ISO Guide 35: Reference Materials

Definitions: Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement

Uses:

- Calibration of a measurement system
- Assessment of a measurement procedure
- Assigning values to other materials
- Quality control

Examples from the VIM:

- Water of stated purity, the dynamic viscosity of which is used to calibrate viscometers
- Human serum without an assigned quantity value for the amount-of-substance concentration of the inherent cholesterol, used only as a measurement precision control material;
- DNA compound containing a specified nucleotide sequence

Vocabulaire International de Métrologie (VIM)

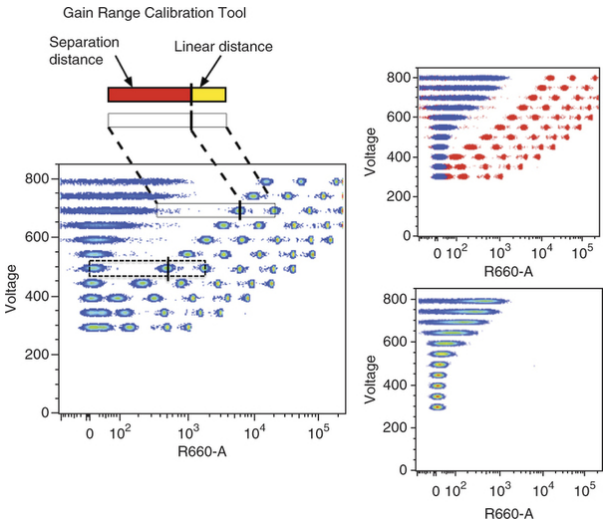
Standards/Calibration material

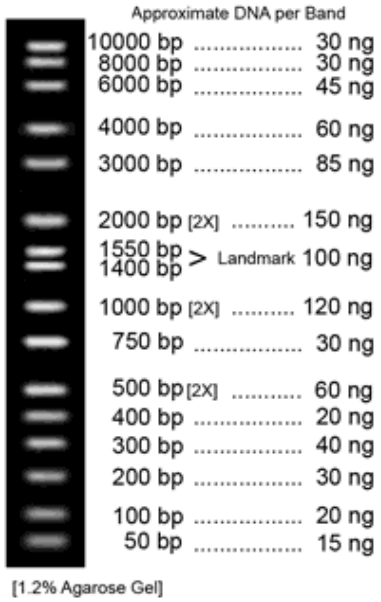
- Compare end product
- Validate process
- Infer comparisons
- Find new uses
- Infer new information
  - Traffic
  - Honesty
  - Route

DULLES AIRPORT TAXI INC.
PART OF WASHINGTON FLYER
CAB #167
Date 04/18/2015
FROM: 19:46 TO: 20:07
TRIP # 4621
DIST 19.49 mi
FARE.....$ 45.62
TOTAL.....$ 45.62
THANK YOU FOR USING US
703-661-8230

- Need a common platform
- Need information/data base
- Need someone to monitor
- Need someone to provide

Standards/Calibration material





- Compare end product
- Validate process
- Infer across labs or groups
- Find new uses
- Infer new information
  - Migration rates
  - RNA run rate vs DNA
  - Instrument quality

- Need a common platform
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STEM CELLS TRANSLATIONAL MEDICINE®

PERSPECTIVES

Enabling Consistency in Pluripotent Stem Cell-Derived Products for Research and Development and Clinical Applications Through Material Standards

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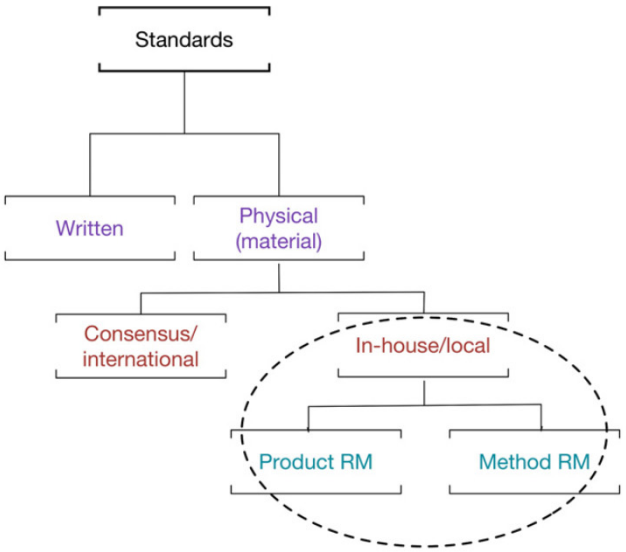
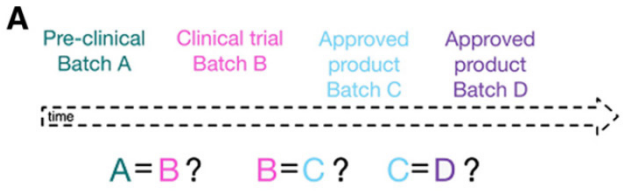
Table 1. Organizations concerned with the generation and/or oversight of reference materials. List of major organisations that play a role in the production, guidance and/or directives concerning reference materials for small molecule drugs and biologics.

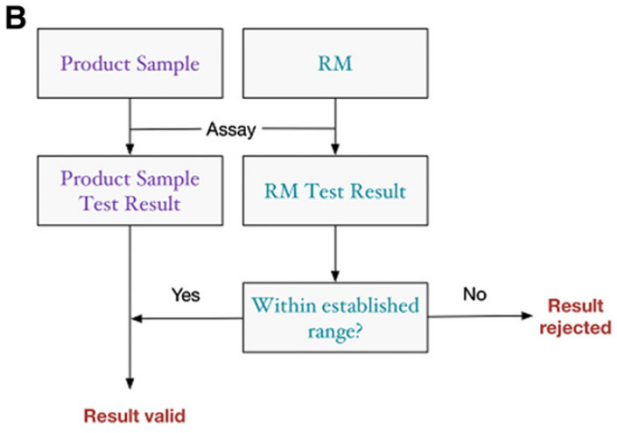
Organization	Region	Description	Hyperlink
National Institute for Biological Standards and Control (NIBSC)	UK	The leading World Health Organisation (WHO) International Laboratory for Standards, is responsible for >90% of global WHO Standards.	(http://www.nibsc.org)
U.S Pharmacopeia Convention (USP)	US	The official organization that sets standards and generates reference materials implemented by the FDA as law in the United States, used globally in	(http://www.usp.org/about-usp)
World Health Organization (WHO)	International	Publishes the International Pharmacopoeia (Ph. Int.) which aims to harmonize global pharmaceutical standards and administers the establishment of	(http://www.who.int/medicines/publications/pharmacopoeia/overview/en/).
European Directorate for the Quality of Medicines & Healthcare (EDQM)	Europe	Responsible for the European Pharmacopoeia (Ph. Eur.) commission, the evaluation of manufacturer's quality dossiers for certification, and market	(http://www.edqm.eu/en/edqm-homepage-628.html)
Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ)	Japan	Produces and distributes Japanese Pharmacopoeia Reference Standards as prescribed in the Japanese Pharmacopoeia (published by Pharmaceuticals and	http://www.pmrj.jp/hyojun/html/frm031.php?lang=e

To Develop a standard/calibration material



- Test the standard to ensure consistency
- Ensure people accept it

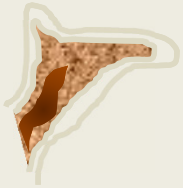
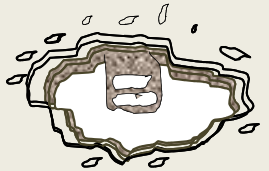




- Need a common platform
- Need information/data base
- Need someone to monitor
- Need someone to provide

Our MSC story

But could be functionally the same- Calibration material would tell us

	BM-MSC	UCB-MSCs	AT-MSC	DP-MSCs	PMSCs
					
Isolation	Gradient separation	Enzyme/mechanical dissociation/centrifugation	Enzyme/mechanical dissociation	Fresh tissue dissection/enzyme	Membrane separation (optional), tissue dissection, enzyme, centrifugation
Markers	CD105, CD73 and CD90, CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II	BM-MSCs markers + Oct4, Nanog, Sox-2 (low levels)	BM-MSCs markers + higher levels of CD146 ; STRO-1 negative	BM-MSC markers + STRO-1 ,CD146	BM-MSC markers + SSEA-1, SSEA-4, Oct4, Nanog; Higher levels of CD49d, CD10, and CD56 than BM-MSCs
Limitations	Rare cell type	Low yield	Heterogeneous populations	Low yield; fresh processing	?

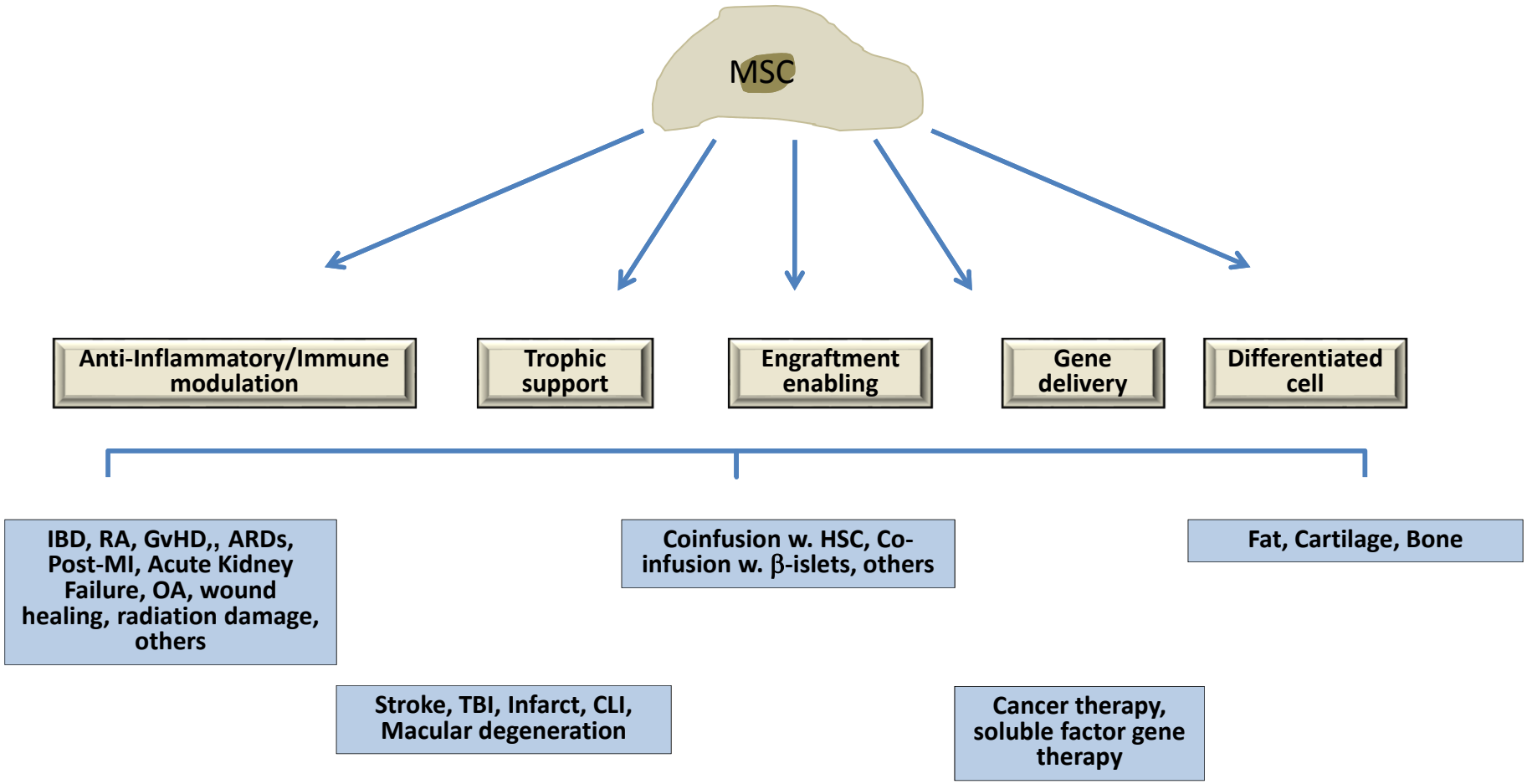
BM-MSCs – Bone Marrow MSCs, UCB-MSCs – Umbillical Cord Blood MSCs (Wharton's Jelly), AT-MSCs – Adipose Tissue MSCs, DP-MSCs –Dental Pulp MSCs, PMSCs-Placental MSCs

Are they the same or different and do we care?

Companies	Commercial Products	Description of Product	Indication
AlloSource (USA)	Allostem	Allogeneic bone matrix with adipose derived MSCs	Orthopedics applications
Cytori (USA)	Celultion System	CE marked-Device for autologous adipose SC (POC)	Reconstructive surgery
Osiris (USA)	Prochymal	BM- MSCs allogeneic	Pediatric GvHD (Canada/New Zealand)
Medipost (S.Korea)	CariStem	UCB-MSCs allogeneic	Degenerative arthritis
Pharmicell-FB (S.Korea)	Hearticellgram-AMI	BM-MSCs autologous	AMI
Stempeutics	Stempeucel	Pooled BMSC	CLI

SC – Stem Cells, POC -Proof-of-concept, BM-Bone Marrow, UCB- Umbillcal Cord Blood, AMI – Acute Myocardial infaraction

Figure 1 – Multiple Modes of Action Attributed to MSCs



IBD – Inflammatory Bowel Disease, RA- Rheumatoid Arthritis, GvHD – Graft versus Host Disease, ARDs – Acute Respiratory Distress Syndrome, OA – Osteoarthritis, TBI – Traumatic Brain Injury, CLI – Critical Limb Ischemia, HSC – Hematopoietic Stem Cells

This Definition turns out not to be enough

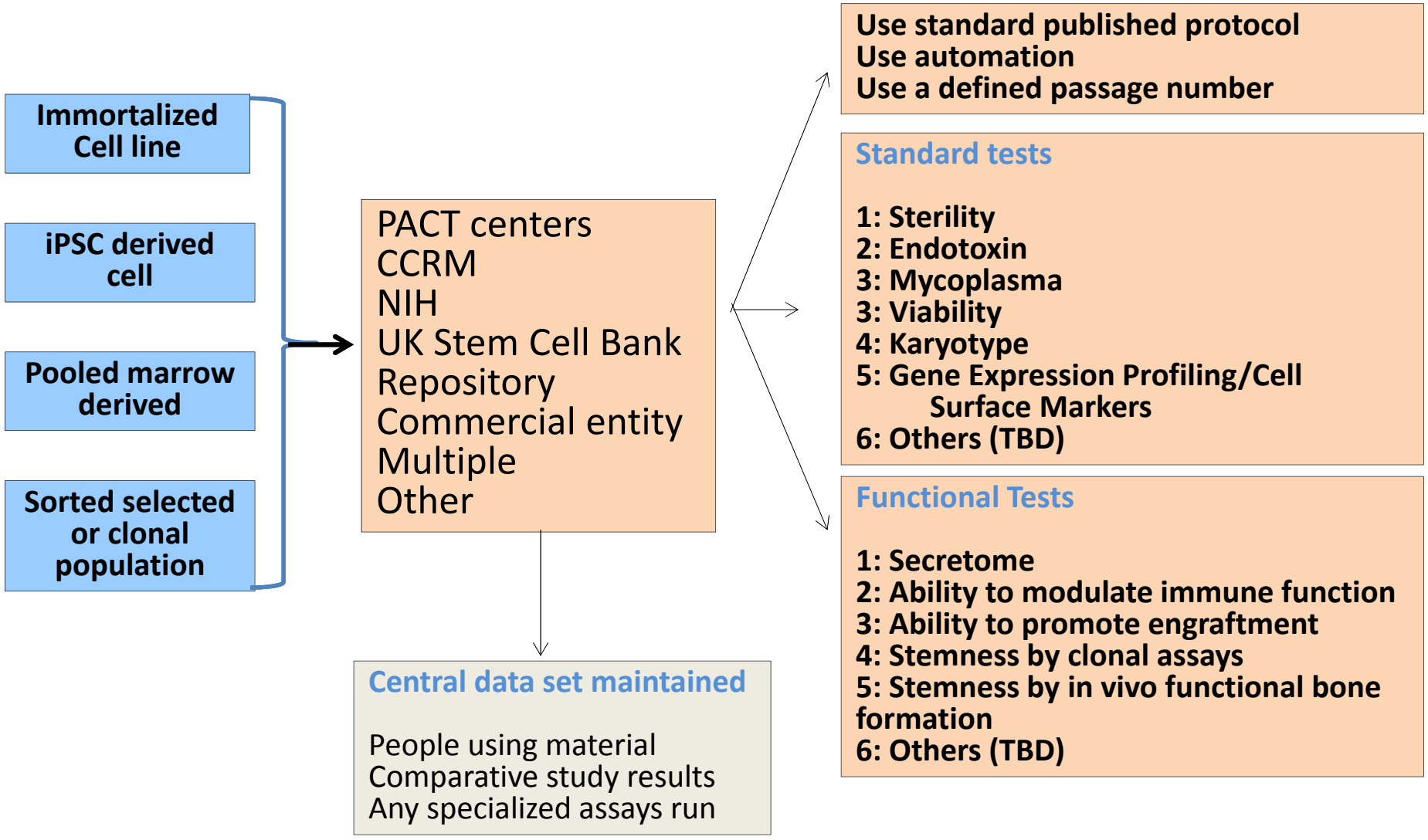
Criteria	Reference
plastic adherence	Dominici et al., 2006
CD105, CD73 and CD90, CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II	Dominici et al., 2006
Anti-STRO-1, anti-CD146, anti-CD271, anti-nestin positive, CD45 negative cells	Gronthos, et al., (1994) Blood; Saccheti et al.,(2007) Cell; Quirici N et all (2002) Exp Hematol; Mendez-Ferrer (2010) Nature
In vitro tri-lineage differentiation	Dominici et al., 2006
In vitro immuno-plasticity assay of MSCs activated by IFN-γ ± TNF-α	Krampera et al., (2013) Cytotherapy
IDO or iNOS activation in primed MSCs	Meisel, R (2011); Meisel, R (2004), Ren, G (2008)
In vitro clonal propagation (CFU-F)	Bianco P.. Methods Enzymol. 2006;419:117.
In vivo ossicle formation	Saccheti et al.,(2007) Cell

- Need a common platform
- Need information/data base
- Need someone to monitor
- Need someone to provide
- Need range

- Darwin Prockop
- ISCT

IFN-g –Interferon Gamma, TNF-a –Tumor Necrosis Factor –alpha, IDO -indoleamine 2,3,-dioxygenase, iNOS – inducible nitrix oxide synthase, CFU-F- colony formation unit fibroblast

Need uniform well characterized unbiased source



iPSCs – induced pluripotent stem cels; PACT – Production Assistance for Cellular Therapies; CCRM – Center for Commercialization of Regenerative Medicine, NIH – National Institutes of health, TBD – To be determined

Table 3- Advantages and disadvantages of different MSC reference lines

	Clonal Population (, for e.g., from placental tissue or MAPCs)	**Mixed population (pooled donors, for e.g, BM)**	**Immortalized Cell Line (for e.g., MSCs w. hTERT)**	**iPSC derived Mesodermal cells**
Pros	• Homogenous • Advantages of clonality	• Heterogeneous • No license issues • Maybe more predictive • Increase time between replacements	• Homgeneous • Renewable resource • Cheap • Easy to maintain • Reporters and engineering possible • Controlled immortalization possible	• Unlimited supply • Multiple types of cells in same background • Easy to engineer to make subclones • Reporters possible • Can piggyback on investments being made
Cons	• Limited choices that allow for sufficient expansion • Replacement of clone an issue • Disadvantages of clonality • Stability and senescence issues	• Cannot be engineered to make reporters and subclones • Relatively expensive • Will require renewal • Manufacturing difficulty • In vivo use as a control may be difficult	• Immortalization process may alter properties • May have patent/license issues on constructs • Multiple lineages from same population not possible	• More expensive • May have patent or license issues • Differentiation may not provide a pure population

Reference Material while making cGMP iPSC lines

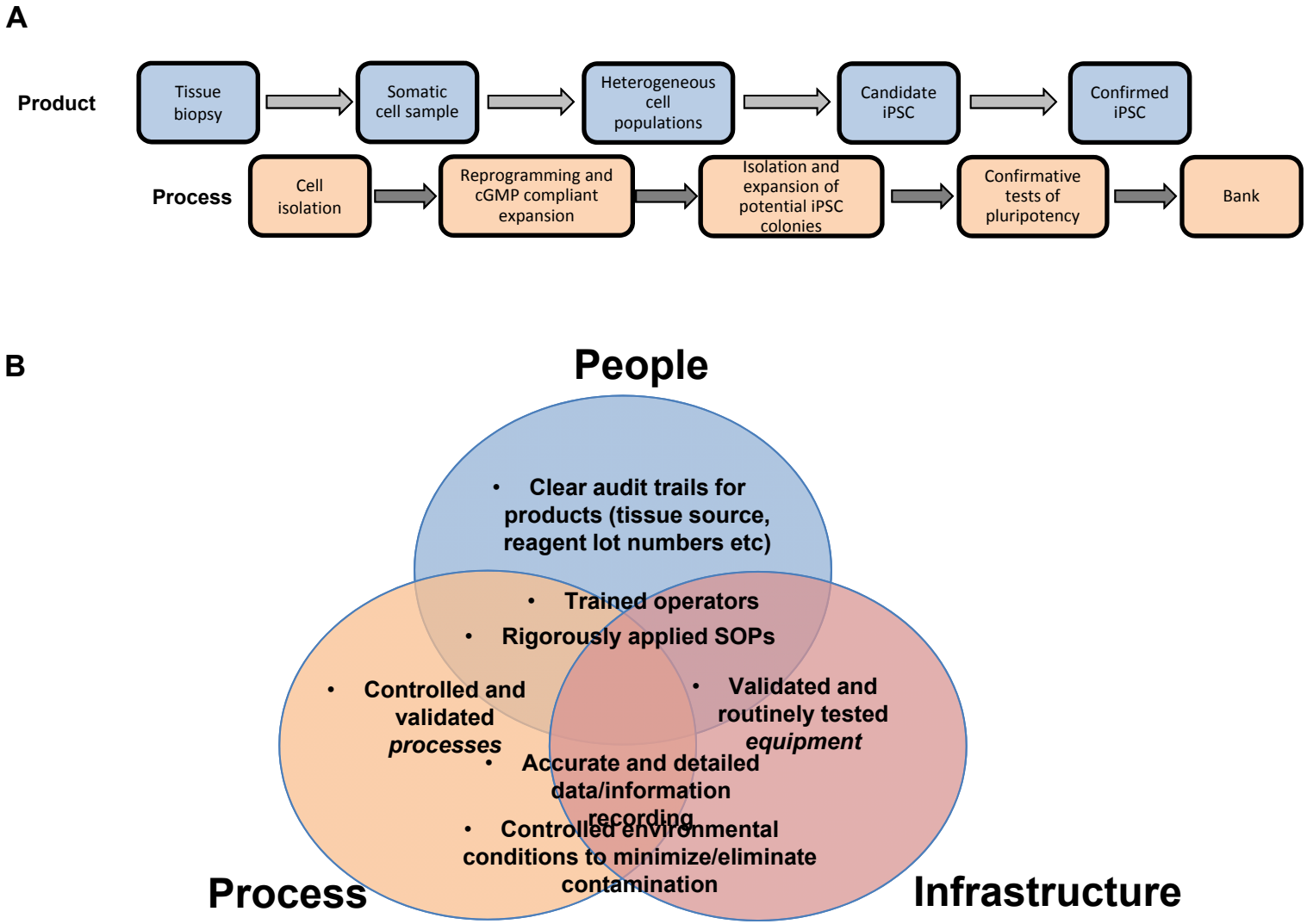


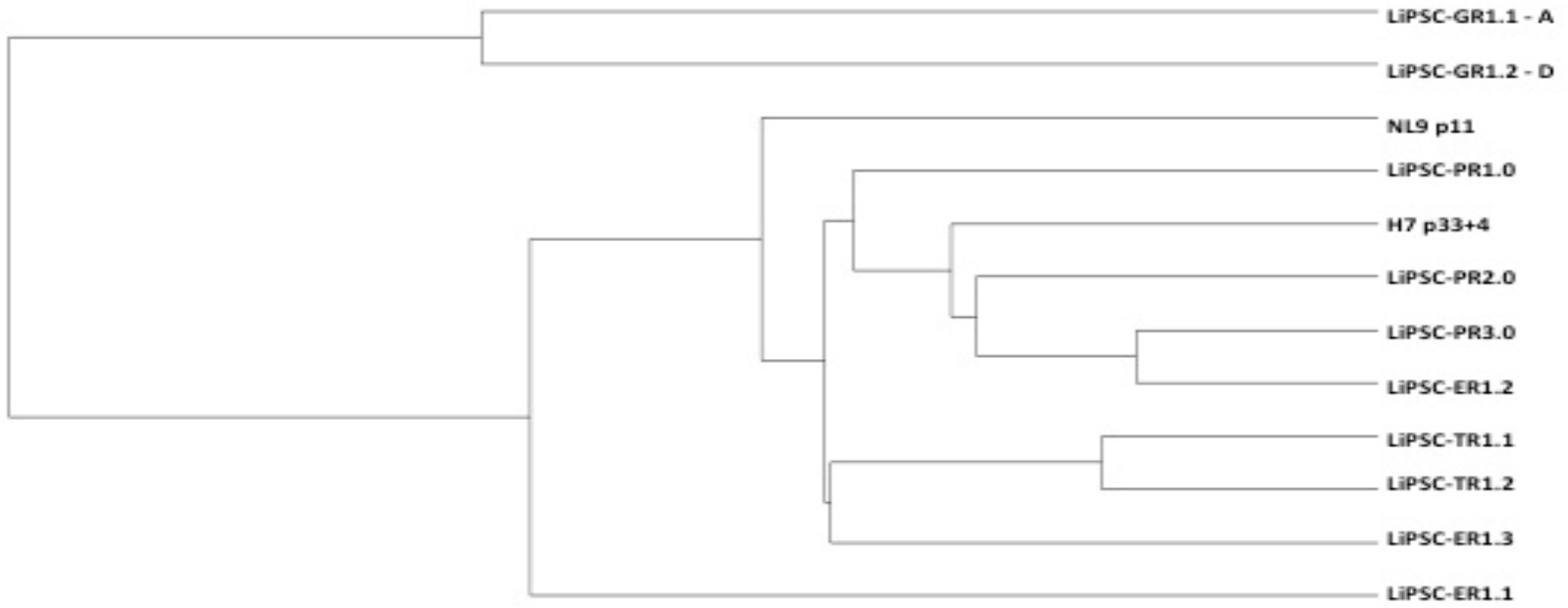
Figure 1. High level process map (A) outlining the transition of of cell samples from *raw* input material to *final* product. In the case of the iPSC product, it is then itself an input material for differentiation processes. The process is then presented to highlight the unit operations required to deliver the downstream product. The relationship between operators, process and infrastructure (B) is critical in the development of robust, standardized manufacturing strategies that reproducibility deliver material of consistent quality.

Table 2. Potential approaches to generating reference materials for PSC-derived products.

In-house reference material (RM) for hPSC-derived products will enable the analysis and qualification of consistency and promote reproducibility. Product RM are used to ensure that a product batch is representative of an intended product and to identify process drift. Method RM validate data derived from specific assays, define assay acceptance criteria and are a tool to detect method drift.

RM category	RM description	Type	Explanation
Product	Primary/ secondary	Cellular	Generated as per product. Primary and secondary RM. Secondary RM is used as the working material which, when depleted is replace with product from a new batch and qualified against the primary RM.
Product	Pooled	Cellular	Generated as per product. RM are produced from a pooled bank of cells, a potential benefit is that variability is averaged across the population.
Product/ Method	Biological equivalent	Cellular	For a limited number of cell types that can be harvested from donors non-invasively e.g. from blood, biological equivalent cell populations can be used as a RM e.g. expression of CD4 levels on peripheral blood T cells and on PSC-derived T cells.
Method	Cell lines	Cellular	Cells lines may have application in a number of characterization assays.
Method	Non-cellular	Non-cellular	Samples such as fixed cells for cell surface marker staining, DNA samples for sequencing or genotyping and RNA for expression profiling.
Not a physical RM	'Virtual'	Non-cellular	Use of transcriptome, proteome, phosphoproteome, or epigenetic mapping to generate a complex data set that when computational algorithms are applied identifies a product 'signature' e.g. concept from PluriTest, PSC scorecard (Mueller et al., 2011, Bock et al., 2011).

Using a well characterized line and microarray analysis as a comparator

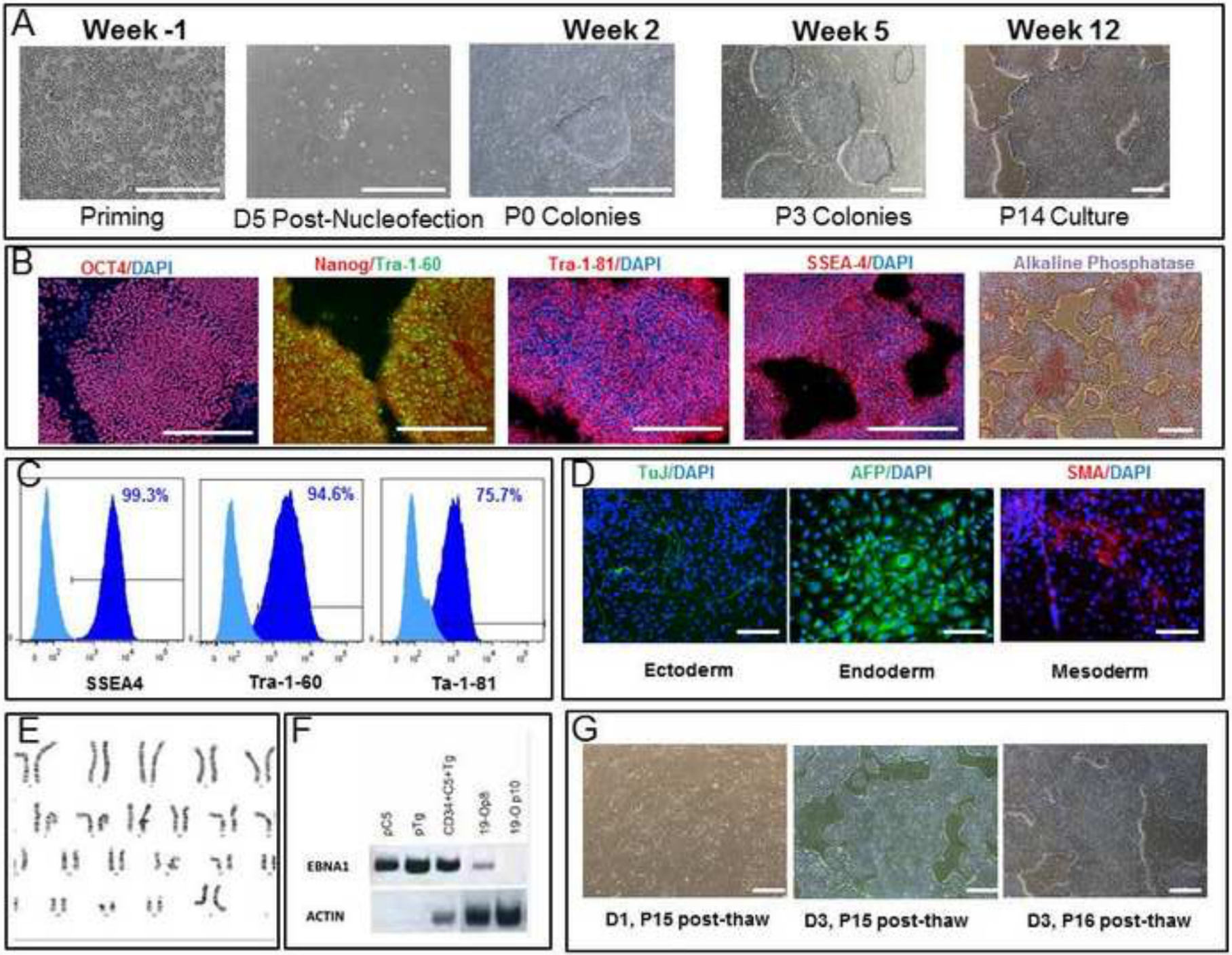


NL9- widely available
Well characterized
Microarray database available
Compared samples to develop a range
Identified invariant markers

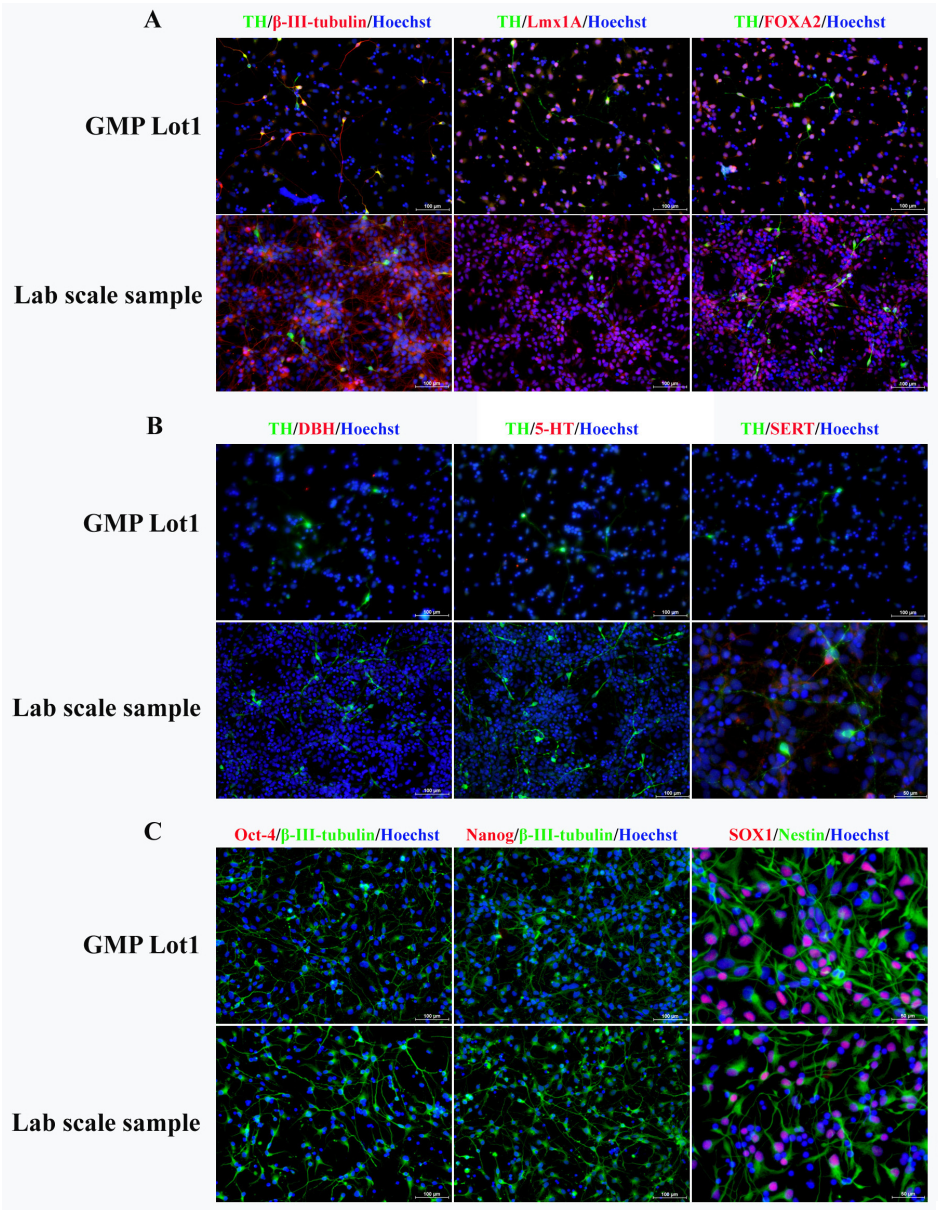
The same cell line can be used for other assays and can therefore provide a link between different tests

Using NIHCRM 9 and microarray analysis as a comparator

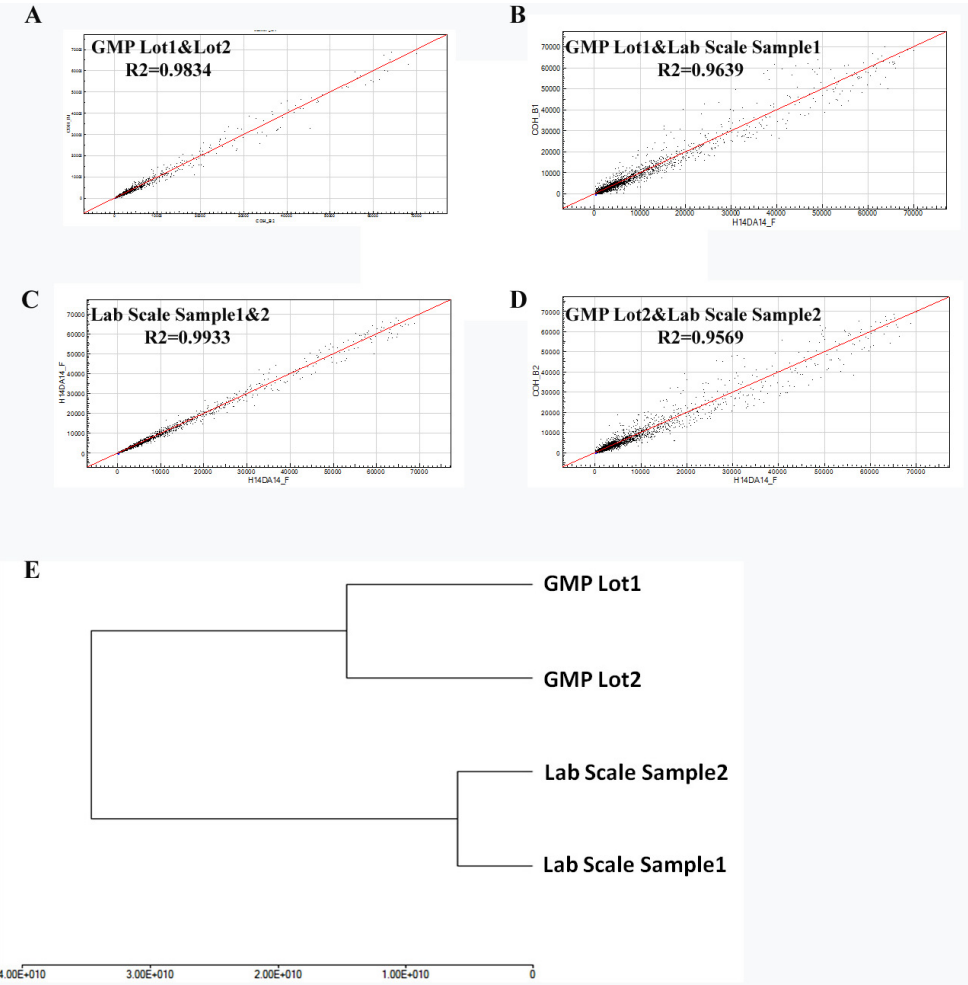
R^2	LiPSC-PR1.0	LiPSC-PR2.0	LiPSC-PR3.0	LiPSC-TR1.1	LiPSC-TR1.2	LiPSC-ER1.1	LiPSC-ER1.2	LiPSC-ER1.3	H7 P37	NL9 p11	LiPSC-GR1.1	LiPSC-GR1.2
LiPSC-PR1.0	1.000	0.978	0.982	0.973	0.982	0.949	0.974	0.977	0.979	0.977	0.949	0.956
LiPSC-PR2.0	0.978	1.000	0.985	0.978	0.985	0.974	0.983	0.980	0.983	0.977	0.949	0.948
LiPSC-PR3.0	0.982	0.985	1.000	0.972	0.984	0.974	0.990	0.977	0.983	0.982	0.953	0.954
LiPSC-TR1.1	0.973	0.978	0.972	1.000	0.990	0.961	0.970	0.979	0.974	0.970	0.929	0.939
LiPSC-TR1.2	0.982	0.985	0.984	0.990	1.000	0.966	0.982	0.980	0.980	0.980	0.942	0.948
LiPSC-ER1.1	0.949	0.974	0.974	0.961	0.966	1.000	0.982	0.964	0.975	0.956	0.931	0.928
LiPSC-ER1.2	0.974	0.983	0.990	0.970	0.982	0.982	1.000	0.976	0.983	0.979	0.952	0.949
LiPSC-ER1.3	0.977	0.980	0.977	0.979	0.980	0.964	0.976	1.000	0.980	0.965	0.941	0.950
H7 P37	0.979	0.983	0.983	0.974	0.980	0.975	0.983	0.980	1.000	0.973	0.951	0.954
NL9 P11	0.977	0.977	0.982	0.970	0.980	0.956	0.979	0.965	0.973	1.000	0.952	0.950
LiPSC-GR1.1	0.949	0.949	0.953	0.929	0.942	0.931	0.952	0.941	0.951	0.952	1.000	0.966
LiPSC-GR1.2	0.956	0.948	0.954	0.939	0.948	0.928	0.949	0.950	0.954	0.950	0.966	1.000


A
Week -1
Week 2
Week 5
Week 12
Priming
D5 Post-Nucleofection
P0 Colonies
P3 Colonies
P14 Culture
B
OCT4/DAPI
Nanog/Tra-1-60
Tra-1-81/DAPI
SSEA-4/DAPI
Alkaline Phosphatase
C
99.3%
94.6%
75.7%
SSEA4
Tra-1-60
Ta-1-81
D
TuJ/DAPI
AFP/DAPI
SMA/DAPI
Ectoderm
Endoderm
Mesoderm
E
F
pC5
pTg
CD34+C5+Tg
19-Op8
19-O p10
EBNA1
ACTIN
G
D1, P15 post-thaw
D3, P15 post-thaw
D3, P16 post-thaw

The process has been successfully transferred to a cGMP facility and the cells produced by the cGMP facility are similar to the cells produced in our lab (Two dry runs: Lot 1: 165 vials, 2.5 million/vial; Lot 2: 90 vials, 4 million/vial)



Vial	1 (COH)	2 (COH)	3 (COH)	1 (Buck)	2 (Buck)	3 (Buck)
Viability	79%	84%	76%	76.5%	75.3%	73%



Validating the tests and the Process- We use NIHCRM lines

Table 1. Assays used to characterize hiPSCs manufactured under cGMP condition

Release Assays			
Pluripotency Markers	Identity & Purity	SSEA-4 >70%, Tra-1-60 >70%, Tra-1-81 >70%, Oct3/4 >70%; Purity: CD34 <5%	Release assay
Karyotype Analysis	Safety	46, XX or 46, XY	Release assay
Mycoplasma Testing	Safety	Negative	Release assay
Sterility Testing	Safety	Negative	Release assay
Endotoxin Testing	Safety	Standard QC release (<0.5 EU/ml)	Release assay
Vector Clearance	Safety	No trace of episomal plasmid DNA detected	Release assay
STR Genotyping	Purity & Identity	STR Profile of starting population and iPSC line are identical	Release assay
Cell Count & Viability	Viability	% viability >50; minimum cell number/vial	Release Assay
Viral Panel Testing	Safety	Standard MCB Release Panel	Release Assay
Characterization Assays			
EB Formation	Identity & Potency	Detection of at least one marker per germ layer	FIO*
Gene Array Analysis	Identity	Clustering with established hPSCs	FIO*
Colony morphology	Identity & Purity	Characteristic morphology of culture/colonies; lack of spontaneously differentiated cells	FIO*
Post-thaw Plating	Thawing efficiency and Viability	20+ colonies / vial (after 7 days or 50% confluency)	FIO*

* For Information Only (FIO)

References or controls make a difference and the NIH's efforts

Regen Med 7(6)89-92 Wagner

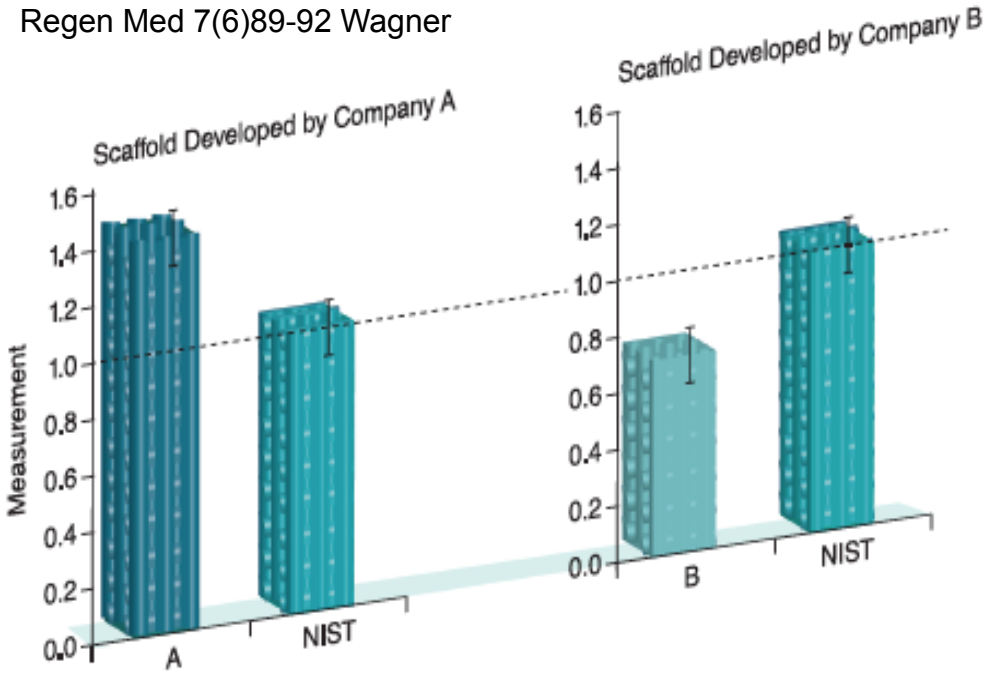


Figure 2. Reference material scaffolds. Reference material scaffolds are being developed that can serve as a calibration point for comparing scaffold measurements between different laboratories. The first-generation reference scaffolds have been deployed and focus on scaffold structure and porosity. A second-generation reference scaffold is under developement that will focus on measuring cell response (adhesion and proliferation) to 3D scaffolds. NIST: National Institute of Standards and Technology.

Rulers work

Allow for comparison without physically having all the samples in one place

Standards agencies can help

Reference iPSC lines may be a way to get rulers for the stem cell field

Rulers don't mandate their use and they are not gold standards to aspire to

We have obtained reference/calibration lines from Dr. Yamanaka, Dr. Thomson, NIHCRM, (and soon Welcome Trust) and deposited at Rutgers

Conclusions

- Need to compare
- Need some kind of widely used material to compare- Cells, data, other material
- This material allows you to validate the instruments being used, the process being used and the end product
- A material data is only as good as the data set available to validate it and a database of SOP's protocols, methods along with results is a complementary requirements