Organotypic Slice Culture of Embryonic Brain Sections

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[Abstract] This technique will allow using brain slices to study several aspects of cortical development (*i.e.* neurogenesis), as well as neuronal differentiation (*i.e.* neuronal migration, axon and dendrite formation) *in situ*. This protocol is suitable for various embryonic stages (de Anda F *et al.,* 2010; Ge *et al.,* 2010).

Material and Reagents

1. Agarose Type VII (low melting) (Sigma-Aldrich, catalog number: A9045-100G)
2. Neurobasal (Life Technologies, Gibco®, catalog number: 21103-049)
3. B27 (Life Technologies, Invitrogen™, catalog number: 17504-04)
4. Glutamine (Life Technologies, Gibco®, catalog number: 25030)
5. Penicillin/streptomycin (Sigma-Aldrich, catalog number: P4458)
6. Horse serum (Life Technologies, Invitrogen™, catalog number: 16050-122)
7. N2 (Life Technologies, Invitrogen™, catalog number: 17502-048)
8. 1x PBS
9. Krazyglue or similar glue

Equipment

1. Vibratome (Leica, model: VT1000 S)
2. Millicell, sterilized culture plate insert (EMD Millipore)
3. Dissecting tools (Ring forceps, Serrated forceps, Fine scissors)
4. Curved spatula
5. 30 mm petri dish

Procedure

1. Remove the head of electroporated mouse embryos (see *"In utero* Electroporation of

Mouse Embryo Brains" (Ge, 2011) and place it into ice-cold 1x PBS.

2. Dissect out the whole brain and place it into ice-cold PBS. Do not let the brain more that 30 min in ice-cold PBS. Faster the brain is processed; better brain slices quality will be obtained.
3. Fill a 30 mm petri dish with 3% low-melting agarose prepared in 1x PBS (use enough agarose to cover the brain) pre-warmed at 40 °C in a microwave. Monitor the temperature with a thermometer while steering the agarose solution with it. The brain will be put into the agarose when the temperature drops to about 35 °C-37 °C, try to use fresh 3% low-melting agarose every time you prepare brain slices.
4. Take out the brain from the ice-cold PBS with a curved spatula and carefully remove the PBS solution from the tissue with a Kimwipe. Use the Kimwipe as a wick. Critical step to maintain the structure of the brain in culture: Try to remove as much PBS solution as possible from the tissue. Avoid the Kimwipe to adhere to the brain in order to prevent tissue damage. Place the Kimwipe as close as you can to the brain to absorb, as much PBS solution as possible. A "PBS solution-free" brain is better embedded into the agarose solution. Thus, once the brain is sliced the brain sections do not detach from the agarose.
5. Place the brain into 3% low-melting agarose (35 °C-37 °C) in a petri dish (olfactory bulb facing down, Figure 1A).

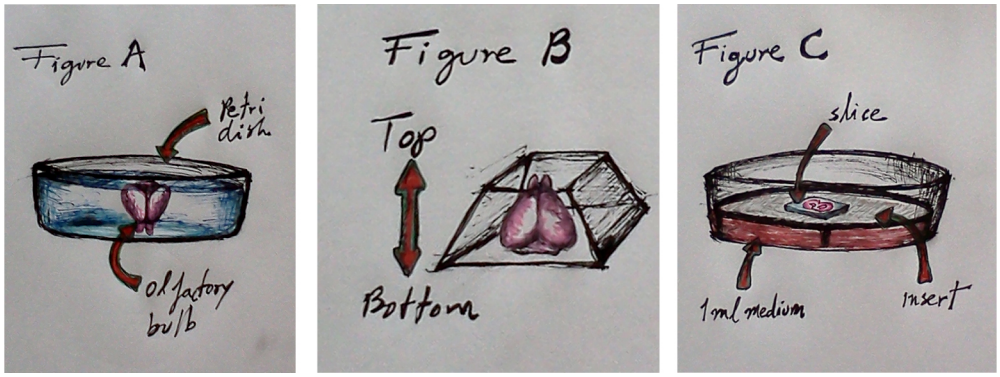

Figure 1. (A) Brain is embedded, with the olfactory bulb facing down, in a preti dish with 3% low-melting agarose. (B) Once the agarose solidifies cut the agarose around the brain with a pyramidal shape in such a way that the base of the brains (side of the cerebellum) is at the base of the pyramid and olfactory bulb faces the apex of the pyramid. (C) Transfer coronal sections from the brain on top of the Millicell culture insert system containing 1 ml medium.

6. Put the petri dish on ice and wait until the agarose solidifies. Carefully keep the position of the brain with the spatula meanwhile the agarose solidifies.

7. Cut the agarose around the brain and keep the brain in the middle of the agarose block. Try to cut the block with a pyramidal shape in such a way that the base of the brain (cerebellum) is the base of the pyramid and the olfactory bulb faces the apex of the pyramid (Figure 1B). This agarose block configuration will allow a steady position of the block when glued at the vibratome tray's holder (the base of the agarose pyramid faces the vibratome holder).
8. Glue the agarose block containing the brain onto the vibratome tray with Krazyglue or similar glue.
9. Add ice-cold 1x PBS into the vibratome tray to cover the agarose block.
10. Slices the agarose block. Slice thickness: 240 µm (tissue flatten over time with thinner slices). Vibratome settings: speed 4-5; vibration 6.
11. Collect slices with curved spatula carefully to avoid the tissue to detach from the agarose. Transfer the slices into a Millicell culture insert system containing 1 ml medium (Figure C; Neurobasal supplemented with B27, N2, 0.5 mM Glutamine, 1% penicillin/ streptomycin, and 5% horse serum). Put as many slices as you can put on top of the membrane insert (generally 4-5 slices per insert).
12. Remove carefully the solution that surrounds the slice with pipette tip. Tissue must be exposed to air.
13. Place the culture insert dish with slices into tissue culture incubator (37 °C) for 1-2 h
    The slices are ready to use (*i.e.* Time-lapse imaging). This culture system has been used for 48 h time-lapse imaging (de Anda F., *et al.*, 2010; Ge *et al.*, 2010). Also is possible to use this culture system for pharmacological treatments (de Anda F., *et al.*, 2010; Ge *et al.*, 2010).

Recipes

1. Neurobasal (250 ml)

50x B27	4.5 ml
200 mM Glutamine	2.5 ml
50x Penicillin / Streptomycin	2.5 ml
Horse serum	12.5 ml
100x N_2	2.5 ml (it should be added to the medium the day of the experiment; 10 µl/1 ml medium)
Total	24.5 ml/250 ml Neurobasal

   This media can be stored at 4 °C protected from light for several months.

Acknowledgments

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References

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