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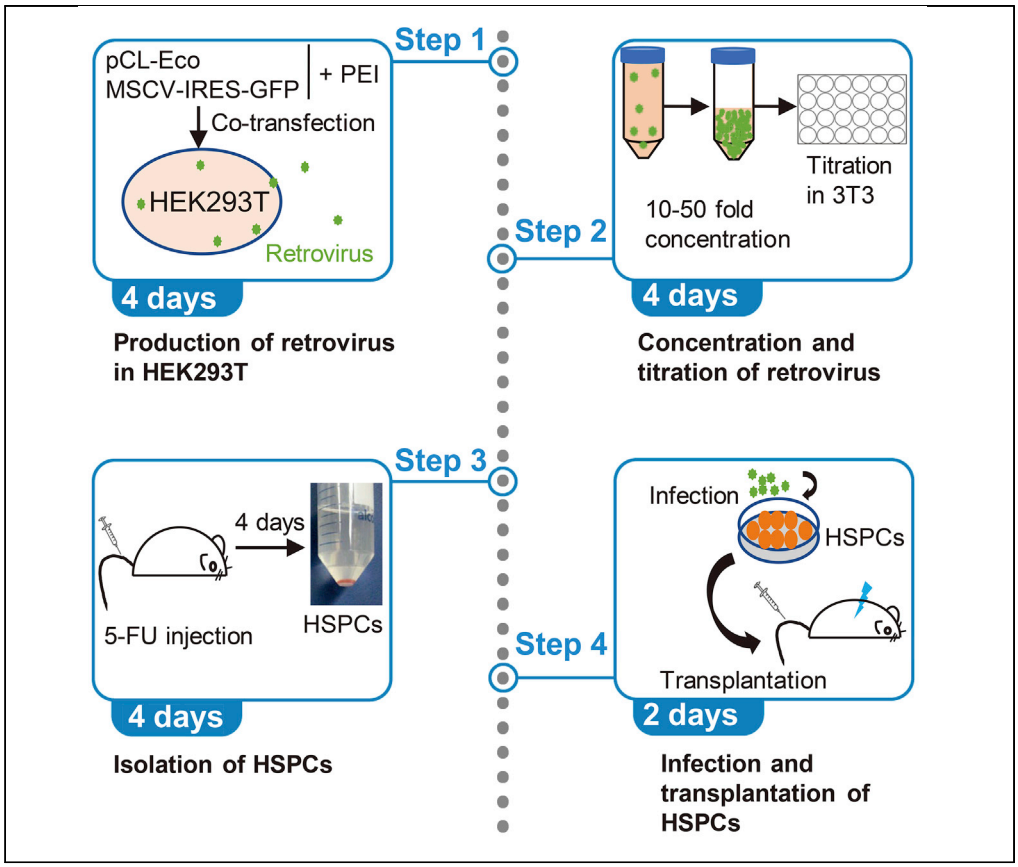

Regulating gene expression through retroviral infection has been widely used in mouse bone marrow transplantation (BMT) to test the capacity of self-renewal, as well as multi-lineage differentiation of hematopoietic stem and progenitor cells (HSPCs). However, it remains challenging to achieve high transduction efficiency in bone marrow cells as transduction of these cells subsequently leads to transplantation failure. Here, we present a modified protocol to overcome this issue, enabling reproducible and high-efficient retroviral transduction of HSPCs for BMT.

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Highlights

Step-by-step details for production of high-titer retrovirus

Isolation and *in vitro* culture of mouse HSPCs for retrovirus infection

Methods for mouse bone marrow transplantation

Qu & Yang, STAR Protocols 2, 100459
June 18, 2021
https://doi.org/10.1016/j.xpro.2021.100459

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Protocol to produce high-titer retrovirus for transduction of mouse bone marrow cells

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https://doi.org/10.1016/j.xpro.2021.100459

SUMMARY

Regulating gene expression through retroviral infection has been widely used in mouse bone marrow transplantation (BMT) to test the capacity of self-renewal, as well as multi-lineage differentiation of hematopoietic stem and progenitor cells (HSPCs). However, it remains challenging to achieve high transduction efficiency in bone marrow cells as transduction of these cells subsequently leads to transplantation failure. Here, we present a modified protocol to overcome this issue, enabling reproducible and high-efficient retroviral transduction of HSPCs for BMT.

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

BEFORE YOU BEGIN

Timing: 1–2 weeks

1. To obtain high titer retrovirus supernatants, high quality, and endotoxin free packaging plasmids are essential. We amplify and isolate the plasmids from Top10 E. Coli cells using an endotoxin free Plasmid isolation Kit.
2. Low passage and good condition of HEK293T cells are critical for high yield production of retrovirus. We thaw and grow healthy HEK293T cells in DMEM media containing 10% fetal bovine serum for at least 3 passages before seeding for transfection.
3. Certified class II biological safety cabinets must be used and personal protective equipment (PPE) including double gloves, lab coats, and masks must be worn when working with retroviruses. Culture medium and other virus contaminated materials such as dishes, plates, and tubes should be autoclaved prior to disposal.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Mouse recombinant SCF	PeproTech	250-03
Mouse recombinant Flt-3	PeproTech	250-31
Mouse recombinant IL-6	PeproTech	216-16
Mouse recombinant IL-3	PeproTech	213-13
5-Fluorouracil	Sigma-Aldrich	F6627

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bovine Serum Albumin	Sigma-Aldrich	V900933
PBS (pH7.4)	Gibco	20012019
Polyethylenimine (PEI)	Polysciences	23966-2
StemSpan SFEM II	Stem Cell Technologies	09655
DMEM	Gibco	41966052
RPMI 1640	Gibco	21870076
Opti-MEM	Gibco	31985047
FBS	Gibco	10099141C
Polybrene	Sigma-Aldrich	H9268
EDTA	Sigma-Aldrich	60004
NaCl	Sigma-Aldrich	31434
Ammonium chloride	Thermo Fisher Scientific	ICN19462380
Hygromycin	Thermo Fisher Scientific	10687010
Puromycin	Thermo Fisher Scientific	A1113803
Potassium bicarbonate	Thermo Fisher Scientific	ICN15255780
Enrofloxacin (Baytril)	Sigma-Aldrich	17849
Retro-X™ Concentrator	Clontech	631455
Bacteria and cell line		
TOP10	Invitrogen	C404003
HEK293T	ATCC	ATCC® CRL-3216™
NIH 3T3	ATCC	ATCC® CRL-1658™
Experimental models: organisms/strains		
C57BL/6 mouse	Charles River	C57BL/6NCrl
Ly5.1 mouse	Charles River	B6.SJL-*Ptprc*^{a}*Pepc*^{b}*/BoyCrl*
Software		
FlowJo	LLC	https://www.flowjo.com
Recombinant DNA		
MSCV-IRES-GFP	Addgene	20672
pCL-Eco	Addgene	12371
Other		
Plasmid Maxi Isolation Kit	QIAGEN	12163
CD117 (c-Kit) MicroBeads	Miltenyi Biotec	130-091-224
LS Columns	Miltenyi Biotec	130-042-201
Falcon™ Cell Strainers 40 μm	Corning	352340
24-Well plate	Corning	3738
6-Well plate	Corning	3516
Falcon 5 mL tube	Corning	352003
10 mL Syringe	Thermo Fisher Scientific	15879152
0.22 μm PES filter	Thermo Fisher Scientific	566-0020
0.22 μm PES microfilter	Thermo Fisher Scientific	721-1320
Kimwipes	Thermo Fisher Scientific	06666
150-mm Tissue culture dish	Thermo Fisher Scientific	150468
Forceps	Sangon Biotech	F519023
Scissors	Sangon Biotech	F519231
1 mL Syringe	BD Biosciences	309659
25G 1 inch Needle	BD Biosciences	305125
27G 0.5 inch Needle	BD Biosciences	305109
Flow Cytometer	BD Biosciences	LSRFortessa
Refrigerated centrifuge	Eppendorf	5804R

MATERIALS AND EQUIPMENT

Dissolve the Baytril in alkaline water (pH 9.0–10.5) to make 100 × stock solution. Dilute it into 1 × into drinking water when use. The stock solution can be saved at 4°C for 2–3 months. Sterilization is not necessary.

Bone marrow flushing and FACS buffer		
Reagent	Final concentration (%)	Amount
FBS (Heat inactive)	2%	10 mL
PBS (pH7.4)	n/a	490 mL
Total	n/a	500 mL
Mix well and sterilize by filtration through 0.22 μm filter. Store the solution at 4°C for 1–2 months.		

ACK red blood cell lysing buffer		
Reagent	Final concentration (mM)	Amount
Ammonium chloride	155	8.3 g
Potassium bicarbonate	10	1 g
EDTA (0.5M, pH7.4)	0.1	0.1 mL
H_2O	n/a	500 mL
Total	n/a	500 mL
Autoclave and store the solution at 4°C for 4–6 months.		

5-FU solution		
Reagent	Final concentration	Amount
5-FU	10 g/L	0.5 g
NaCl	0.9%	0.45 g
H_2O	n/a	50 mL
Total	n/a	50 mL
Dissolve the 5-FU in a 55°C water bath with vigorous vortexing as described (Daadi, 2019). Sterilize by filtration through 0.22 μm filter and store aliquots at −70°C.		

100 × Baytril solution		
Reagent	Final concentration (g/L)	Amount
Baytril	20	1.0 g
H_2O	n/a	50 mL
Total	n/a	50 mL

Cytokine solution (to make stock solution of cytokines)		
Reagent	Final concentration (%)	Amount
BSA	2	0.2 g
PBS (pH7.4)	n/a	10 mL
Total	n/a	10 mL

Mix well and sterile filter. Dissolve the cytokines in a proper volume to make 100 μM stock solution. Store the aliquots at −20°C freezer.

1000 × Polybrene stock solution (8 g/L)		
Reagent	Final concentration (g/L)	Amount
Polybrene	8	0.08 g
PBS (pH7.4)	n/a	10 mL
Total	n/a	10 mL
Mix well and sterilize by filtration through 0.22 μm filter. Store the solution at −20°C.		

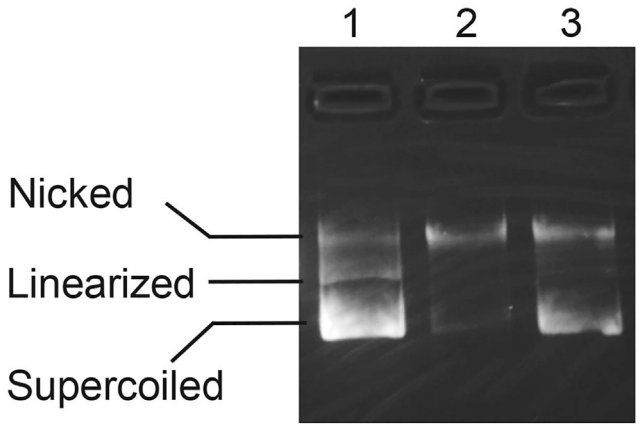

Figure 1. Representative plasmid DNA isoforms
Agarose gel electrophoregrams for 3 batches (lane 1–3) of plasmids with identical construction. Supercoiled, linearized, and nicked isoforms are labeled in left.

STEP-BY-STEP METHOD DETAILS

Retroviral particles production and concentration

Timing: 4 days

Package and concentrate retrovirus from HEK293T cells

1. Late afternoon, seed 6.0 × 10^6 HEK293T (293T) cells per 150-mm dish containing 10% FBS in 20 mL high glucose and sodium pyruvate DMEM medium (293T medium). Gently mix the cells and culture medium before returning dish into incubator (37°C, 5% CO_2). Keep culturing for 16–20 h.

2. PEI transfection (Early in the morning)
   a. 293T cells should be 60%–70% confluent and well attached. To increase the transfection efficiency, add 5 mL fresh and warm 293T culture medium containing 25 μL 25 mM chloroquine (1000 × stock, final concentration is 1 mM) per dish at 1 h before starting the transfection. Gently rock the dishes from side to side and return them to incubator.
   b. Take out the transfection plasmids and PEI aliquot from freezer. Per 150-mm dish, prepare transfection mixture A (25 μg retroviral plasmid and 20 μg pCL-Eco helper plasmid in 1 mL Opti-MEM) and mixture B (add 135 μL 1 mg/mL PEI into 1 mL Opti-MEM). Transfer the mixture B to A, vortex gently for a short period of time, and incubate the transfection mixture for 20 min at 20°C–25°C without disturbance.

   Note: Check plasmids integrity and isoforms by running agarose gel prior to starting transfection (Figure 1). The supercoiled plasmid has a higher transfection efficiency than other isoforms.

   c. Add 2 mL plasmids/PEI mixture to each 150-mm dish in a dropwise manner. Rock the dish gently from side to side before returning it to incubator.
   d. Twelve hours after transfection, carefully aspirate the old medium, pipet 20 mL fresh 293T medium slowly against the side wall of 150-mm dish and return plates to incubator.

⚠ CRITICAL: The transfection medium should be replaced with fresh 293T medium 12–14 h post transfection because of the toxicity of PEI and chloroquine.

Note: PEI has a high transfection efficiency in 293T cells. More importantly, it costs much less than the transfection reagent that we used previously (Yang et al., 2019).

3. Collect and concentrate viral particles
   a. Twenty-four hours after changing medium (36 h post transfection), collect supernatant from each 150 mm dish into 50 mL tube.
   b. Centrifuge at 1000 × *g* for 10 min at 4°C to remove cells and debris. Carefully transfer the supernatant into a new 50 mL tube.
   c. Add 1 volume of Retro-X Concentrator for 3 volumes of supernatant. Gently mix by inverting the tubes every 10–20 min during the first hour of incubation at 4°C.
   d. Incubate mixture for 10–16 h at 4°C refrigerator.
   e. Centrifuge the samples at 1,500 × *g* for 45 min at 4°C. The white pellet at the bottom of 50 mL tube will be visible. Discard as much as possible of the supernatant.
   f. Add 1/10 to 1/50 of the original volume of RPMI medium supplied with 2% heat inactivated FBS. Gently shake the tube on ice until the pellet completely goes into suspension.

Note: To avoid damaging virus, do not vortex white pellet. Because the viral pellet is difficult to resuspend if adding too little medium, we recommend resuspending the pellet with more than 1/50 of the original volume medium.

   g. Titrate the concentrated virus immediately or freeze the aliquots at −80°C.

Titration of retrovirus

Timing: 4 days

4. Plate 50,000 healthy NIH 3T3 cells in 500 μL of complete DMEM (DMEM+10% FBS) per well of a 24 well plate. To ensure that each well has same number of cells as much as possible, we resuspend 1.3 × 10^6 cells in 13 mL complete DMEM and then aliquot 500 μL into each well. Return the plate into incubator and keep culturing for 16–20 h.

5. Trypsinize 3 wells from the plate and count cell number individually. Record average number as (#) cell plated.
   a. Aspirate old medium from each well and add 450 μL complete DMEM containing 8 μg/mL polybrene (for boosting infection efficiency).
   b. Thaw a vial of frozen retrovirus (or fresh viral supernatant) in 37°C water bath and prepare a series of 10 fold dilution of the tested virus as follow:
      i. 100 μL non-diluted viral supernatant.
      ii. 10^{-1}: 18 μL of non-diluted viral supernatant + 172 μL complete DMEM.
      iii. 10^{-2}: 18 μL of 10^{-1}dilution + 172 μL complete DMEM.
      iv. 10^{-3}: 18 μL of 10^{-2} dilution + 172 μL complete DMEM.
      v. 10^{-4}: 18 μL of 10^{-3} dilution + 172 μL complete DMEM.
   c. Distribute 50 μL /well of each virus supernatant dilution in triplicate. Leave 3 wells as a non-viral infection control (add 50 μL/well complete DMEM).

Note: Be sure to keep virus and cells warm, as a cold shock will decrease infection efficiency.

   d. Gently pipet up and down 3 times using a 1 mL pipette. Return the plate into incubator.

6. Twenty-four hours after infection, replace medium containing the viral supernatant with 500 μL fresh complete DMEM.
7. Forty-eight hours after infection, trypsinize and resuspend the 3T3 cells from each well in FACS buffer individually. Analyze the percentage (%) of GFP^+ cells using Flow Cytometer (Figure 2). For a well with 1%–10% of GFP^+ cells, calculate virus titer as follow:

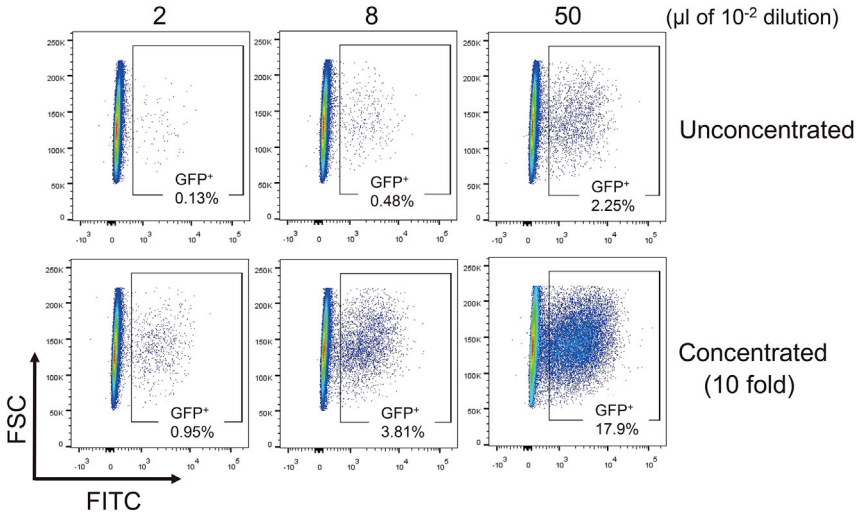

Figure 2. FACS plots showing 3T3 cells transduced with retroviral supernatants

3T3 cell was infected with 2 μL, 8 μL, and 50 μL of 10^{-2} diluted viral supernatants (unconcentrated and 10-fold concentrated) individually. The percentage of positive transduced cells (GFP^+) was measured by flow cytometry at 48 h after infection.

Virus infectious units per mL (IFU/mL) = (%) GFP^+ × (#) cell plated (right before infection) × dilution factor/ 0.05 mL. The average value of triplicate wells represents the viral titer.

Note: The method provided above is suitable for fluorescent labeled and non-transmission (replication defective) virus. For the non-fluorescent retrovirus, please switch to other retroviral titration methods or commercial kit such as Retro-X qRT-PCR Titration Kit (Takara, Cat#: 631453).

Isolation of murine HSPCs

Timing: 4 days

8. In order to enrich hematopoietic and progenitor stem cells (HSPCs), we intravenously inject (iv) 300 μL 10 mg/mL 5-Fluorouracil (5-FU) for a 20 g adult mouse (150 mg/kg) at 4 days prior to viral infection (Yang et al., 2019).
9. Euthanatize the mice through cervical dislocation at 4 days after administration of 5-FU. Rinse shortly with 70% ethanol, and then place the mouse on paper towel and then transfer it into a biosafety cabinet.
10. Cut the hind and front limbs (Figure 3A) into a 100-mm dish containing 10 mL PBS. Disconnect the joint and remove the muscles as much as possible. Place the bones in a new 100-mm dish (Figure 3B).
11. Flush out bone marrow into a new 50 mL tube with bone marrow flushing buffer using a 10–20 mL syringe and 25G 1 1/2 inch needle.
12. To dissociate the clumps of cells, pipet up and down twice by the same syringe and needle. Spin down the cells at 400 × *g* for 5 min at 4°C. Aspirate supernatant.
13. To lyse the red blood cells, add 5 mL ACK lysis buffer and resuspend the cell pellet into a new 15 mL tube. Keep the tube on ice until the lysis becomes clear.
14. Add 8 mL more flushing buffer into the tube to stop the lysis. Inverse the tube 3–5 times and then centrifuge at 400 × *g* for 5 min at 4°C.
15. Resuspend the bone marrow with 10 mL flushing buffer. To remove tissue and cell cluster, pass the suspension through a 40 μm strainer.

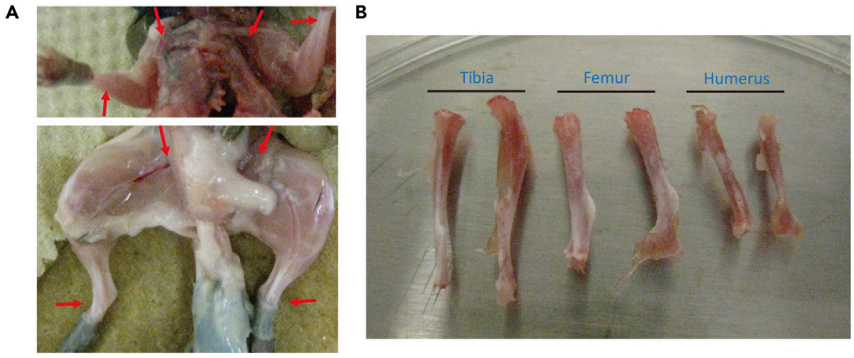

Figure 3. Cell isolation from mouse bone marrow

Cutting sites were marked by red arrows (A). After disconnecting joints and removing meat, clean bones (B) were transferred into a new dish.

16. Enrich c-Kit^{+} HSPCs using CD117 magnetic beads and LS column following manufacturer's instruction (https://www.miltenyibiotec.com/US-en/products/cd117-microbead-kit-human.html) (optional).
17. Centrifuge at 400 × *g* for 5 min at 4°C. Aspirate supernatant.
18. Add 1 mL RPMI 1640 medium (containing 20% FBS, 20 ng/mL SCF, 20 ng/mL Flt-3, 10 ng/mL IL3 and 10 ng/mL IL6) per mouse to resuspend HSPCs cells into one well in 6 well plate. Count the cell number.

Note: Typically, 5–10 × 10^{6} HSPCs, if there are no further enrichments, could be obtained from a mouse at 4 days after 5-FU injection. To obtain enough cells for viral infection and subsequent transplantation, pool cells isolated from 2–3 mice in one well.

19. Incubate the cells for 20–24 h in 37°C incubator supplied with 5% CO_2.

Optional: A commercial HSPCs culture medium (StemSpan) is an option for growing HSPCs.

Retroviral infection of HSPCs

Timing: 1 day

20. Thaw one vial (200 μL aliquot) of frozen retrovirus and frozen polybrene in 37°C water bath.
21. Add 200 μL of viral suspension and polybrene (final concentration is 8 μg/mL) in one well of 6-well plate containing 2 mL cell suspension. Pipet up and down 3 times to briefly mix. Return the plate into incubator.
22. After a 30 min incubation, initiate spinoculation by centrifuging the plate at 500 × *g* for 90 min at 32°C.
23. Carefully return the plate into incubator and keep culturing for 2–4 h.
24. Repeat spinoculation once by adding 200 μL more concentrated retrovirus in same well as descried above. Return the plate into incubator.
25. After 1–2 h incubation, collect cells in a 15 mL tube and count cell number.
    a. Aliquot approximately 10,000 cells in same medium and keep culture for 24 h. Next day, check transduction efficiency by FACS if the virus expresses fluorescence (e.g., GFP). For non-fluorescence expressing virus, estimate cell viability by antibiotics resistance gene screening (such as hygromycin or puromycin selection). Calculate cell viability as follow:
       % cell viability = cell (#) after incubation with antibiotics for 24 h / cell plated (#) (here 10,000) ×100%

b. Wash the cells that used for transplantation to remove virus and polybrene with RPMI medium supplied with 2% heat inactivated FBS.
c. Place the tube on ice until injection into murine recipients.

Note: For each retroviral infection of HSPCs, we recommend performing preliminary experiment to estimate the transduction efficiency or measure the functional effect through colony formation assay at 24 h after infection. Since pluripotency of HSPCs decreases with prolonged culture time, the cells should be transplanted into recipient mouse as early as possible (24–48 h post infection).

Bone marrow transplantation

Timing: 1 day

26. Depending on the purpose of experiment, irradiate the recipient mice lethally (1100 Rads, 3 h apart) or sub-lethally (450 Rads once) from an X or gamma irradiator. Note that lethal irradiation leads to complete myeloablation which generally follows reconstitution of the whole hematopoietic system by hematopoietic stem cell transplantation.
27. Return irradiated mice into cages with fresh food and drinking water supplied with 0.2 mg/mL Baytril.
28. Resuspend cells with 10 mL cold RPMI 1640 medium containing 2% heat inactive FBS and filter the suspension through 40 μm Strainers. Centrifuge at 400 × *g*, 4°C for 5 min. Aspirate supernatant by vacuum.
29. Pipet up and down of cells with a proper volume of cold RPMI 1640 medium containing 2% heat inactive FBS.
30. Count and adjust cell number into a concentration of 0.2–1 × 10^6 cells/0.2 mL.

Note: Cell number that used for transplantation is dependent on the cell transduction efficiency and the purpose of experiment. Thus, we recommend performing a preliminary experiment to check transduction efficiency in similar condition (e.g., same batch of virus). The transduced cell number for each mouse could be calculated as: Transduced cell (#) = transplanted cell (#) × (%) transduction. For recipient mice with lethal irradiation, co-transplant with 0.2–0.5 × 10^5 healthy bone marrow cells as helper.

31. Inject 0.2 mL cell suspension into each irradiated recipient mouse via tail vein using a 1.0 mL syringe and 27G 1/2 inch needle.
32. Change cages with fresh food and drinking water (containing 0.2 mg/mL Baytril) one week post transplantation.

Note: Recipient mice are immunocompromised for the first two weeks post BMT, and therefore they should be housed under strict barrier conditions. The Baytril added in the drinking water can protect mice from bacterial infection.

Optional: After irradiation, the recipient mice could also be maintained on acidic (adjust to pH 3.0 by hydrochloric acid) drinking water for 2 weeks.

Expected outcomes

In this protocol, to produce high titer retrovirus with less cost, we use PEI as a mediator to deliver retroviral packaging plasmids (MSCV-IRES-GFP and pCL-Eco) into HEK293T cells, which produced comparable retroviral particles (up to 3 × 10^7/mL) after 20 fold concentration.

We usually obtain 40–60 × 10^6 mononuclear bone marrow cells from each 8–12 weeks old mouse by following the protocol as mentioned above and else (Yang et al., 2019; Yang and Jiang, 2020). 5-FU

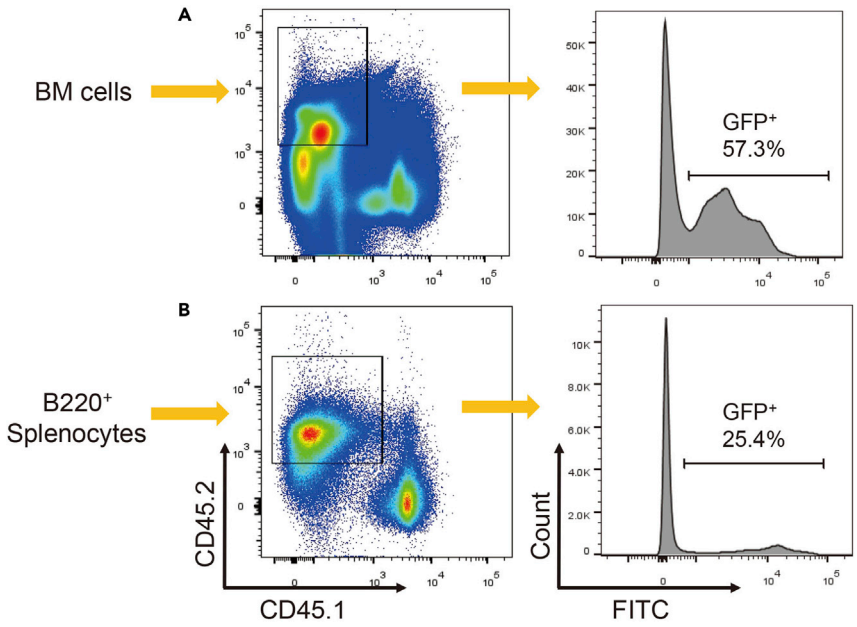

Figure 4. Donor contribution of retroviral transduced cells (GFP$^+$) in total bone marrow cells and splenocytes

Wild type bone marrow cells were isolated and transduced with retrovirus expressing GFP. Donor contribution of (GFP$^+$) in total BM cells (A) and B220$^+$ spleen cells (B) were estimated by flow cytometry at 5 weeks post transplantation.

is a nucleotide analogue that is incorporated into DNA during the S-phase of the cell cycle. Thus, it induces cell death of cycling cells and enriches HSPCs that have slow cell division in vivo. To enrich HSPCs, Intraperitoneal injection (IP) of 5-FU is mentioned in other publications (Zhu et al., 2016; Chen et al., 2019). However, we found that intravenous (iv) injection is more powerful and reliable for enrichment of HSPCs. For each mouse, around 5–10 × 10^6 mononuclear BM cells could be isolated at 4 days after iv injection of 5-FU. Those cells could be directly transduced with retrovirus or be further enriched by surface marker based enrichments such as c-Kit conjugated beads or flow mediated selection.

Herein, four days after administration of 5-FU, we further enriched c-Kit positive BM cells using a commercial CD117 magnetic beads and pooled cells isolated from 3 mice in one. After transduction with retrovirus expressing GFP, 0.4 × 10^6 total cells (CD45.2$^+$) together with 0.2 × 10^6 competitor BM cells (CD45.1$^+$) were transplanted into each lethal irradiated recipient. Percentages of GFP$^+$ cells in bone marrow and splenocytes were estimated by flow cytometry at 5 weeks post-transplantation. Results were analyzed by FlowJo software (Figure 4). More than half of CD45.2$^+$ BM cells, 25% B220$^+$; CD45.2$^+$ spleen cells were GFP$^+$, suggesting a significant number of HSPCs were transduced.

LIMITATIONS

This protocol was designed to produce retrovirus expressing fluorescent marker (e.g., GFP). The virus titer and transduction efficiency could be easily measured by flow cytometer. Therefore, researcher may optimize this protocol if using a non- fluorescent expressing retrovirus. Moreover, the use of CD117 magnetic beads to collect mouse c-Kit$^+$ cells (step 16), for some experiments, may not sufficient to enrich HSPCs. In this condition, a rigorous method (Yang et al., 2016) is needed.

TROUBLESHOOTING

Problem 1

Low production of retrovirus (step 2 of step-by-step method details)

Potential solution

Healthy and low passage (<20 passages) of 293T cells are essential for the production of high titer of retrovirus. In addition, check the integrity and isoforms of retroviral plasmids by agarose gel before transfection. High quality of plasmid (Figure 1) greatly improves the viral production.

Problem 2

Low yield of HSPCs (step 18 of step-by-step method details)

Potential solution

With 5-FU injection, around 5–10 × 10^6 HSPCs could be obtained from each adult mouse. Thus, cell recovery of less than 5 × 10^6 HSPCs per mouse is defined as low yield. To avoid this problem, we recommend: (1) Control the 5-FU dose at ratio of 150 mg/kg of body weight and sacrifice mouse at 4 days after administration; (2) Flushing out cells from all tibia, femur, and humerus; (3) lyse red blood cells on ice using ACK buffer, stop lysis immediately when it becomes clear by adding flushing buffer or equal volume of 2 × PBS prior to centrifuging.

Problem 3

Bacteria contamination in HSPCs (step 19 of step-by-step method details)

Potential solution

Autoclave all the materials including scissors, forceps, and Kimwipes that used in BM isolation. Furthermore, we recommend that rinsing the mouse in 70% ethanol completely for at least 1 min after sacrifice. And then performing the cell isolation in a biosafety cabinet as mentioned above.

Problem 4

Low retroviral transduction efficiency in HSPCs (step 25 of step-by-step method details)

Potential solution

Check the viral titer before BM transplantation. Since low temperature reduces transduction efficiency, we recommend warming the frozen viral aliquot in 37°C water bath in a short time before adding them into cell culture. To minimize dilution of the culture media, in particular of the cytokines, the viral supernatant should not exceed 1/10 volume of the total medium. In addition, we highly recommend that spinoculation twice in 2–4 h apart and transplantation of transduced cell be performed in same day.

Problem 5

Recipient mice die after irradiation/BM transplantation (step 32 of step-by-step method details)

Potential solution

Recipient mice are quite fragile in the first two weeks after irradiation. Thus, they need to be housed under strict barrier conditions. In addition, we add antibiotic (Baytril) in the drinking water to prevent bacterial infection. To help mice reaching food, put some chow in cage and soften them by spraying a little drinking water containing Baytril. For lethal irradiation mice, co-transplant with healthy BM cells as competitor which will protect mouse from BM reconstitution failure.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Zhenhua Yang (zhenhua@hust.edu.cn).

Materials availability

No unique reagent was generated in this study.

Data and code availability

No unique datasets or code was generated in this study.

ACKNOWLEDGMENTS

This work was supported partly by startup fund from Huazhong University of Science and Technology, National Natural Science Foundation of China (82070171) to Z.Y.

AUTHOR CONTRIBUTIONS

J.Q. performed the experiments and analyzed data. Z.Y. analyzed the data and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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