Preparation of Lentivirus (from Alex Van Slyke)

Purefection protocol link: https://www.systembio.com/wp-content/uploads/PureFection_protocol_ver2-1.pdf

Day-by-day schedule

1. Day 1
    1. Plate HEK293Ts in 6 well plate (approx. 400-500k to be 50% confluent the next day)
2. Day 2
    1. Remove 1mL of media and add 1mL fresh media to HEK293Ts a few hours prior to transfection
    2. Mix vectors and purefection, add to HEK293Ts
3. Day 3
    1. Replace HEK293T media with fresh media
4. Day 4: Plate target cells (approx. 150-250k for U2OS, MDA, MCF10a, MCFCA1a
5. Day 5: Collect HEK293T media with virus, add polybrene, spin, add to target cells at between 1:2 and 1:20 dilution (diluted with normal cell culture media)

1. Trypsinize HEK293T cells.
2. Plate approx. 400-500k in a 6 well dish to be 50% confluent the next day
3. Sometime (at least a couple of hours before transfection) remove 1mL of the media and replace with 1mL of fresh media
4. Transfection prep-
   -Mix the following in a sterile 1.5 mL Eppendorf tube:
   -200uL Opti-MEM
   -1:1:1 molar ratio of DNA:
   2 ug psPax2
   1 ug PMD2.G
   X ug of desired lentiviral vector (where X gives it a proper molar ratio)
   -8uL of purefection
   -Vortex for 10s
   -Let sit for 15 min at room temperature
   -Add in a dropwise fashion to HEK293T cells
   -Gently shake plate to stir it around and return to incubator
5. The following day remove the media and replace with fresh media
6. Approx. 48 to 72 hours after initial transfection the media will need to be harvested, plan accordingly.
7. On the day before you plan on harvesting media containing viral particles, plate target cells in 6 well plate (approx. 150-250k cells for U2OS, MDA, MCF10A, MCFCA1a)

PureFection™ Nanotechnology-based Transfection Reagent

Catalog Number: LV750A-1, LV750A-5

Product Description

PureFection™ Nanotechnology-based Transfection Reagent delivers more DNA and siRNAs to cells than the leading lipid-based transfection kits.

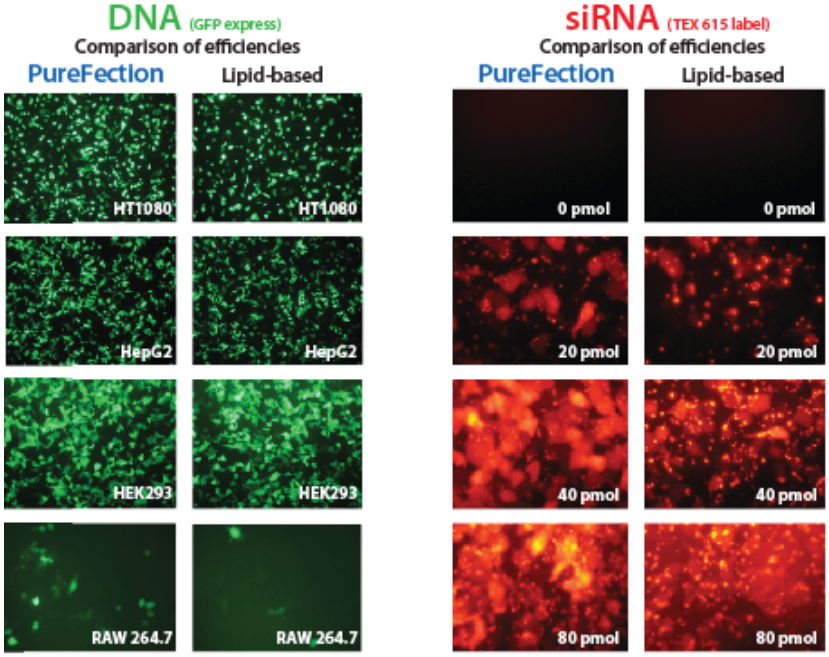
PureFection™ is a powerful, broadly applicable transfection reagent for effective and reproducible transfections.

The PureFection reagent self-assembles nanoparticles in the presence of DNA and RNA. These complexes are readily taken up by target cells for efficient gene delivery. No media changes are required as PureFection works in the presence of antibiotics and serum. The easy-to-use protocol with rapid, one-step incubation for 15 minutes before adding directly to target cells makes PureFection well-suited for high-throughput transfection experiments.

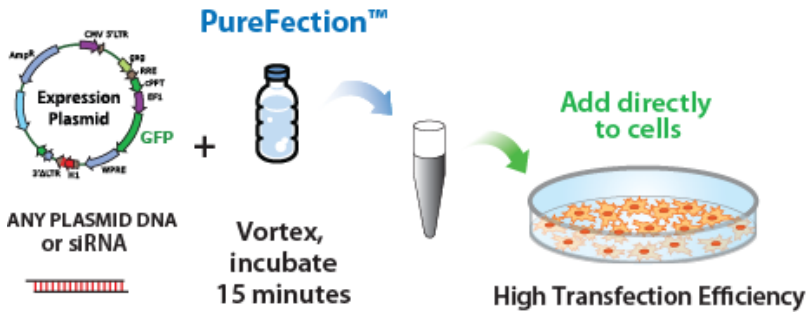
Advantages:

- Highly effective transfection technology - works with most cell types
- Cost-effective alternative to lipid-based products
- Nano-based gene delivery with low toxicity
- Rapid 15 minute protocol makes PureFection ideal for high-throughput transfections
- Works with both Plasmid DNA and siRNAs
- Package virus with high titer, low cost and simple procedure

Comparison of Transfection Efficiency



Transfection Procedure Summary



Important Guidelines for Transfection

For high transfection efficiency and lower toxicity, transfect cells at high density. 50-80% confluency is highly recommended

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the cell density reaches 50~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 2 hours before transfection.

Step II. Preparation of PureFection/DNA Complex and Transfection Procedures:

The following protocol is given for transfection in a 24-well plate, refer to **Table 1** for transfection in other culture formats.

1) For each well, add 0.5 ml of complete medium with serum and antibiotics (antibiotic does not influence the result) freshly 2 hours before transfection.

2) For each well, dilute 0.5 µg of DNA into an Eppendoff tube with 50 µl of serum-free DMEM, and Mix gently.

3) Add 1 µl of PureFection reagent into the same tube. Vortex 5-10 seconds and spin down briefly to bring drops to the bottom of the tube.

4) Incubate for ~15 minutes at room temperature to allow PureFection/DNA complexes to form.

5) Add the 50 µl PureFection/DNA mix drop-wise to the cells in each well and homogenize by gently swirling the plate.

6) Return the plates to the cell culture incubator.

7) Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture

Culture Dish	Surface Area (cm^2)	Cell Number	Volume (ml)	Plasmid (µg)	Pure-Fection (µl)	Diluent Volume (µl)
96-Well	0.3	1-1.7x10^4	0.1	0.1	0.2	10
48-Well	1	2.5-5x10^4	0.25	0.25	0.5	20
24-Well	2	0.5-1x10^5	0.5	0.5	1-2	50
12-Well	4	1-2x10^5	1	1	2-4	100
6-Well/35 mm	9.5	2-4x10^5	2	2.5	4-8	200
60 mm/T25	28	5-10x10^5	5	6-8	12-24	300
100 mm/T75	79	1.5-3x10^6	10	15-20	30-40	500
150 mm/T150	153	5-9x10^6	20	25-40	40-60	1000

Note:
For different cell types, the optimal ratio of PureFection (µL): DNA (µg) is around 2:1. We recommend the PureFection (µL):DNA (µg) ratio of 2:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity, however the amount of PureFection may be adjusted from 1 to 4 µl per µg of DNA depending on the cell line to be transfected. To ensure the optimal size of PureFection/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PureFection Reagent.

Procedures for Packaging Lentivirus

1. 18 to 24 hours prior to transfection, seed **7-8X10^6** 293TN or 293FT cells in per 150 cm^2 cell culture plate* in 20ml of normal culture medium (without antibiotics) so that the cell density reaches to 60~80% confluency at the time of transfection.
2. Add **1-1.6ml** of DMEM (serum free) to an autoclaved 2 ml Eppendorff tube.
3. Add **45µl** of pPACKH1 (Cat#LV500A-1) and **4.5µg** of your plasmid construct to the same tube of DMEM. Mix by pipetting.
4. Then add **55µl** of PureFection into DMEM-Plasmid mixture. Mix well by vortex 10 seconds.
5. Incubate DMEM-Plasmid-PureFection mixture at room temperature for 15 minutes.
6. Add DMEM-Plasmid-PureFection mixture drop-wise into the dish, and swirl the dish to disperse evenly in the plate.
7. Return the dish to cell culture incubator at 37°C with 5% CO_2.
8. Change to fresh medium 12-24 hours after transfection (optional).
9. Collect medium that contains lentiviruses at 48 hours and 72 hours after transfection into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000rpm for 15 minutes at room temperature to pellet cell debris. Transfer the viral supernatant into a new tube.
10. For fresh viral supernatant, aliquot the supernatant into sterile 1.5-ml microfuge tubes and store them at -80°C.
11. To concentrate virus, add a quarter volume of 5X PEG-it (Cat# LV810A-1) to the viral supernatant (volume of PEG-it vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture to 4°C refrigerator over night and spin the virus pellet down next day. Please refer the user manual for details.

*If you use 10cm plates, seed 3-4X10^6 cells/ dish in 9 ml normal culture medium without antibiotics.
In step 2, add 0.8ml of serum free medium per 10 cm plate.
In step 3, add 20µl of pPACKH1 and 2 µg plasmid per 10 cm plate.
In step 4, add 24 µl of PureFection per 10cm plate.

Protocols for siRNA Transfection on 24-well Plate Format

1. Seed 0.5-1X10^5 cells/well in 0.5 ml of culture medium 16-24 hours before transfection. The cell density should reach 40-60% confluency according to the cells' growth rate and the purpose of the experiment.

2. **Prepare siRNA:** Dilute siRNA with RNase-Free Buffer (pH 7.5) to the working concentration of 10 μM. Heat solution to 94°C for 2 minutes. Cool it at room temperature. Product will be fully resuspended in a stable, double-stranded form. Aliquot enough amount for transfection. Store the remaining at -20°C.

3. **Prepare Purfection-SiRNA complex:** Add serum-free DMEM 100 μl to an Eppendoff tube. Add SiRNA 20, 40, and 80 pmol to each tube to test the optimal amount for your cells. Add 1 μl of PureFection to each tube. Vortex for 5 sec, then spin down briefly. Incubate at room temperature for 15 minutes.

4. Add the PureFection-SiRNA mixture drop-wise into each well.

5. Return the plate to cell culture incubator at 37°C with 5% CO_2. Incubate for 1-3 days depending on the purpose of experiment.

8. Collect media from HEK293T cells in 2mL tubes and add polybrene to a final concentration of 8ug/mL (1250x dilution). Discard HEK293T cells (noting that they contain viral particles)
9. Spin down media+polybrene at 3000xg for 5 minutes.
10. Collect supernatant (viral particles are suspended in this)
11. Dilute viral particle containing media at between 1:2 and 1:20 with normal media and add to cells. Return to incubator.
12. Swap media with fresh media the following morning.
13. After 24 hours the cells should now be transduced and ready to move to a larger container for antibiotic selection or to grow out for FACS (make sure nothing gets 100% confluent during this process)