Plasmid-Transfection of murine testicular cell lines (GC-1spg and MSC-1) with Biontex K2® Transfection system.

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Materials and Methods.

Cell culture.

Murine Spermatogonial Germ cells (GC-1 spg; ATCC-CRL-2053) and Murine Sertoli Cells (MSC-1; Peschon, 1992) were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St Louis, MO, USA), supplemented with 10% inactivated fetal bovine serum (GIBCO/BRL 235 Life Technologies, Grand Island, NY, USA) and 1% HEPES. Cell lines were cultured in the presence of 50 mg of penicillin, 50 mg of streptomycin and 100 mg of neomycin per millilitre of media (GIBCO) and maintained in a humidified incubator at 37ºC and 5% CO2.

Transfection.

Twenty-four hours before transfection, 2.5 × 105 cells/well of each cell line were plated in 24-well (16mm) plates. After 24 hrs cells reached a 95% confluency and were used for transfection. The plasmid DNAs were transiently transfected using either K2 Transfection System or TurboFect (Thermo Scientific) according to manufacturers instruction. Briefly, GC-1spg and MSC1 cells were co-transfected with 150ng of *Photinus* luciferase expression plasmid (pGL3-CMV, Promega) and 150ng of *Renilla* luciferase expression plasmid (pRL-CMV, Promega) in order to obtain both luciferase activities in the same cells. In another hand, GC-1spg and MSC1 cells were transfected with negative control plasmids pGL3-basic (150ng) and pRL-null (150ng, Promega). Grown cells into wells with 250 µl of DMEM were treated with 2.5µl of K2 multiplier reagent for two hours. For each well, 300ng of plasmids were mixed in 15µl of DMEM without SFB and in other microtube 1.2 µl of transfection reagent was mixed in 15µl of DMEM without SFB. Plasmid solutions were added to transfection solution, mixed by soft pippeting and were incubated for 15 minutes at RT. Next, mix was added to cells in each well by gently dropping. The medium was replaced 24h post-transfection and the cells were harvested at 48 hours and lysed. Cells lysates were subjected to luciferase activity assays using the Dual-Luciferase Reporter Assay System.

Results

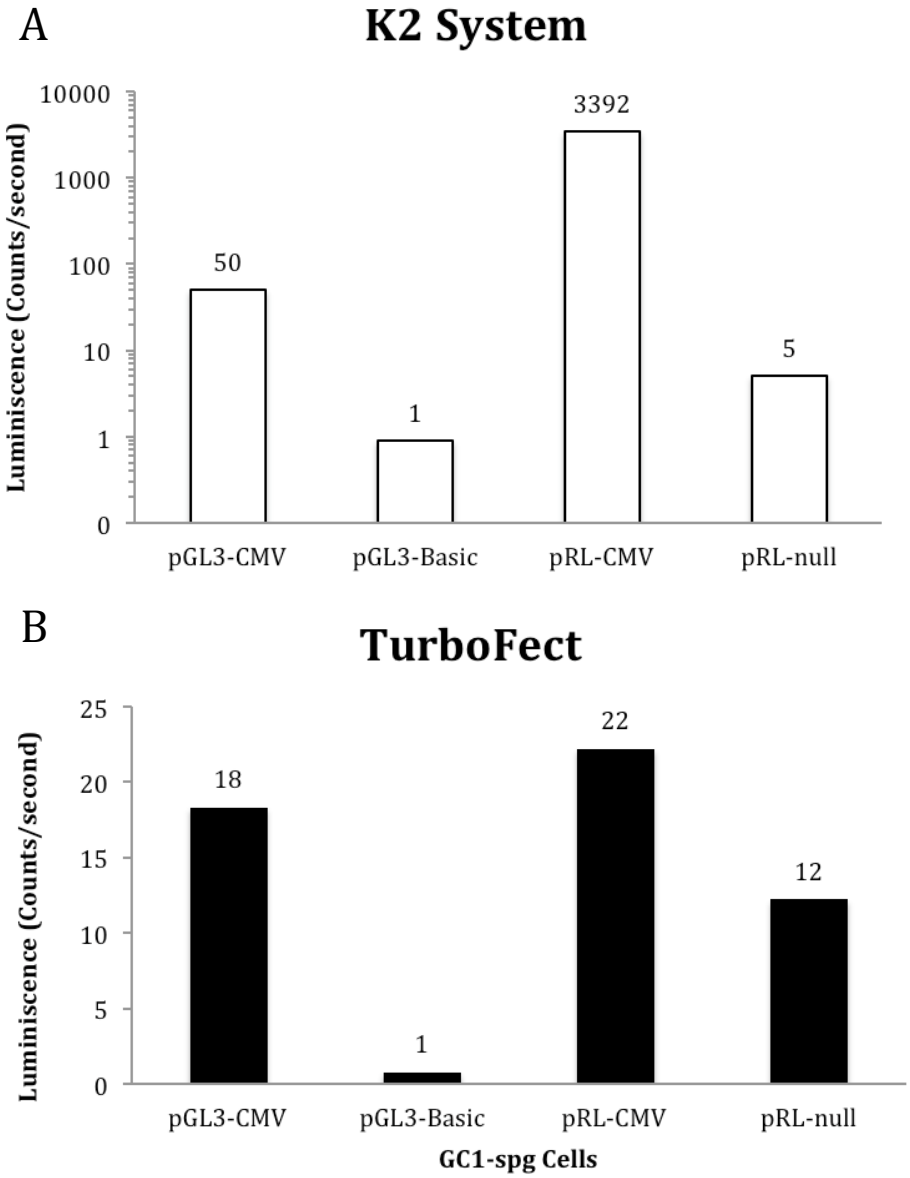

Figure 1. Luciferase activity in transfected GC-1spg cells. Comparison between K2 System vs. Turbofect transfection reagents: A)GC-1spg cell line was transfected using K2 system (open bars) with expression Luciferase plasmids (pGL3-CMV, pRL-CMV) and their control plasmids without promoter (pGL3-basic and pRL-null). B) Transfection of GC-1spg using TurboFect reagent (solid bars) with the same plasmids.

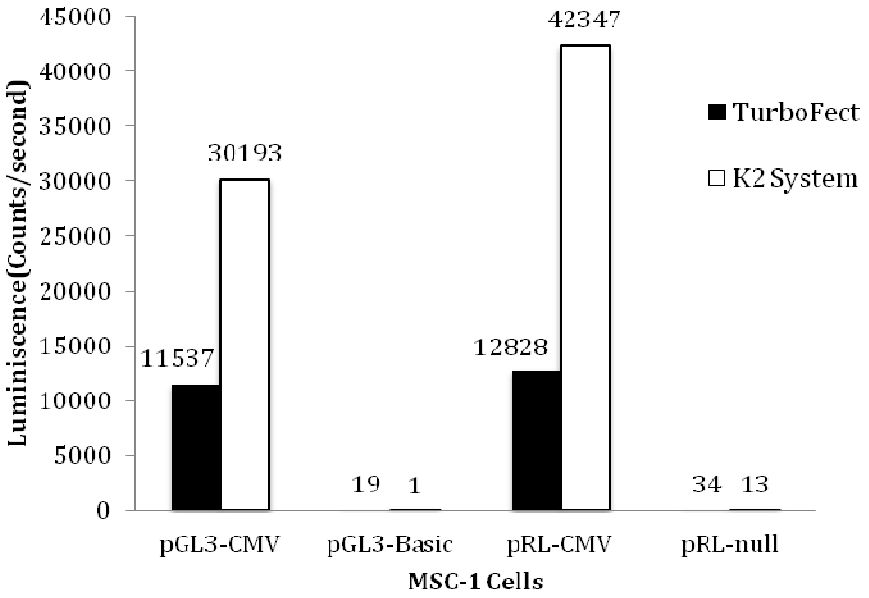

Figure 2. Luciferase activity in transfected MSC-1 cells. MSC-1 cells were transfected with *Photinus* and *Renilla* luciferase expression plasmids and their control plasmids without promoter. Transfections were performed with the K2 system (open bars) and Turbofect (solid bars), luciferase activities were determined with dual luciferase assay (Promega) after 48 hours of transfection.

The expression plasmids for *Photinus* and *Renilla* Luciferases in GC-1spg cell line, had an increased transfection with the K2 system in comparison with Turbofect which had low efficiency of transfection in GC-1spg cell line. The highest luciferase activity was observed with *Renilla* luciferase expression plasmid (pRL-CMV) under the Cito-Megalo-Virus promoter transfected in GC-1spg by the K2 system (Fig.1 A), but not with Turbofect (Fig. 1B). The background luciferase activity was very low in the two transfection systems when negative controls (without promoter plasmids) were used.

We observed a better transfection in MSC-1 cell line, which corresponds to Sertoli cells. The K2 system increased transfection over three times than Turbofect transfection system (Fig.2) and low background activity was mantained in negative controls.

Conclusions.

We consider that K2 system is a better transfection system for luciferase expression plasmids in testicular cell lines like GC-1spg and MSC-1. Both luciferase expression plasmids are usually used for analysis of promoters and indirect transcriptional activity by determination of luciferase assay. These plasmids in combination with K2 system might allow the examination of testis specific promoters with better rates of transfection and a low background in the luciferase assays.