Transfection ready cryopreserved cells can be efficiently used for reverse transfection instantly after thawing using a polymer based transfection reagent

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introduction

The use of transiently transfected cells is a common approach in high throughput drug discovery when a recombinant cell line cannot be established due to time restrictions or feasibility. Various protocols have been developed to furnish screening campaigns with the required amount of transfected cells. However, all these protocols suffer from a low reproducibility when multiple batches of transfected cells need to be prepared.

We here present a strategy to overcome this restriction by using cells which are cryopreserved in a highly receptive state. These cells can be transfected instantly after thawing by conventional transfection methods without any prior cultivation or expansion. We demonstrate that transfection ready cells are as efficient as cells from a continuously passaged culture and provide a better reproducibility and reliability when different transfection attempts are compared.

To further optimize our protocols, we used Viromer® RED, a novel polymer based transfection reagent, which improves the availability of plasmid DNA in the transfected cells. We established a convenient and straight forward protocol of reverse transfection where the complexed plasmid DNA is simply mixed with the freshly thawed transfection ready cells in bulk suspension which results in a strong and reliable expression of the transgene.

preparation of transfection ready cells (TRCs)

To prepare cryopreserved cells which remain highly receptive for transfection, the cells are expanded under optimal culture conditions and strict passage control. It is essential to keep the cells in the logarithmic growth phase during expansion in order to obtain optimal results. After the controlled expansion the cells are harvested enzymatically with formulations which are more gentle than regular trypsin, e.g. Accutase® or TrypLE™ Express. For subsequent cryopreservation the cells are suspended in optimized freezing medium containing 5% DMSO or less for cryoprotection. For improved reproducibility the cells are automatically dispensed into vials using a Fill-It® (TAP) or a BioFill® (Fluidix) and frozen in a controlled-rate freezer to minimize the impact of crystallisation heat. For long-term storage cryopreserved transfection ready cells are kept in vapour phase of liquid nitrogen.

Fig. 1: Production of cryopreserved transfection ready cells (TRCs)

comparison of different transfection reagents

To find the best way of transfecting TRCs, different reagents were tested. Transfection ready cells from CHO-K1 (10 million cell/vial) were thawed and seeded into a 96-well plate at 10.000 cells per well. After over night cultivation the cells were transfected with a luciferase expression vector using Viromer® RED (Lipocalyx), Viromer® Yellow (Lipocalyx), Genejuice® (Millipore), and Lipofectamine® (LifeTechnologies) according to the manufacturer's protocol.

24 hours after transfection Luciferase expression was quantified using the Firefly Luc One-Step Glow Assay Kit (Pierce).

With Viromer Red the expression of luciferase was significantly higher than with other transfection reagents. Therefore Viromer RED was used for subsequent experiments.

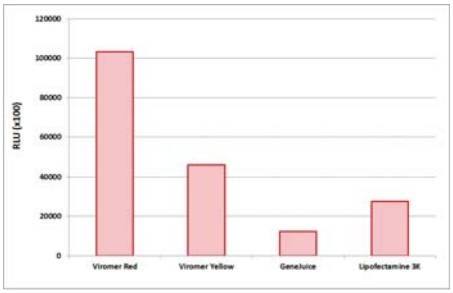


Fig. 2: Comparison of transfection reagents. Expression of Luciferase in TRCs from CHO-K1 transfected with four different reagents.

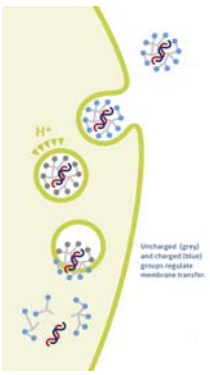


Fig. 3: Principle of Viromer transfection

Viromer is a polymer based transfection reagent which mimics the infection pathway of Influenza virus A. Virus hemagglutinin includes a pH sensitive domain which inserts into the endosome membranes as soon as it gets protonated and enable an active escape from the vesicle. By designing the polymer accordingly using a mix of alkyl (grey) and pH sensitive (red) groups the Viromers act the same way and provide a better availability of transfected DNA.

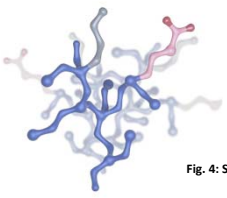


Fig. 4: Structure of Viromer

performance of transfection ready cells

Transfection ready cells (TRCs) prepared from four different cell lines which are frequently used as expression hosts, i.e. CHO-K1, HEK293, MDCK, and Vero, were compared with respective cells from a continuously passaged culture. These fresh cells were detached from a sub- confluent tissue culture flask after five regular passages. Transfection ready cells were thawed from a cryopreserved aliquot and washed once in assay medium. Both cell samples were seeded into a 96-well plate (10.000 cells/well) and let attach over night. The cells were transfected using Viromer RED with expression vectors for firefly luciferase or GFP. 24 hours after transfection luciferase expression (Fig. 5) and GFP expression (Fig. 6a+b) was analysed.

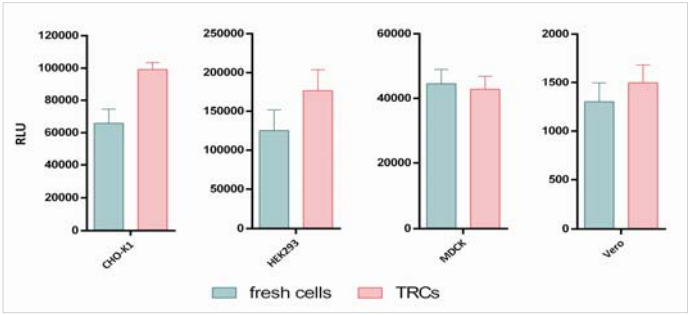


Fig. 5: Expression of Firefly Luciferase in continuously cultivated cells (green bars) and TRCs (red bars).

As expected, the four cell lines expressed Luciferase at different levels. While HEK293 and CHO-K1 showed a strong expression, the Luciferase activity detected in transfected MDCK or Vero cells was significantly lower. However, when TRCs were compared with fresh cells. Luciferase expression was higher in transfection ready cells or at least equal (MDCK) to the freshly passaged cells (Fig. 5).

GFP expression detected by FACS was slightly higher in transfection ready CHO-K1 cells as well. While from a continuously passaged culture (Fig 6A) 87 % of the cells could be transfected, almost 93% of CHO-K1 TRCs (Fog 6B) express GFP (green scatter plots) compared to mock transfected cells (grey scatter plot).

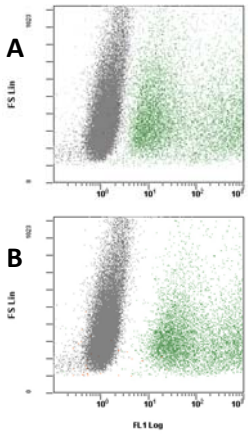
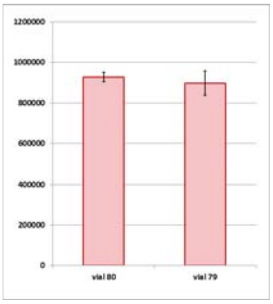


Fig. 6: Expression of GFP in continuously cultivated CHO-K1 cells (A) and CHO-K1 TRCs (B).

one day reverse transfection protocol

Transfection ready cells develop the most advantage when they are used instantly for the transfection. We therefore used an one-day reverse transfection protocol that does not require the seeding of the cell beforehand but allows a direct addition of the cells to the transfection reaction:

1. *Thaw cells, wash and re-suspend in transfection buffer*
2. *Let cell recover for 30 minutes at room temperature*
3. *Mix plasmid DNA and Viromer to initiate complex formation*
4. *Add cell suspension to the DNA-Viromer solution and mix carefully.*
5. *Seed transfected cell into tissue culture plates.*
6. *Incubate 24 to 48 hours until optimal expression is obtained.*



Transfection ready CHO-K1 cells transfected with the reverse one-day protocol (Fig. 7) expressed Luciferase at similar levels as obtained with the two-day protocol that is allowing the attachment of the cells before transfection (Fig. 5). Batches of transfection ready cells display a very high consistency. When independent vials from the same batch were compared the assay variation from vial to vial is within the range of the intra-assay standard deviation (Fig. 7) .

Fig. 7: Vial to vial reproducibility of TRCs. Luciferase expression in CHO-K1 TRCs from two independent vials transfected by Viromer RED using the one-day reverse protocol.

discussion

Cryopreserved cells which are frozen in a state of high receptivity for DNA, can be used directly after thawing in a reverse transfection protocol where the DNA-transfection solution is mixed with the cell suspension and a prior seeding of the cells is omitted. The cells show similar, if not better expression levels and transfection efficacy than cells which derive from a continuously passaged culture. In particular when a high consistency of individual assays is important, transfection ready cells become a valuable tool. Even over an extended period of time identically performing cells from the same batch are available which increases the reproducibility of assays based on transiently transfected cells. Because TRCs are instantly available from cryostorage in any quantity, even large scale screening approaches don't require an extended lead time to get started. For other applications like transient protein production, virus production, or infection assays transfection ready cells may be of similar advantage.

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