BioSpotlight

Transferring Embryos with Ease

Minimizing pain and discomfort for animals used in research studies is a growing concern for many scientists. Advances that make working with animals in the lab faster and easier while at the same time reducing their stress levels can have a significant impact on research studies. Up to now, scientists conducting gene transfer or targeted gene modifications have had to perform small animal surgery to implant embryos successfully, which creates the potential for complications arising from the use of anesthesia or post-surgical infection. B. Spears and colleagues at the University of Kentucky address this problem by creating a new instrument specifically designed for the transcervical delivery of embryos into female mice without the need for surgery. Their prototype device, which the authors call the non-surgical embryo transfer (NSET) device, consists primarily of a P2 pipet tip connected to catheter tubing. A speculum is inserted into the mouse vagina and positioned through the cervix to guide the NSET device to the proper location. The embryos can then be dispensed into the uterine horn without the need to anesthetize the animal.

Once trained in the technique, the entire procedure takes only seconds to perform. In a series of experiments, the authors present evidence demonstrating that the NSET device results in similar embryo transfer efficiencies as the traditional surgical procedure. The authors go on to examine embryo transplantation at different developmental stages; they found that the blastocyst stage (embryonic day 3.5) resulted in the highest number of pup births (33% of those implanted) using the NSET device, which is a result similar to findings for the traditional surgical procedure. Experiments also suggest that the NSET device can be used to create chimeric mice from embryos containing embryonic stem cells. In the end, the new NSET device should make transcervical embryo transfer easier, safer, and more efficient for all, as well as open the technique to a greater range of researchers by eliminating the need for complicated, time-consuming surgical procedures.

See “A device for the simple and rapid transcervical transfer of mouse embryos eliminates the need for surgery and potential post-operative complications” on page 919.

Uninhibited Profiles

In the world of forensic DNA analysis, samples often come in small amounts mixed with substances that inhibit template amplification by PCR. This creates a problem for criminologists as they must often extensively purify samples prior to DNA profiling—even at the risk of further template loss. P. Radstrom and colleagues tackle this problem by exploring the use of alternative polymerases and buffers to amplify these problem templates without the need for additional DNA purification steps. Since *Taq* DNA polymerase is known to be susceptible to PCR inhibitory substances, the authors tested nine different DNA polymerases and buffer systems (including different versions of *Taq* polymerase) for their ability to amplify low-copy number DNA templates from crime scene saliva samples that, in prior attempts, did not yield amplification products using standard approaches and polymerases. To analyze and compare the results of their tests, the authors developed a statistical model to evaluate the effects of various chemicals, polymerases, and conditions on forensic DNA profiles. Using these nine polymerases in combination with their model, the authors found that 20 out of the 32 previously inhibited samples showed a significant improvement in the DNA profile when amplification was performed with three of the alternative polymerases. When the DNA profiles were then ranked by an experienced crime scene investigator, 28

of the samples were categorized as being of higher quality than profiles generated with the same samples using standard *Taq* DNA polymerase kits. The use of such alternative polymerases for the amplification of crime scene DNA should increase the likelihood of generating a usable DNA profile, even with previously hard-to-work samples, thus giving another tool to specialists in the field. In addition, the authors note that the model they have developed will aid in both the quality control of forensic sample analysis now and the evaluation of novel forensic DNA analysis procedures in the future.

See “Improved forensic DNA analysis through the use of alternative DNA polymerases and statistical modeling of DNA profiles” on page 951.

Covalently Closed Circles in the Cytoplasm

Transgenic animals are commonly created by microinjection of linearized DNA into the pronuclei of mammalian fertilized eggs. Although effective, this approach requires extensive technical expertise as well as expensive micromanipulation equipment. Since pronuclei are easily damaged, injection often leads to a large proportion of lysed zygotes; while in some species, pronuclei are difficult to even visualize due to an abundance of colored lipids in the zygote. To identify the pronuclei in bovine eggs, a researcher often needs to

centrifuge the zygotes, which can reduce their viability. In this issue, a research team from the Friedrich Loeffler Institute (Greifswald, Germany) led by W. Kues provides a simple, but elegant solution to these challenges. The authors show that injection of covalently closed circular (ccc) plasmids directly into the cytoplasm of fertilized bovine and murine eggs results in a high ratio of plasmid-expressing blastocysts. Their data indicate that more than 80% of bovine zygotes were viable in culture following injection with conventional plasmids, plasmids containing a scaffold/matrix attachment region, or with bacterial artificial chromosomes. Co-injection of two plasmids was equally successful, suggesting that microinjection success rates could be monitored by co-injection with a separate plasmid encoding a fluorescent marker. Following injection, plasmids were translocated to the nucleus within 12 hours and were maintained epistemally in the cultured zygotes. Promoters controlling green fluorescent protein (GFP) expression showed species-specific onset of transcription at the expected times during embryogenesis regardless of the timing of injection, an indication that plasmids were properly processed by the cellular machinery. The onset of transcription was dependent upon the methylation status of the plasmid, where unmethylated promoters led to expression of GFP in mouse embryos approximately 12 hours after injection. Although the methylated plasmids did not undergo demethylation

Expression of fluorescent proteins encoded by BAC and S/MAR proteins.

with the rest of the embryonic genome, the expression of GFP matched the expected developmental pattern of the associated promoter. These results demonstrate that cytoplasmic microinjection of ccc plasmids is an efficient and effective alternative for ectopic expression of foreign DNA in mammalian embryos and has the potential to be useful in cellular reprogramming studies during early ontogenesis.

See “Cytoplasmic injection of circular plasmids allows targeted expression in mammalian embryos” on page 959.

Shedding Inhibitions

PCR inhibitors in DNA samples can cause reduced amplification efficiency or reaction failure, and are especially problematic in reactions with degraded or exceedingly low amounts of template such as ancient or forensic DNA. In such cases, it is necessary to detect and accurately measure the level of PCR inhibition. For quantitative PCR, this can be done using two methods: (i) determining the difference in quantification cycle (C_q) of an exogenously added internal positive control (IPC) DNA in sample versus control reactions, or (ii) analysis of amplification efficiency based on the slope of amplification plots. While these methods have been applied separately in the past, C. King, H. Poinar, and colleagues at McMaster University (Ontario, Canada) demonstrate that a combination of both approaches is better for characterizing and

measuring PCR inhibition, especially in reactions with low-copy number templates. They tested their new approach on a group of ancient DNA samples from permafrost soil, mammoth hair and bones, and packrat paleofeces. Extracted DNA from these samples was added to qualitative PCR (qPCR) reactions along with a human β -2-microglobulin cDNA IPC template

not present in any of the ancient DNA samples. Inhibition of PCR was measured by comparison of IPC amplification in the sample qPCR reaction relative to the control qPCR reaction containing only IPC using the two methods. First, the ΔC_q between the sample and control reactions was determined, and then the Hill slope of a sigmoidal curve fitted to the raw fluorescence data for each reaction was calculated and the “amplification efficiency” (E) was defined as the sample’s Hill slope expressed as a percentage of the control’s Hill slope. Using these two measurements, the authors examined PCR inhibition in the ancient DNA samples. While the expected inhibition pattern of high ΔC_q and low E was observed, deviations from this pattern (i.e., high ΔC_q with high E or low ΔC_q with low E) were detected with their approach that would not have been observed using either approach alone. Their inhibition testing method also proved useful for optimizing combinations of PCR facilitators such as BSA, additional *Taq* polymerase, and dilution of the sample DNA to maximize DNA yields and qPCR accuracy in subsequent reactions.

See “A quantitative approach to detect and overcome PCR inhibition in ancient DNA extracts” on page 941.

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A mummified specimen of a young woolly mammoth, found in Siberian permafrost. Institut Royal des Sciences Naturelles de Belgique, Bruxelles. Image reprinted with permission © 2007 GNU Free Documentation License.