102. 利用第二型腺病毒伴隨病毒載體轉染建立攜有綠螢光蛋白質與新黴素耐受基因之小鼠胎體成纖維細胞株

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本研究之目的係利用第二型腺病毒伴隨病毒載體（adeno-associated viral vector type 2, AAV2）進行 STO 小鼠胎體成纖維細胞（STO mouse embryonic fibroblast）之基因轉殖，期建立攜有外源綠螢光蛋白質（green fluorescent protein, GFP）及新黴素耐受基因（neomycin resistant gene, Neo^r）之 STO 小鼠胎體成纖維細胞株，以做為豬胚幹細胞之供養層細胞。試驗結果顯示，利用 G418 進行 STO 細胞之選殖試驗，最適之選殖濃度為 400 μg/ml，其選殖 7 天後的死亡率為 41.8 ± 11.6%。利用 AAV2 載體轉染 STO 小鼠胎體成纖維細胞後，轉染率可達 50.9 ± 17.1%，利用於培養液中添加 400 μg/ml 的 G418 進行轉染後選殖，經過 4 次之繼代選殖後，純度達 70.2 ± 12.0%，再配合以流式細胞分析法（flow cytometry）進行 GFP 表現陽性（GFP$^+$）細胞分離，分離後之 STO/GFP$^+$小鼠胎體成纖維細胞之純度可達 93.6 ± 2.8%。經過轉染後的 STO/GFP$^+$細胞株，用於培養豬胚幹細胞，能維持幹細胞群落之未分化狀態，顯示 STO/GFP$^+$細胞株可做為豬胚幹細胞之供養層細胞。因為 STO/GFP$^+$細胞株攜有外源性 GFP 及 Neo^r，未來可作為豬與其他物種的胚幹細胞進行基因轉殖試驗後，以 neomycin 選殖時之供養層細胞，為胚幹細胞之基因轉殖試驗提供有價值的應用基礎。

關鍵語：綠螢光蛋白質、新黴素耐受基因、STO 小鼠胎體成纖維細胞

TRANSFECTION OF MOUSE EMBRYONIC FIBROBLAST CELL LINE WITH GFP AND NEOMYCIN RESISTANT GENES BY ADENO-ASSOCIATED VIRAL VECTOR TYPE 2

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The purpose of this study was to establish a mouse embryonic fibroblast cell line that carrying green fluorescent protein (GFP) and neomycin resistant (Neo^r) genes Murine embryonic fibroblasts from STO cell line were transfection with adeno-associated viral vector type 2 (AAV2) followed by cultured in G418 selection medium. The results showed that the optimal concentration of G418 selection was 400 μg/ml and its mortality was 41.8 ± 11.6% after 7 days of selection culture. The transfection rate of STO cells using AAV2 was 50.9 ± 17.1%, determined by GFP expression. Following the selection with G418-containing medium (400 μg/ml) and flowcytometry sorting, the proportion of GFP-expressing STO cells was promoted to 70.2 ± 12.0% and 93.6 ± 2.8%, respectively. The transfected STO/GFP$^+$ cell line could support the maintenance of undifferentiated status for the porcine embryonic stem cells. This STO/GFP$^+$ cell line which carrying Neo^r gene might provide as a valuable source of feeder layer for cultivation and selection of transgenic ES cells, which were transfected with Neo^r gene as a selection marker.

Key Words: Green fluorescent protein, Neomycin resistant, Mouse embryonic fibroblasts