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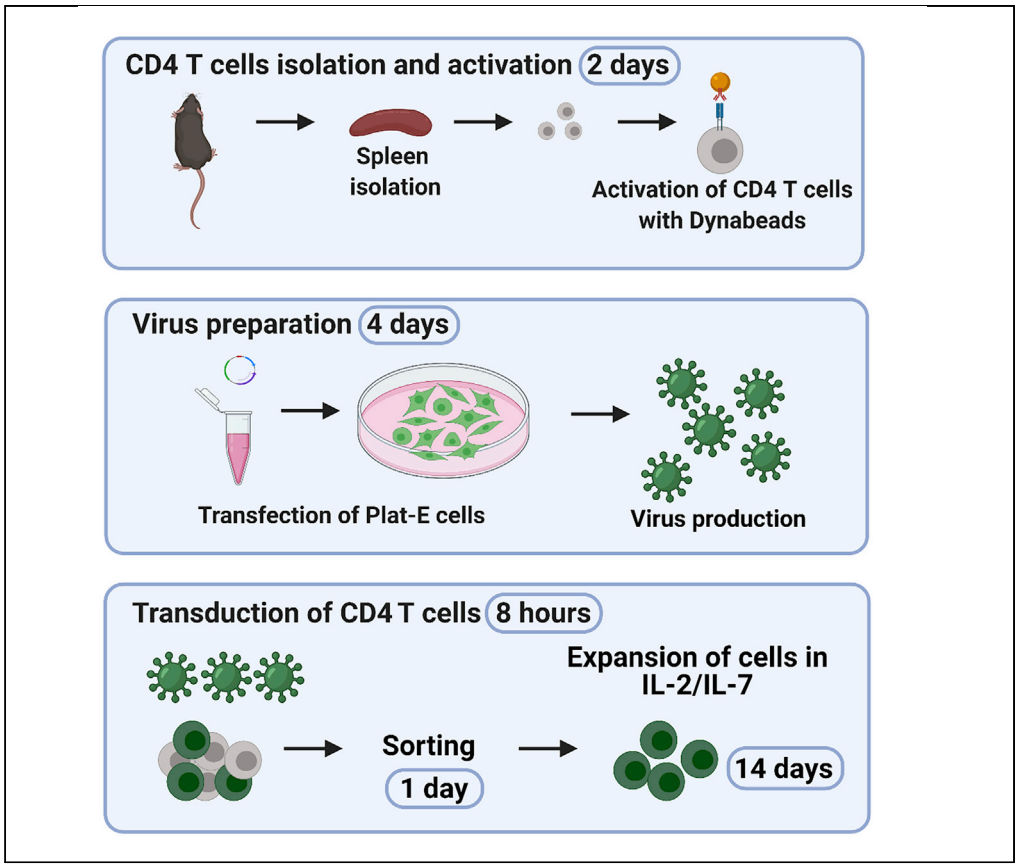
An optimized protocol for the retroviral transduction of mouse CD4 T cells



Transduction of primary T cells has become prominent with the introduction of chimeric antigen receptor T-cell therapy. Although there are many protocols for the transduction of human T cells, it remains a challenge to transduce murine T cells. We present an optimized protocol for the retroviral transduction of murine CD4 T cells, which overcomes major challenges including large-scale production and long-term culturing of transduced cells. The optimized protocol combines high transduction efficiency with a low rate of cell death.

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Highlights

Detailed description of retroviral transduction of murine CD4 T cells

Large-scale production of murine CD4-transduced T cells

Enhanced proliferation and viability of murine CD4-transduced T cells with IL-2/IL-7

Eremenko et al., STAR Protocols 2, 100719 September 17, 2021
https://doi.org/10.1016/j.xpro.2021.100719

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An optimized protocol for the retroviral transduction of mouse CD4 T cells

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SUMMARY

Transduction of primary T cells has become prominent with the introduction of chimeric antigen receptor T-cell therapy. Although there are many protocols for the transduction of human T cells, it remains a challenge to transduce murine T cells. We present an optimized protocol for the retroviral transduction of murine CD4 T cells, which overcomes major challenges including large-scale production and long-term culturing of transduced cells. The optimized protocol combines high transduction efficiency with a low rate of cell death.

For complete details on the use and execution of this protocol, please refer to Eremenko et al., 2019.

BEFORE YOU BEGIN

This protocol describes an efficient method for the transduction of CD4 murine T cells, based on retroviral (RV) delivery of genes of interest. It presents as a useful resource for the generation of mouse CAR-T cells, enabling researchers to drive this area of research forward.

The common protocol includes several steps such as, viral packaging, isolation and preparation of CD4 T cells for viral transduction and expansion of the transduced cells in culture. This optimized protocol enables the large-scale production of RV-transduced T cells, previously presented as a major challenge, owing to the limited proliferation capability and poor cell survival. Our protocol utilizes the packaging cell line, Platinum-E (Plat-E) cells, to produce retroviral particles, which can stably produce retroviruses with an average titer of 1×10^7 cfu/mL (Morita et al., 2000).

Although it is widely accepted that IL-2 is necessary for T cell cultures (Zhang et al., 2020), recent studies indicate the benefit of adding other cytokines, such as IL-7 and IL-15 (Xu et al., 2014; Alvarez-Fernández et al., 2016; Hurton et al., 2016; Alizadeh et al., 2019).

IL-7, was found to maintain the survival of human T cells in vitro (Rathmell et al., 2001). We found that the combination of IL-2 and IL-7 increased the long-term survival of mouse CD4-transduced T cells.

When working with RV supernatants and RV-transduced samples it is important to follow the universal precautions. All experiments should be carried out in at least a class II biological safety cabinet with appropriate protective equipment, including a lab coat, protective sleeves, and double gloves. Detergents or ethanol can readily inactivate retroviruses owing to the retrovirus being surrounded by a lipid membrane derived from the virus-producing cell (Coffin et al., 1997).

Culturing plat-E cells

Timing: 2 weeks

1. Thaw Plat-E cells, routinely stored at −80°C.
2. Suspend in 15 mL 293T medium and incubate in a 75 cm^2 flask at 37°C in a humidified 5% CO_2-containing incubator.
3. Once the cells reach a confluence of around 85%–90%, detach cells by adding 2 mL trypsin-EDTA 0.05% and incubate at 37°C in a humidified 5% CO_2-containing incubator for 3 min.
4. Collect the cells into a 15 mL conical tube followed by washing the flask with 8 mL 293T medium.
5. Centrifuge at 500 × *g* for 5 min at 22°C.
6. Remove the supernatant and resuspend the cells in Plat-E medium. Divide the cells to two new 75 cm^2 flasks and make up to 15 mL with Plat-E medium.
7. Incubate at 37°C in a humidified 5% CO_2-containing incubator.
8. Treat cells biweekly, as described above.

Mice

9. Ideally use male or female, 8–12 weeks old C57BL/6 mice.

Note: Mice are housed under standard conditions, in a 12-h light/dark cycle with food and water supplied *ad libitum*. All procedures are performed under the approval of the Ben-Gurion University of the Negev Animal Care and Use Committee. All efforts are made to minimize suffering of the animals.

Plasmid generation

Timing: 2 h

10. Prepare plasmid stocks using standard molecular biology techniques following the manufacturer instructions of the Purelink™ HiPure Plasmid Filter Maxiprep Kit (Invitrogen) or an alternative preferred kit.
11. Determine the DNA concentration of the plasmid using NanoDrop spectrophotometer.

Note: Ensure there is sufficient plasmid concentration for the experiment.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Poly-D-lysine	Sigma-Aldrich	P6407
PureLink™ HiPure Plasmid Filter Maxiprep Kit	Invitrogen	K210016
Trypsin-EDTA 0.05 %, phenol red	Gibco	25300054
DMEM Medium	Gibco	11965092

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Blasticidin (10 mg/mL) in HEPES Buffer	InvivoGen	Ant-bl-05
Puromycin (10 mg/mL) in H_2O	Sigma-Aldrich	P9620
Penicillin-Streptomycin Solution (100×)	Biological Industries	03-031-1B
Isoflurane	Primal Critical Care	NDC66794-017
pMP71 Gpre GFP plasmid	Kindly provided by Professor Wolfgang Uckert, Max Delbrück Center for Molecular Medicine, Berlin, Germany	N/A
Lipofectamine™ Transfection Reagent	Invitrogen	18324012
ACK Lysing Buffer	Gibco	A1049201
Normal Rat Serum	STEMCELL Technologies	13551
EDTA 0.5 M, pH 8.0	Bio-Lab	009012230100
Water, Cell Culture Grade	Biological Industries	03-055-1A
Human IL-2	PeproTech	200-02-10UG
Mouse Recombinant IL-7	STEMCELL Technologies	78054
Fetal Bovine Serum	Gibco	10500-064
RPMI Medium 1640	Gibco	21875
HEPES Buffer Solution (1M)	Biological Industries	03-025-1B
MEM Non-Essential Amino Acids Solution (10 mM)	Biological Industries	01-340-1B
Sodium Pyruvate (100 mM)	Biological Industries	03-042-1B
β-Mercaptoethanol (14.3 M)	Sigma-Aldrich	M3148
Hank's Balanced Salt Solution (HBSS), no Phenol Red	Biological Industries	02-016-1A
Dulbecco's Phosphate Buffered Saline (PBS), without Calcium and Magnesium	Biological Industries	02-023-1A
Bovine Serum Albumin	MP Biomedicals	160069
Cell Trace Blue Cell Proliferation Kit	Invitrogen	C34568
eBioscience Fixable Viability Dye eFlour 780	Invitrogen	65-0865-14
Experimental models: Cell lines		
Plat-E Cells	Cell Biolabs	RV-101
Experimental models: Organisms/strains		
C57BL/6	Jackson	000664
Other		
Biological Safety Cabinet	NuAire	NU 425-600E
FACSAria™ III Cell Sorter	BD	648282
Centrifuge	Eppendorf	5810R
NanoDrop One Microvolume UV-Vis Spectrophotometer with Wi-Fi	Thermo Scientific™	ND-ONE-W
Counting Chamber	Paul Marienfeld GmbH & Co.KG	0610010
15 mL Conical Tubes	Corning	188261
50 mL Conical Tubes	Corning	227270
10 cm Cell Culture Petri Dish Nunclon™ Delta Surface	Nunc™	150350
Non-Treated Multidishes 24 wells	Nunc™	144530
Cell-Culture Treated Multidishes 24 wells	Nunc™	142475
96 Well Round (U) Bottom Plate	Nunc™	163320
Serological Pipette 10 mL Sterile Individually Wrap	Bar-Naor	BNP10S1IPG
Serological Pipette 5 mL Sterile Individually Wrap	Bar-Naor	BNP5S1IPG
1.5 mL Eppendorf Tubes	Axygen	MCT-150-C
70 µm Cell Strainer	SPL	93070
3 mL Disposable Syringe without Needle	MediPlus	KL 3ML
DynaMag™-2 Magnet	Invitrogen	12321D
EasySep™ Magnet	STEMCELL Technologies	18000
CytoFLEX LX Flow Cytometer	Beckman Coulter	
Dissecting Scissors	Kent Scientific	INS600393-G
Forceps	Kent Scientific	INS650914-4

Alternatives: In theory, all reagents and resources listed in the 'key resources table' can be substituted with equivalent items from other suppliers, with exception to the critical commercial assays and Nunc™ Non-Treated Multi-dishes 24 wells; however, it should be noted that the protocol has been calibrated to the reagents listed in the above table and alternatives have not been tested on the protocol performance.

MATERIALS AND EQUIPMENT

293T Medium		
Reagent	Final concentration	Amount for 500 mL
Fetal Bovine Serum	10% (v/v)	50 mL
Penicillin-Streptomycin Solution (100×)	1 mg/mL Streptomycin, 100 U/mL Penicillin	5 mL
DMEM Medium	n/a	To 500 mL
Store at 4°C for up to one month.		

Plat-E Medium		
Reagent	Final concentration	Amount for 500 mL
Fetal Bovine Serum	10% (v/v)	50 mL
Penicillin-Streptomycin Solution (100×)	1 mg/mL Streptomycin, 100 U/mL Penicillin	5 mL
Puromycin (10 mg/mL) in H_2O	1 μg/mL	50 μL
Blasticidin (10 mg/mL) in HEPES buffer	10 μg/mL	500 μL
DMEM Medium	n/a	To 500 mL
Store at 4°C for up to one month.		

Complete RPMI Medium		
Reagent	Final concentration	Amount for 500 mL
Fetal Bovine Serum	10% (v/v)	50 mL
Penicillin-Streptomycin Solution (100×)	1 mg/mL Streptomycin, 100 U/mL Penicillin	5 mL
HEPES Buffer Solution (1M)	10 mM	5 mL
MEM Non-Essential Amino Acids Solution (10 mM)	0.1 mM	5 mL
Sodium Pyruvate (100 mM)	1 mM	5 mL
β-mercaptoethanol (14.3 M)	50 μM	1.75 μL
RPMI Medium 1640	n/a	To 500 mL
Store at 4°C for up to one month.		

EasySep™ Mouse CD4 T-cell Isolation Kit Recommended Medium		
Reagent	Final concentration	Amount for 100 mL
Fetal Bovine Serum	2% (v/v)	2 mL
EDTA 0.5 M, pH 8.0	1 mM	200 μL
Dulbecco's Phosphate Buffered Saline (PBS), without Calcium and Magnesium	n/a	To 100 mL
Aliquot and store at - 20°C for up to one year.		

Dynabeads™ Mouse T-Activator CD3/CD28 for T-Cell Expansion and Activation Buffer		
Reagent	Final concentration	Amount for 100 mL
Bovine Serum Albumin	0.1% (w/v)	0.1 g
EDTA 0.5 M, pH 8.0	2 mM	400 µL
Dulbecco's Phosphate Buffered Saline (PBS), without Calcium and Magnesium	n/a	To 100 mL
Aliquot and store at - 20°C for up to one year.		

Cell Sorting Medium		
Reagent	Final concentration	Amount for 500 mL
Fetal Bovine Serum	20% (v/v)	100 mL
Penicillin-Streptomycin Solution (100×)	2 mg/mL Streptomycin, 200 U/mL Penicillin	10 mL
RPMI Medium 1640	n/a	To 500 mL
Store at 4°C for up to one month.		

STEP-BY-STEP METHOD DETAILS

Retroviral virus production

Timing: 4 days

Plat-E cells were transfected with a DNA plasmid to produce the retroviral particles

Day 1: Plat - E seeding

1. Before starting, ensure you have enough plasmid for transfection, step 8.
2. Coat 10 cm plates by adding 5 mL of 50 µg/mL poly-D-lysine in sterile water. Incubate at 22°C for 45 min. Wash twice with sterile water to ensure no residues remain.
3. Detach Plat-E cells, previously cultured in Plat-E medium in a 75 cm^2 flask. First, aspirate the culture medium and carefully add 2 mL pre-warmed trypsin-EDTA 0.05%. Incubate at 37°C in a humidified 5% CO_2-containing incubator until the cells are detached, 3–5 min. Wash flask out with 8 mL medium and collect the supernatant in a 15 mL conical tube. Wash cells twice by centrifuging at *500 × g* for 5 min and replacing the supernatant with fresh medium.
4. Count the cells using a hemocytometer.
5. Seed Plat-E cells in the coated plates, 3 × 10^6 cells per plate.
6. Incubate for 16 h at 37°C in a humidified 5% CO_2-containing incubator.

Day 2: Transfection of Plat-E cells

7. Once Plat-E cells are 85%–90% confluent, replace the medium with 10 mL DMEM medium supplemented with 10% (v/v) FBS and 1 mg/mL streptomycin, 100 U/mL penicillin.
8. Prepare the materials for transfection by adding the required volume of DMEM, without supplements, to each 1.5 mL Eppendorf tube. In one tube add 15 µg of the DNA plasmid [in this case pMP71Gpre GFP (Engels et al., 2003)] and in the other tube add 45 µL Lipofectamine.

   Note: The final volume in each Eppendorf tube should be 250 µL.

   Note: The concentration of the plasmid is checked prior to transfection.

9. Transfer the diluted Lipofectamine to the diluted DNA solution and immediately pipette up and down 3–4 times.

10. Incubate at 22°C for 20 min to allow Lipofectamine/DNA complexes to form.
11. After 20 min, mix well and add (dropwise) to the Plat-E plates.
12. Gently swirl the plate and incubate for 16 h at 37°C in a humidified 5% CO_2 containing incubator.
13. Replace the medium with DMEM medium supplemented with 10% (v/v) FBS and 1 mg/mL streptomycin, 100 U/mL penicillin.

Isolation and activation of CD4 T cells

Timing: 1 day

CD4 T cells were isolated from C57BL/6 mice spleens and subsequently activated with Dynabeads™ Mouse T-Activator CD3/CD28

Day 3: Isolation of CD4 T cells from mouse spleens and activation with Dynabeads™ Mouse T-Activator CD3/CD28

14. Check the fluorescence of the transfected Plat-E cells to determine transfection efficiency.
15. The day after transfection, sacrifice male or female C57BL/6 mice, aged 8–12 weeks, with an overdose of isoflurane and harvest the spleens. Collect the spleens to a 10 cm petri-dish filled with 5 mL HBSS.
16. In a BSC2 hood, wash the spleens in 5 mL HBSS in a 10 cm plate.
17. Transfer the washed spleens to a 70 μm cell strainer, placed within a 10 cm plate, with 4 mL HBSS, and using the ridged side of a 3 mL syringe plunger mash the spleens through the cell strainer (Figure 1). Rinse with 10 mL HBSS and transfer the cell suspension to a 15 mL conical tube.

Note: You will get approximately 80 × 10^6 leukocytes per spleen.

18. Centrifuge at *500* × *g* for 5 min at 4°C.
19. Remove the HBSS and add 300 μL ACK lysing buffer per spleen. Gently mix with the pellet and incubate for 1 min at 22°C. Next, add 10 mL HBSS and centrifuge at *500* × *g* for 5 min at 4°C. If the pellet remains red after centrifugation, remove the HBSS and add 150 μL ACK lysing buffer per spleen and incubate at 22°C for 45 s. Next, add 10 mL HBSS and centrifuge at *500* × *g* for 5 min at 4°C.
20. Remove the HBSS and wash twice by adding 10 mL HBSS and centrifuge at *500* × *g* for 5 min at 4°C.
21. Isolate CD4 T cells using the EasySep™ Mouse CD4 T Cell Isolation Kit as per manufacturer's instructions.

Note: You will get approximately 10% CD4 T cells from the total splenocytes.

22. Count the cells using a hemocytometer and bring to a concentration of 1 × 10^6 cells per mL in complete RMPI medium.
23. Activate the cells with Dynabeads™ Mouse T-Activator CD3/CD28.

First, wash the Dynabeads™ in preparation for use, as follows; Transfer the required volume of Dynabeads™ to a 1.5 mL Eppendorf tube and add an equal volume of washing buffer [PBS supplemented with 0.1% (w/v) BSA and 2 mM EDTA, pH 7.4], or at least 1 mL, and pipette five times. Place the Eppendorf on the DynaMag™ Magnet for 1 min and remove the supernatant. Remove the Eppendorf from the DynaMag™ Magnet and suspend the Dynabeads™ in the same volume of culture medium as the initial volume of Dynabeads™ taken. Add 25 μL of Dynabeads™ per 1 × 10^6 CD4 T cells.

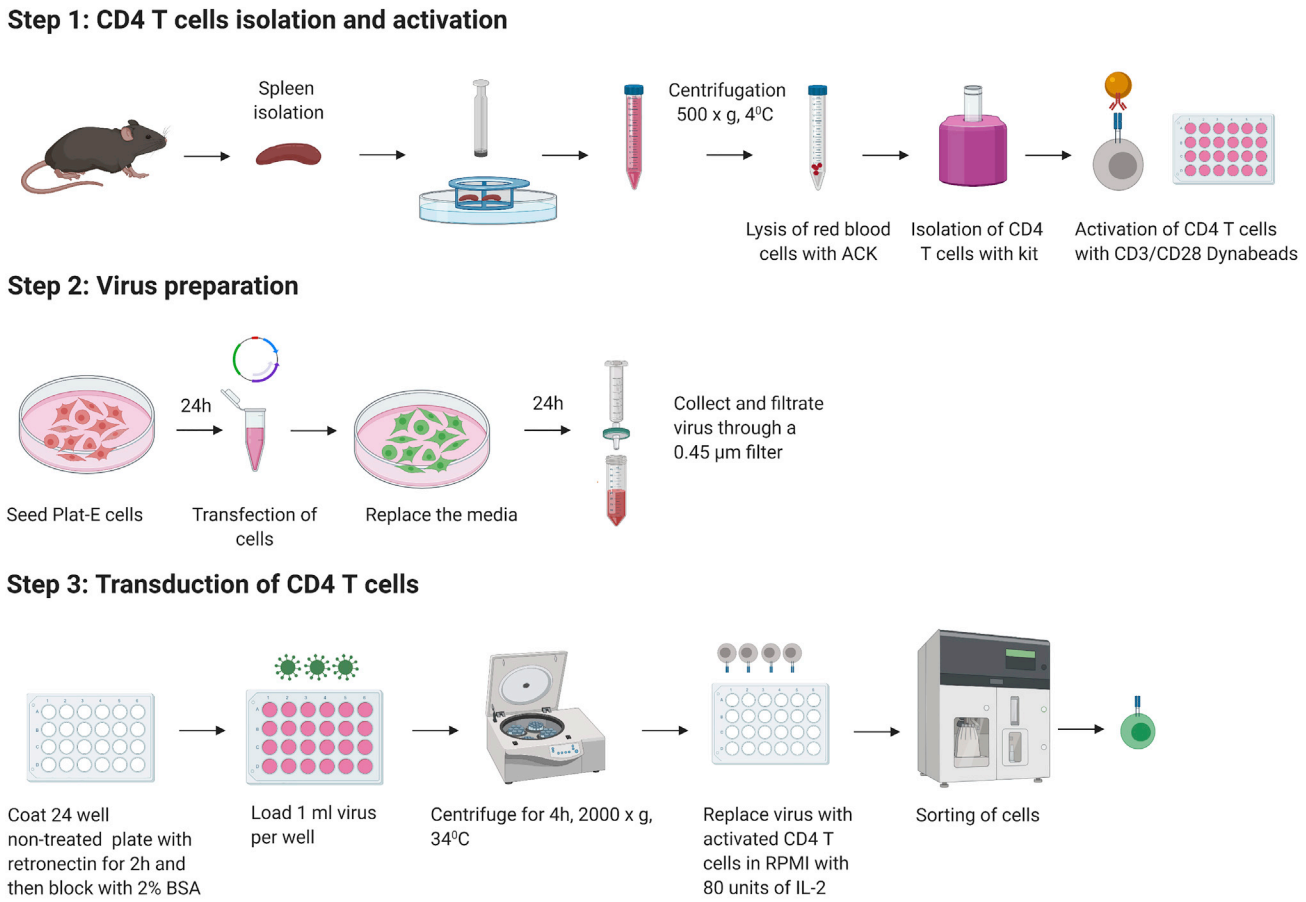

Figure 1. Schematic overview of the retroviral transduction protocol

Step 1: CD4 T cells are isolated from spleens of C57BL/6 mice; Step 2: The retroviral particles are produced using the Plat-E packaging cell line; and Step 3: The CD4 T cells are transduced with the retrovirus.

24. Seed 1 × 10^6 cells per well in a 24 well plate and incubate at 37°C in a humidified 5% CO_2-containing incubator for 16–18 h.

Retroviral transduction of CD4 T cells

Timing: 8 h

CD4 T cells were retrovirally transduced with the viral supernatant

Day 4: Transduction of CD4 T cells

25. Coat non-treated 24-well plates with 350 µL per well of PBS containing 20 µg/mL RetroNectin®.

Pause point: The RetroNectin® coated plates can be stored at 4°C for up to 1 week.

26. Incubate plates for 2 h at 37°C in a humidified 5% CO_2-containing incubator.
27. During the incubation, harvest the viral supernatant from the transfected Plat-E cells and centrifuge at *800 × g* for 10 min. Filtrate through a 0.45 µm filter.

Pause point: The viral supernatant can be stored at 4°C for up to 3 days.

28. Replace with 500 μL per well of PBS supplemented with 2% (w/v) BSA. Incubate at 20°C–22°C for 30 min. Wash twice with PBS.

Caution: Ensure BSA is properly washed from the plate by first removing all of the BSA from each well and then washing the wells twice with 1 mL of PBS. Finally ensure all liquid is removed from the wells.

29. Remove the PBS from the washed plates and add 1 mL of the viral supernatant per well.
30. Wrap the plates with plastic wrap and centrifuge at *2000* × *g* for 4 h at 32°C.
31. Two hours prior to the end of centrifugation, add 80 international units (IU)/mL of recombinant IL-2 to the CD4 T cells.
32. After 2 h, collect the cells to 15 mL conical tubes and centrifuge at *500* × *g for* 5 min. Collect the supernatant and store at 22°C, for not more than 1 h, for use in step 34.
33. Remove the Dynabeads™ using a DynaMag™ Magnet.
34. Suspend the cells in the collected supernatant.
35. Remove the viral supernatant from the plate and add 1 mL of the CD4 T cell suspension per well, i.e., 1 × 10^6 cells per well.
36. Re-wrap the plate in the plastic wrap and centrifuge at *800* × *g* for 30 min at 32°C.
37. Remove the plastic wrap and store at 37°C in a humidified 5% CO2-containing incubator.

Culture and expansion of transduced CD4 T cells

Timing: 10–14 days

The transduced CD4 T cells were expanded in culture in complete RPMI medium supplemented with 20 IU of IL-2 and 10 ng/mL IL-7.

Day 6: Treatment of transduced cells

38. After 48 h, remove 500 μL from each well and add 500 μL complete RPMI medium supplemented with 20 IU of IL-2 and 10 ng/mL IL-7.

Day 7: Cell sorting of transduced cells

39. 72 h post transduction, collect the cells and sort for the positive population using the 70 μm nozzle of the FACSAria™ III Cell Sorter (Figure 2).

Note: Use medium for cell sorting.

Note: The timing of cell sorting can vary but should take place more than 5 days post transduction. It is important to check the fluorescence of the cells before cell sorting.

40. Seed sorted cells, 1 × 10^6 cells per well, in a 24-well plate in complete RPMI medium supplemented with 20 IU of IL-2 and 10 ng/mL IL-7.

Day 9: Activation of sorted cells

41. 48 h post cell sorting, activate the cells with Dynabeads™ Mouse T-Activator CD3/CD28 in complete RPMI medium, as described in step 23.

Day 11: Treatment of cells

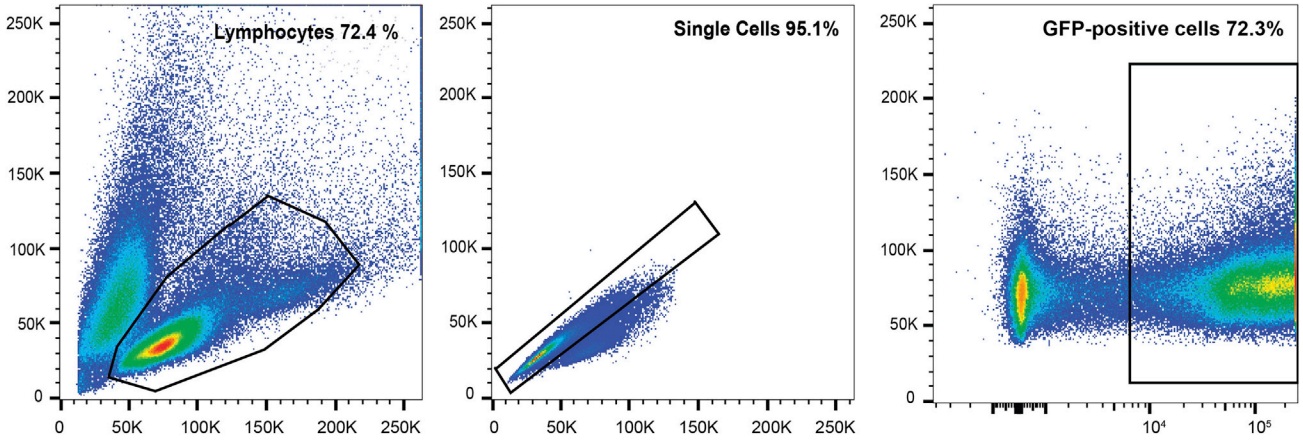

Figure 2. Representative flow cytometry plots outlining the sorting of the transduced GFP-positive CD4 T cells

Plots (left to right) show the percentage of lymphocytes, gating on single cells and the mean fluorescent intensity (MFI) of GFP-positive cells

42. 48 h post cell activation, remove 500 µL of the medium and replace with 500 µL of complete RPMI supplemented with 20 IU of IL-2 and 10 ng/mL IL-7.

Note: In order to optimize the protocol and enhance the proliferation and survival of the transduced cells, 10 ng/mL IL-7 was added to the standard RPMI medium supplemented with 20 IU of IL-2 (Figure 3).

43. Every 2 days, while cells are proliferating, carefully pipette and split one well to two. Add 500 µL of fresh complete RPMI supplemented with 20 IU of IL-2 and 10 ng/mL IL-7.
44. Once the cells are resting, replace half of the medium with fresh medium every second day.
45. Activate cells with Dynabeads™ every 7–10 days, as required.

EXPECTED OUTCOMES

Activation of murine CD4 T cells was performed by co-culturing CD3/CD28 Dynabeads™ with the CD4 T cells for 16–18 h prior to transduction. Coating plates for viral integration with RetroNectin® significantly increased the yield of transduced CD4 T cells. We also observed an increased yield of transduced CD4 T cells by incubating the cells with 80 IU of recombinant IL-2 two hours prior to viral transduction. Evaluation of GFP expression by flow cytometry demonstrated an exclusive gene transfer into murine CD4 T cells. Supplementing the transduced murine CD4 T cells with IL-2 and IL-7 increased their viability and proliferation capacity (Figure 3).

LIMITATIONS

Since the majority of lentiviruses poorly transduce murine T cells, RV are the favored choice for gene delivery into murine T cells (Kerkar et al., 2011). One of the main restrictions of RV is the transduction of proliferating cells, unlike lentiviruses that can transduce also non-proliferating cells (Cepko and Pear, 2001). Since it is essential to transduce the CD4 T cells during proliferation, we activate the cells with Dynabeads™ Mouse T-Activator CD3/CD28 16–18 h prior to transduction and supplement with 80 IU of recombinant IL-2 for 2 h prior the transduction. It is thus important to validate cell proliferation prior to transduction.

The vector size can influence the transduction efficiency, with both retroviral and lentiviral vectors having limited cargo capacity (Simmons and Alberola-Ila, 2016). The limit of the DNA fragment size for retroviral gene transfer is around 11 kb, with DNA fragments larger than 8 kb presenting

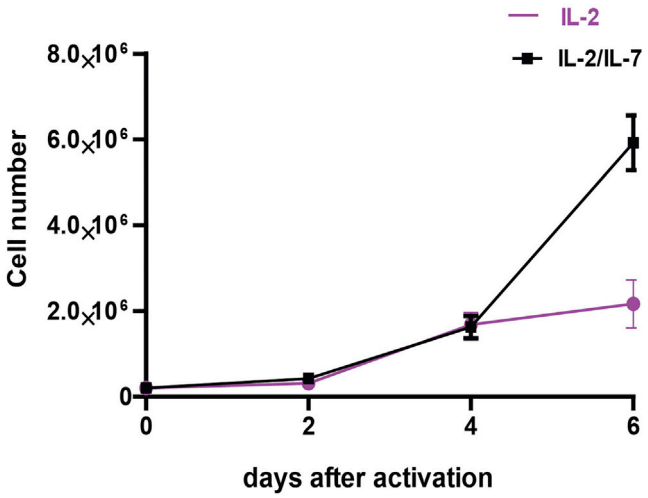

Figure 3. Proliferation of retroviral-transduced CD4 T cells in the presence of IL2-/IL-7 medium

CD4 GFP T cells were seeded in a 24 well plate (0.5 × 10^6 cells per well) in RPMI medium supplemented with either 20 IU IL-2 and 10 ng/mL IL-7 or the control 20 IU RPMI. Cells were counted every two days post activation with Dynabeads™. Each condition had four wells per experiment and the experiment was repeated three times. Error bars represent mean ± SEM.

difficulties to work with (Current protocols in molecular biology; Simmons and Alberola-Ila, 2016). The titer of virus produced is in inversely proportional to construct size (Kumar et al., 2001).

TROUBLESHOOTING

Problem 1

Viability of the donor spleens. (step 15)

Potential solution

We recommend using an overdose of isoflurane to sacrifice the donor mice. An alternative euthanasia is CO_2 overdose, but this can cause necrosis of the spleen and therefore affect the quantity and viability of the splenocytes.

Problem 2

Low RV transduction efficiency. (steps 2, 8, 14, 25, and 30)

Potential solution

Coat tissue culture plates with poly-D-lysine before seeding Plat-E cells to enhance adherence. Otherwise, they are loosely attached and easily dislodged.

Check the concentration of the plasmid prior to transfection.

Check fluorescence of the Plat-E cells post transfection.

Use non–coated plates for spin transduction to increase transduction efficiency.

Use RetroNectin to facilitate adsorption of RV to target cells.

Keep cells above 22°C to maintain activity. Set centrifuge to 32°C for spin-transduction.

Problem 3
Insufficient activation of CD4 T cells. (steps 24 and 31)

Potential solution
Transduce cells 16–18 h post activation with Dynabeads^{TM}.

Add IL-2 two hours prior to transduction. IL-2 should be aliquoted and frozen in appropriate volumes as freeze/thaw cycles can reduce the efficacy of IL-2.

For activation, seed cells at a density of 1×10^6 cells per well in a 24-well plate.

Problem 4
Viability of the CD4 T cells post transduction. (steps 28 and 36)

Potential solution
Carefully wash plates after blocking with BSA by first removing all the BSA and then by washing the well twice with 1 mL of PBS. Finally remove all of the liquid.

Do not exceed the recommend centrifugation time of CD4 T cells to viral-coated plates. We recommend 30 min.

Problem 5
Poor viability of the CD4 T cells after cell sorting. (steps 39 and 41)

Potential solution
Perform cell sorting in cell sorting medium.

Allow sorted cells to rest for at least 48 h before the following activation.

Problem 6
Limited fluorescence of CD4 T cells.

Potential solution
Transduce the CD4 T cells twice to increase the transduction efficiency.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ekaterina Eremenko (eremenkoem@gmail.com).

Materials availability
This study did not generate new unique reagents.

Data and code availability
This protocol did not generate/need datasets.

ACKNOWLEDGMENTS
This research was supported by funding from the National Institute for Biotechnology in the Negev Ltd. and the Ministry of Science and Technology (grant # 3-16148). The graphical abstract and Figure 1 were created with BioRender.com.

AUTHOR CONTRIBUTIONS

E.E. and Z.V.T. performed the experiments. E.E., Z.V.T., A.P., and A.M. wrote the manuscript. All authors read, revised, and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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