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TECHNOLOGIES FOR STEM CELL MEDICINE

Kinetic Stem Cell (KSC) Counting:

A brief introduction to the technology

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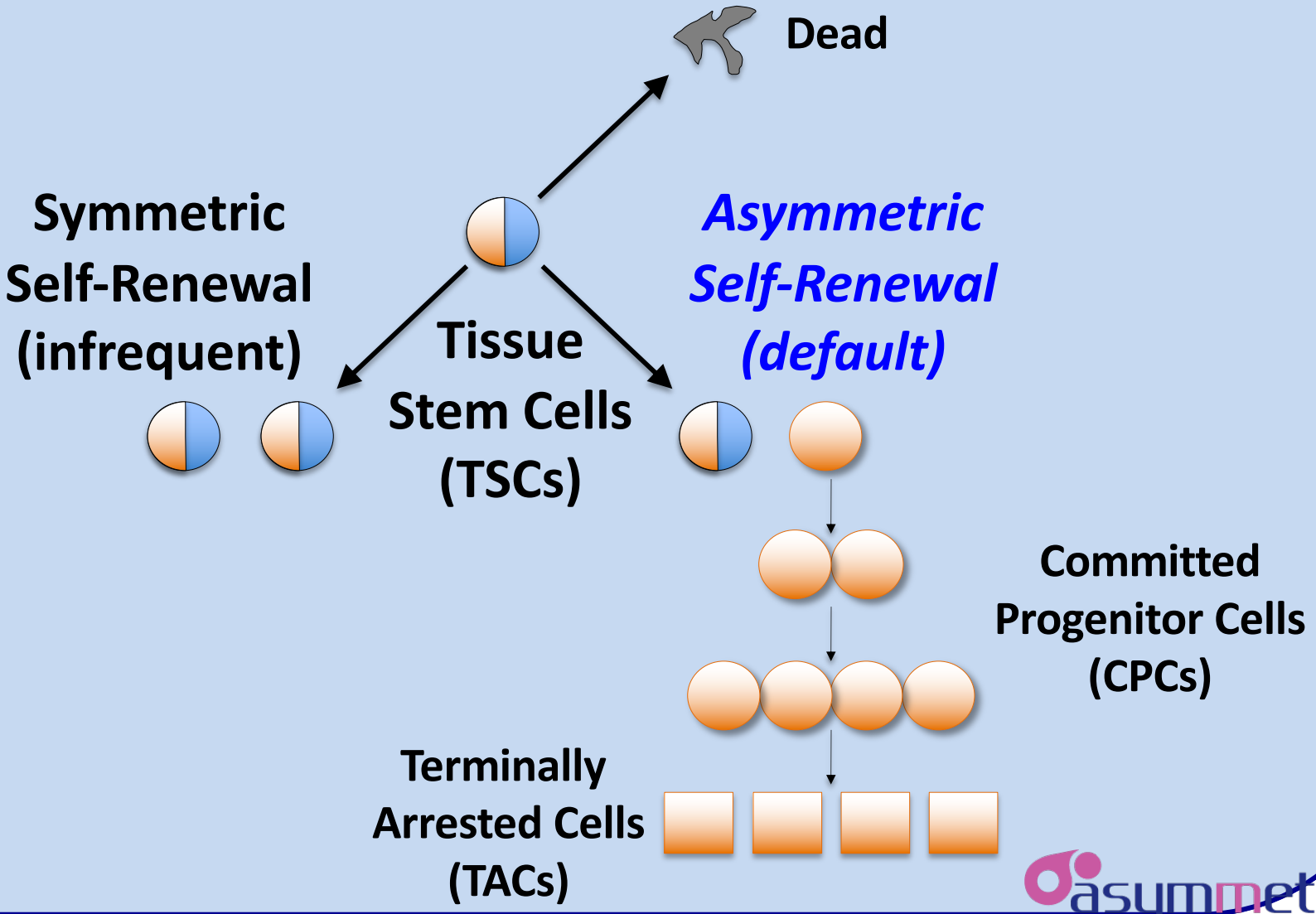
KSC Counting Principle I

In vivo TSC kinetics continue in cell culture.



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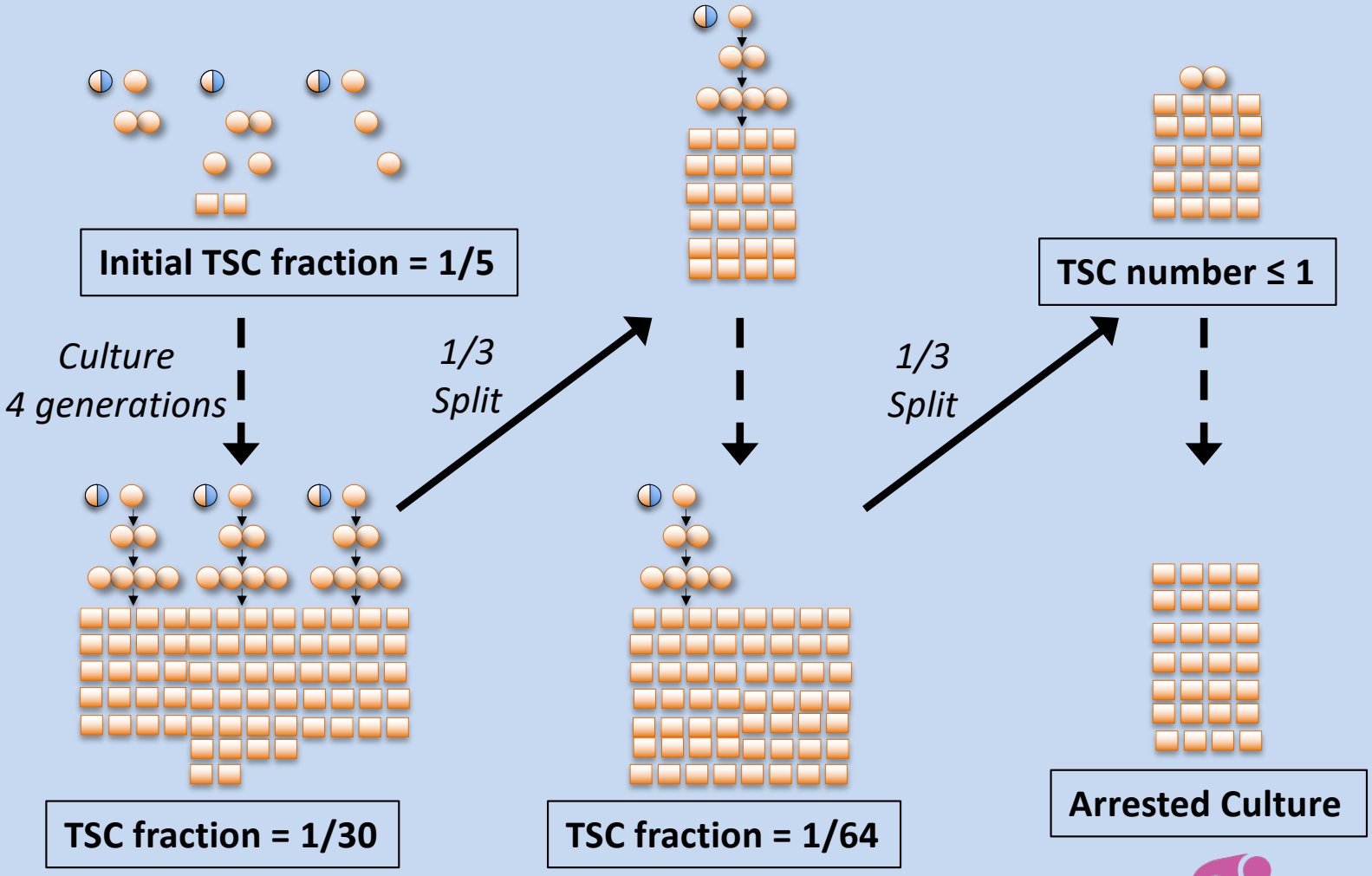
KSC Counting Principle II

Primary tissue cell cultures' total cell output depends on TSC-specific fraction and cell kinetics.



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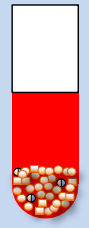
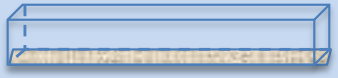
KSC Counting Begins with Serial Culture



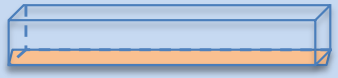
Tested
Tissue Cells

(Adherent
or
Suspended)

1. Initiate culture

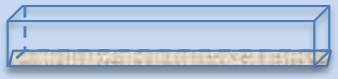


2. Culture

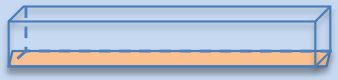


3. Count and passage

4. Culture

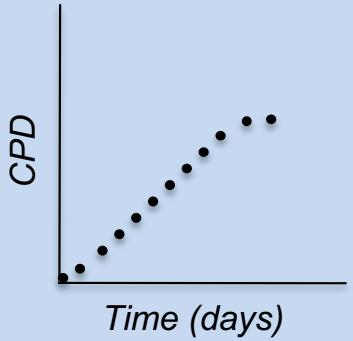


5. Repeat steps 3 and 4 until
no division or no cells detected



A measure of the total output of serial culture

6. *Transform cell count data to CPD*



CPD,
cumulative population doublings

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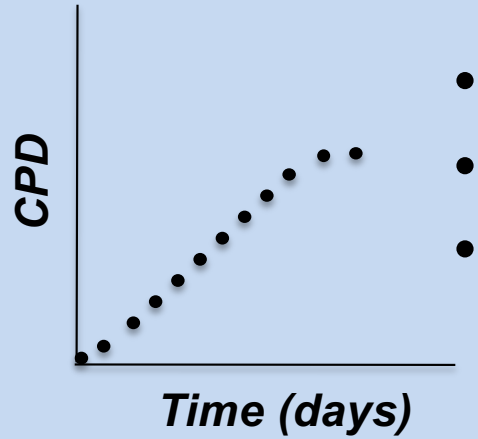
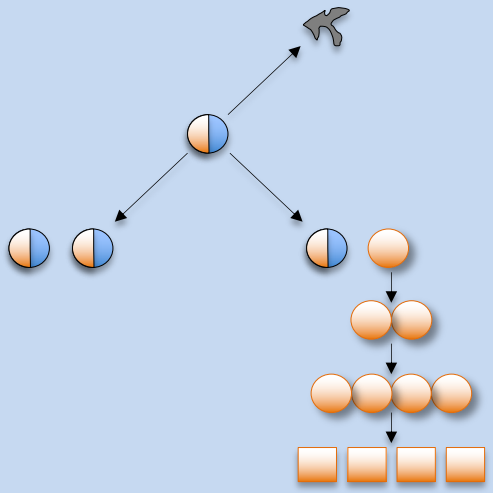
CPD Kinetics Depend on Two Sets of Factors



- Rate
- Maximum
- Time of arrest

Known Culture Factors

Input cell number
Split interval
Split fraction
Cell viability



***Unknown* Cell Kinetic Factors**

TSC Number
CPC Number
TAC Number

TSC Viability
CPC Viability
TAC Viability

TSC Asymmetric CC Time
TSC Symmetric CC Time
CPC CC Time

TSC Symmetric Rate
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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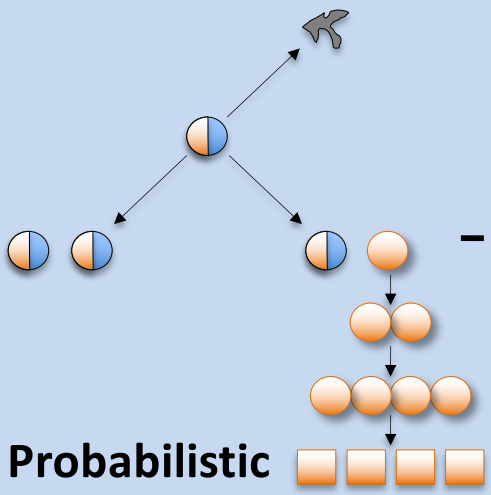
KSC Counting – A computational simulation modeling approach



Probabilistic Stem Cell Kinetics Model

KNOWNS: Measured Culture Factors

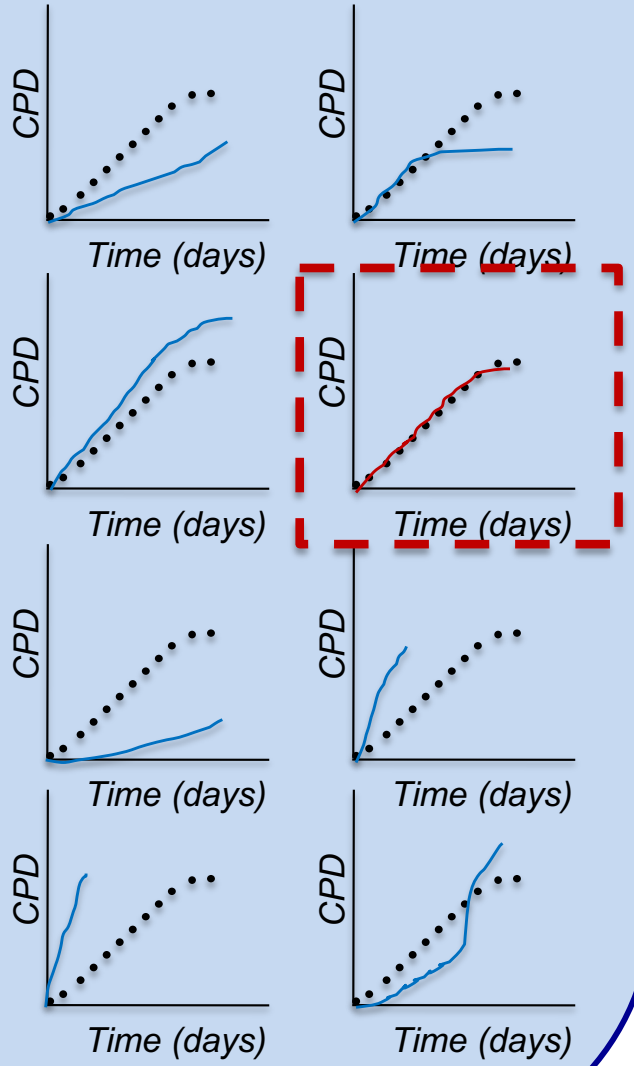
Input cell number
Split interval
Split fraction
Cell viability

TORTOISE Test Computer Simulation Software

(10,000 trials)

***COMPUTATIONAL SEARCH FOR UNKNOWNS*: Vary Cell Kinetic Factors**

TSC Number
CPC Number
TAC Number
TSC Viability
CPC Viability
TAC Viability
TSC CC Times (Asymmetric & Symmetric)
CPC CC Time
TSC Symmetric Rate
CPC Division Number (TDN)



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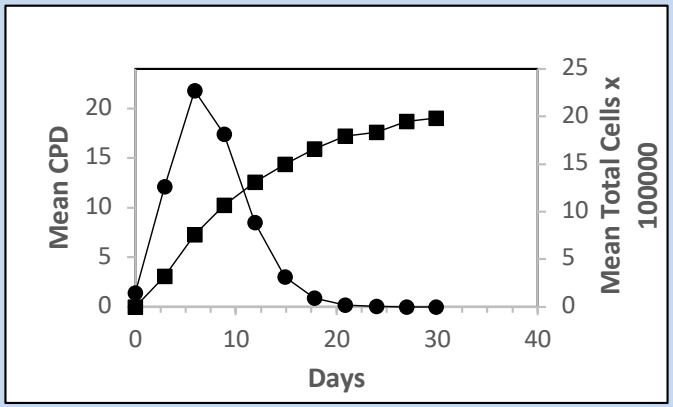
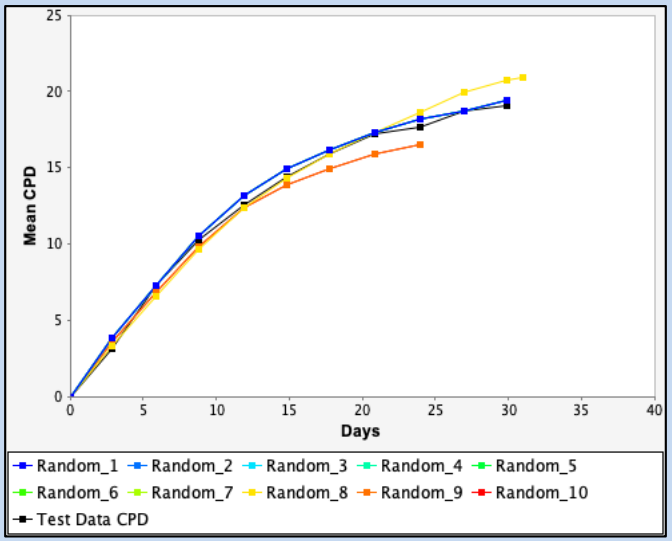
The KSC Counting Process

1.



Serial Cell Count Data

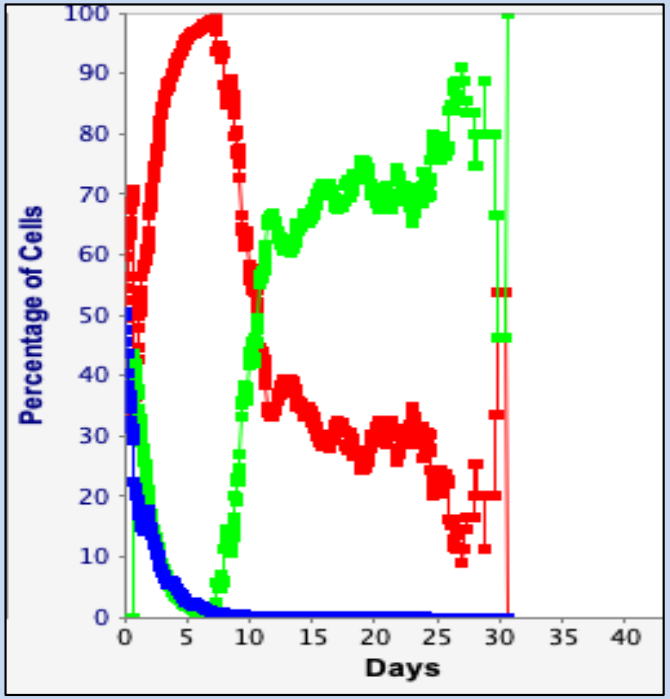
2.



TORTOISE Test Simulation

Example of 10 independent simulations

3.



KSC Counting Cell Subtype Kinetics

Blue – Tissue stem cells (**TSCs**)
Red – Committed progenitor cells (**CPCs**)
Green – Terminally arrested cells (**TACs**)

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

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KSC Counting Validations

I. Comparison to independent detection of asymmetric self-renewal divisons[1]

II. Analysis of effects of tissue stem cell-active agents (positive and negative factors)[1]

III. $CD34^+$ fractionation analyses for HSCs[1,2]

IV. *Comparison to SCID mouse repopulating cell assays for HSCs*[2]

1. 2020. Dutton *et al*., *OBM Transplantation* 4(3):24; doi:10.21926/obm.transplant.2003117.
2. 2022. Dutton *et al*., submitted.

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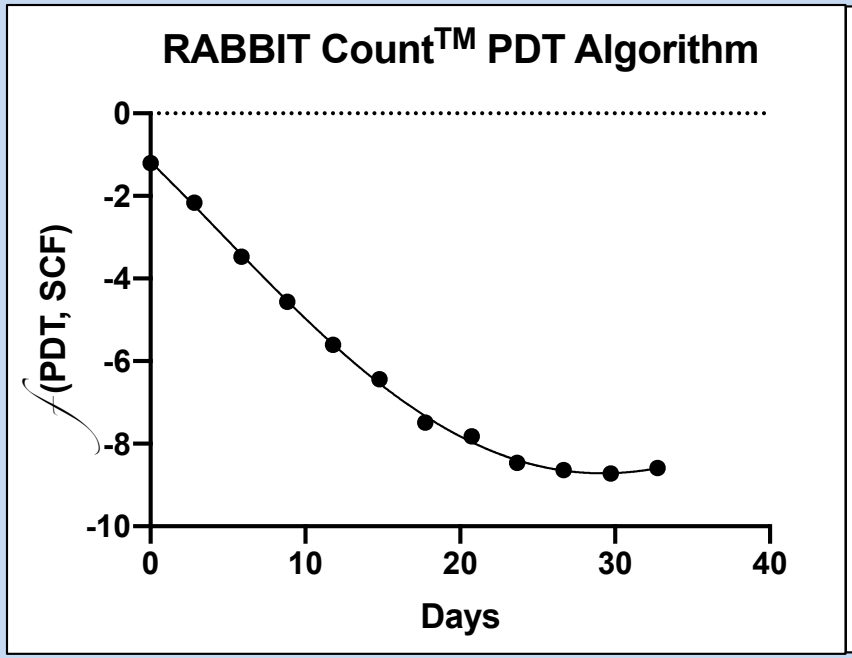
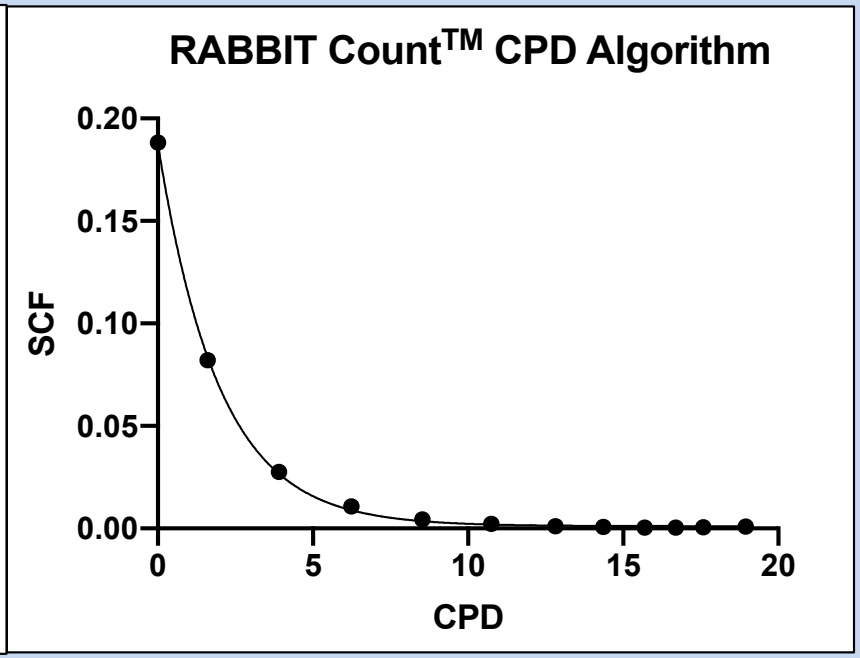
Rapid-Counting Algorithms

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; **PDT**, population doubling time; **CPD**, cumulative population doublings*

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Example of Online Rapid-Counting Calculator Portal

Result: 0.40667518566225075

Asymmetrex® Rabbit Algorithm For Human Adipose-Derived Mesenchymal Stem Cells

This calculator allows determination of the tissue stem cell-specific fraction (SCF) of primary human adipose-derived mesenchymal stem cells when cultured in the following commercial cell culture medium:
Thermo Fisher MesenPro RS™ Basal Medium supplemented with MesenPro RS™ Growth supplement (Kit Cat#1276012), 2 mM L-glutatmine, and pen/strep.

Culture and counting procedures

1. In a 6-well plate, initiate evaluation cultures with ideally 50,000 to 100,000 total viable cells per 5.0 mLs of the culture medium specified above. Fewer cells can be used, but more cells should not be used. Recommend preparing 6 replicate wells.
2. Use 3 of the cultures to determine the mean total cell count 4-5 hours after plating. This analysis sets time = 0 hours.
3. Approximately 72 hours later, determine the mean total cell count in the remaining 3 cultures, time = "72 hours." This period should not be less than 66 hours and not more than 78 hours (i.e., range in fractional days from 2.75 to 3.25).

Note: Accurate tissue stem cell counting requires an increase in total cell number during the period of this analysis.

Calculator Procedure

1. Enter, **d**, the number of days of serial culture from the first cryopreservation of the initial isolated primary tissue cell preparation (e.g., 0, 3.0, 6.0). Entry of fractional days is recommended for increased accuracy.
Important Note: For **d > 0**, subsequent culturing must have occurred in the same culture medium as prescribed above. (*Calculators can be provided for other cell culture media and conditions. Please inquire.*)

d 0.0

2. Enter **N0**, the total number of cells (live and dead) in the evaluation cultures at time = 0.

N0 52000.0

3. Enter **N72**, the total number cells (live and dead) after approximately 72 hours of culture.

N72 305500.0

4. Enter, in units of fractional days, **T**, the actual period of time between the N0 cell count and the N72 cell count.

T 3.2

Calculate

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Human tissue stem cells counted to date

- Bone marrow hematopoietic stem cells[1]
- Mobilized peripheral blood hematopoietic stem cells[1,2]
- Umbilical cord blood hematopoietic stem cells*[1,2]
- 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)
- Liver hepatic stem cells
- Lung interstitial stem cells
- Corneal stem cells
- Amniotic membrane stem cells

***Available for preview now**

1. $CD34^+$-selected
2. Unfractionated

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KSC Counting Applications

- Quantity tissue stem cell-specific fraction in research studies
- Monitor tissue stem cell-specific fraction during expansion culture
- Optimize tissue stem cell fraction for biomanufacturing
- Certify the tissue stem cell fraction of manufactured products
- Certify the stability and viability of cryopreserved tissue stem cells *specifically*
- Certify the potency of tissue stem cell culture medium and growth factors
- Determine the tissue stem cell-specific dosage of treatment preparations
- Evaluate drug candidates for tissue stem cell-specific effects:
  Positive – expansion factors; potential healing therapeutics
  Negative – early preclinical identification of tissue stem cell toxicity that causes chronic organ failure

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For additional information:

https://asymmetrex.com/tortoise-test/

https://asymmetrex.com/rabbit-count/

https://asymmetrex.com/stem-cell-counting-center/

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