Institut des Neurosciences de Montpellier

Inserm U1051 : Déficits sensoriels et moteurs

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Manuscript submission

Title: Multicolor labeling using *in utero* electroporation of MAGIC Markers to individualize cortical mouse astrocytes

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Dear Dr Myers,

It is my pleasure to submit the manuscript by Dumas et al. to your consideration for publication in *Journal of Visualized Experiments*.

I have been invited by Isabel Martinez Garay, guest editor of "Standard and novel methods in the study of corticogenesis" to contribute to this method collection with a methods video featuring the techniques we use to study lineage tracing during cortical development.

For this purpose, my co-authors and I have put together a manuscript that details the key steps of multicolor labeling based on *in utero* electroporation (IUE) to single out astrocytes in the mouse cerebral cortex, and analyze their volume and morphology with a user-friendly image analysis pipeline.

Therefore, we present here a protocol to label cortical astrocytes with the multicolor MAGIC Markers strategy, which relies on piggyBac/Tol2 transposition and Cre/*lox* recombination to stochastically express distinct fluorescent proteins (FP) in nearby cortical progenitors using IUE. This results in combinations of color markers expressed by cortical progenitors prior to the start of gliogenesis, which enable to track their descent, including astrocytes, at the individual cell level, from embryonic to adult stages. Semi-sparse labeling is achieved by adjusting the concentration of electroporated MAGIC Markers vectors while color contrasts resulting from distinct FP expression in astrocyte processes permit to collect key information about their territorial volume (using IMARIS) and complex morphology (using Vaa3D). In summary, our present work provides a comprehensive experimental workflow including the details of the electroporation procedure, multichannel imaging with confocal image acquisition and computer-assisted 3D segmentation.

Importantly, this convenient and robust multicolor strategy helped us to uncover key features of cortical astrocyte development (Clavreul et al, 2019). Indeed, this technique gives an easy and quick access to individualized cortical astrocyte surface and morphology in the mouse cerebral cortex at various developmental stages. Finally, this powerful approach will be useful to the research community as it offers an alternative way to analyze cortical astrocyte anatomical properties in various experimental conditions without resorting to complex crosses with transgenic reporter lines.

Given the above elements, I thank you for considering our manuscript for publication in *Journal of Visualized Experiments.*

Sincerely yours,

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