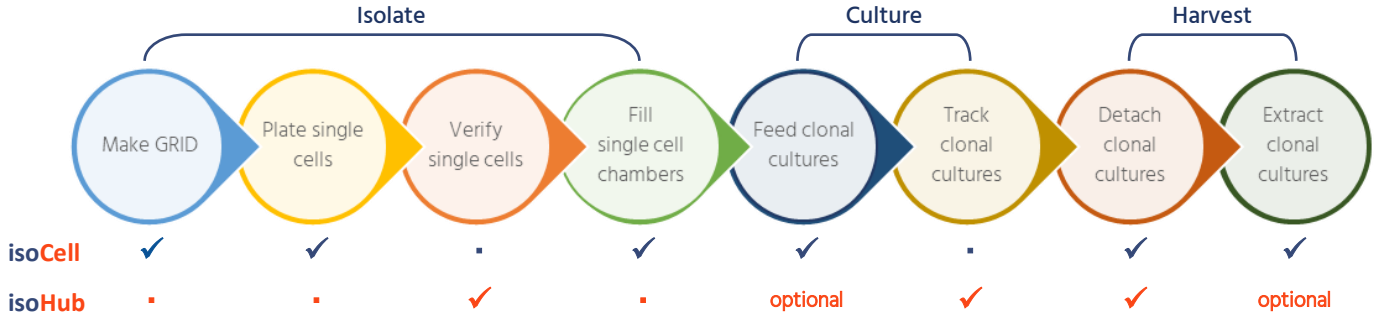
Cloning Platform Training Guide

Introduction

The **cloning platform** is comprised of two instruments: **isoCell** and **isoHub**. The system provides the user with an automated and consistent workflow for single-cell cloning, from isolating single-cells into a novel culture vessel, to feeding your cells in less than 1 µL of medium, tracking outgrowth and harvesting clonal cultures of your choice. It is recommended that users complete this training guide at least once prior to running live experiments on the cloning platform. This document will guide you through self-training on the **cloning platform** workflow, consisting of the following steps:



Before Starting

Prior to commencing training, please ensure that: ***The guided isoCell installation has been completed*** and you are familiar with the **Customer Portal** – especially workflow ***animations*** and ***isoCell manual*** (see support section below).

iotaSciences' supplied materials

- cloneG kit-A-Trn (IOTA-10032), which contains:

Content	Quantity
Colour-coded 60 mm tissue culture treated polystyrene dishes	6
1.5 mL RNAse/DNAse-free Polypropylene Eppendorf Tubes	25
Pipetting aid	1
8 Tube PCR Strip (200 µL)	12 strips
FC40^{STAR}	40 mL

User supplied materials

- Medium
- Cells supplied in a 1.5 mL Eppendorf at 5,000 cells/mL

⚠ *Refer to the "Single-cell Suspension Preparation Guidelines" document in the customer portal for how to prepare this.*

- Optional: 1 mL of Trypan Blue diluted to 0.04% in media in a 1.5 mL Eppendorf.

⚠ *The use of Trypan Blue is to aid visualisation only as part of this training. It is not used as part of the routine single-cell cloning workflow.*

- 70% Ethanol
- PBS
- Deionised water (sterile)
- 1 mL pipette and sterile pipette tips

Support

Support will be available to you within 15 minutes of request throughout the entire pre-scheduled installation and training, which typically takes 2 to 4 hours. All manuals, protocols and workflow animations can be found on our website via accessing the customer portal: http://iotasciences.com/login-only.

Please contact **Tech support** for all installation and training queries:

Email: techsupport@iotasciences.com

Phone: +44 (0)1865 309630

Alternatively, submit a tech support request via the customer portal: http://iotasciences.com/login-only

Single-cell isolation using the cloning platform

Turn isoCell on

1. Turn on **isoCell** by pressing the power button on top of the instrument.
2. Wait for the screen to turn on and run "**Startup routine**" to prepare the **isoCell** for use. Follow on-screen instructions.
3. Once prompted, select ✓ to continue to the home screen.

⚠ *Do not skip startup routine.*

Make a GRID – Fabricate a GRID of 256 isolated chambers

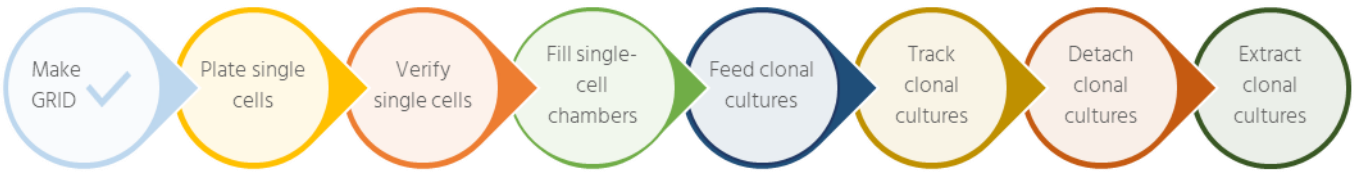
1. Add 2 mL of medium to the 60 mm tissue culture dish provided, ensuring the entire surface of the dish is wet.
2. Tilt the dish and collect excess medium using a 1 mL pipette, and respective tip. Avoid leaving air-bubbles on the dish.
3. **Without delay**, place pipetting aid on the dish. Pipette 2 mL FC40^{STAR} on the ribbed side of the pipetting aid to allow FC40^{STAR} to gently flow onto the inner rim of the dish.
4. **Without delay**, remove pipetting aid and align the dish on the **isoCell**.

⚠ *Ensure dish is placed on **isoCell** without a lid for correct alignment. Align by first slotting in the dish notch and then rotate dish gently left and right and press downwards until firmly in place.*

5. **Without delay**, on the **isoCell** interface, select "**Isolate**" and "**GRID**" to proceed to make a GRID. Setup the **isoCell** bed as illustrated on the interface and follow on-screen instructions. With a marker, label dish lid as "Dish ①".
6. Create a second GRID by repeating steps 1 to 5. With a marker, label dish lid as "Dish ②".

GRID fabrication

- To fabricate GRIDs, it is important you proceed to each step without delay and follow specific protocols for different media/coatings. For protocols, please visit the customer portal: http://iotasciences.com/login-only.
- It is always best to prepare GRIDs shortly before plating cells. Once a GRID is made, it remains stable throughout the entire workflow and you can handle as required.



⚠ The use of dye for dish ① is recommended during initial installation of the instrument as a systems check and to aid visualisation of the GRIDs during training.

Dish ① with dye →

Dish ② with cells →

Plate dye into GRID chambers (Dish ① only)

1. First, we will use Trypan Blue as a surrogate for a single-cell suspension. To begin, on the isoCell select "**Isolate**" then "**Plate**" and follow on-screen instructions.

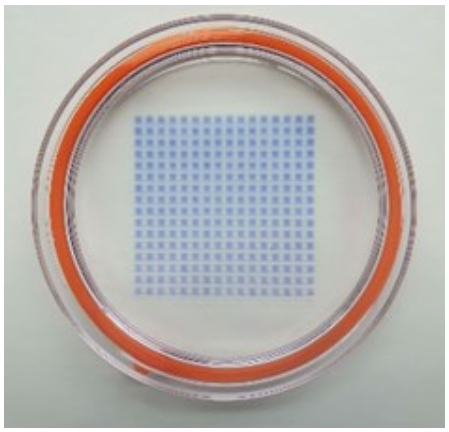


Figure 1
Appearance of dish ① after plating of trypan blue into GRID chambers. Each chamber contains 200 nL of fluid.

2. When the isoCell prompts for "**Cells**", place the 1.5 mL Eppendorf containing 1 mL of diluted trypan blue onto the isoCell bed and resume program. This will add 200 nL of the solution to each chamber.
3. Once the program has completed, confirm your dish looks as in Figure 1. If this is not the case, please contact iotaSciences.

Single-cell isolation

- The dye plating exercise is for training purposes only to facilitate user understanding of how to visualize a GRID correctly for single-cell verification using the isoHub with a 10x objective.
- In a normal workflow using cells, only prepare your cells once GRIDs are ready. Please refer to the "*Single-cell suspension preparation guidelines*" document on the customer portal for further guidance: http://iotasciences.com/login-only.

Check GRID/Single-cell verification (Dish ①)

1. Turn on the isoHub and place dish in the dish holder on the isoHub stage. Ensure the 10x objective is selected.

⚠ *Ensure dish is sitting flat in the isoHub dish holder. Align by first slotting in the dish notch and then rotate dish gently left and right and press downwards until firmly in place.*

2. Look down the eyepiece and scan through the GRID chambers using the isoHub controller to ensure that individual chambers are isolated from each other and the GRID is free of any defects such as connected chambers. If this is not the case, please contact iotaSciences.

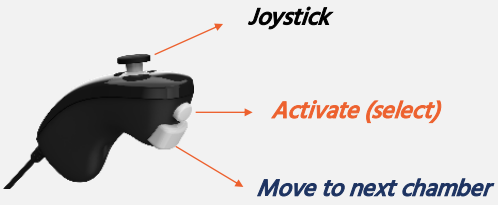
⚠ *For free movement of the stage with the isoHub controller ensure interface is displaying the home screen.*

3. On the isoHub interface, select "**Identify**" to proceed to identify chambers of interest (in a normal workflow, these would be those which contain a single cell).
4. To create a user, select "+" and then name the user - this can either be your name or the name of the experiment. In this case, use "Training" and then select a dish colour (the same colour as the ring around the dish that you are using).
5. Looking down the eyepiece, focus on the chamber walls to ensure you are at the appropriate focal depth (note in-focus chamber walls in Figure 2). In a normal workflow using cells, ensure you focus on the chamber walls rather than on the cells, otherwise you may not be at the correct focal depth.
6. If chamber is not central in the field of view, select on the screen and use the joystick to move the chamber so it is central. Select "**set as chamber centre**" to update the calibration.

⚠ *Centering is not required for every single dish, only when a chamber is no longer in the centre of the field of view.*

7. Using the isoHub controller, scan through the GRID to activate (select) chambers of interest. In this training exercise, we recommend activating 30 random chambers in Dish ①. When a chamber is activated, its icon will change to indicate its status (see chamber states information box on page 8). For more information on the controller, see the isoHub controller information box above.
8. Once all 256 chambers have been viewed, select on the isoHub screen to save your data.

isoHub controller



- **Activate/deactivate chambers:** top button.
- **Move to next chamber:** lower button.
- **Move within chamber:** Joystick.
- **Move to previous chamber:** Move joystick to left and press lower button.
- **Continuous scan:** Hold lower button and select ⚙ to change between 3 scan speeds.
- **Invert motion controls:** Select ⚙ to change the direction the stage moves horizontally and/or vertically.

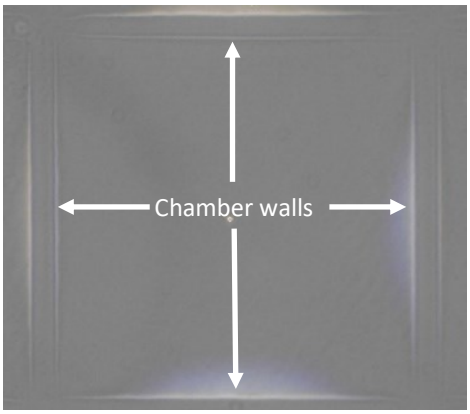


Figure 2 In-focus view of a single GRID chamber. Note the clear, solid lines around the edge.

Fill (Dish ①)

1. On the isoCell interface, select "**Culture**" then "**Fill**" to proceed to top up activated (selected) single-cell chambers to a full culturing volume of 600 nL.
2. Select the "Training" user and relevant dish colour (the same colour as the ring around the dish that you are using). Set up the isoCell bed as illustrated on the interface, but for this training exercise substitute medium for Trypan Blue. Follow on-screen instructions.

⚠ *Filled chambers should be apparent as they contain more volume than non-filled chambers (see Figure 3).*

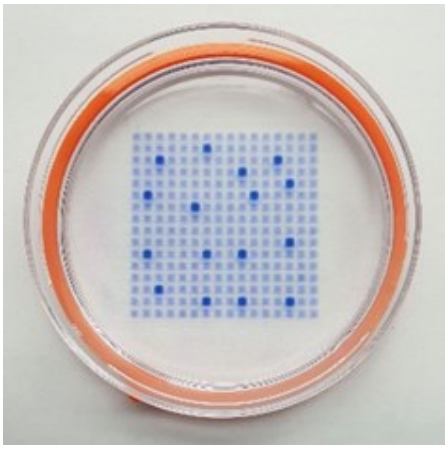


Figure 3

A GRID containing Trypan Blue which has been plated into every chamber. Activated (selected) chambers contain a higher volume and are a darker blue due to the addition of 400 nL of Trypan Blue solution.

Feed (Dish ①)

1. On the isoCell interface, select "**Culture**" then "**Feed**" to proceed to exchange medium from activated chambers.
2. Select the "Training" user and correct colour of dish, verify visually on the interface that the isoCell is going to feed the 30 chambers you activated earlier and select ✓ to exchange the medium.
3. Set up the isoCell bed and follow the on-screen instructions. For this step, make sure to use medium without Trypan Blue, or alternatively PBS so that it becomes obvious visually which chambers have been fed.

⚠ *After feeding you should not have any dark blue chambers remaining. The active chambers will now be light blue in colour, as chamber contents were exchanged with medium or PBS.*

Track (Dish ①)

1. On the isoHub interface, select "**More**" then "**Data**" to view a summary of information about your GRID.
2. Place dish on the isoHub stage, select the "Training" user and "**Dishes**" before choosing the relevant dish colour to review the state of individual chambers and also the number of chambers in each state. You can use the isoHub controller to move between chambers. Alternatively, pressing on an individual chamber on the interface of the isoHub when in the "**Data**" program will move the dish holder so that you can view that specific chamber down the eyepiece. This program can be useful when you wish to visually check your cells, or specific chambers. For more information on what each chamber state icon represents, see page 8 of this training guide.

Detach (Dish ①)

⚠ *When working with suspension cells, skip the detach step and instead run the "**Prep Extract**" program on the **isoHub**.*

1. On the **isoHub** interface, select "**Prep Detach**" to select chambers to detach.
2. Place dish on the **isoHub** stage, select the "Training" user and relevant dish colour, then select eight chambers to detach using the **isoHub** controller.
3. Select ✓ to save your data.

⚠ *You can select more than 16 chambers on the **isoHub**, but will only be able to detach 16 per run on the **isoCell**. Selecting >16 will result in several sequential rounds of the detach and extract programs being ran.*

4. On the **isoCell** interface, select "**Harvest**" then "**Detach**" to proceed to detach your cells.
5. Place dish on the **isoCell** bed, select the "Training" user and relevant dish colour, then set up the **isoCell** bed as illustrated on the interface. Follow the on-screen instructions.

⚠ *For this training you can substitute your dissociation reagent for PBS, if desired.*

⚠ *When using cells, place dish in an incubator after completion of the program until cells are fully detached. Incubation times vary depending on cell type and therefore this may need to be optimised.*

⚠ *When using cells, you can assess whether they have detached before moving to the next step by making use of the "Data" programme on the **isoHub** (see Track on the previous page).*

Extract (Dish ①)

1. Manually add 120 µL of medium to each tube of an 8-tube PCR strip.
2. On the **isoCell** interface, select "**Harvest**" then "**Extract**" to proceed to extract detached clonal colonies.
3. Select the "Training" user and relevant dish colour. You will see the previously detached chambers selected. Follow the on-screen instructions.
4. Once the program has been completed, you may dispose of Dish ① and proceed to using Dish ② with a single-cell suspension (see next page).

Plate cells into GRID chambers (Dish ②)

1. Just prior to starting this section of the training, ensure you have prepared a single-cell suspension by following the instructions in the "*Single-cell suspension preparation guidelines*" document. Keep cells in suspension until the isoCell prompts you to input "**Cells**" downstream.
2. On the isoCell interface, select "**Isolate**" then "**Plate**" to proceed to plate cells. Follow the on-screen instructions.
3. When the isoCell prompts for "**Cells**", place the 1.5 mL Eppendorf containing your single-cell suspension onto the isoCell bed and resume the program. This will add 200 nL of your cell suspension to each chamber.
4. Once the program has completed, proceed to verifying single cells without delay.

Single-cell verification & filling (Dish ②)

1. Using the isoHub, you should now scan through the GRID to activate all chambers containing only a single cell. See page 4 of this training guide for instructions on how to identify chambers containing only a single cell, remembering to select the correct colour of Dish ② on the isoHub interface.
2. Once all 256 chambers have been viewed, select  on the isoHub screen to save your data.
3. You should then proceed to fill the activated chambers with your cell culture medium. For details on how to do this, see page 5 of this training guide. Remember to select the correct colour of Dish ② on the isoCell interface when running the **"Fill"** program.

⚠ *Once single-cell chambers have been activated, the isoCell will only work with those chambers downstream. You will still be able to use programs on these chambers, but you will not be able to use any new chambers that were not initially selected at this stage.*