

## ACHIEVING SUCCESSFUL FISH TRANSGENESIS WITH ESCO CO<sub>2</sub> INCUBATORS

### Abstract

Fish transgenesis serves as a platform for the development of fish species with faster growth and increased disease resistance. One of the most efficient and safest ways to deliver genes into fish spermatogonial cells is through the use of nanomaterials. Such technique requires cell culture methods and Esco CO<sub>2</sub> incubators prove to be just the right incubators to achieve success in nanomaterial gene transfection.

### Introduction

Transgenesis is the introduction of a foreign DNA, which could be transmitted to subsequent generations, into an organism. This technique has become a useful tool in gene expression and gene function analyses.<sup>1</sup> Moreover, a number of large, complex, and biologically active recombinant proteins have been successfully expressed in transgenic mammals.<sup>2</sup> Fish transgenesis has particularly gained significance in the last decade due to its benefits for aquaculture— that is, fish species have been developed with faster growth and increased disease resistance. Aside from its commercial use, transgenic fish studies have been developed as models for biomedical research experiments. Transgenic fishes are great candidates as alternative to vertebrate laboratory models.

One of the most commonly used fish species for transgenic studies is the tilapia fish (*Oreochromis niloticus*). The tilapia is a significant group in global aquaculture as it is the 2<sup>nd</sup> most important food fish. Aside from its significance in aquaculture, the tilapia is also an excellent laboratory animal, being relatively robust, disease-free, and fast growers.<sup>3</sup> For these reasons, the tilapia fish is a suitable subject for transgenic experiments.

Transgenic studies are considered successful when there is high efficiency of gene expression and low cell death rates during the delivery methods. Yet, some current methods cause high cell death rates, such as those using commercial reagents and electroporation. Other methods just fail to efficiently transfect cells, especially cells from primary culture.<sup>4</sup> To address these issues, a recent study used nanomaterials to transfect genes into the tilapia fish, thus demonstrating that transgenesis could be done in a more efficient manner.

### Gene Transfection in Nile tilapia using Nanomaterials

Researchers have recently demonstrated that nanomaterials such as multiwalled carbon nanotubes (MWCNTs), nanographene oxide (NGO), and gold nanorods (NRs) could be functionalized and complexed to the cyan fluorescent protein gene to achieve a more efficient gene delivery into Nile tilapia spermatogonial stem cells (SSCs). SSCs are considered difficult-to-transfect cells, especially when using the common commercial reagents for transfection or through electroporation. Thus, SSCs are such good models to demonstrate the efficiency of transfection with nanomaterials.<sup>4</sup>

<sup>1</sup> Rembold et al. 2016.

<sup>2</sup> Zbikowska 2002.

<sup>3</sup> Maclean et al. 2002.

<sup>4</sup> Tonelli et al. 2016.

The researchers first isolated SSCs from tilapia testes and then fragmented in pieces and treated with several reagents to obtain an enriched spermatogonia cell suspension. The cell suspension was then plated on 6-well dishes. Nanomaterials were separately synthesized. The cyan fluorescent protein AmCyan1 gene plasmid DNA was complexed to the functionalized nanomaterials. The SSCs plated on the 6-well plates were then exposed to the nanomaterials and incubated for 24 hours at 28°C and 5% CO<sub>2</sub> using an Esco CO<sub>2</sub> incubator.<sup>4</sup>

After 24 hours of incubation, assessment and analysis showed that nanomaterials were able to deliver plasmid DNA to the tilapia SSCs and cyan fluorescence was observed. Moreover, there were significantly lower cell death when SSCs were transfected with nanomaterials than when commercial reagents were used.<sup>4</sup>

The study was able to show that functionalized nanomaterials could be used to transfect Nile tilapia SSCs with foreign DNA. The gene delivery was efficient with significantly low cytotoxicity. The use of functionalized nanomaterials in SSCs presents the feasibility of nanobiotechnology as a useful tool in fish transgenesis.<sup>4</sup>

### **Fish Stem Cell Culture with Esco CO<sub>2</sub> Incubators**

The study tapped the competence of an Esco CO<sub>2</sub> incubator for the incubation of the spermatogonial stem cells with the nanomaterials. Esco CO<sub>2</sub> incubators are built to satisfy different facets of biomedical research, from fish stem cell research to cancer research and drug discovery.

For fish stem cell culture such as that demonstrated in the abovementioned study, the Esco CelCulture® with Integrated Cooling System is best to be used for culture of samples (Fig.1). This model of the CelCulture® is built with a highly efficient Peltier cooling system that provides precise heating and cooling inside the chamber, maintaining a uniform environment that is safe for your samples. The wide temperature range of this model, 8°C below ambient to 60°C, equates to a wide range of applications, including fish stem cell culture. Fish cell lines thrive over a wide temperature range, depending on the species, from 0-28°C.<sup>5</sup> This temperature range is considerably lower than that of used in mammalian cell culture, which is usually at 37°C.



Fig. 1. The CelCulture® CO<sub>2</sub> Incubator with Integrated Cooling System for fish stem cell culture.

<sup>5</sup> Bols and Lee 1991.

The CelCulture® CO<sub>2</sub> Incubator is equipped with complete contamination control methods, such as the 90°C moist heat decontamination cycle, ULPA filter, Isocide™ antimicrobial powder coating, and 0.2 micron inlet filter for gas inputs, to protect your samples from contaminants. These features prove that the CelCulture® CO<sub>2</sub> Incubator is the perfect CO<sub>2</sub> incubator for fish stem cell research.

## Conclusion

Fish transgenesis could be done more efficiently and with lower cytotoxicity via gene delivery using functionalized nanomaterials. This technique involves the incubation of fish spermatogonial stem cells exposed to the nanomaterials in a CO<sub>2</sub> incubator. The Esco CelCulture® with Integrated Cooling System is a competent candidate for such cell culture due to its wide temperature range, precise parameter control, and superior contamination control.

The successful use of nanobiotechnology in fish transgenesis poses a number of implications to the future of biomedical research and Esco CelCulture® CO<sub>2</sub> incubators are ready to be your partner in this new era of cell science.

## References

Bols, N.C. and Lee, L.E.J. (1991). Technology and uses of cell cultures from the tissues and organs of bony fish. *Cytotechnology* 6: 163-187.

Houdebine, L.M. and Chourrout, D. (1991). Transgenesis in fish. *Experientia* 47: 891-897. Maclean, N., Rahman, M.A., Sohm, F., Hwang, G., Iyengar, A., Ayad, H., Smith, A., Farahmand, H. (2002). Transgenic tilapia and the tilapia genome. *Gene* 295: 265-277.

Rembold, M., Lahiri, K., Foulkes, N.S., Wittbrodt, J. (2006). Transgenesis in fish: efficient selection of transgenic fish by co-injection with a fluorescent reporter construct. *Nature Protocols* 1(3): 1133-1139.

Tonelli, F.M.P, Lacerda, S.M.S.N., Paiva, N.C.O., Lemos, M.S., de Jesus, A.C., Pacheco, F.G., Correa-Junior, J.D., Ladeira, L.O., Furtado, C.A., França, L.R., and Resende, R.R. (2016). Efficient and safe gene transfection in fish spermatogonial stem cells using nanomaterials. *RSC Adv.* 6: 52636- 52641.

Zbikowska, H.M. (2003). Fish can be first – advances in fish transgenesis for commercial applications. *Transgenic Research* 12: 379-389.