RAW264.7 Transfection Optimization
Comparison of K4 Transfection System and Lipofectamine 3000

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Transfection reagents were used according to the instructions of the manufacturers. Transfection optimization table for a 24 well plate:

Reagent Used (volume)	DNA Amount	Incubation time	Transfection %
K4 Transfection System 2µl	1µg	6h	5% (Fig.1)
K4 Transfection system 2µl	1µg	24h	2%
K4 Transfection system 3µl	1µg	6h	2%
K4 Transfection system 3µl	1µg	24h	1%
P3000 Reagent 1 µl Lipofectamine 3000 0.75 µl	0.5 µg	6h	0%
		24h	0%
P3000 Reagent 1µl Lipofectamine 3000 1.5µl	0.5 µg	6h	0%
		24h	0%

Transfection percentage was determined by eye after 24 hours and best condition were achieved using K4 Transfection System 1:2 (µg/µl) replacing the media after 6 hours of incubation. Detailed protocol as following:

1. Plate 2 x 10^5 RAW264.7 cells each well of 24 well plate in 0.5ml of RPMI-1640 containing pen/strep, FBS 10%, L-glutamine, HEPES 10mM and Na-Pyruvate 1mM.
2. Incubate cells overnight at 37°C in 5% CO2 incubator. The day after the well surface should be covered about 90%.
3. Bring the stock solutions of K4® Transfection System and DNA to room temperature and agitate gently to ensure they are evenly mixed.
4. 1/2 h before adding the lipoplex, replace complete growth media without antibiotics and pipet 5 µl K4® Multiplier (1% related to growth medium without pen/strep) into each well of cells to be transfected.
5. Prepare the following solutions in a polypropylene vessel. Always place the medium in the vessel first to prevent the reagent and DNA solutions from coming into direct contact with the vessel material. Solution A: 1 µg DNA to 25 µl serum-free medium (OPTIMEM) B: 2 µl K4® Transfection Reagent to 25 µl serum-free medium (OPTIMEM).
6. Mix each solution by gently pipetting up and down once.
7. Combine the solutions (A to B), mix by gently pipetting up and down once, and incubate the mixture at room temperature for 15–20 min.
8. Immediately after the 15–20 min of incubation add the 50 µl of DNA-lipid complex (A+B) to the cells, mix gently by agitating the cell culture vessel and incubate in a CO2 incubator at 37°C.

9. Remove the transfection mixture after 6 h and replace with fresh complete growth medium.
10. Verify the transfection efficiency of pEGFP-N1 24h after the transfection (fig 1).

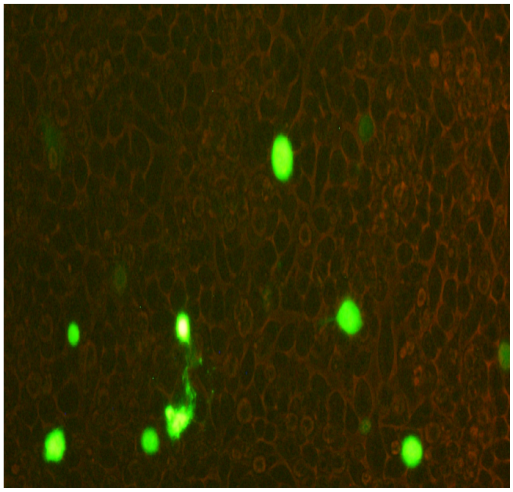

Fig. 1: pEGFP-N1 Transfection efficiency evaluation using Nikon Eclipse TE2000U.

In conclusion the K4 transfection system revealed to be the best performing regent to transfect the RAW264.7 cell line without any apparent toxic effect after 6 hours of incubation. We also previously tested other transfection methods such as electroporation or Fugene HD and Extreme gene 9 without success.

The optimized transfection protocol using the K4 Transfection System is going to be used to generate a KO cell line by Cas9n/CRISPR technology via pSpCas9n(BB)-2A-Puro(PX462) plasmid.