Methods Article

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Generation of stably expressing IRF5 spliced isoform in Jurkat cells

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Abstract: Lentiviral transduction enables the generation of gain-of-function of a targeted gene in mammalian cells. Single cell cloning through limiting dilution can establish a population of cells with homogenous transgene expression for exploring protein function. Here, we describe step by step optimized protocols for generating clonal stably expressing using crude lentiviral supernatant in Jurkat cells. Although the protocol is for general use, we will detail how to create stable cell lines based on Jurkat cells expressing IRF5 spliced isoform. These protocols will be broadly useful for researchers seeking to apply overexpression by viral transduction and generation of stable clone to study gene function in mammalian cells.

Keywords: Lentiviral; IRF5; viral transduction; transgene; Jurkat cells

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INTRODUCTION

IRF5 has a diverse role in the induction of pro-inflammatory cytokines and chemokines downstream to various signalling pathways contributing to the pathogenesis of various autoimmune and inflammatory diseases [1-3]. In humans, *Irf5* gene exists as multiple spliced variants that give rise to at least nine isoforms [2-4]. Out of these nine isoforms, four are known as the functional isoforms (v1, v3, v4, v5 and v6), the rest are either transcriptionally inactive or lack certain functional elements, resulting in mutant IRF5 [4-6]. We utilised one of the predominant functional IRF5 spliced isoforms; IRF5 variant 4 (IRF5v4) in our study. The IRF5v4 is among the first to be cloned variant, and it exhibits similar characteristics to another spliced variant, IRF5v3 in terms of identical deletion pattern in exon6, encodes identical polypeptide sequences as well as their functions [4-7]. The IRF5v4 are widely characterised in early studies in defining IRF5 roles in regulating type 1 interferons in response to viral infection [8, 9]. Besides that, IRF5v4 was found to be involved in ubiquitination, a post-transcriptional process critical for the nuclear translocation and target gene regulation of IRF5 [10].

Gene overexpression is a method to produce many targeted gene products for several applications. These include studying gene function, recombinant protein production, screening tools for drug targets, connecting genes in biological pathways, and finding genetic modifiers overexpression phenotypes [11]. The use of mammalian cells as expression systems has been widely established to study the function of gene and production of recombinant protein [12]. Delivery of exogenous genetic materials (e.g., DNA, siRNA) can be accomplished using viral or non-viral vectors that act as vehicles to carry genes of interest into host cells. Viral vectors consist of viruses such as adenoviruses, retroviruses, and lentiviruses, which deliver nucleic acids efficiently into target cells through infection [13-16].

Lentiviral vector derived from HIV has been widely used as a gene delivery tool. It provides stable and long-term gene expression in various mammalian cells, including non-dividing primary cells like neurons [17]. It also serves several benefits, such as carrying large inserts and exhibiting low immunogenicity after some modification in the vector design. Gene transfer mediated by viruses is highly efficient due to their natural ability to infect cells. However, concern over safety and toxicity issues due to the possibility of producing wild-type infectious viruses has led researchers to explore various strategies to improvise the use of viruses as a vector by generating a recombinant viral vector. Generation of viral vector particles depends on multiple plasmid proteins of which the genes are genetically separated, for instance, a) packaging plasmids that consist of *gag, pol*, *tat,* and *rev* genes that are required viral particle formation, b) transfer vector bearing the expression cassette for

transgene insert, c) envelope glycoprotein for infectivity [18-20].

To date, three-generation systems of HIV type 1 based lentiviral vector have been generated. The first generation system closely resembles the wild-type HIV genome except that the packaging systems are modified whereby the helper plasmid that encodes *gal-pol* and the envelope plasmid are driven by heterologous promoter rather than the viral LTR. The HIV glycoprotein (gp120) envelope is usually replaced with Vesicular stomatis virus glycoprotein (VSV-G) to target broader host cells [18, 21]. The second and third-generation vectors are designed so that the necessary components for virus production are split for increased biosafety of the use of lentiviral in the laboratory. In the second generation systems, the accessory genes are removed from the packaging systems, whereas in the third generation, the packaging systems are separated into two plasmids, and *tat* gene is removed; (*gal* and *pol*) and (*rev*) [18, 19, 21, 22]. Besides, the transfer vector in the third generation is modified such that a chimeric 5'LTR is fused to a heterologous promoter such as cytomegalovirus (CMV) or Rovs sarcoma virus (RSV), and the U3 3'LTR is deleted from the viral genome to create a self-inactivating vector which is replication-incompetent [15, 20-22]. Of note, the replacement of a strong viral promoter or enhancer with a hybrid heterologous constitutive promoter reduces insertional mutagenesis and immune genotoxicity because of the absence of virulence factors. The third generation lentiviral systems are considered safe, even to be used in clinical studies [22].

The lentivirus production is accomplished through trans-complementation, whereby by change, all three plasmids get contained into a single cell and together express all the viral proteins that assemble into infectious lentiviral particles [23]. The 293 cells are derived from human embryonic kidney cells, which were transformed by adenovirus type 5 fragments [24] followed by the insertion of a temperature-sensitive version of the simian virus 40 (SV-40) large T tumor antigen [25, 26]. The permissibility of transfection of 293T cells and SV40T, which aids in the extra-chromosomal amplification of HIV-1 plasmid containing SV40 origin of replication, makes it suitable for the production of HIV-1 based lentiviral vector particles [26].

Crude viral supernatant is usually sufficient for in vitro transduction [27-30]. The titer of crude viral supernatants usually ranged from 1 to $5x10^7$ infectious particles [31]. Although several studies have employed the technique of concentrating lentivirus and titer, this is particularly important for some difficult to transfect cells such as primary cells and the use in vivo experiment that requires control of transduction rate [30, 32-35]. Nonetheless, many studies have reported the use of fresh viral supernatant to transduced cells [30, 32, 33, 35]. Moreover, the use of virus supernatant is a faster and simpler approach to transduce cells. Most of the existing protocols used polybrene to enhanced transduction efficiency [32, 36-38]. This is because both cells and virus lipid confers net negative charges, and polybrene a cationic polymer function as counteracting repulsive electrostatic effect, which mediates virus adsorption to the cells, thereby increasing the transduction rate [39].

In this study, we used pLenti CMV GFP Puro (Addgene 17448), a third-generation lentiviral vector to carry the IRF5 cDNA construct into Jurkat cells to achieve stably expressing Jurkat cells. This lentiviral vector is a tat independent and self-inactivating lentiviral vector with enhanced green fluorescence protein, (GFP) under CMV promoter and confers *Puro* gene for puromycin antibiotic selection. To do so, DNA fragment encoding GFP was removed and replaced with IRF5v4. The production of recombinant lentivirus was carried out by transient co-transfection of three plasmids; pLenti expressing GFP or pLenti expressing IRF5v4 was co-transfected with packaging plasmid, psPAX2 (Addgene 12260) and envelope plasmid, pmD2G (Addgene 12259) into HEK 293T cells, mediated by Trans-IT X2 transfection reagent (Mirus Bio LLC). Production of experimental lentiviral constructs of IRF5v4 was done in parallel with lentiviral containing GFP in separate flasks. Unlike the transfer vector that carries GFP in its backbone, no reporter gene was conjugated in these experimental lentiviral constructs of IRF5v4. To ensure that the experimental conditions during this transient transfection experiment were conducive, the expression of GFP was evaluated in the cells transfected with pLenti-GFP. Prior to generating stably expressing IRF5v4 in Jurkat cells, the transduction protocol was optimized using pLenti-GFP.

This methodology article aims to present the optimized protocol for evaluating the use of crude viral supernatant for generating stably expressing IRF5v4 in Jurkat cells. Moreover, this experiment was conducted to investigate whether the virus packaging was an indeed successful and infectious virus was released into the culture medium. For this experiment, the easy-to transduce cells, HEK 293T cells, were used for transduction with lentivirus expressing GFP. Aside from checking the infectious virus produced, we also titer different volumes of the viral supernatant to test an adequate amount for transduction without causing any toxicity. Following the optimized protocol, the transduction of lentiviral IRF5v4 construct was done parallel to lentiviral encoding GFP that served as a positive control. The success of the generation of stable clones of IRF5v4 was validated through western blot analysis. The flow of the experiment is illustrated in Figure 1.

METHOD DETAILS

Construction of lentiviral vector carrying IRF5v4

Primers for human IRF5 coding sequences [8] were designed with restriction enzymes *Bam*HI and *Sal*I, incorporated at the 5' and 3' ends, respectively. Amplified fragments of IRF5v4 (1467 bp) were cloned into pCR Blunt II TOPO vector (Invitrogen) and subsequently cloned into pLenti GFP Puro (Addgene 17448), replacing the GFP fragment.

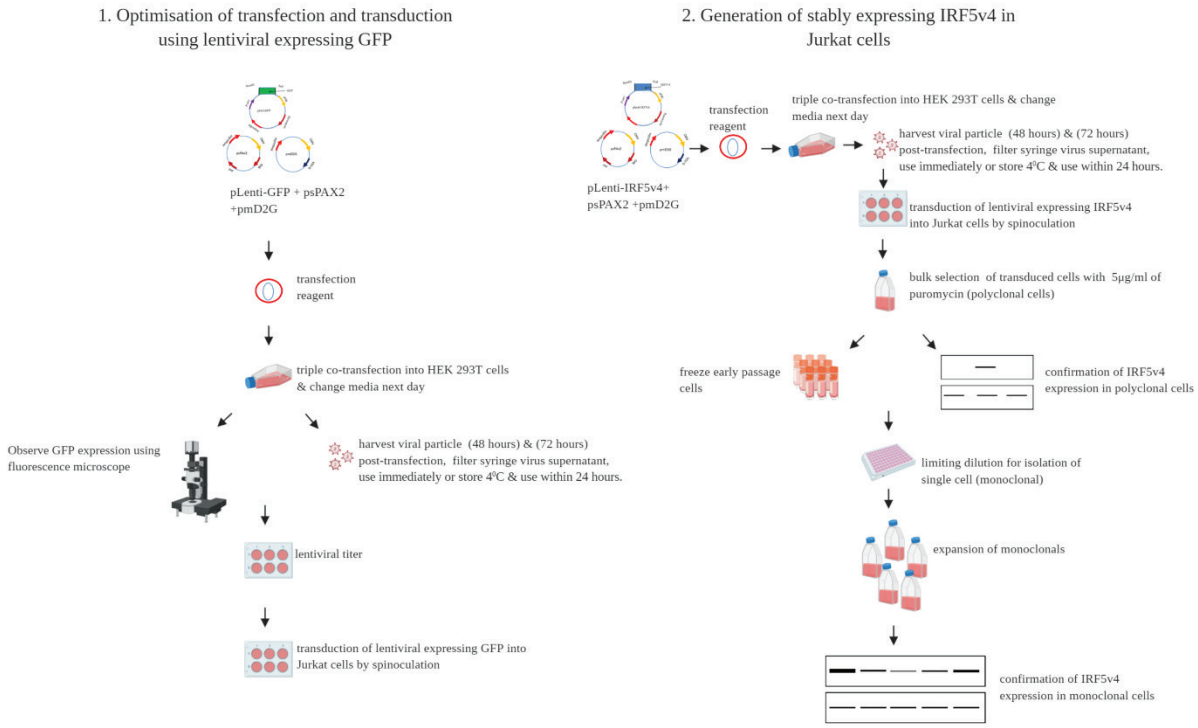

Figure 1. The schematic diagram for the flow of the experiment

Cell line and conditions

Human embryonic kidney (HEK) 293T cells (generously provided by Dr. Leong Chee Onn, International Medical University) were maintained in DMEM (Cellgro Mediatech, US) supplemented with 4.5g/L glucose, L-glutamine, sodium pyruvate, 10% heat-inactivated fetal bovine serum, FBS (Sigma Aldrich), and 1% penicillin/ streptomycin (Gibco, Thermo Fisher Scientific, US). Jurkat cells (obtained from cell culture bank of University of Nottingham Malaysia Campus) were cultured in RPMI medium (Cellgro Mediatech, US) supplemented as above, with an additional component of 25mM HEPES.

Determination of infectivity and optimal inoculum of crude supernatant for transduction using lentiviral expressing GFP

Materials

- Serological pipettes, pipettor
- Pipettes, tips
- microcentrifuge tubes and rack
- falcon tubes and holder
- DMEM (Cellgro Mediatech, US)
- OptiMEM (Gibco, Thermo Fisher Scientific, US)
- Heat inactivated FBS (Sigma Aldrich)
- 0.25 % trypsin-EDTA (Gibco, Thermo Fisher Scientific, US)
- 1xPBS without Ca^{2+} and Mg^{2+} (Cellgro Mediatech, US)
- Mirus TransIT-X2 Dynamic Delivery System
- Plasmids (pLenti-GFP, psPax2, pmD2G)
- Petri dish
- 30 ml syringes
- 0.45µm PES filter
- T-75 flask
- 6 well plate

a) Procedure for recombinant lentivirus production

1. One day before transfection, seed $5x10^6$ HEK293T cells in T-75 cell culture flask containing 15 ml of growth media, DMEM +10% FBS media (*no antibiotic) to achieve 70-80% of confluency on day of transfection.

   **Notes: Antibiotic may interfere with a transfection complex*

2. Add 1.85 ml of serum-free media OptiMEM into falcon tube followed by all three plasmid DNA as below:

Plasmids	Concentration	Ratio
Transfer vector (pLenti-GFP)	3750 ng	4
Packaging vector (psPAX2)	2812.5 ng	3
Envelope vector (pmD2G)	937.5 ng	1

3. Add 45 µl of Mirus TransIT-X2 by dropwise into the falcon tube and gently mix with the diluted plasmids and incubate for 30 minutes undisturbed for complex formation.

4. Aspirate the mixture gently* and add dropwise into the flask using 1000 µl pipette. Rock flask front-back and sideways to distribute the complex evenly over the cells. Incubate the cells at 37°C, 5% CO_2 incubator for 16 hours.

   **Note: Do not pipette up and down this time as it will dislodge the formed complex between the plasmids and transfection reagent.*

5. After 16 hours of post-transfection, remove media and replace* with fresh 15 ml of complete media. Return the flask into the incubator for 24 hour incubation.

   **Note: It is essential to do all media changes with extreme gentleness, as the cells have been sensitized and can easily detach from the plate. If needed, use two serological pipettes, to add media (first 10 ml and then 5 ml, to prevent cells detaching, insert the serological pipette fully inside the flask and add media to edge of flask, dropwise).*

6. At day two post-transfection (48 hours after transfection). Observe cells for GFP expression using a fluorescence microscope and capture images to evaluate transfection efficiency.
7. Harvest the supernatant containing viral particles by gently aspirating the medium from the flask with a serological pipette into a 50 ml centrifuge tube. Replace 15 ml of complete media into the flask and put back in the incubator.
8. Get rid of debris* from the supernatant containing viral particles by filter syringe. This can be done by gentle aspiration of supernatant from the petri dish with a syringe, invert syringe several times to mix the solution well. Pass supernatant through 0.45 μM filter and drain the filtered supernatant into a falcon tube.

   **Notes: Ideally, there should be no debris of cells in the virus supernatant. If there are some small cells, gentle centrifugation of 1500 rpm for 5 minutes at 4^0C before filtering the supernatant can be done to prevent the filter from getting blocked by the cells' debris.*

9. On day three post-transfection (72 hours after transfection). Repeat step 6-7.
10. Pool the 48 and 72 hours of viral supernatant collection. Use immediately or store in 4^0C for not more than 24 hours and proceed with transduction. Avoid freeze-thaw as it can cause a lower transduction rate.

    **Notes: The ratio of transfer plasmids to packaging plasmid and envelope plasmid used for transfection is 4:3:1 was adapted from previous studies[29,33]. The ratio of plasmids DNA to transfection reagent used is 1:2:4 and the seeding number of cells was determined in preliminary expreiment done in out lab. The steps shown here is for T-75 flask, to scale down to 6 wells, divide by 2.5. Number of cells for 6 wells to use: 0.5x10^6 cells/well.*

b) Procedure of lentiviral titer using crude viral supernatant

1. Prepare complete media containing polybrene to a final concentration 8 μg/ml.
2. Seed 2x10^5 HEK 293T cells into 6 wells of the 6 well culture plate containing 2 ml of growth media and polybrene.
3. Bring virus supernatant to room temperature* and mix gently. Add 0.6ml, 0.8ml, 1ml, 1.5ml, and 2ml of virus supernatant into seeded HEK 293T cells (Figure 2).

   **Notes: Thawing of virus supernatant from 4^0C can be done by keeping it in the biosafety cabinet to reach room temperature. Be careful not to leave the tube containing virus supernatant in the water bath too long as high heat can reduce viral infectivity resulting in poor transduction.*

4. Incubate the cells at 37°C, 5% CO_2 incubator for 24 hours.
5. After 24 hours of incubation, transfer change media with complete fresh media. Incubate cells at 37°C, 5% CO_2 incubator for another 48 hours.
6. At day three post-transduction (72 hours after transduction). Observe cells for GFP expression using a fluorescence microscope and capture images to evaluate transduction efficiency.

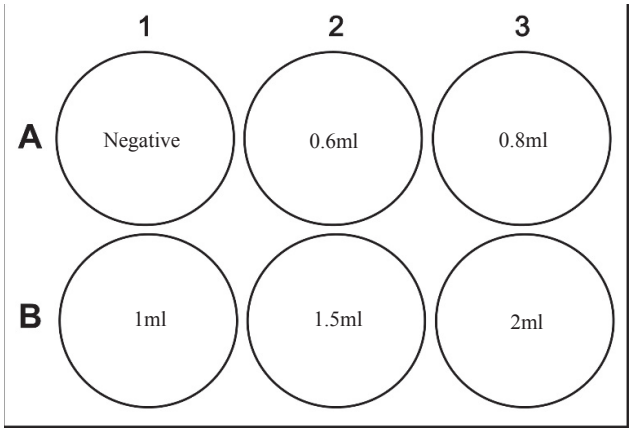

Figure 2. A layout of 6 well- plate for optimization of transduction. A1: Control 1: non-transduced, A2: 0.6ml viral supernatant, A3: 0.8ml viral supernatant, B1:1ml viral supernatant, B2: 1.5ml viral supernatant, B3: 2ml of viral supernatant.

Generation of stable clones expressing IRF5v4

Materials

- Serological pipettes, pipettor

- Pipettes, tips
- Multichannel pipettor
- 1.5 ml microcentrifuge tubes and rack
- 15 ml falcon tubes and holder
- RPMI medium (Cellgro Mediatech, US)
- FBS (Sigma Aldrich)
- Penicillin/ streptomycin (Gibco, Thermo Fisher Scientific, US)
- Hexadimethrine bromide is known as polybrene (Sigma-Aldrich).
- 6 well plate
- T-25 flask

Procedure for transduction in Jurkat cells

1. Prepare recombinant lentiviral particles harboring IRF5v4 coding sequence following steps 1a in parallel with lentiviral particle harboring GFP to monitor experimental conditions.
2. Prepare two sets of 6 well plates. Seed $2x10^5$ Jurkat cells into 6 wells of 6 well plate cell culture flask containing 2ml of growth media and polybrene. In one 6 well plate label (IRF5v4) and another one as (GFP).
3. Bring virus supernatant to room temperature and mixed gently. Transfer 2 ml of virus supernatant into each well. One plate containing lentiviral particles harboring GFP and another plate for lentiviral harboring IRF5v4.
4. Seal the plate with paraffin film and spin the cells* at 1,200 x g for 90 minutes at 25^0C without a break. Incubate the cells at 37°C, 5% CO_2 incubator for 24 hours.
   **Notes*: *Spinoculation can be performed in a falcon tube if a plate adapter for centrifugation is not available. If the falcon tube is used, resuspend the mixture gently and transfer it into the 6 well plates.*
5. The next day, change the media by centrifugation at 1000 x g for 10 minutes at 25^0C and replace it with fresh 2ml of complete media into each well. Incubate the cells at 37°C, 5% CO_2 incubator for another 48 hours.
6. At day 3 post-transduction (72 hours after transduction), observe cells for GFP expression using a fluorescence microscope, and capture images to evaluate transduction efficiency.

Procedure for selection of transduced cells and isolation for monoclonal

1. Collect the transduced cells from each well and pool them, and split into four T-25 flasks, with each flask containing 3ml of cells suspension.
2. Add 6ml (1:3 ratio) of complete media containing 5 µg/ml puromycin into each well and culture the transduced cells * for two weeks to generate polyclonal cells.

   **Notes: Selection of transduced Jurkat cells in puromycin containing medium, typically takes two weeks to achieve the population of cells that contain only integrated transgene as any unintegrated cells would have died. These bulk cells that survived the antibiotic selection are referred to as polyclonal cells.*
3. After two weeks, split the cells and freeze down some polyclonal cells to have early passage cells that serve as storage and back up. Use the remaining cells to check for the presence of the transgene.
4. Using the early passage cells, perform limiting dilution to isolate a single cell (monoclonal) to generate a homogenous population of cells.
5. To do so, dilute $1 x10^4$ transduced cells in 20 ml media.
6. Transfer 5 µl of cells into a tube containing 5ml of complete media supplemented with 5 µg/ml puromycin and 5 ml of self-conditioned media*.

   **Notes: The self-conditioned media is isolated from healthy growing Jurkat cells (non-transduced cells). To spin down the cells and collect the supernatant into a sterile tube and filter using 0.22 µm polyvinylidene fluoride (PVDF) filter.*
7. Transfer 100 µl of the diluted cells suspension into each well using a multichannel pipettor.
8. Incubate cells undisturbed at 37^0C in 5% CO_2 for 2 hours.
9. Inspect wells and label wells containing only a single cell.
10. Incubate plate undisturbed at 37^0C in 5% CO_2 for three weeks with regular inspection for confluency.
11. Once cells reach about 30-50% confluency, transfer the cells to 24 wells and expand to 6 wells and later to T-25 flask.
12. Check the expression level* and freeze down early passage cells to be used in subsequent experiments.
    **Notes: Routinely check expression level by reverse transcription PCR (RT-PCR) to ensure the transgene is not lost over the time of culture, a common gene silencing phenomenon associated with transgene under CMV promoter. If transgene exhibit toxic to cells resulting in extended lag phase cell growth, an alternate viral vector under inducible promoter can be used.*

METHOD VALIDATION

Confirmation of successful transient transfection for recombinant lentivirus

Since GFP fragments were removed in order to construct experimental transfer vectors pLenti-IRF5v4; by inserting IRF5v4 DNA fragment in replacement of GFP DNA fragment, there was no reporter marker to track these constructed experimental vectors during transient transfection for recombinant lentivirus production. Therefore, the production of recombinant lentivirus harboring IRF5v4 was conducted in parallel with pLenti-GFP in separate T-75 flasks to ensure the setting for successful transfection for virus production. As seen in Figure 3(a), co-transfection of pLenti-GFP along with the two helper plasmids (psPAX2 and pmD2G) in HEK 293T cells exhibited about 60-70% of green fluorescence at day 2 of post-transfection, similar to the results obtained in the preliminary experiment conducted as described above. Also, the morphology of the transfected cells was examined under the bright-field view. The photo bright-field of transfected cells shows the presence of syncytia represented by the multi-nucleated cells (in black arrow) that were

caused by the translation of glycoprotein of VSV-G (envelope protein), indicating successful transfection and sign for lentivirus production [35, 40]. The third day of post-transfection, fluorescent microscopy showed GFP was expressed about 80-90%, as seen in Figure 3 (b). Based on these results, it was deduced that the production of lentivirus was successful and ready to be used for the transduction experiment.

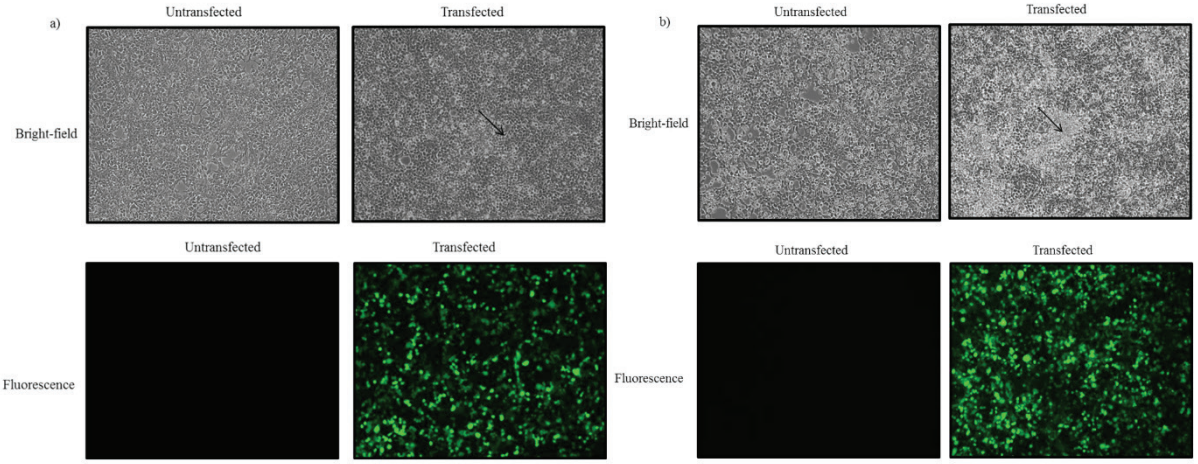

Figure 3. Detection of GFP expressions from recombinant lentivirus expressing GFP from post-transfection days.(a) On day 2, post-transfection. (b) On day 3, post-transfection. Photos were taken under Nikon Inverted Microscope Eclipse TS2R under bright field or fluorescent light (x200 magnification). The exposure time of fluorescent images were 600 milliseconds. The black arrowhead shows the presence of syncytia in transfected cells.

Determination of the optimal amount of viral supernatant for transduction

The presence of GFP expression visualized by a fluorescence microscope after transfection reveals the efficiency of transfection, but it does not display the lentivirus's infectivity. It was necessary to ensure the recombinant lentivirus harvested was infectious for proper gene delivery to target cells [41]. It was also essential to make sure that there was no cell toxicity from a viral infection that could cause massive cell death, resulting in a lower transduction rate. Based on the results, the minimal volume of lentiviral supernatant inoculum that showed some GFP expression was 0.6ml (Figure 4 (b)), and the highest volume, 2 ml tested showed greater transduction efficiency of more than 90% GFP expression seen (Figure 4(f)). Moreover, about 95% of viable cells were observed by evaluating trypan blue, indicating no cell toxicity from a viral infection with the amount of virus supernatant used for transduction. This data provided the evidence that the recombinant lentivirus produced was infectious and good transduction efficiency was achieved with crude viral supernatant.

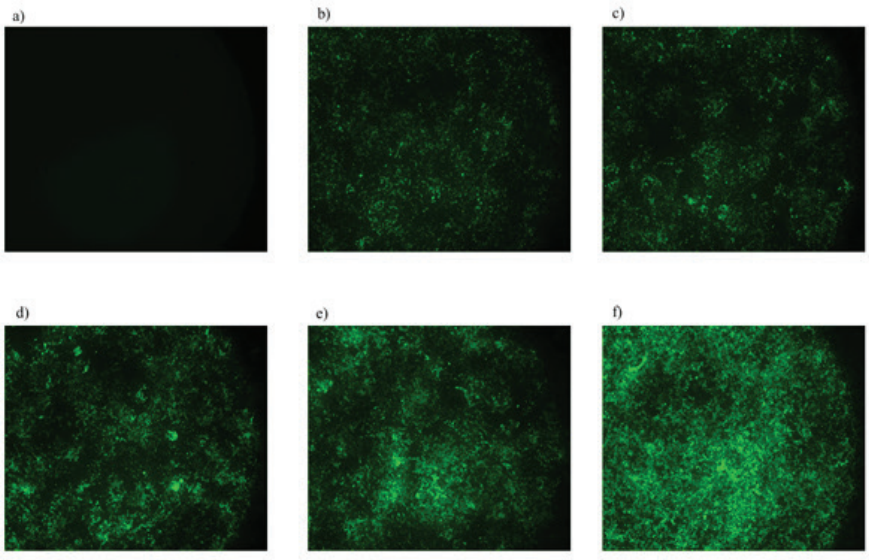

Figure 4. Titers of lentivirus expressing GFP on HEK 293T cells. (a) non-transfected cells, (b) 0.6 ml of lentivirus, (c) 0.8ml of lentivirus, (d) 1ml of lentivirus, (e) 1.5ml and (f) 2ml of lentivirus. Photos were taken under Nikon Upright Microscope Eclipse Ei under fluorescent light (x100 magnification).

Confirmation of successful transduction in Jurkat cells

Transduction is more permissible in the adherent cell line. Thus, spinoculation is not required transduction in HEK 293T cells. But this is not the same for suspension cells like Jurkat cells. Numerous researchers have conducted transduction of Jurkat cells by spinoculation and the presence of polybrene, which have shown to enhance the transduction of lentiviral vector by virus binding to the cells [32, 36-38]. The experiment was conducted in parallel with the control transfer vector, pLenti-GFP in a separate culture flask to track the successful transduction experiment. After 72 hours of transduction, GFP expression was accessed by a fluorescence microscope. As seen in Figure 5, the bright field photo of transduced cells revealed that infection with lentivirus did not alter Jurkat cells' morphology, implying no cell toxicity from the lentiviral transduction. Moreover, Jurkat cells' transduction with virus supernatant obtained from the transfection of pLenti vector expressing GFP displayed about 70-80% of green fluorescence. As expected, no GFP expression was observed in non-transduced cells.

Meanwhile, the transduced cells showed an excellent transduction efficiency of more than 70% of GFP expression accessed by a fluorescence microscope. Furthermore, no cell toxicity from viral infection was noticed as the evaluation of trypan blue observed about 95% of viable cells. From these data, it was deduced that the infectious lentiviral constructs efficiently transduced Jurkat cells.

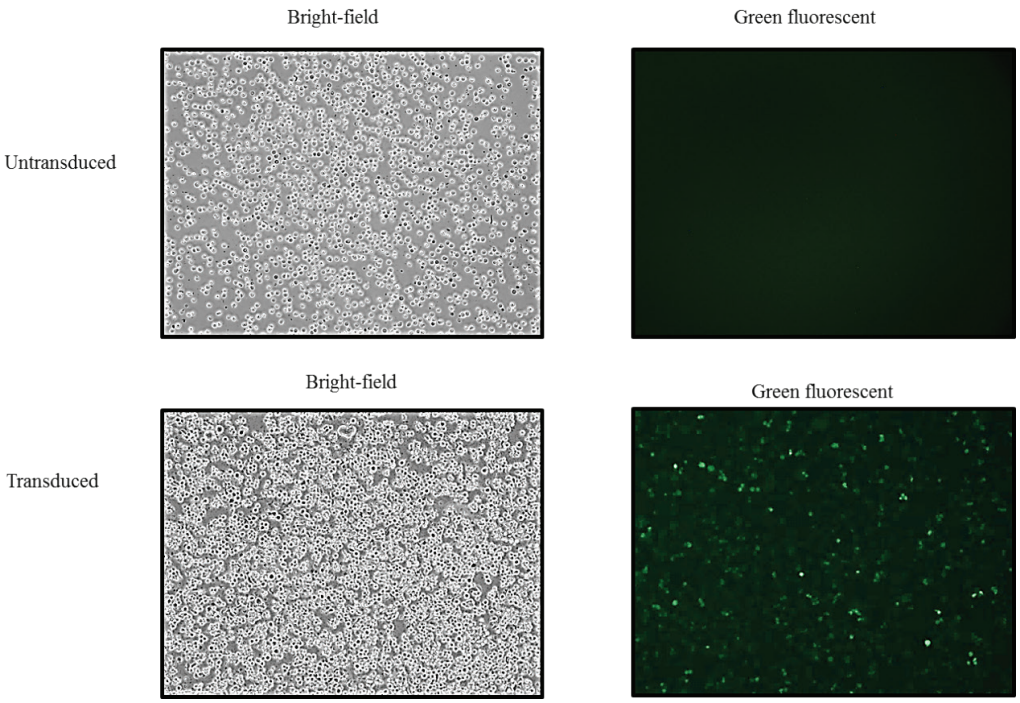

Figure 5. Detection of GFP expressions from transduced Jurkat cells with lentivirus harbouring GFP at day 3 post-transduction. Photos were taken under Nikon Inverted Microscope Eclipse TS2R under bright field or fluorescent light (x200 magnification).

Confirmation of IRF5v4 overexpression in polyclonal Jurkat cells

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes, which cause premature chain termination during translation [42, 43]. The pLenti-GFP and the engineered pLenti-IRF-v4 encode puromycin-N-acetyl transferase gene (Puro) in their plasmid backbone constitutively express it. Therefore, during the antibiotic selection, those cells with stably integrated transgene and constitutively expressed the resistant gene, Puro, will survive when grown in a medium containing puromycin. Those non-transduced cells and cells that uptake plasmid transiently will not survive the antibiotic selection. The presence of IRF5v4 in the polyclonal cells generated from the experimental lentiviral constructs was checked western blot analysis. As seen in Figure 6, no IRF5 was detected in non-transduced Jurkat cells. Similarly, no IRF5 was detected in Jurkat cells transduced with pLenti-GFP, which indicates that infection with lentiviral without IRF5 spliced variant in the backbone did not lead to an upregulation of endogenous IRF5. Therefore, detecting IRF5 in stably expressing cells indicates the *de novo* synthesis of integrated IRF5v4 in Jurkat cells. In line with this, approximately 60 kDa bands corresponding to the IRF5v4 protein were detected from polyclonal cells, as shown in Figure 6. From these results, it was deduced that experimental lentiviral constructs had successfully integrated the transgene of IRF5v4 in Jurkat cells.

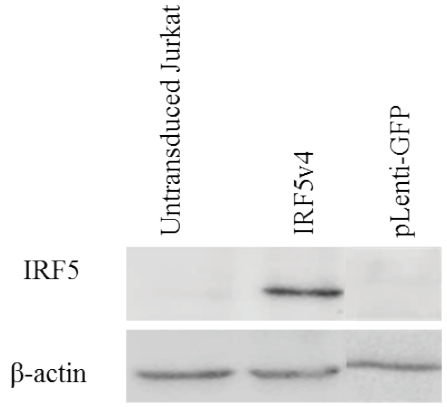

Figure 6. Confirmation of overexpression of IRF5v4 in polyclonal cells. Western blot analysis for detection of IRF5v4 (approximately 60kDa). Housekeeping protein actin act as the loading control (43kDa) was included as part of the analysis. The chemiluminescence signal was captured by Gel Doc imager.

Confirmation of IRF5v4 in monoclonal cells

Polyclonal cells constitute of heterogeneous transgene expression level, which means the population of cells containing both high and low expression levels of the transgene [35]. Over time, the transgene population of polyclonal cells may drop because cells with high transgene levels may have a slower growth rate. Therefore the rapidly growing low-level transgene expression may take over the culture. To avoid the drift effect towards low transgene expression and obtain a homogenously higher level of expression of the IRF5v4, the early passage of polyclonal cells was used to isolate single cells (monoclonal) limiting dilution method. Limiting dilution is a traditional method for obtaining clonally identical cells (monoclonal) of stable expressing transgene derived from single isolated cells cultured in multi-well plates [44]. Several monoclonal cells were isolated and expanded in culture with a complete medium containing 5 µg/ml of puromycin. The clones were screened for the presence of overexpression of IRF5v4 using western blot analysis. From the result seen in Figure 7, two clones expressing IRF5v4 (referred to as B8, F9) were found to have stable high IRF5 expression. While two clones, D7 and E6, showed a substantial level of IRF5v4. Another clone E4 had a low expression of IRF5v4.

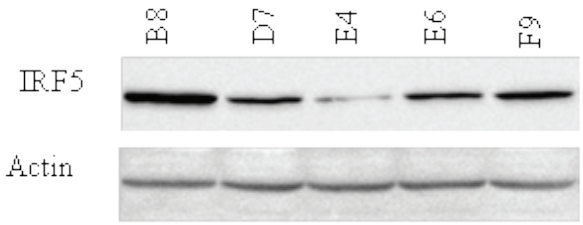

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Figure 7. Confirmation of overexpression of IRF5v4 in monoclonal cells. West ern blot analysis for detection of IRF5v4 (approximately 60kDa). Housekeeping protein actin act as the loading control (43kDa) was included as part of the analysis. The chemiluminescence signal was captured by Gel Doc imager.

CONCLUSION

In conclusion, the present study shows the optimization protocol for generating IRF5v4 stable clones using recombinant lentiviral transduction. Stably overexpressing IRF5v4 was successful achieved through lentiviral transduction. Five monoclonal of IRF5v4 expressing Jurkat cells were obtained. The GFP expression from the control lentiviral vector helped evaluate whether the experiments carried out following the protocol designed were ideal and successful.

Authors Contributions

AK and C-MF performed the literature review and manuscript writing. C-MF conceptualizes the research project.

Conflict of Interest

The authors declare that there is no conflict of interest in this work.

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