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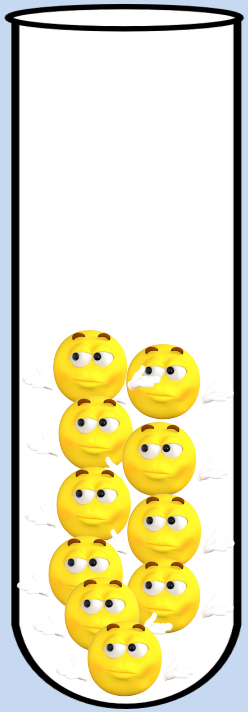
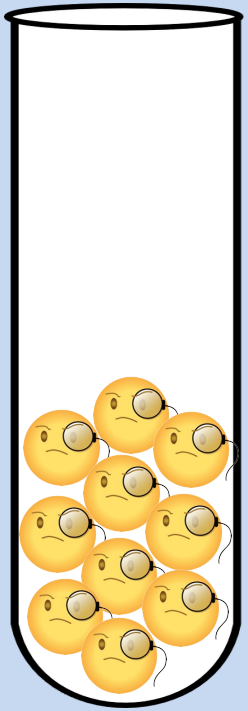
TECHNOLOGIES FOR STEM CELL MEDICINE

Kinetic Stem Cell (KSC) Counting

Technologies for
specific quantification and analysis
of therapeutic tissue stem cells

THE CURRENT PROBLEM

Homogeneous  *or*  Impossible

How many people think about the dosage of therapeutic tissue stem cell preparations

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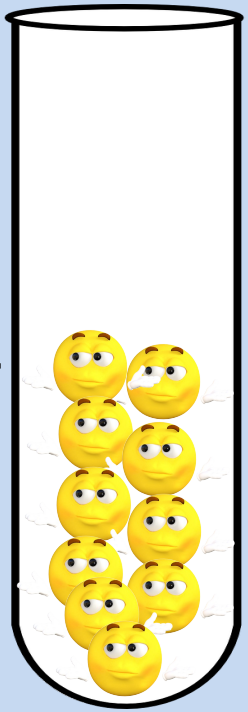
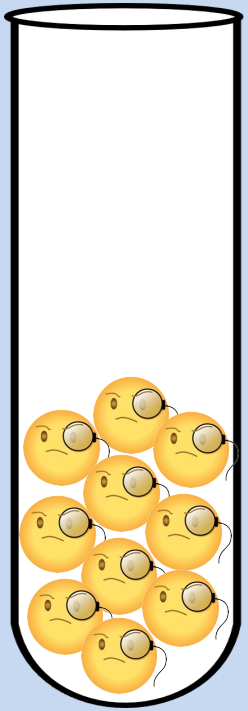
THE CURRENT PROBLEM

Homogeneous

Incorrect



or



Impossible

Previously

How many people think about the dosage of therapeutic tissue stem cell preparations

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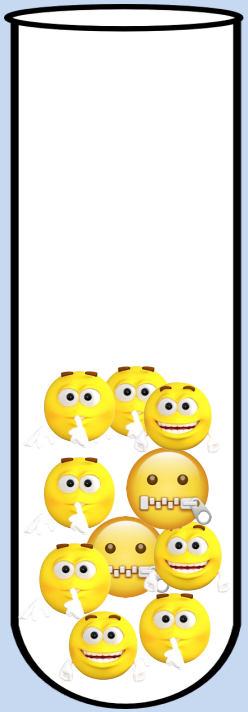
THE ASYMMETREX VISION

The Reality



Heterogeneous with difficult to quantify
low tissue stem cell fraction

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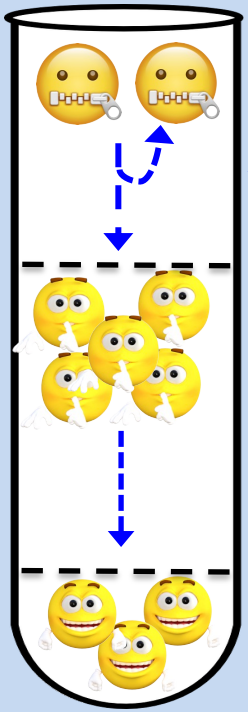
THE ASYMMETREX VISION

Tissue Stem Cells

Committed Progenitor Cells

Terminally-Arrested Cells



Asymmetric* (and Symmetric) *Cell Kinetics

Symmetric Cell Kinetics

Arrested

The Asymmetrex Solution:
Cell Kinetics Distinctions

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OUTLINE

I. Tissue stem cell number determines treatment potency
II. Previous ineffective technologies
III. Kinetic Stem Cell (KSC) Counting
IV. KSC Counting Applications

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I. Tissue stem cell number determines treatment potency

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Tissue Stem Cell Number Is An Important Potency Factor

?

For stem cell therapies (*e.g.*, HSC transplantation medicine)

Tissue stem cell-specific dosage is a critical quality attribute for:

- Optimizing and reproducing treatment outcomes
- Designing clinical trials
- Normalization for evaluating clinical trial effectiveness

For stem cell-engineered gene therapies (*e.g.*, HSC gene editing)

Monitoring stem cell fraction during engineering

Tissue stem cell-specific dosage is a critical quality attribute

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II. Previous Ineffective Technologies*

Technology	Effective?	Problems
Flow cytometry	No	Specificity: TSC = CPC
SCID mice	Only for HSCs	$, mice, 16 wks, unreliable
CFU	No	Specificity: TSC = CPC
Metabolic	No	Specificity: TSC = CPC

* Except for muscle satellite stem cells (nuclear Pax6, Pax7)
TSC = tissue stem cells
CPC = committed progenitor cells

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III. Kinetic Stem Cell (KSC) Counting

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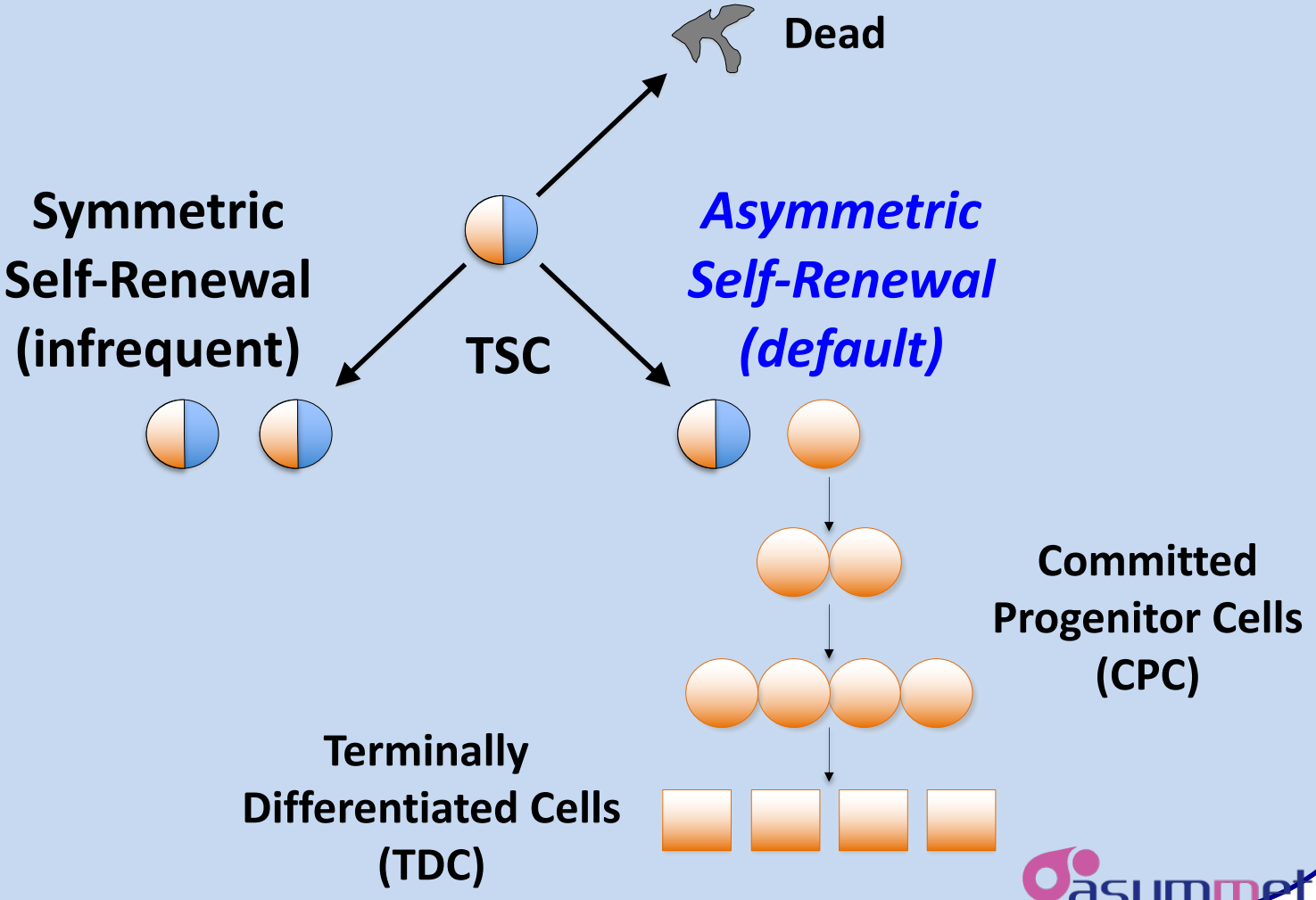
KSC Counting Principle I

In Vivo TSC Kinetics Continue in Cell Culture



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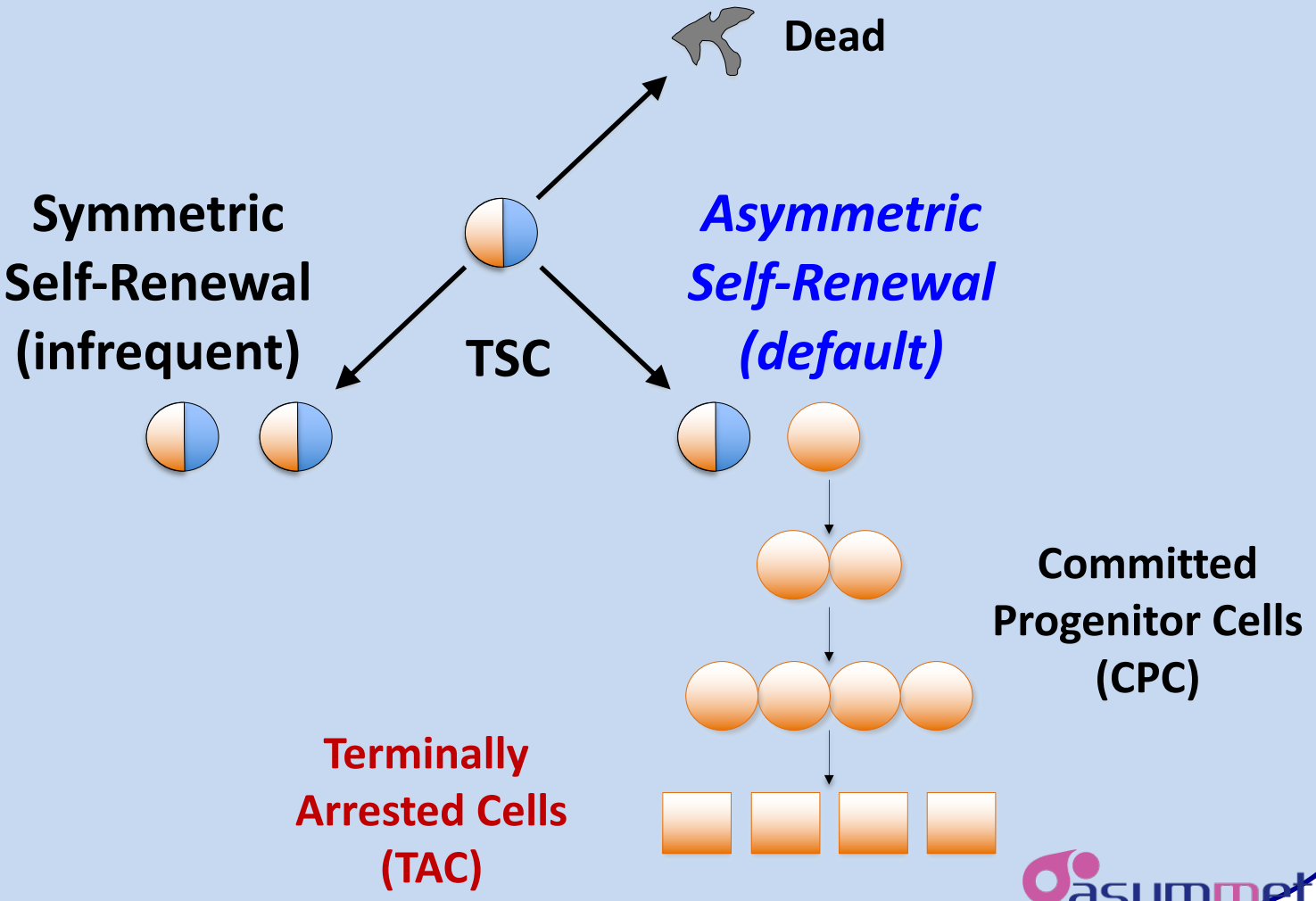
KSC Counting Principle I

In Vivo TSC Kinetics Continue in Cell Culture*



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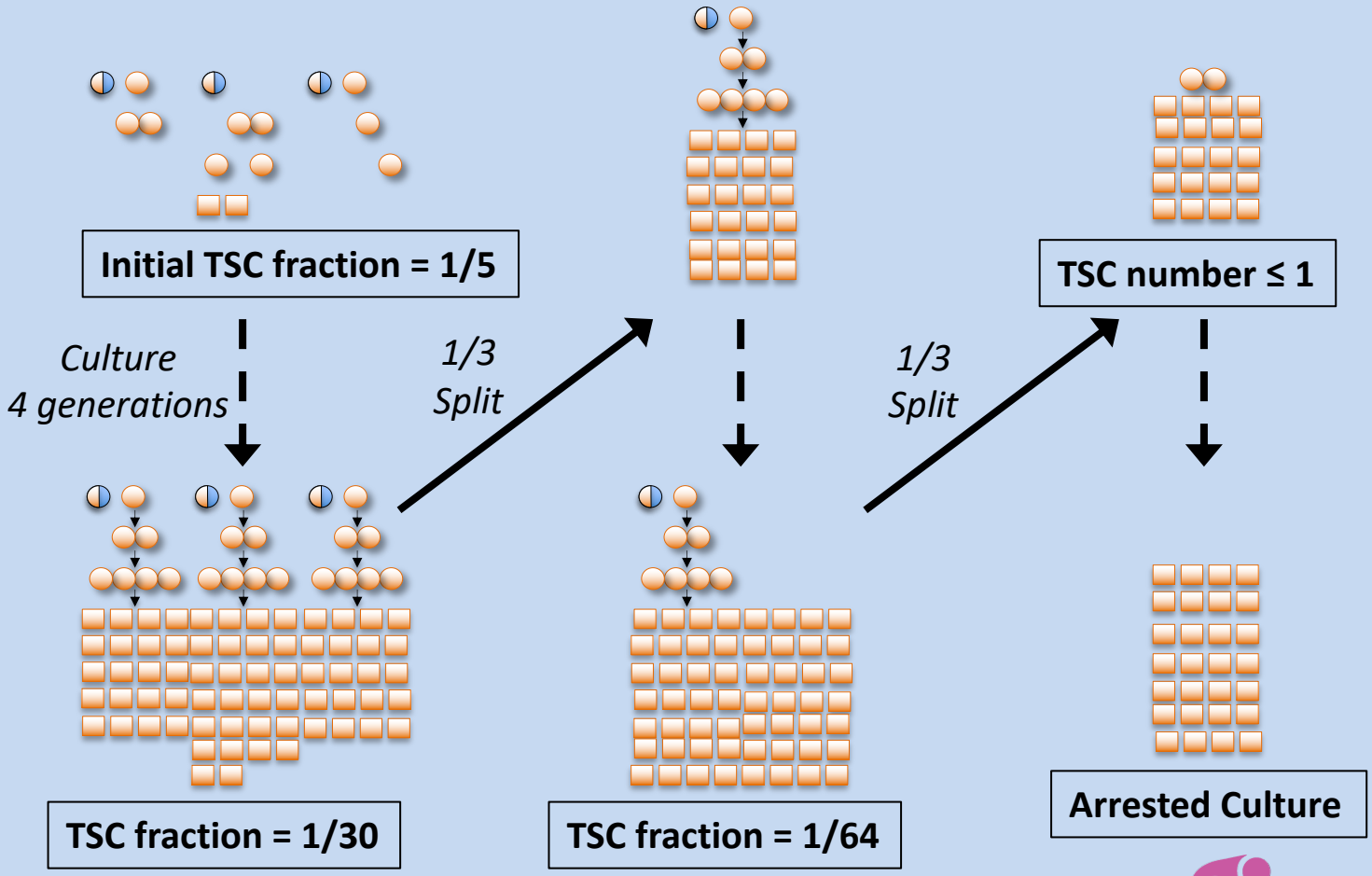
KSC Counting Principle II

Therefore, culture total cell output depends on TSC-specific fraction and cell kinetics.



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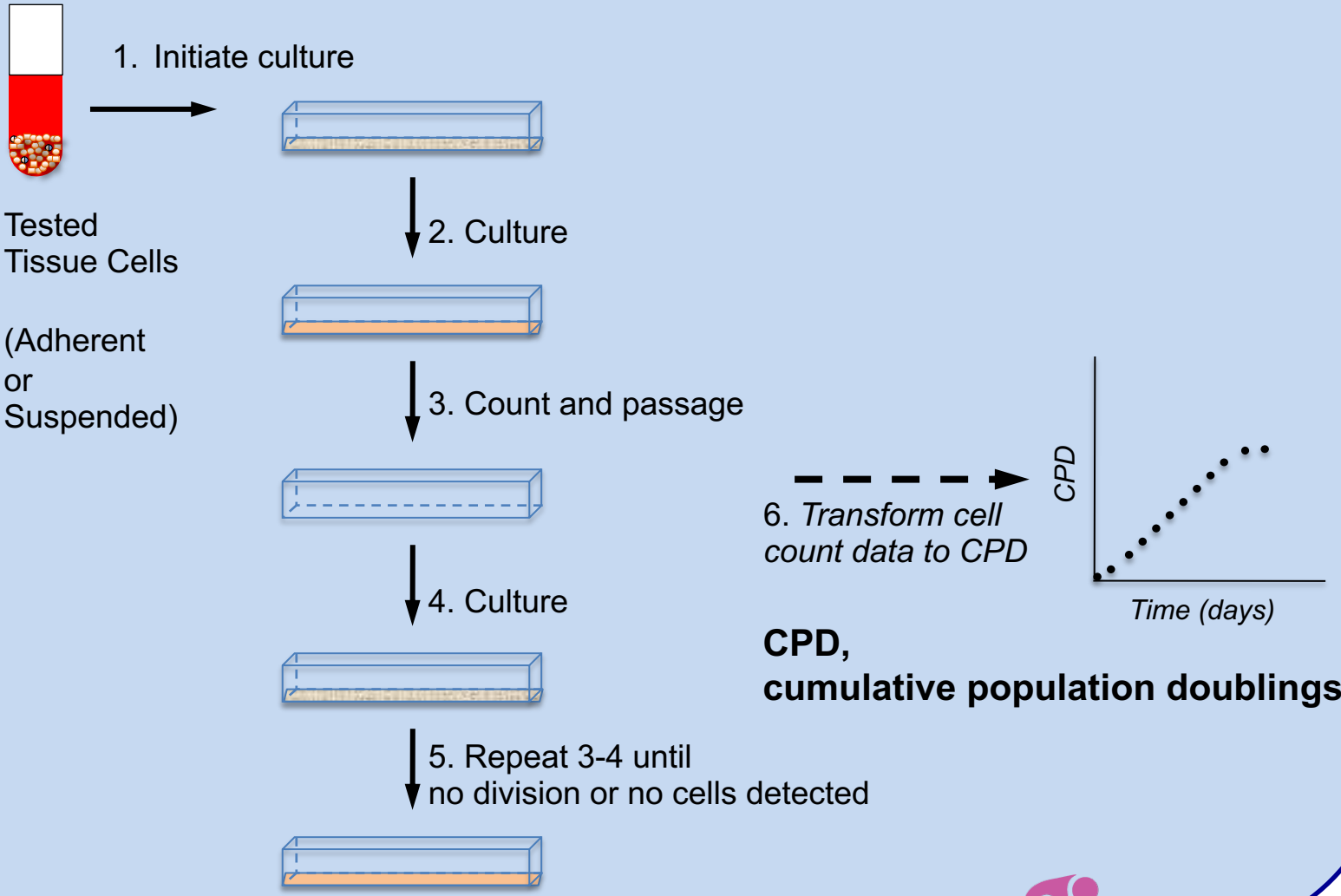
KSC Counting Begins with Serial Culture



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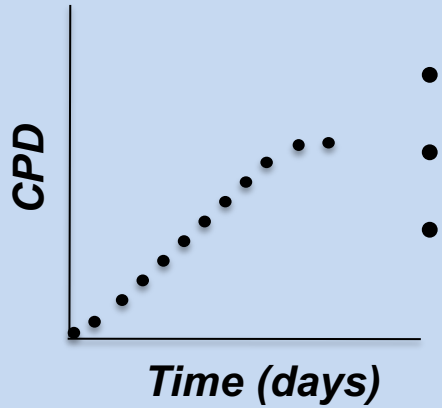
CPD Curve Culture Dependencies



- Rate
- Maximum
- Time of arrest

Culture Factors

Input cell number
Split interval
Split fraction
Cell viability

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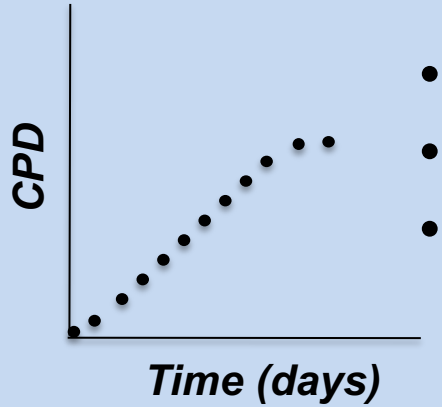
CPD Curve Cell Kinetics Dependencies



- Rate
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Culture Factors

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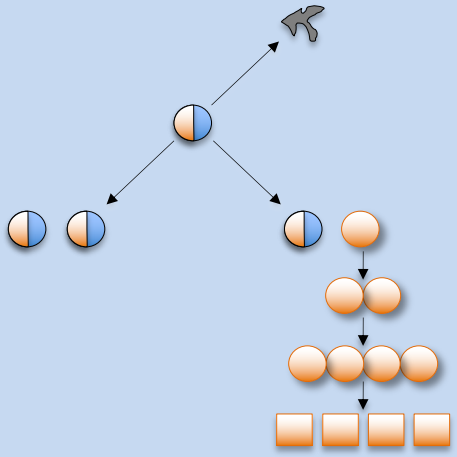

Cell Kinetic Factors

TSC Number
CPC Number
TAC Number
TSC Viability
CPC Viability
TAC Viability
TSC Symmetric Rate
TSC CC Times (Asym & Sym)
CPC CC Time
CPC Division Number

TSC, tissue stem cell; CPC, committed progenitor cell;
TAC, terminally-arrested cell; CC, cell cycle;
Division Number = number of divisions before producing TACs

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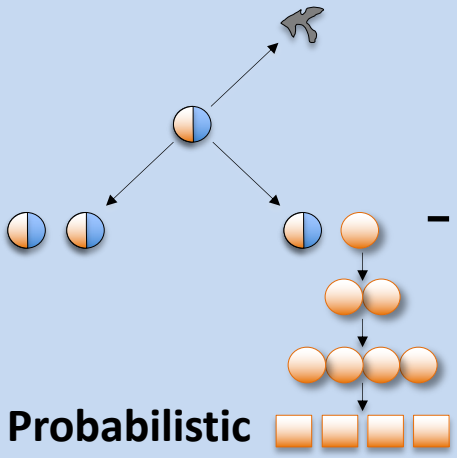
KSC Counting: A computational simulation modeling approach



Probabilistic Stem Cell Kinetics Model

KNOWNS: Measured Culture Factors

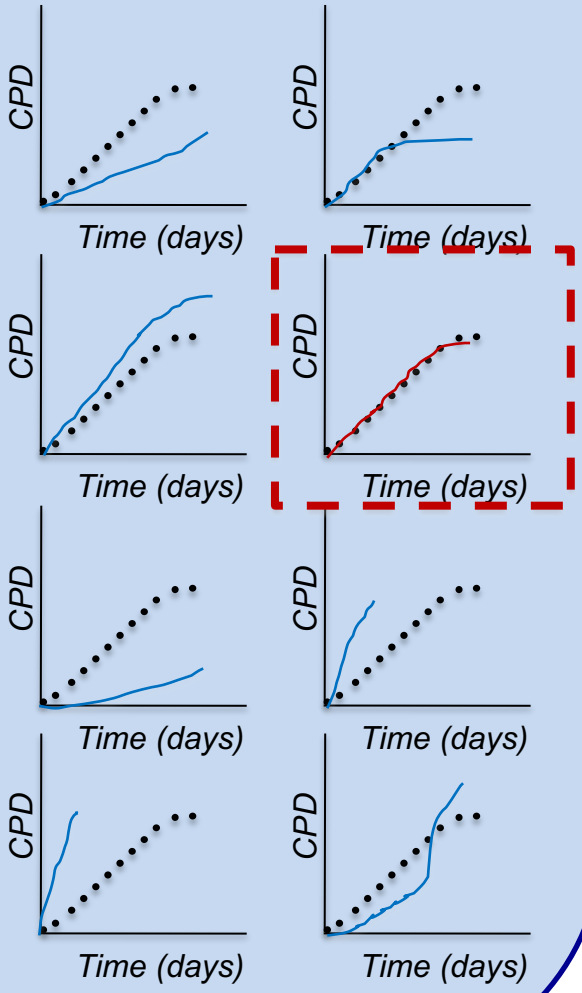
Input cell number
Split interval
Split fraction
Cell viability

TORTOISE Test™ Computer Simulation Software

(10,000 trials)

UNKNOWNS: Varied Cell Kinetic Factors

TSC Number
CPC Number
TAC Number
TSC Viability
CPC Viability
TAC Viability
TSC Symmetric Rate
TSC CC Times (Asym & Sym)
CPC CC Time
CPC Division Number



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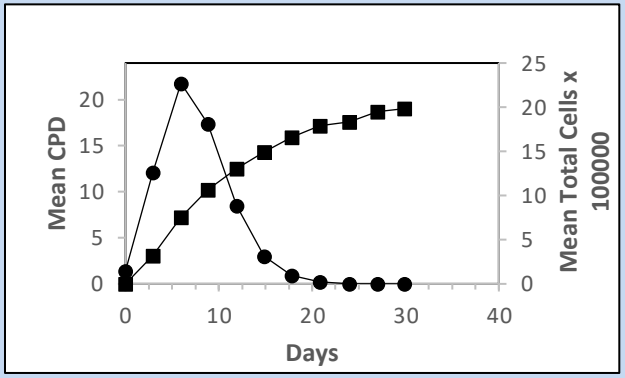
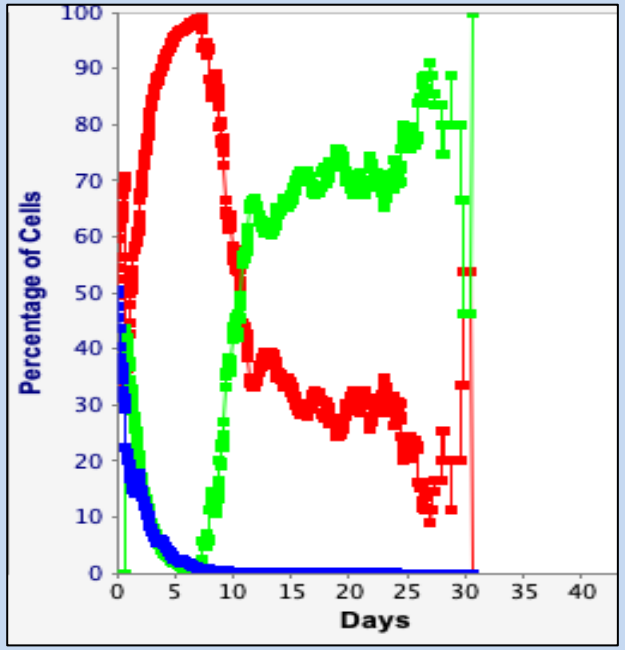
The KSC Counting Process



Serial Cell Count Data

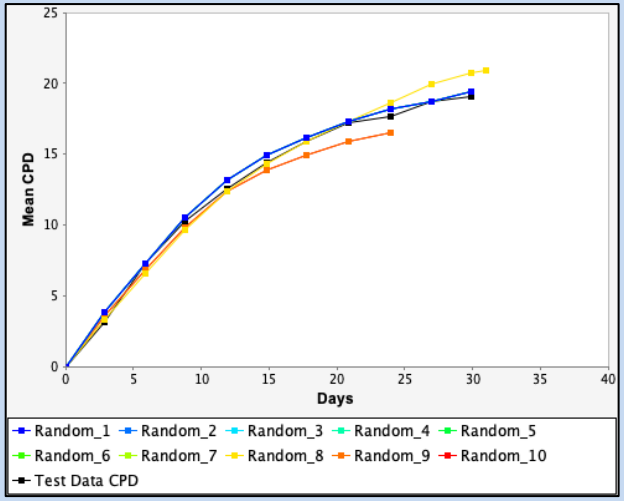


KSC Counting Outputs

Blue – Tissue stem cells

Red – Committed progenitor cells

Green – Terminally-arrested cells



TORTOISE Test™ Simulation

Average of 10 best from 10,000 trials

***Note**: Analysis for CD34⁺ UCB cells*

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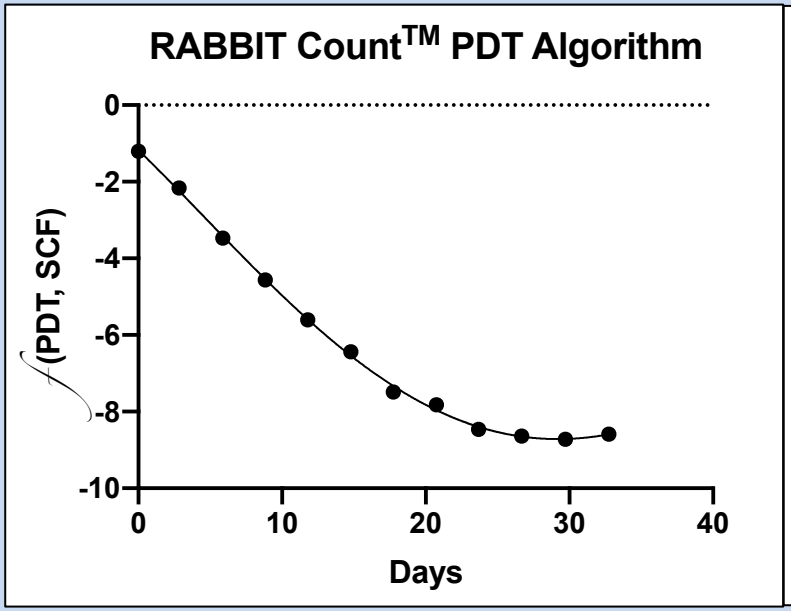
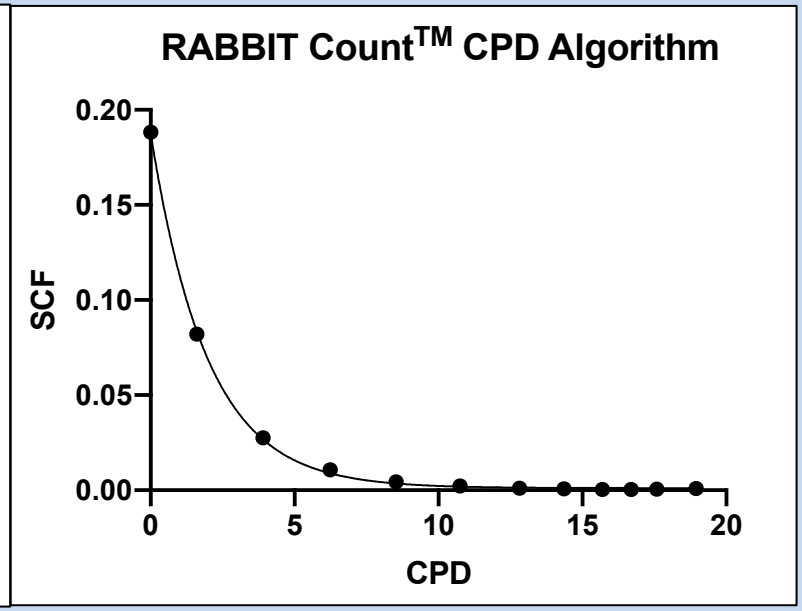
KSC Counting
Rapid Counting Algorithms

For any day of serial culture, if you know the culture's PDT,



…you know the SCF.

For any future cell culture, if you know the culture's CPD,



…you know the SCF.

***Note**: Data for CD34⁺ umbilical cord blood HSCs*
***SCF**, stem cell-specific fraction*

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CD34$^+$ Selection Validations

[Mean SCF (95CI)]

Source	CD34$^-$	CD34$^+$
UCB	0.0001 (0.001-0.001)	0.63 (0.36-0.90)

Source	Unfractionated	CD34$^+$
UCB-1a	0.03 (0.02-0.05)	
UCB-1b	0.02 (0.01-0.02)	
UCB-2		0.18 (0.14-0.22)
UCB-3		0.72 (0.58-0.85)
MPB-1	0.02 (0.01-0.03)	0.78 (0.61-0.96)
MPB-2	0.15 (0.06-0.24)	
MPB-3		0.83 (0.76-0.91)

SCF, stem cell-specific fraction; **95CI,** 95% confidence interval
UCB, umbilical cord blood; **MPB**, mobilized peripheral blood

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Human tissue stem cells
KSC counted to date

- Liver hepatic stem cells
- Lung interstitial stem cells
- Corneal stem cells
- Bone marrow hematopoietic stem cells
- Mobilized peripheral blood hematopoietic stem cells
- Umbilical cord blood hematopoietic stem cells
- Umbilical cord tissue mesenchymal stem cells
- Bone marrow-derived mesenchymal stem cells
- Adipose-derived mesenchymal stem cells
- Oral-derived mesenchymal stem cells
  (bone, gingival, dental pulp)
- Amniotic membrane stem cells

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IV. KSC Counting Applications

A. Cord Blood Applications

- **1.** Complement CFU for better potency determinations
- **2.** Complement MNC for unit selection for increased yield
- **3.** Complement CFU for more rapid unit stability evaluations

B. Stem cell therapy and stem cell-engineered gene therapy dosage

C. Stem cell-based cell and tissue biomanufacturing (*e.g.*, MSCs)

D. Drug evaluations

- **1.** Tissue stem cell toxicity evaluations to avoid chronic organ failure
- **2.** Regenerative medicine drug evaluations

E. Environmental chemical analysis for tissue stem cell toxicants (*e.g.*, carcinogens)

F. Tissue cell research and stem cell biology research

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For more information

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