



Determination of TREC Copy Numbers from Dried Blood Spots using Digital PCR

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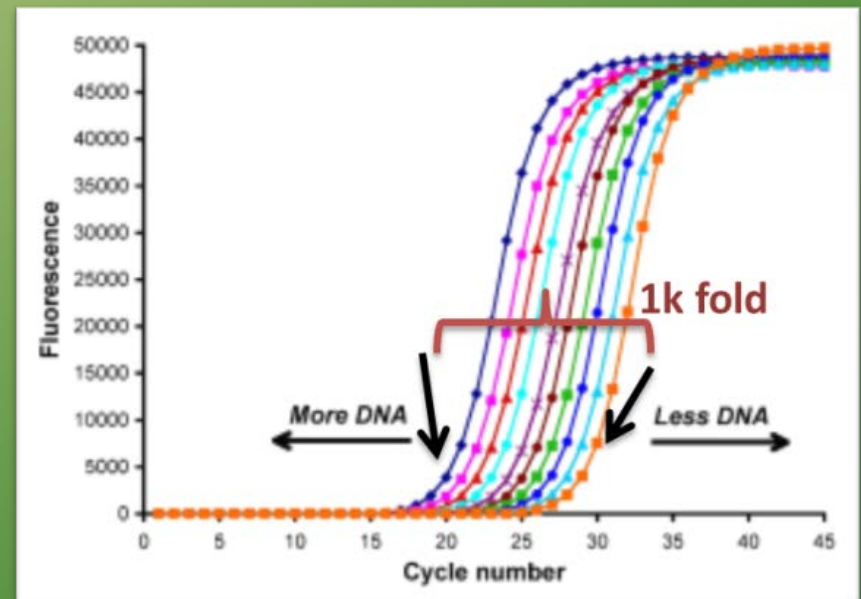
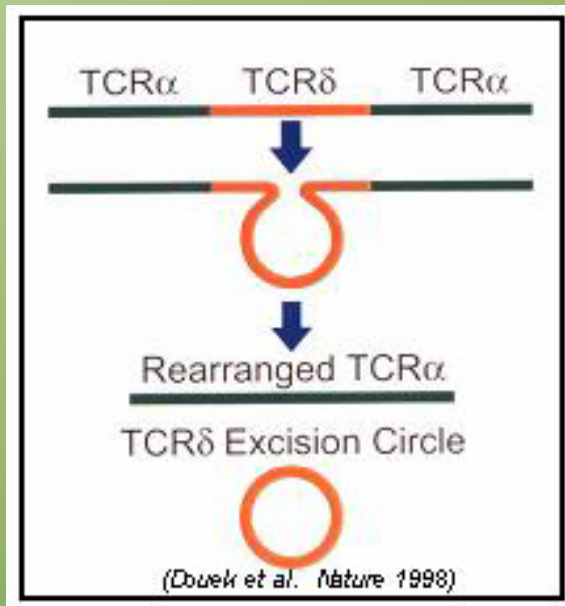
**State Hygienic Laboratory at
The University of Iowa**

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Severe combined immunodeficiency (SCID), T-cell receptor excision circles (TRECs), and polymerase chain reaction (PCR)

- SCID caused by absent/reduced T-cells and subsequent impaired B-cell function
- SCID screen based on presence/absence of TREC DNA; functional T-cell genomic marker
- Real time PCR used to detect TRECs

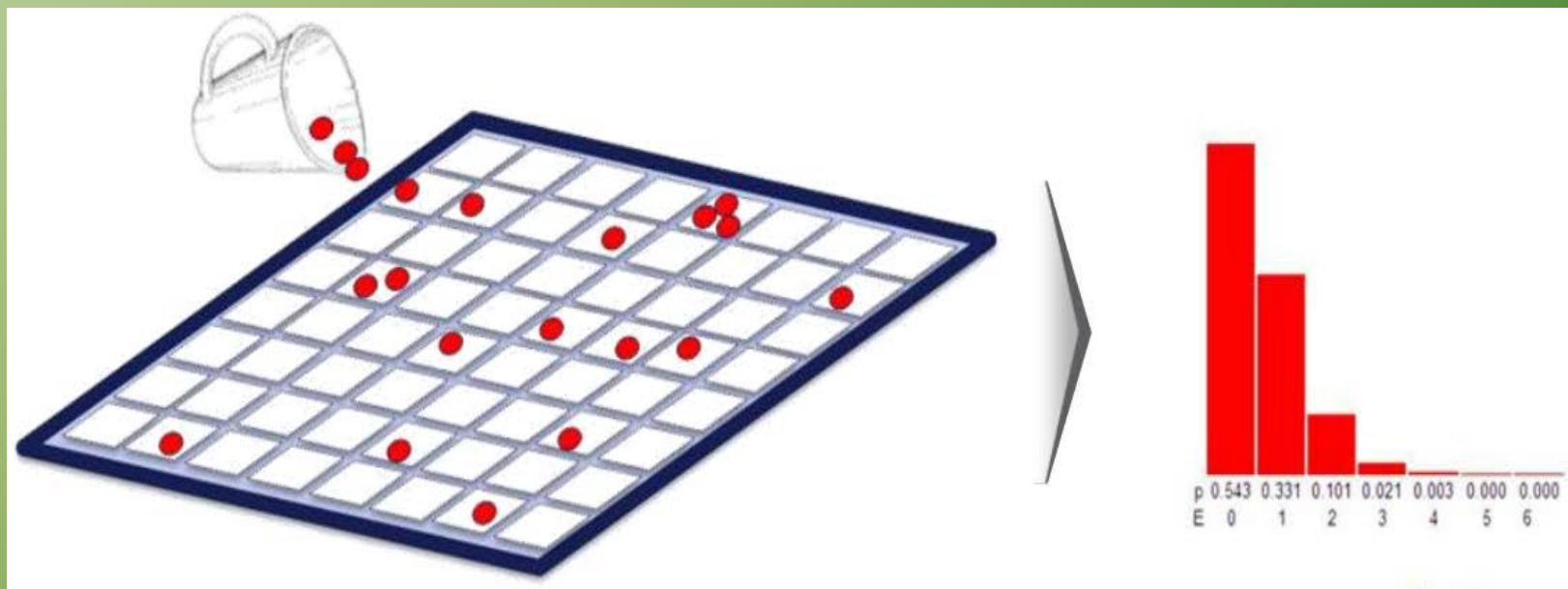


Digital PCR advantages

- Eliminate error in application of standard curve
- Accurate quantification relies on similar efficiency of samples and standards
- No “standard” standard-each lab reports different copy numbers
- Digital PCR may provide means to calculate TREC copies based on Poisson distribution which does not require a standard curve-only ratio of positive to negative wells

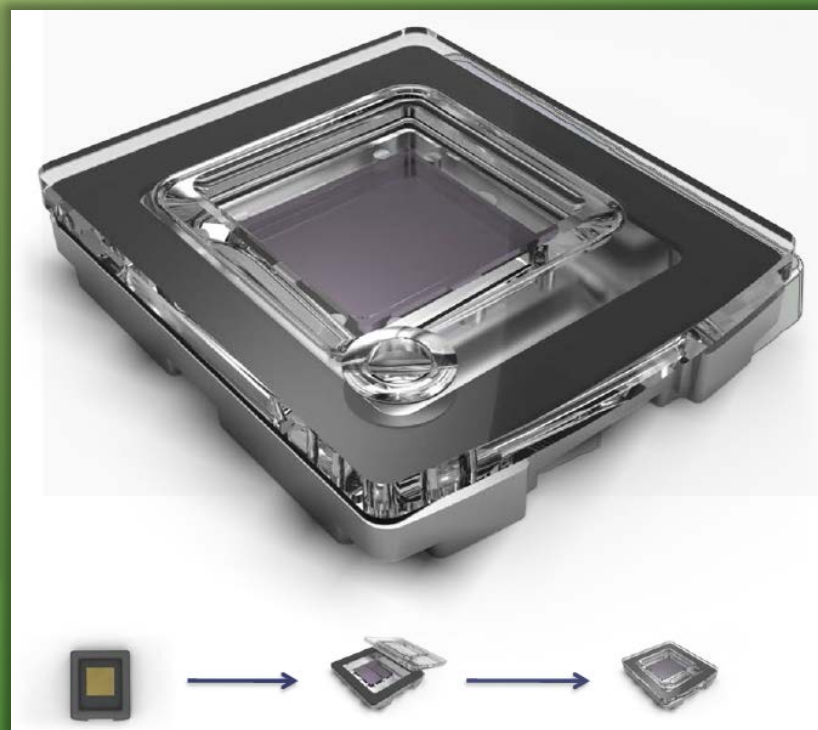
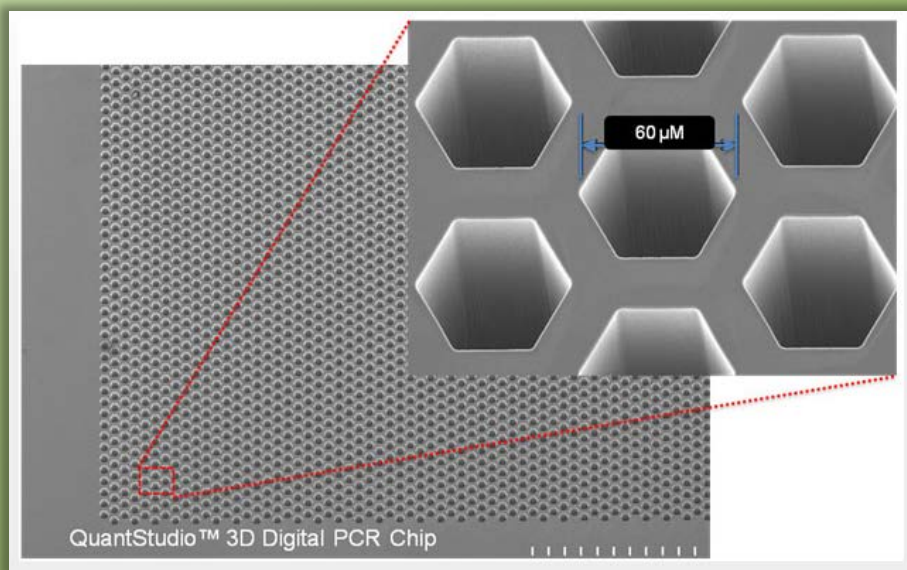
Digital PCR

- Random assortment of DNA during reaction preparation
- Poisson distribution describes the probability of a reaction containing “n” target molecules and also corrects for reactions containing more than one target molecule

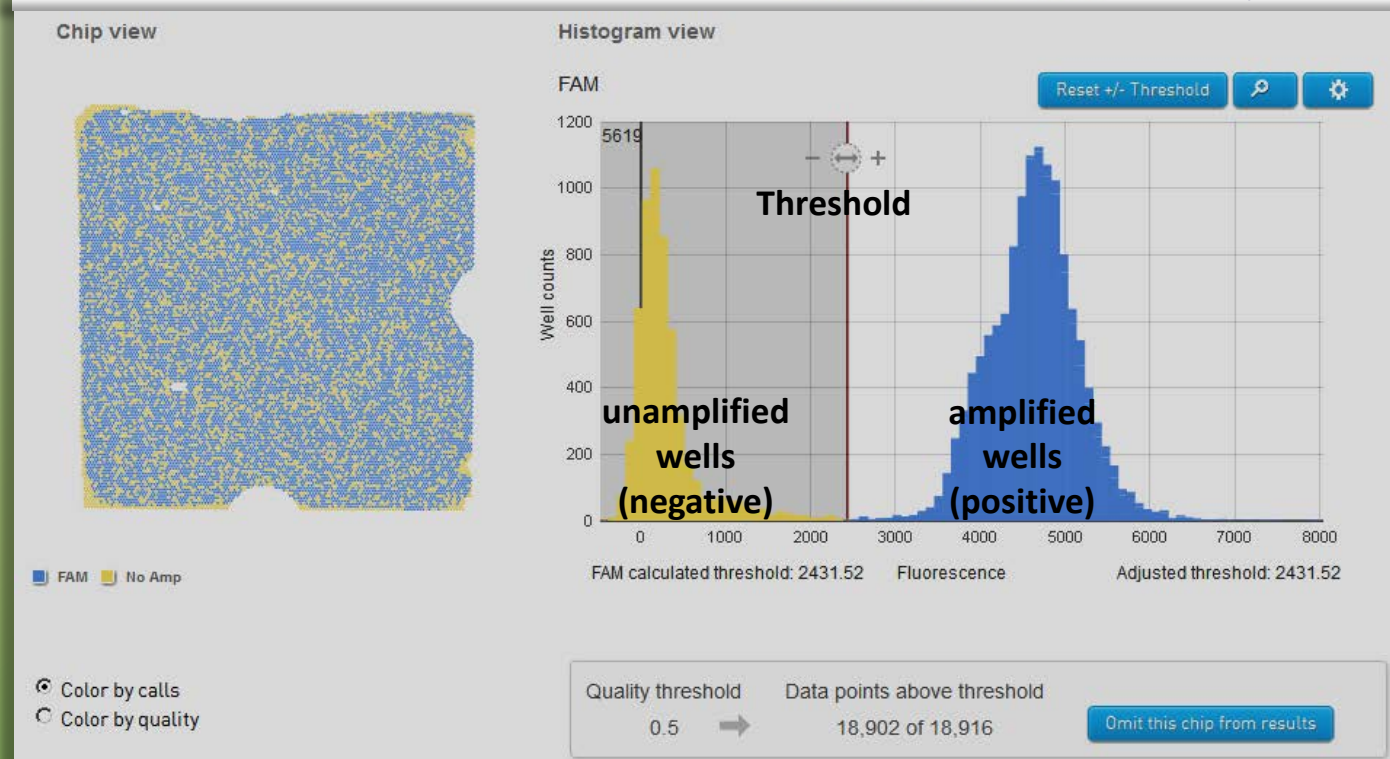


Digital PCR

- QuantStudio 3D digital PCR platform
- Each 10 mm² chip contains 20,000 individual reaction wells
- Large number of reactions equates to greater statistical power



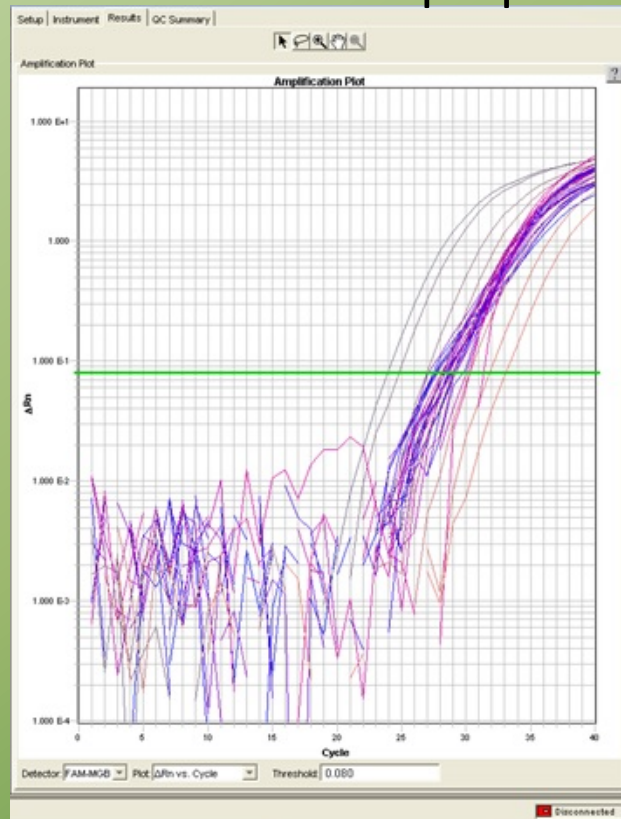
Digital PCR



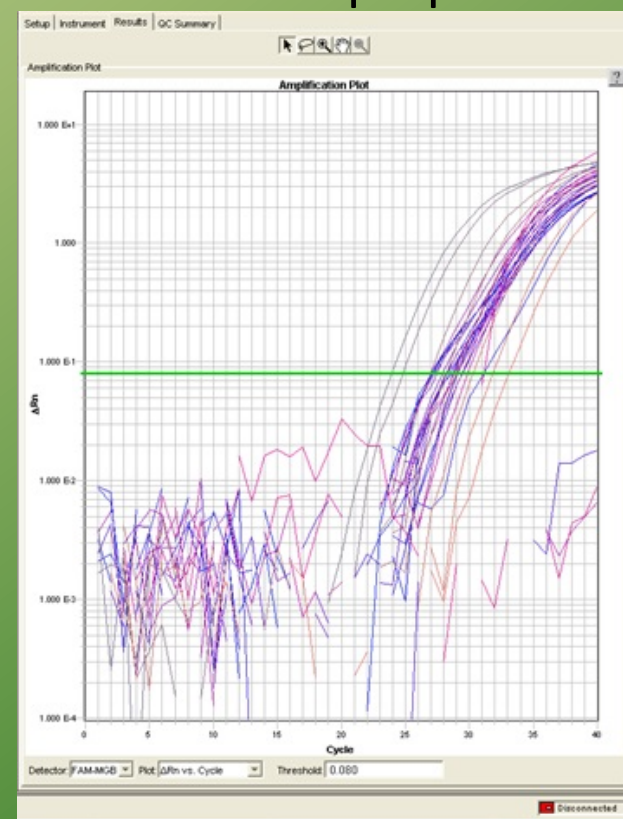
qPCR of DNA extracts

- Amplification of DNA eluted using varying DNA extraction methods indicated similar performance

DNA elution prep

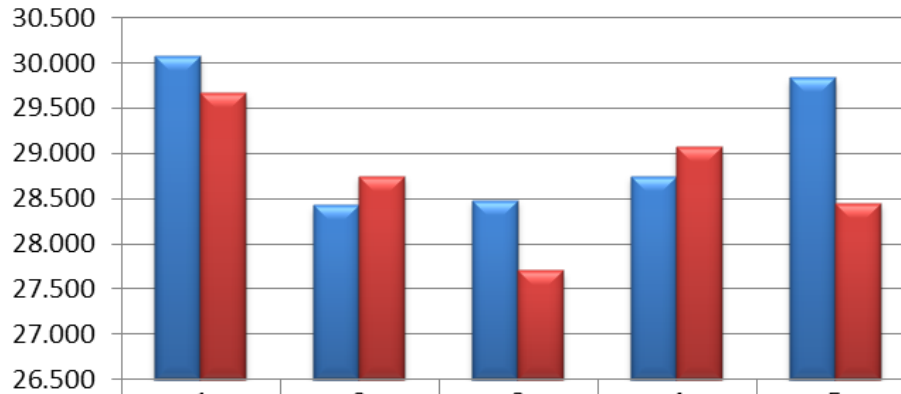


In situ prep



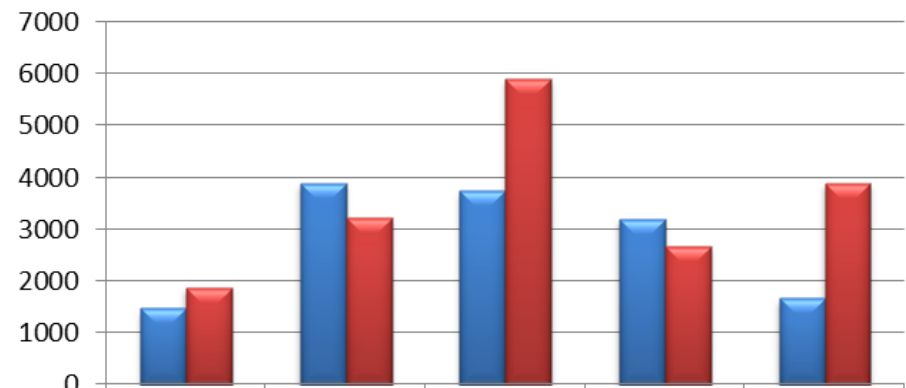
qPCR of DNA extracts

TREC Cqs



	1	2	3	4	5
DNA elution	30.070	28.425	28.476	28.743	29.840
In situ	29.676	28.749	27.706	29.067	28.448

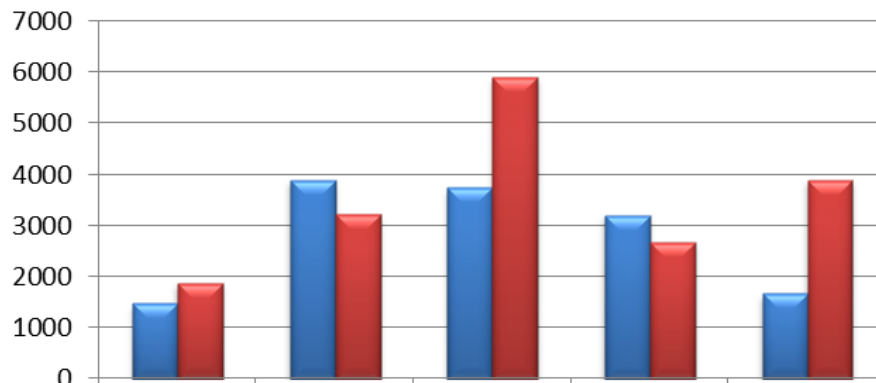
TREC copies (per μ l blood)



	1	2	3	4	5
DNA elution	1477	3890	3748	3206	1665
In situ	1871	3219	5916	2677	3890

qPCR of DNA extracts

TREC copies (per μ l blood)



■ DNA elution	1477	3890	3748	3206	1665
■ In situ	1871	3219	5916	2677	3890

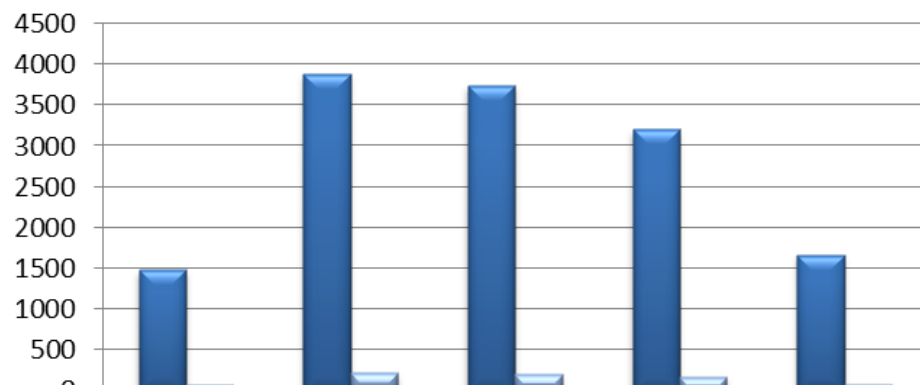
Std curve
1

Slope	-3.872
Y-Intercept	39.191
R ²	0.998

Std curve
2

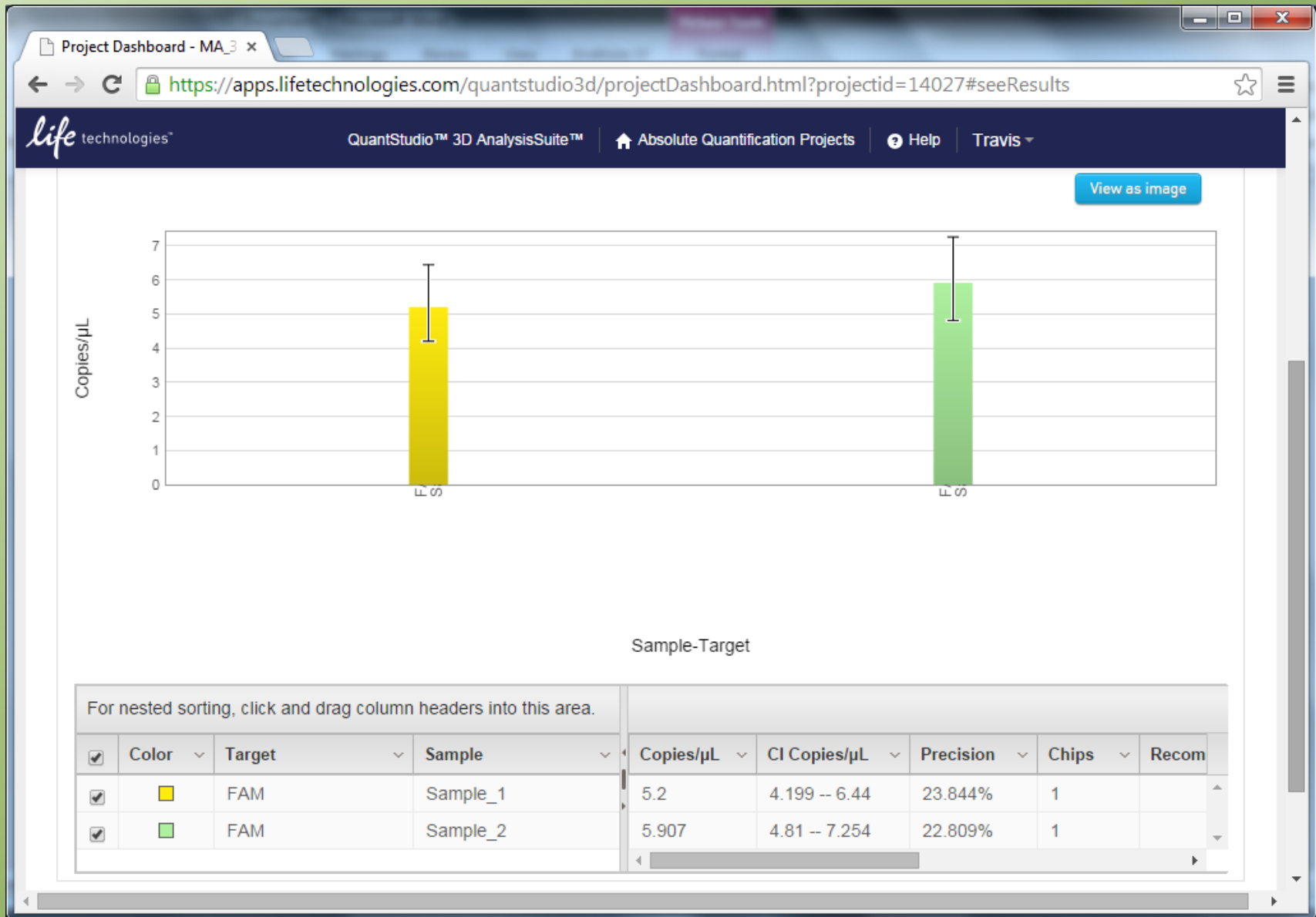
Slope	-3.469
Y-Intercept	36.523
R ²	0.998

TREC copies (per μ l blood)

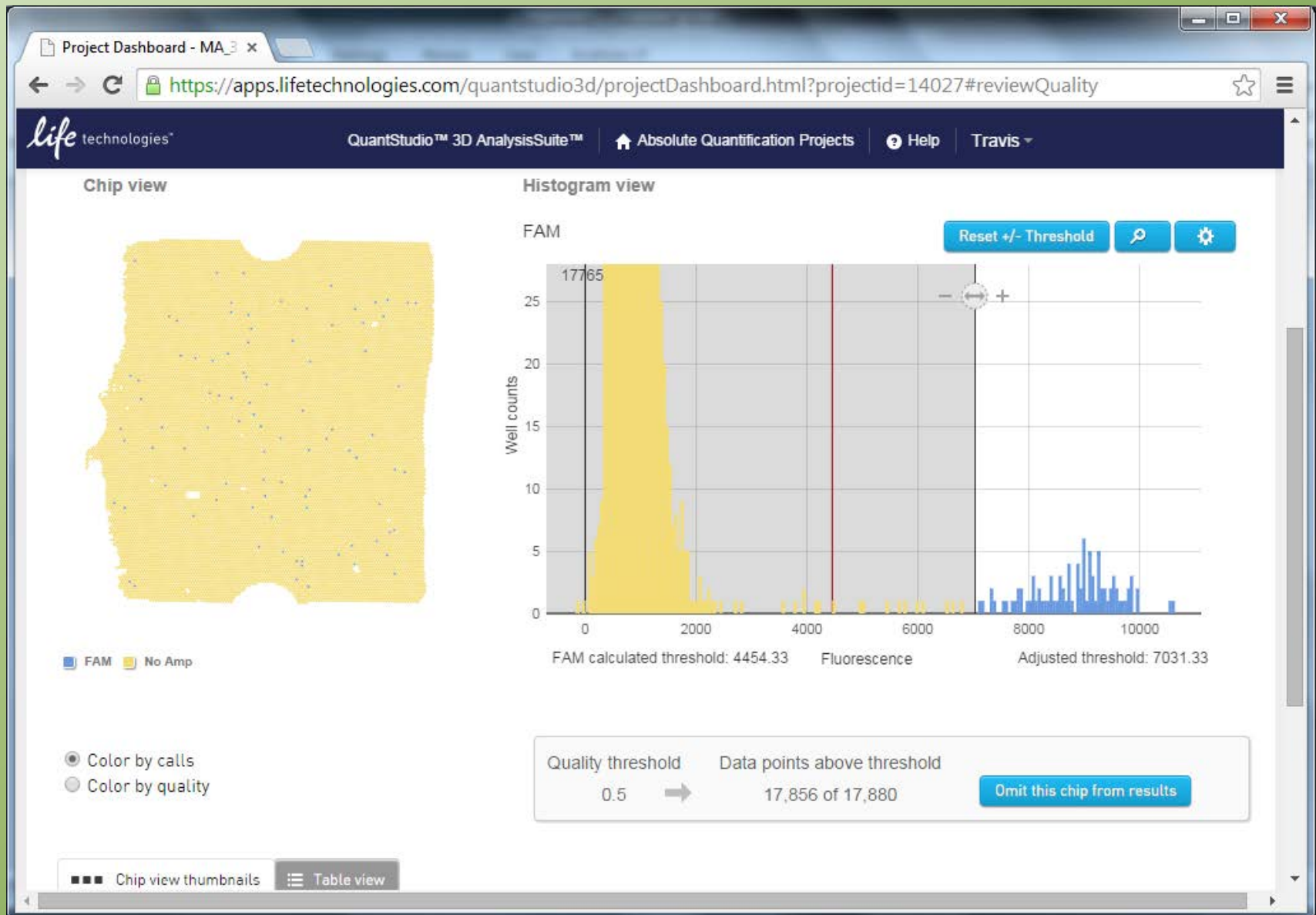


■ Std curve 1	1477	3890	3748	3206	1665
■ Std curve 2	73	218	204	175	85

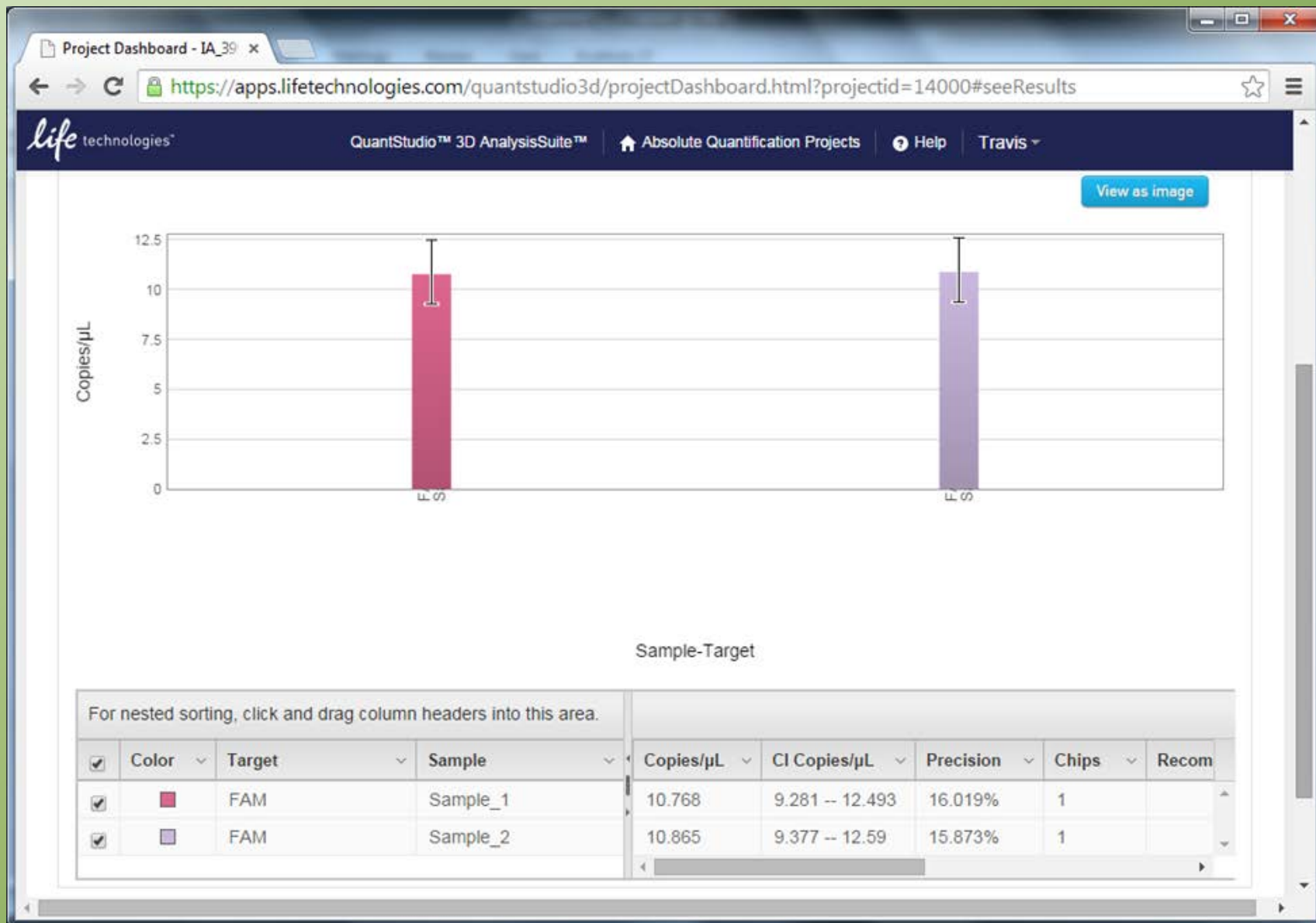
Digital PCR data-DNA elution sample



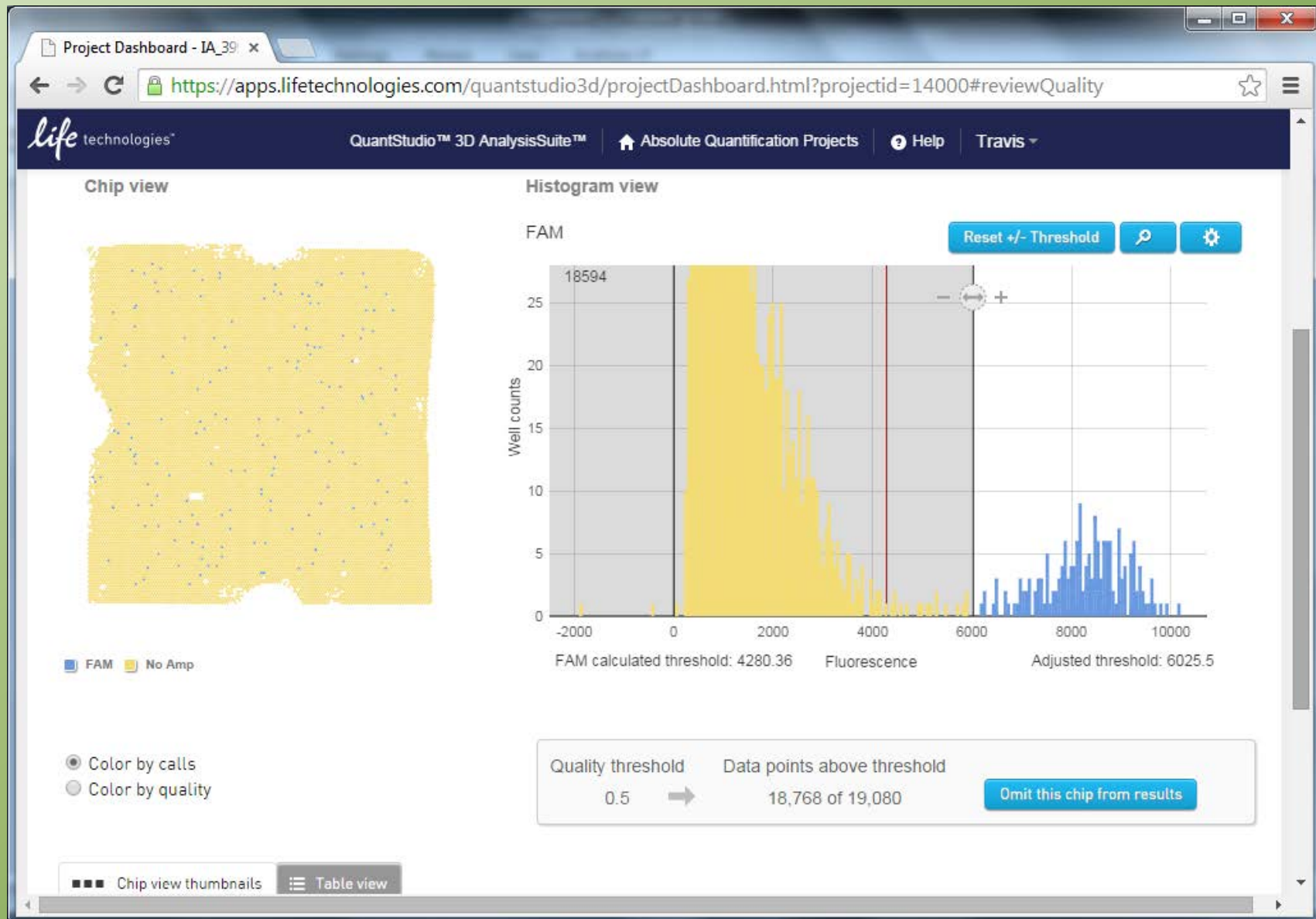
Digital PCR data-DNA elution sample



Digital PCR data-*in situ* sample

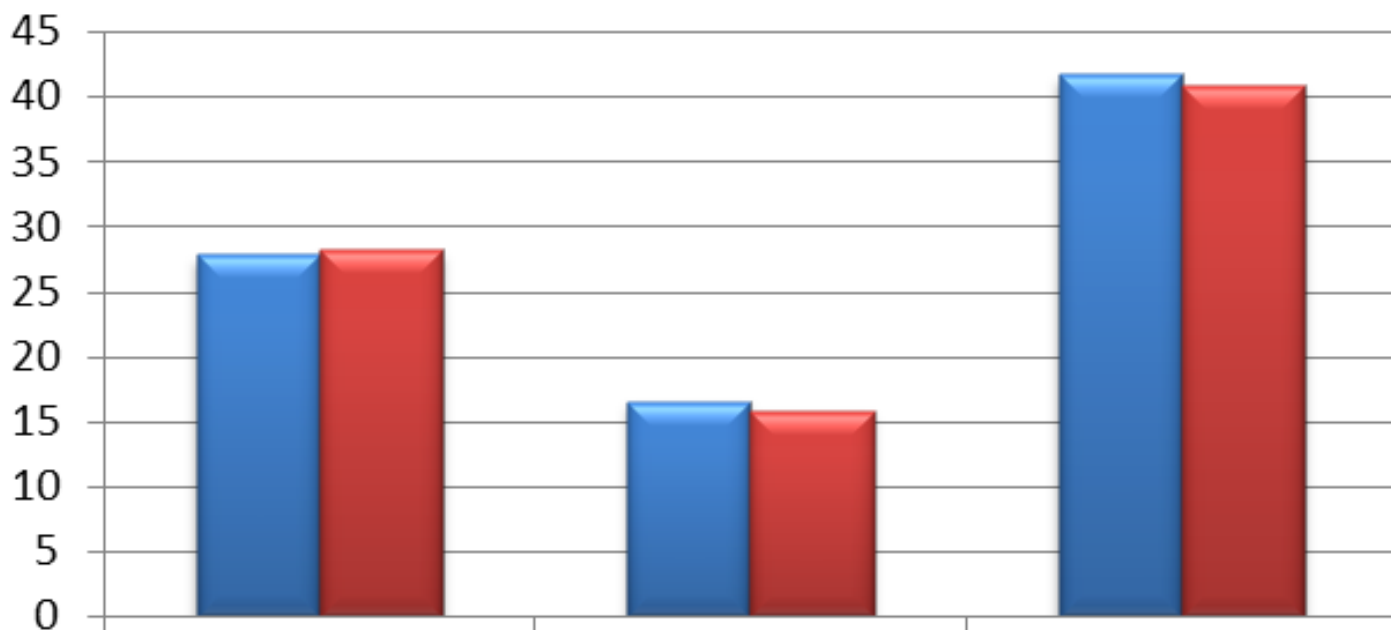



Digital PCR data-*in situ* sample



Digital data results comparison

Digital TREC copies (per μl blood)




 DNA elution

27.8

16.5

41.7

 In situ

28.2

15.8

40.8

Conclusions

- Digital PCR can provide TREC copy numbers without the need for a standard curve
- Digital PCR may provide standardization of results regardless of assay method (extraction, reagents, and instruments)
- Digital PCR may provide added resolution for PIDs with T-cell involvement that are not true SCID (atypical, leaky SCID)



Acknowledgements

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