Digital PCR as a Novel Way to Assess Chimerism of Humanized Immune System Mice

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Introduction

Humanized immune system (HIS) mice contain human and murine immune cells. Analysis of chimerism in HIS mice is typically accomplished via flow cytometry of peripheral blood, but this requires collecting a 75 µL blood sample and access to a flow cytometer. Repeated bleeding can negatively affect the health of HIS mice, limiting serial analysis. Digital PCR offers a novel method for chimerism analysis, which uses much smaller blood volumes (as little as 10 µL) and can be performed on clotted or frozen samples, sample types unsuitable for flow cytometry.
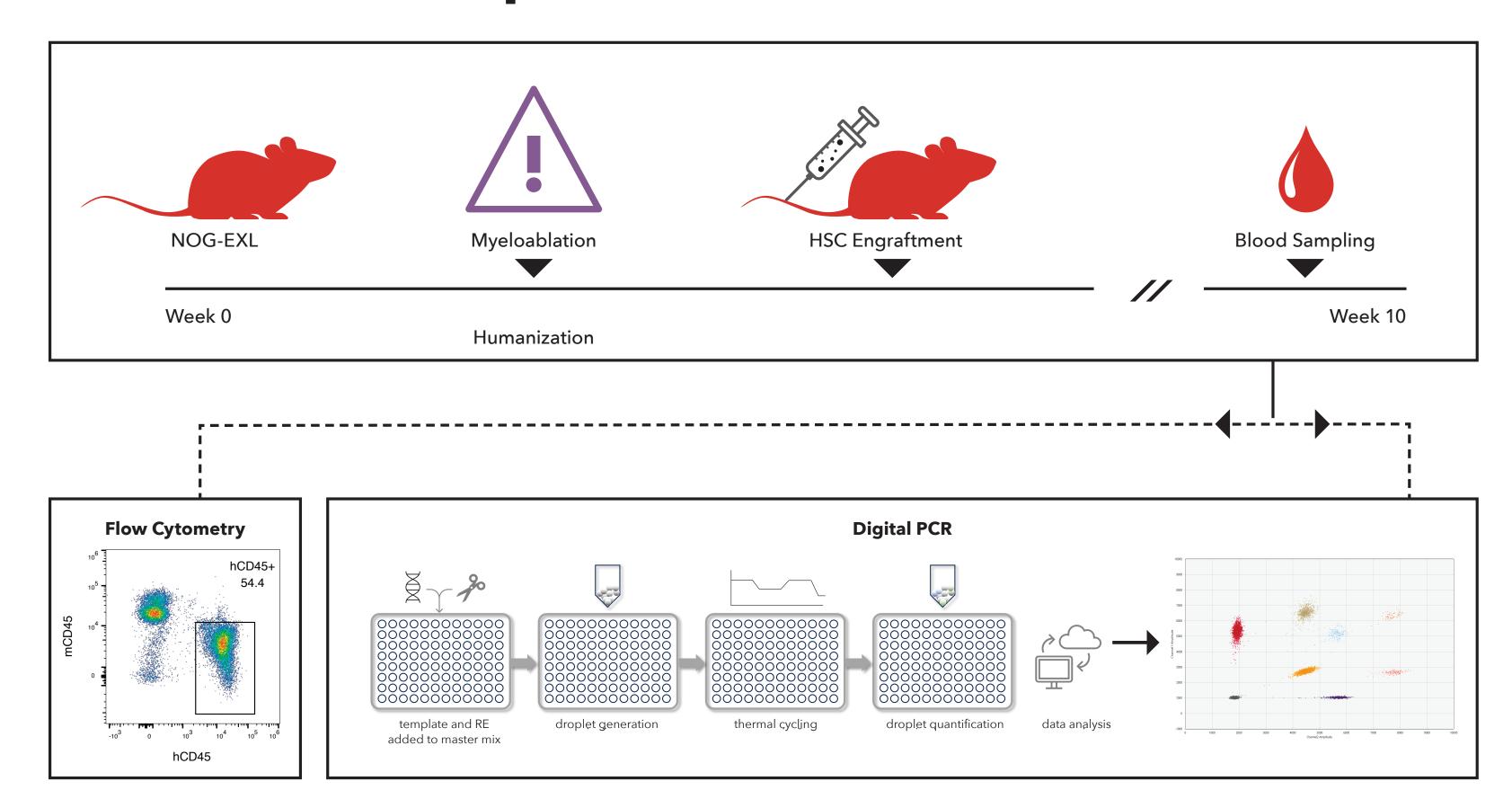
Experimental schematic

Fig 1. Mice were humanized using standard protocols, then peripheral blood was sampled at 10 weeks post-engraftment and used for parallel analysis by flow cytometry and digital PCR.

Methods

A digital PCR assay directly comparing the quantification of three human genes and one murine gene was developed. This assay was validated in the huNOG-EXL HIS mouse engrafted with CD34+ hematopoetic stem cells from 3 unique donors via blind comparison with chimerism data from flow cytometry.

Juvenile NOG-EXL females were myeloablated and engrafted with cord blood-derived human hematopoietic stem cells. At 10 weeks post-engraftment, peripheral blood was sampled for further analysis via flow cytometry at the humanization site. 75 µL of the peripheral blood was stained with an antibody panel including anti-murine CD45 (mCD45) and anti-human CD45 (hCD45) antibodies to identify murine and human immune cells, respectively. Samples were run on the Attune NxT Flow Cytometer and analyzed using FlowJo. The percent human was calculated as the percent of hCD45 positive, mCD45 negative cells of total single cells.

A remaining aliquot of whole blood (20-50 µL) was frozen in a 1.5 mL Eppendorf tube. Whole blood samples were shipped to IDEXX BioAnalytics for analysis on the Bio-Rad QX200 Droplet Digital PCR System. Total nucleic acids were extracted from each sample following a standard protocol. Multiplexed droplet digital PCR was performed to quantify human SRSF4, human SF3A1, human IPO8 and mouse B2m gene targets. Bio-Rad QX Manager software applied necessary Poisson statistics to determine fractional abundance of each human gene compared to mouse gene present within the sample. This method utilizes the proportion of positive partitions relative to the total number of partitions and their known volume to compensate for the fact that more than one amplicon may exist within a single partition.

Digital PCR results were then paired to flow cytometry data for each individual animal. Linear regression and mean of difference was calculated and compared by GraphPad Prism.

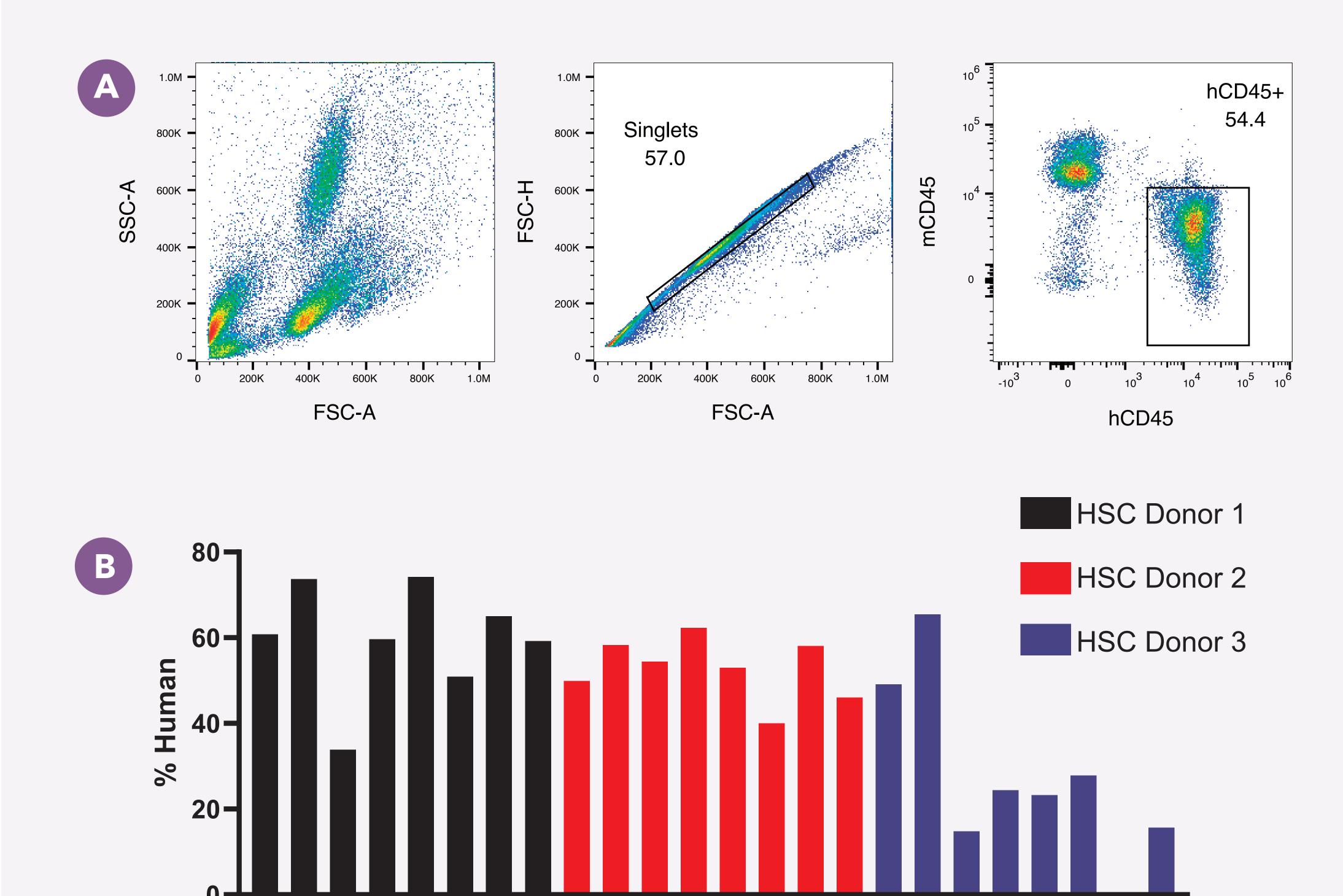## Results

Flow Cytometry Gating and Results

Fig 2.
A) Example gating strategy for human chimerism by flow cytometry. Single cells were gated based on forward scatter height (FSC-H) vs forward scatter area (FSC-A) to exclude doublets and cell debris. Human cells were identified as hCD45 positive and mCD45 negative.
B) Percent human as assessed by flow cytometry. % Human was calculated as the percentage of human cells of the total single cells. Black bars represent animals engrafted with HSC donor 1, red bars represent HSC donor 2, and blue bars represent HSC donor 3.

Fractional abundance of all three human genes provides consistent results across HIS samples
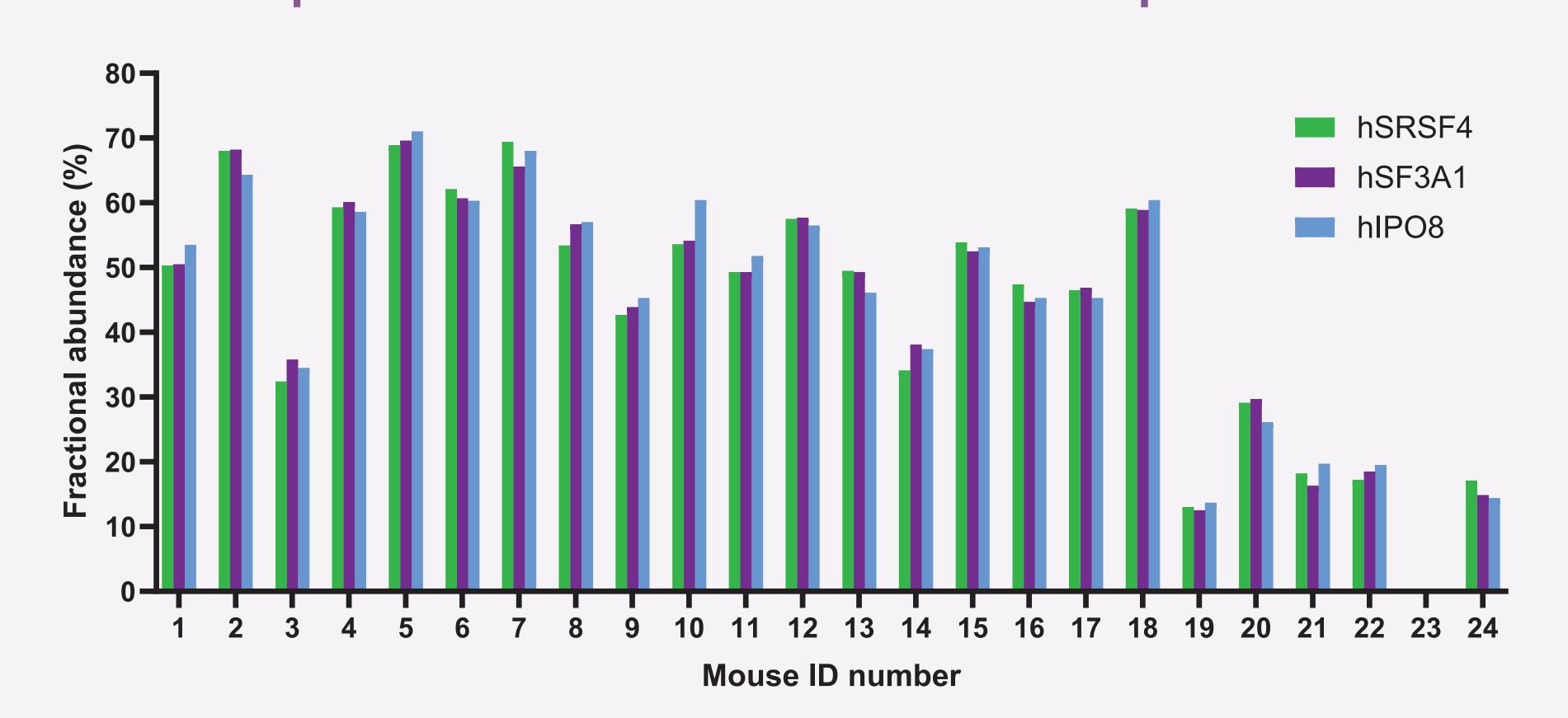

Fig 3. Fractional abundance of each of three human gene targets is represented for each individual mouse. Droplet digital PCR was performed on individual samples in multiplexed format. Each of three human gene targets were individually compared to the same mouse gene target. Poisson statistics were applied by Bio-Rad QX Manager software to report fractional abundance plotted as percent (%) human.

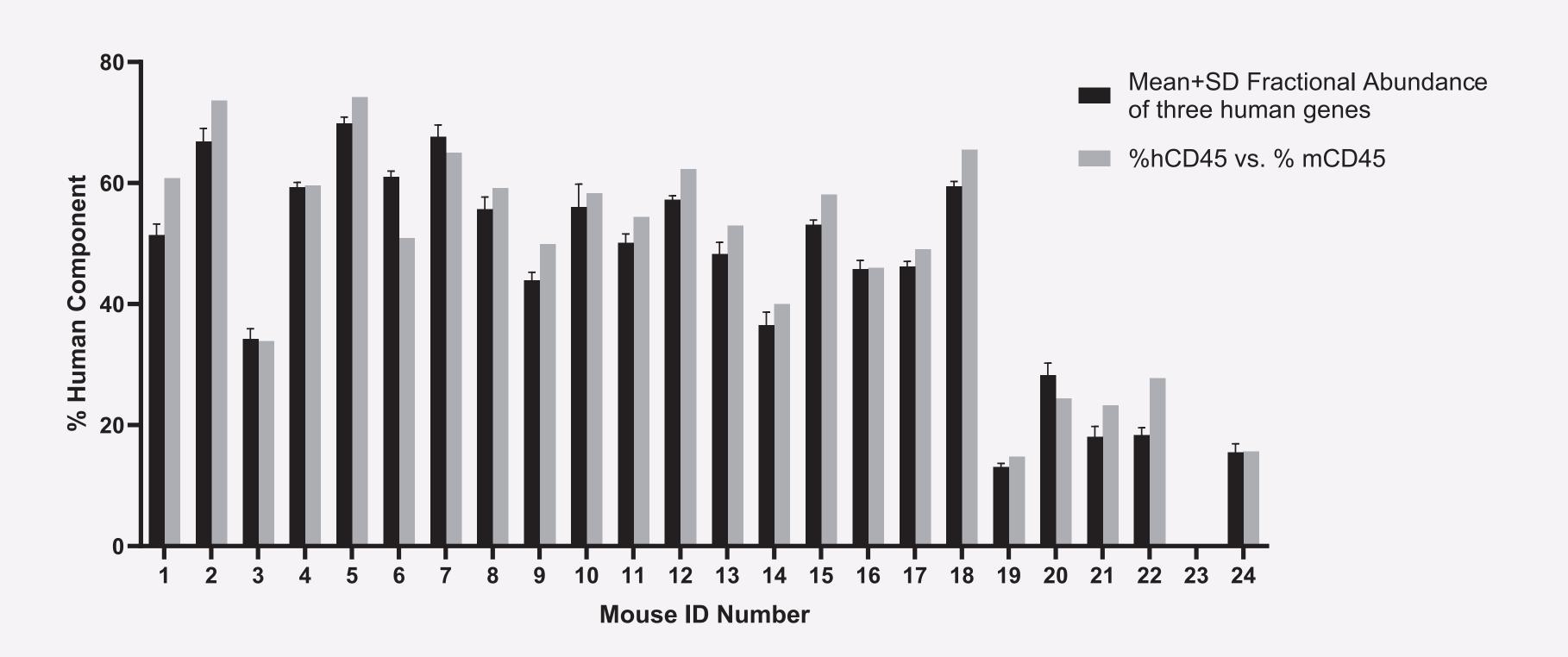### Human gene measurements align with flow cytometry results

Fig 4. Percent human component of whole blood, as assessed by droplet digital PCR is compared to flow cytometry for individual HIS animals. Black bars represent the mean plus standard deviation of calculated fractional abundance of three human gene targets. Gray bars represent the % human component as determined by flow cytometry comparison of %hCD45 compared to %mCD45.

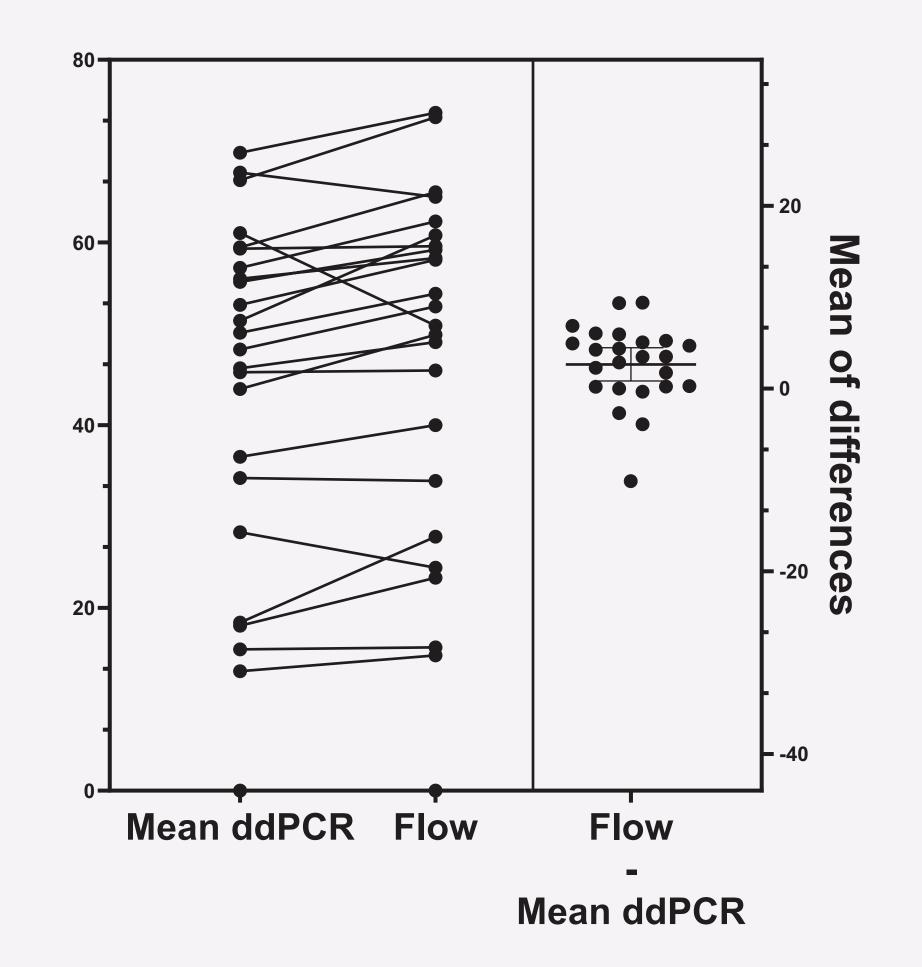### Comparison between sampling methods

Fig 5. Estimation plot comparing each animal's paired testing results from the ddPCR method and flow cytometry. The mean of differences between the two methods is shown on the right, plotted with a 95% CI. The mean difference is 2.65% with flow cytometry reporting a higher percentage of human component. The correlation coefficient (r) was 0.9762, p value (one tailed) <0.0001 indicating significant agreement between the two methods.

Fractional abundance correlates tightly with chimerism as measured by flow cytometry
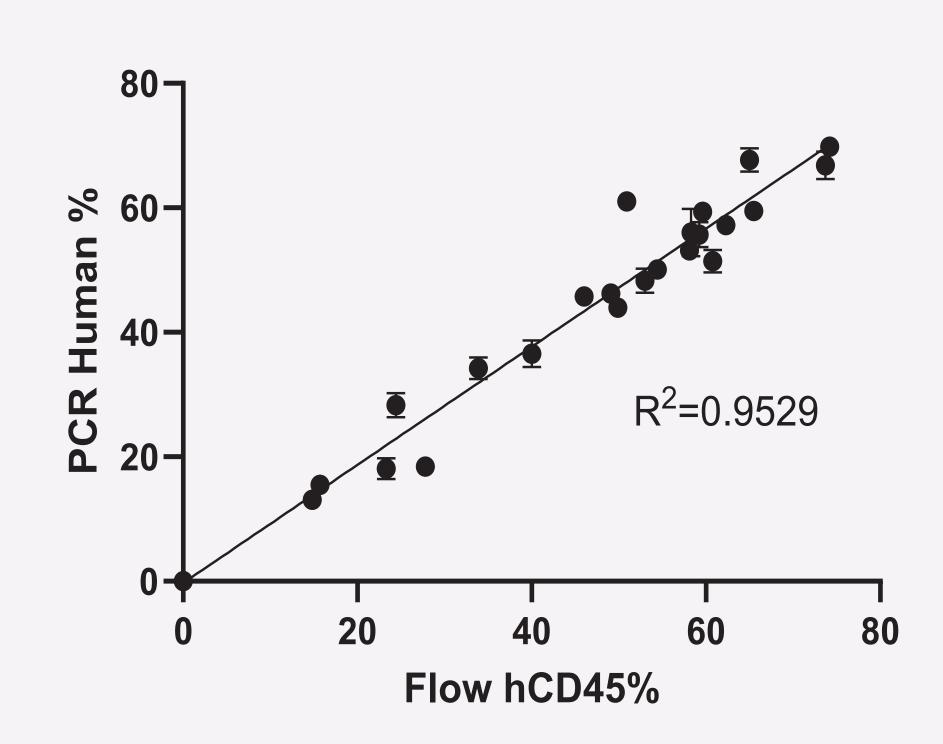

Fig 6. Linear regression of flow cytometry data versus the mean of three human gene measurements of fractional abundance as measured by digital PCR. R^2=0.9529

Conclusions

- The digital PCR assay shows excellent correlation for all three human genes against chimerism as measured by flow cytometry of the peripheral blood at 10 weeks post engraftment

- This assay represents a new, user-friendly, rapid tool for the analysis of HIS mice and may permit serial chimerism measurement using as little as 10 µL whole blood

- This assay may be useful to investigate other mouse-human chimera models, including mice harboring functional human hepatocytes, tumors, and other cells

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