# ST: Reactions in the Proplate updated protocol

Protocols used can be found here.

## Day 1 of reactions in Proplate

The protocol presumes 4 slides with active positions C, D, and E have been loaded into a Proplate (<https://gracebio.com/product/proplate-multi-array-slide-system-204860/)>. The Proplate will be placed at position 6 on the Bravo and never moved during the protocol.

**The Proplate outline on a 96 wells system:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  |
| D | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  |
| E | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  | Sample | Sample |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

All tip boxes (but the last one in step F) should be prepared in advance with every third columns removed to fit the Proplate layout. These plates are prepared with Bravo protocol “Make 7 tipboxes Part 0.pro”.

### Attach the proplate slide holder onto the Bravo, position 6.

**Both black and silver metal plates underneath proplate holder!**

### Start Bravo.

Start Bravo parts in the following order:

1. Computer
2. Temp box.
3. Bravo.
4. Stackers
5. Minihub

Note: Do not touch the Bravo parts while they are being started up.

### Prepare tip boxes

1. Load 5 full tip boxes in stacker 2.
2. Load empty tip boxes (stored underneath Bravo in cabinet) on positions 1:5, 4:1, 4:2 and 4:5 in minihub.
3. Start protocol “Make 7 tipboxes Part 0.pro”.

### Prepare reaction plates

Only load reaction mixes to the wells which corresponds to a well on the proplate. Prepare master mixes according to [sheet](https://docs.google.com/spreadsheets/d/1tXzbVIHQmUWtnK8qkzLjvGxKJYre32USgARHSlaYewk/edit#gid=0).

**Permeabilization plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Exo I  Buffer  130 ul |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F | Pepsin  85 ul |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**cDNA/Release plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | cDNA mix  85ul |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F | Release mix  90ul |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**MV7 oil plate**

* **If release: 150 ul**
* **If cDNA/USER mix: 80 ul**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Oil |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

### Restart Bravo

Turn every Bravo part including computer off, and put them back on according to the order in A.

### Start fixation of your slide.

### Load reaction plates, WB3 reservoir and waste plate

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Empty tip box | Pos2: partly filled tip box |  |
|  |  |  |
| Pos7: Waste deepwell plate |  |  |

**Stacker - remember to close the gate!**

|  |  |
| --- | --- |
| **Stacker 1** | **Stacker 2** |
| 6 partly filled tip boxes (the ones prepared in step C) |  |

**Minihub**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** |
| **Top** | Oil 96 well plate |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  | Empty tip box |  |  |
| **Bottom** | Reservoir WB3 | Empty tip box |  |  |

### Start HE staining of your slide

### Initialize Bravo

1. Open the ST form.
2. From the ST form, select Part0\_withTissueRemoval1.
3. Select 12 columns
4. Double check that the 2 boxes indicating testing mode are unchecked.
5. Click start protocol.

Bravo initializes.

1. Click “Ignore and continue” on the first pop up window.
2. Click “Retry” on the second pop up window.
3. Click ok on the remaining pop up windows which appears.

Bravo starts to cool/heat temp blocks on positions 4 and 6.

### Go scan on the microscope.

### Load your slide onto the Bravo

1. Let slide get completely dry.
2. Put slide in a proplate mask. Check underneath that the rubber is tight to the glass to prevent leakage during run
3. Place proplate mask in proplate holder. Try to align masks with the other masks and tape to keep masks in place.
4. Once the Bravo has cooled/heated, a pop up window appears telling you this. Then it is time to load the final plates onto the Bravo (NOTE; load the proplate slide holder lastly since pos6 is warm!):

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Empty tip box  (already loaded) | Pos2: Full tip box  (already loaded) |  |
| Pos 4: cDNA/Release plate | Pos 5: Permeabilization plate | Pos6: Proplate slide holder |
| Pos7: Waste plate  (already loaded) |  |  |

1. Click OK on the pop up window → Protocol starts.

## Day 2 of reactions in Proplate

### Prepare reaction plates

Only load reaction mixes to the wells which corresponds to a well on the proplate. Prepare PrK mix according to sheet.

**Tissue removal plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | PrK  190ul |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F | PrK  190ul |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**WB1 plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | WB1  200ul |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F | WB1  200ul |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**WB2 plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | WB2  200ul |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F | WB2  200ul |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

### Load reaction plates on minihub

**Minihub**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** |
| **Top** | Oil 96 well plate | Tissue removal plate |  |  |
|  |  |  |  |  |
|  | WB1 |  |  |  |
|  | WB2 | Empty tip box |  |  |
| **Bottom** | Reservoir WB3 | Empty tip box |  |  |

### Collect cDNA/USER samples

1. If running with cDNA/USER mix, collect samples from the slide by pipetting 70ul underneath the oil (Remove the proplate from the Bravo, tip it and aspirate 70 ul from the upper left corner).
2. Put the proplate back on position 6.

### Continue with protocol

A pop up window appears after the cDNA synthesis is done. Press continue when ready to proceed with tissue removal, washes and release.

### Collect cDNA released material

1. After 2 hours release, collect samples from the slide by pipetting 70ul underneath the oil (Remove the proplate from the Bravo, tip it and aspirate 70 ul from the upper left corner).
2. Press “Pause all” on the Bravo.
3. Press “Abort protocol”
4. Press “Yes” when asked if to release stacker.
5. Part 0 program is now finished.
6. Turn computer and all the Bravo accessories off. Do not save any of the protocols when asked.

### Start Bravo Part 1

1. Remove the proplate slide holder on position 6 and replace it with the 96 well Eppendorf temp holder.

**Only black metal plate underneath Eppendorf temp holder!**

1. Prepare master mixes depending on the number of columns you are loading according to sheet.
2. Prepare three (3) 50ml tubes with 80% EtOH and pour into “EtOH” reservoir.
3. Pour H2O into the “Water” reservoir.
4. Prepare bead plates:
   1. One plate with **100 ul** Ampure RNAclean beads per sample well.
   2. One plate with **81 ul** Ampure RNAclean beads per sample well.
5. Turn on Bravo in the order stated in B.

### Load Bravo

1. Open form “Spatial transcriptomics” and select to start Part 1.
2. Select the numbers of columns to run.
3. Press Display setup to view the starting setup on the Bravo Part 1.
4. Load Bravo, Stacker and Minihub:
   1. Pay special attention to the number of tip boxes to load.

\*\*\* LOAD ONLY FULL TIP BOXES, I.E WITH 96 TIPS \*\*\*

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Deepwell waste plate | Pos2: Full tip box |  |
|  |  |  |
| Pos7: Magnet | Pos8: Empty tip box |  |

**Stacker - remember to close the gate!**

|  |  |
| --- | --- |
| **Stacker 1** | **Stacker 2** |
| X full tip boxes (number depending on the number of columns to run) |  |

**Minihub**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** |
| **Top** | 100 ul bead plate | 81 ul bead plate |  |  |
|  |  |  | Final PCR plate |  |
|  |  | New PCR plate |  |  |
|  | Reservoir Water |  |  |  |
| **Bottom** | Reservoir EtOH |  |  |  |

1. Press Start protocol

Bravo is initializing.

1. Click “Ignore and continue” on the first pop up window.
2. Click “Retry” on the second pop up window.

Bravo is cooling both temp holders.

1. A pop up window appears when temp holders reached 4C degrees.
2. Spin down master mix and sample plate.
3. Load master mix and sample plate onto the Bravo:

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Deepwell waste plate | Pos2: Full tip box |  |
| Pos4: |  | Pos6: |
| Pos7: Magnet | Pos8: Empty tip box |  |

1. Click continue on the pop up window in 8) to start the protocol.

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* NOTE \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

**Part 1 takes approx. 17-18 hours (depending on the number of columns) before new EtOH must be added. PLAN YOUR TIME ACCORDINGLY!**

## Day 3

### Add EtOH

1. Before the bead clean up after IVT in the morning, new EtOH must be added. Prepare 2-3 50ml tubes with 80% EtOH and pour into the EtOH reservoir without disrupting the minihub.
2. While waiting for the bead clean up to finish, prepare for Part 2.

### Prepare Bravo Part 2

1. Prepare master mixes depending on the number of columns you are loading according to sheet.
2. Prepare bead plates:
   1. Two plates with **54 ul** Ampure RNAclean beads per sample well.
3. Prepare a plate with 2.5 ul aRNA adapter per sample well.

### Start Bravo Part 2

1. Once Bravo part 1 has ended, samples are stored on pos6 at 4C degrees.
2. Put your aRNA adapter plate on position 6 (also at 4C degrees). Transfer 8ul of aRNA from pos4 to pos6 to each well on your aRNA adapter plate. Seal aRNA adapter plate and put on ice.
3. Save remaining ~2ul of aRNA plate (from pos6) on ice if running BioA Pico.
4. Click continue on the pop up window saying that Part 1 protocol has ended.
5. Turn off all Bravo accessories including computer.
6. Empty deepwell waste plate.
7. Turn on Bravo in the order stated in B.
8. Open form “Spatial transcriptomics” and select to start Part 2.
9. Select the numbers of columns to run.
10. Press Display setup to view the starting setup on the Bravo Part 2.
11. Load Bravo, Stacker and Minihub:
    1. Pay special attention to the number of tip boxes to load.

\*\*\* LOAD ONLY FULL TIP BOXES, I.E WITH 96 TIPS \*\*\*

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Deepwell waste plate | Pos2: Full tip box |  |
|  |  |  |
| Pos7: Magnet | Pos8: Empty tip box |  |

**Stacker - remember to close the gate!**

|  |  |
| --- | --- |
| **Stacker 1** | **Stacker 2** |
| X full tip boxes (number depending on the number of columns to run) |  |

**Minihub**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** |
| **Top** | 54 ul bead plate | 54 ul bead plate |  |  |
|  |  | Final sample collection PCR plate |  |  |
|  | New PCR plate |  |  |  |
|  | Reservoir Water |  |  |  |
| **Bottom** | Reservoir EtOH |  |  |  |

1. Press Start protocol

Bravo is initializing.

1. Click “Ignore and continue” on the first pop up window.
2. Click “Retry” on the second pop up window.

Bravo is cooling both temp holders.

1. While Bravo is cooling down, incubate 10.5 ul aRNA + aRNA adapter plate at 70C degrees for 2 min in an Eppendorf PCR machine.
2. Load incubated 10.5 ul aRNA + aRNA adapter plate and master mix plate onto the Bravo:

**Bravo**

|  |  |  |
| --- | --- | --- |
| Pos1: Deepwell waste plate | Pos2: Full tip box |  |
| Pos4: |  | Pos6: |
| Pos7: Magnet | Pos8: Empty tip box |  |

1. Click continue on the pop up window to start the protocol.

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* NOTE \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

**Part 2 takes approx. 5.5 hours (depending on the number of columns) before new EtOH must be added. PLAN YOUR TIME ACCORDINGLY!**

### Add EtOH

1. Before the final bead clean up, new EtOH must be added. Prepare 2-3 50ml tubes with 80% EtOH and pour into the EtOH reservoir without disrupting the minihub.

### End Part 2

1. Once Bravo part 2 has ended, samples are stored on pos6 at 4C degrees and a pop up window has appeared. Store the final plate with cDNA and press continue.
2. Press “Yes” when asked if to release stacker.
3. Turn computer and all the Bravo accessories off. Do not save any of the protocols when asked.