

SAINT-MIX™ for the transfection of Embryonic Stem cells

In collaboration with the Division of Medical Biology, University of Groningen.

Knock-out animals (and transgenes) are a well described and indispensable tool in fundamental research in the unraveling of the function of genes. The production of these animals is not easy and one of the primary hurdles to overcome is the transfection of Embryonic Stem cells (ES cells). One way to transfect these cells is by electroporation, which has certain drawbacks in the amount of DNA needed, the almost 90% mortality of these hard to grow cells and the lack of viability after the transfection. New methods are investigated to make transfection of these stem cells easier, more reliable and reproducible and less expensive in cells, DNA and direct costs.

Since SAINT-MIX™ proved to be a very efficient transfection system for the transfection of several

eukaryotic cell lines (see references) we undertook to investigate whether SAINT-MIX™ could be successfully used to transfect ES cells.

In this preliminary report Saint BV would like to discuss briefly some of the data.

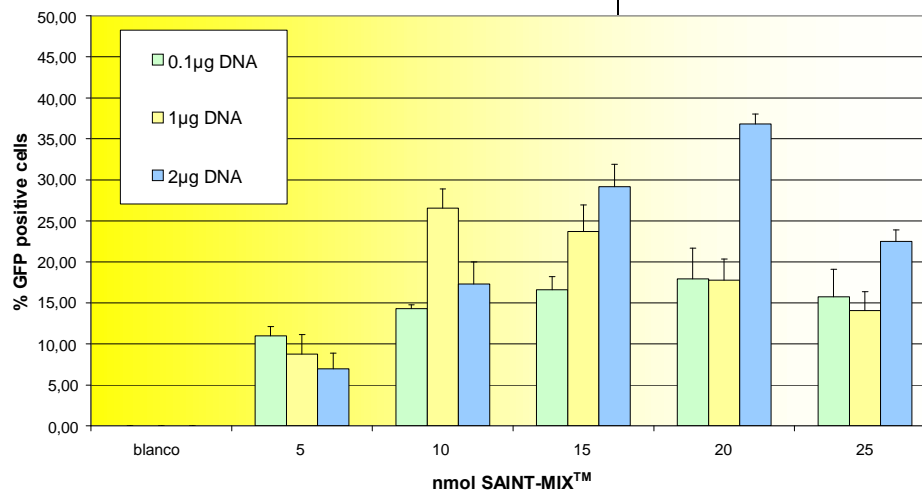
ES cells (HM1, derived from E14) were grown on a fibroblast feeder layer and transfected with a CMV-GFP construct using SAINT-MIX™ in several concentrations.

Transfection efficiencies were highly reproducible and > 35% when only 2 µg DNA was used with 20 nmol SAINT-MIX™ in a well of a 6 well plate (2.5x10⁵ cells per well).

Also downscaling to 96 wells (1.5x10⁴ cells), to

reduce the amount of cells did not affect the efficacy!. SAINT-MIX™ allows transfection on gelatin plates which allows immediate analysis!.

ES transfection with SAINT-MIX™ (6-well plate)



Graph 1: transfection efficacy as a result of different concentrations of SAINT-MIX™ combined with different amounts of DNA. The optimal ratio (2 µg DNA with 20 nmol SAINT-MIX™) resulted in transfection efficacies

of more than 35%

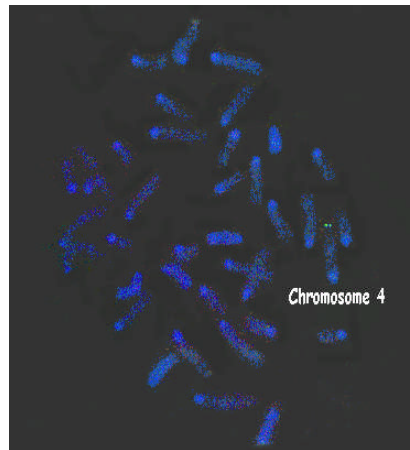
In the production of knock-out animals or transgenes, huge numbers of trials are needed before the right gene is knocked out or a gene is inserted on the right place without insertional problems. Every single trial requires millions of cells and several micrograms of DNA.

Using SAINT-MIX™ the amount of cells can be reduced to thousands in 96 wells plates, accordingly the amount of DNA needed can be reduced. These material reductions combined with a high transfection efficacy which results in more clones to chose from plus a higher reproducibility of the

separate experiments make SAINT-MIX™ very

suitable for ES cell transfections

Picture:
Fluorescence In Situ
Hybridisation
analysis showed
integration in
chromosome 4



SAINT references:

- 1 **van der Woude et al:** Novel pyridinium surfactants for efficient, nontoxic in vitro gene delivery. *Proc Natl Acad Sci USA*, 1997 Feb vol 94: 1160-1165
 - 2 **van Leeuwen EB, et al:** Transfection of small numbers of human endothelial cells by electroporation and synthetic amphiphiles. *Eur J Vasc Endovasc Surg*, 1999 Jan vol 17: 9-14
 - 3 **Audouy S, et al,** In Vivo evaluation of the safety and toxicity of a new cationic liposome formulation for human gene therapy applications. *AACR meeting, April 10-14 1999.*
 - 4 **Oberle V, et al:** Lipoplex formation under equilibrium conditions reveals a three-step mechanism. *Biophys J* - 2000 Sep 79: 1447-1454
 - 5 **Audouy S. et al:** Serum as a modulator of lipoplex-mediated gene transfection: dependence of amphiphile, cell type and complex stability. *J Gene Med* 2000; 2: 465-476.
 - 6 **Audouy S, et al:** Cationic lipid-mediated transfection in vitro and in vivo (Review) *Molecular Membrane Biology*, 2001, 18, 129-143
 - 7 **van Leeuwen E.B.M.,** AN SV40 large T-antigen immortalized human umbilical vein endothelial cell line for anti-endothelial cell antibody detection. *Clin. And Exp. Rheum.* 2001;19:283-290
 - 8 **Shi F, et al:** Efficient cationic lipid-mediated delivery of antisense oligonucleotides into eukaryotic cells: down-regulation of the corticotropin-releasing factor receptor. *Nucleic Acids Res*, 2001, vol. 29, No. 10, 2079-2087
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