Large Scale Viral Vector Production

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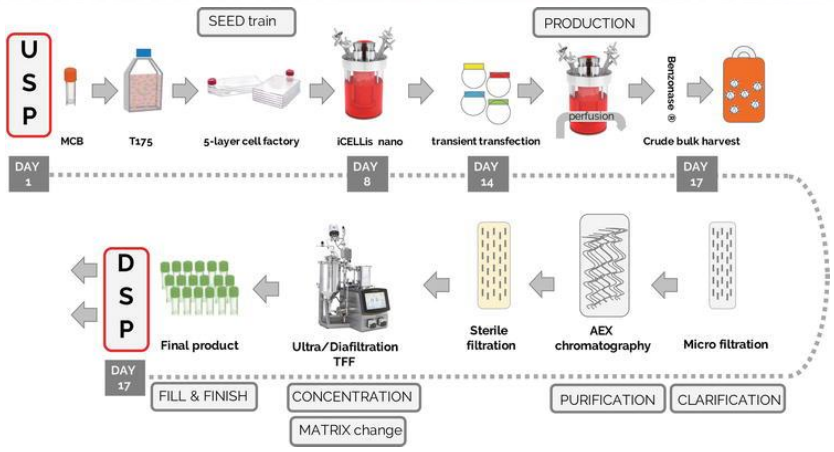
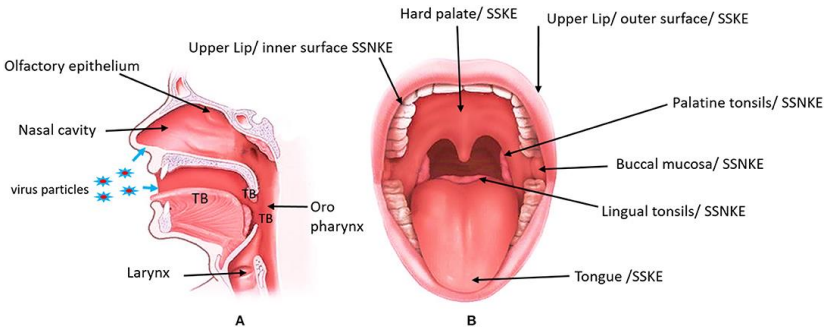
Recombinant Virus Production



Lentiviral Vectors Come of Age? Hurdles and Challenges in Scaling Up Manufacture
Juan C. Ramirez, DOI: 10.5772/intechopen.81105

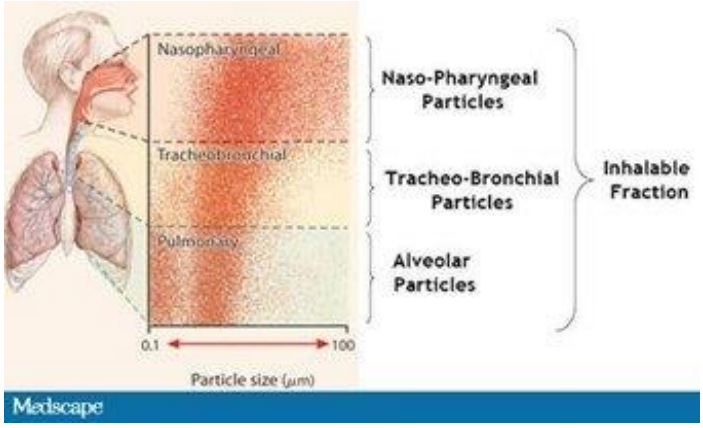
Routes of Entry

- Mucous Membranes



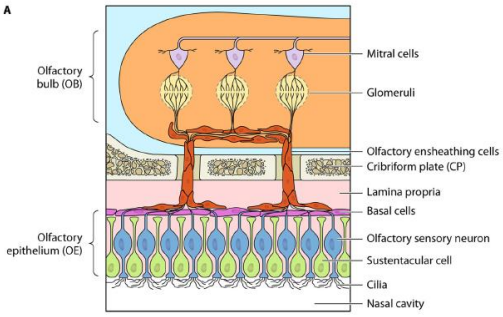
Routes of Entry

- Respiratory Aerosols



Routes of Entry

- Naso-Pharyngeal Fraction
  - ***S. pneumonia***
  - ***N. meningitidis***
  - ***H. influenza***
  - **VEE, WEE, EEE**
  - **Herpesviruses**
  - **Influenza A**
  - **Paramyxoviruses**
  - **Rabies virus**



Samantha J. Dando, Alan Mackay-Sim, et al, Clinical Microbiology Reviews Oct 2014, 27 (4) 691-726;

Viral Vector Risks

- Viral Vectors
  - **Lentivirus**
    - Risk of chromosomal integration & insertional mutagenesis (1) (2) (3)
    - Risk of healthy worker genome modification- multiple gene copies

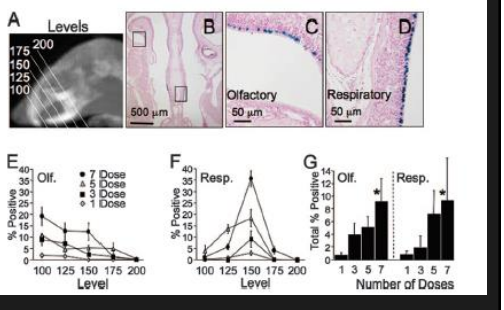
1. Bushman F, et al. Genome-wide analysis of retroviral DNA integration. *Nat Rev Microbiol.* 2005;3(11):848–858.
2. Hacein-Bey-Abina S, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest.* 2008;118(9):3132–3142.
3. Schlimgen R, Howard J, Wooley D, et al. Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. *J Occup Environ Med*. 2016;58(12):1159-1166. doi:10.1097/JOM.0000000000000879

Lentivirus Vector Can Be Readministered to Nasal Epithelia without Blocking Immune Responses

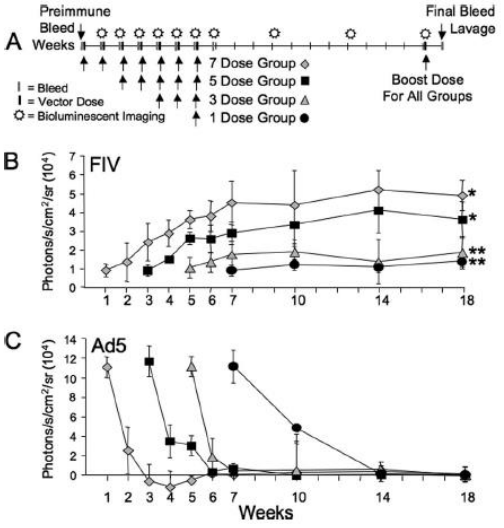
- SINN ET AL, JOURNAL OF VIROLOGY,
- Vol. 82, No. 21,Nov. 2008, p. 10684–10692.

Acute Repeat Administration of Lenti Vector

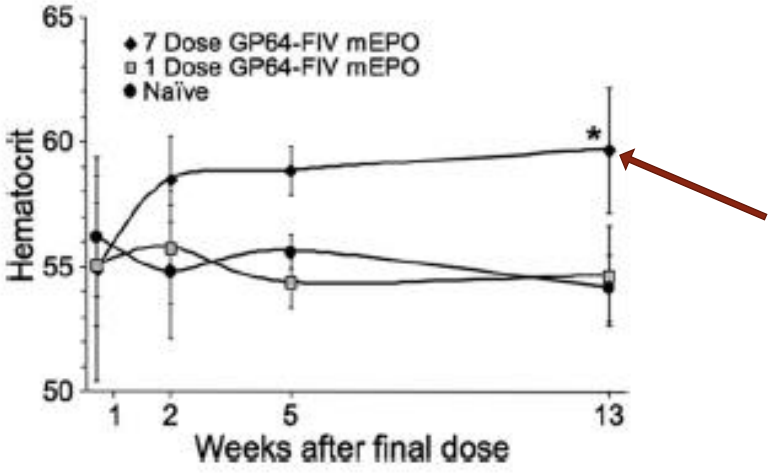
delivered to mice following the same protocol as Fig. 1A. Four weeks following the final dose, sections were collected from five standardized levels based on anatomical landmarks, as shown schematically (A). One level is ~30 μm. Low-power photographs (B) of both olfactory (C) and respiratory (D) epithelia were collected from each level. Using ImagePro software, the percentages of β-Gal-positive surface epithelial cells were determined for both olfactory (Olf. [E]) and respiratory



FIV vs Ad5 Intranasal Dosing



FIV-mEPO Dosing vs Hemocrit Changes



Viral Vector Risks

- AAV (Adeno Associated Virus)
  - **Hepatocellular carcinoma**
    - Clonal integration of wt-adeno-associated virus type 2 (AAV2) in 11 of 193 HCCs.
    - These AAV2 integrations occurred in known cancer driver genes, namely CCNA2 (cyclin A2; four cases), TERT (telomerase reverse transcriptase; one case), CCNE1 (cyclin E1; three cases), TNFSF10 (tumor necrosis factor superfamily member 10; two cases) and KMT2B (lysine-specific methyltransferase 2B; one case). (1)
    - Because no liver tumors have been observed to date in patients treated using AAV vectors for gene therapy, the risk of HCC is probably very low, if it exists at all. However, we must consider the two independent mouse models of AAV vectorization that developed HCC through clonal oncogenic insertion. (2)

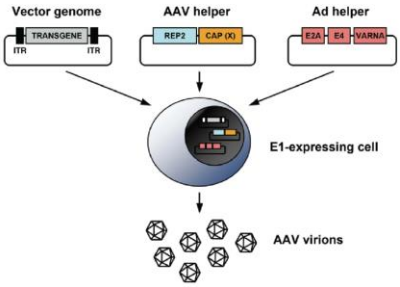
1. Berns KI, Byrne BJ, Flotte TR, et al. Adeno-Associated Virus Type 2 and Hepatocellular Carcinoma?. *Hum Gene Ther*. 2015;26(12):779-781. doi:10.1089/hum.2015.29014.kib
2. Nault JC, Mami I, La Bella T, et al. Wild-type AAV Insertions in Hepatocellular Carcinoma Do Not Inform Debate Over Genotoxicity Risk of Vectorized AAV. *Mol Ther*. 2016;24(4):660-661. doi:10.1038/mt.2016.47

Adeno-Associated Virus (AAV) Risk

- AAV viral vectors were produced by combining three plasmids (Rep/Cap; pXR2 helper; TReGFP), HEK 293 cells and PEI Max into a WAVE bioreactor
- Using continuous harvest, the system produced greater than 1E14 viral genomes per L at 48 hour harvest.

AAV Production using HEK293 Cells, Plasmids and Polyethyleneimine Max

Adeno-Associated Virus (AAV) Risk



- ..enhanced its plasmid DNA delivery efficiency 21 times *in vitro*, as well as 10,000 times in mice with a concomitant 1,500-fold enhancement in lung specificity."
- Evaluate potential risk to workers for transfection reagents.
- Thomas et al, PNAS 2005, V102, N16, pp 5679-5684

AAV Production using HEK293 Cells, Plasmids and Polyethyleneimine Max

2006 Apr;100(4):658-63.

Risk of Aerosol Generation

Agent Concentration	1.00E+09				
Accident	Spray Factor	Potential Release CFU/M^3	Potential Release CFU/L	Breathing Exposure 1 minute CFUs	Breathing Exposure 10 minutes CFUs
Blocked peristaltic pump	2.59E-06	2590.00000	2.59000	38.85000	388.50000
15 mL spill 0.9 m	1.04E-06	1040.00000	1.04000	15.60000	156.00000
Antifoam Failure	2.20E-03	2200000.00000	2200.00000	33000.00000	330000.00000
Pipe Failure	1.40E-04	140000.00000	140.00000	2100.00000	21000.00000
Glass Vessel Shatter	3.80E-05	38000.00000	38.00000	570.00000	5700.00000
CSA 8 Centrifuge Leak	2.50E-04	250000.00000	250.00000	3750.00000	37500.00000
Metal Vessel Rupture	1.10E-03	1100000.00000	1100.00000	16500.00000	165000.00000
Sonicator, max	1.00E-04	100000.00000	100.00000	1500.00000	15000.00000
Sonicator, min	5.00E-07	500.00000	0.50000	7.50000	75.00000
Single Drop falling 1 M	2.00E-06	2000.00000	2.00000	30.00000	300.00000
Centrifuge Rotor	4.60E-06	4600.00000	4.60000	69.00000	690.00000
Centrifuge Bucket	1.70E-05	17000.00000	17.00000	255.00000	2550.00000
Shaking Incubator	1.28E-06	1280.00000	1.28000	19.20000	192.00000
Steaming Sample Valve	2.00E-06	2000.00000	2.00000	30.00000	300.00000
Smashed Flask	5.50E-05	55000.00000	55.000000	825.000000	8250.000000

Bennett A, Parks S. Microbial aerosol generation during laboratory accidents and subsequent risk assessment. J Appl Microbiol. 2006 Apr;100(4):658-63.

Potential Risk of Gene Over-Expression

Gene Therapy Target

- ADA-SCID- Adenosine deaminase loss
- VEGF-165- Cardiac vascularization
- H-Coagulation factor IX- Hemophilia B
- H-Fibroblast growth factor-4
- ATP7-beta- Copper accumulation
- Micro-dystrophin

Target Gene Over-Expression

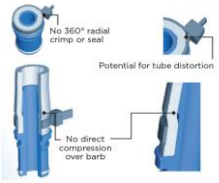
- Hemolytic anemia
- Increased tumorigenicity
- Deep vein thrombosis
- Overexpressed in cancers
- Copper excretion into bile
- 100-fold cardiac overexpression causes cardiac abnormalities

Process Step Risk Review

Tubing Connections and Peristaltic Pumps

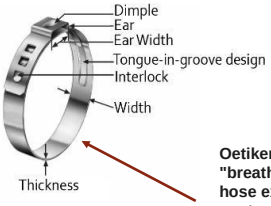
Tubing Clamps

Plastic band clamps do not “breathe”; Create leak path at latch; require second clamp latch 180 degrees opposite first



Gear clamps do not “breathe; serations cut tubing creating leaks

Clamp must be place next to barb to prevent slippage



Oetiker ear type clamps "breathe" and adapt to hose expansion and contraction from heating or aging-- no need to retighten.

Sanitary Connections

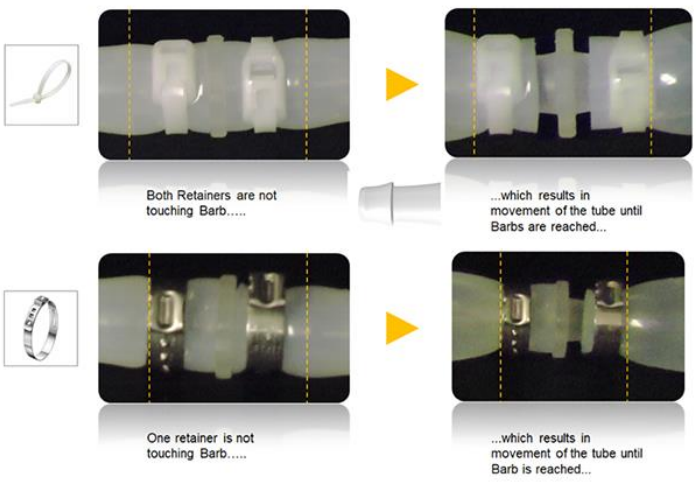
Molded sanitary-tubing connections

Single use sanitary-tubing connections for custom tubing construction

Sanitary connection gasket

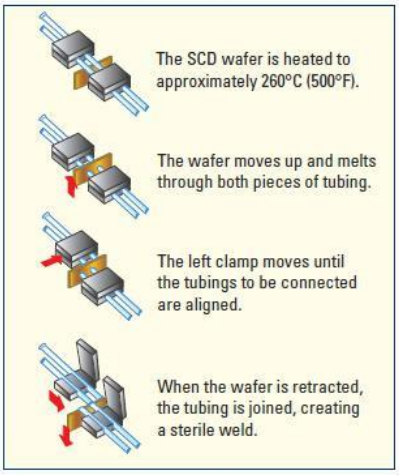
Constant torque (spring-loaded) sanitary clamp preferred if connection will be autoclaved and/or cooled. Prevents Teflon gasket ‘creep’, which results in leaks.

Tubing Clamps- Location

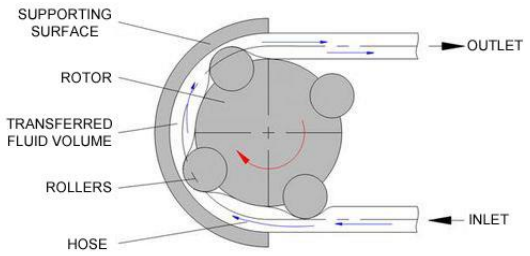


Thermal Tubing Welders and Sealers

- Has regular preventative maintenance and calibration been performed?
  - **The welding mechanism requires very tight tolerances to prevent tubing leaks**
  - **Weld debris can cause jamming or misalignment**



Peristaltic Pumps



- Rules not to forget-
  - Correct tubing (manufacture specific)
  - Maximum operating pressure decreases with increased flow rate
  - Correct pump rotation direction
  - Open clamps and valves

Process Step Risk Review

Bioreactor

Single Use Bioreactors

- **Prevent vessel overpressure**
- Max pressure = 7 psig (0.5 bar)
- Overpressure will:
  - Rupture tubing connection, releasing aerosol

Single Use Bioreactors

- **Prevent vessel overpressure**
- Exhaust filter line vertical for drainage
- Filter heater to prevent condensation

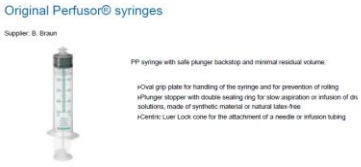
Single Use Bioreactors

- **Prevent vessel overpressure**
- Pressure control unit for sparge and overlay gas
- Relieves at 7 psig
- Install before inlet gas filters

Single Use Bioreactors

Sampling

- Clave IV connector for syringe sampling
  - **Syringe plunger pullout**
    - Use syringe with plunger backstop to prevent removal

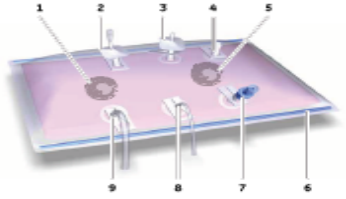


WAVE Bioreactor

WAVE Bioreactor

Illustration of Cellbag bioreactor

The illustration shows a general Cellbag bioreactor. The configuration of your Cellbag bioreactor may vary from the configuration shown below.



Port	Description
1	pH bag sensor port, located on the underside of the bag
2	Outlet vent filter with pressure control valve
3	Inlet vent filter
4	Addition port
5	DO bag sensor port, located on the underside of the bag
6	Cellbag rod
7	Clave™ sampling port
8	Addition port
9	Addition/harvest port

Note: The inlet and outlet vent filters are distinguished by the pressure control valve on the outlet filter.

Single Use Bioreactors

Prevent vessel overpressure

- Bag pressure sensor monitors and alarms
- Exhaust condenser, filter heaters and vertical position reduce risk

Single Use Bioreactors

Probe Installation

- Damage to bag fittings may cause in-process leaks
- Accidental impact can shear off bag connection
- Vendor notices for damaged bag lots miss-communicated
  - **50L SUB leaked at probe ports, releasing BSL-2 vaccine to floor**

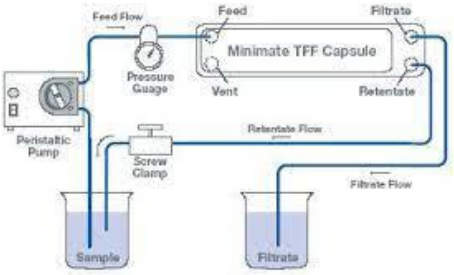
During the filling process and fermentation process, small quantities of liquid may penetrate the probe adapter.

Process Step Risk Review

MF/UF/ Depth Filtration

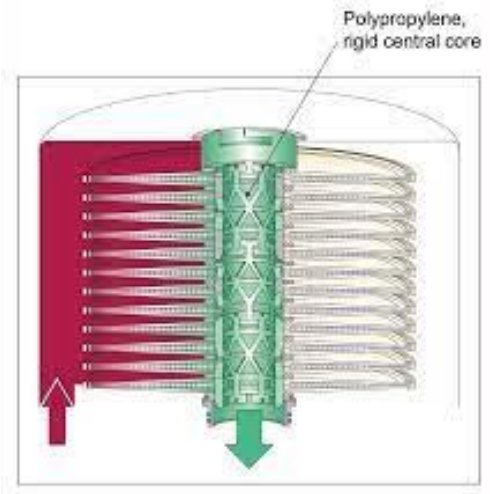
MF/UF Filtration

- Microfiltration- 0.2 microns
- Ultrafiltration- molecular weight cut-off
- Concentrate virus or change media composition



Depth Filtration

- Remove
  - **Lysed cells**
  - **Coagulated DNA**
  - **Particles from chromatography**



MF/UF/Depth Safety Points

- Develop emergency shutdown procedure, including remote power shutdown location.
- Flow waste container shall have filtered vent and located in a secondary catch container to control overflow.
- Pressure transducer test and calibration before each campaign.
- Consider pressure alarms at 80% max pressure of column or tubing, whatever is weakest.
- Institute pressure hold tests before introducing virus.
- Place the unit inside a plastic tray to catch spillage. Install a drain connection to a carboy. Consider a conductive rope detector to alarm for leakage.

MF/UF/Depth Safety Points

- Consider a negative pressure BioBubble. Goal is to control spray. If not feasible, cover filter with plastic sheeting. Ends are tucked into spill tray
- When loading from methods library, use two-person signature to reduce choice error.
- Tag cassettes with pressure max, methods permitted.

Process Step Risk Review

Chromatography

Chromotography

- Removes
  - **Nucleic acids**
  - **Proteins**
- Concentrate virus

Chromatography Safety Points

- Develop emergency shutdown procedure, including remote power shutdown location.
- Develop emergency plan to recover virus charge if pump fails for overpressure
- All waste containers have filtered vent and placed in a secondary container. Seal wash fluid drain tube placed under disinfectant.
- Pump seals maintenance critical. Depending on risk, change before each campaign.
- Pressure transducer test and calibration before each campaign.
- Consider pressure alarms at 80% max pressure of column or tubing, whatever is weakest.
- In-line filters changed before each virus campaign.

Chromatography Safety Points

- If tubing connectors are plastic nut with ferrule, consider more frequent changes.
- Institute pressure hold tests before virus use.
- Locate unit in a plastic tray to catch spillage. Place drain connection in vent-filtered carboy. Consider conductive rope leak detector.
- A BioBubble should be considered to surround the unit.
- Consider virus-free runs to confirm elution gradients do not cause precipitation, hence overpressure.
- When loading via methods library, use two-person signature to reduce choice error.
- Tag columns with pressure max, methods permitted.

THANK YOU!!!!!