Adenoviral Reference Material Working Group
Bid Submission Form
Viral Bank Production and Testing Donation
RFP 4.0

Item for Submission

Production of an Ad5 Wild-type Virus Bank in lysate form to support production of 10 lots of adenovirus reference material. The bidder will also provide a Certificate of Analysis summarizing characterization as called for below. The delivered virus bank will need to at a minimum be 5×10^{13} total particles in at least 20 aliquots.

General Requirements for Bidding

The Virus Bank will need to be produced under well-documented conditions (not necessarily equivalent to CGMP). Virus stock and cells used for bank production will be supplied from other bid activities in this process. Indicate your minimum requirements / concerns for acceptance of these materials (cell bank vials and viral seed material). The bid should indicate the amount of time required from receipt of the cell bank vials and viral material for the production and release of the virus bank.

The institution will need to provide a brief statement describing the proposed method for production of this bank, including proposed vial configuration. The institution should include their proposed specifications, including information on the proposed test methods, and a proposed Certificate of Analysis addressing the following points:

- ?? Identity
- ?? Particle concentration
- ?? Functional activity (such as infectivity)
- ?? Sterility (USP or 21CFR610.12)
- ?? Mycoplasma
- ?? *In vitro* adventitious viral agents, or equivalent
- ?? *In vivo* adventitious viral agents
- ?? Human pathogens:
  - EBV
  - HIV 1& 2
  - HTLV 1& 2
  - HBV
  - HCV
  - CMV
  - Parvo B19
  - AAV
- ?? Bovine virus – Certificate of Analysis where applicable (raw material or final vial test) (per 9CFR113.47)

?? Porcine parvovirus – Certificate of Analysis where applicable (raw material trypsin or final vial test)

The proposal should include details of the proposed method/container for shipping to ensure integrity of the viral bank vials upon arrival at the production facility for the purified reference material.

Documentation Requirements

A description of the method proposed for production of the virus bank.

A proposed Certificate of Analysis should be provided addressing the characterization described above along with the proposed specifications, and information on the proposed test methods,

Proposed details related to shipping of virus bank vials to production facility.

In addition to these specific documentation requirements, each institution bidding should include a brief statement describing their experience and capacity to perform such activity and a description of the facility in which the work will be performed. The facility description should address procedures to ensure segregation during viral bank production.

Please complete the following fields:

Contact Information – RFP 4.0

Contact Individual:	Beth Hutchins
Institution:	Canji, Inc. CANJI Inc.
Address:	3525 John Hopkins Ct. San Diego, CA 92121
Phone Number:	Direct: 858-646-5930 Reception 858-597-0177
Email Address:	beth.hutchins@canji.com

Viral Bank Production and Testing Donation Information – RFP 4.0

Please indicate if your institution is also submitting proposals for the other activities:

- ☐ Donation of Cell Bank
- ☒ Ad5 Wild-type Virus Bank Production
- ☒ Ad5 Wild-type Purified, Formulated Bulk Production
- ☐ Donation of Repository Services
- ☐ Vialing of Ad5 Wild-type Reference Material
- ☐ Donation of Supplies/Other Services

Please attach:

Proposed Certificate of Analysis, specifications, and summary of test methods
Description of production of viral bank including vial configuration
Facility information
Information on shipping

Submit this completed form and all attached information for receipt **by February 28, 2001** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about March 31, 2001. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please include an estimated cost and market value of all goods and services donated.

Williamsburg BioProcessing Foundation
Attn: Adenovirus Reference Material Working Group
4015 Killam Avenue
Norfolk, VA 23508

PH: 757-423-8823
FAX: 757-423-2065

EMAIL: advector@wilbio.com

ATTACHMENT – RFP 4.0 Ad5WT Virus Bank
Submission From Canji, Inc.

Proposed Method of Production of Viral Bank (including vial configuration)

Canji proposes to produce the Adenovirus 5 WT Virus Bank using a microcarrier-based bioreactor process. Cells will be expanded in flat stock culture from the frozen cell bank vial(s) provided. The cell line is likely to be 293 cells and this is the method described. However a similar production method exists at Canji for an A549 cell-based production of adenovirus.

The 293 cells are expanded in DMEM with high glucose supplemented with 10% fetal bovine serum (irradiated) until a total of 2×10^9 cells are available. Cells are then placed in a 5 L bioreactor vessel with microcarriers, for seeding the cells onto the microcarriers. The cells are expanded within the bioreactor for approximately 10 days until achieving a cell density of at least 7×10^6 cells/mL. The bioreactor is then infected with Adenovirus 5 source lysate at approximately 1×10^7 particles/mL. Viral production is monitored beginning at 24 hours via particle concentration determined using an in-process version of the Resource Q HPLC Assay (reference Shabram *et al.*). When the particle concentration remains the same for two consecutive time points or starts to decline, the reactor is harvested. Adenovirus product is released from the cells through 3 consecutive freeze-thaw steps. Product release is monitored using the Resource Q HPLC assay. After clarification, the lysate is treated with Benzonase (200 units per mL for 1 hour) and filtered through a 0.2 µm filter prior to vialing at a 10 mL fill into 30 mL sterile PETG bottles. PETG bottles are relatively impermeable to CO_2, preventing inactivation of the adenovirus when shipped on dry ice or stored at or below –55°C. Additional vials will be filled to allow for testing, disposition, and retention. A minimum of 100 x 10 mL filled containers at a minimum concentration of 1×10^{11} particles per mL will be available for production of reference material batches after disposition for a minimum total viral bank size of 1×10^{14} particles.

The cell bank and Adenovirus 5 source materials provided through the bid process will be accepted by Canji on the basis of the characterization or certificate information requested in RFPs 1.0 and 2.0. At a minimum this should include identity, sterility, and mycoplasma test results, along with. viable cell number (cell bank) and adenoviral particle concentration (Ad5 WT source material).

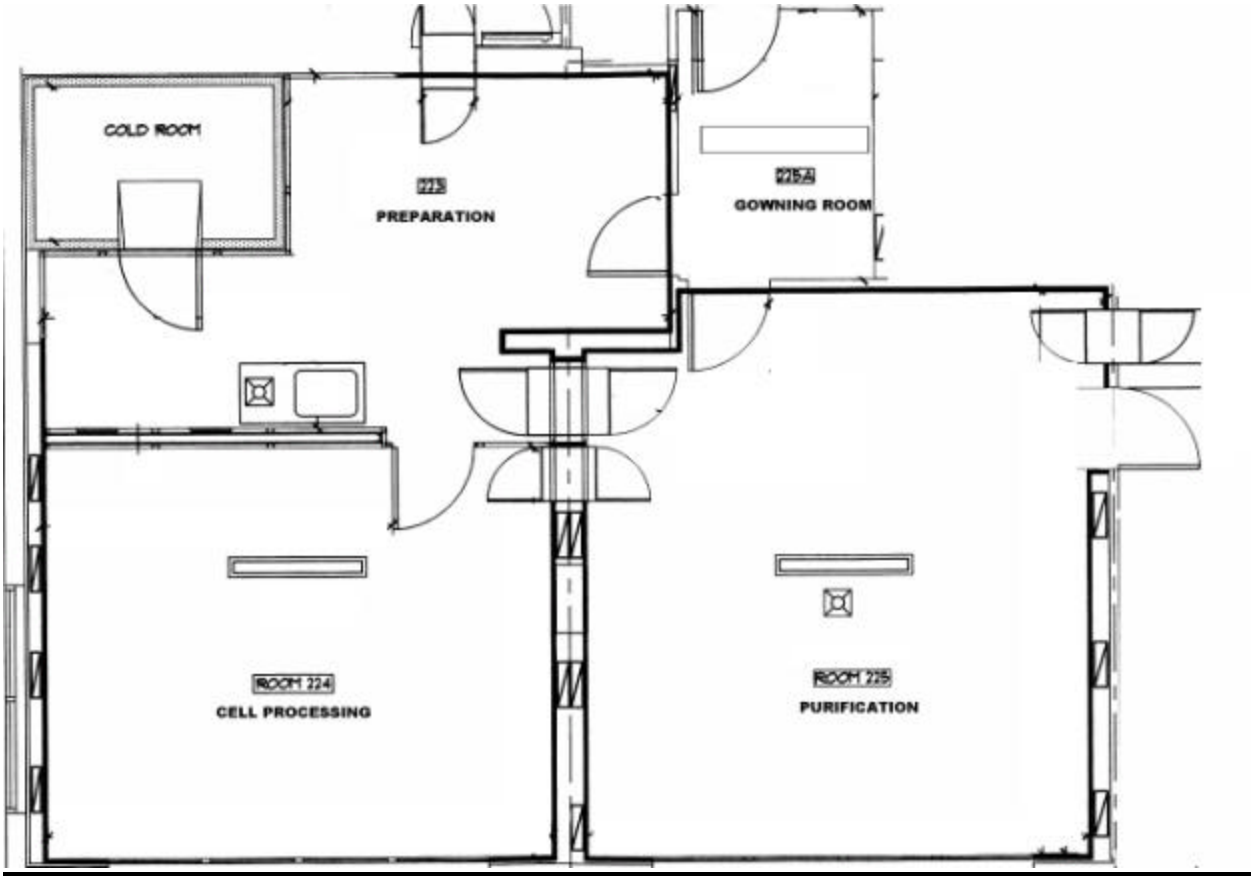
Canji estimates that it will take approximately 5 weeks from receipt of the materials to produce the virus bank. It will take an additional 5 weeks for completion of all testing with the exception of the *In Vivo* Detection of Viruses. Completion of the *In Vivo* Detection of Viruses test will take an additional 4 to 5 weeks. From start to finish, we expect the production and release of a Master Virus Bank, consisting of a minimum of 100 x 10 mL filled containers at a minimum concentration of 1×10^{11} particles per mL, to take 13 to 14 weeks.

Production and testing would take place under CGMP. Personnel involved in production of an adenovirus viral bank have previously produced such banks using the process described as well as adenoviral banks using cell factory viral culture methods. Lead personnel are Paul Shabram, Manufacturing Supervisor, and Daniel Giroux. Canji has produced adenovirus materials since 1994 under their direction. The Resource Q HPLC (anion exchange HPLC) analytical method was created at Canji (reference Shabram *et al*.). Canji methods have been transferred to Canji's corporate parent, Schering Plough, and Canji is considered a leader in the field of adenovirus production and analytical characterization. QC/Bioanalytical personnel are well versed in the adenovirus-specific test methods such as measurement of particle concentration and infectious titer and identification via RFLP and Western blot. Production will take place using batch records, a copy of which will be provided for archive.

Canji's Viral Production Facility consists of a suite of rooms in approximately 1200 square feet shown in Figure 1. There is an entry gowning room that has doors leading into either a preparation room or into a purification room. The preparation room has a door leading to the cell and viral culture room. There is an emergency exit door out of the purification room (required by California). The suite contains a number of pass-throughs for moving materials into, within, and out of the Viral Production Facility. The gowning, preparation, and cell/viral culture rooms have a designation of Class 100,000, while the purification room has a designation of Class 10,000. There are Class 100-designated laminar flow hoods within both the cell and viral culture and the purification rooms. Cell and viral culture would take place in the cell/viral culture room. Benzonase treatment, filtration, and vialing would take place in the purification room with vialing in the Class 100 hood. Access to the facility is controlled by key-card and limited to production and quality unit personnel. An active environmental monitoring program is supported. With the exception of the glass bioreactor vessel, all other containers and tubing with which product could be in contact are disposable. The reactor vessel is washed and autoclaved and then media tested for sterility prior to inoculation with cells during production. The Viral Production facility operates under CGMP. All activities within the Viral Production facility are scheduled and production activities are campaigned so that only one viral construct is in the facility at a time.

Figure 1. Canji Viral Production Facility.

Hallway



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Emergency exit

Canji's proposed Certificate of Analysis and Specification is contained in the electronic file "Canji VirusBank CofA specs.doc". We do not propose to perform the "*In Vitro* Adventitious Agent Assay" as Adenovirus is known to cause a positive result in the assay. We will submit Certificates of Analysis for the serum and trypsin used in production. These Certificates will include testing for bovine viruses per 9CFR113.47 and porcine parvovirus.

Description of Test Methods:

Identity via RFLP. Described in the electronic file labeled "Canji Ad5WT characterizaton.doc".

Identity via Western Blot for Expression of Adenovirus Proteins. Described in the electronic file labeled "Canji testmethods.doc".

Particle Concentration. The Resource Q HPLC Assay (analytical anion exchange HPLC assay) was developed at Canji and is described in detail in Shabram *et al.* which is attached as an electronic file labeled "Canji ResQ Shabram et al.pdf". A copy of the SOP is contained in the electronic file labeled "Canji ResQ SOP.doc". The assay has been extensively qualified in cross validation studies with Schering Plough and through internal studies at Canji.

Infectivity. Infectivity is measured via a FACS-based method monitoring expression of hexon using a FITC-labeled monoclonal antibody after infection of A549 cells. This method is a slight variation on the Canji method described in the electronic file labeled "Canji InfectAssay 293 SOP.doc", a FACS-based infectivity assay that utilizes 293 cells. Except for the change in cell line, the methodology is otherwise the same.

Safety Tests. All other tests are to be performed at a contract laboratory. Sterility will be performed per 21CFR610.12. Mycoplasma will be performed per Points to Consider. The *In Vivo* Detection of Adventitious Viruses Assay will be performed per ICH Q5. Conventional PCR will be performed to determine the presence of AAV, Parvo-B19, HIV-1, HIV-2, Hepatitis B, Hepatitis C, Epstein Barr Virus, Cytomegalovirus, HTLV-I, and HTLV-II.

Procedure for Shipping Adenovirus

The following method as been used successfully to ship both lysate and purified Adenovirus within the United States. It complies with the current DOT and IATA regulations covering the shipment of infectious agents affecting humans. [Up to 4 L total volume may be shipped in one box using this procedure.]

Materials

- ?? Adenovirus material(s) in primary container(s)
- ?? Absorbent (with capacity to exceed volume being shipped)
- ?? Plastic container with threaded lid (secondary container)
- ?? Dry Ice
- ?? Large Styrofoam shipper with cardboard over pack box
- ?? "Dry Ice", "Infectious Substances affecting Humans", "Inner Packages Comply" labels
- ?? "Cargo Aircraft Only" labels
- ?? Inventory sheet describing samples
- ?? Internal and External labels with both consignee and shipper names
- ?? FedEx (or courier) Airbill and Dangerous Goods Manifest

Procedure

1. Fill out the Inventory sheet, external and internal shipping labels and FedEx documents.
2. Label the plastic (secondary) container with both the consignee's and shipper's names, addresses, and phone numbers.
3. Prior to placing samples in the plastic (secondary) container, pre-chill the plastic container(s) to prevent the thawing of the material when transferred into it.
4. Place virus materials in the cold plastic (secondary) container with absorbent material.
5. Seal the container (secondary container) and place it in the large styrofoam box containing a small amount of dry ice.
6. Immediately, fill the styrofoam shipper with dry ice.
7. Place the lid on the box but do not seal.
8. Place inventory and attachments on top of the closed shipper.
9. Tape the cardboard over pack box closed. Do not seal all edges so as to allow gaseous CO_2 to escape.
10. Label the over pack box with an external label with both consignee, shipper and infectious substance information, a dry ice label indicating the weight of dry ice, an infectious substance label, and an "inner packages comply " label.
11. Apply a "Cargo aircraft Only" label when the volume is greater than 50 mL.
12. Fill out the airbill and dangerous goods forms according to courier requirements.
13. Inform the recipient that the shipment is in route.

References

?? Huyghe, B. G., Liu, X., Sutjipto, S., Sugarman, B.J., Horn, M.T., Shepard, H.M., Scandella, C.J., and Shabram, P. (1995) "Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 by Column Chromatography," *Human Gene Therapy 6*: 1403-1416

?? Shabram, Paul W., Daniel D. Giroux, Ann M. Goudreau, Richard J. Gregory, Mark T. Horn, Bernard G. Huyghe, Xiaodong Liu, Mary H. Nunnally, Barry J. Sugarman, and Suganto Sutjipto (1997) "Analytical Anion-Exchange HPLC of Recombinant Type-5 Adenoviral Particles," *Human Gene Therapy 8:* 453-465

?? Nyberg-Hoffman, Cassandra, Paul Shabram, Wei Li, Daniel Giroux, and Estuardo Aguilar-Cordova (1997) "Sensitivity and reproducibility in adenoviral infectious titer determination" *Nature Medicine 3:* 808-811

?? Hutchins, Beth (1998) "Making the (Clinical) Grade with Adenoviral Gene Therapy Vectors," *The Quality Assurance Journal 2:* 119-127

Patents:

Shabram, Paul W., Bernard G. Huyghe, Xiaodong Liu, and H. Michael Shepard. Viral Purification Process. United States Patent No. 5,837,520. Issued November 17, 1998. A method for purifying viral vectors containing therapeutic genes for use in gene therapy. (Also describes the anion exchange analytical method.)

Giroux, Daniel D., Ann M. Goudreau, Muralidhara Ramachandra, and Paul W. Shabram. Viral Production Process. United States Patent No. 5,994,134. Issued Nov. 30, 1999. A method producing recombinant viral vectors at high titers during viral culture.

Approximate Value of Donation:
$ 68,000 for testing including raw materials
$ 20,000 for production
$ 88,000 Approximate Total Value of Donation