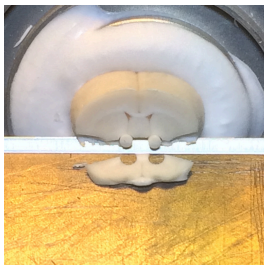
Feb 14, 2020

Fresh Frozen Mouse Brain Preparation (for Single Nuclei Sequencing)



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The Single Cell Ninjas

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

1 Mice are anesthetized with 3% isoflourane prior to cardiac perfusion with ice cold HEPES-Sucrose Cutting Solution:

HEPES-Sucrose Cutting Solution

	MW	final concentration (mM)	for 1L (in g or ml)	for 2L (in g or ml)
NaCl	58.44	110	6.43	12.86
HEPES	238.3	10	2.383	4.766
glucose	180.2	25	4.505	9.01
sucrose	342.3	75	25.6725	51.345
$MgCl_2$ $6H_20$	203.31	7.5	7.5 ml (1M stock)	15 ml (1M stock)
KCl	74.56	2.5	2.5 ml (1M stock)	5 ml (1M stock)

adjusted to pH 7.4 with NaOH

Store cutting solution at 4^{o}C

2 Their head is removed by bisecting between c2 and c3 with large scissors. The brain is removed immediately as follows:

3 The cranial and facial skin and fat pads are peeled forward and the splenius capitis muscle is removed with a forceps exposure the dural sheath covering the fossa between the foramen magnum and the first cervical vertebra. A small pointed scissor is used to pierce the sheath in a rostral-caudal direction and then the 1 or 2 cervical vertebra are cut along the dorsal midline.

4 The same scissors are then inserted carefully 1-2mm into the foramen magnum with the lower blade pressing upward against the inner edge of the skull cavity. Proceed to snip the skull along the midline keeping constant gentle upward pressure so as not to cut or piece or otherwise damage the brain. Cut sagittally all the way up along the midline through the intra-orbital area over the olfactory bulbs.

5 Using a small toothed forceps, grab the midline edge of the bone covering the cerebellum and gently push the entire hemisphere of cut bone away from the midline. It will break, and only part of it will be removed from over the underlaying brain tissue. Repeat the process until the bone from the foramen magnum up to the orbit is removed. Then do the other side. Be most careful of the cerebellar parafloculus - that lobe nestles inside of a little fossa of the temporal bone along the lateral side of the cerebellum (ie, behind the ear) and we don't want to tear it off.

6 The bone around the cribiform plate and orbit is particularly thick - this must be carefully picked away around the olfactory bulbs which are inside of it. Once the bulbs are fully exposed on the dorsal aspect they can be gently eased caudally using a blunt instrument like a small weighing spatula. As you release the olfactory bulbs, continue to “roll” the brain up and back from the inner rostral skull. The optic nerves will appear under the brain next - use the small scissors to cut them leaving the chiasma intact (this makes a great landmark later). Next comes the carotid artery - cut as well - then towards the

posterior the basilar artery and some of the small cranial nerves appear. Try to cut vs. tear these if you can.

7 The entire operation thus far should have been less than ten minutes from start of anesthesia. If you are taking longer, you just need practice, don't waste your time using the RNA from practice brains.

8 Now the brain is ready to freeze. Wash in cold PBS to remove any hair, blood or misc. debris. Blot gently the excess PBS, not dry, but not swimming in liquid that will turn to ice in the next steps. Watch the movie.

9 Keep the brain frozen at -80 until use. We store whole mouse brains in a small 5 ml tube that has 2 ml of OCT frozen in the bottom (in advance). This OCT serves as a moisture reservoir that keeps the brain from sublimation and drying during long term storage. Desiccated brain tissue has poor RNA quality.