COSMO BIO CO., LTD.
Inspiration for Life Science

Adenovirus CMV Expression System

Complete Adenovirus CMV Expression System	A100
Partial Adenovirus CMV Expression System	A110
Partial Adenovirus CMV Expression System	A120
Supplemental Kit for All Adenovirus Systems	A097
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All Adenoviral Expression Systems provided by abm Inc. include the following safety features:

• The entire E1 gene is deleted from the adenovector (pAdeno). Expression of the E1 protein is required for the expression of the other viral genes, and thus viral replication only occurs in cells that express E1, like 293 cells (Graham et al., 1977; Kozarsky and Wilson, 1993; Krougliak and Graham, 1995).

• Recombinant adenovirus produced from abm Inc.'s adenoviral cloning systems is replication-deficient in mammalian cells that do not express the E1 proteins (Graham et al., 1977; Kozarsky and Wilson, 1993; Krougliak and Graham, 1995).

• Since recombinant adenovirus is replication-incompetent, the presence of the viral genome will be transient and will eventually be degraded or diluted out as cell division occurs.

• Adenovirus does not integrate into the host genome upon transduction, avoiding any possible insertional mutations.

Despite the safety features discussed above, it is highly recommend- ed that all manipulation with adenoviral vectors, including viral production and transduction, be performed under Biosafety Level 2 (BL-2). All published BL-2 guidelines with proper waste decontamination should be strictly followed. In addition, exercise extra caution when creating adenovirus carrying potentially harmful or toxic genes (e.g. oncogenes). For more information about the BL-2 guidelines and adenovirus handling, refer to "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition. This may be downloaded at: www.cdc.gov/biosafety/publications/bmbl5/index.htm

The genomic copy of E1 in all 293 cell lines contains some homol- ogous regions of overlap with our pAdeno vectors. In rare instances, it is possible for homologous recombination to occur between the E1 genomic region in 293 cells and the viral DNA, causing the gene of interest to be replaced with the E1 region, and resulting in generation of a "wild-type," replication-competent adenovirus (RCA) (Lochmuller et al., 1994). This event is most likely to occur during large-scale preparations or amplification of virus, and the growth advantages of the RCA allow it to quickly overtake cultures of recombinant adenovirus. To reduce the possibility of generating RCA contaminated adenoviral stocks:

• Use caution when handling all viral preparations, and treat as BL-2 (see the previous page for more details).

• Screen for the presence of wild-type RCA contamination following large-scale viral preparations. Methods for RCA screening include PCR screening (Zhang et al., 1995) or supernatant rescue assays in non-E1 expressing cells (Dion et al., 1996).

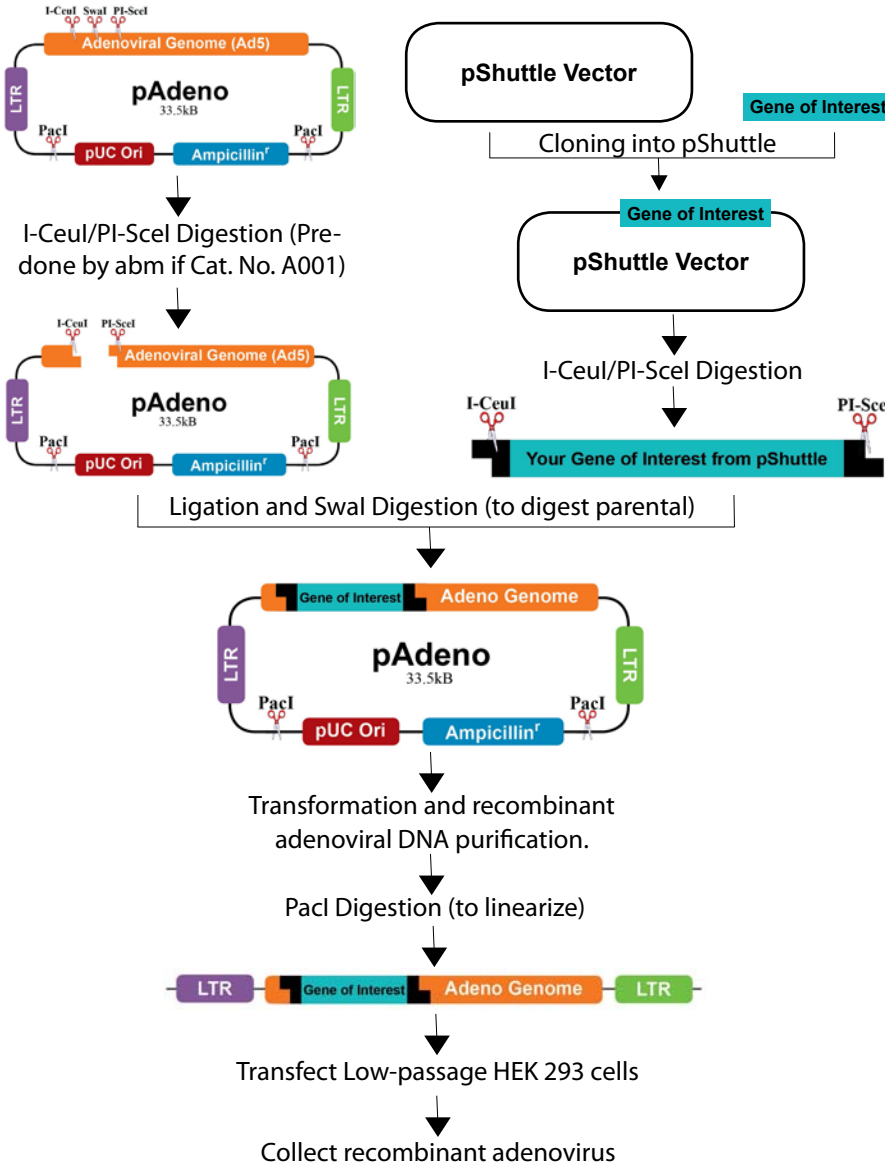

Figure 1: Experimental flow chart of entire experiment, depending on your situation all or part of the chart may apply.

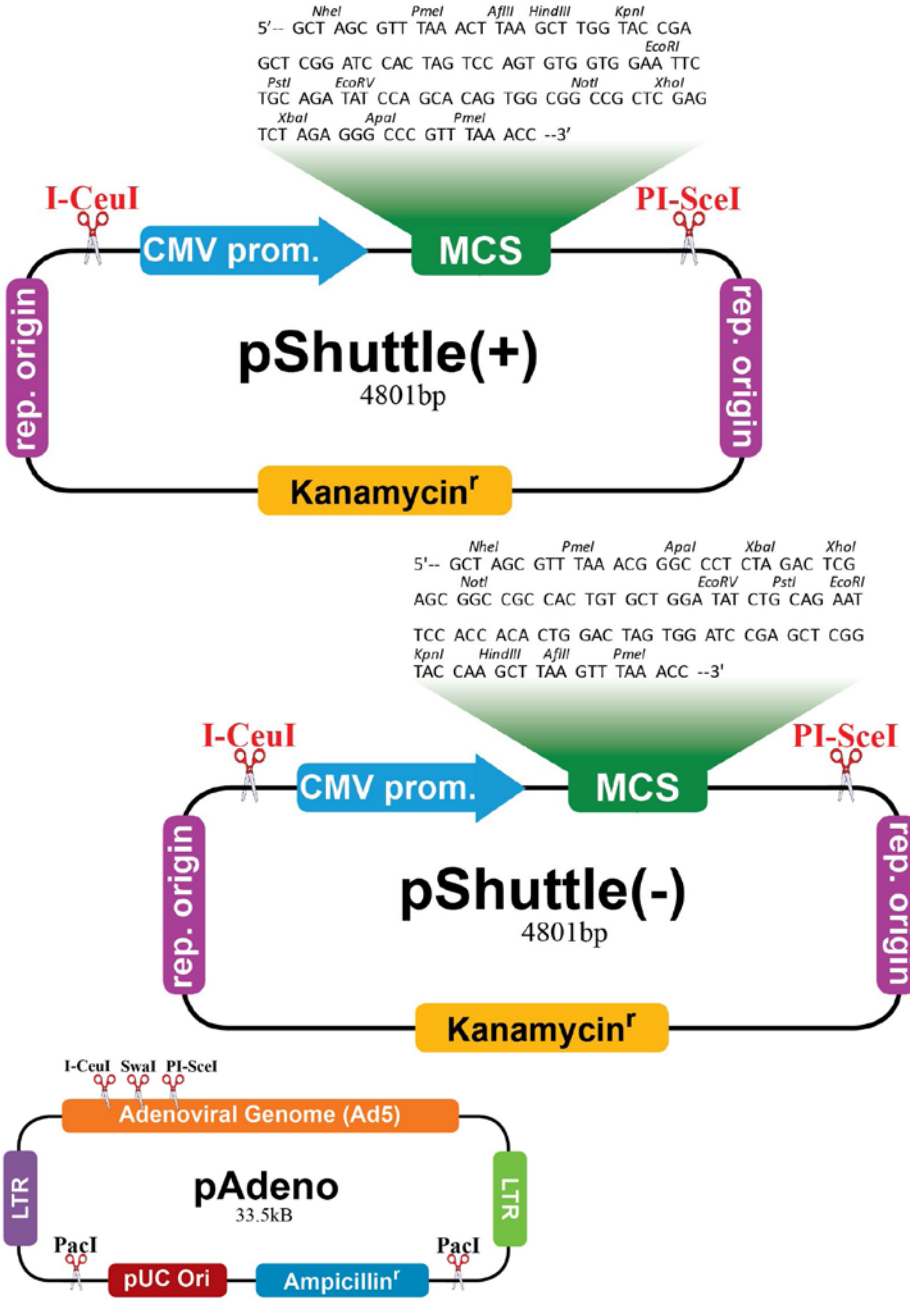

Figure 2: Maps of pShuttle(+), pShuttle(-) and pAdeno.

abm Inc.'s Adenovirus CMV Expression System provides an ef-ficient method for constructing recombinant adenoviruses. Our procedure uses *in vitro* ligation to subclone your gene of interest into a replication-incompetent (-E1/-E3) human adenoviral type 5 (Ad5) genome. This approach enables you to produce recombinant adenoviruses quickly (in less than 3 weeks) and reliably. Also, our pShuttle(+/-) vectors have numerous cloning sites for simplified subcloning manipulation.

How Adenovirus Gene Expression Works

Adenoviruses enter target cells by binding to the Coxsackie/Adeno-virus Receptor (CAR) (Bergelson et al., 1997). After binding to the CAR, the adenovirus is internalized via integrin-mediated endocytosis (Russell, 2000) followed by active transport to the nucleus. Once in the nucleus, early genes, such as the E1 and E2, are expressed. This is followed by expression of the adenoviral late genes and viral genomic DNA replication. The presence of E1 protein is required for the expression of all late genes. For the generation of recombinant adenovirus using this kit, E1 is provided in trans by 293 packaging cells. For more information about the adenovirus life cycle and adenovirus biology, refer to published reviews (Russell, 2000).

Recombinant Protein Expression

After the adenovirus is translocated into the target cell's nucleus, it does not integrate into the host genome. Therefore, expression of your recombinant protein of interest is detectable within 24 hours post infection and is transient in nature. The expression will persist as long as the viral genome is not degraded, which is about 1-2 weeks depending on the cell type. Longer expression can be observed in slow dividing cells such as neurons.

In vivo Gene Delivery

Recombinant adenoviruses have been shown to be the most efficient system for *in vivo* gene delivery applications. Many groups have successfully used adenoviral vectors to deliver target genes to a variety of tissues including the lungs, heart, brain and skeletal muscle. For detailed information about target genes that have been successfully expressed *in vivo* using recombinant adenovirus-based vectors, refer to published reviews (Russell, 2000; Wang and Huang, 2000; Wivel, 1999).

Materials

Table I. Adenovirus CMV Expression Kits

Component	Cat. No.	Quantity	Kit Cat. No. A100	A110	A120	A097	A098	A099
Linearized pAdeno DNA	A001	5ug	✓	✓	✓			
pShuttle(+) Vector	A002	5ug	✓	✓				
pShuttle(-) Vector	A003	5ug	✓		✓			
pShuttle-LacZ Control	A004	5ug	✓	✓	✓			
Enzyme Pack w/ Buffers	A005	2 x 50ul	✓			✓	✓	
PCR Screening Sets	A006	2 x 100ul	✓			✓	✓	
293 Cells	A007	$1x10^6$					✓	
Ad-competent Cells	A014	10 x 50ul						✓
Glycogen 20mg/ml	A013	100ul						✓

- Store Ad-Competent cells at -70°C and all other components at -20°C.
- Spin all items briefly before use to recover contents.
- Avoid repeated freeze-thaw cycles; make smaller aliquots if required.

Additional Materials Required

The following materials and reagents are required but not provided:

- Kanamycin (Cat. No. G022) and Ampicillin (Cat. No. G021)
- LB Broth/Liquid (Cat. No. G246) and LB Agar (Cat. No. G247)
- Glycogen, 20mg/ml (Cat. No. G024) and Cell Freezing Medium
- Agarose (Cat. No. G060-1 or G060-2)
- Sterile, deionized H_2O, 100% Ethanol and 70% Ethanol.
- 10M (saturated solution) Ammonium Acetate (NH4OAc) or 3M sodium acetate (NaOAc; pH 5.2)
- Electro- or Chemically Competent DH5a *E. coli* Cells (Cat. No. A014)
- Restriction Endonuclease SwaI, PacI, I-CeuI and PI-SceI (N.E.B.)
- T4 DNA Ligase (Invitrogen)
- Mini DNA Purification Kit (Cat. No. D024)
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 293 cell line (Cat. No. A007) and Trypsin-EDTA (Cat. No. TM050 or TM051)
- Dulbecco's Modified Eagle's Medium (Invitrogen Cat: 11995)
- Fetal bovine serum (FBS) (Cat. No. TM99-100 or TM999-500)
- Solution of 10,000 units/ml Penicillin G sodium and 10,000 µg/ml Streptomycin sulphate (Sigma Cat. No. P0781)
- Transfection reagent, and tissue culture plates and flasks
- Standard Phosphate-Buffered Saline (PBS) and Dulbecco's Phosphate Buffered Saline (DPBS; VWR Cat. No. 82020-066)

Protocol

NOTE: The following protocol has been broken down into sections for convenience. However, time should be taken to familiarize oneself with the full procedure before attempting the experiment.

A. Cloning Your Gene Into pShuttle

Note: Check for the following restriction sites in your gene of interest.

Although I-CeuI and PI-SceI sites are relatively rare, their presence will conflict with the subcloning of your gene of interest from the pShuttle vector into the pAdeno vector. SwaI is used to eliminate background clones during the subcloning from pShuttle to pAdeno. PacI is used to linearize the recombinant adenoviral DNA before transfection into packaging 293 cells. Site-directed mutagenesis will need to be performed for gene inserts where any of these restriction sites exist.

I-CeuI Recognition Sequence

5'–TAACTATAACGGTCCTAAGGTAGCGA–3'
3'–ATTGATATTGCCAGGATTCCATCGCT–5'

PI-SceI Recognition Sequence

5'–ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGGCA–3'
3'–TAGATACAGCCCACGCCTCTTTCTCCATTACTTTACCGT–5'

PacI Recognition Sequence

5'–TTAATTAA–3'
3'–AATTAATT–5'

SwaI Recognition Sequence

5'–ATTTAAAT–3'
3'–TAAATTTA–5'

Once it has been determined that there are no I-CeuI, PI-SceI, SwaI, nor PacI sites in your gene of interest, subclone it into the pShuttle using any of the unique restriction sites located in the MCS region. Refer to Figure 2 (pg.4) for the availability of restriction sites. For more detailed information on molecular cloning, see Sambrook & Russell (2001) and Ausubel et al. (1995).

B. Producing Recombinant Adenoviral DNA

1. PI-SceI/I-CeuI digestion of recombinant pShuttle:

a. Prepare a 40µl PI-SceI/I-CeuI double-digest reaction of the pShuttle vector.

b. Combine the reagents shown in Table II in a sterile 1.5ml microcentrifuge tube.

Table II. PI-SceI/I-CeuI Double Digest of pShuttle DNA

Reagent	Volume
pShuttle or pShuttle-LacZ DNA	2-3ug
10X Double Digestion Buffer	4ul
PI-SceI Restriction Enzyme (1U/ul)	2ul
I-CeuI Restriction Enzyme (1U/ul)	2ul
10X BSA	4ul
ddH_20	Variable
Total Reaction Volume	**40ul**

c. Mix well and spin briefly to collect liquid.

d. Incubate at 37°C for exactly 3 hours.

e. Perform a phenol:chloroform:isoamyl alcohol (25:24:1) ex-traction using the protocol in Appendix A (page 16).

2. Subclone the expression cassette of the pShuttle vector into the pAdeno genome:

a. Combine the reagents shown in Table III in a sterile 1.5ml mi-crocentrifuge tube in the order as shown.

Table III. Ligation of Recombinant Adenoviral DNA

Reagent	Volume
PI-SceI/I-CeuI Digested Vector (Provided)	4ul
PI-SceI/I-CeuI Digested pShuttle	3ul
5X DNA Ligation Buffer	2ul
T4 DNA Ligase	1ul
Total Reaction Volume	**10ul**

b. Gently mix, then spin briefly in a microcentrifuge.

c. Incubate at room temperature for 1.5 hours.

d. To the ligation product, add the reagents listed in Table IV.

Table IV. Linearization of Remaining Parental Vector

Reagent	Volume
Ligation Product	10ul
10X SwaI Digestion Buffer	1.5ul
10X BSA	1.5ul
SwaI Enzyme	1ul
ddH_2O	1ul
Total Reaction Volume	**15ul**

e. Mix well and incubate at room temperature for an hour.

f. Perform a phenol:chloroform:isoamyl alcohol (25:24:1) ex-traction using the protocol in Appendix A (page 16).

3. Transform E. coli with ligation products:

a. Transform electro- or chemically competent DH5a cells or Ad-competent (Cat No. A014) cells with the SwaI digestion (3-5µl).

b. Select for ampicillin-resistant (Amp^r) transformants by plat-ing the transformation mixture on a LB agar/Amp plate (100µg/ml ampicillin). Incubate overnight at 37°C.

c. Pick up 10 colonies and inoculate into 100µl LB/Amp medium in a sterile Eppendorf tube in the early morning.

d. After 5-6 hours of incubation, screen for positive clones by PCR with Adeno screening primer mix, see Appendix B (page 17).

e. When a bacterial clone carrying the desired recombinant has been identified, inoculate 100-500ml of LB/Amp medium with all the bacteria (100µl) from the 1.5 ml Eppendorf tube.

f. Incubate the culture at 37°C overnight.

g. Purify the plasmid using a Column Pure Plasmid Maxi-Prep Kit or an equivalent kit from Qiagen. Expected yield: 30-50µg plasmid DNA/100ml culture.

Note: pAdeno is a large plasmid (>30kb) that is susceptible to damage and rearrangement in E.coli. For best results, use fresh, log-phase cultures for purification of recombinant pAdeno DNA. Do not store culture at room temperature, 4°C, or on ice for long periods (i.e. >24 hours) before purification.

4. Analyze the Adenoviral DNA by PCR or restriction digestion.

C. PI-SceI and I-CeuI Restriction Analysis

The presence of the expression cassette can be verified by double digestion with PI-SceI and I-CeuI as shown in Table V. Analyze the digestion by electrophoresis on a 0.8–1.0% agarose/EtBr gel. The digestion will give rise to two fragments. The larger fragment is the pAdeno vector and the other is your gene insert cut out by PI-SceI and I-CeuI. Note: the insert fragment might appear much bigger than its actual predicated size due to the migration retardation by the binding of restriction enzymes of gene insert.

1. **Set up the 30µl PI-SceI/I-CeuI double digests by combining the** reagents in Table V in a sterile 1.5 ml microcentrifuge tube.

Table V. Restriction Analysis of Recombinant Adenoviral DNA

Reagent	Volume
Adenoviral DNA	2-3ug
10X Double Digestion Buffer	3ul
PI-SceI Restriction Enzyme (1U/ul)	2ul
I-CeuI Restriction Enzyme (1U/ul)	2ul
10X BSA	3ul
ddH_20	Variable
Total Reaction Volume	**15ul**

2. **Mix well and spin briefly to collect contents**.
3. **Incubate at 37°C for exactly 3 hours.**
4. **Verify the insert by electrophoresis on a 0.8-1.0% agarose/Safe-**view™ (Cat. No. G108) gel. ***Important:*** *Since I-CeuI and PI-SceI tend to remain bound to DNA after digestion, use a gel loading buffer that contains SDS (final concentration after combining with sample: 0.1%), heat to 65°C for 10 minutes before loading to dissociate the enzymes from the DNA.*

D. PCR Analysis

Also, you can screen pAdeno DNA for the presence of pShuttle de-rived expression cassettes by PCR with Adeno forward and reverse PCR primers, see Appendix B (page 17). These primers specifically amplify a 350bp sequence that spans the multiple cloning site of pAdeno. Only recombinant Adenoviral templates are amplified since non-recombinants lack the pShuttle sequence needed for annealing with the forward primer.

E. Preparing Adenoviral DNA for Transfection

1. **In a sterile 1.5 ml microcentrifuge tube, combine the reagents in Table VI.**

Table VI. PacI Digestion of Recombinant Adenoviral DNA

Reagent	Volume
Recombinant Adenoviral DNA	5ug
10X PacI Digestion Buffer	4ul
10X BSA	4ul
PacI (10U/ul)	2ul
ddH_20	Variable
Total Reaction Volume	**40ul**

2. **Mix contents well and spin the tube briefly.**
3. **Incubate at 37°C for 3 hours.**
4. **Perform phenol:chloroform extraction, Appendix A (page 16).**

F. Generating Adenovirus in 293 Packaging Cells

1. **Plate 293 cells at 70% confluency in a 6-well plate 12-24 hours** before transfection.
2. **Incubate the plate(s) at 37°C in a humidified atmosphere main-**tained at 5% CO_2.
3. **Transfect each 6 well culture plate with 5-7µl of PacI-digested** recombinant Adenoviral DNA. Use DNAfectin™ (Cat. No. G061) to transfect DNA into 293 cells.
4. **Subculture cells into a T75 flask using trypsin 3-5 days later.**
5. **Check periodically for cytopathic effect (CPE). Note: It normally** takes 7-12 days to see CPE.
6. **When >90% of the cells have detached from the plate, prepare** viral stock by following steps 7-10. Name this stock "Primary Amplification" and store at -20°C.

   • Primary Amplification stock can be used for infecting target cells. We suggest evaluating the functionality of this viral stock before preparing a high-titre stock.

   • The presence of the recombinant construct can be verified by PCR or Western blotting.
7. **Centrifuge the suspension at 1,500xg for 5 minutes at 23°C.**

8. **Resuspend the pellet in 500µl sterile PBS.**
9. **Lyse cells with three consecutive freeze/thaw cycles. Freeze cells** in dry ice/ethanol bath, thaw cells by placing the tube in a 37°C water bath. Do not allow suspension to reach 37°C.
10. **After the third cycle, briefly centrifuge to pellet the cell debris.** Transfer the lysate to a clean, sterile centrifuge tube and store the lysate at -20°C.
11. **Determine the adenoviral titre. The Adenovector Rapid Titre Kit** (Cat. No. A1-20T) enables determination of the adenoviral titre by using an anti-Hexon antibody-based immunocytochemical stain.

G. Amplification of Recombinant Adenoviral Vectors

The viral titre from the primary stock will be relatively low, further rounds of amplification are needed for 100% transduction in most target cells. In general, up to 2-3 rounds of amplification will give rise to titre of 10^6 pfu/ml from 293 supernatant, which is enough for the transduction of most target cells *in vitro* at 70~100% efficiency. However, large-scale virus preparation is needed for most *in vivo* gene transfers. For detailed protocol of adenoviral amplification, please refer to Appendix C (page 18).

H. Determining Adenoviral Titre

There are three protocols for determining adenoviral titre:

1. Plaque Assay (requires ~2–3 weeks)
2. End-Point Dilution Assay (requires ~10 days)
3. OD_{260} Assay (requires ~ 1 hour)

Methods 1 and 2 are biological assays; they measure the number of infectious viral particles. Method 3 on the other hand, is a physical assay; it measures the concentration of viral DNA and viral protein. Therefore, it does not distinguish between infectious and non-infectious viral particles.

For detailed information about adenoviral titre assay, please refer to our online technical support:

http://www.abmgood.com/Adenovirus-Plaque-Assay.html

I. Transduction Procedure

If the virus is to be used in an *in vitro* transduction, double CsCl purification is not required as the viral supernatant will provide 100% gene transduction efficiency in most human cell lines. For *in vivo* studies (i.e. animal studies), purification is essential to remove defective particles, cell debris, and small amounts of media components, since these contaminants can induce significant immune responses. In addition, CsCl purification will concentrate the virus to a level suitable for *in vivo* injections.

1. **Prepare target cells in a 6-well plate or 10cm at 70% confluency** one day prior to transduction.

2. **Aspirate the culture medium and overlay with viral culture** supernatant (1ml for a 6-well plate and 4-5ml for 10cm dishes) to cover the cells for one hour in an incubator.

3. **Remove the media containing the virus and replace it with** fresh complete media.

4. **Gene transduction can be evaluated 48-72 hours after transduction** by different assays, such as microscope observation, if there is a colour-emitting reporter gene, Western blotting or qPCR analysis.

Problem	Possible Cause	Solution
No Colonies After Transformation of pShuttle	Incorrect antibiotic used or antibiotic concentration too high.	Use Kanamycin at 50ug/ml of LB agar medium.
	Poor transformation efficiency.	Check transformation efficiency using a control plasmid like pShuttle.
No Colonies After Transformation of pAdeno	Incorrect antibiotic used or antibiotic concentration too high.	Use Ampicillin at 100ug/ml of LB agar medium.
	Poor transformation efficiency.	Check transformation efficiency using pAdeno.
	Unsuccessful ligation.	Over digestion of pShuttle by PI-SceI or I-CeuI. Check digested products on agarose gel. Reduce the amount of enzyme or shorten the digestion period if a "smear" of bands is observed.
Too Many Background Colonies	Incomplete SwaI digestion.	Digest pAdeno with SwaI followed by transformation. Incubate longer or use more SwaI enzyme in the digestion.
Restriction Analysis of DNA Prepared from Large-Scale Culture Reveals More Bands than Expected	Mutation or recombination occurred.	pAdeno is a large plasmid and prone to recombination. Select a different clone or Maxi-DNA purification.
Adeno DNA Resistant to Enzyme Digestion	Incompatible *E. Coli* strains were used for transformation.	Use an authentic DH5a strain for transformation.
No Virus Particles Produced	Poor transfection efficiency.	Check the transfection efficiency by a reporter gene, such as a GFP construct. A minimum 20-30% transfection is required. Adjust seeding density of cells to optimize confluency at time of infection.
	293 cell culture used for transfection might be a late passage or too dense.	Start a fresh culture of 293 cells at low passage (p<30). Transfect 293 cells at about 70% confluency.
	Low quality pAdeno DNA.	Check the purity (A_{260}/A_{280}) and identity of the plasmid DNA used for transfection.
	Too little or too much pAdeno DNA used.	Titrate the amount of PacI digested recombinant Adeno DNA to achieve maximal transfection efficiency. In general, 2-3ug of DNA is good for a 6-well size plate.
High Rate of Cell Death	Insert gene is toxic to 293 cells.	Make sure to use 293 cells from abm Inc. (Cat. No. A007). These cells have been optimized to be more tolerable to usually toxic genes.

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Appendix A: Phenol:Chloroform:Isoamyl Alcohol Extraction

1. **Top up the sample to 100µl with TE buffer (pH 8.0), then add 100µl** phenol:chloroform:isoamylalcohol(25:24:1).
2. **Vortex thoroughly for 20 seconds.**
3. **Spin the tube in a microcentrifuge at 14,000rpm for 2 minutes at** room temperature to separate the phases.
4. **Carefully transfer the top aqueous layer to a clean 1.5ml tube.**
5. **Add 400µl 100% ethanol, 30µl 10M NH_4OAc (or 1/10 volume of 3** M NaOAc), and 1µl glycogen (20 mg/ml).
6. **Vortex thoroughly for 20 seconds.**
7. **Spin the tube at 14,000rpm for 5 minutes at room temperature.**
8. **Remove and discard the supernatant.**
9. **Carefully overlay the pellet with 400µl 70% ethanol.**
10. **Spin the tube at 14,000rpm for 2 minutes at room temperature.**
11. **Carefully aspirate off the supernatant.**
12. **Air-dry the pellet for approximately 3-5 minutes at room tem-** perature to evaporate the residual ethanol.
13. **Dissolve the DNA in 10µl sterile 1X TE Buffer (pH 8.0) for down-** stream applications or store at -20°C until use.

1. Set up the reaction as follows using 2x PCR Taq MasterMix (Cat. No. G013) or your own Taq DNA polymerase. Make sure to include a negative control for each assay.

Table VII. PCR Components

Reagent	Volume
Template DNA (Bacterial or Purified)	1ul
Mixture of Forward & Reverse Primers	2ul
ddH_20	22ul
2X PCR Taq Mastermix	25ul
Total Reaction Volume	**50ul**

2. Perform PCR as follows:

Table VIII. Thermocycler Conditions

Process	Temperature	Time
Denaturation	95°C	4 minutes
PCR Cycles (30-35)	94°C	30 seconds
	55°C	30 seconds
	72°C	1 minute
Elongation	72°C	5 minutes

3. After PCR, perform DNA agarose gel electrophoresis. These prim- ers specifically amplify a 350-bp sequence that spans the cloning site in pAdeno. Only recombinant Adenoviral templates are amplified since non-recombinants lack the pShuttle sequence needed for annealing with the forward primer.

1. **Depending on the required viral amount, one will need to grow up** different quantities of 293 cells. For example, if 50ml viral supernatant is needed, five 10cm plates should be prepared. To achieve this, we recommend customers start growing 293 cells in one well of a 6-well plate and in one 10cm dish.
2. **When cells are approximately 60-70% confluent in the 6-well plate,** add 100µl of primary adenovirus stock to 0.5ml of complete culture medium. Aspirate the culture medium from the 6-well plate and then add the diluted virus onto the 293 cells slowly without dislodging the cells. Return the plate to the 37°C 5% CO_2 incubator for 1-2 hours before adding another 1.5ml of complete culture medium into the well. It will take 4-6 days to see over 95% of the cells are detached.
3. **While adenoviral vectors are being replicated in the 6-well plate, sub-**culture the 10cm dish to five 10cm dishes. When the 293 cells reach 70% confluence in the 10cm dishes, add 300-400µl of crude viral stock from the previous 6-well plate directly into the 10cm culture dishes.
4. **It will take another 4-5 days before the completion of CPE. Collect all** cells and culture medium into a 50ml culture tube. Freeze and thaw 3 times to release the viral particles from cells. Pellet the cell debris by centrifugation at 2,000g for 10 minutes.
5. **Then the supernatant can be used for most *in vitro* transductions. Store** the remaining virus at 4°C if you are going to use it for transduction or the next round of amplification (next step) within a couple of weeks. Store the virus at -70°C if you are not planning to use the virus for a while. **Note:** Adenoviral vectors are more stable at 4°C or at room temperature than lentiviral or retroviral vectors. After repeated testing, we found there is no significant loss of titre when stored at 4°C or room temperature for up to 72 hours. Virus will still be viable after one year of storage at 4°C, but long-term storage at -70°C in the presence of 5% glycerol is needed to minimize the stock titre loss.
6. **For *in vivo* transduction using adenoviral vector, even greater virus** production is needed. Up to fifty 15cm dishes of adenovirus can be amplified. The virus preparation can then be purified and concentrated by filter-based purification kit (A900) or CsCl banding.
7. **Adenoviral vector titres are generally within the following ranges from** different preparations. ***Note:*** *Titres will be lower if gene is toxic to cells.*

Table IX. Viral Titres from Different Sample Preparations

Titre	Preparation
10^6	Original culture supernatant.
10^7	Original culture (medium and cells) after freeze/thaw cyles (up to 3 cycles).
10^8-10^{12}	Purification by filter-based methods.
10^9-10^{12}	Purification by CsCl banding.

Notes

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