

Other factors affecting the success of nuclear transplantation-based transgenesis

1. Egg quality contributes significantly to the level of post-gastrula development which is obtained from sperm nuclear transplantation. To obtain good post-gastrula development, eggs must be generally healthy. In particular, they should have even pigmentation and should be firm enough to hold their shape well after dejellying. In addition it is important that they do not become activated before they are injected with nuclei. When egg quality is poor, a fraction of the embryos will show morphogenetic defects resulting in incomplete blastopore closure during gastrulation. This problem is often compounded by expressing genes at high levels during the gastrula stages. Therefore embryos expressing genes from the CMV promoter are more likely to show non-specific gastrulation defects than embryos expressing genes from strong promoters that are turned on after gastrulation.
2. Transplantations should be performed and eggs incubated after transplantation at temperatures no higher than 22°C. We have found that transplantation and early incubation of activated eggs at elevated temperatures (24-25°C) lowers the frequency with which plasmids are expressed in batches of nuclear transplantation-derived embryos. Embryos in these batches also frequently express plasmids in only one quarter to half of the expected cells. We believe that acceleration of the first cell cycle which occurs at 24-25°C may give these embryos inadequate opportunity to integrate introduced plasmids prior to first cleavage, thus resulting in more chimeric and non-expressing embryos.
3. It may be important to note that although this technique is very efficient and workable, it involves several steps; all of which are critical for its success. Therefore we suggest that anyone trying to learn the technique does so in steps, rather than all at once. For example, one should first learn to isolate sperm nuclei and transplant them into eggs. Once this can be done successfully, resulting in normal development, then one can then determine whether sperm nuclei, swollen in extracts gives normal development. If swelling of sperm in extract has no adverse effects on the level of development obtained after transplantations, one can add plasmid and enzyme to the reaction, thus reconstituting the whole transgenesis procedure.
4. Do not be fooled by pseudocleavage in eggs that have been activated but did not receive a nucleus. Activated eggs try to cleave at around the correct time after injection (often slightly earlier than eggs receiving nuclei), but the cleavage furrow is usually very shallow and never completely cleaves through the egg. Activated eggs continue to try to cleave every thirty minutes or so, but are always unsuccessful. Unfortunately activated eggs can sometimes be mistaken for embryos that later die. Make sure that the cleavage furrows in your embryos go all the way into the vegetal hemisphere. Also collect only embryos with healthy and robust cleavage furrows in general.