



# Stem Cells: Overcoming the Safety & Regulatory Challenges of Clinical Application

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 BioReliance

# FDA Guidance

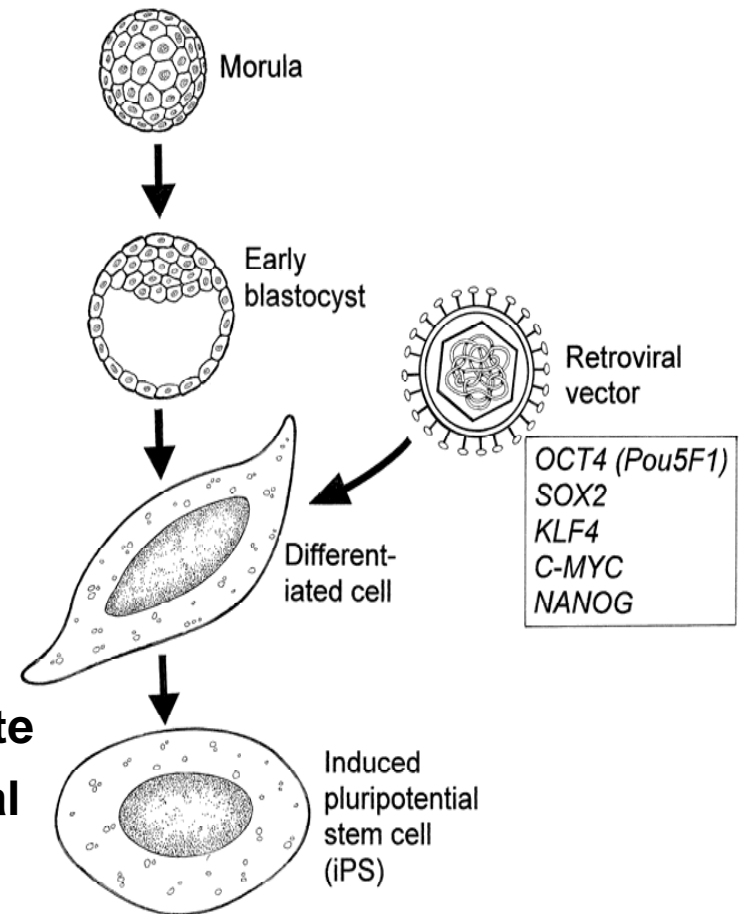
- [Guidance for Industry: Current Good Tissue Practice \(CGTP\) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products \(HCT/Ps\)](#)  
1/16/2009
- [Guidance for Industry: Regulation of Human Cells, Tissues, and Cellular and Tissue-Based Products \(HCT/Ps\) - Small Entity Compliance Guide](#)  
8/24/2007
- [Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products](#)  
8/8/2007

# Recent & forthcoming changes to regulations EU

- Advanced Therapy Medicinal Products (ATMPs) are medicinal products for human use, and are based on gene therapy, somatic cell therapy or tissue engineering. They offer groundbreaking new treatment opportunities for diseases and injuries of the human body. The regulatory framework for ATMPs is established by [Regulation \(EC\) No 1394/2007 on advanced therapy medicinal products](#)
- Human cell-based medicinal products  
CHMP/410869/06. Sep. 2008
- New ATMP committee proposed
- Certification procedure
- Hospital exemption

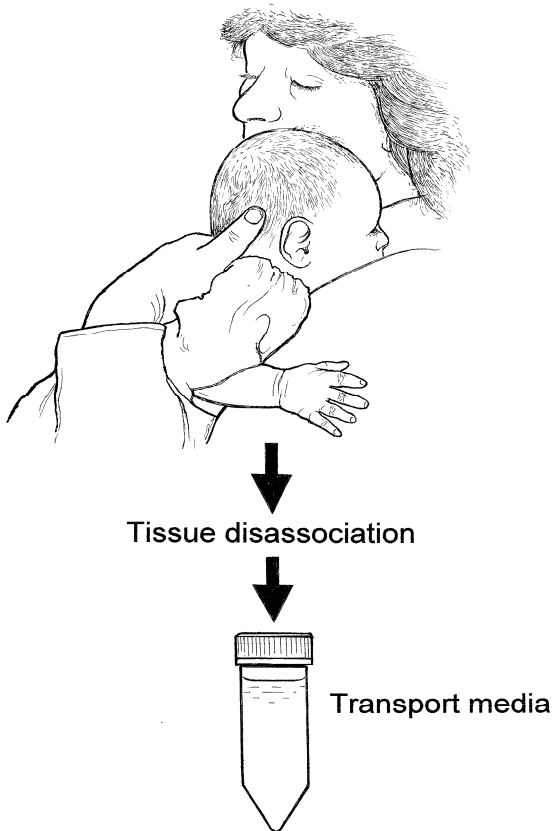
# Tests Required for Stem Cells and Cell Therapy

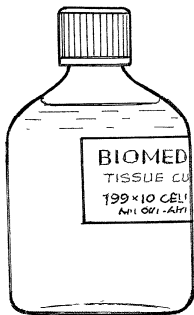
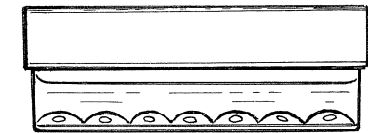
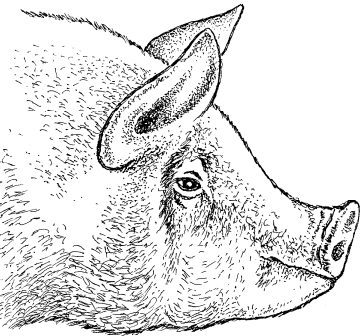
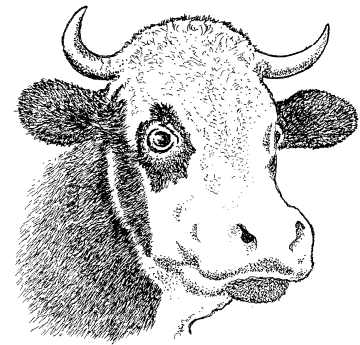
- **Pathogen and contaminant screening**
- **General CMC issues**
  - Cell or tissue sourcing
  - Media testing
  - Matrix or feeder cell tests
  - Cell characterization
- **Measures of identity**
  - HLA
  - STR
- **Measures of Long term stability, including genomic and epigenetic stability, X-chromosome inactivation**
- **Measures of quality and ability to differentiate**
- **Measures of contamination (feeders, residual tumorigenic stem cells)**
- **Functional assays (potency, efficacy & lot to lot variability)**



# Donor or donor mother screening

- **Donor or donor mother screened according to**
  - Advisory Committee on the Microbial Safety of Blood and Tissues, 2000; American Association of Tissue Banks, 2002; European Medicines Evaluation Agency, 1999).
- **Viruses**
  - HIV 1 & 2,
  - Hepatitis virus B & C
- **Antibody to:**
  - *Treponema pallidum*
  - hCMV
- **Test For**
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*
  - *Trypanosoma cruzi*
- **History**
  - Familial CJD and related conditions





### Serum

- BPyV
- Anelloviruses
- BCV
- BTV
- EHDV
- BHV
- BAV
- Papillomaviruses

### Trypsin

- PPV, PCV
- Anelloviruses
- Hepatitis-E
- Hokoviruses
- EMCV

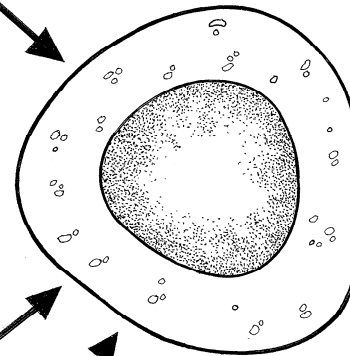
### Feeder Cell

- Dependant on species
- X-MuLV

### Media and Matrices

- MVM
- Spiroplasma

- Somatic Stem Cell
- hES
- iPS



**Viruses From Donor**

# General Manufacturing Assays

## Pathogen and contaminant screens

Bacteria and Fungi, 21 CFR

Mycoplasma, PTC

Bovine pathogens, 9 CFR

PCR for bovine polyoma virus

PCR for bovine circovirus

PCR for bovine herpesvirus 1&4

PCR for bovine papillomavirus

PCR for Borna disease virus

MAP for murine pathogens; LCMV challenge

Porcine pathogens, 9 CFR

PCR for Porcine EMCV

PCR for Porcine HEV

PCR for Porcine Circovirus, anellovirus and hokovirus

MAP for murine pathogens; LCMV challenge

PCR for Borna disease virus

*In vitro* for adventitious agents

## Pathogen and contaminant screens, cont

*In vivo* for inapparent viruses

TEM for viruses and retroviruses

FPERT

Induction with IdUR, AzaC and detection of murine retrovirus

PCR for SV40

PCR for human immunodeficiency virus 1&2

PCR for human T-cell lymphotropic virus 1&2

PCR for hepatitis A, B, C, E

PCR for human Adenoviruses

PCR for cytomegalovirus

PCR for herpes simplex virus 1&2

PCR for EBV

PCR for HHV, 6, 7 & 8

PCR for human parvovirus B19

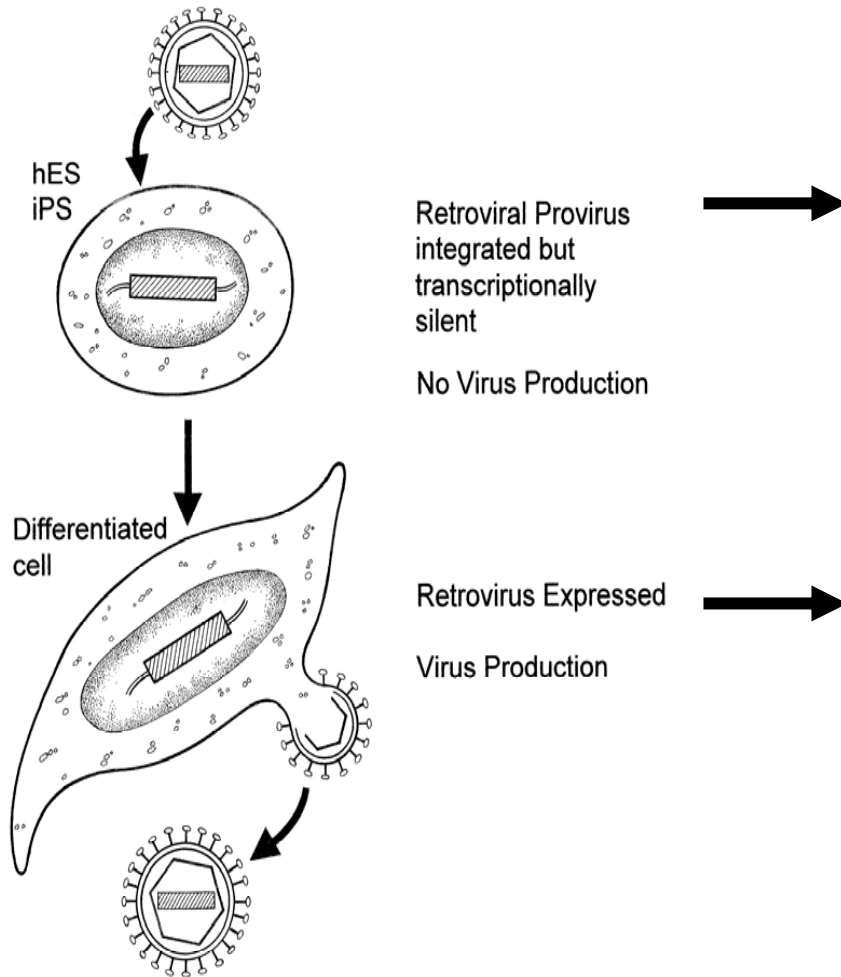
PCR for human polyomaviruses, BK, JC, Wu, Ki, and Merkel

PCR for Anelloviruses (TTV)

PCR for papillomaviruses for certain cells

*In vitro* infectivity MRC-5 & vero

# Identity and adventitious agent testing can be conducted at different stages



- Test of identity conducted here
  - Surface antigens
  - Key gene expression
  - miRNA profile/ epigenetic status
- Adventitious agent testing
  - Sufficient cells expanded for tests.
  - Viruses latent in stem cells may be expressed in differentiated cells
  - PCR, infectivity and transcriptome

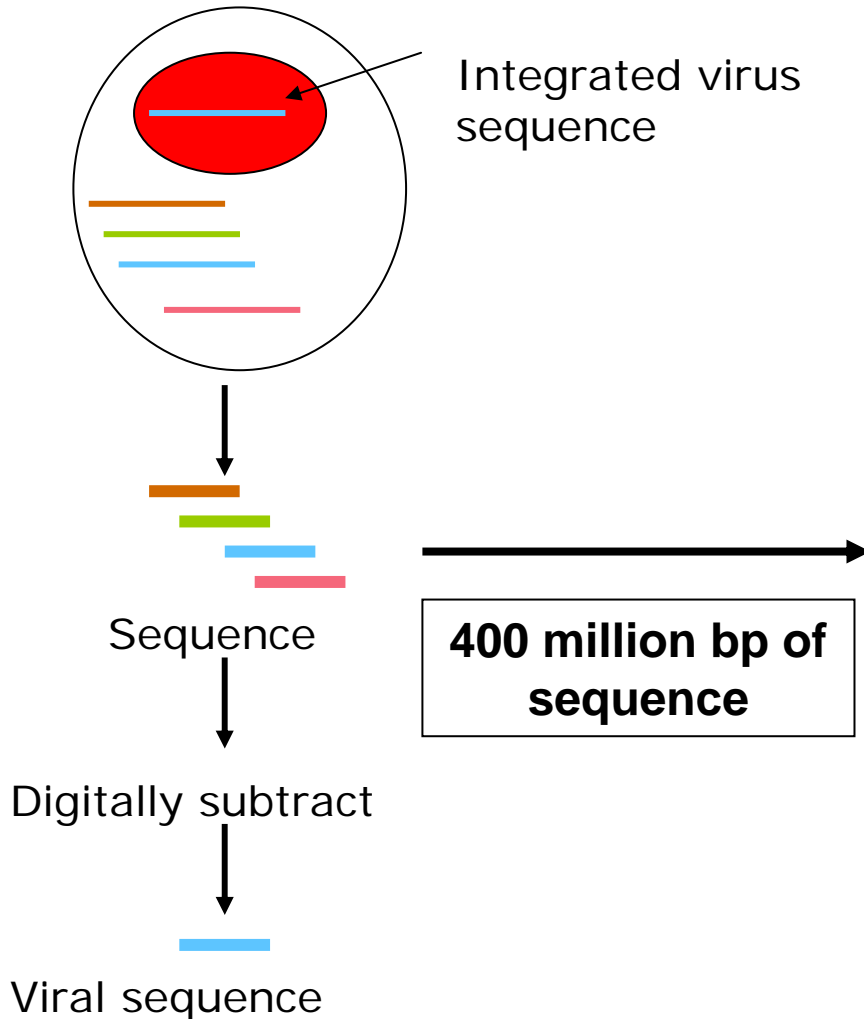
# High Throughput Massively Parallel Sequencing

- We have developed high throughput, deep, sequencing to detect adventitious agents using digital subtraction
- Typically, ~1M reads of 300 to 600 bases per O/N run
- Application for stem cell characterisation & safety
  - To detect virions
  - To detect integrated viral sequences
  - Characterise expression profile
  - Can be used for miRNA profiling and epigenetic status

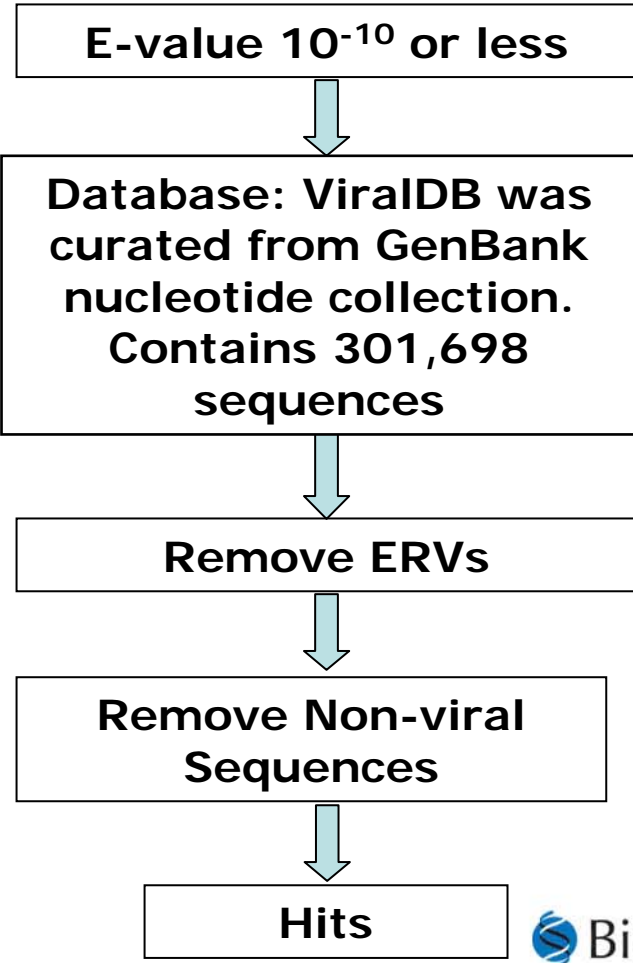


# Deep sequencing is now being used to exclude any latent or integrated contaminants

## Deep Sequencing



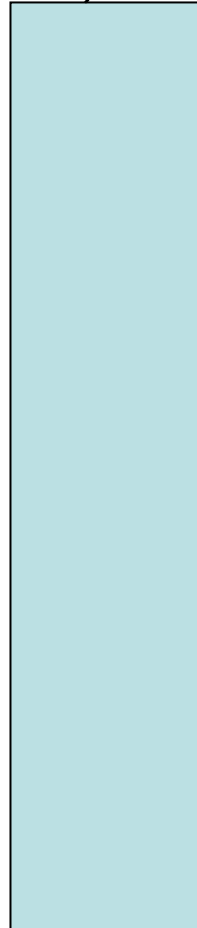
## Reliant Algorithm



# Why you need bioinformatics

**140 Mbp output printed on A4 sheets would be 3.5 metres high!**

3.5 metres 30,000 pages



Bioinformatics

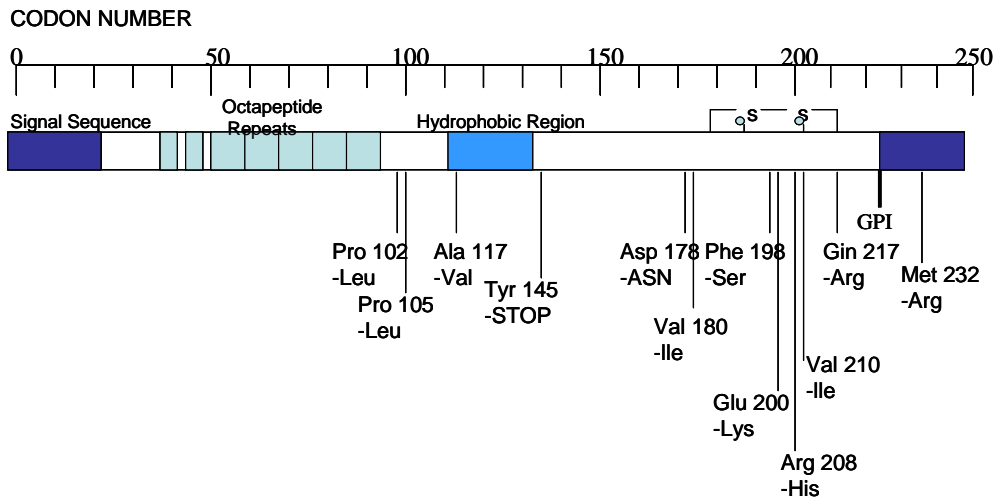


10 pages



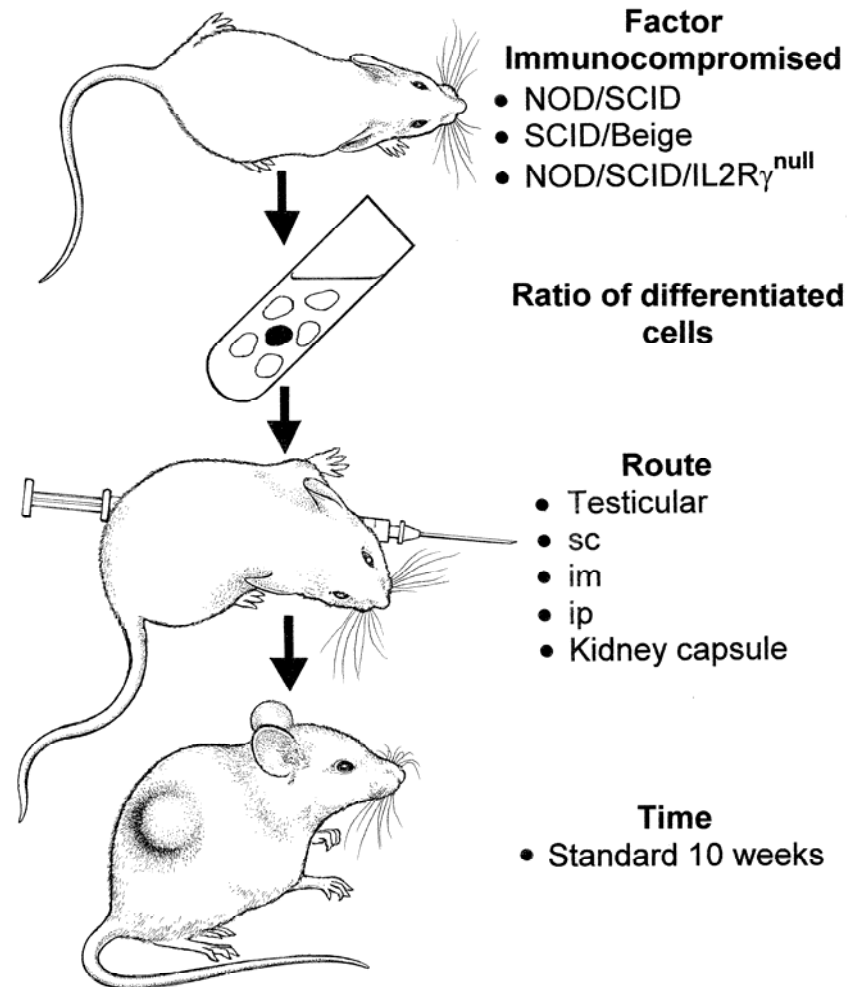
# Sequenece PrP alleles to exclude familial CJD

- **Both alleles sequenced to exclude:**
  - Point mutations associated with familial TSEs
  - Octapeptide repeat insertions



# Residual tumorigenic cells are one of the major concerns in stem cell therapy

- A characteristic of hESC is the ability to form teratomas in immunocompromised mice.
- The concern is the presence of residual tumorigenic cells in differentiated cells used in therapy.
- Dependent on several factors sensitivity may be of the order of 1 tumour cell in 4000 normal cells (Hentze et al stem Cell Res 2: 198 2009)
- Additional studies based on miRNA profiling, SKY karyotyping & CD99 and CD3 aneuploid marker detection.



# Tests to Monitor Stability of Stem Cell Lines



Stability
Tert expression (qPCR)
Mitochondrial function assessment
D-loop sequencing (qPCR)
Mitochondrial genomic DNA ratio (PCR)

Genomic Stability
Karyotyping
Spectral Karyotyping
FISH
SNP genotyping
Cell surface aneuploidy marker detection (e.g., CD99 and CD30)

Epigenetic Stability
Methylation analysis (bead array)
miRNA profiling (NCODE array)
X-chromosome inactivation (PCR, ICC)

***Several measures of cell stability can be assessed***

# Summary

- The safety of stem cells and tissues therapies depends on :
  - Rigorous adventitious agent testing
  - Determination that tumorigenic cells are absent
  - Assessment of the characteristics and long term genetic stability of the cells