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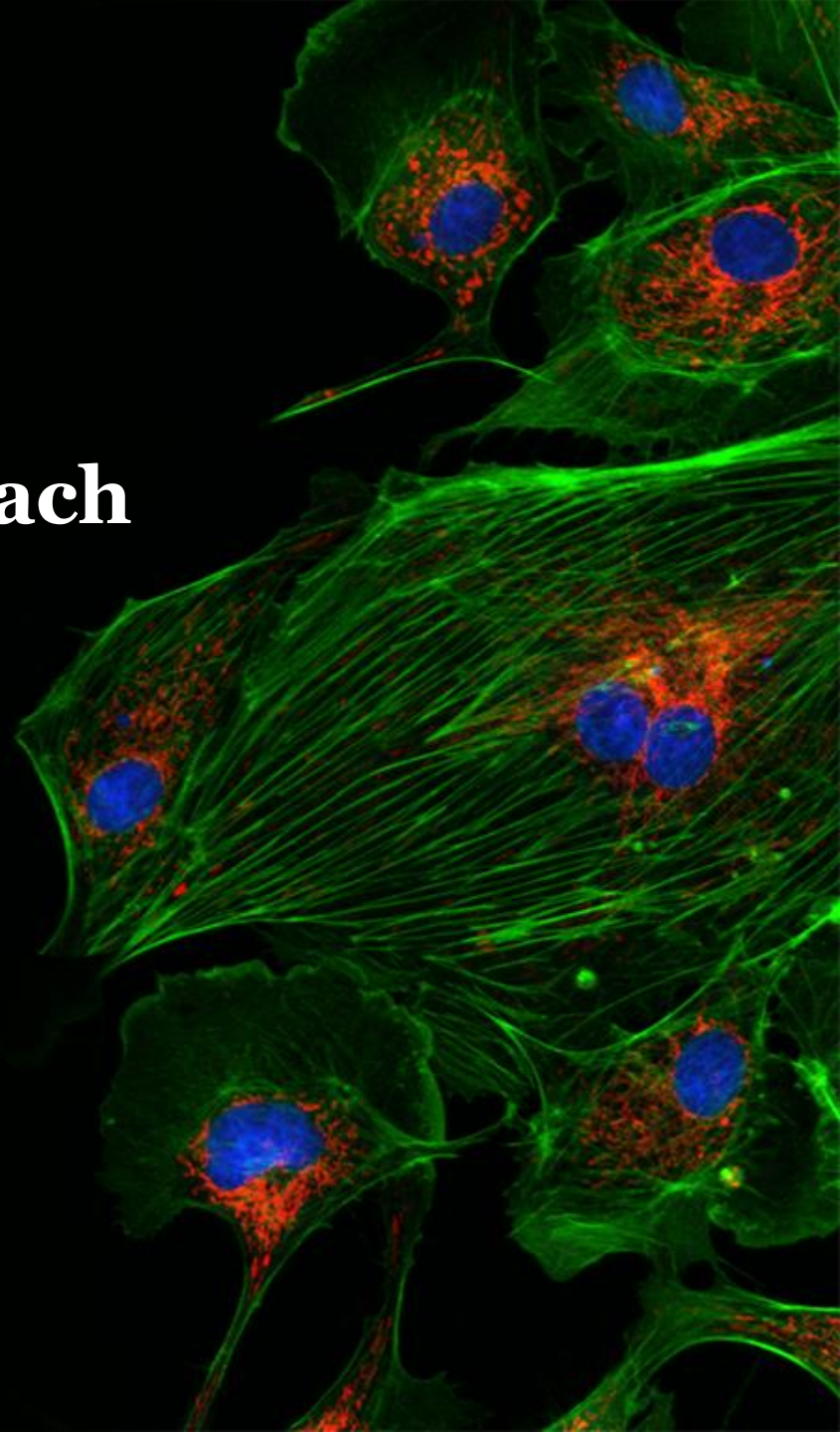
Cell and Gene Therapy

Multiparametric Approach for Defining Product Quality



Dr Damian Marshall

Bioprocessing Summit

August 2016

The Cell and Gene Therapy Catapult

£70m Development Facility

- 1,200m² Custom designed cell and gene therapy development facility
- Prime location in the heart of the London clinical research cluster
- 120 permanent staff

£55m large scale manufacture center

- 7,200m² manufacturing centre designed specifically for cell and gene therapies
- Located in the Stevenage biocatalyst
- Opening 2017

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The Cell and Gene Therapy Catapult

Therapy industrialisation

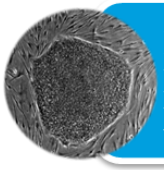
- Process development
- Analytical development
- GMP process proving
- Supply chain
- Late clinical phase manufacturing

Business

- Business development
- Business models
- Health economics

Clinical trial and regulatory

- Regulatory
- Clinical trial sponsor
- Clinical operations
- Pre-clinical safety

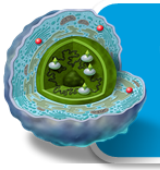


ES and iPS cells
HSC's
MSC's

10

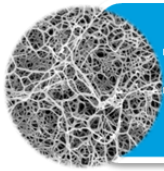
T cells
T Regs
Dendritic cells

7



Somatic cells
- Islet cells
- Fibroblasts

3



Tissue / Scaffold
Tech development

8

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Product Characterisation

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Product characterisation

Characterization of a biological product (includes determination of physiochemical properties, biological activity, immunochemical properties, purity and impurities) by appropriate techniques is necessary to allow relevant specifications to be established

ICH 6QB - Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

Why characterise cell and gene therapy products ?

- Product characterisation demonstrates control of the manufacturing process
- Establishing specifications during manufacture helps ensure the quality and lot-to-lot consistency of the final product
- Product parameters can be used to anticipate sub-optimal manufacture runs
- Characterisation can help assess product integrity and stability

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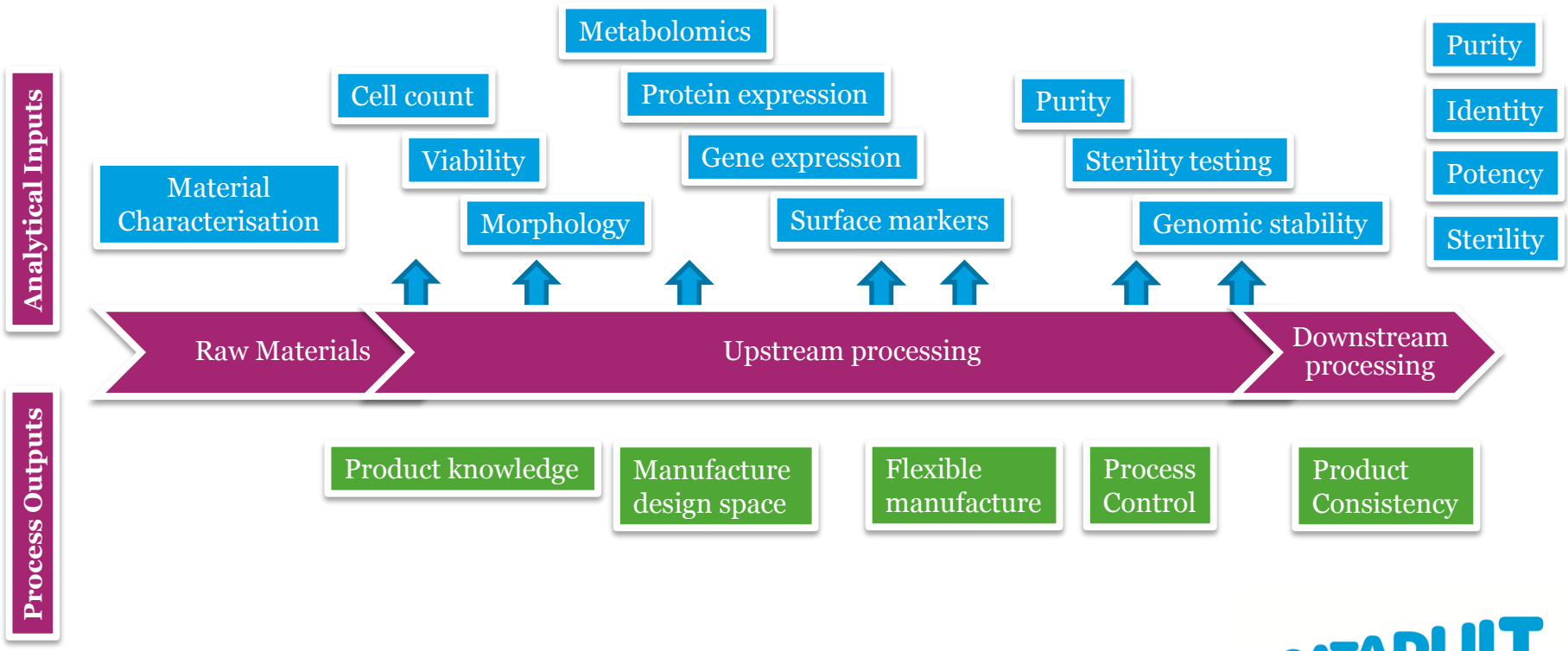
Product characterisation challenges

- What are the relevant parameters to measure?
- Can cell quality be measured and monitored? What is cell quality?
- Impact of the manufacturing process on cells – what is affected?



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Product characterisation challenges

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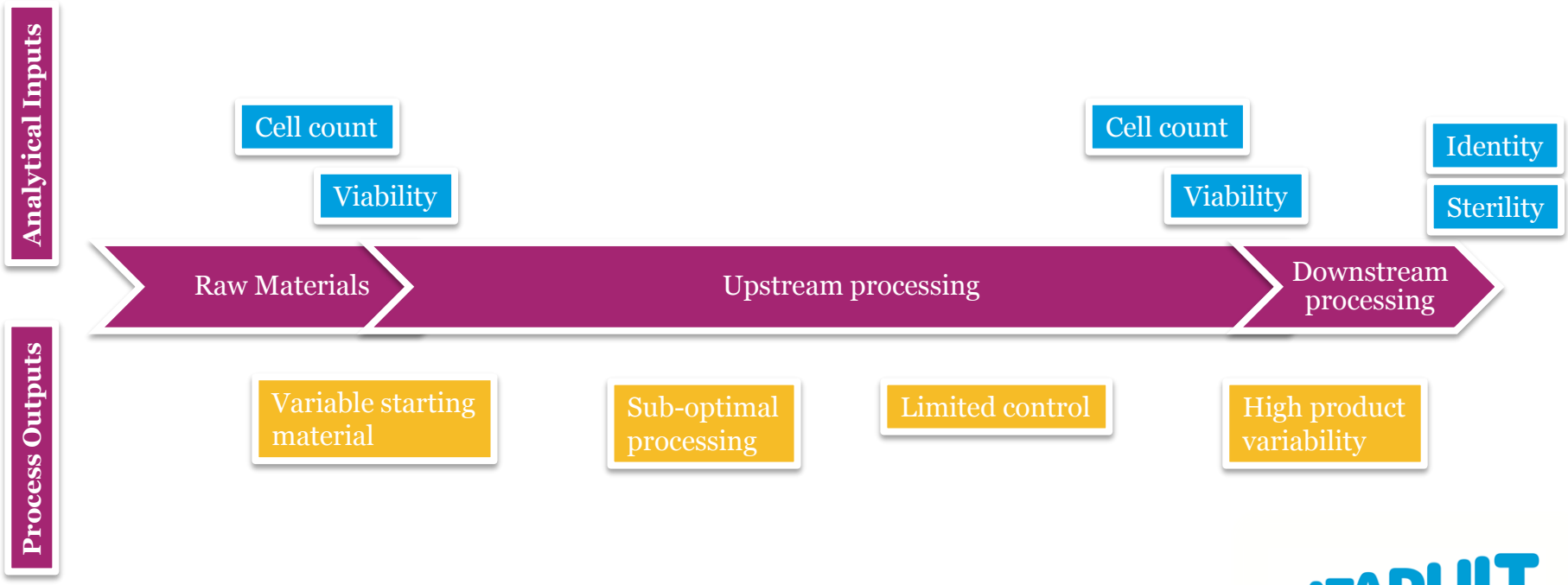

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The CGT Analytical Platform

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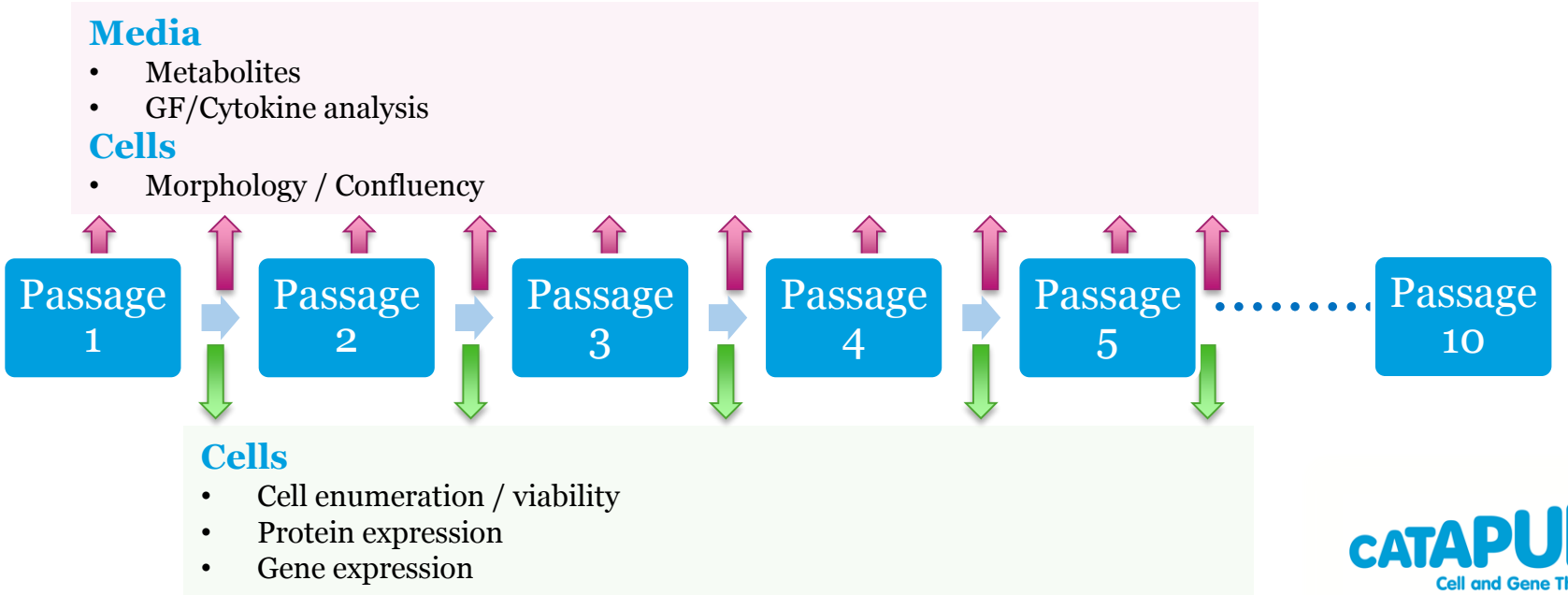
iPS cell manufacture

Development of an automated iPS cell expansion process

- iPS cell expansion maintained in adherent culture for 40 days
- Cells undergo approximately 20-25 population doublings

Challenge – Development of an IPC framework which can monitor cell quality and predict process success in manual and automated systems



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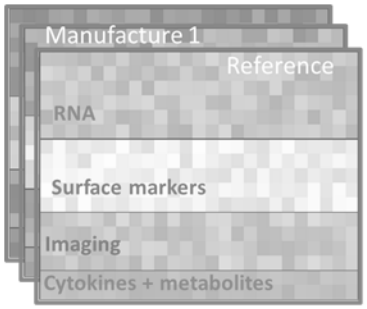
Analytical Platform

1 Analytical Design

- QbD Analysis
- Analytical strategy based on:
  - *Key unit operations*
  - *Sample Availability*
  - *Technology suitability*

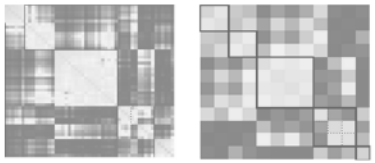
2 Analytical testing

- Screening strategy
- Integrated analysis
- Augment Information
- Stress testing



3 Biological Profiling

- Cluster Analysis
- Data Reduction
- Multivariate Analysis



4 Customised QC

- Custom Marker Selection
- Reference materials
- Automated analysis
- Design space

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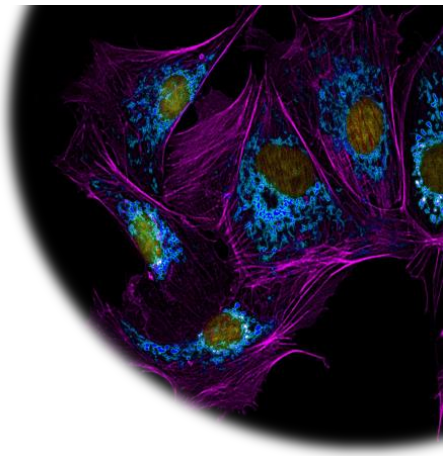
Quality by Design

- The cell and gene therapy catapult uses a quality by design (QbD) approach to establish the appropriate analytical strategy for a product



- QbD is a risk based framework for process design which relates product and process attributes to product quality

- The approach is based on knowledge of product biology and the engineering used during its manufacture

- By establishing the critical quality attributes (biological, physical, biochemical) for a product a design space can be established which defines the expected range of each characteristic during manufacture

QbD is usually guided by the quality of the final product……..

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QbD Toolkit

Analytical Design

CATAPULT Cell and Gene Therapy

Project Code _____

Number of Failure modes 211
Mean RPN 189

Priority grid # reference	Process section	Potential failure mode	Potential failure effects	Severity	Mechanism of failure	Occurance	Current process controls	Detection	RPN	RPC	Potential controls	Method of implementation	Ease of implementation	Process benefit
1	Manual expansion	Confluene at harvest - criteria is too high	Reduced viability, reduced average growth, differentiation	4	Harvest criteria based on time or based on confluence but poorly defined	3	current harvest criteria is when cells cover most of the well (4-5 days)	7	84	MH	Define harvest criteria based on confluence. Automate confluence measurements Initiate operator training to improve confleuncy assessment	Documentation changes Training image analysis software	2	3
2														

Transfer

Step 1

10 minutes

Put in detail

(Unclassified)

Assay or Donor data

Decision

No → Rejected Product

Yes → Final Product

CATAPULT Cell and Gene Therapy

Analytical Platform

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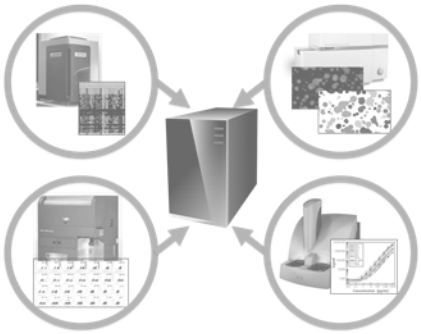
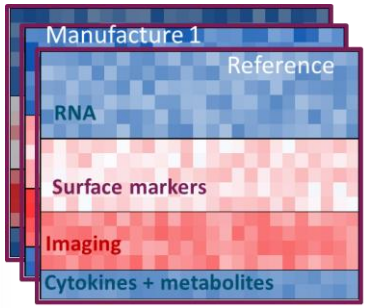


2 Analytical testing

- **Screening strategy**
- **Integrated analysis**
- **Augment Information**
- **Stress testing**



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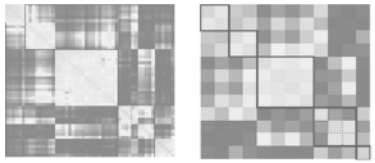

4 Customised QC

- Custom Marker Selection
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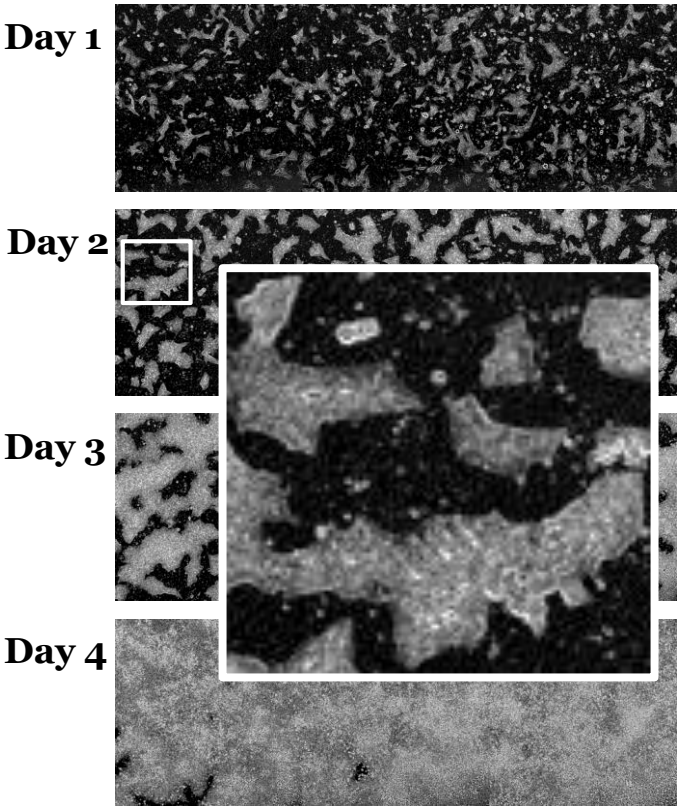
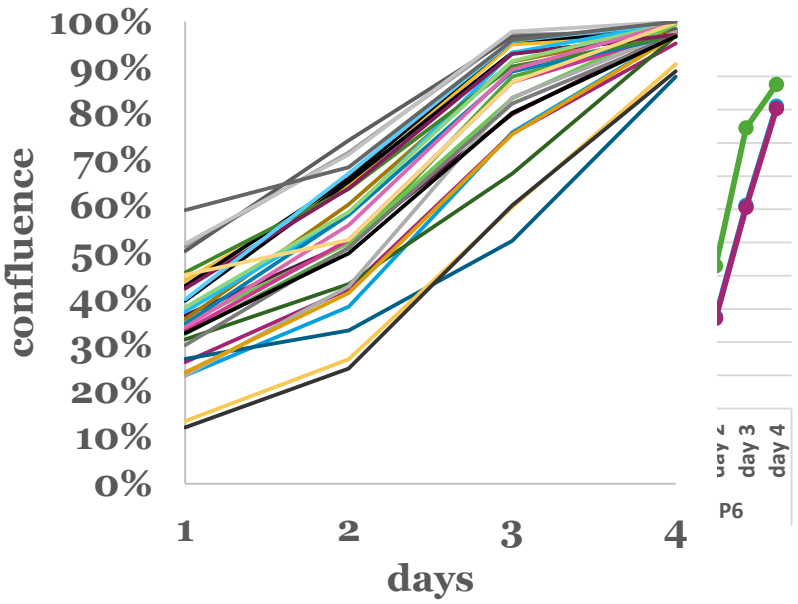
Image analysis

Cell confluence tracking using quantitative imaging



- 3 independent biological replicates
- Cells seeded at the same cell density



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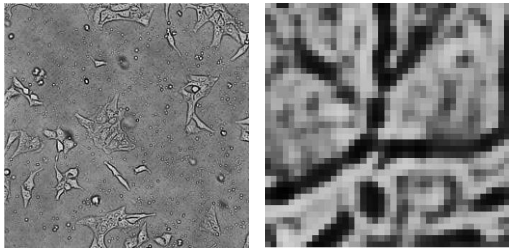
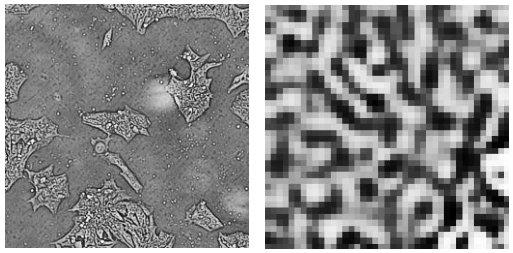
Texture analysis

Texture of colonies

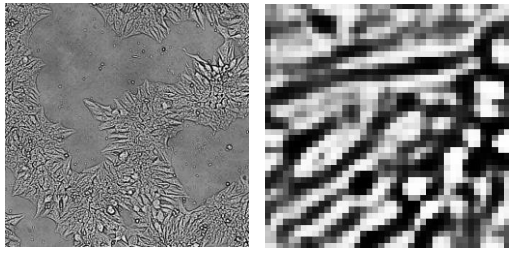
Day 1



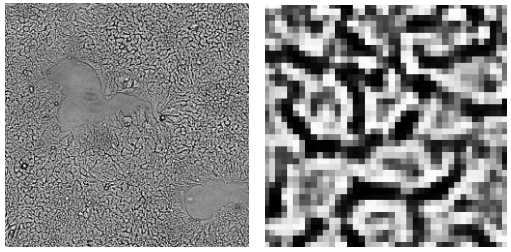
Day 2

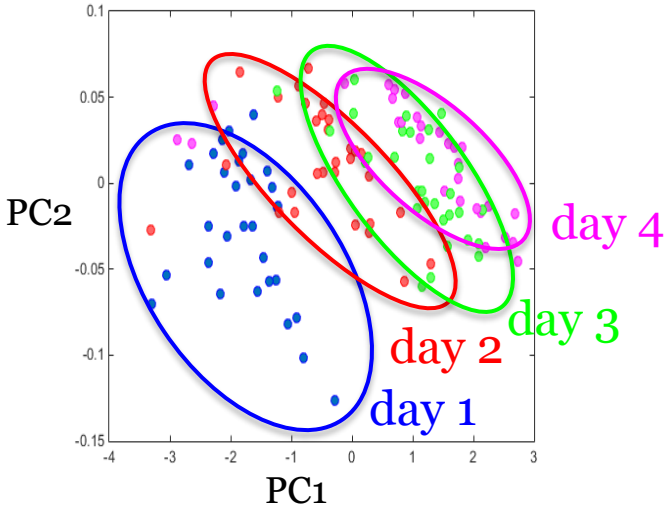


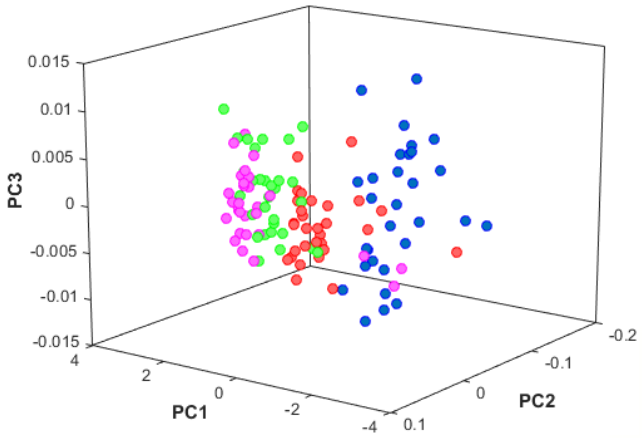
Day 3



Day 4







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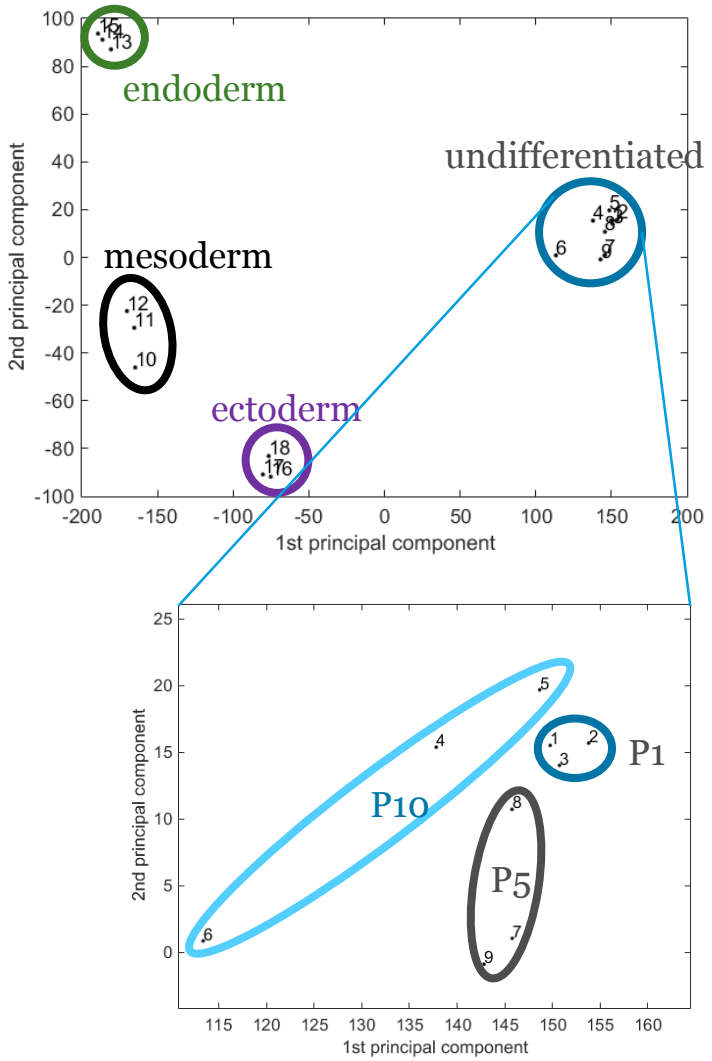
Flow cytometry analysis

	Passage 2			Passage 4			Passage 6			Passage 8			Passage 10		
FUNCTION	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Stress	0	0	0	0.11	0.09	0.12	0.13	0.08	0.05	0	0	0	0.01	0.06	0.04
Stress	0.11	0.03	0.18	0.11	0.22	0.18	0.21	0.13	0.06	0.73	2.2	1.38	0.48	0.33	0.33
Stress	0.04	0.06	0.03	0.17	0.13	0.13	0.37	0.58	0.37	0.01	0.08	0.03	0.02	0.02	0
Stress	0.1	0.06	0.07	0.15	0.36	0.33	0.23	0.28	0.17	2.96	3.64	2.09	10.2	11.4	13.9
Stress	0.17	0.09	0.08	0.19	0.19	0.13	0.35	0.28	0.1	0.93	2.34	1.79	1.06	0.94	1.22
Pluripotency	95.6	96.4	95.5	86.4	86.2	85.4	72.4	75.8	75.9	86.2	89.3	83.4	79.6	76.9	80.1
Pluripotency	99.2	99	98.4	99.8	99.6	99.8	99.5	99.5	99.7	98.9	98.4	98.4	97.9	98.6	98.3
Pluripotency	99.9	99.9	99.4	100	99.9	100	100	99.9	99.9	99.5	99.6	99.6	99.8	99.8	99.7
Pluripotency	5.29	5.25	5.55	3.8	2.87	2.92	1.03	1.29	0.94	5.66	5.87	2.74	2.43	2.38	2.24
Pluripotency	7.6	5.75	4.79	45.3	31.4	33	74.8	80.8	86.8	43.3	40.5	46.7	31	20.7	26.1
Pluripotency	96.9	97.5	97.9	90	88	87.6	96.1	96.5	96.8	98.8	97.9	99	88	86.1	84.1
Pluripotency	70	77.7	72	85.9	86.7	85.6	92	91.8	93.6	67.3	66.6	68.5	69.5	67.4	69.3
Pluripotency	37.8	47.2	42.3	50.8	35.6	39.8	35.6	43.5	47.4	35.7	36.9	27.2	19.9	25.6	22.8
Pluripotency	9.87	14.3	12.2	20.2	19.4	20.8	61	59.7	61.4	57.1	63.1	63.2	48.9	46	42
Pluripotency	66.2	65.2	62.4	60.3	57	61.1	76.3	79.1	78.4	36.1	44.4	33.5	43.6	44.7	44.8
Pluripotency	79.8	83.9	83.5	97.8	97.2	96.7	95	95.4	96.5	92.4	89.3	86.1	81.5	79.6	74.4
Pluripotency	63.3	63.2	55.6	95.8	96.1	97.6	96.7	95.8	96.9	53.4	58	43	10.3	9.74	12.2
Pluripotency	1.08	1.19	0.93	0.97	0.92	0.93	1.07	2.36	1.4	0.88	0.83	0.54	1.17	1.28	2.28
Ecotoderm	20.1	76.6	85.8	93.6	85.7	11.7	75.5	74.4	75.3	84.7	80.1	82.6	92.5	92.6	93.2
Ecotoderm	20.5	98.3	98.8	95.3	91.2	81.2	85.5	81.8	82.9	89.2	83.9	83.1	95.1	94.7	94.8
Ecotoderm	1.69	85	91.2	62.7	35.6	14.5	71.7	64.8	42.3	90.3	83.3	82.9	67.1	61.2	57.1
Ecotoderm	96	87.1	84	12.3	10.3	7.6	7.13	21.9	17	51.8	51.5	47.4	42.1	34.9	21.2
Ecotoderm	99.9	99.2	99.4	75.2	70.2	74.4	99.8	98.1	98.4	97.8	96.8	96.6	99.5	99.9	99.9
Mesoderm	1.26	0.42	1.49	0.47	0.62	0.54	1.9	9.39	1.81	43.2	67.4	65.1	33.9	23.3	37.6
Mesoderm	4.11	1.91	2.56	14.9	12.4	11.6	11.1	13.3	11.3	10.4	9.47	19.7	10.7	5.1	7.49
Mesoderm	0.28	0.37	0.23	3.94	3.27	2.84	2.91	4.9	6.29	0.75	0.95	0.82	0.58	1.87	1.07
Mesoderm	0.13	0.11	0.26	0.22	0.25	0.11	0.17	0.25	0.19	0.18	0.21	0.19	0.16	0.22	0.19
Mesoderm	1.47	1.29	1.16	2.57	1.87	1.5	1.61	1.95	1.31	0.46	0.47	0.49	3.82	2.31	3.78
Mesoderm	1.01	2.53	0.69	0.66	0.55	0.84	0.92	1.14	1.3	4.2	4.63	2.79	2.87	1.72	3.53
Mesoderm	0.83	0.8	0.69	0.93	0.97	0.72	0.53	0.47	0.46	0.3	0.28	0.31	0.64	0.59	0.73
Endoderm	0.22	0.13	0.36	0.24	0.37	0.27	0.65	0.75	0.36	0.53	0.57	0.41	1.94	15.4	7.09
Endoderm	75.3	83.1	78.6	73.1	67.2	70	28	42.4	26.1	1.43	1.13	1.52	1.29	0.55	2.19
Endoderm	3.9	10.7	6.32	2.16	1.43	0.5	0.28	0.47	0.44	5.37	5.94	2.15	6.38	4.19	4.93
Differentiation	2.04	2.89	3.17	7.02	7.36	7.43	6.18	8.99	9.97	4.97	4.86	4.65	7.03	7.9	7.44
Differentiation	2.7	0.65	0.76	1.18	0.76	1.09	0.71	0.72	0.74	3.67	4.5	5.62	7.87	16.6	4.89
Differentiation	15.4	14.5	17.1	2.28	2.27	1.14	18.9	27.9	26.9	92.7	92.3	91.9	90.7	82.8	67.7
Differentiation	0.05	0.06	0.04	0.13	0.14	0.14	0.01	0	0	0	0	0	0	0	0
Differentiation	0.68	0.78	0.43	1.17	1.05	0.81	0.72	0.77	0.94	0.31	0.26	0.44	0.3	0.26	0.24
Differentiation	0.58	0.52	0.54	0.21	0.23	0.19	0.19	0.23	0.07	8.27	7.79	13.9	15.1	9.7	11.7
Differentiation	0.16	0.24	0.31	0.11	0.13	0.08	0.1	0.11	0.11	2.43	5.23	1.98	12.9	8.59	13.8
Differentiation	2.28	3.64	3.92	3.22	3.71	2.85	7.91	8.27	6.88	15.7	24.3	29	12.6	10.8	6.15
Differentiation	7.29	1.71	0.47	0.12	0.17	0.18	1.81	2.15	0.99	89.1	89.3	91.7	56	55.2	58.5

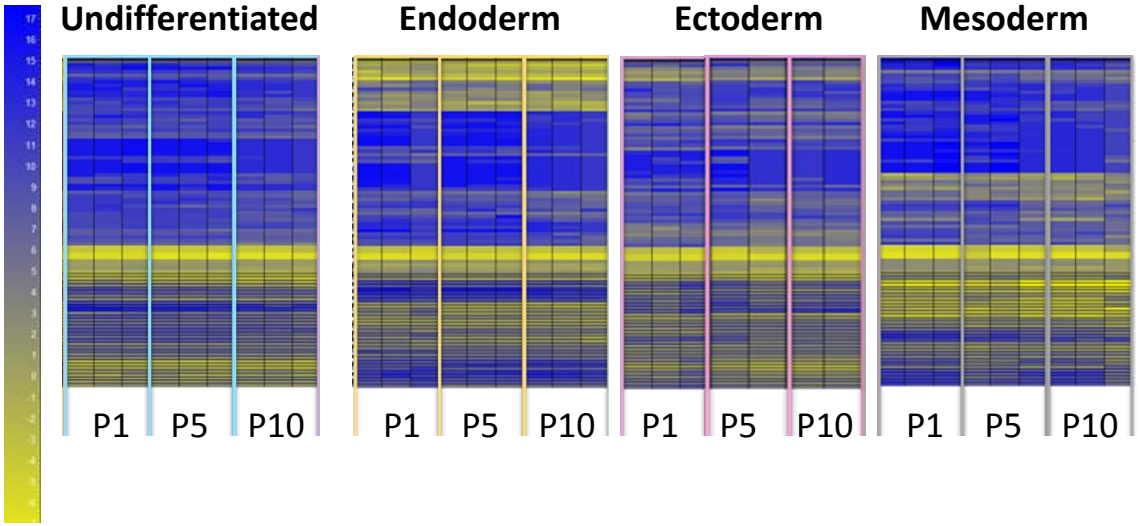


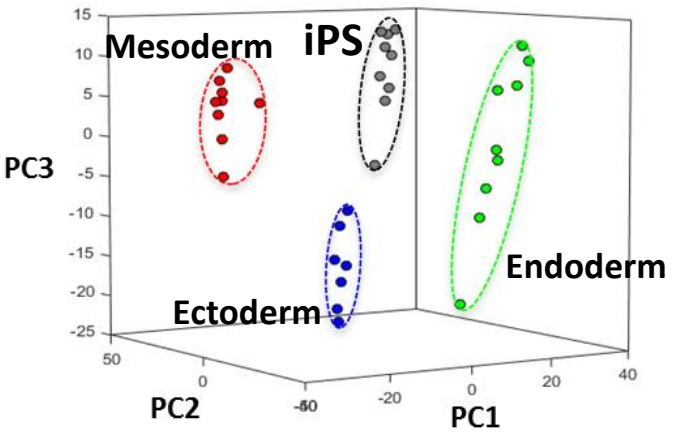
RT-qPCR analysis

2 Analytical testing

- Commercial screening technologies can be hard to interpret
- Reference samples may not be fit for purpose

- Established in house panels for ES and iPS cell manufacture
- Panel based on pluripotency, self renewal, differentiation and stress markers





CATAPULT Cell and Gene Therapy

Extended metabolic screen

2 Analytical testing

- Use of LC/MS to screen for metabolic profile
- Wide range screen
- Automated sample preparation and analysis

Internal Standard	Amino Acids and Derivatives	Vitamins
2-Isopropylmalic acid	2-Aminoadipic acid	4-Aminobenzoic acid
	4-Aminobutyric acid	Ascorbic acid
	4-Hydroxyproline	Ascorbic acid 2-phosphate
	5-Glutamylcysteine	Biotin
Sugars	5-Oxoproline	Choline
Gluconic acid	Alanine	Cyanocobalamin
Glucosamine	Alanyl-glutamine	Ergocalciferol
Hexose（Glucose）	Arginine	Folic acid
Sucrose	Asparagine	Folinic acid
Threonic acid	Aspartic acid	Lipoic acid
	Citrulline	Niacinamide
	Cystathionine	Nicotinic acid
	Cysteine	Pantothenic acid
Nucleic acids	Cystine	Pyridoxal
Adenine	Glutamic acid	Pyridoxine
Adenosine	Glutamine	Riboflavin
Adenosine monophosphate	Glutathione	Tocopherol acetate
Cytidine	Glycine	
Cytidine monophosphate	Glycyl-glutamine	
Deoxycytidine	Histidine	
Guanine	Isoleucine	**Others**
Guanosine	Kynurenine	2-Aminoethanol
Guanosine monophosphate	Leucine	2-Ketoisovaleric acid
Hypoxanthine	Lysine	3-Methyl-2-oxovaleric acid
Inosine	Methionine	4-Hydroxyphenyllactic acid
Thymidine	Methionine sulfoxide	Citric acid
Thymine	N-Acetylaspartic acid	Ethylenediamine
Uracil	N-Acetylcysteine	Fumaric acid
Uric acid	Ornithine	Glyceric acid
Uridine	Oxidized glutathione	Histamine
Xanthine	Phenylalanine	Isocitric acid
Xanthosine	Pipecolic acid	Lactic acid
	Proline	Malic acid
	Serine	O-Phosphoethanolamine
	Threonine	Putrescine
Antibiotics	Tryptophan	Pyruvic acid
Penicillin G	Tyrosine	Succinic acid
	Valine	

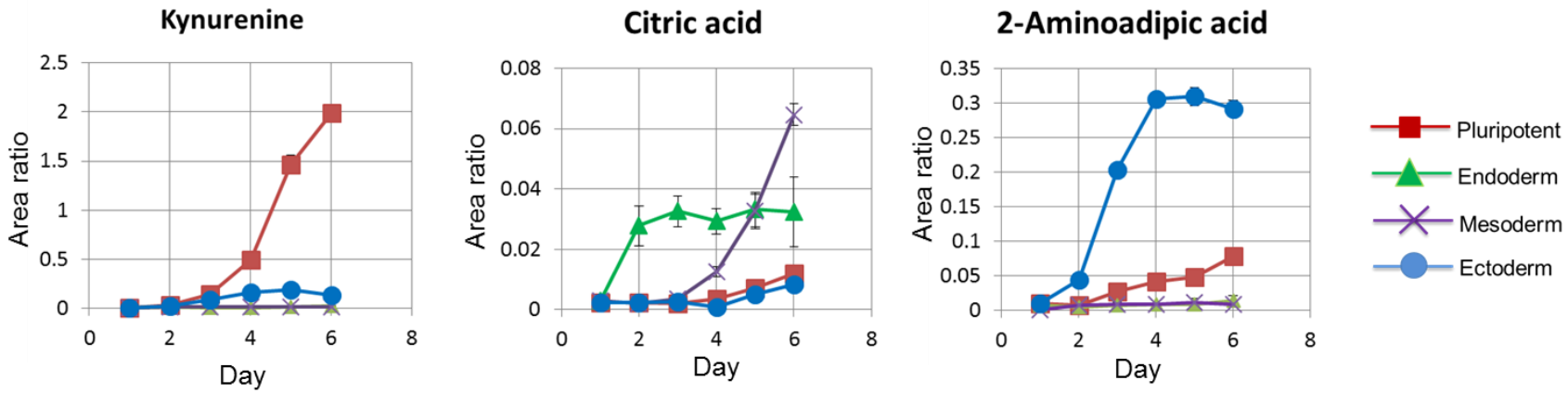
TEL TOKYO ELECTRON

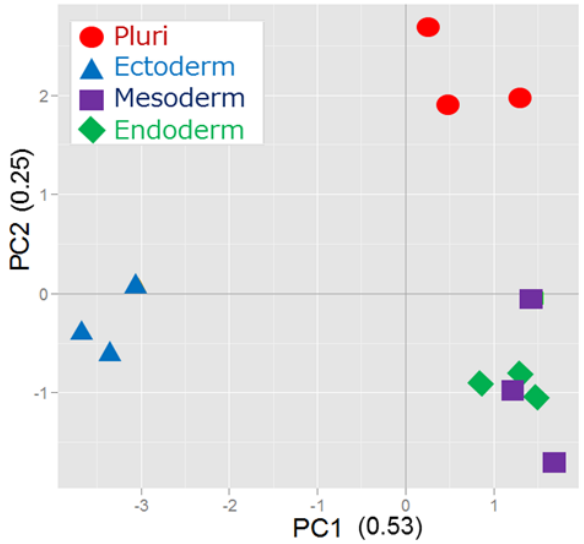
SHIMADZU

CATAPULT Cell and Gene Therapy

Extended metabolic screen

2
Analytical testing





TEL TOKYO ELECTRON

SHIMADZU

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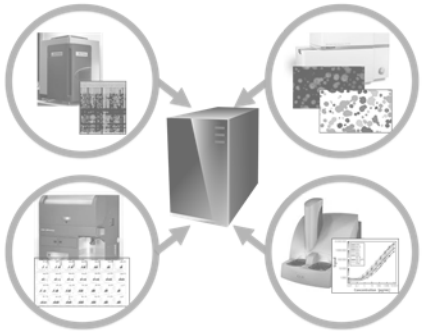
Analytical Platform

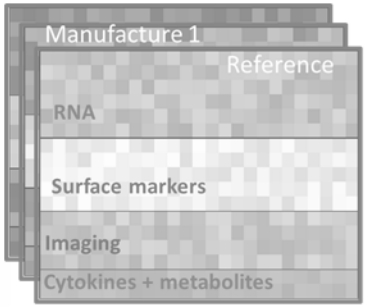
1 Analytical Design

- QbD Analysis
- Analytical strategy based on:
  - *Key unit operations*
  - *Sample Availability*
  - *Technology suitability*



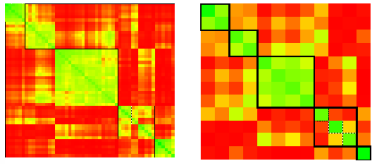
2 Analytical testing

- Screening strategy
- Integrated analysis
- Augment Information
- Stress testing



3 Biological Profiling

- **Cluster Analysis**
- **Data Reduction**
- **Multivariate Analysis**



4 Customised QC

- Custom Marker Selection
- Reference materials
- Automated analysis
- Design space

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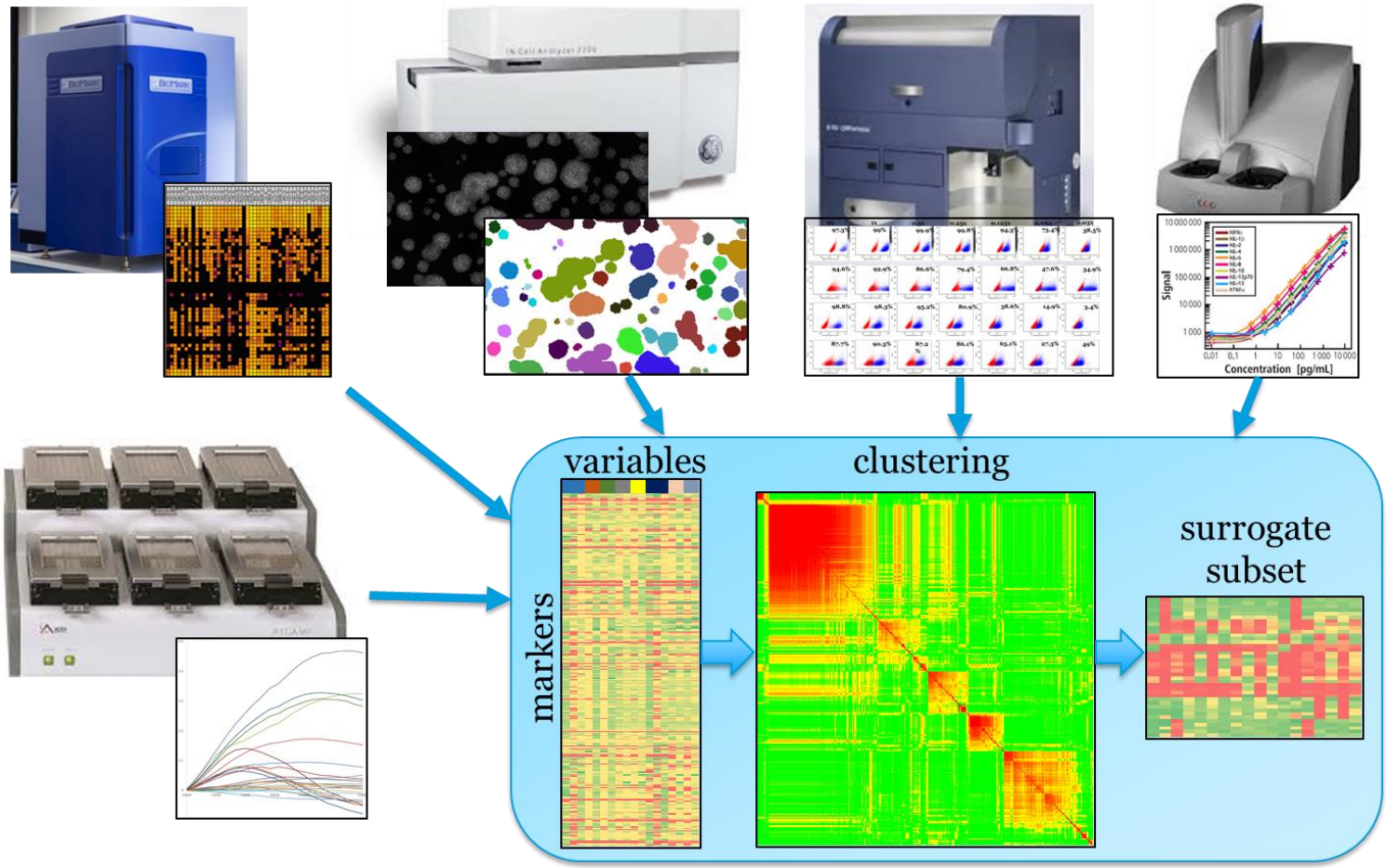
Data integration

3

Biological Profiling



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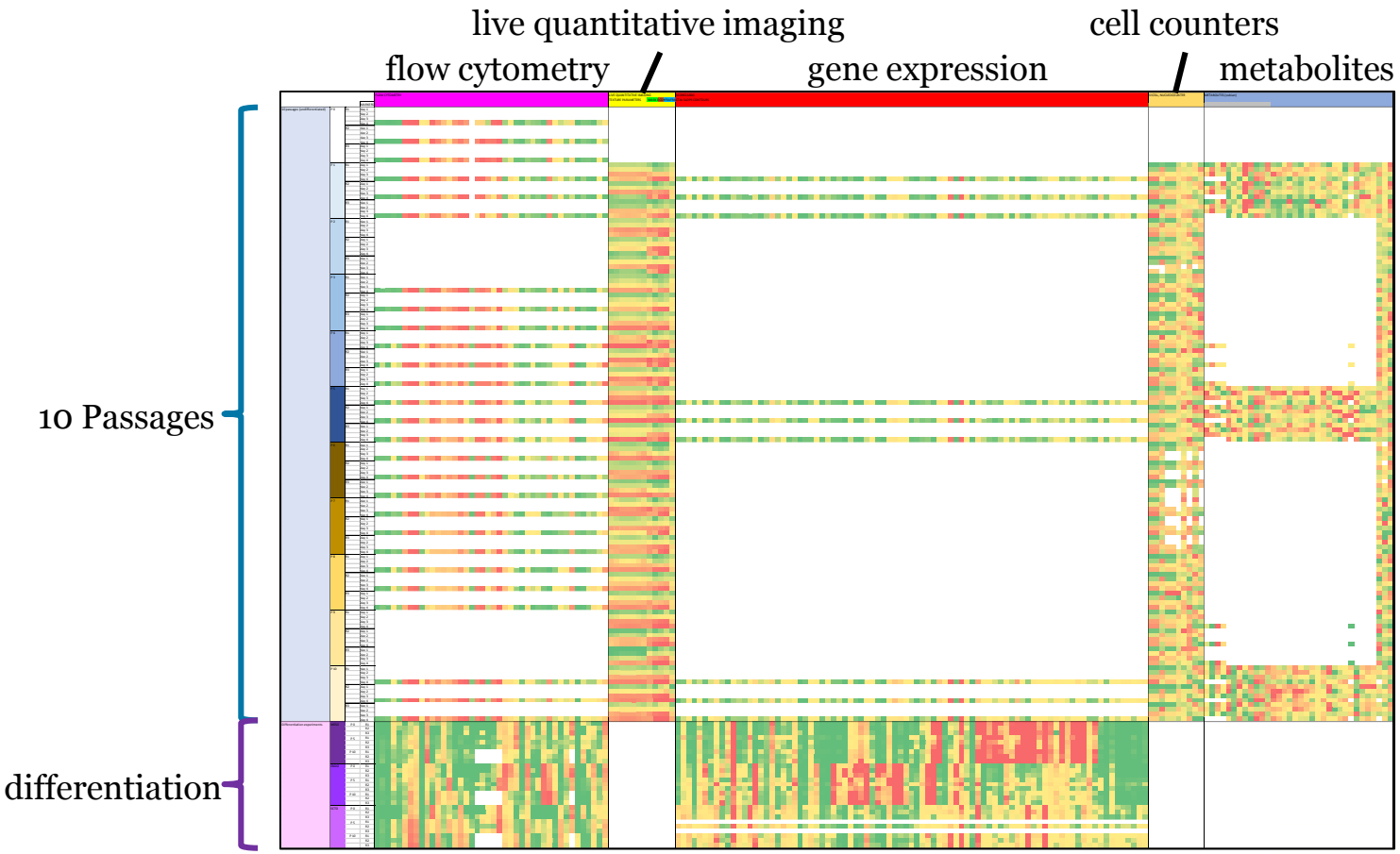
Multi-parametric data structure

183 parameters in total, 159 time points/conditions



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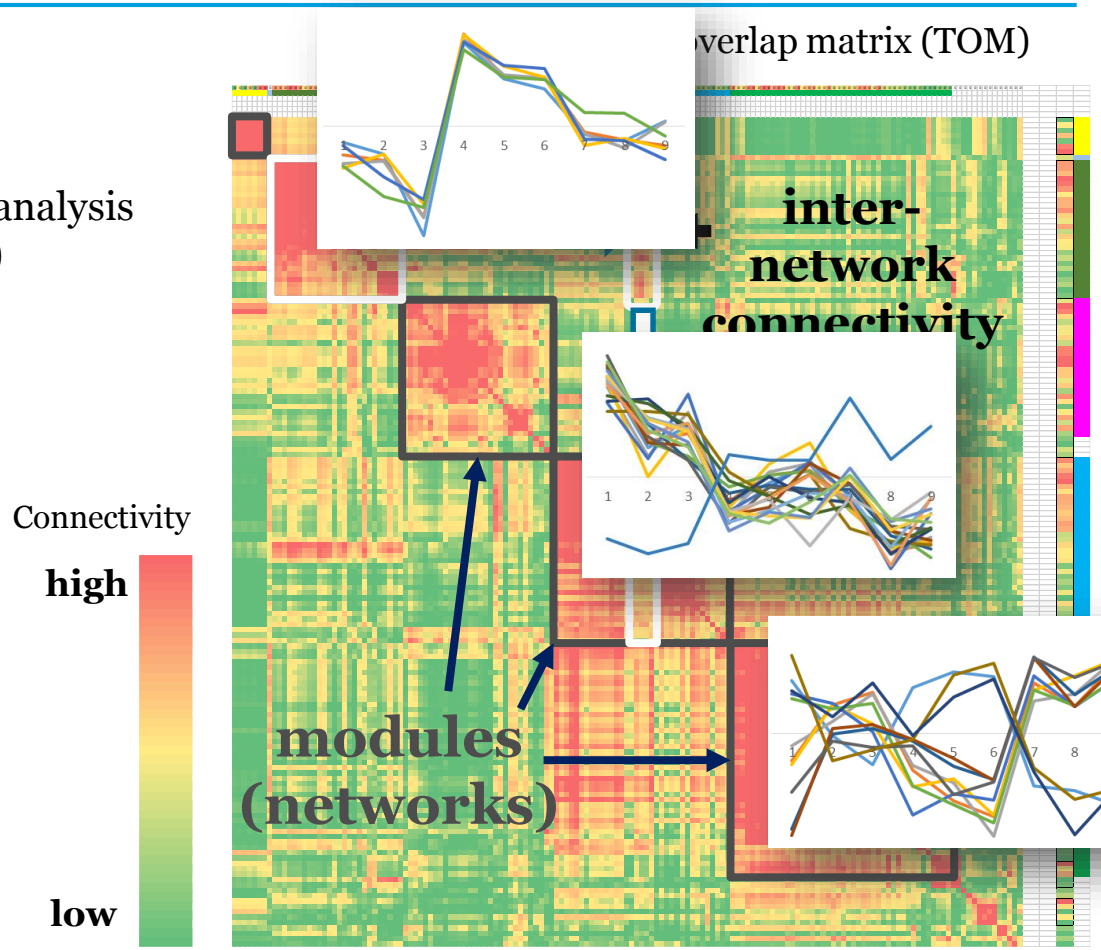
Weight Network Expression Analysis

183 parameters in total,
159 time points/conditions

157 parameters filtered for network analysis (Topological Overlap Matrix – TOM)

Modules = networks:
parameters with similar expression patterns across conditions, suggesting functional relationships.

Connectivity:
- *Intra-network* = clusters along diagonal
- *Inter-network* = red clusters away from diagonal



Within a particular module, similar AND different types of markers are mixed up and connected

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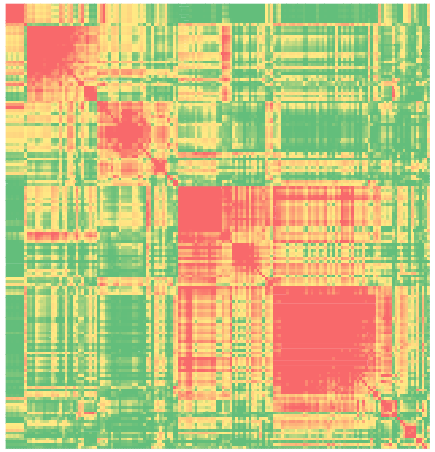
Data reduction

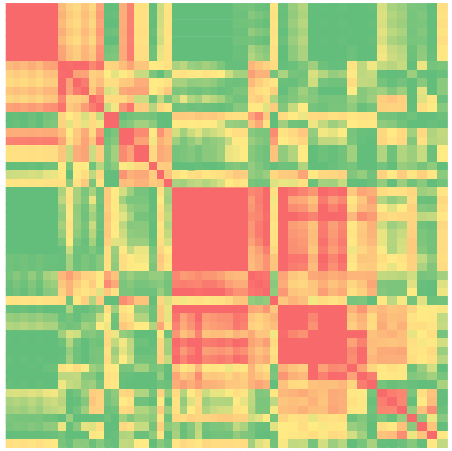
3 Biological Profiling

The least connected markers are removed to leave a low resolution set of markers which has the same overall connectivity as the full original dataset

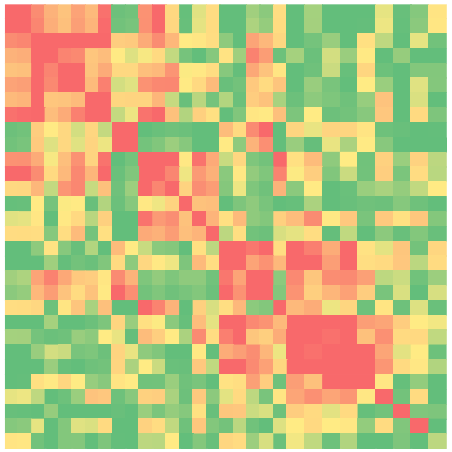
original TOM



157 markers

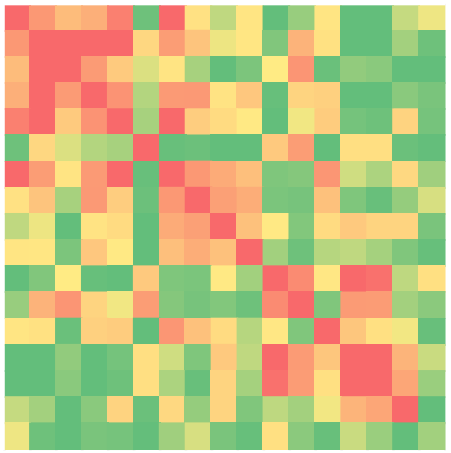


53 markers



30 markers

reduced TOM



17 markers

Texture / morphology = **17**
Metabolites = **8**
Cell paramerters= **10**
Genes = **80**
Membrane markers = **42**

Data reduction

Conservation of information
Relationships between markers as well as network connectivity is maintained

Texture / morphology = **1**
Metabolites = **3**
Cell count= **1**
Genes = **6**
Membrane markers = **6**

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Inferential markers

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Analytical Platform

① Analytical Design

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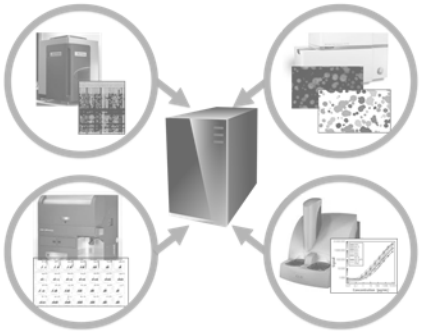
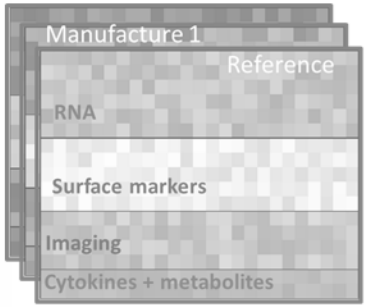


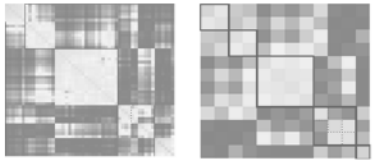
② Analytical testing

- Screening strategy
- Integrated analysis
- Augment Information
- Stress testing



③ Biological Profiling

- Cluster Analysis
- Data Reduction
- Multivariate Analysis



④ Customised QC

- **Inferential analysis**
- **Reference materials**
- **Automated analysis**

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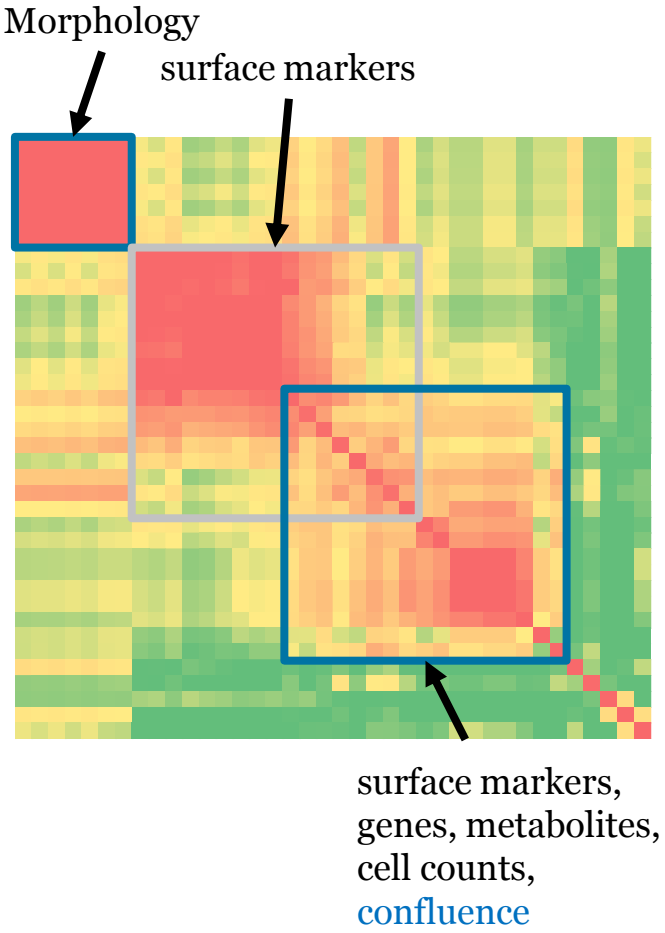
Network Analysis –surrogate markers

- Potential inferential markers can be identified based on their connectivity with other markers.
- By looking for strong overlap between modules it is possible to identify coordinated expression patterns – consistent throughout the production process
- This allows the expression of one marker to be used to indicate the expression of others



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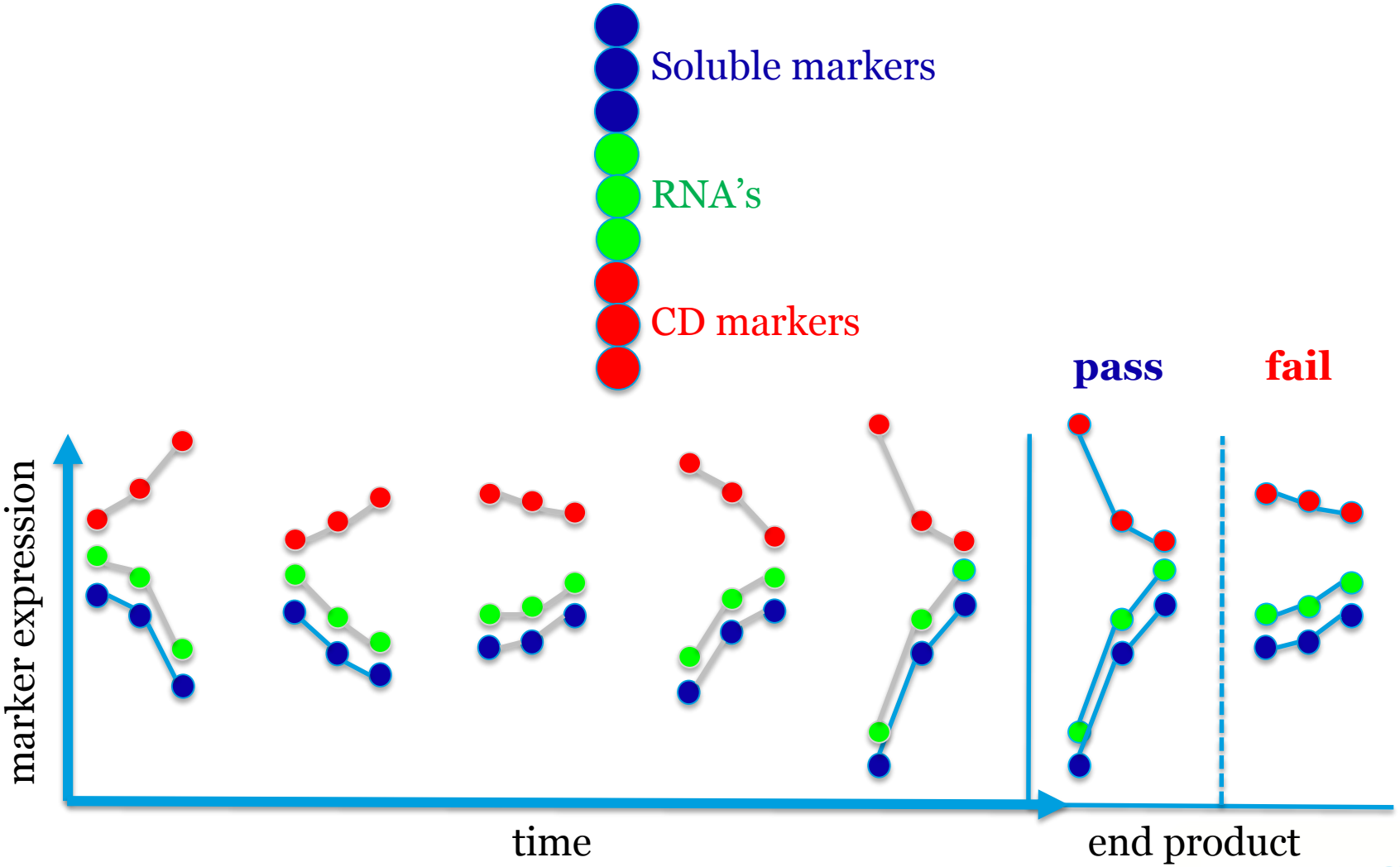
Inferential markers application



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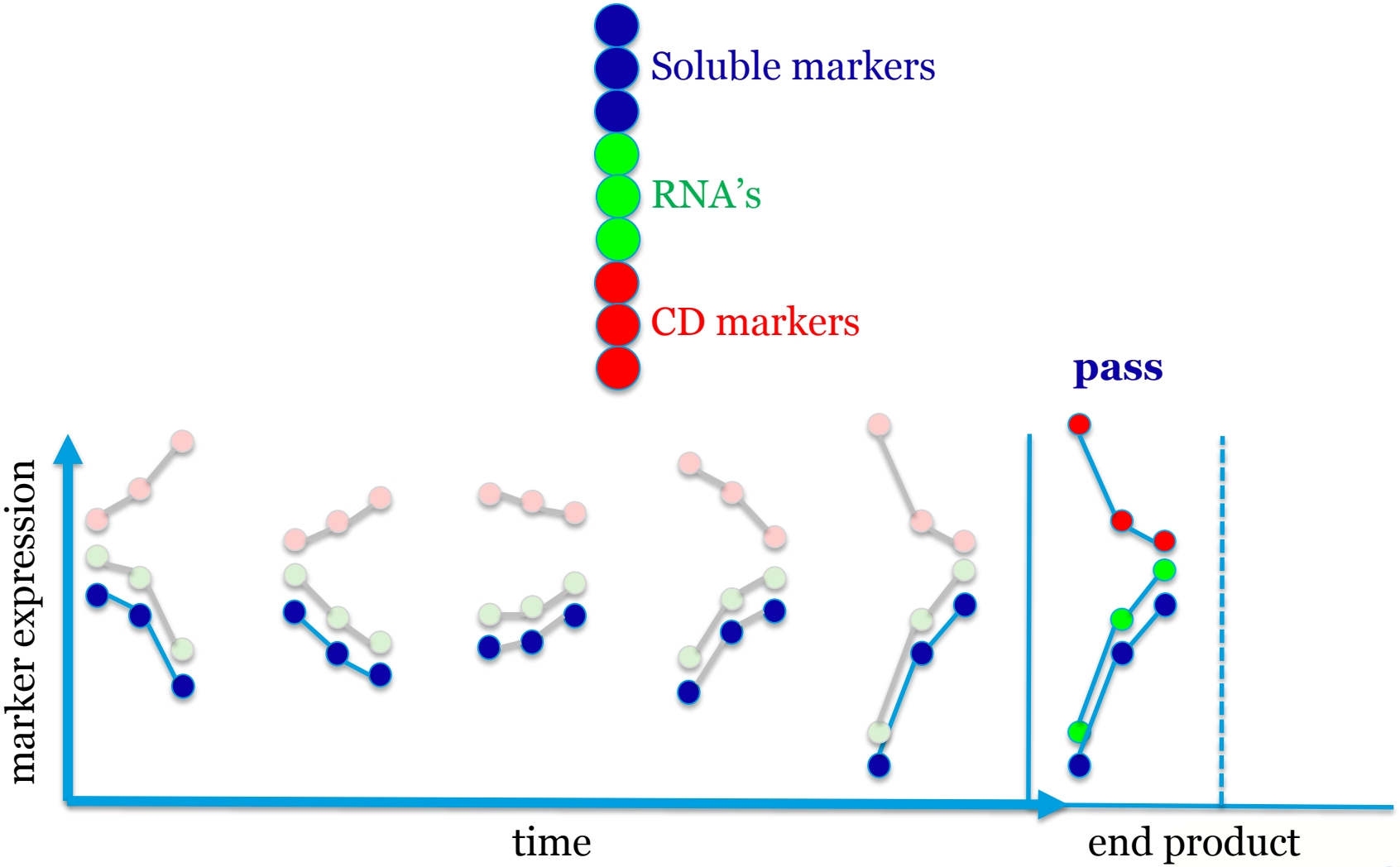
Surrogate markers application



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Summary

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Analytical platform at the CGT

Build quality in the process by

Using a systematic approach to understanding product quality and define what characteristics to measure in order to…..

- Allow control of the manufacturing process
- Support process optimisation to ensure the quality and lot-to-lot consistency of the final product
- Have the analytical capacity to understand and control (or terminate) the manufacture process

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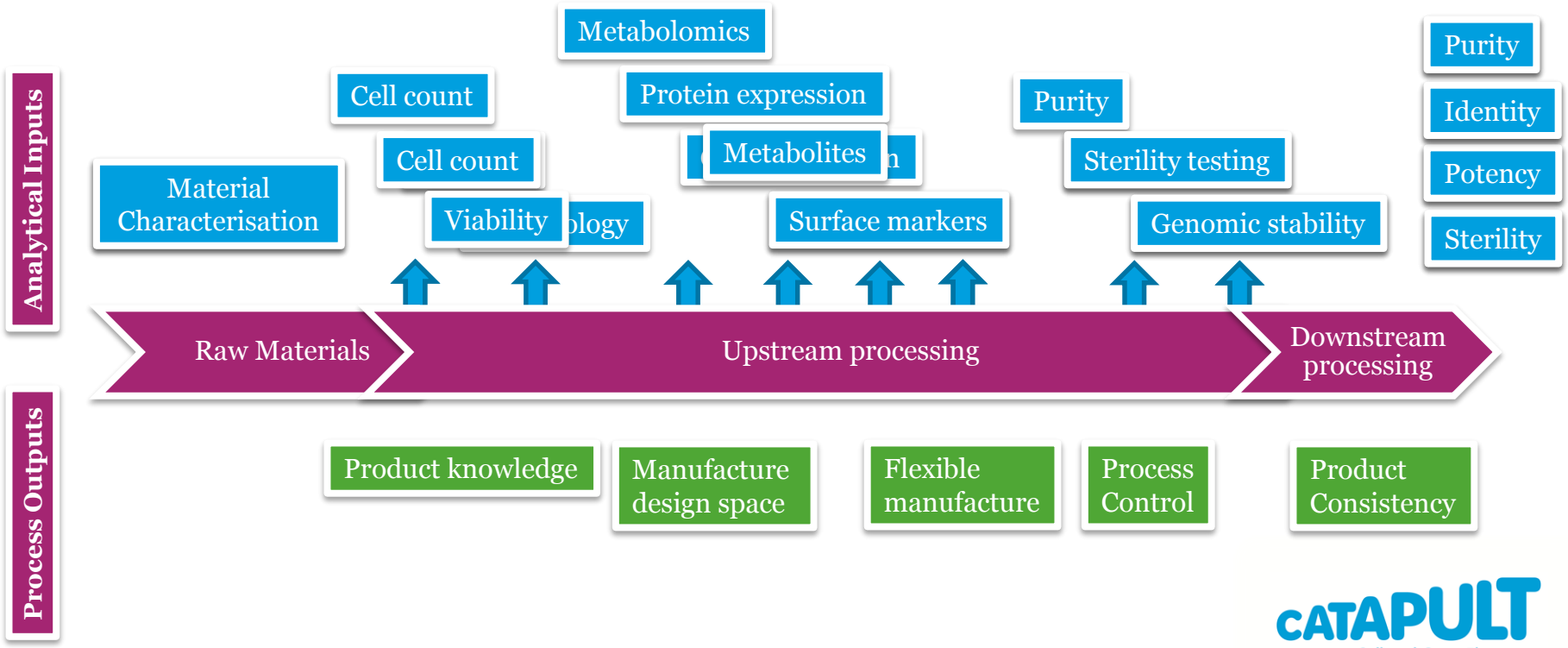
Summary – CGT analytical platform

Moving QC into the process

Providing the analytical platform for targeted characterisation to ensure that a product is of intended quality, based on information collected during the manufacturing process



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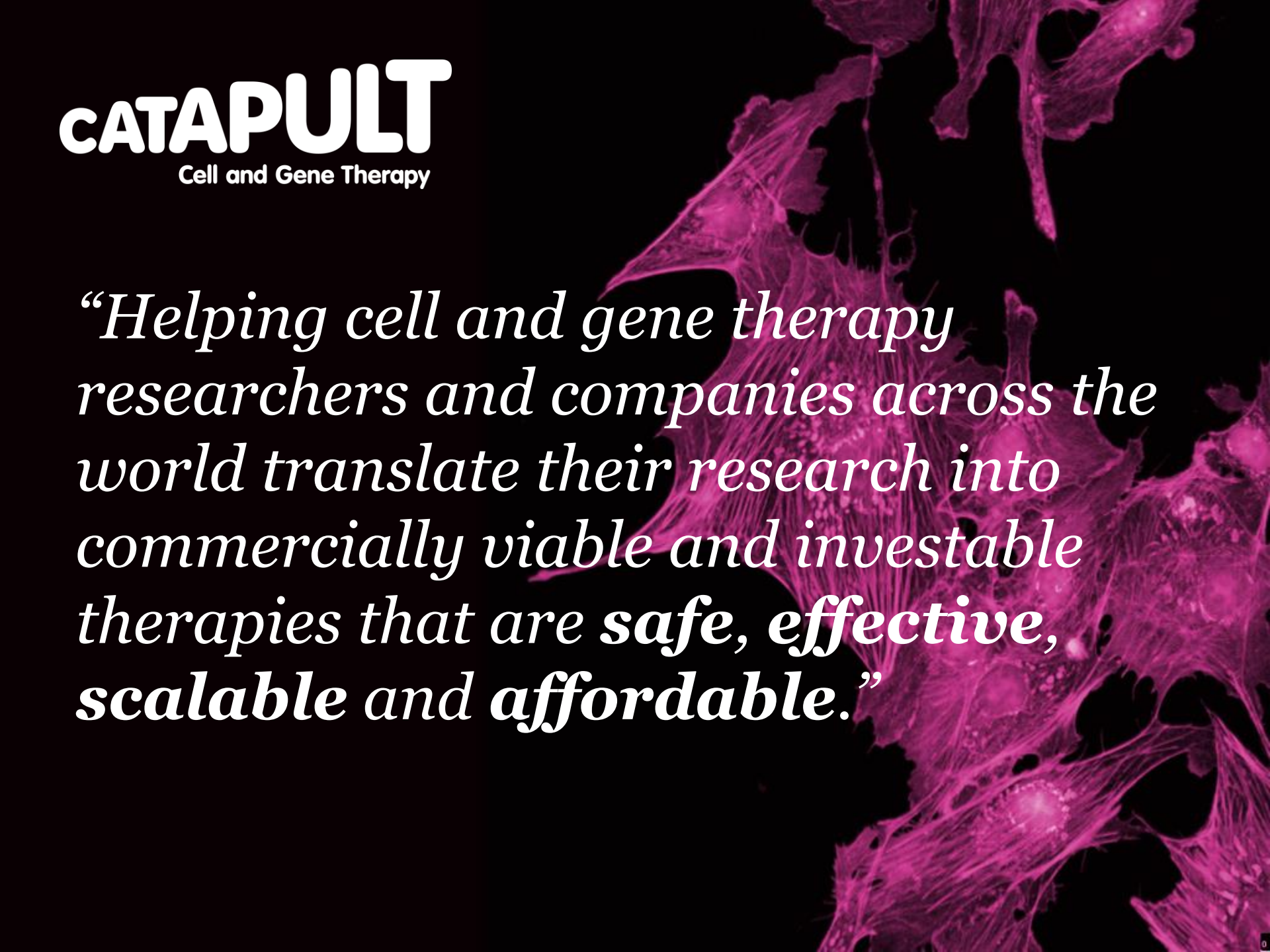

*"Helping cell and gene therapy researchers and companies across the world translate their research into commercially viable and investable therapies that are **safe**, **effective**, **scalable** and **affordable**."*