - For samples where the cargo comprises >10% the total reaction volume, the osmotic concentration should be rebalanced by using a 10X PBS or 5M NaCl solution as described in Appendix 3.
   - Include an extra cargo reaction for a “no boost” control.
   - When preparing the same cargo master mix for multiple samples, make at least 10% more to allow overage.

1.4 Cell Preparation

1. Ensure the delivery buffer is warmed to room temperature[13].
2. Collect cells from the incubator and transfer into a conical tube.
   - If a high degree of cell aggregation is suspected, cells may be passed through a 40 µm mesh strainer to remove large aggregate or debris.
3. Count cells with a cell counting device and note cell diameter.
4. Centrifuge to collect (specific spin speeds and times can be found in individualized protocols).

5. Resuspend cells in the delivery buffer at the desired master mix concentration for a final 1X concentration within the recommended range in Table 3.2.

[13] Warming delivery buffer to 37°C may improve viability performance.

1.5 Cell Boost

1. Ensure complete media is warmed to 37°C.
2. Prepare 1.5 mL tubes for collection of each sample after it has passed through the Booster cartridge. Prepare additional tubes for mixing samples prior to passing through the Booster cartridge and collecting wash buffer between samples.
3. Prepare an **untreated** control sample by mixing the cell master mix with an appropriate volume of delivery buffer to bring cells to 1X concentration in a 1.5 mL tube.
4. Prepare a **"no boost" control** sample by mixing the cell master mix with the cargo master mix in a 1.5 mL tube.
5. Set the pressure on the Gateway system to the desired boost pressure[14]. Allow the gauge to settle (<1 min). Adjust the pressure if necessary.
6. Follow the below steps for each sample.
   a. Check to ensure the desired pressure is reflected on the Gateway system and adjust pressure if necessary. The purple indicator light will illuminate when the system is pressurized and ready.
   b. Place a 1.5 mL collection tube in the holder on the Gateway system and secure the cap by placing it on the side of the holder, using caution not to contaminate the inside of the lid.
   c. Mix the cell master mix with the cargo master mix (or an appropriate volume of delivery buffer for a "no cargo" control, if desired). For optimal performance, only mix cells and cargo immediately prior[15] to loading the Booster cartridge.
   d. Transfer the cells + cargo solution into the top of the Booster cartridge.
   e. Screw the Booster cartridge onto the Gateway system until secure, ensuring that it is not overtightened. Lift the collection tube into place below the Booster cartridge.
   f. Press the illuminated button to pass the sample through the Booster cartridge and hold the button until the entire solution has come out of the Booster cartridge.
      - If the indicator light turns off, wait for it to illuminate before you can boost.
      - If the indicator light turns off before the entire sample has passed through the Booster, wait for it to re-illuminate, then press the illuminated button to allow the remaining sample to pass through the Booster.
   g. Remove the Booster cartridge from the Gateway system by unscrewing and remove the collection tube from the holder.
      - Handle the collection tube gently at this time; do not tap or spin the collection tube.
   h. Carefully add complete media to the 1.5 mL tube. Depending on the desired downstream application, add a minimum of 1:1 V:V and up to 1.5 ml final volume.
      - If any of the sample has splashed up onto the sides of the tube, complete media can be washed down the side of the tube when pipetting to collect all boosted material. A quick spin should not be performed at this step.
7. Optional: wash the Booster cartridge with 100 µL of delivery buffer if desired. Collect wash in a fresh 1.5 mL tube to discard.
8. Repeat steps 6-7 for each sample.
   a. The same Booster cartridge can be used for multiple samples in succession, provided that the cell type is the same.
   b. Portal recommends washing twice in between samples with different cargo.
   c. Portal recommends using a new Booster cartridge after 5 samples, although variables including cell concentration may impact the number of samples that can be passed through the Booster.
9. When all samples have been collected, transfer all samples to an appropriately sized tissue culture plate to rest for 1-2 hrs (non-adherent cell types only- for adherent cells, skip this step).
10. Move to cell processing for downstream applications and/or analysis. This may include but is not limited to preparing cells for culturing, flow cytometry analysis, or microscopy.
    a. For same day readout, transfer desired number of cells to a 96-well V-bottom plate for flow cytometry (see Appendix 5 for flow cytometry protocol).

b. To culture cells, follow the below steps:
   - Count the cells to determine the cell concentration and cell number.
   - If cargo removal is desired, transfer the cells to 1.5 mL tubes and spin to collect. Resuspend the cells to a desired final concentration in complete media.
   - Transfer cells into a tissue culture plate of appropriate size in complete media for overnight culturing in a 37°C incubator with 5% CO_2.

[14] See Booster Specifications table 3.2 for recommended pressure for optimal performance. A pressure sweep may be performed to optimize performance for specific use cases.
[15] Delaying mixing until immediately prior to passing the sample through the Booster cartridge is most important for sensitive cargo (i.e., RNA).

Notes and Tips

- Contact us! We're happy to help (info@portal.bio)
- Results may be donor-dependent.
- For optimal cell performance, experiments should be concise and completed in a short timeframe (ideally <30 minutes) to reduce cell stress.
- It is ideal to add sensitive cargo (e.g., mRNA) to cells immediately prior to passing cells through the Booster cartridge for optimal performance.
- Temperature can affect performance. Lower temperatures are more harsh on cells but can increase delivery. Pre-warming all media and buffers can help preserve cell health.
- At research scale, Portal has found cell concentration has little to no impact on performance; however cargo concentration can impact delivery performance and when in doubt a cargo titration should be performed. When cargo is in excess (in the recommended ranges), there is no need to change the cargo concentration when cell concentration changes, or when multiplexing cargos.

Microbooster Size Specifications

Size	Part
3 µm	02050350312-01
4 µm	02050350416-01
4.5 µm	02050354516-01
5 µm	02050350520-01
5.5 µm	02050355520-01
6 µm	02050350624-01
6.5 µm	02050356524-01
7 µm	02050350728-02
7.5 µm	02050357528-01
8 µm	02050350830-01
9 µm	02050350930-01
10 µm	02050351030-01
11 µm	02050351133-01
12 µm	02050351236-01
13 µm	02050351339-01
14 µm	02050351442-01
15 µm	02050351545-01

Guidelines and Troubleshooting

Portal's Boost Guidelines & Troubleshooting

Don't see a solution to your challenges here? Contact us! We're happy to help (info@portal.bio)

Tracer Recommendation:

Portal recommends using Cascade blue 3 kDa dextran (Thermo Fisher, D7132) co-delivery in all samples as a tracer for delivery efficiency

- Dextran is inert and won't react with other cargo
- Allows an immediate readout as a control for delivery performance when analyzing data with flow cytometry
- This fluorescent color fits nicely into large flow panels

Key Performance Variables:

For optimal cell performance, experiments should be concise and completed in a short timeframe (ideally < 30 min) to reduce cell stress

- Good starting cell health and gentle handling protocols maintain higher performance characteristics
- Lower temperatures increase harshness & delivery
- It is ideal to add sensitive cargo (e.g., mRNA) to cells immediately prior to boosting for optimal performance. This reduces potential for sensitive cargo degradation and endocytosis of cargo by some cell types

Understanding the Principles that Drive Efficiency:

- Mechanoporation efficiency is driven by cell speed which is controlled through pressure or flow rate
- Optimization of the appropriate speed for desired efficiency may be required
- Efficiency is non-linear: as cell speed increases, efficiency increases to a plateau which allows for a range of effective conditions (speeds) which can achieve desired efficiency before excessive harshness reduces efficiency
- The delivery and viability relationship is inversely related but non-linear: as delivery efficiency increases with increasing speed, viability may drop until the plateau zone is achieved at which viability and delivery remain constant until speed becomes too harsh

Buffer Considerations:

- Delivery buffer can affect performance
  - Membrane closure is a calcium-dependent process; calcium-containing buffers will facilitate membrane closure (and cell health), while calcium-free buffers will keep membranes open for longer periods of time
  - Growth factor support can help support cell health
  - Serum is not recommended as an additive for delivery buffer
- Cargo buffer & volume can affect performance
  - High delivery is a function of proper buffer osmolarity
  - It is preferred to maintain a balanced osmolarity by ensuring cargo resuspended in low salt buffers remains <10% of the total reaction volume
  - If cargo comprises >10% of the total reaction volume, add PBS or NaCl to compensate: a 10X PBS stock solution can be used at 10% the cargo volume to balance the salt content

Recommended Buffers:

- Portal typically uses Opti-MEM (Gibco 31985062) or other minimal media for the delivery buffer
  - Opti-MEM is typically used for PBMCs and subtypes
  - Basal media is typically used for iPSCs
- Complete media supplemented with cytokines is recommended for T cell or PBMC culturing
  - Including supplements and cytokines in the media help support cell health
- mTESR Plus (StemCell 100-0276) is recommended for iPSC culturing; Supplementing with Y27632 (Rho kinase inhibitor) helps support survival following single cell workflow.

Troubleshooting Guide

Observation/ Challenge	Possible Reason	Recommended Solution
Low cell viability	Poor starting cell health	Ensure cells are handled well in processing prior to boost; follow gentle isolation and processing procedures; freeze in DMSO and thaw quickly Rest cells in complete media for 30-60 minutes prior to boosting
	Poor boost processing	Minimize time that cells are out of the incubator; ensure experiment time is ≤30 minutes for optimal performance
	Poor post-boost cell processing	Spin cells at ≤400 RCF; use flick method from plate Minimize washing & handling Recover in complete media; optimize growth factors to best support cell health & growth
	Improper conditions	Include no contact negative control (cells alone with no cargo or boost, handled in the same manner as experimental samples)
	Boost is too harsh	Decrease working pressure or increase core size Adjust cell concentration (typical optimal concentrations are 1-5 x 10^7 cells/ml
	Delivery buffer optimization	Ensure serum-free media is used; complex buffers & additives can be ok in certain cell types- try first with minimal media Minimal medias are ideal since they are simple, but can negatively impact viability; more complex media can support viability
	Cargo concentration is too low	Ideal cargo concentration is between 0.01 mg/ml - 1 mg/ml, with 0.1 mg/ml as a standard starting point
	Improper conditions	Include an endocytosis control sample (cells + cargo, no boost) as negative control
	Boost is too gentle	Increase working pressure or decrease core size Adjust cell concentration (typical optimal concentrations are 1-5 x 10^7 cells/ml)

Troubleshooting Guide

Observation/ Challenge	Possible Reason	Recommended Solution
Low cell viability	Delivery buffer optimization	Calcium-containing medias facilitate membrane closure Minimal medias are ideal since they are simple, but can negatively impact viability (see above)
	Improper buffer osmolarity	Use a balanced salt-containing buffer for delivery buffer Ensure low salt buffers are <10% of the total reaction volume, or are properly balanced by compensating with PBS or NaCl (see above) High salt buffers are typically not ideal for cell performance
High background signal	Too much delivery material still present	Add more wash steps prior to analysis
	Stickiness or endocytosis of cargo	Add endocytosis control sample (cells + cargo, no boost) as negative control
Low cell retention	Cell concentration is too low	Adjust cell concentration (typical optimal concentrations are 1-5 x 10^7 cells/ml)
	Volume is retained in the Booster™ cartridge	Ensure the boost button is held down until all of the reaction volume is recovered; if the Gateway™ system de-pressurizes before all the volume has been recovered, the button may need to be pressed again

[16] Solution may splash on the side of the 1.5 mL tube while boosting. Complete media may be used to wash boosted sample from the side of the tube; avoid spinning the tube until after complete media is added.

troubleshooting: tank is refilling in the middle of my experiment... then you prime it to give max time following these directions
1. Before boosting cells, wash the MicroBooster cartridge with 100 µl delivery buffer at desired boost pressure13 as follows:
   1. Set the pressure on the Gateway system by turning the purple knob until the pressure gauge indicates the desired pressure. Wait until the purple indicator light is ready. Press and hold until all the liquid comes out the purple button until the light turns off to empty the tank. Wait for the pressure tank to refill and the indicator light to turn back on14.
      * Emptying the tank is an important step to ensure sufficient and consistent pressure is available for the duration of the boost. The tank should be emptied each time a new pressure is set.
   2. Place a collection tube in the holder on the Gateway system and secure the cap by placing it on the side of the holder, using caution not to contaminate the inside of the lid.
   3. Pipette 100 µL of delivery buffer into the top of the MicroBooster cartridge.
   4. Connect the MicroBooster cartridge to the Gateway system by screwing into the luer lock on top until secure. Be careful not to over-torque.
   5. Raise the collection tube below the Booster cartridge.
   6. Press and hold the purple button to pressurize the system until the indicator light turns off. The delivery buffer should flow through the Booster cartridge.
   7. Discard the delivery buffer.

Mechanoporation Citations

1. Non-endocytic delivery of functional engineered nanoparticles into the cytoplasm of live cells using a novel, high-throughput microfluidic device (Nano Letters, 2012)

The ability to straightforwardly deliver engineered nanoparticles into the cell cytosol with high viability will vastly expand the range of biological applications. Nanoparticles could potentially be used as delivery vehicles or as fluorescent sensors to probe the cell. In particular, quantum dots (QDs) may be used to illuminate cytosolic proteins for long-term microscopy studies. Whereas recent advances have been successful in specifically labeling proteins with QDs on the cell membrane, cytosolic delivery of QDs into live cells has remained challenging. In this report, we demonstrate high throughput delivery of QDs into live cell cytoplasm using an uncomplicated microfluidic device while maintaining cell viabilities of 80–90%. We verify that the nanoparticle surface interacts with the cytosolic environment and that the QDs remain nonaggregated so that single QDs can be observed.

2. A vector-free microfluidic platform for intracellular delivery (PNAS, 2013)

Intracellular delivery of macromolecules is a challenge in research and therapeutic applications. Existing vector-based and physical methods have limitations, including their reliance on exogenous materials or electrical fields, which can lead to toxicity or off-target effects. We describe a microfluidic approach to delivery in which cells are mechanically deformed as they pass through a constriction 30–80% smaller than the cell diameter. The resulting controlled application of compression and shear forces results in the formation of transient holes that enable the diffusion of material from the surrounding buffer into the cytosol. The method has demonstrated the ability to deliver a range of material, such as carbon nanotubes, proteins, and siRNA, to 11 cell types, including embryonic stem cells and immune cells. When used for the delivery of transcription factors, the microfluidic devices produced a 10-fold improvement in colony formation relative to electroporation and cell-penetrating peptides. Indeed, its ability to deliver structurally diverse materials and its applicability to difficult-to-transfect primary cells indicate that this method could potentially enable many research and clinical applications.

3. Plasma membrane recovery kinetics of a microfluidic intracellular delivery platform (Integrative Biology, 2014)

Intracellular delivery of materials is a challenge in research and therapeutic applications. Physical methods of plasma membrane disruption have recently emerged as an approach to facilitate the delivery of a variety of macromolecules to a range of cell types. We use the microfluidic CellSqueeze delivery platform to examine the kinetics of plasma membrane recovery after disruption and its dependence on the calcium content of the surrounding buffer (recovery time ~ 5 min without calcium vs. ~ 30 s with calcium). Moreover, we illustrate that manipulation of the membrane repair kinetics can yield up to 5× improvement in delivery efficiency without significantly impacting cell viability. Membrane repair characteristics initially observed in HeLa cells are shown to translate to primary naïve murine T cells. Subsequent manipulation of membrane repair kinetics also enables the delivery of larger materials, such as antibodies, to these difficult to manipulate cells. This work provides insight into the membrane repair process in response to mechanical delivery and could potentially enable the development of improved delivery methods.

4. Ex Vivo Cytosolic Delivery of Functional Macromolecules to Immune Cells (PLOS ONE, 2015)

Intracellular delivery of biomolecules, such as proteins and siRNAs, into primary immune cells, especially resting lymphocytes, is a challenge. Here we describe the design and testing of microfluidic intracellular delivery systems that cause temporary membrane disruption by rapid mechanical deformation of human and mouse immune cells. Dextran, antibody and siRNA delivery performance is measured in multiple immune cell types and the approach's potential to engineer cell function is demonstrated in HIV infection studies.

5. Microfluidic squeezing for intracellular antigen loading in polyclonal B-cells as cellular vaccines (Scientific Reports, 2015)

B-cells are promising candidate autologous antigen-presenting cells (APCs) to prime antigen-specific T-cells both in vitro and in vivo. However to date, a significant barrier to utilizing B-cells as APCs is their low capacity for non-specific antigen uptake compared to "professional" APCs such as dendritic cells. Here we utilize a microfluidic device that

employs many parallel channels to pass single cells through narrow constrictions in high throughput. This microscale “cell squeezing” process creates transient pores in the plasma membrane, enabling intracellular delivery of whole proteins from the surrounding medium into B-cells via mechano-poration. We demonstrate that both resting and activated B-cells process and present antigens delivered via mechano-poration exclusively to antigen-specific CD8+T-cells and not CD4+T-cells. Squeezed B-cells primed and expanded large numbers of effector CD8+T-cells in vitro that produced effector cytokines critical to cytolytic function, including granzyme B and interferon-γ. Finally, antigen-loaded B-cells were also able to prime antigen-specific CD8+T-cells in vivo when adoptively transferred into mice. Altogether, these data demonstrate crucial proof-of-concept for mechano-poration as an enabling technology for B-cell antigen loading, priming of antigen-specific CD8+T-cells and decoupling of antigen uptake from B-cell activation.

6. Live-cell protein labelling with nanometre precision by cell squeezing (Nature Communications, 2016)

Live-cell labelling techniques to visualize proteins with minimal disturbance are important; however, the currently available methods are limited in their labelling efficiency, specificity and cell permeability. We describe high-throughput protein labelling facilitated by minimalistic probes delivered to mammalian cells by microfluidic cell squeezing. High-affinity and target-specific tracing of proteins in various subcellular compartments is demonstrated, culminating in photoinduced labelling within live cells. Both the fine-tuned delivery of subnanomolar concentrations and the minimal size of the probe allow for live-cell super-resolution imaging with very low background and nanometre precision. This method is fast in probe delivery (~1,000,000 cells per second), versatile across cell types and can be readily transferred to a multitude of proteins. Moreover, the technique succeeds in combination with well-established methods to gain multiplexed labelling and has demonstrated potential to precisely trace target proteins, in live mammalian cells, by super-resolution microscopy.

7. Cell engineering with microfluidic squeezing preserves functionality of primary immune cells in vivo (PNAS, 2018)

The translational potential of cell-based therapies is often limited by complications related to effectively engineering and manufacturing functional cells. While the use of electroporation is widespread, the impact of electroporation on cell state and function has yet to be fully characterized. Here, we use a genome-wide approach to study optimized electroporation treatment and identify striking disruptions in the expression profiles of key functional transcripts of human T cells. These genetic disruptions result in concomitant perturbation of cytokine secretion including a 648-fold increase in IL-2 secretion ($P < 0.01$) and a 30-fold increase in IFN-γ secretion ($P < 0.05$). Ultimately, the effects at the transcript and protein level resulted in functional deficiencies in vivo, with electroporated T cells failing to demonstrate sustained antigen-specific effector responses when subjected to immunological challenge. In contrast, cells subjected to a mechanical membrane disruption-based delivery mechanism, cell squeezing, had minimal aberrant transcriptional responses [0% of filtered genes misregulated, false discovery rate (FDR) $q < 0.1$] relative to electroporation (17% of genes misregulated, FDR $q < 0.1$) and showed undiminished effector responses, homing capabilities, and therapeutic potential in vivo. In a direct comparison of functionality, T cells edited for PD-1 via electroporation failed to distinguish from untreated controls in a therapeutic tumor model, while T cells edited with similar efficiency via cell squeezing demonstrated the expected tumor-killing advantage. This work demonstrates that the delivery mechanism used to insert biomolecules affects functionality and warrants further study.

8. Engineered red blood cells (activating antigen carriers) drive potent T cell responses and tumor regression in mice

Activation of T cell responses is essential for effective tumor clearance; however, inducing targeted, potent antigen presentation to stimulate T cell responses remains challenging. We generated Activating Antigen Carriers (AACs) by engineering red blood cells (RBCs) to encapsulate relevant tumor antigens and the adjuvant polyinosinic-polycytidylic acid (poly I:C), for use as a tumor-specific cancer vaccine. The processing method and conditions used to create the AACs promote phosphatidylserine exposure on RBCs and thus harness the natural process of aged RBC clearance to enable targeting of the AACs to endogenous professional antigen presenting cells (APCs) without the use of chemicals or viral vectors. AAC uptake, antigen processing, and presentation by APCs drive antigen-specific activation of T cells, both in mouse in vivo and

human in vitro systems, promoting polyfunctionality of CD8+ T cells and, in a tumor model, driving high levels of antigen-specific CD8+ T cell infiltration and tumor killing. The efficacy of AAC therapy was further enhanced by combination with the chemotherapeutic agent Cisplatin. In summary, these findings support AACs as a potential vector-free immunotherapy strategy to enable potent antigen presentation and T cell stimulation by endogenous APCs with broad therapeutic potential.

9. Engineered RBCs Encapsulating Antigen Induce Multi-Modal Antigen-Specific Tolerance and Protect Against Type 1 Diabetes

Antigen-specific therapies that suppress autoreactive T cells without inducing systemic immunosuppression are a much-needed treatment for autoimmune diseases, yet effective strategies remain elusive. We describe a microfluidic Cell Squeeze® technology to engineer red blood cells (RBCs) encapsulating antigens to generate tolerizing antigen carriers (TACs). TACs exploit the natural route of RBC clearance enabling tolerogenic presentation of antigens. TAC treatment led to antigen-specific T cell tolerance towards exogenous and autoantigens in immunization and adoptive transfer mouse models of type 1 diabetes (T1D), respectively. Notably, in several accelerated models of T1D, TACs prevented hyperglycemia by blunting effector functions of pathogenic T cells, particularly in the pancreas. Mechanistically, TACs led to impaired trafficking of diabetogenic T cells to the pancreas, zinduced deletion of autoreactive CD8 T cells and expanded antigen specific Tregs that exerted bystander suppression. Our results highlight TACs as a novel approach for reinstating immune tolerance in CD4 and CD8 mediated autoimmune diseases.

10. Microfluidic Squeezing Enables MHC Class I Antigen Presentation by Diverse Immune Cells to Elicit CD8+ T Cell Responses with Antitumor Activity

CD8+ T cell responses are the foundation of the recent clinical success of immunotherapy in oncologic indications. Although checkpoint inhibitors have enhanced the activity of existing CD8+ T cell responses, therapeutic approaches to generate Ag-specific CD8+ T cell responses have had limited success. Here, we demonstrate that cytosolic delivery of Ag through microfluidic squeezing enables MHC class I presentation to CD8+ T cells by diverse cell types. In murine dendritic cells (DCs), squeezed DCs were ~1000-fold more potent at eliciting CD8+ T cell responses than DCs cross-presenting the same amount of protein Ag. The approach also enabled engineering of less conventional APCs, such as T cells, for effective priming of CD8+ T cells in vitro and in vivo. Mixtures of immune cells, such as murine splenocytes, also elicited CD8+ T cell responses in vivo when squeezed with Ag. We demonstrate that squeezing enables effective MHC class I presentation by human DCs, T cells, B cells, and PBMCs and that, in clinical scale formats, the system can squeeze up to 2 billion cells per minute. Using the human papillomavirus 16 (HPV16) murine model, TC-1, we demonstrate that squeezed B cells, T cells, and unfractionated splenocytes elicit antitumor immunity and correlate with an influx of HPV-specific CD8+ T cells such that >80% of CD8s in the tumor were HPV specific. Together, these findings demonstrate the potential of cytosolic Ag delivery to drive robust CD8+ T cell responses and illustrate the potential for an autologous cell-based vaccine with minimal turnaround time for patients.

Portal Patents, Patent Applications, and Licenses

In addition to wholly owned assets, Portal has a license to legacy SQZ IP and other IP through its agreement with Stemcell Technologies

App. No.	Title/Info
PCT/ US2024/017527	Membrane mechanoporation devices and methods for transfecting cells
PCT/ US2024/017531	Membrane electro-mechanoporation devices and methods for transfecting cells
PCT/ US2024/038369	Centrifuge devices and methods for intracellular delivery
PCT/ US2024/029330	Cellular delivery of gene editing complexes and combinations thereof
PCT/ US2024/059370	Silicon filters for cell mechanoporation
PCT/ US2024/059377	Cell mechanoporation cartridges and methods of use thereof
PCT/ US2024/059380	High-throughput systems for intracellular delivery and components and uses thereof
US 29/940,972	Design patent application directed to Portal's cell mechanoporation cartridge
35/003,333	
US 29/941,018	Design patent application directed to Portal's tabletop cell mechanoporation system
35/003,334	
63/778,054	Provisional Application
63/746,756	Provisional Application
63/814,890	Provisional Application
PCT/ US2017/03093	Intracellular delivery of biomolecules to induce tolerance
PCT/ US2018/066295	System for delivery of a payload into a cell
PCT/ US2017/030933	Intracellular delivery of biomolecules to induce tolerance
PCT/ US2019/054586	Intracellular delivery of biomolecules to enhance antigen presenting cell function
PCT/ US2020/015098	Anucleate cell-derived vaccines
PCT/ US2017/013055	Intracellular delivery of complexes
PCT/ US2021/048771	Methods to stimulate hla-agnostic immune responses to proteins using nucleated cells
PCT/ US2020/026891	Cartridge for use in a system for delivery of a payload into a cell
PCT/ US2016/050287	Intracellular delivery of biomolecules mediated by a surface with pores
PCT/ US2016/050288	Intracellular delivery of biomolecules to cells comprising a cell wall
PCT/ US2022/023356	Reprogramming of cells and uses thereof
PCT/ US2020/020194	Delivery of biomolecules to pbmcs to modify an immune response
PCT/ US2022/028546	Methods for delivering genome editing molecules to the nucleus or cytosol of a cell and uses thereof
US13/139,044	Filter apparatus and filter plate system
PCT/ US2019/021705	Intracellular delivery of biomolecules to modify immune response
PCT/ US2021/043567	Methods to stimulate immune responses to mutant ras using nucleated cells
PCT/ US2021/043568	Methods to stimulate immune responses to mutant ras using nucleated cells
PCT/ US2022/074273	Methods to generate enhanced tumor infiltrating lymphocytes through microfluidic delivery
PCT/ US2022/079843	Methods to generate enhanced tumor infiltrating lymphocytes through microfluidic delivery
PCT/ US2023/060116	Modified hematopoietic stem cells and uses thereof
PCT/ US2023/062998	Methods of producing glial cells and uses thereof
PCT/ US2023/023375	Reprogramming of cells with self-amplifying rna
PCT/ US2023/02356	Cartridges and devices for use in a system for delivery of a payload into a cell
PCT/ US2023/071263	Polynucleotides encoding linked antigens and uses thereof

Terms and Conditions

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1. Agreement Terms

1.1 General Terms. These terms and conditions ("Terms"), our quotation (if any) and Supplementary Terms, if any, comprise the agreement ("Agreement") between you and Portal Biotechnologies. Unless your order is subject to a valid, written, executed agreement between you and Portal Biotechnologies, in which case such agreement applies, you agree to accept and be bound by the Agreement by ordering products on portal.bio or if you receive ordering or sales documents that reference these Terms. This Agreement is the complete and exclusive contract between us with respect to your purchase of the products.

1.2 Supplementary Terms. Some of our products are subject to additional software licenses, limited use label licenses or other written contract terms that you will not find here ("Supplementary Terms"). You will find any Supplementary Terms that apply to your purchase in our quotation to you, on portal.bio, or in literature that accompanies the product. You can also obtain copies from Customer Services.

1.3 Terms Conflict. If any conditions within the Agreement documents conflict with each other, we will give them the following priority: (a) the quotation; (b) any applicable Supplementary Terms; and © finally these Terms. We expressly reject any different terms or provisions contained in any document you provide, and if the terms and conditions in this Agreement differ from the terms of your offer, this Agreement will serve as the governing terms for our contract.

1.4 When Agreement takes Effect. The Agreement between us is created when you receive email confirmation that we have accepted your order.

2. Price

2.1 Determining Price. We may change our prices at any time without notice. Prices we quote you are valid for 30 days, unless we state otherwise in writing. If no price has been specified or quoted to you, the price will be the product price on portal.bio in effect at the time we accept your order.

2.2 Taxes and Fees. Our product prices do not include any taxes (including VAT), duties, levies or other government fees that may apply to your order. If they apply, it will be your responsibility to pay them. If we pay them, we will add them to your invoice. If you claim any exemption, you must provide a valid, signed certificate or letter of exemption for each respective jurisdiction.

2.3 Delivery Fees; Freight Policy. You are also responsible for standard delivery and handling charges, if applicable, and our product prices do not include such charges unless expressly stated. If we pay such charges, we will also add these to your invoice.

3. Cancellation and Changes

Once you have placed your order, you cannot cancel or change it without our written consent.

4. Payment

4.1 Payment Terms. We will invoice you for the product price and all other charges due when we ship you the products. Unless we have agreed otherwise in writing, you will pay us within 30 days from your receipt of invoice. Each order is a separate transaction, and you may not off-set payments, including from one order against another. We reserve the right to require you to make full or partial payment in advance, or provide other security to our satisfaction, if we believe in good faith that your financial condition does not justify the payments terms otherwise specified. You will make all payments in the currency specified in our invoice to you. You may make payments via ACH or other electronic interface that directly exchanges funds between your bank account and ours; checks mailed to one of our lockbox remittance locations; or a credit card at the time of purchase. We will not accept credit card payments made after the time of your purchase.

4.2 Late Payment. If you are late in making payment then, without affecting our other rights you will make payment to us, upon our demand, of a late-payment charge. The late payment charge will be calculated as interest on the sums due from the payment due date until you make payment in full, at the rate of 1.5% per month, or, if less, the maximum amount allowed by law and will also include our reasonable costs of collection (including collection agency fees and attorneys' fees). We also reserve the right to cancel or stop delivery of products in transit and withhold shipments in whole or in part if you do not pay us when due, or if you otherwise do not perform your obligations in this Agreement.

5. Delivery

5.1 Delivery. We will ship products to the destination you specify in your order, FCA Incoterms 2010 our shipping point. By agreeing to these Terms, you (i) give your consent for us to arrange for carriage for all products supplied hereunder on your behalf; and (ii) waive your right to arrange carriage or to give us any specific instructions regarding carriage. We may, in our discretion, make partial shipments and invoice each shipment separately. Our shipping dates are approximate only, and we will not be liable for any loss or damages resulting from any delay in delivery. You may not refuse delivery or otherwise be relieved of any obligations as the result of such delay. If our delivery of a product to you is delayed due to any cause within your control, we will place the delayed products in storage at your risk and expense.

6. Risk of Loss and Title

Excluding software incorporated within or forming part of a product, which we or our licensors continue to own, title to and risk of loss of the products will pass to you when we load them onto the commercial carrier at our facility.

7. Returns and Shortages

7.1 Returns. Customer Services must pre-authorize all product returns. Customer Services will approve return of any product that is damaged or defective on receipt, provided you contact

Customer Services within five days after receiving the product and provided such damage or defect has not been caused by any failure by you or the carrier to handle or store products using reasonable care or as otherwise indicated on the label. If you do not contact us within this five day period, we will deem the product to be accepted, but you will not lose any warranty rights.

7.2 Product-Credit Eligibility. If we exercise our discretion to authorize a product for return then the product must arrive at our facilities in a condition satisfactory for resale. Any return not due to our error is subject to a restocking charge of 25% of the sale price. We do not credit shipping charges. You will not receive credit for any product returned without our prior consent.

8. Warranties

8.1 Limited Warranties for Consumables and General Labware. Unless a different warranty is included in applicable Supplementary Terms or product literature or on the relevant portal.bio product pages, we warrant that each consumable and item of general labware will meet its specifications in our published catalogs or associated Supplementary Terms. This warranty lasts from the time we ship the consumable or item of general labware until the earlier of: (a) the consumable's or item of general labware's expiry or "use by" date; and (b) its specified number of uses. If we do not specify the expiry date, the number of uses, or a different warranty period, the warranty will last for twelve (12) months from the date we ship the product.

8.2 Limited Warranties for Instruments. Unless a different warranty is included in applicable Supplementary Terms, or in the applicable quotation, we warrant that instruments will be free of defects in materials and workmanship, when subjected to normal, proper and intended usage by properly trained personnel, for twelve (12) months from the date we ship the instrument to you, or in the case of instruments that require installation by our personnel, twelve (12) months from installation, but in no event longer than fifteen (15) months from the date we ship the instrument to you.

8.3 Limited Warranty for Spare Parts. We also warrant that spare parts you purchase from us and that we install, or are installed by a company we have certified as an authorized installer, will be free of defects in materials and workmanship for three (3) months from the date we deliver them, or, if longer, the original warranty period of the instrument in which the part is installed. We do not provide warranties for parts that you do not purchase from us or that we do not install. These parts are provided "as is".

8.4 Exclusions. In addition to our exclusion for third party products as set out in Section 8.7 of these Terms, our warranties do not apply to (a) normal wear and tear; (b) accident, disaster or event of force majeure; © your misuse, fault or negligence; (d) causes external to the products such as, but not limited to, power failure or electrical power surges; (e); instruments sold to you as 'used' products; (f) installation, removal, use, maintenance, storage, or handling in an improper, inadequate, or unapproved manner by you or any third party (including the carrier), such as, but not limited to, failure to follow our instructions or operating guidelines, or protocols, operation outside of stated environmental or use specifications, or operation or contact with unapproved software, materials, chemicals or other products; or (g) products manufactured in accordance with specifications you gave us. ADDITIONALLY, ANY INSTALLATION, MAINTENANCE, REPAIR, SERVICE, RELOCATION OR ALTERATION TO OR OF, OR OTHER TAMPERING WITH, THE PRODUCTS PERFORMED BY ANY PERSON OR ENTITY OTHER THAN US WITHOUT OUR PRIOR WRITTEN APPROVAL, OR ANY USE OF REPLACEMENT PARTS WE HAVE NOT SUPPLIED, WILL IMMEDIATELY VOID AND CANCEL ALL WARRANTIES WITH RESPECT TO THE AFFECTED PRODUCTS.

If we determine that products for which you requested warranty services are not covered by the warranty, or if we provide repair services or replacement parts that are not covered by this warranty, you will pay or reimburse us for all costs of investigating and responding to such request at our then prevailing time and materials rates.

8.5 Limitations.

(A) OUR WARRANTIES EXTEND ONLY TO YOU, THE ORIGINAL PURCHASER AND YOU CANNOT TRANSFER THEM. OUR OBLIGATION TO
REPAIR OR REPLACE A PRODUCT IS YOUR SOLE REMEDY.
(B) EXCEPT AS OTHERWISE STATED, WE DISCLAIM ALL OTHER WARRANTIES, WHETHER EXPRESS OR IMPLIED, ORAL OR WRITTEN, WITH RESPECT TO THE PRODUCTS, INCLUDING WITHOUT LIMITATION ALL IMPLIED WARRANTIES

(a) OF MERCHANTABILITY;

(b) OF FITNESS FOR ANY PARTICULAR PURPOSE; AND/ OR

© THAT THE PRODUCTS ARE ERROR-FREE OR WILL ACCOMPLISH ANY PARTICULAR RESULT.

8.6 Remedies. During the applicable warranty period only, for products not meeting our warranty, we agree, in our sole discretion, to repair or replace the non-conforming product and/ or provide additional parts as reasonably necessary to comply with our warranty obligations, but you must first promptly notify us in writing when you discover any defect or nonconformance, and include in the notice clear details of your warranty claim. After our review, assuming we authorize the product return, we will provide you with service data and/or a Return Material Authorization ("RMA"), which may include biohazard decontamination procedures and other product-specific handling instructions that you must follow. For valid product warranty claims timely made in accordance with this Agreement, you must return the non-conforming products to us, unless we agree otherwise, and we will prepay the shipping costs. For instruments only, we may choose to provide you with new or refurbished replacement parts. All replaced parts will become our property. We will ship your repaired or replacement products according to our Delivery terms in Section 5 of these Terms.

8.7 Third Party Products. We do not support or make any warranties about products manufactured or supplied by third parties that you purchase through any of our sales channels. When you buy a third party product, we will let you know that this purchase is governed by the third-party's own contract terms. You must look directly to the relevant third-party manufacturer for product support, warranties, and to make warranty claims. We agree, however, to assign to you any warranty rights we may receive from the original manufacturer or third party supplier, to the extent the original manufacturer or third party supplier allows.

9. Indemnification

9.1 Our Indemnity.

(A) Our Infringement Indemnity. We will defend and indemnify you against infringement damages finally awarded in any legal action brought by a third party against you alleging infringement of any intellectual property rights owned by third parties arising directly and solely from a product, as manufactured and provided by us to you, but always excluding use and/or combination of such product with other products or components. This infringement indemnity does not apply to (a) claims that arose based on your failure to comply with the Agreement; (b) claims that arose based on your failure to acquire any applicable additional intellectual property rights related to your use of the products ("Additional Rights"); © products that we made, assembled or labeled in reliance upon your instructions, specifications, or other directions; (d) your use or resale of products; (e) modifications made by you or any third party; or (f) products originating from third parties.

THIS INDEMNITY IS OUR ONLY LIABILITY TO YOU, AND, SUBJECT TO SECTION 11.4 OF THESE TERMS, YOUR ONLY REMEDY, FOR ANY INFRINGEMENT OR CLAIMED INFRINGEMENT OF THIRD PARTY INTELLECTUAL PROPERTY RIGHTS BY OR IN CONNECTION WITH ANY PRODUCT.

(B) Conditions to Our Indemnity. As a condition to our indemnification obligations you must (a) notify us in writing, as soon as you become aware of any claim; (b) not admit any liability or take any other action in connection with the claim that could affect the defense; © allow us to solely control the defense or settlement of the claim; (d) give us your reasonable information, co-operation and assistance; and (e) take all reasonable steps to mitigate losses incurred, including allowing us to exercise any and all of options set out in Section 11.4 of these Terms.

9.2 Your Indemnity of Us. If a third party makes a claim against us for infringement of its intellectual property rights based on (a) our manufacture or sale of a product or custom product we make under your instructions, specifications, or other directions, or using materials that you provide to us (b) your failure to comply with the Agreement, © your failure to acquire any applicable Additional Rights, or (d) your modification, use or resale of a product, then you will indemnify and hold us harmless from and against any and all claims, losses, damages, liabilities and expenses (including reasonable attorneys' fees and other costs of defending and/or settling any action) that we may have to pay as a result of the claim.

10. Software

10.1 Definitions. With respect to any software products incorporated in or forming a part of our products, you understand and agree that we are licensing such software products and not selling them, and that the words "purchase", "sell" or similar or derivative words are understood and agreed to mean "license", and that the word "you" is understood and agreed to mean "licensee". We, or our licensor, as applicable, retain all rights and interest in software products we provide to you.

10.2 License. We hereby grant to you a royalty-free, non-exclusive, nontransferable license, without power to sublicense, to use software we provide to you under this Agreement solely for your own internal business purposes on the hardware products we provide you hereunder, and to use the related documentation solely for your own internal business purposes. This license will automatically terminate when your lawful possession of the associated hardware products provided hereunder ceases, unless earlier terminated as provided in this Agreement.

10.3 Restrictions. You agree to hold in confidence and not to sell, transfer, license, loan or otherwise make available in any form to third parties the software products and related documentation provided hereunder. You may not disassemble, decompile or reverse engineer, copy, modify, enhance or otherwise change or supplement the software products provided hereunder without our prior written consent. We will be entitled to terminate this license if you fail to comply with any term or condition herein.

10.4 Return of Software and Documentation. You agree, upon termination of this license, immediately to return to us all software products and related documentation provided hereunder and all copies and portions thereof.

11. Intellectual Property

11.1 Use Limitations. As between you and us, we exclusively own all intellectual property rights relating to our products and services. Unless we expressly state otherwise in Supplementary Terms, our sale of products to you grants you only a limited, nontransferable right under our intellectual property to use the quantity of products purchased from us for your internal research purposes. No right to transfer, reverse engineer, decompile, disassemble, distribute, or resell our products or any of their components is conveyed expressly, by implication, or by estoppel. Unless expressly permitted by us in writing, you will not modify, change, remove, cover or otherwise obscure any of our brands, trade or service marks on the products. Nothing in the Agreement limits our ability to enforce our intellectual property rights.

11.2 Commercial Applications; Additional Rights. Unless we expressly state otherwise in Supplementary Terms, we give no rights to use our products in any commercial application, including manufacturing, quality control, commercial services such as reporting the results of your activities for a fee or other consideration, or in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals. If you need commercial use rights in respect of our products (including the right to perform fee-for services), please contact us at partnering@portal.bio. Where your use of our product is outside the scope of the Agreement, it is solely your responsibility to acquire Additional Rights.

11.3 Intellectual Property Ownership. Unless otherwise specified in applicable Supplementary Terms, we exclusively own all intellectual property rights in any inventions (patentable or otherwise), discoveries, improvements, data, know-how, or other results that are conceived, developed, discovered, reduced to practice, or generated by or for us, or jointly by you and us, in relation to processes, designs and methods utilized in manufacture of a custom product. You agree to transfer and assign to us all your right, title, and interest in and to any joint intellectual property. At our request and at our expense, you will help us secure and record our rights in such intellectual property.

11.4 Intellectual Property Infringement. We want to avoid claims of intellectual property infringement. If we believe a product we have sold to you may be subject to a claim for intellectual property infringement, you must allow us (at our option) to either (a) secure for you the right to continue using the product; (b) substitute the product with another suitable product with similar functionality; or © tell you to return the product to us and we will refund to you the price you paid. In the case of instruments, we will take off a reasonable amount for the instrument's use, damage or because it is now out of date or out of use.

12. Custom Products

12.1 Declining to Make or Deliver. If you ask us to manufacture a custom product, we may decline to design or manufacture that product at any stage of the process if the product is unsuitable or commercially impractical to manufacture as specified. If so, we will notify you, and you will not be obligated to pay any fees for any expenses we incurred in connection with the declined product. If a custom component or material fails, we may delay or cancel a custom product's delivery without liability to us.

12.2 Your Responsibilities. By submitting an order for a custom product, you represent and agree that you (a) have given us all information you know of regarding any biological, radiological, and chemical hazards associated with the handling, transport, exposure to, or other use of the materials you supply to us; and (b) have the requisite rights, including but not limited to any necessary intellectual property rights, to instruct manufacture of such product.

13. Instrument-Related Services

When you purchase an instrument, we may install it and provide training, maintenance, repairs, or any other services that you and we expressly agree on ("Instrument Services"). We also offer annual and other instrument-service plans. All Instrument Services are subject to our Instrument Services Supplementary Terms. For full details of our instrument-service plans and to obtain a copy of our Instrument Services Supplementary Terms, please check our website and/or contact Customer Services.

14. Limitations and Exclusions of Liability

(A) TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, WE WILL NOT BE LIABLE UNDER ANY LEGAL THEORY (INCLUDING BUT NOT LIMITED TO CONTRACT, NEGLIGENCE, STRICT LIABILITY IN TORT OR WARRANTY OF ANY KIND) FOR ANY INDIRECT, SPECIAL, INCIDENTAL, PUNITIVE, MULTIPLE, EXEMPLARY OR CONSEQUENTIAL DAMAGES (INCLUDING BUT NOT LIMITED TO ALL COSTS OF COVER, LOST PROFITS, LOST DATA, LOSS OF BUSINESS, LOSS OF GOODWILL OR LOSS OF REVENUE) THAT YOU MIGHT INCUR UNDER THE AGREEMENT, OR THAT MAY ARISE FROM OR IN CONNECTION WITH OUR PRODUCTS OR SERVICES, EVEN IF WE HAD NOTICE OF THE POSSIBILITY OF SUCH DAMAGES.

(B) IN ADDITION, OUR MAXIMUM AGGREGATE LIABILITY ARISING OUT OF OR IN CONNECTION WITH THE AGREEMENT, OR ANY PRODUCT UNIT OR SERVICE, IS LIMITED TO 120% OF THE AMOUNT YOU PAID TO US FOR THE SPECIFIC PRODUCT PURCHASED THAT GAVE RISE TO THE LIABILITY.

(C) THE PROVISIONS ABOVE IN THIS SECTION 14 DO NOT LIMIT OUR LIABILITY THAT CANNOT BE LIMITED BY LAW, INCLUDING BUT NOT LIMITED TO LIABILITY FOR FRAUD AND DEATH OR PERSONAL INJURY CAUSED BY OUR NEGLIGENCE.

15. Export Restrictions

15.1 Items. You acknowledge that each product and any related software and technology, including technical information we supply you, including those contained in product documents (collectively "Items"), is subject to U.S., EU and local government export controls.

15.2 Export Controls. The export controls may include, among others, those of the Export Administration
Regulations of the U.S. Department of Commerce (the "EAR"), which may restrict or require licenses for the export of Items from the United States and their re-export from other countries.

15.3 Compliance Requirements. You must comply with the EAR, and all other applicable laws, regulations, treaties, and agreements relating to the export, re-export, and import of any Item. You must not, directly or indirectly, without first obtaining the required license to do so from the appropriate U.S. government agency; (a) export, re-export, distribute or supply any Item to (a) any restricted or embargoed country or to a person or entity whose privilege to participate in exports has been denied or restricted by the U.S. government; (b) any person or entity who is involved in improper development or use of nuclear weapons or of chemicals/biological weapons, or missiles, or in terrorist activities. You will, if we request, provide information on the end user and end use of any Item you export or plan to export.

15.4 Audit Cooperation. You will cooperate fully with us in any official or unofficial audit or inspection related to applicable export or import control laws or regulations, and will indemnify and hold us harmless from, or in connection with, your or your consultants', agents' or employees' violation of this Section 15.

16. Miscellaneous

16.1 No Assignment. You may not delegate any duties nor assign any rights or claims hereunder without our prior written consent, and any such attempted delegation or assignment will be void.

16.2 Governing Law. The Agreement and performance under it will be governed by the laws of (a) the state of
Massachusetts, if you are located in the USA or Canada; or (b) the laws of the country where the selling entity (as specified on your order confirmation from us) is located, if you are not located in the USA or Canada. In the event of any legal proceeding between you and us relating to the Agreement, neither party may claim the right to a trial by jury. Any action arising under the Agreement must be brought within one year from the date that the cause of action arose. The U.N. Convention on Contracts for the International Sale of Goods is hereby expressly excluded.

16.3 Regulatory Restrictions. In addition to the restrictions set out in Section 11 of these Terms: (a) you must use our products in accordance with our instructions; (b) you are solely responsible for making sure that the way you use our products complies with applicable laws, regulations and governmental policies; © you must obtain all necessary approvals and permissions you may need; and (d) it is solely your responsibility to make sure the products are suitable for your particular use.

16.4 Uncontrollable Circumstances. We will not be responsible or liable for failing to perform our obligations under the Agreement to the extent caused by circumstances beyond our

reasonable control. In certain situations, we may use our reasonable judgment and apportion products then available for delivery fairly among our customers.

16.5 No Waiver; Invalidity. Our failure to exercise any rights under the Agreement is not a waiver of our rights to damages for your breach of contract and is not a waiver of any subsequent breach. If any provision or part of the Agreement is found by any court of competent jurisdiction to be invalid or unenforceable, such invalidity or unenforceability will not affect the other provisions of the Agreement. No person other than you or us will have any rights under the Agreement.

16.6 Headings. Headings are for convenience only and shall not be used in the interpretation of these Terms.

16.7 Confidentiality. You agree to keep confidential any non-public technical information, commercial information (including prices, without limitation) or instructions (including any gene sequences, oligo types or sequences) received from us as a result of discussions, negotiations and other communications between us in relation to our products or services.

16.8 Notices. Any notice or communication required or permitted under these Terms must be in writing and will be deemed received when personally delivered, or 3 business days after being sent by certified mail, postage prepaid, to a party's specified address.

16.9 Requirement to Reduce to Writing. No waiver, consent, modification, amendment or changes to the terms of the Agreement will be binding unless in writing and signed by both of us. Our failure to object to terms contained in any subsequent communication from you will not be a waiver or modification of our Agreement.

16.10 Severability. Any provision of the Agreement which is prohibited or which is held to be void or unenforceable shall be ineffective only to the extent of such prohibition or unenforceability without invalidating the remaining provisions hereof.

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PORTAL