USE OF ADENOVIRUS VECTORS, SITE SPECIFIC RECOMBINASES AND MOLECULAR SWITCHES TO REGULATE GENE EXPRESSION

INTRODUCTION AND OVERVIEW


Control of gene expression from Ad vectors.

Although there are numerous systems for regulating gene expression, and many have been used in Ad vectors, most if not all of these are leaky, and allow at least a low level of expression from cassettes in the “off” mode. As a consequence certain cytotoxic genes cannot be rescued into Ad vectors. Furthermore, even gene products that are not necessarily cytotoxic can be inhibitory when over expressed from Ad vectors and may result in the cassette being difficult or impossible to rescue into an Ad vector. An example is the Rabies glycoprotein which could not be rescued as a cassette regulated by the strong MCMV IE gene promoter plus an intron but was readily rescuable when gene expression from the same cassette was down regulated by using the lac repressor (Matthews, D. A., Cummings, D., Evelegh, C., Graham, F. L. and Prevec, L. Development of a modified 293 cell line expressing the lac repressor and its use in the rescue of recombinant adenoviruses expressing high levels of rabies glycoprotein (G). J. Gen. Virol. 80: 345-53, 1999).

Molecular “switches” based on excision of a DNA segment inserted between a promoter and a cDNA can be engineered to be extremely “tight”, as the “stuffer” DNA can include transcriptional and translational stop signals that prevent gene expression unless the stuffer DNA segment is excised. When the intervening stuffer is excised expression can be turned on to very high levels if a strong promoter such as the HCMV or MCMV promoter is used. Examples of vectors containing a cassette whose expression is dependent on Cre recombinase mediated excision are AdfloxlacZ1 (Figure 1 A) and Adfloxluc1 (Figure 1 B).
Figure 1. Indicator vectors for expression of β-galactosidase or luciferase under control of a Cre regulatable switch

Adfloxreporter

Fragment of AdfloxLacZ1
9000 bp (molecule 36684 bp)

Fragment of AdfloxLuc1
6000 bp (molecule 34785 bp)
Expression of the reporter gene can be switched on by infecting a host cell that expresses the Cre recombinase (cf. Chen, L., Anton, M. and Graham, F. L. Production and characterization of human 293 cell lines expressing the site-specific recombinase Cre. Somat. Cell and Molec. Genet. 22: 477-488, 1996.) or by coinfection of cells with a second Ad vector that expresses Cre (Anton and Graham J. Virol. 69:4600-4606, 1995). Examples of vectors expressing Cre recombinase are AdCre1 (Figure 2A) and AdCreM1 (Figure 2B) which express Cre under the control of the HCMV IE gene promoter and the MCMV IE gene promoter respectively.

**Figure 2. AdCre1 and AdCreM1: Ad5 vectors expressing Cre recombinase under the control of CMV promoters**

A

**AdCre1**  
\[± 36000 \text{ bp}\]

Fragment of AdCre1

B

**AdCreM1**  
\[32795 \text{ bp}\]

Fragment of AdCreM1
illustrates the switch on of β-galactosidase expression in 293 cells coinfected with AdfloxLacZ1 and AdCre1 or AdCreM1 (both AdCre vectors give similar results). 293 cells were infected with AdfloxLacZ1 alone (moi 1 PFU/cell) (Figure 3A) or coinfected with 1 PFU/cell of each of AdfloxLacZ1 and AdCre1 or AdCreM1 then fixed and stained with X-gal 24 h later. In singly infected cells no blue staining was evident but after coinfection with AdfloxLacZ1 and either of the AdCre vectors intense staining was apparent in all cells.

Figure 3. Lac Z expression in 293 cells 24 h after (A) infection with AdfloxLacZ1 (B) coinfection with AdfloxLacZ1 plus AdCre

Control of gene expression in mammalian cells containing floxed DNA sequences by transduction with AdCre.

An important use of Ad vectors expressing Cre recombinase takes advantage of the many useful properties of Ad vectors, namely growth to high titres and ease of purification, efficient gene transfer resulting in high level gene expression in virtually any mammalian cell type and efficient gene delivery to a variety of different tissues when injected into animals. An additional property of gene delivery by “first generation” (FG) Ad vectors, not usually considered an advantage, is the transient nature of gene expression which is actually advantageous in the examples described below. Because FG Ad vectors are E1 deleted they are highly attenuated and can be used to transduce human cells without inducing cpe (if the moi is low). Examples of the use of AdCre1 to induce rearrangements of floxed DNA segments in human cells were described by Wang et al., (Somatic
They used AdCre1 to transiently express Cre in human cell lines with chromosomal loxP sites and showed that expression of Cre could induce high efficiency excision (nearly 100%) of floxed DNA segments and could also catalyze site specific integration of a cotransfected plasmid having a loxP site into chromosomal lox P sites.

Because Ad vectors can be used for efficient gene delivery into many tissues in most animals the use of AdCre in vivo makes possible an entirely new approach to using transgenic animals in research: “somagenics” in which delivery and expression of recombinases can be used to remodel the genome in somatic tissues of transgenics carrying floxed DNA (Figure 4). The possible applications are too numerous to list but for a few examples of studies that have used AdCre in transgenic animals see Wang, Krushel and Edelman, Proc. Natl. Acad. Sci US 93: 3932-3936, 1996; Rohlmann et al., Nature Biotech. 14: 1562-1565, 1996.

Figure 4. In vivo gene expression from a molecular switch regulated by Cre recombinase expressed by AdCre
293 cells grow in monolayer, preferably in plastic petri dishes, unless adapted to growth in suspension. They are particularly sensitive to the way they are handled (or mishandled). They should never be allowed to become overconfluent, should not be seeded too thinly, should have regular medium changes between splits (twice weekly if they are not growing rapidly enough to permit splitting every 2-4 days). Probably any standard growth medium is acceptable. For many years they had been grown in Joklik's modified MEM plus 10% heat inactivated horse serum but now are maintained in MEM or α-MEM plus heat inactivated new born bovine serum. Fetal calf serum should also be suitable.

Microbix 293 cells are low passage 293 cells that have been maintained under optimal conditions for strong adherence to plastic. They are particularly well suited for adenovirus plaque assays and DNA transfections. To ensure that these properties are retained it is essential that they be cultured appropriately as described below. We recommend that a sufficient number of ampoules of the cells be frozen and stored in liquid N2 to permit initiation of new cultures when the passage number of the lab stocks reaches 40-45 passages (including the passage number at which the cells were obtained from Microbix) or when the cells are no longer behaving well under agar overlays. Higher passage or poorly adherent cells may be suitable for growth of virus but the properties of the cells are more critical for plaque assays and transfections.

**Procedure for Splitting Cells**

Remove the medium from the dishes. Rinse dish (containing cells at approximately 90% confluency), 2 times with 1X Citric Saline (approximately 5 ml for a 150 mm dish). Remove all but a trace of Citric Saline (enough to cover the cell monolayer, approximately 0.5 - 1 ml). Leave dish for approximately 15 min., examining frequently, until cells start to round up and lift off. (This can be done at room temperature. Cells will detach faster at 37 but conditions may be more difficult to control.) When essentially all the cells appear to be loosened, knock the cells off the plastic by tapping the dish sideways against a solid surface. Do not attempt to detach cells by scraping or by vigorous pipetting. We do not recommend the use of trypsin. Resuspend the cells in medium containing serum and distribute to new dishes. We recommend that you not dilute the cells more than 1:3, but the usual split ratio should be 1:2. Incubate the cells at 37°C and refresh the medium every 3 days if the cells are not ready to passage. (This is more likely to happen when the cells are initially started from a frozen ampoule.) Although it may seem that rapidly growing 293 cells are desirable we have found that they may be less suitable for transfections and plaque assays. **Never allow the cells to become completely confluent or overgrown, never allow them to pile up, and never leave them unattended for long periods. They will never forgive you!**

**10X Citric Saline**
50g KCl
22g Na Citrate
Dissolve in H2O and bring to 500 ml.
Sterilize by autoclaving for 15 min. at 15 lb./sq. in. pressure

**Growth Medium**

MEM + 10% Heat Inactivated Newborn Bovine Serum or Fetal bovine serum.
MEM = GIBCO Catalogue Number 61100-061
To 400ml of medium, add Penicillin/Streptomycin (as recommended by manufacturer), and 2 mM L-Glutamine. If fungal contamination is a problem Fungizone (Gibco) can be added to 0.25 µg/ml.

**Phosphate buffered saline**

Prepared as follows: To make solution A, dissolve 80 g NaCl, 2 g KCl, 11.5 g Na2HPO4, and 2 g KH2PO4 in H2O to a final volume of 1 liter. To make solution B, add 1 g CaCl2H2O to 100 ml H2O. To make solution C, add 1 g MgCl26H2O to 100 ml H2O. Sterilize solutions separately by autoclaving. For 100 ml PBS++, mix 88 ml sterile H2O with 10 ml solution A, followed by 1 ml each of solutions B and C.

For PBS–: Mix 10 ml solution A with 90 ml sterile H2O.

For PBS++ + 10% glycerol: Add 10 ml sterile glycerol (sterilized by autoclaving) to 90 ml PBS++.

**Freezing cells for storage in liquid N2.**

Detach as above, maintaining volume of suspension to a minimum. Resuspend cells in 100% fetal bovine serum. Add 1/10 volume sterile dimethylsulfoxide (DMSO). Freeze in ampoules using standard methods at a density of about 1/3 of a 150mm dish per ampoule. Optionally the cells can be resuspended in medium, centrifuged, and resuspended in an appropriate volume of 90% FBS - 10% DMSO.

**Thawing frozen cells.**

Thaw cells rapidly by dipping the lower half of the ampoule in a 37C bath. As soon as the sample is melted add the cells to 25 ml warm growth medium in a 75 cm sq plastic flask that has been equilibrated in a CO2 incubator. Optionally, the cells can be diluted in 10 ml complete medium, centrifuged, and resuspended in fresh medium before seeding into flasks. Leave undisturbed in the incubator for 2-3 hrs then gently remove the flask and examine the cells in the microscope. If the majority of the cells have attached, gently remove the medium and add fresh growth medium. If a significant number of cells are still in suspension it is possible to seed a second flask by transferring the medium and incubating overnight.

**PLAQUE PURIFICATION OF FG VECTORS**
It is strongly recommended before growing up large scale preparations of any Ad vector that
the virus first be taken through a plaque purification on 293 cell monolayers. Subsequent
amplifications for large scale vector production and purification should then proceed from plaque
isolates. The main purpose of this is to minimize the risk of outgrowth of RCA (replication
competent adenoviruses that inevitably and unavoidably arise due to homologous recombination
with Ad 5 sequences in the host 293 cells).

1. Set up appropriate 293 cells in 60 mm dishes one day prior to use. Arrange to have cells at about
80-90% confluency when used. About 6-8 60mm dishes can be set up from each 150mm dish of
293s. Whenever seeding cells into 60mm dishes for any purpose be sure to distribute evenly by
vigorously agitating tray (not in a circular motion, however) just before leaving it undisturbed in the
incubator. HOWEVER, DO NOT SLOSH MEDIUM OVER EDGE.

2. Next day: prepare virus dilutions in PBS++. Remove medium from dishes (do not aspirate more
than 20-30 dishes at a time or the cells may dry out), add 0.2 ml virus/dish. (can use same pipette
for all dilutions if you start with most dilute virus and go up but be sure to change pipettes while
preparing the dilution series).

3. Tilt dishes to spread virus, adsorption is fairly rapid so 30-60' at room temp or 37 is adequate for
routine plaque assays. Tilt once more during adsorption. If virus has been added undiluted in
medium then you must incubate in a CO2 incubator to maintain pH.

4. Add overlay which has been prepared beforehand and has equilibrated in a 44°C waterbath.

5. Allow the agarose to solidify at room temperature then place dishes in an appropriate (virus)
incubator.

6. On 293 cell monolayers plaques should be visible within 4-5 days and can be counted at 6-8 days.
Plaques on 293s can be counted by eye without staining the cells (preferred) or you can flip out the
overlay and stain the monolayer with crystal violet. Plaques appear as holes in the monolayer.

OVERLAY

Prepare 400ml 2x F11 + antibiotics + 8ml 5% yeast extract. This can be stored a few weeks
at 4°C.

For 200 ml complete overlay (20 dishes) prepare 100ml 2xF11 + 10ml inactivated HS, and
autoclave 100ml H2O + 1g agarose. Bring agarose and F11 to 44°C before mixing and use within
about 1hr.

SCREENING ADENO PLAQUE ISOLATES FROM PLAQUE PURIFICATIONS
The simplest method of analysis for identification of FG vectors and to distinguish the vector from RCA or other potentially occurring rearrangements is via restriction enzyme digestion and agarose gel electrophoresis using diagnostic enzymes, making use of an accurate vector DNA restriction map or preferably a complete DNA sequence. The protocol below is designed for 293 cells.

1. Set up 60mm dishes of 293s as for plaque assays or cotransfections, ie to be about 80-90% confluent at time of use. The denser and older the cell monolayer the longer it takes for virus cpe to reach completion. Use dishes the next day or after 2 days.

2. Pick well isolated plaques from titrations done as above by punching out agar plugs using a sterile Pasteur pipette and transfer mashed agar to 1 ml of PBS++ plus 10% glycerol. This can be stored at -70°C until results of the subsequent DNA analysis are available.

3. Remove medium from 293 dishes and add 0.2 ml virus suspension from step 2. Distribute over monolayer and adsorb at room temp for 30-60 min occasionally rocking the dishes to spread the inoculum over the cells. Add 5 ml F11 + 5%HS and incubate at 37°C.

4. Depending on size of the plaque and growth properties of the recombinant virus you should start to see cpe within 2-3 days. Do not attempt to harvest before cpe is absolutely complete, ie essentially all cells rounded and many floating (usually 3-4 days).

5. Process dishes with complete cpe as follows: leave dishes undisturbed in a laminar flow hood for 20-30 min to allow any cells in suspension to settle. Gently remove medium with a pipette and save about 4ml or so in a sterile glass vial containing 0.5 ml sterile glycerol, for storage at -70°C. Remove residual medium by suction. If all this is done carefully the majority of loose cells will be left behind in the dish. The recovered medium should contain significant amounts of virus at titres at least 10⁷ and 10⁸ PFU/ml and can be used in preliminary experiments or for further virus expansion.

6. Add 0.5 ml pronase-SDS (see below) and digest at 37°C for 3-4 hrs or overnight.

7. Transfer viscous lysate to a 1.5 ml Eppendorf tube and extract once with 0.5 ml phenol (saturated with buffer). Collect the aqueous phase with a P1000 Eppendorf or Gilson pipetter and transfer to a fresh tube.

8. Add 1 ml 96% ethanol, vortex lightly or mix by tipping tube. You should get an easily visible fibrous precipitate. Spin and wash 2x with 96% ethanol to remove phenol.

9. Dry completely in a 37°C warm room or heating block and redissolve DNA in 50ul 0.1 x SSC or 10mM Tris 1mM EDTA. It is advisable to tap the tube sharply to suspend the DNA then leave the sample overnight at 4C to allow the DNA to dissolve.

10. Digest 5-10 µl with any suitable restriction enzyme (Hind III is often the best all purpose diagnostic enzyme) for 3-4 hrs or preferably overnight. The careful worker will have already used the computer and DNA analysis software such as Vector NTi to create predicted maps and diagnostic restriction patterns for comparison with the observed patterns.
11. Run on 1% agarose gel with an appropriate marker.

Comments: If cpe was complete you should get a relatively “pure” preparation of viral DNA (roughly 50% viral DNA - 50% cellular) with a background of cellular DNA running as a smear (some enzymes, eg Pvu I, cut human DNA infrequently in which case the cellular DNA will remain near the top of the gel). There should be very little RNA. If Hind III was used there will be a band of cellular repetitive DNA at around 1.8kb (derived from a LINE-1 element), not to be confused with viral DNA. Determination of the fragment sizes using molecular weight markers and comparison with a computer generated gel (if the complete vector DNA sequence is available) should unequivocally confirm the structure of the vector if the appropriate restriction enzyme(s) are chosen.

Pronase stock solution: 5mg/ml in 0.01 M Tris, pH7.5: preincubate at 56°C for 15 min followed by 37°C for 1hr. Aliquot and store at -20°C. Add to pronase-SDS working buffer (0.01 M Tris, pH7.5, 0.01 M EDTA, 1% SDS) before use (this dilute pronase solution can be used for about a month if stored at 4°C).

PREPARATION OF HIGH-TITER VIRAL STOCKS (CRUDE LYSATES) FROM CELLS IN MONOLAYER

General comments. Because most of the virus remains associated with the infected cells until very late in infection ie until the cells lyse, high-titer stocks can be prepared easily by concentrating infected 293 cells as described here.

1. Set up 150-mm dishes of 293 cells to be 80-90% confluent at time of infection. We generally use eight or more dishes for each virus.

2. To prepare high-titer stocks, remove medium from the 293 cells and infect at a multiplicity of infection (MOI) of about 1 PFU per cell (1 ml virus suspension per 150-mm dish). For the initial stock preparation, we dilute virus (from the untitered 4 ml sample stored at -70°C after the first amplification of an isolated plaque) 1:8 with PBS++. To minimize the probability of E1+ replication competent virus (RCA), prepare subsequent high-titer stocks from this same original viral screening sample.

3. Adsorb for 30-60 min, then refeed with complete MEMF11 + 5% HS. Incubate at 37°C, and examine daily for signs of cytopathic effect.

4. When cytopathic effect is nearly complete, i.e., most cells rounded but not yet detached, harvest by scraping the cells off the dish, combining the cells plus spent medium (if it contains significant numbers of floating cells), and centrifuging at 2000 g for 15 min. Aspirate the medium, and resuspend the cell pellet in 2 ml PBS++ + 10% glycerol per 150-mm dish. Freeze (-70°C) and thaw (37°C) the crude virus stock three times prior to titration. Store aliquots at -70°C.
Solutions

$PBS^{++}$, $PBS^{++} + 10\%$ glycerol, and complete MEM + 5\% HS

PREPARATION OF HIGH-TITER CRUDE VIRAL STOCKS FROM CELLS IN SUSPENSION

General comments. Many experimental studies can be carried out using virus in the form of crude infected cell lysates. The following protocol results in high titre virus preparations. Recombinant Ads can be produced using either monolayer (see above) or suspension cultures. However, due to the greater ease of handling suspension cultures, these are preferable for the production of large amounts of high-titer viral stocks. Suspension adapted 293 cells (293N3S) are available from Microbix.

1. For infection with replication-defective viruses (E1 insertion recombinants) grow 293N3S cells in spinner culture to a density of 2-4 x $10^5$ cells/ml in 4 liters complete Joklik's modified MEM + 10\% HS. For growth of wt adenovirus or replication-proficient E1$^+$ viruses (eg E3 insertion recombinants), grow 4 liters KB cells to a density of 5-6 x $10^5$ cells/ml in complete Joklik's modified MEM + 10\% NBS. Centrifuge cell suspension at 750 g for 20 min, saving half of the conditioned medium. Resuspend the cell pellet in 0.1 vol fresh medium, and transfer to a sterile 500-ml bottle containing a sterile stir bar.

2. Add virus at an MOI of 10-20 PFU/cell and stir gently at 37$^\circ$C. After 1 hr, return the cells to the 4-liter spinner flask and bring to the original volume using 50\% conditioned medium and 50\% fresh medium. Continue stirring at 37$^\circ$C.

3. Monitor infection twice daily by inclusion body staining as follows:
   a. Remove a 5-ml aliquot from the infected spinner culture. Spin for 10 min at 750 g and resuspend the cell pellet in 0.5 ml of 1% sodium citrate.
   b. Incubate at room temperature for 10 min; then add 0.5 ml Carnoy's fixative and fix for 10 min at room temperature.
   c. Add 2 ml Carnoy's fixative, spin 10 min at 750 g, aspirate, and resuspend the pellet in a few drops of Carnoy's fixative. Add one drop of fixed cells to a slide and air-dry for about 10 min; then add one drop orcein solution and a coverslip and examine in the microscope. Inclusion bodies appear as densely staining nuclear structures resulting from accumulation of large amounts of virus and viral products at late times in infection. A negative control should be included in initial tests.

4. When inclusion bodies are visible in 80-90\% of the cells (2 to 3 days depending on the input MOI), harvest by centrifugation at 2000 g for 20 min in sterile 1-liter bottles. Combine pellets in a small volume of medium, and spin again. Resuspend pellet in 20 ml $PBS^{++}$ and 10\% glycerol. Freeze and thaw 2-3 times then aliquot and store at -70$^\circ$C until use. Titrate by plaque assays on 293
monolayer cells.

Solutions

1. Complete Joklik's modified MEM + 10% HS (or 10% NBS): Add 50 ml HS (or NBS) to 450 ml complete Joklik's modified MEM (described in Section B). Store at 4°C.

2. 1% sodium citrate: Dissolve 1 g trisodium citrate dihydrate in H2O to a final volume of 100 ml.

3. Carnoy's fixative: Add 25 ml glacial acetic acid to 75 ml methanol.

4. Orcein solution: Add 1 g orcein dye to 25 ml glacial acetic acid plus 25 ml H2O. Filter through Whatman No. 1 paper.

PREPARATION OF PURIFIED HIGH-TITER VIRAL STOCKS BY CSCL BANDING

General comments. Many experimental studies can be carried out using virus in the form of crude infected cell lysates prepared as described above. However, for some experiments, particularly for animal work it is usually desirable to use purified virus. The following protocol is adapted from Hitt et al. (1998).

Recombinant Ads can be purified from crude lysates of either monolayer or suspension cultures. Suspension cultures are easier to harvest, but it is easier to determine the correct time to harvest (i.e., complete or near complete cpe) when using monolayer cultures. A subline of 293 cells, 293N3S cells, adapted to growth in suspension is available from Microbix. The following protocol describes a method for banding virus obtained from 3-liters of infected suspension culture, or about 30 x 150-mm dishes.

1. Prepare crude cell lysate from infected cells:

   a) For 3-liter spinner cultures: when inclusion bodies are visible in 80-90% of the cells, harvest by centrifugation at 750 x g for 20 min in sterile 1-liter bottles. Combine pellets in a small volume of medium, and spin again. Resuspend pellet in 15-30 ml 0.1M Tris-HCl, pH 8.0: if wild-type levels of virus are expected, resuspend in 30 ml, if lower yields are expected (some vectors may replicate to lower yields than others), use 15 ml. Store sample at -70°C.

   b) For 30 x 150-mm dishes: when all infected cells are rounded and most are floating, scrape dishes, then transfer cells and supernatant to 50-ml centrifuge tubes and spin 10 min at 750 x g. Resuspend cell pellet in 15 ml 0.1M Tris-Cl, pH 8.0. Store sample at -70°C.

2. Thaw sample and add 1.5 ml 5% Na deoxycholate for each 15 ml of cell lysate. Mix well and incubate at room temperature for 30 min. This results in a relatively clear, highly viscous suspension.

3. Add 150 µl 2 M MgCl₂ and 75 µl DNase I solution for each 15 ml of cell lysate, then mix well.
Incubate at 37°C for 30-60 min, mixing every 10 min. The viscosity should be reduced to only slightly more than that of water.

4. Spin 15 min, top speed, at 5°C in the Beckman table top centrifuge.

5. Meanwhile, prepare CsCl step gradients (one SW41 ultraclear tube for each 5 ml of sample) in the virus hood: Add 0.5 ml of 1.5g/cc solution to each tube. Gently overlay with 3.0 ml of 1.35g/cc solution. Gently overlay this with 3.0 ml of 1.25g/cc solution. Do not disturb gradients once they are formed.

6. Apply 5 ml of supernatant from step 4 to the top of each gradient. If necessary, top up tubes with 0.1M Tris-Cl, pH 8.

7. Spin at 35K rpm in SW41 rotor, 10 °C, for 1 hour (accelerate/decelerate set to 1).

8. Collect the viral bands (should be at the 1.25g/cc-1.35g/cc interface) and pool. (One can collect by sealing the top of the tube with a piece of parafilm, puncturing the parafilm with a needle, then puncturing the bottom of the tube with a red hot 16 gauge needle, controlling the flow of solution out the bottom with a gloved finger over the top hole.)

9. Transfer the pooled virus to a SW50.1 ultraclear tube (using the 1.35g/cc CsCl solution to top up the tube), mix well, and centrifuge in the SW50.1 rotor at 35K rpm, 4°C, for 16-20 hr. (Alternatively, the pooled virus can be centrifuged in the SW41 rotor at 35K rpm, 10°C, 16-24 hr.)

10. Collect the virus band in the smallest volume possible (usually 0.5 to 1 ml), transfer to a Slide-A-Lyzer dialysis cassette, and dialyze at 4°C against 3 changes of 500 vol (or more) 10 mM Tris-HCl, pH 8.0, for at least 24 hr total.

11. After dialysis, add sterile glycerol to a final concentration of 10%. Store the purified virus at -70°C in small aliquots.

**Materials**

The following special equipment is required: Beckman SW 41 rotor, SW50.1 rotor, ultraclear tubes for each rotor, Pierce Slide-A-Lyzer dialysis cassettes, sterile 1-liter beaker with stir bar, syringes and needles.

**Solutions**

1. 0.1 M Tris-HCl, pH 8.0: Add 1.2 g Tris base to 80 ml H2O. Adjust pH to 8.0 with HCl. Adjust volume to 100 ml and autoclave.

2. 5% Na deoxycholate: Add 5 g Na deoxycholate to 100 ml H2O.

3. 2M MgCl2: Add 40.6 g MgCl2.6H2O to 100 ml H2O and sterile filter.
4. **DNase I solution:** Dissolve 100 mg bovine pancreatic deoxyribonuclease I (DNase I) in 10 ml of 20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, and 50% glycerol. Store in small aliquots at -20°C.

5. **CsCl solutions for banding:**

<table>
<thead>
<tr>
<th>density</th>
<th>CsCl</th>
<th>10 mM Tris, pH8</th>
</tr>
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<tbody>
<tr>
<td>1.50 g/cc</td>
<td>90.8 g</td>
<td>109.2 g</td>
</tr>
<tr>
<td>1.35 g/cc</td>
<td>70.4 g</td>
<td>129.6 g</td>
</tr>
<tr>
<td>1.25 g/cc</td>
<td>54.0 g</td>
<td>146.0 g</td>
</tr>
</tbody>
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Add 10 mM Tris to indicated amount of CsCl and stir to dissolve. Verify the density by weighing 1.0 ml of each solution. Sterile filter and store at room temperature.

6. **Dialysis buffer:** 10 mM Tris, pH 8.0. Use at 4°C.

7. **Sterile glycerol:** Prepare by autoclaving.

**REFERENCES**

1. **293 cells**


2. **Development and use of AdCre vectors**


3. Miscellaneous articles on Ad vectors

Packaging capacity of Ad vectors


Ad vector construction using the two plasmid method


5. General


Patent Information

Kit C is covered by US patent no. 6,140,087; AdMax(TM) is covered by US patent no.6,379,943 (plus patents pending); Kit G is covered by US patent no. 6,120,764 (plus patents pending).
**Quality Control Information**

*Storage:* Viral vectors are shipped frozen on dry ice. These viral vectors should be stored frozen at <-70°C.

*Hazards:* This product contains live virus. Biosafety Level 2 practices and containment should be employed while handling this product. This material should be handled only by qualified personnel using generally accepted good laboratory practices appropriate for biological reagents.

*Stored In:* PBS++ and 10% glycerol
AdCreM2: an Ad5 vector expressing high levels of Cre recombinase containing a nuclear localization signal

The expression cassette is based on a modified AdMax™ shuttle plasmid, pDC516(i), containing an intron 5' of the polycloning site for super high levels of expression. The Cre coding sequences have been modified to contain a nuclear localization signal for enhanced Cre activity. AdCreM2 induces approximately 100 fold more Cre activity in transduced cells than AdCreM1 and replaces both AdCre1 and AdCreM1.

Note that because AdCreM2 was rescued using the AdMax™ system it contains an frt (FLP recombination) site 5' of the pIX coding sequences.