|  |  |
| --- | --- |
| **Date** |  |
| **Setup** | Lysis binding mix  PCR clean 1.5ml tubes – Labeled  Cutting blade  Slicing blade  Plus slides – Labeled  Forceps  Punching tool  Vortex  RNAzap, EtOH, Kimwipes  Dry ice + Tissue storage box |
| **Start time** |  |
| **Temperature** | chamber (-20°C) stage (-12°C) |
| **RNA Zap** | Blade, brush, forcep |
| **Ethanol** | Chamber |
| **End time** |  |

**Dissected brains**

|  |  |  |
| --- | --- | --- |
| **Brain ID** | **Chuck ID** | **Note** |
|  |  | **Log - Brain out of the freezer /Brain in the cryostat / Slicing start time / Punching start time / Lysis start time** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

**Brains to punch Decision final – 07/08/2019**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **#** | **Cohort** | **Mouse (sample ID)** | **Mouse (sample ID)** | **Mouse (sample ID)** |
| **1** | **105** | **6** | **7** | **11** |
| **2** | **106** | **2** | **3** | **5** |
| **3** | **107** | **5** | **8** | **11** |
| **4** | **108** | **5** | **8** | **11** |
| **5** | **109** | **1** | **5** | **7** |
| **6** | **110** | **2** | **6** | **11** |
| **7** | **112** | **1** | **6** | **12** |
| **8** | **113** | **2** | **9** | **11** |
| **9** | **114** | **5** | **8** | **11** |
| **10** | **115** | **3** | **9** | **11** |
| **11** | **116** | **3** | **10** | **11** |

**Punching order**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **1** | **105-11** | | **18** |  | |
| **2** | **106-5** | | **19** |  | |
| **3** | **107-5** | | **20** |  | |
| **4** | **108-8** | | **21** |  | |
| **5** | **109-5** | | **22** |  | |
| **6** | **110-11** | | **23** |  | |
| **7** | **112-12** | | **24** |  | |
| **8** | **113-2** | | **25** |  | |
| **9** | **114-8** | | **26** |  | |
| **10** | **115-9** | | **27** |  | |
| **11** | **116-10** | | **28** |  | |
| **12** |  | | **29** |  | |
| **13** |  | | **30** |  | |
| **14** |  | | **31** |  | |
| **15** |  | | **32** |  | |
| **16** |  | | **33** |  | |
| **17** |  | |  |  | |
| **Date** | |  | | |
| **Dissected brains** | |  | | |
| **Setup** | | Lysis binding mix  PCR clean 0.5ml tubes – Labeled  Cutting blade  Slicing blade  Plus slides – Labeled  Forceps  Punching tool  Vortex  RNAzap, EtOH, Kimwipes  Dry ice + Tissue storage box | | |
| **Start time** | |  | | |
| **Temperature** | | chamber (-20°C) stage (-12°C) | | |
| **RNA Zap** | | Blade, brush, forcep | | |
| **Ethanol** | | Chamber | | |
| **End time** | |  | | |
| **Autoclave** | |  | | |

**mirVana Lysis binding mix**

|  |  |  |
| --- | --- | --- |
| **Prep date/time** |  | |
| **Batch ID** |  | |
| **Lysis buffer (99ul/tube)** | **ul** |  |
| **2-Mercaptoethanol (1ul/tube)** | **ul** |  |
| **Total**  **(100ul/tube)** | **ul** |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Brain ID** | | |  | | |
| **Thaw start time** | | |  | | |
| **Slicing thickness** | | | **um** | | |
| **Slicing start time** | | | Cleaned ( ) | | |
| **Microdissection end time** | | | Cleaned ( ) | | |
| **1** | **mPFC** |  | **7** | **CA1** |  |
| **2** | **NAcc Core** |  | **8** | **VMH** |  |
| **3** | **NAcc shell** |  | **9** | **CA3** |  |
| **4** | **LS** |  | **10** | **MEA** |  |
| **5** | **BNST** |  | **11** | **BLA** |  |
| **6** | **mPOA** |  | **12** | **VTA** |  |
| **Lysed** | | |  | | |
| **5 min incubation** | | |  | | |
| **Lysates storage location** | | |  | | |
| **Slides storage location** | | |  | | |
| **Comments** | | | | | |

**Backup – subordinate Brains to punch**

**Decision final – 08/15/2019**

|  |  |  |
| --- | --- | --- |
| **#** | **Cohort** | **Subordinate** |
| **1** | **105** | **9** |
| **2** | **106** | **6** |
| **3** | **107** | **1** |
| **4** | **108** | **6** |
| **5** | **109** | **11** |
| **6** | **110** | **9** |
| **7** | **112** | **8** |
| **8** | **113** | **4** |
| **9** | **114** | **2** |
| **10** | **115** | **1** |
| **11** | **116** | **2** |

**Sample ID assignment**

|  |  |
| --- | --- |
| **34** |  |
| **35** |  |
| **36** |  |
| **37** |  |
| **38** |  |
| **39** |  |
| **40** |  |
| **41** |  |
| **42** |  |
| **43** |  |
| **44** |  |