Instant Use of Cryopreserved Cells for Transient Transfection

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Introduction

Transient transfected cells are a useful alternative if the generation of a stably transfected recombinant cell line is not feasible for various reasons. For primary screening campaigns often high amounts of assay cells of consistent quality are required in a timely fashion. While this need can easily been met by the use of assay ready frozen cells or automated cell culture the supply of large bulks of transiently transfected cells is sill challenging. Although there are good technologies available for bulk transfection the provision of the required amount of host cells is still a bottle neck.

Here we show that frozen cells, if prepared in a proper way, can be used for transient transfections directly after thawing.

Preparation and Use of Transfection Ready Cells (TRCs)

Cells have been cultivated on 500cm^2 cell culture dishes or CellSTACK® (Corning) and were harvested at a cell density below 1E5 cells/cm^2 by using TrypLE™ Express (Life Technologies). Cells were pelleted by centrifugation at 180xg for 5 min and were resuspended in cryopreservation medium (culture medium + 10% DMSO) at 2.5E6 cells/ml. The cell suspension was frozen in various containers (Fig.1) at 1°C per minute using a controlled rate freezer. The frozen cells were stored at -80°C until use and kept on dry ice while handled.

To thaw the cells the containers were placed into a 37°C water bath and carefully agitated until the cell suspension was completely thawed (Tab.1). After thawing the cell suspension was pelleted at 180xg for 5 min to remove the DMSO and the cells were resuspended in their respective culture medium or buffer to perform subsequent experiments.

	Volume	Time to Thaw
Cryovials (2ml, Greiner Bio One)	2 ml	2 min
Conical Centrifugation Tubes (50 ml, Corning)	30 ml	15 min
Freezing Bags (50 ml, CryoMACS®, Miltenyi)	50 ml	5 min

Tab.1: Freezing and thawing of TRC transfection ready cells in bulk aliquots

Fig1: Different containers for freezing of assay ready cells

Transient Bulk Transfection of Transfection Ready Cells

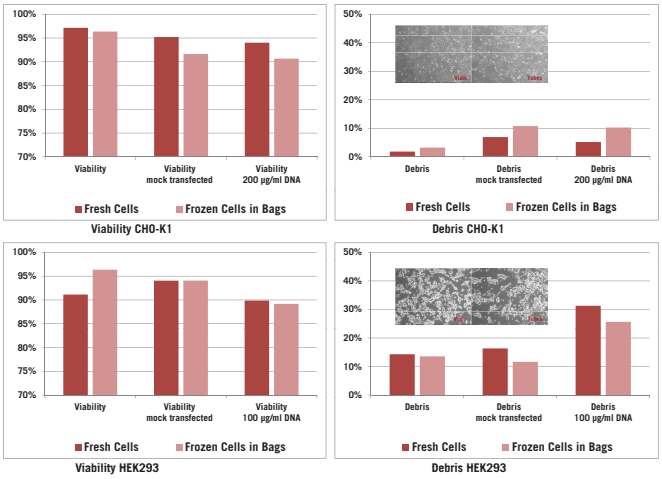

Fig.2: Transient Bulk Transfection of Transfection Ready Cells
Transfection Ready Cells frozen in cell bags were thawed and resuspended in electroporation buffer at 1E8 cells/ml. The cell suspension was mixed with plasmid DNA at a final concentration of 100 (HEK) or 200 (CHO) µg/ml and filled into the electroporation cassettes of the MaxCyte® STX™. Electroporation was performed by using the cell type specific electroporation settings provided by the instrument. Viability (A+C) and debris (B+D) were determined before electroporation as well as 24 hours after electroporation with DNA or without DNA (mock).

Transfection Efficacy of Frozen Instant Cells

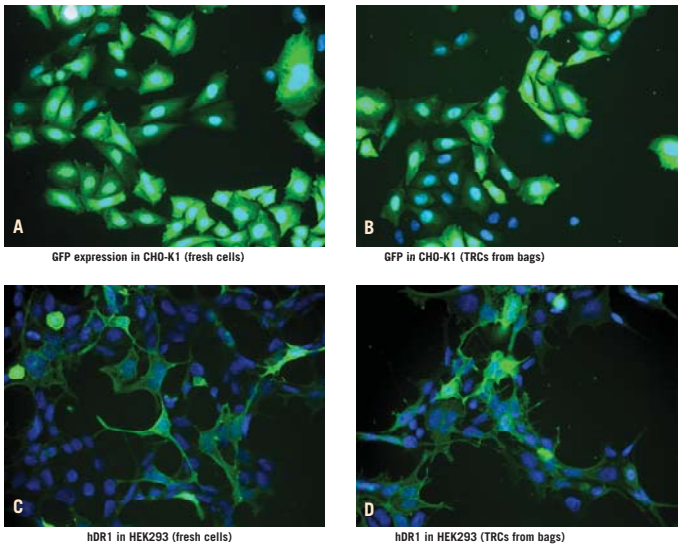

Fig.3: Bulk Transfection of TRC -Transfection Ready Cells®
Either fresh cells from a growing culture (A+C) or Transfection Ready Cells from freezing bags (B+D) were thawed and resuspended in EP buffer at 1E8 cells/ml. The cell suspension was mixed with an expression vector for GFP (A+B) or for human Dopamine receptor D1 (C+D) at a final concentration of 100 (HEK) or 200 (CHO) µg/ml and filled into the electroporation cassettes of the MaxCyte® STX™. Transfected cells were recovered and seeded onto glass cover slips. The other day the cells had been fixed and the expression of the recombinant target was either analysed by direct GFP fluorescence or immunofluorescence labelling using a monoclonal antibody against hDR1(Santa Cruz).

Transient Protein Expression in TRC's

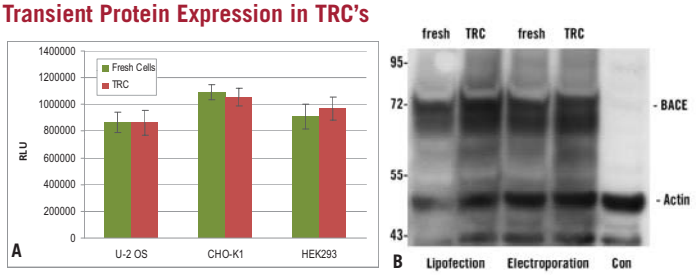

Fig.4: Transient Expression of SEAP (A) and BACE (B) in Transfection Ready Cells
A: Transfection ready cells (red bars) or cell from a continuously growing culture (green bars) have been transiently transfected with an expression vector for secreted embryonic alkaline phosphatase (SEAP) by electroporation using MaxCyte® STX™. The expression of the reporter gene was detected by SEAP activity in the cell culture supernatant using CSPD, a luminescent substrate. In all cell lines SEAP was expressed to the same level in transfection ready frozen cells as well as in fresh cells.
B: Transfection Ready Cells (TRC) or fresh cells from HEK293 have been transfected with an expression vector for BACE protease either by Lipofectamine or by electroporation. BACE expression has been detected by Westernblot using a monoclonal antibody against BACE (SantaCruz).

Discussion

We showed that TRC - Transfection Ready Cells® which had been prepared and frozen in bulky aliquots can be directly used in transient transfection without further cultivation. Although the amount of debris is increasing in HEK293 cells within 24 hours when transfected with DNA, this was observed with cells from a continuously growing culture as well. This just demonstrates the greater sensitivity of HEK2193 cells to the electroporation procedure. Transfection efficacy of both cell lines is very good for fresh cells as well as for transfection ready cells which had been frozen in freezing bags. Not only GFP but also a recombinant transmembrane receptor (hDR1) could be transfected with an efficacy greater than 80%.

Secreted protein like SEAP or BACE can be efficiently produced in Transfection Ready Cells applying either electroporation or lipofection.

So TRCs are a versatile tool to supply host cells for transient transfection and can be used for cell based screening as well as for large scale protein production. The time-consuming step of upscaling the cells can be omitted.

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