

Automated Analysis of Embryoid Bodies Enables Correlation of Size and Differentiation Potential

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Abstract

Size and shape analysis of spheroids growing in suspension culture is typically performed manually on a small fraction of the entire population. This approach is time consuming, inconsistent due to inherent person to person variability, and often does not accurately characterize the entire population. The production of embryoid bodies (EBs) is used as an *in vitro* assay to characterize the pluripotency of ESC/iPSCs and is a common intermediate during the *in vitro* differentiation of these cells into specialized cell types. The size distribution of EBs plays a significant role in the efficiency of differentiation and in production yields. The Celigo™ Colony Counting: Embryoid Body application accurately analyzes EB populations of all shapes and sizes in a non-destructive manner so that these EBs may be subsequently used in differentiation experiments. The system records whole well images of multi-well (384W to 6W) plates enabling the tracking of live EB characteristics for correlation with self-renewal, pluripotency, and final differentiation patterns. Results show that that the Colony Counting application can be used to efficiently characterize EBs derived from hiPSCs in a high throughput manner and was used to determine the optimal EB size for differentiating these cells into neurons, cardiomyocytes, and hepatocytes.

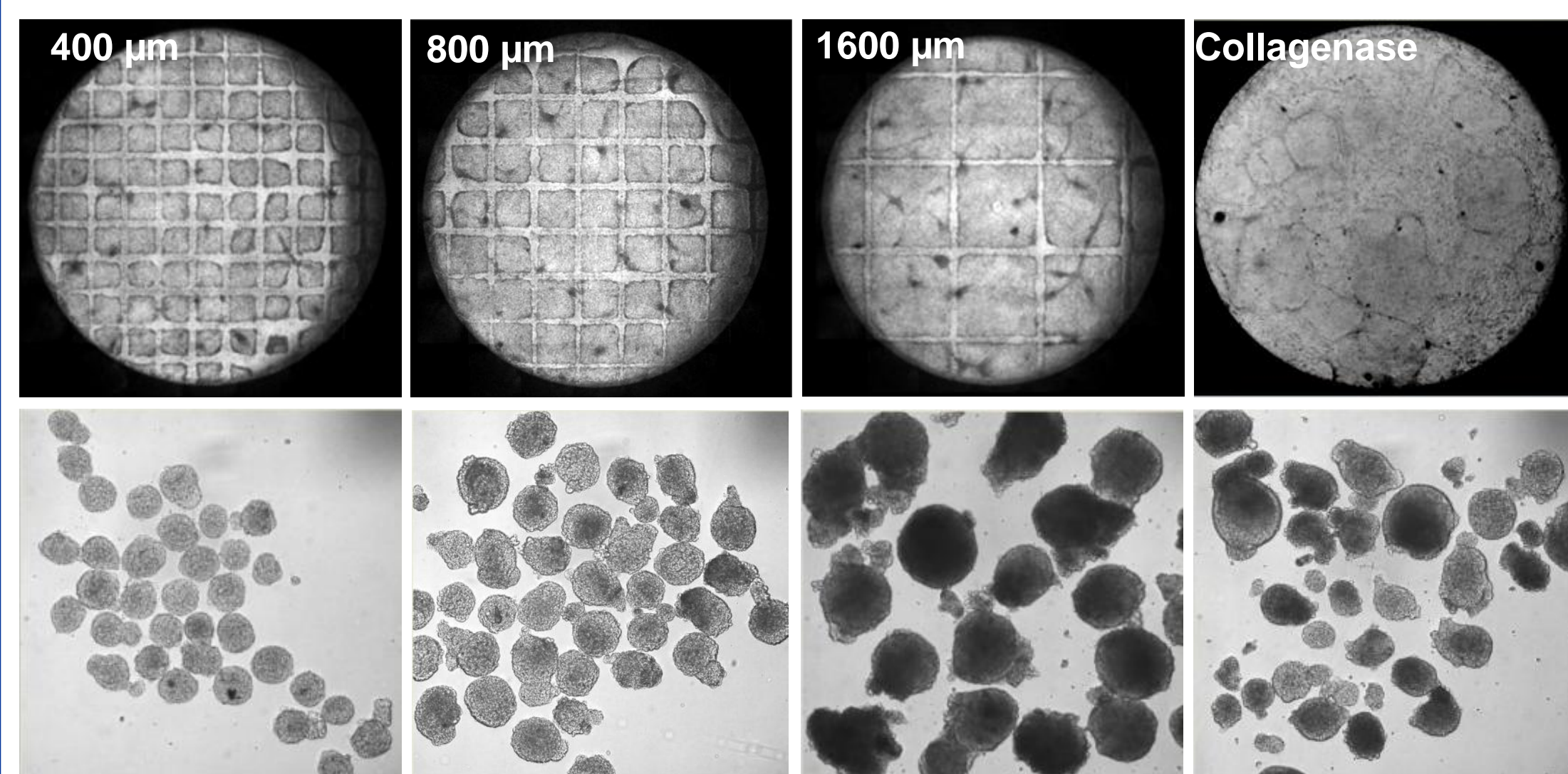
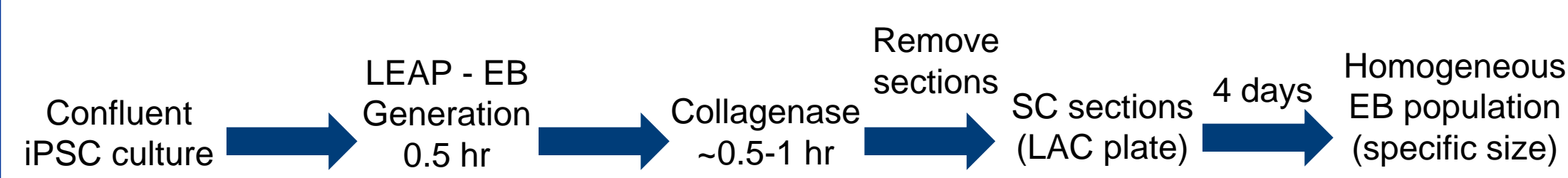
Embryoid Body Generation

LEAP™ Workstation

Typical Methodology (Collagenase)



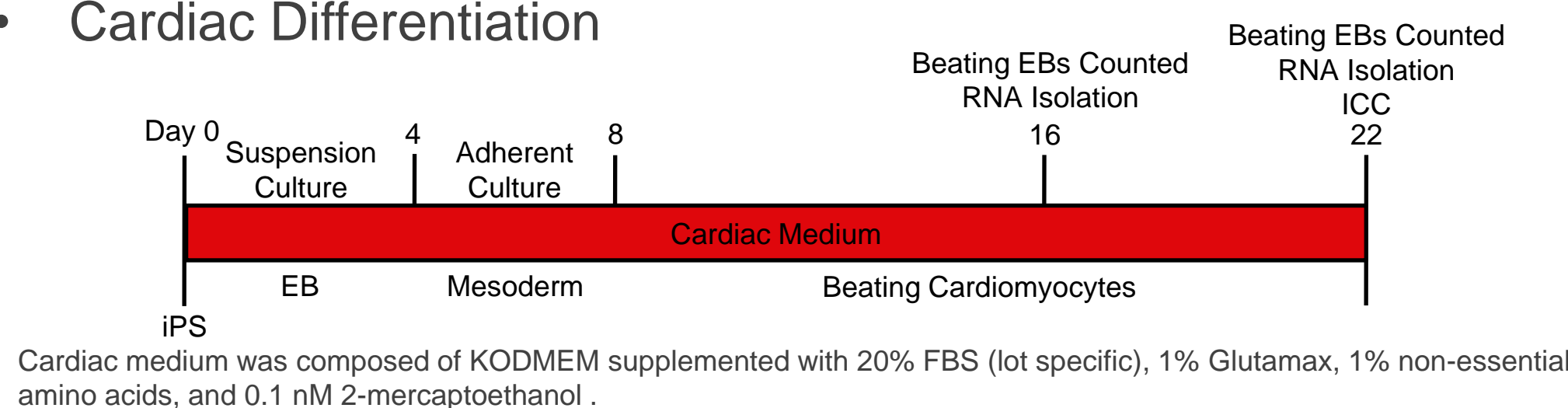
LEAP Methodology



- Typical methods for EB production result in heterogeneous EB populations
- Homogeneous EBs of specific size can be generated using the LEAP Workstation

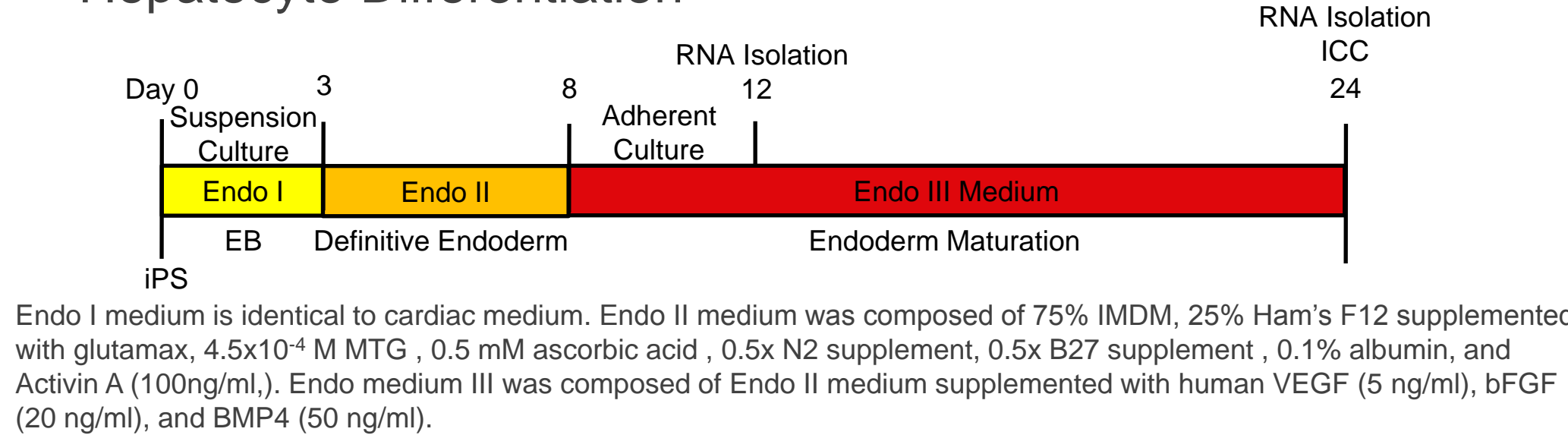
Experimental Design

- EB analysis
EB characteristics were analyzed on day 4 of suspension culture (cardiac medium)
- Cardiac Differentiation



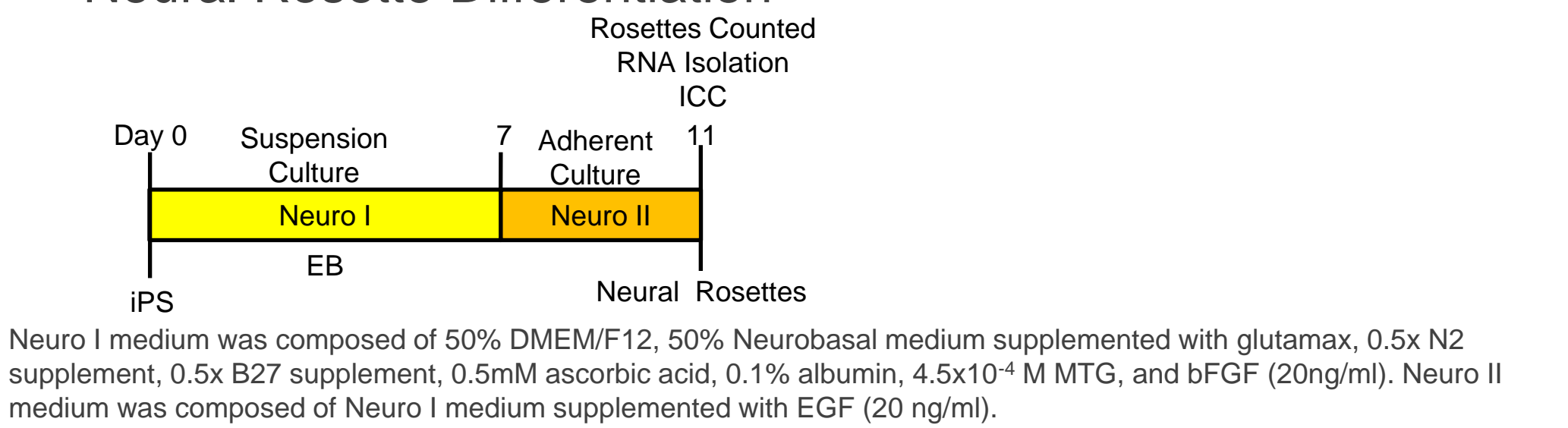
Cardiac medium was composed of KODMEM supplemented with 20% FBS (lot specific), 1% Glutamax, 1% non-essential amino acids, and 0.1 nM 2-mercaptoethanol.

- Hepatocyte Differentiation



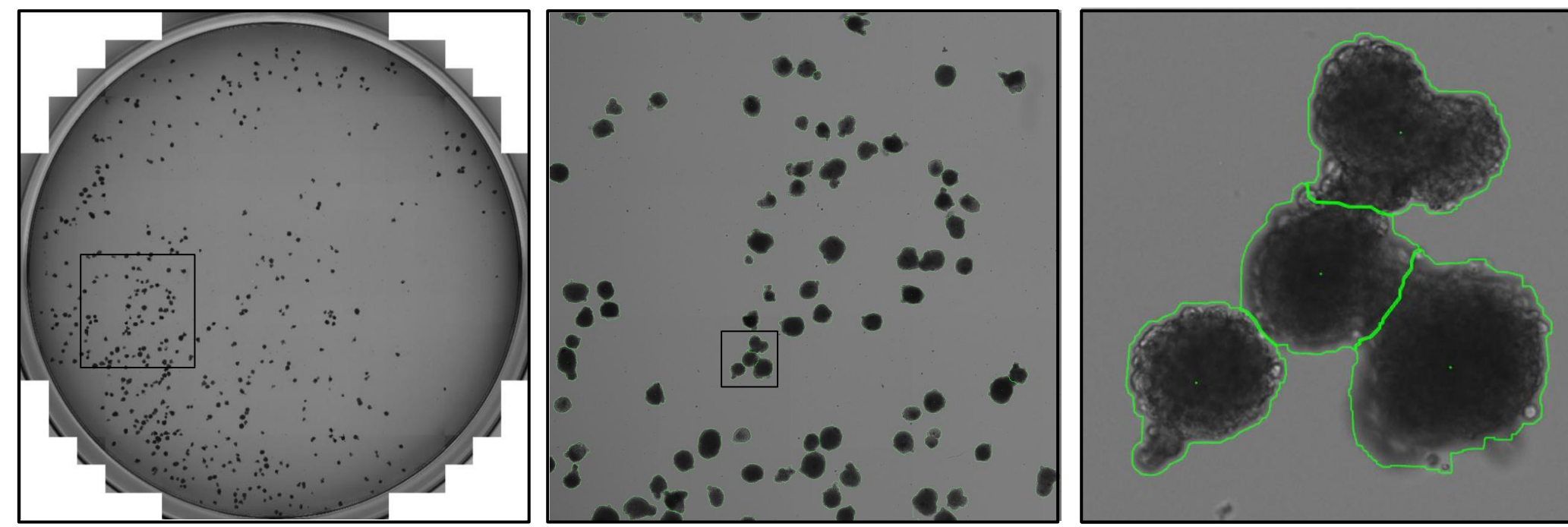
Endo I medium is identical to cardiac medium. Endo II medium was composed of 75% IMDM, 25% Ham's F12 supplemented with glutamax, 4.5x10⁻⁴ M MTG, 0.5 mM ascorbic acid, 0.5x N2 supplement, 0.5x B27 supplement, 0.1% albumin, and Activin A (100ng/ml). Endo medium III was composed of Endo II medium supplemented with human VEGF (5 ng/ml), bFGF (20 ng/ml), and BMP4 (50 ng/ml).

- Neural Rosette Differentiation

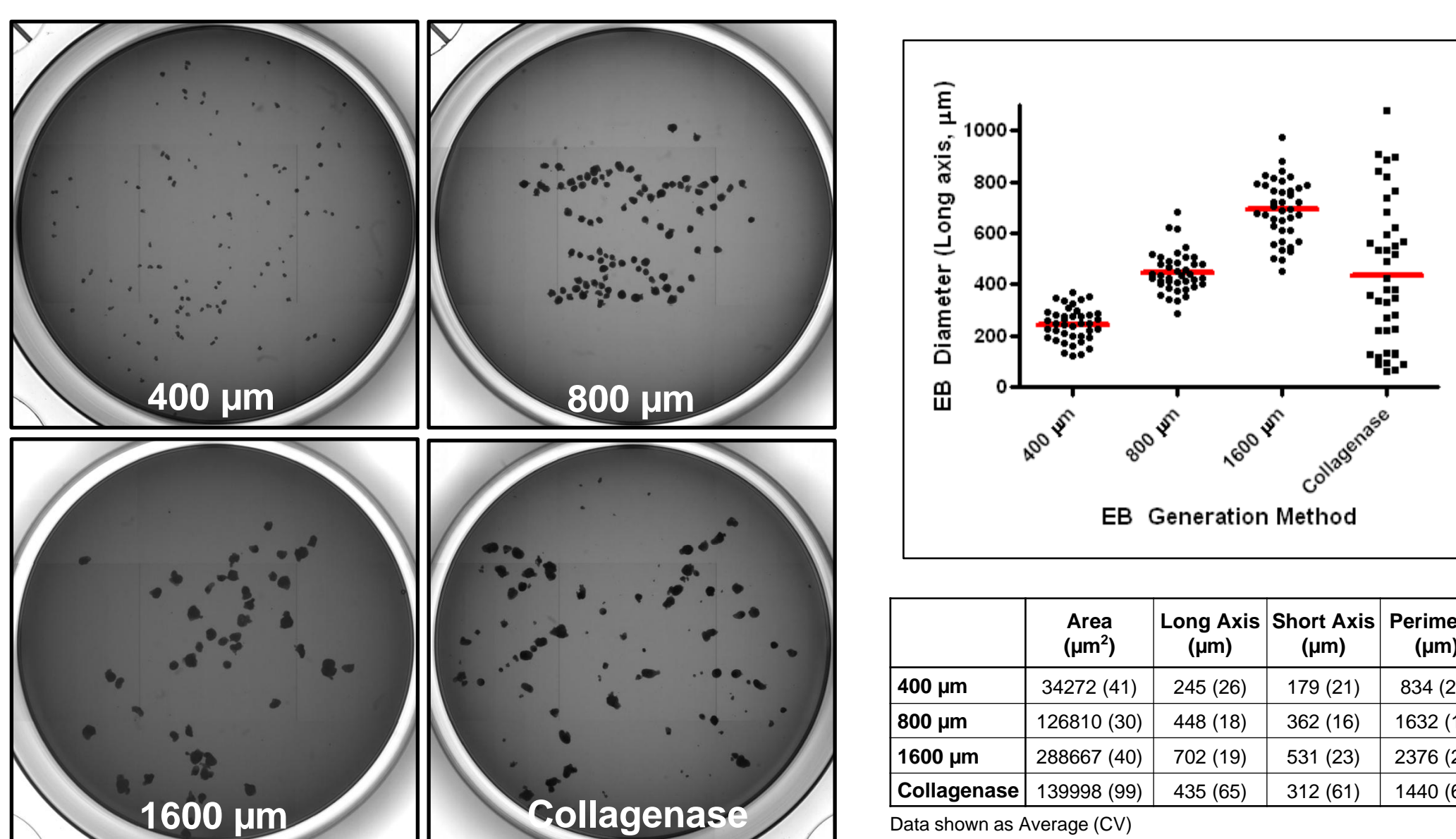


Neuro I medium was composed of 50% DMEM/F12, 50% Neurobasal medium supplemented with glutamax, 0.5x N2 supplement, 0.5x B27 supplement, 0.5mM ascorbic acid, 0.1% albumin, 4.5x10⁻⁴ M MTG, and bFGF (20ng/ml). Neuro II medium was composed of Neuro I medium supplemented with EGF (20 ng/ml).

Automated Characterization of EBs

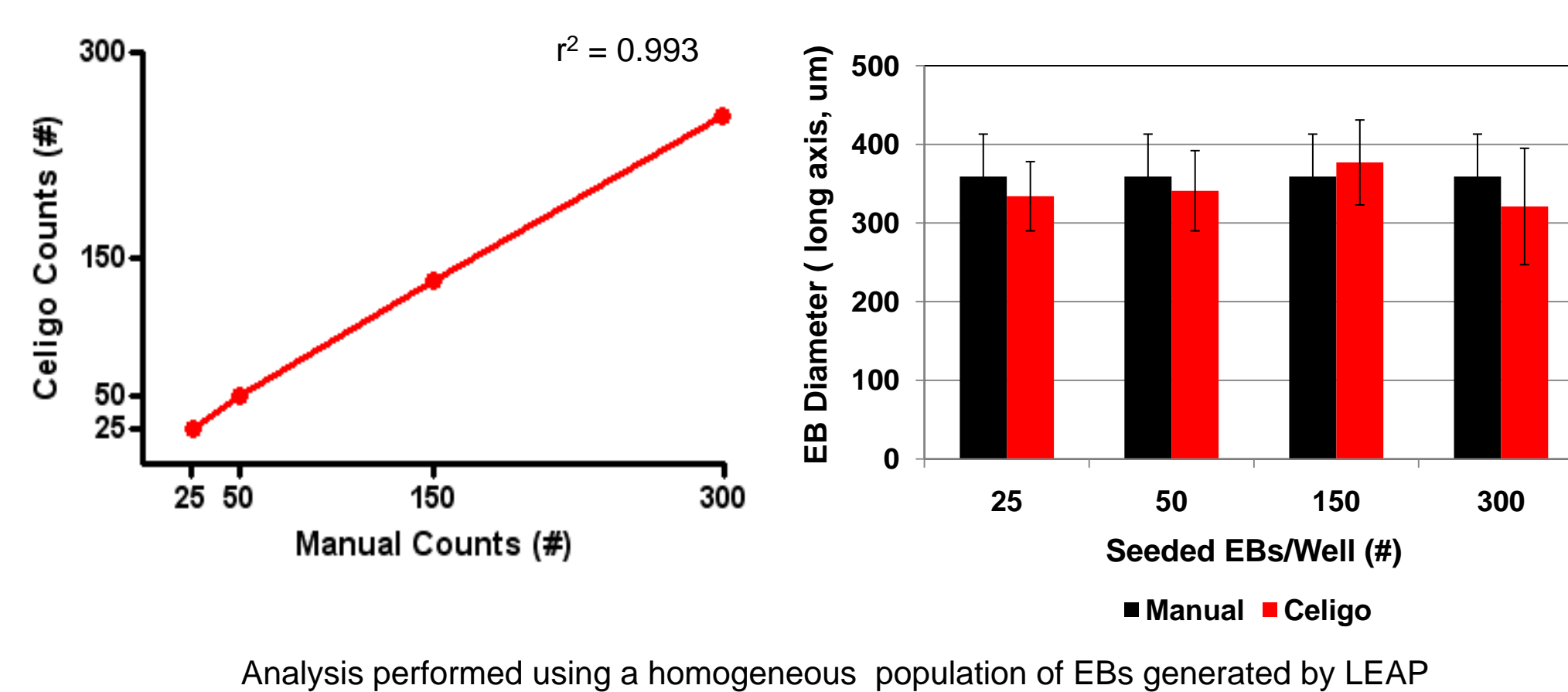


- EB populations (day 4) analyzed *in situ* using brightfield images (6 well image above; 12 well image below).



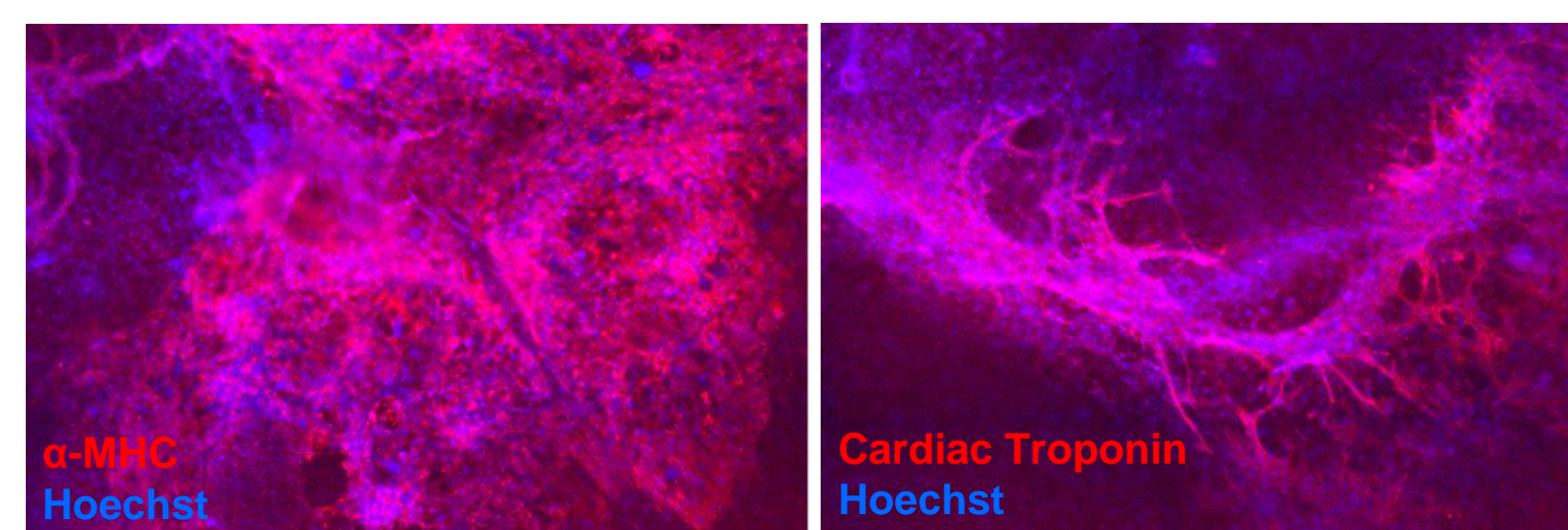
- EB characteristics were quantified using the Celigo Colony Counting: Embryoid Body application.
- Characteristics included: EB count, area, long axis diameter, short axis diameter, equivalent diameter, perimeter, form factor, smoothness, aspect ratio, EB density, and nearest neighbor distance.
- Average, standard deviation, and CV are reported for each characteristic

Brightfield EB Counting Validation

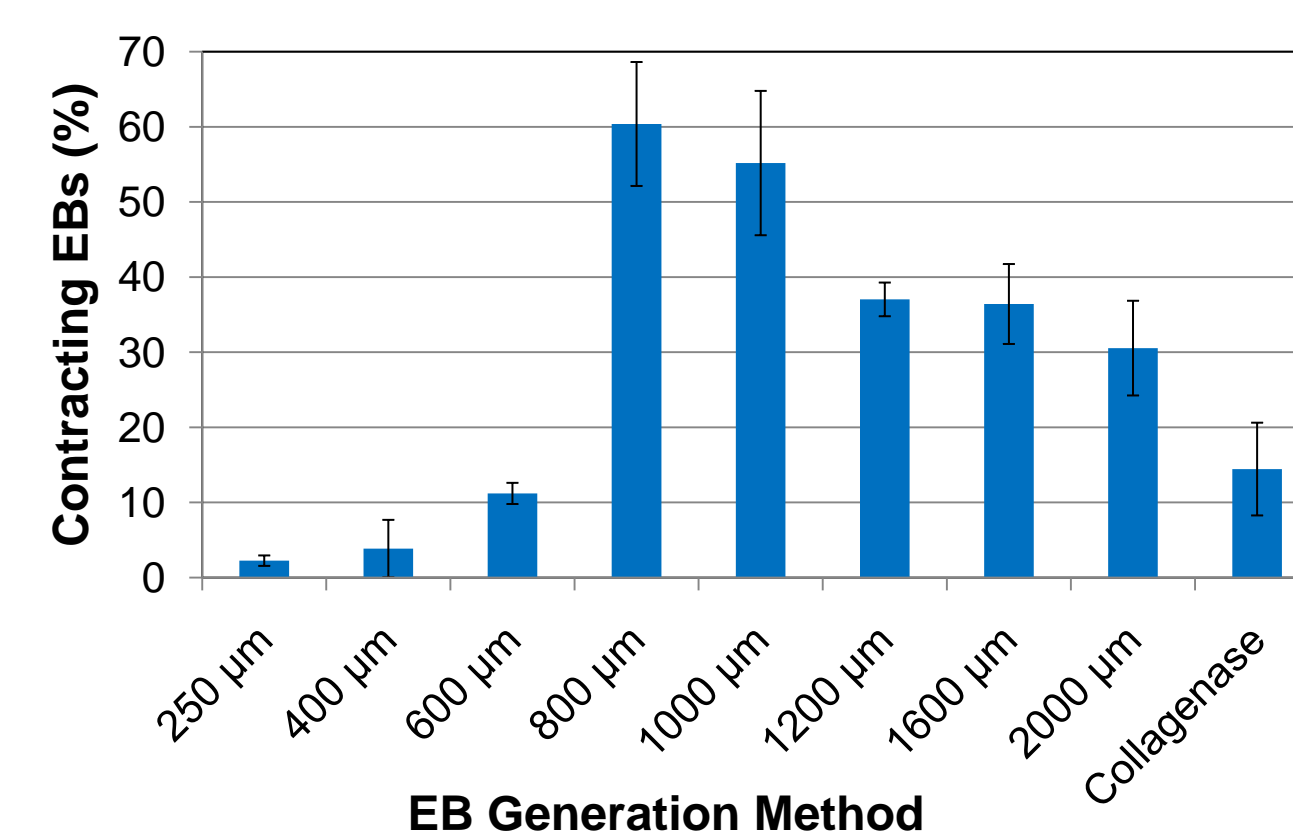


- Good correlation between automated EB counts on Celigo and manual counts using whole well images
- Good correlation between automated EB diameter measurement of the entire population on Celigo and manual measurement of 25 EBs using single EB images

Size-specific EBs Differentiate More Efficiently into Cardiomyocytes

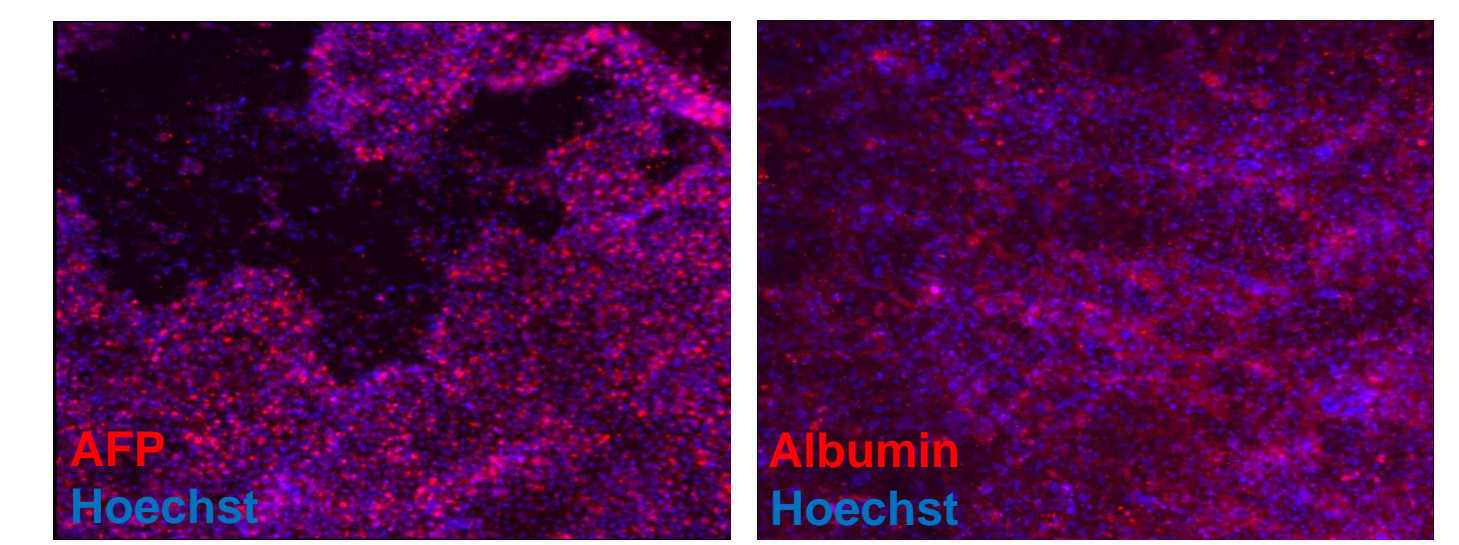


Day 22 of Cardiac Differentiation

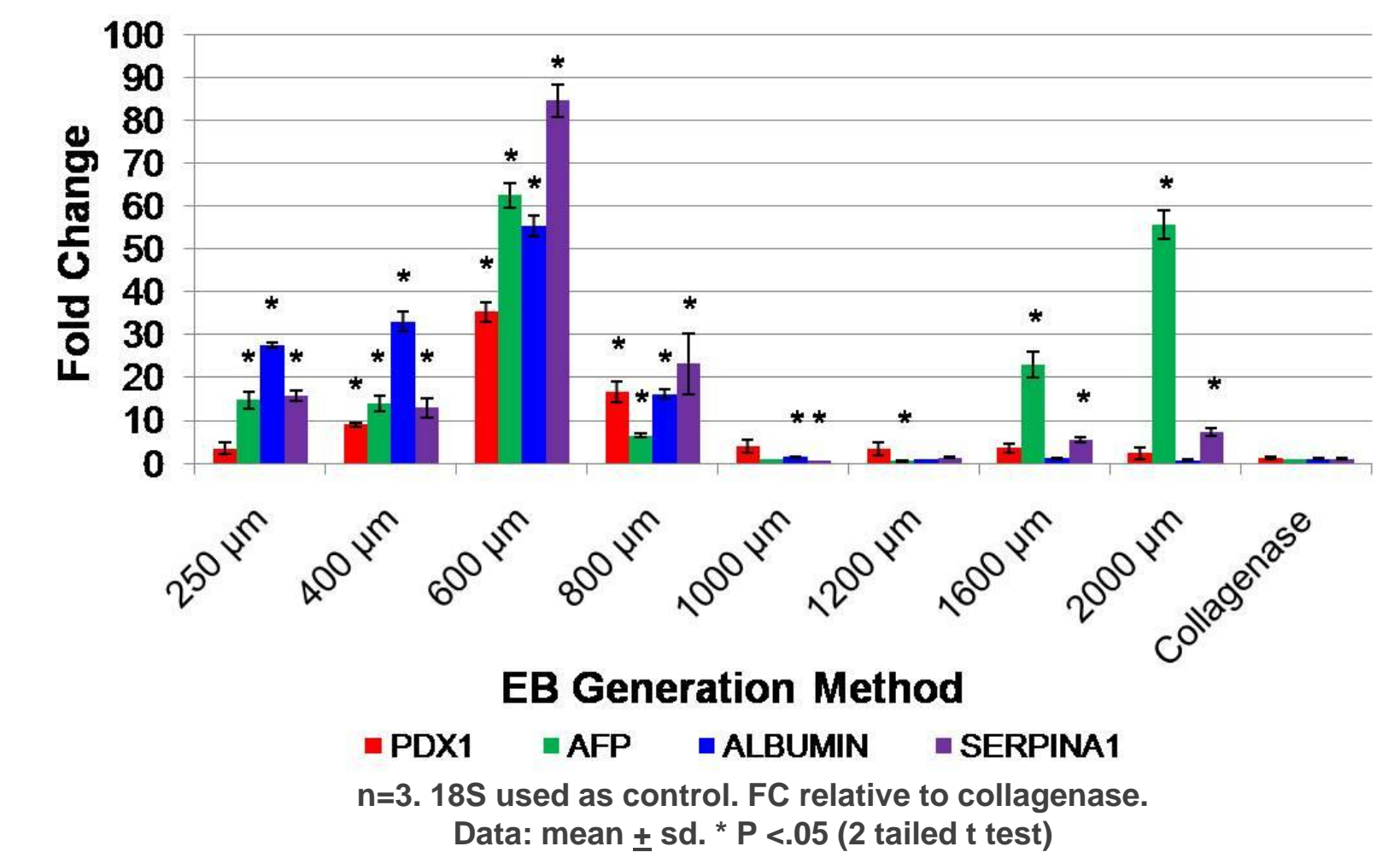


- EBs generated from 800 μm sections using LEAP hold greater differentiation potential into cardiomyocytes

Size-specific EBs Differentiate More Efficiently into Hepatocyte-Like Cells

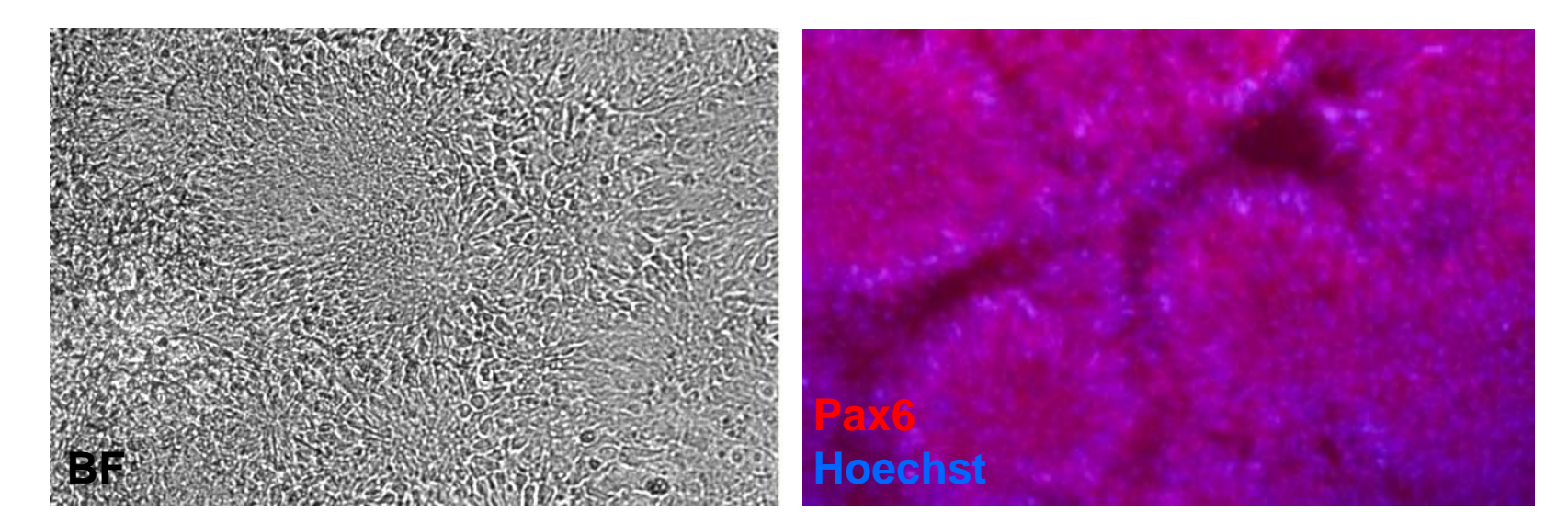


Day 24 of Hepatocyte Differentiation

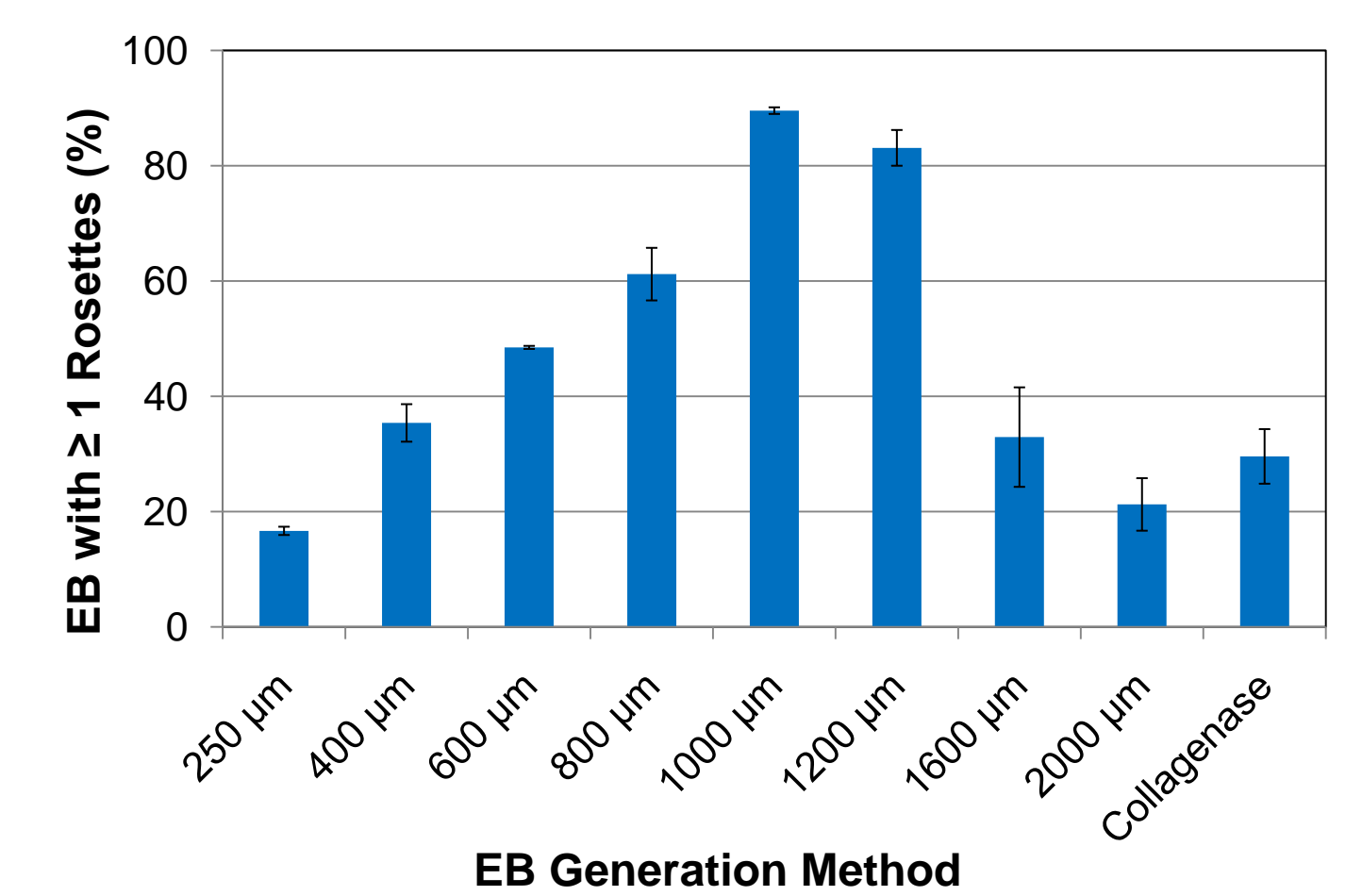


- EBs generated from 600 μm sections using LEAP hold greater differentiation potential into hepatocyte-like cells

Size-specific EBs Differentiate More Efficiently into Neural Rosettes

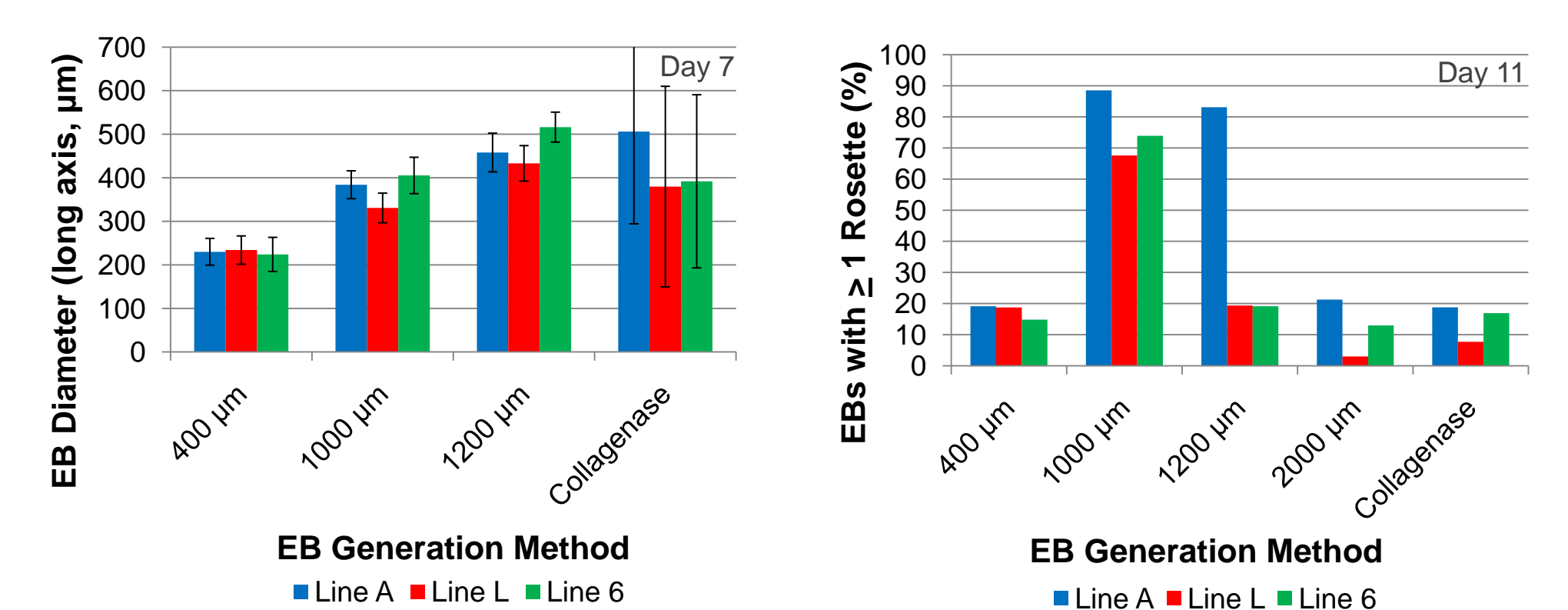


Day 11 of Neural Differentiation



- EBs generated from 1000 μm sections using LEAP hold greater differentiation potential into neural rosettes

Analysis of Varying hiPSC Lines



- EBs generated from different hiPSC lines are similar in size as analyzed by Celigo EB Counting Application
- EBs from different hiPSC lines (LEAP-1000 μm sections) hold greater differentiation potential into neural rosettes

Conclusion

These data demonstrate that the EB Colony Counting application can be used to efficiently characterize EBs derived from hiPSCs in a high throughput manner and was used to determine the optimal EB size for differentiating these cells into cardiomyocytes and neurons. In addition, data show that automated analysis of EB populations can be used to evaluate different human ES/iPS cell lines. The Colony Counting: EB application should also be able to characterize neurospheres and tumor sphere populations. The Colony Counting: Embryoid Body application on Celigo provides an efficient, reproducible, automated method for assessing the number, size, and shape of live EBs, within multiwell plates.

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