National Research Council
Biotechnology Research Institute
Animal Cell Technology and
Downstream Processsing Group
Adenoviral Vector Production Facility

Expertise

The group has some 30 scientific and technical experts all dedicated to Integrated process development for the production of recombinant proteins and viral vectors using animal cell. The group has developed expertise in cell and metabolic engineering, scale-up of serum-free suspension cultures, bioreactor operations, on-line monitoring and process control.

With a multidisciplinary dedicated team, the group has a demonstrated ability to operate transient as well as perfusion based processes for production of recombinant proteins and viral vectors from 3-150 L scale.

The group has gained a world wide reputation, in the past 10 years, in collaboration with the biopharmaceutical industry and the largest academic centers. It is concentrating its research activities on production strategies for recombinant proteins and viral vectors to be used in gene therapy applications. In addition, the group has broadened its range of skills in high cell density (fed-batch and perfusion productions), which is used with mammalian cells and advanced process control and monitoring operations, as well as various purification methods in Downstream Processing.

Infrastructure, equipment and analytical support

- A 1,830-square-foot large scale Biosafety Level 2 production and purification suite.
- 4 fully-equipped and dedicated laboratories for upstream and downstream processing delopment.
- 3-to-180 L scalability for adenoviral vectors and reagents for gene transfer projects at various levels of maturation.
- Quality assurance and quality control system in place to ensure product traceability.
- Analytical support (HPLC, FACS…)
- A range of related expertise and state-of-the-art equipment for pharmaceutical and biotechnology processes.

Progress/achievements :

***Mammalian cell technology and Adenoviral Vectors*:**

In the last 4 years, more than 50 adenovirus fed-batch productions at 20L scale have been performed to generate research material. The main vectors produced were: GFP, Lac-Z, Utrophin, Dystrophin, and RSV-phophorylase. Under specific conditions, productions ranged from 2000-4000 infectious particles per cell at cell densities as high as 1.5 million cells per mL.

A serum-free suspension culture has been developed using standard bioreactors for the production of adenoviral vectors for gene transfer experiments.

A clonal cell line (293SF-3F6) has been deposited at ATCC and is being patented. Use of serum-free-medium has shown increased cellular aggregation and low specific production. A new serum free medium, NSFM13, has been successfully developed here to tackle this issue. Yield and cell viability comparable to serum supplemented medium are now achieved. Extensive experience in this area was acquired and application to human cell lines complementing the adenovirus vectors has placed the group in the forefront of process development for the production of gene therapy vectors. Through a NRC major initiative fund, an animal cell culture Biosafety Level 2 pilot plant production facility was completed. The process can now be scaled-up to 150L.

A technique for the fast, accurate measurement of infectivity is very important to the process, particularly in downstream processing after the culturing of the viruses. The green fluorescent protein (GFP) has been an excellent tool to rapidly assess levels of infectious viruses using FACS analysis. This, combined with HPLC which gives a total virus concentration, provides pertinent information on overall viral bioactivity versus total viral load. This GFP approach allows researchers to create an optimal model process for the production of adenoviral vehicles carrying therapeutically valuable genes. The superiority of the new UNO Q column lies in its capacity to measure total virus particles from cell lysates without any additional steps such as Benzonase treatment.

The critical steps in the infection process were experimentally studied to create a mechanistic model. This approach will help in predicting the kinetic characteristics of any new therapeutic vector. As such, diffusion, absorption and internalization using a CY5 dye were studied. Protein synthesis and DNA replication kinetics will be evaluated using different promoters. An in-situ, on-line GFP probe, was developed and used to monitor the overall expression kinetics. An on-line capacitance probe was also used to identify physiological events during the infection/expression process.

A large-scale purification process based on an anion exchange column eluted with a NaCl gradient to purify adenoviral vectors has been implemented. Good separation between the intact virions, the individual viral proteins and cellular DNA, with 55 % recovery and purity higher than 90% was achieved. Screening of four different matrices helped increase the yield, the purity and the binding capacity up to 10^{12} viral particles per mL of

gel. Moreover, no loss of activity was observed and the process is scaleable to 100L production. Size exclusion chromatography as a polishing step has been evaluated . We have completed an integrated process, from the development of new vectors and packaging cell lines, to the final purified, active adenovirus vector for gene transfer.

Selected papers:

J. Transfiguracion, A. Bernier, N. Arcand, P. Chahal and A. Kamen (2001) Validation of HPLC Assay for the Quantification of Adenovirus Type-5 (Ad5) Particles. Submitted to Journal Of Chromatography B.

Klyushnichenko V., Bernier A., Kamen A.and Harmsen E. Improved HPLC method in the analysis of adenovirus particles, (2000) Journal of Chromatography B. **755**: 27-36

Nadeau I., Jacob D., Perrier M & Kamen A. (2000), 293SF Metabolic flux analysis during cell growth and infection with an adenoviral vector. Biotechnol. Progress. . **16**:872-884

Nadeau I., Sabatie J., Koehl M., Perrier M. and Kamen A (2000), Human 293 cell metabolism in low glutamine-supplied culture: interpretation of metabolic changes through metabolic flux analysis, Metabolic Engineering **2:** 277-292

Gilbert P.A., Garnier A., Jacob D. and Kamen A., (2000) On-line measurement of GFP fluorescence for the monitoring of recombinant adenovirus production. Biotech. Letters **22**: 561-567

Gilbert R., Nalbantoglu J., Petrof B.J., Ebihara S., Guibinga H., Tinsley J. M., Kamen A., Massie B., Davies K. E. and Karpati G., (1999) Adenovirus-mediated utrophin gene transfer mitigates the dystrophic phenotype of mdx mouse muscles, Human Gene Therapy, **10**:1299-1310.

Côté J., Garnier A., Massie B. and Kamen A., (1998) Serum-free production of recombinant proteins and adenoviral vectors by 293SF cells, Biotechnology and Bioengineering **59**: 567-575

Côté J., Bourget L., Garnier A. and Kamen A., (1997), Green Fluorescent Protein as reporter gene for adenovirus production in serum-free 293SF suspension culture. Biotechnology Progress **13**:709-714.

Nadeau I., Garnier A., Côté J., Massie B, Chavarie C. and Kamen A., (1996) Improvement of recombinant protein production with the human-adenovirus/293S expression system using fed-batch strategies. Biotechnology and Bioengineering **51:** 613-623

Garnier, A., Côté, J., Nadeau, I., Kamen, A., and Massie, B. (1994). Scale-up of the adenovirus expression system for the production of recombinant protein in human 293S cells. Cytotechnology, **15**: 145-155.

PATENT

COTÉ, M.T. Johanne; KAMEN, A. Amine; MASSIE, Bernard; Serum-Free Production of Recombinant Proteins and Adenoviral Vectors using 293SF-3F6 cells Application US 09/201,168.

Short CVs of key personnel involved in this project:

Alice Bernier

-BSc in Biochemistry.
-Has worked in the science field for 20 years.
-Has extensive experience in Downstream Processing, including the analytical techniques required to follow a purification process.
-Has worked in a Clean Room for a protein purification procedure under GMP guidelines.
-Is involved in the Adenovirus Project which consists of scaling up the purification of r-Adenovirus Type 5 from 3L to 20L to eventually 100L productions. Has dedicated the last 3 years to this project.

Julia Transfiguracion
-BSc in Biochemistry
-MSc in Biotechnology
-Had worked (1993-95) as a Research Assistant dept of Oncology Mc Gill University
-Had worked (1995-1999) as DSP associate and Quality control manager at Rougier Biotech Ltd. Montreal
- Is working at the Biotechnology Research Institute since 1999 in the field of viral vector quantification and purification.

Normand Arcand
-BSc in Microbiology
-MSc in Applied Microbiology
- Is working at the Biotechnology Research Institute since 1992 in Fermentation process and Enzyme engineering (1992-96); in protein purification (1996-1999)
- Is involved in the Adenovirus Project which consists of scaling up the purification of r-Adenovirus Type 5 from 3L to 20L to eventually 100L productions. Has dedicated the last 3 years to this project.

Rosanne Laurie Tom
-B.Sc., Biochemistry (1986)
-M.Sc.A, Biochemical Engineering (1991)
- Is working at the Biotechnology Research Institute for 12 years in cell culture and recombinant protein production using the Baculovirus Expression Vector System up to the 150-L scale. (co-)authored 18 publications.