Drug-free selection of stable transfectants using rIB4-SAP

(continued from page 1)

mammalian cells, except in humans and Old World monkeys.

Transient expression of EndoGalC should lead to resistance in some transfected cells to isolectin BS-I-B_4 conjugated to saporin (rIB4-SAP; #IT-10; Advanced Targeting Systems Inc.), which causes cell death of α-Gal epitope-expressing untransfected cells, mainly because rIB4-SAP, internalized via specific binding to the cell-surface α-Gal epitope, inhibits protein synthesis.[1,2] During the period (~3 days after transfection) of transient expression of exogenous DNA (pCAG/EndoGalC), expression of α-Gal epitope on the cell surface is lost, allowing the cell to survive rIB4-SAP treatment (as schematically depicted in Fig. 1). Concomitantly, the co-introduced plasmid carrying the GOI has a chance to be integrated into host chromosomes. Untransfected cells continue to express the α-Gal epitope, which is specifically recognized by rIB4-SAP, and are eliminated after about 10 days of cultivation (Fig. 1). Thus, the surviving cell population is expected to express the GOI and the α-Gal epitope, since pCAG/EndoGalC introduced into a cell is lost during the 10-day cultivation after rIB4-SAP treatment. This concept has been previously explored by us,[3] in which we demonstrated that the GOI could be effectively integrated into host chromosomes via the *piggyBac* system after rIB4-SAP treatment.

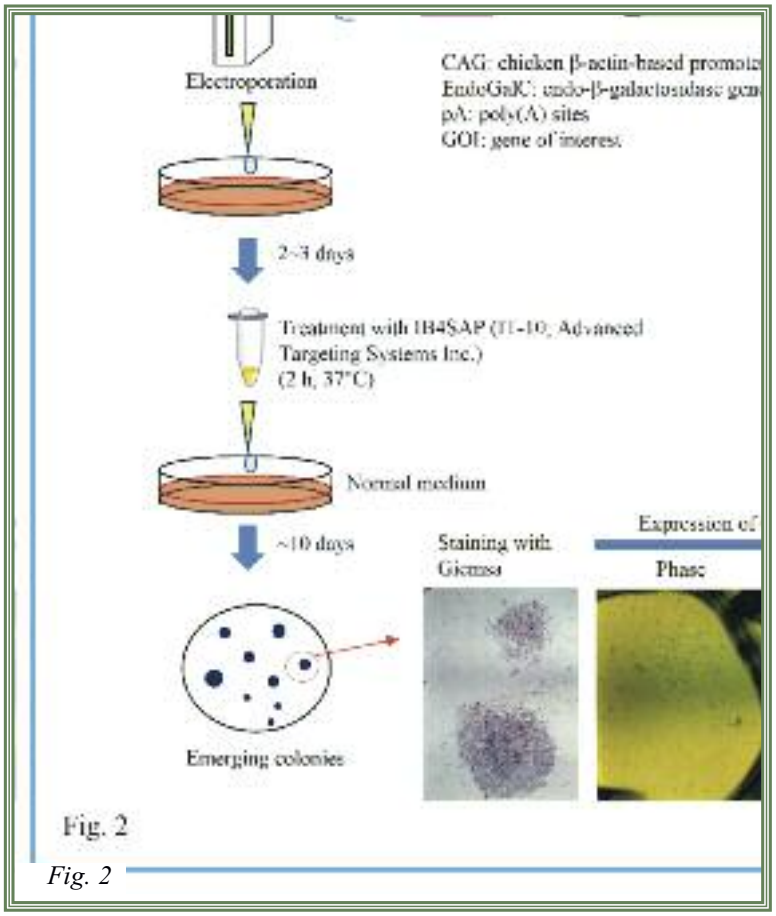

Fig. 2

Fig. 2

This drug-free acquisition of stably transfected cells is a very simple and convenient system. We prepared only two vectors, an EndoGalC-expression vector (in circular form) and a vector (in linearized form) carrying a GOI (Fig. 2). These vectors were mixed with trypsinized porcine fetal fibroblasts and were then delivered to the cells via electroporation. Immediately after electroporation, all transfected cells were seeded onto a dish containing normal medium and cultured for 2–3 days. The cells were then trypsinized and treated with 80 μg/mL rIB4-SAP in a 0.5-mL microfuge tube for 2 h at 37°C. Then, these cells were seeded in normal medium and cultured for ~10 days until colonies developed. The colonies were stained with Giemsa and were seen to express the GOI (tdTomato), as shown in the images at the bottom of Fig. 2. This system does not require selective drugs such as G418. Therefore, it does not require a pilot study to test the effectiveness of the drugs using untransfected cells. Furthermore, it will be useful for gene delivery to cells that are resistant to several selective drugs.

References

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3. Sato M, Inada E, Saitoh I, Matsumoto Y, Ohtsuka M, Miura H, Nakamura S, Sakurai T, Watanabe S. (2015) A combination of targeted toxin technology and the piggyBac-mediated gene transfer system enables efficient isolation of stable transfectants in nonhuman mammalian cells. *Biotechnol J* 10(1):143-153.