Miniproject A – Methods

BIOENG-451 – 2023

Group 1:
Title: “Progenitor Hyperpolarization Regulates the Sequential Generation of Neuronal Subtypes in the Developing Neocortex”

Assignment: In this paper the author study how progressive changes in the membrane potential of neural progenitor coordinate the successive generation of distinct neuronal subtypes. In Figure 1A the authors show that in contrast to the control condition, in which E14.5-born neurons were essentially confined to L4, E14.5-born neurons following Kir2.1 IUE were located more superficially, including within L2/3.
Please provide a comprehensive protocol for the experiment shown in Figure 1A, focusing particularly on the transfection constructs, reagents, and the embryos strain and developmental stages used in the experiment.

Group 2:
Title: "Temporal Plasticity of Apical Progenitors in the Developing Mouse Neocortex".

Assignment: The authors explore the plasticity of various types of neuronal progenitors using a blend of transfection methods and transplantation techniques, as illustrated in Figure 1a.
Please provide a comprehensive protocol for the experiment shown in Figure 1a, focusing particularly on the transfection constructs, reagents, and the embryos developmental stages used in the experiment.

Group 3:
Title: "FASN-Dependent De Novo Lipogenesis is Essential for Brain Development"

Assignment: To examine the role of FASN in human brain development, the authors treated brain organoids with chemicals and subsequently performed lipid staining with 17-ODYA.
Please provide a detailed protocol to replicate the experiment in Figure 5F, beginning with the cultivation of brain organoids and concluding with the specific staining method.

Group 4:
Title: "iPS-Cell-Derived Microglia Facilitate Brain Organoid Maturation Through Cholesterol Transfer".

Assignment: The authors demonstrate the significance of microglial cells in transferring cholesterol to neural progenitors using an organoid co-culture system. In Figure 3 f, g, h, they reveal how iMicro can export cholesterol to neuronal cells in organoids using BODIPY staining.
Please outline a detailed protocol to replicate the experiment in Figure 3 g, starting from the culture of brain organoids and iMicro, and ending with the specific staining method.

Group 5:
Title: "Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment"

Assignment: The authors study how human-specific substitution in NOVA1, which is exclusive to modern humans since divergence from Neanderthals, may have had functional consequences for our species' evolution.
In figure 7A the authors show how the Introduction of NOVA1 archaic genetic variant in modern human brain organoids alters neuronal network activity. Please provide a comprehensive protocol for the experiment shown in Figure 7A. Focus particularly on the transfection construct used for generating the archaic transgenic line, reagents, and the organoids protocol and stage used in the experiment.

Group 6:
Title: "Pyramidal neurons form active, transient, multilayered circuits perturbed by autism- associated mutations at the inception of neocortex".

Assignment: The authors show that during mouse brain development cortical layer 5 pyramidal neurons switch between two distinct active circuit motifs. In figure 7G the authors show how perturbations in autism associated genes lead to alterations in activity levels in Rbp4 positive neurons.
Please provide a comprehensive protocol for the experiment shown in Figure 7G. Focus particularly on the transgenic mouse lines used, microscopy set up, reagents, and the experimental paradigm used in the experiment.

Group 7:
Title: "Antisense oligonucleotide therapeutic approach for Timothy syndrome".

Assignment: The authors demonstrate that patents with Timothy syndrome could potentially be treated using ASO, targeting the aberrant splicing variant. In figure 5 they ultimately demonstrated the efficacy of the ASO therapy in vivo using an innovative patient derived brain organoids transplantation paradigm.
Please provide a comprehensive protocol for the experiment shown in Figure 5E.
Focus particularly on the ASO strategy design, the PSC lines and the specific brain organoids protocol used, the reagents, the transplantation set up and the neuronal recording paradigm used in the experiment.