Adenoviral Reference Material Working Group
Bid Submission Form
Viral Material Donation, RFP 2.0

Item for Submission

Ad5 Wild-type Virus to support production of banks that will allow production of a minimum of 10 lots of adenovirus reference material. A proposal to submit a plasmid capable of generating an Ad5 Wild-type Virus is also an option. At a minimum the amount of virus donated should be 1×10^{12} total particles in at least three aliquots.

General Requirements for Bidding

A description of the material, its history, and information regarding characterization of the material to be donated must be included. The material should be documented to be free from adventitious agents; at a minimum it should be tested for sterility and, for viral material, also for mycoplasma and AAV. For plasmid material endotoxin levels should also be indicated. Characterization should also include information on the material's concentration (particle/mL or µg/mL plasmid), assessment of identity, buffer or media information, and for viral material, infectious activity. It is desirable that the identity testing be based on molecular characterization, such as restriction mapping. If viral material, the material may be either a lysate or a purified material. Plasmid donations should include a molecular map. Proposals containing more complete characterization will be favored.

Materials should be available for transfer approximately 1-2 weeks after the bid is awarded. The proposal should include details of the proposed method/container for shipping to ensure integrity of the viral (or plasmid) material upon arrival at the virus bank production facility.

Documentation Requirements

Characterization information as stated above. Documentation of all testing done on the material will ideally include copies of final reports for assays performed. A brief description of how to use the material should also be included.

Please complete the following fields:

Contact Information – RFP 2.0

Contact Individual:	Beth Hutchins
	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 Material Donation Information – RFP 2.0

Virus/Plasmid description (e.g. Ad5 WT): _____**Adenovirus Type 5 (Wild-Type)**_____

Number of vials to donate: ____**3 x 10 mL vials**_____

Concentration/Volume per vial: ___**10 mL per container; 5.29 x 10^{11} particles/mL**___

Indicate Material Format: ☒ Lysate Ad ☐ Purified Ad ☐ Plasmid

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: Description of material
Characterization information, including final reports
Suggested information for propagation
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 for 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 2.0 Viral Source Material
Submission From Canji, Inc.

CANJI Inc. *Description of material*

Material is labeled: **Ad5WT Lot M1**.

The source material was an ATCC vial of Adenovirus, Type 5 (catalog # VR-5). The material from ATCC was passaged twice by infecting A549 cells (ATCC catalog # CCL-185) and preparing a lysate by freeze-thawing three times. The A549 cells were grown in DMEM supplemented with 10% (v/v) fetal bovine serum in flat stock culture. The resulting lysate was purified by column chromatography (reference Huyghe *et al*.) and designated Ad5WT Lot 28AAL. This lot of purified Ad5WT virus was expanded on A549 cells and a freeze-thaw lysate produced. Using ten-fold serial dilutions from 10^{-4} to 10^{-8}, the lysate was placed in a plaque-forming assay on A549 cells. Three distinct plaques were harvested and the virus expanded three times by infecting A549 cells, the third time using cell factories. The resulting lysate was prepared in DMEM supplemented with 10% (v/v) fetal bovine serum and designated **Ad5WT Lot M1**.

The electronic file labeled "Canji Ad5WT characterization.doc" contains:

- ?? Identity test result via RFLP

The electronic file labeled "Canji Ad5WT char info #2.pdf" contains:

- ?? Copy of ATCC information sheet on Adenovirus Type 5 (2 pages)
- ?? Copy of ATCC information sheet on A549 cells (2 pages)
- ?? Copy of Hyclone Certificate of Analysis for Fetal Bovine Serum (1 page)

The electronic file labeled "Canji Ad5WT char info #3.pdf" contains:

- ?? Copy of Summit Biotechnology Fetal Bovine Serum Certificate of Processing (Irradiation) (2 pages)
- ?? Copy of Summit Biotechnology Fetal Bovine Serum Certificate of Analysis (1 page)
- ?? Copy of Canji Resource Q HPLC Assay data package (4 pages)
- ?? Copy of Canji FACS-Based Infectivity Assay/A549 cells (1 page)
- ?? Copy of Canji Identity Assay RFLP raw data information (3 pages, meant as an attachment to "Canji Ad5WT characterization.doc" file)

Description of Test Methods:

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

Sterility was performed per USP. Mycoplasma was performed per Points to Consider, and the test to determine whether AAV is present was performed using conventional PCR with a limit of detection of approximately 150,000 copies per 0.5 µg of DNA (the amount tested). Sterility, mycoplasma, and testing for AAV were performed by a contract laboratory, MDS PharmaServices, Inc.

The **Ad5WT Lot M1** lysate was assayed for particle number concentration via Resource Q HPLC Assay (reference Shabram *et al*.; a copy of the standard operating procedure is contained in the electronic file: "Canji Ad5WT resource Q.doc") and infectious titer via a flow cytometry-based method on A549 cells. A summary of the infectious titer method is contained in the electronic file "Canji Ad5WT InfectAssay 293.doc". This method is identical to the FACS-based method except that the cell line is 293. **Ad5WT Lot M1** was submitted to a contract laboratory for Sterility testing, Mycoplasma testing, and testing via PCR analysis to detect Adenovirus-Associated Virus (AAV). Ad5WT Lot M1 was used to infect A549 cells and the virus was purified via column chromatography (reference Huyghe *et al*.), resulting in Ad5WT Lot 43AAM. The purified virus was submitted for RFLP analysis and found to be consistent with Adenovirus, Type 5 sequences described in Genbank.

Characterization Summary

Ad5WT M1 Adenovirus Type 5	10 mL lysate per container	5.29×10^{12} viral particles per container
Test	*Method*	*Result*
Identity	RFLP Analysis	Consistent with Adenovirus Type 5
Particle Concentration	Resource Q HPLC Assay	5.29×10^{11} particles per mL
Infectivity	FACS-Based Infectivity Assay on A549 cells	5.07×10^{9} infectious units per mL
Particle to Infectious Titer Ratio	Resource Q HPLC to FACS-Based Infectivity Assay Ratio	104:1
Sterility	Per USP	Awaiting final report from MDS PharmaServices, Inc.
Mycoplasma	Per PTC	Awaiting final report from MDS PharmaServices, Inc.
AAV	PCR Limit of detection: approximately 150,000 copies per 0.5 µg DNA	Awaiting final report from MDS PharmaServices, Inc. from test on 0.5 µg DNA

***Final reports will be sent electronically as soon as provided by MDS PharmaServices. Earthquake affected phone lines and ability to transmit report.

Unofficial laboratory results: Sterility-pass; Mycoplasma-pass; AAV/PCR-negative.

SEE ELECTRONIC FILES WITH CHARACTERIZATION INFORMATION.

CANJI Inc. *Suggested information for propagation*

May be propagated on a number of cell lines including A549 and 293 cell lines.

For 293 cell lines:
For T-225 flasks, seed cells at a seeding density of 2 x 10^6 cells/flask in DMEM with high glucose supplemented with 10% fetal bovine serum.
Infect the cell flasks with 8 x 10^7 p/mL per flask 3-4 days after seeding the cells, prior to the cells becoming 100% confluent.
Harvest the T-225 flasks after seeing evidence of CPE, but prior to complete CPE, usually on day 3 or 4 post-infection.

Seed 2 cell factories with cells at a seeding density of 8 x 10^7 cells/cell factory in DMEM with high glucose supplemented with 10% fetal bovine serum.
Infect the cell factories with 8 x 10^7 p/mL per cell factory 3-4 days after seeding the cells, prior to the cells becoming 100% confluent.
Harvest the cell factories after seeing evidence of CPE, but prior to seeing complete CPE, usually on day 3 or 4 post-infection.

For A549 cell lines:
NOTE: A549 cells are not as readily infectable as 293 cells.

Seed 2 cell factories with cells at a seeding density of 8 x 10^7 cells/cell factory in DMEM with high glucose supplemented with 10% fetal bovine serum.
Infect the cell factories with 8 x 10^7 p/mL per cell factory 3-4 days after seeding the cells, prior to the cells becoming 100% confluent.
Harvest the cell factories after seeing evidence of CPE, usually on day 4 or 5 post-infection.

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 50 mL total volume may be shipped in one box using this procedure. For larger volumes (up to 4 L in one box) an additional restriction and label is required.]

Materials

- ?? Adenovirus sample(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 overpack box
- ?? "Dry Ice", "Infectious Substances affecting Humans", "Inner Packages Comply" 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 to prevent the thawing of the samples when transferred into it.
4. Place samples 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 overpack box closed. Do not seal all edges so as to allow gaseous CO_2 to escape.
10. Label the overpack 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. Fill out the airbill and dangerous goods forms according to courier requirements.
12. Inform the recipient that the shipment is in route.

References

1. 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

2. 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

Approximate Value of Donation:
$6,000.00