LifeCanvas Technologies
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LifeCanvas Curriculum for First SmartBatch+ Experiments

The following is an introductory curriculum for SmartBatch+ to gain experience with the protocol and device.

Contents

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Antibodies of Interest

First, decide which antibodies you want to use for labeling. Select one antibody from each column (you can omit any columns for a simplified experiment). Be mindful of potential host conflicts (i.e. don't choose two antibodies from the same host):

488 channel	561 channel	647 channel
Rabbit Anti-GFP (Thermofisher A-11122)	Rabbit Anti-GFP (Thermofisher A-11122)	Rabbit Anti-GFP (Thermofisher A-11122)
Goat Anti-GFP (Encor GPCA-GFP)	Goat Anti-GFP (Encor GPCA-GFP)	Goat Anti-GFP (Encor GPCA-GFP)
	Goat Anti-ChAT (Millipore AB144P)	Goat Anti-ChAT (Millipore AB144P)
	Rabbit Anti-Dopamine Beta Hydroxylase (DBH) (abcam ab209487)	Rabbit Anti-Dopamine Beta Hydroxylase (DBH) (abcam ab209487)
	Rabbit Anti-Iba-1 (CST 17198S)	Rabbit Anti-Iba-1 (CST 17198S)
	Mouse Anti-NeuN (Encor MCA-1B7)	Mouse Anti-NeuN (Encor MCA-1B7)
	Rabbit Anti-NeuN (CST 24307S)	Rabbit Anti-NeuN (CST 24307S)
	Propidium Iodide (nuclear dye)*	Rabbit Anti-cFos (abcam ab214672)

*Nuclear dyes will contaminate the cup and may stain future samples run in the same cup.

Please review the Validated Antibodies List (link) for purchasing links from the appropriate vendors. You will also need to purchase secondary antibodies which are also listed in that document.

Materials needed

- Primary and secondary antibodies
- Normal Donkey Serum (or Normal Goat Serum, matching secondary antibody host)
- Spoon or spatula for handling samples
- 50mL Conical tubes
- SmartBatch+
- SmartBatch+ accessories
  - Lock rings
  - Sample rings
  - Incubation jar
  - Ring stand
  - Sample bags
  - Sample cup (small for single sample or large for batch)
- Reagents
  - SHIELD-Buffer
  - SHIELD-Epoxy
  - SHIELD-On
  - Delipidation Buffer
  - Conduction Buffer
  - Primary Sample Buffer
  - Primary Device Buffer
  - Secondary Sample Buffer
  - Secondary Device Buffer
  - Easy Index
- PBS + 0.02% Sodium Azide (PBSN)
- Distilled or ultrapure type 1 Water
- 4% PFA

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Protocol Steps

This is meant to serve as a general overview of the protocol and the critical steps. This is a supplement to, not a replacement of, the Full Pipeline Protocol. Please read the Full Active Pipeline Protocol in its entirety and just use this as a reference while conducting your experiment.

Animal perfusion instructions

To prepare samples, transcardially perfuse rodents with ice-cold 1X PBS with 10 U/mL heparin until fluid runs clear, followed by ice-cold 4% PFA. Extract the brain or chosen sample and incubate in 4% PFA solution at 4C for 24 hours with gentle shaking. Wash the sample twice in PBS and then store samples in PBS.

You also may wish to use samples that have already been perfused and dissected, such as from Hilltop Labs. If so, you may skip directly to the SHIELD step.

Step A: SHIELD (protocol v5.04, page 4, starting at Step 5)

- SHIELD-Off
  1. 5mL DI water, 5mL SHIELD Buffer, 10mL SHIELD Epoxy (per brain)
  2. 3 days @ 4°C shaking
- SHIELD-On
  1. 24 hours @ 37°C shaking

Step B: Delipidation in SmartBatch+ (protocol v5.04, page 6)

- Incubate in Delipidation Buffer overnight at 37°C.
- Delipidate in SmartBatch+ using the following materials and settings:
  1. Fill clearing cup with 32mL of Delipidation Buffer
  2. Press “Preset” until it indicates **Clearing Mode**
  3. Entire bottle (450mL) Conduction Buffer in the chamber
  4. 24 hours
- Wash the device (see protocol)

Step C: Labeling in SmartBatch+ (protocol v5.04, page 8)

1. **ASSUMPTION:** This protocol is for labeling one brain in the Single Sample Cup. For batch staining (3-12 whole mouse brains), please refer to the Full Active Pipeline Protocol
2. Incubate in Primary Sample Buffer overnight at room temperature
3. Refresh Primary Sample Buffer in the morning to start labeling ~4 hours later
4. Prepare Sample cup: rinse SDS storage buffer from cup with a gentle stream of tap water followed by a DI water rinse. Leak test the cup by filling with DI water and setting on a paper towel for ~15 minutes
5. Wash sample bag thoroughly with tap water followed by a DI water rinse
6. Prepare SmartBatch+ Device for labeling:
   - Press “Preset” until it indicates **Labeling 1 Mode**

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- Drain water from the reservoir and wipe up any remaining water in the chamber with a paper towel
- Pour 1 bottle of Primary Device Buffer into the chamber
- Set timer to 18:00 (18 hours)

7. Prepare sample:
   - Add 9mL Primary Sample Buffer to cup
   - Add in primary antibodies
   - Add in 200uL Normal Donkey Serum (whole mouse brain)
   - Place sample in bag and then in the sample cup
8. Place cup in the device and turn on Electrophoresis and Timed Shutdown
9. The next morning, check pH inside the cup using pH strip with at least 0.2 sensitivity or a calibrated pH meter
   - pH should be < 8.0 (but above 7.0)
   - For each tenth above 8.0, continue electrophoresis for 1 additional hour (e.g. if pH is 8.1, run for 1 more hour; pH is 8.3, run for 3 more hours)
10. Wash sample with PBSN for 4 hours, two times (removing unbound primaries)
11. Wash the device and cup
12. Fix sample in 4% PFA at room temperature overnight
13. In the morning, place sample in Secondary Sample Buffer at 37°C
14. Refresh buffer 2 hours later and incubate for an additional 2 hours
15. Prepare Sample cup: rinse SDS storage buffer from cup with a gentle stream of tap water followed by a DI water rinse. Leak test the cup by filling with DI water and setting on a paper towel for ~15 minutes
16. Prepare SmartBatch+ Device for labeling:
    - Press "Preset" until it indicates **Labeling 2 Mode**
    - Drain water from the reservoir and wipe up any remaining water in the chamber with a paper towel
    - 1 bottle of Secondary Device Buffer into the chamber
    - Set timer to 4:00 (4 hours)
17. Actively wash in SmartBatch+ (removing excess PFA)
    - Prepare sample:
      - Place sample in bag inside sample cup
      - Add 9mL Secondary Sample Buffer to cup
    - Place sample cup inside of chamber
    - Run electrophoresis for 2 hours, remove sample and refresh buffer in the sample cup, and run for additional 2 hours (leave the same buffer in the device)
18. Prepare sample:
    - Refresh cup with 9mL Secondary Sample Buffer
    - Add in secondary antibodies and 200uL Normal Donkey Serum (whole mouse brain)
19. Place sample cup inside of chamber set timer to 12:00 and preset to **Labeling 2**
20. Turn on Electrophoresis and Timed Shutdown
21. The next morning, actively wash in SmartBatch+ (removing unbound secondaries)
    - Refresh cup with 9mL Secondary Sample Buffer

- Run electrophoresis for 3 hours, remove sample and refresh buffer, and run for additional 3 hours

22. Wash in PBSN for remainder of the day
23. Wash device
24. Fix in 4% PFA at room temperature overnight
25. Wash in PBSN, refreshing at least once over the course of a day

Step D: Index Matching (protocol v5.04, page 17)

- Incubate in 50% EasyIndex for 24 hours at 37°C
- Incubate in 100% EasyIndex for 24 hours at 37°C

Suggested Timetable

NOTE: All times are approximate and just show a possible schedule for the week. For exact timings and steps, please refer to the protocol

Day	Time	Step
Friday	Anytime	SHIELD-Off
Monday	3:00PM	SHIELD-On
Tuesday	3:00PM	Delipidation Buffer incubation
Wednesday	9:00AM	Clear in SmartBatch+
Thursday	9:00AM	PBSN wash
Friday	4:00PM	Primary Sample Buffer incubation
Monday	9:00AM	Refresh Primary Sample Buffer
Monday	3:00PM	Begin primary labeling
Tuesday	9:00AM	Check pH (continue electrophoresis or wash in PBSN)
Tuesday	1:00PM	Refresh PBSN
Tuesday	5:00PM	PFA fix
Wednesday	9:00AM	Wash in Secondary Sample Buffer
Wednesday	11:00AM	Refresh Secondary Sample Buffer
Wednesday	1:00PM	Pre-secondary wash
Wednesday	3:00PM	Refresh buffer, continue wash
Wednesday	5:00PM	Add antibodies, continue secondary
Thursday	9:00AM	Post-secondary wash
Thursday	11:00AM	Refresh buffer, continue wash
Thursday	1:00PM	PBSN wash

Thursday	5:00PM	PFA fix
Friday	9:00AM	PBSN wash (refresh at least once)
Friday	5:00PM	50% EasyIndex
Monday	9:00AM	100% EasyIndex
Tuesday	After 24 hours	Check sample transparency & image