BTX Applications

Tech-Trends
Volume 1, Series 5

Transfection of Primary Lymphocytes

High Efficiency Transfection of Primary Human & Mouse T Lymphocytes Using RNA Electroporation

ECM 830/ HT 200 System

Introduction

The difficulty associated with nonviral gene transfer methods in primary lymphocytes can result in low gene transfer efficiency and high transfection related toxicity. High efficiency gene transfection and low transfection related toxicity was achieved by electroporation using *in vitro* transcribed mRNA. RNA electroporation may be used to engineer T cells with new biological functions, providing a new and powerful tool for altering T cell biology where long term transgene expression is not necessary or desirable [1].

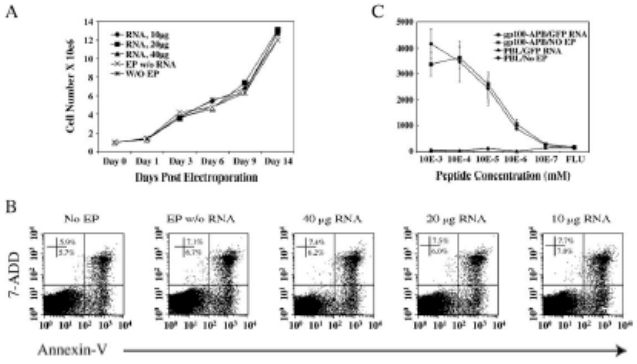

FIG.1.Effects of electroporation on T cell growth and function. (A) 5 x 106 stimulated PBLs were electroporated with increasing amounts of IVT GFP RNA as indicated. Samples were collected at different times postelectroporation and the cell numbers were counted and growth curves plotted. (B) PBLs were electroporated as in (A). Three days after electroporation, the numbers of annexin V- and 7-AAD- positive cells (% of total cells) were determined. The results shown were plotted without gating (representative of tw experiments). (C) The melanoma tumor antigen gp100-specific TCR-transduced PBL line (gp100-APB) or nontransduced PBLs as control (PBL) were electroporated with GFP (gp100-APB/GFP RNA) and compared to PBLs electroporated without RNA (gp100- APB/NO EP). Serially diluted gp100 peptide-pulsed T2 cells were cocultured with the electroporated PBLs. IFN-g secretion was detected by ELISA (representative of two experiments).

TABLE 1: Optimization of electroporation conditions for resting and stimulated T cells

	Resting human PBLs[a]			Stimulated human PBLs[b]			Resting murine splenocytes[c]	
Settings	% GFP	MFI	Viability (%)	% GFP	MFI	Viability (%)	% GFP	Viability (%)
200 V/50 ms	44	170	33	91	746	60	21	9
200 V/20 ms	69	176	62	95	538	78	18	41
200 V/10 ms	69	129	62	83	212	86	17	50
300 V/10 ms	95	286	57	nd	nd	nd	52	28
300 V/5 ms	nd	nd	nd	nd	nd	nd	49	41
360 V/5 ms	88	214	47	nd	nd	nd	56	15
360 V/2 ms	nd	nd	nd	78	437	70	nd	nd
360 V/1 ms	nd	nd	nd	90	153	81	29	52
400 V/0.5 ms	30	24	68	93	100	82	15	57
500 V/2 ms	nd	nd	nd	58	236	54	nd	nd
500 V/1 ms	nd	nd	nd	90	227	63	nd	nd
500 V/0.5 ms	nd	nd	nd	94	123	70	nd	nd
No EP	0.5	8	73	0.6	4	82	0.5	72

GFP expression of CD3+ cells and cell viability (by PIexclusion) were detected 24 h postelectroporation. nd, not determined. Data are representative of two independent experiments. a Cryopreserved PBMCs (resting PBLs) were thawed, washed twice with OPTI-MEM, and electroporated with IVT GFP RNA at the settings indicated. b PBLs were stimulated with OTK3 for 3 days. Electroporation was conducted at the settings indicated. c Fresh murine splenocytes were electroporated with IVT GFP RNA at the settings indicated.

Results

1. The RNA electroporation system improved transfection efficiency and reduced or eliminated transfection related toxicity. Over 90% transfection efficiency was achieved for human PBLs and murine T cells stimulated with ConA or antigen-specific pepetide [1].
2. Optimization experiments using different electroporation parameters for stimulated human PBLs showed that the viability of transfected T cells was in the 63-86% range 24 hours after electroporation with a transgene expression of 99% 3 days after electroporation. “Nucleofection, a modified electroporation method, has a number of limitations. With this technology special buffers (supplied only by the manufacturer) are required to be used with a specific electroporation apparatus and preset programs limit the user’s ability to develop alternative electroporation conditions”, Yanbing Zhao [1].
3. Cells stimulated from 2 to 18 days showed similar efficiencies indicating that the post stimulation time length doesn’t greatly influence RNA electroporation [1].
4. RNA electroporation shows advantages over plasmid DNA based electroporation. The plasmid must enter the nucleus before DNA can be transcribed into mRNA following electroporation. Transcription efficiency is dependent on the plasmid vector promoter which can be influenced by target cell typespecific transcription factors, ultimately determining transgene expression efficiency. Transcription is bypassed by introducing mRNA directly into the cytoplasm, and the target gene can be immediately translated into protein. GFP expression was detectable within 30min after electroporation. A synthetic molecule that mimics native mRNA’s is introduced during RNA electroporation. Bacterial based plasmids contain signal receptors that can lead to alterations in T cell biology [1].
5. Optimized electroporation conditions did not induce adverse effects on T cell viability and proliferation, or cause apoptosis [1].

Conclusion

RNA electroporation is a highly efficient tool to introduce genes into human and murine primary T lymphocytes. This technology may be used to engineer T cells with new biological functions. The availability of a BTX 96 well electroporation system makes this technology compatible with high throughput screening protocols, and scalable genetic modifications of T lymphocytes required for clinical applications. There are possible candidates which can be transferred into T cells for the adoptive immunotherapy of cancer and infectious diseases.

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Transfection of Primary Lymphocytes (cont.)

Protocol ECM ® 830 HT/96 Well

Preparation:
Human PBL's were stimulated with anti-OKT3 (30ng/ml) and IL-2 (50cu/ml) for three days, counted and washed 1 X in Opti-MEM. The cell pellet was re-suspended in Opti-MEM to a final cell density of 2.5 X 107c/ml

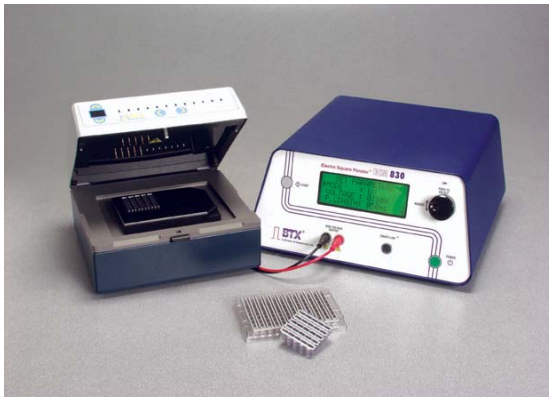
Electrical Settings:

Choose Mode:	LV
Set Voltage:	500V
Electrode Gap:	4mm
Pulse Length:	800µsec
No. of Pulses:	1
Electrode Type:	4mm Gap 96 well Plate
Field Strength:	1250 V/cm

Electroporation Procedure:

Volume:	200ul
RNA Amount:	10µg (2µg/106cell)
Temperature:	Room temperature
Pulse:	Press Automatic **Start** to activate Charge and Pulse Sequence

Post Pulse Treatment: Immediately following electroporation the cells were transferred to culture media



Results:
70-90% Cell Viability, 90% of transgene expression

References:
Zhao, Y., Zheng, Z., Cohen, C., Gattinoni, L., Palmer, D., Restifo, N., Rosenburg, S., Morgan, R., (2005). High Efficiency Transfection of Primary Human and Mouse T Lymphocytes Using RNA Electroporation. *Molecular Therapy 668.*

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