

IS A REDUCTION IN EMBRYO LYSIS POSSIBLE USING THE MICRO-EPORE (MEP)?

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CRISPR-Cas9 gene editing technology has increased our ability to produce Genetically Modified mice not only in a highly specific manner but also quickly and on demand. Establishments can easily design and produce the CRISPR guides and donors needed to engineer their mice. However despite these advances in the field there is still the need for the highly technical task of Pronuclear or Cytoplasmic microinjection to deliver the reagents. These methods can require a large number of embryos in order to reach the required number of founders, one factor contributing to this high embryo usage is lysis due to damage to the embryo by the injection needle.

We have used the MEP to aid cytoplasmic and pronuclear microinjection of one and two cell embryos and to see if it helped to reduce embryo lysis rates. A chloride silver wire is inserted into the injection needle ensuring it reaches the injection mix, then on pressing the MEP foot switch just before injection the MEP then delivers a localized voltage signal to the site of injection which aids needle entry into the embryo with minimal damage.

If you are familiar with a microinjection rig then the MEP is an easy setup however inserting the filament into the injection needle and mix can be tricky and time consuming. Our injection needles are pulled on a pipette puller and cut to an exact length, then filled by capillary action and further backfilled using a microloader to ensure the filament makes contact with the CRISPR mix.

Audible and visual alarms are built into the MEP giving distinctive tones and flashes of green or red colours on the MEP monitor when pressing the foot switch, this ensures that microinjection is carried out properly. We used the MEP for Crispr-CAS9 targeted deletion, point mutations (oligo donors) and more complex projects (long single stranded donors). Our data shows intermittent reduced lysis rates, no difference in birth rates, mutation rates and on-target mutation rates when using the MEP for both pronuclear and cytoplasmic delivery but it is still a useful tool to try to reduce lysis rates particularly for people who are new to microinjection.