Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Evaluating droplet digital PCR for the quantification of human genomic DNA: converting copies per nanoliter to nanograms nuclear DNA per microliter

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DATA

Results were obtained for the three human DNA components of a candidate certified reference material (CRM). The components are labeled “A”, “B”, and “C”.

Each component was assessed with ten human nuclear DNA (nDNA) assays, each assay designed to be specific to a unique genetic locus and optimized for droplet digital PCR (ddPCR). The assay indices and codes are: 1) 2PR4, 2) POTP, 3) NEIF, 4) R4Q5, 5) D5, 6) ND6, 7) D9, 8) HBB1, 9) ND14, and 10) 22C3.

Five sets of results were obtained for each component for each of the ten assays. Each set consisted of two technical replicates per assay. Within each set, all results reflect subsamples from the same independently prepared 1:4 dilution of the CRM component. Results were collected from a total of five 96-well plates, each plate providing one set of results for each of the three components plus non-template controls. The plate indices and codes are: 1) d20170711, 2) d20170712a, 3) d20170712b, 4) d20170713, and 5) d20170714.

Table S1 lists the data used in in this study.

Table S1 λ/F, Dilution-Adjusted Copies per Microliter

			ddPCR Plate									
	Assay		1) d20170711		2) d20170712a		3) d20170712b		4) d20170713		5) d20170714	
Sample	Index	Code	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
A	1	2PR4	12.25	12.48	12.26	12.78	12.04	12.25	13.87	14.25	11.74	11.69
	2	POTP	12.16	12.38	11.66	12.51	12.32	12.04	13.73	13.34	11.27	10.32
	3	NEIF	12.11	12.23	12.33	11.86	11.87	11.93	14.36	13.98	10.74	11.26
	4	R4Q5	11.72	12.19	11.97	12.95	11.46	11.66	13.07	13.75	10.97	11.16
	5	D5	12.16	12.37	12.77	12.12	11.82	12.14	14.14	15.61	11.30	11.65
	6	ND6	11.97	12.11	12.25	12.33	11.84	11.68	13.68	13.13	10.96	11.14
	7	D9	12.16	12.14	12.46	12.15	11.15	11.81	13.40	13.55	11.23	11.30
	8	HBB1	12.08	11.84	12.91	12.86	11.44	11.63	13.30	13.22	11.38	11.62
	9	ND14	12.09	12.55	13.01	12.96	11.79	11.94	13.70	13.65	11.12	11.13
	10	22C3	11.35	11.78	12.95	12.92	12.31	11.88	14.18	14.27	11.48	*a*
B	1	2PR4	13.73	14.40	15.98	16.40	13.30	13.27	13.11	13.38	12.63	12.99
	2	POTP	13.59	13.09	16.18	16.85	13.70	13.23	12.75	13.32	12.88	12.48
	3	NEIF	12.56	12.54	15.96	16.29	13.27	13.13	13.07	13.33	13.03	13.17
	4	R4Q5	13.64	13.13	*a*	15.27	13.80	12.99	13.48	12.86	13.06	12.79
	5	D5	13.58	13.44	16.39	16.11	13.40	13.15	13.18	13.58	13.25	13.19
	6	ND6	13.44	13.42	15.39	14.95	12.99	12.27	12.50	12.57	12.63	12.44
	7	D9	13.68	13.66	14.32	15.51	13.31	12.86	13.22	13.07	13.24	12.81
	8	HBB1	13.41	13.73	14.49	15.60	12.96	12.80	12.97	13.00	13.48	12.82
	9	ND14	13.75	13.03	15.61	15.18	13.71	13.09	12.89	12.68	12.72	12.60
	10	22C3	13.05	13.98	15.48	15.03	13.34	13.71	13.17	13.33	13.54	*a*
C	1	2PR4	11.98	12.53	11.02	11.00	10.67	10.46	10.80	10.61	10.92	10.71
	2	POTP	11.73	11.58	11.58	11.84	9.90	10.27	10.37	10.49	10.45	10.72
	3	NEIF	11.57	11.30	11.36	11.47	10.25	9.92	11.11	10.57	10.57	10.76
	4	R4Q5	11.69	11.43	11.64	11.36	10.60	10.72	10.67	10.79	10.58	10.79
	5	D5	11.83	11.81	11.59	11.23	10.64	11.77	11.52	11.20	11.13	10.71
	6	ND6	11.68	11.38	11.75	10.86	10.75	10.55	10.88	10.46	10.35	10.38
	7	D9	11.53	11.55	11.66	11.05	10.30	10.53	10.60	10.72	10.80	10.84
	8	HBB1	11.50	12.35	11.17	11.40	10.83	10.89	10.51	10.78	10.67	10.50
	9	ND14	11.49	10.97	10.87	10.57	10.77	10.89	10.38	10.37	10.77	10.48
	10	22C3	11.38	12.16	11.11	10.98	10.58	11.52	10.92	10.87	10.02	10.23

a) Technical failure

Fig. S1 provides a schematic image of the relationships among the study’s factors.

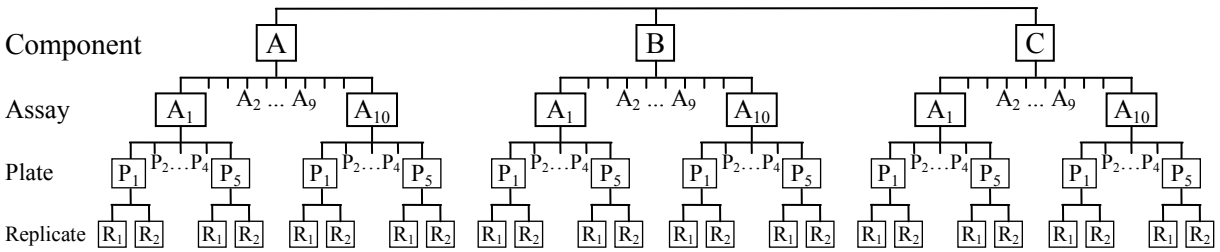

Fig. S1 Relationships Among Experimental Factors

OPENBUGS ANALYSIS OF VARIANCE

Using a Markov Chain Monte Carlo Bayesian approach [1,2] to analysis of variance, we model the measurement results as

$$y_{ijk} \sim N(\gamma_{ijk}, \sigma_{ij}^2),$$
$$\gamma_{ijk} \sim N\left(\alpha_{ij}, \sigma_{plate_{ij}}^2\right),$$
$$\alpha_{ij} \sim N(\mu_i, \sigma_{component_i}^2).$$

where "~" signifies "distributed as", $N(\mu, \sigma^2)$ represents a Gaussian distribution with mean μ and variance σ^2, i indexes the CRM components, j the assays, and k the plates. The variance components are $\sigma_{component_i}^2$ which measures the between-assay variability for component i, $\sigma_{plate_{ij}}^2$ which measures the between-plate variability for each component and assay combination, and σ_{ij}^2 which measures the repeatability variability.

The data was analyzed using Bayesian MCMC with non-informative Gaussian priors for the means and either Gamma priors for the variance components or Uniform priors for standard deviations when Gamma variance priors would not converge.

OpenBUGS is a freely available open-source software package for performing Bayesian inference Using Gibbs Sampling [3]. The user defines a statistical model that states the relationships between related variables and controls the evaluation of the data using the model. Please see http:// http://www.openbugs.net/w/FrontPage on how to obtain 1) the package and 2) the User's Manual.

The following provides the complete code, initialization values, and data required to estimate the values of interest.

Definition of Terms

```
# Outputs
# c0[3]..... Between-plate %CV for each component
# c1a[3,5]..... Between assay %CV for each component-plate combination
# c1aA[3]..... Mean within-component between-assay %CV
# c1aGA..... Global mean between-assay %CV
# c1b[3,5]..... Repeatability %CV for each component-plate combination
# c1bA[3]..... Mean within-component repeatability %CV
# c1bGA..... Grand mean repeatability %CV
#
# d1[3,10] ..... % difference from mean for each component-assay combination
# d1A[3]..... Mean % difference from mean for each component
# d1GA ..... Grand mean % difference
#
# m0[3] ..... Grand mean for each component
# m1[3,5] ..... Mean for each plate for each component
```

```
# m1P[3,10] ....  Prediction mean for each assay for each component
# m2[3,5,10] ...  Mean for each assay for each plate for each component
#
# Indices
# i..... components (A, B, C)
# j..... assays (2PR4, POTP, NEIF, R4Q5, D5, ND6, D9, HBB1, ND14, 22C3)
# k..... plates (dd20170711a, dd20170711b, dd20170712, dd20170713, dd20170714)
# l..... Technical replicates (Rep1, Rep2)
#
# Intermediate variance-related variables initialized as uniform distributions
# s1a[3,5] ..... Between assay standard deviation for each component-plate combination
# s1P[3,10]..... Prediction between plate standard deviation for each component-assay combination
# v1a[3,5]..... Between assay inverse-variance for each component-plate combination
# v1P[3,10] ..... Prediction between plate inverse-variance for each component-assay combination
#
# Intermediate variance-related variables requiring initialization
# v0[3] ..... Between-plate inverse-variance for each component
# v1b[3,5] ..... Repeatability inverse-variance for each component-plate combination
#
# Data
# lamb1[3,10,5,2]... dilution-adjusted copies per droplet arranged by component/plate/assay/technical replicate
# lamb2[3,10,5,2]... same as lamb1; required for predicting the differences (BUGS doesn't allow data re-use)
```

OpenBUGS Code

```
Anova_Begin{
#
# Define distributions
for(i in 1:3){m0[i]~dnorm(0,1.0E-5);v0[i]~dgamma(1.0E-5,1.0E-5);c0[i]<-100/(m0[i]*sqrt(v0[i]))}
for(i in 1:3){for(k in 1:5){m1[i,k]~dnorm(m0[i],v0[i])
 s1a[i,k]~dunif(0,3);v1a[i,k]<-1/pow(s1a[i,k],2);c1a[i,k]<-100/(m0[i]*sqrt(v1a[i,k]));
 v1b[i,k]~dgamma(1.0E-5,1.0E-5);c1b[i,k]<-100/(m0[i]*sqrt(v1b[i,k]))}}
for(i in 1:3){for(j in 1:10){for(k in 1:5){m2[i,j,k]~dnorm(m1[i,k],v1a[i,k])}}}
#
# Define average %CV
c0A<-mean(c0[]);c1aGA<-mean(c1a[,]);c1bGA<-mean(c1b[,])
for(i in 1:3){c1aA[i]<-mean(c1a[i,]);c1bA[i]<-mean(c1b[i,])}
#
# Parameterize distributions
for(i in 1:3){for(j in 1:10){for(k in 1:5){for(l in 1:2){lamb1[i,j,k,l]~dnorm(m2[i,j,k],v1b[i,k])}}}}
#################
# Prediction phase
#################
# Define the between-assay differences
for(i in 1:3){for(j in 1:10){m1P[i,j]~dnorm(0,1.0E-5);s1P[i,j]~dunif(0,5);v1P[i,j]<-1/pow(s1P[i,j],2)}}
for(i in 1:3){for(j in 1:10){d1[i,j]<-100*(m1P[i,j]/m0[i]-1)}}
#
# Define average difference
d1GA<-mean(d1[,]);for(j in 1:10){d1A[j]<-mean(d1[,j])}
#
# Parameterize prediction-phase distributions
for(i in 1:3){for(j in 1:10){for(k in 1:5){for(l in 1:2){lamb2[i,j,k,l]~dnorm(m1P[i,j],v1P[i,j])}}}}
}End_Anova
```

Initialization Values

```
list(v0=c(1,1,1),
v1b=structure(.Data=c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1),.Dim=c(3,5)))
```

Data

```
list(lamb1=structure(.Data=c(
12.25,12.48,12.26,12.78,12.04,12.25,13.87,14.25,11.74,11.69,
12.16,12.38,11.66,12.51,12.32,12.04,13.73,13.34,11.27,10.32,
12.11,12.23,12.33,11.86,11.88,11.93,14.36,13.98,10.75,11.26,
11.72,12.19,11.97,12.95,11.46,11.66,13.07,13.75,10.97,11.16,
12.16,12.37,12.77,12.12,11.82,12.14,14.14,15.61,11.30,11.66,
11.97,12.11,12.25,12.33,11.84,11.68,13.68,13.13,10.96,11.14,
12.16,12.14,12.46,12.15,11.15,11.81,13.40,13.55,11.23,11.30,
12.08,11.84,12.91,12.86,11.44,11.63,13.30,13.22,11.38,11.62,
12.09,12.55,13.01,12.97,11.79,11.94,13.70,13.65,11.12,11.13,
11.35,11.78,12.95,12.92,12.31,11.88,14.18,14.27,11.48, NA ,
13.73,14.40,15.98,16.40,13.30,13.27,13.11,13.38,12.63,12.99,
13.59,13.09,16.18,16.85,13.70,13.23,12.75,13.32,12.88,12.48,
12.56,12.54,15.96,16.29,13.27,13.13,13.07,13.33,13.03,13.17,
13.64,13.13, NA ,15.27,13.80,12.99,13.48,12.86,13.06,12.79,
13.58,13.44,16.39,16.11,13.40,13.15,13.18,13.58,13.25,13.19,
13.44,13.42,15.39,14.95,12.99,12.28,12.50,12.57,12.63,12.44,
13.68,13.66,14.32,15.51,13.31,12.86,13.22,13.07,13.24,12.81,
13.41,13.73,14.49,15.60,12.96,12.80,12.97,13.00,13.48,12.82,
13.75,13.03,15.61,15.18,13.71,13.09,12.89,12.68,12.72,12.60,
13.05,13.98,15.48,15.03,13.34,13.72,13.17,13.33,13.54, NA ,
11.98,12.53,11.02,11.00,10.67,10.46,10.80,10.61,10.92,10.71,
11.73,11.58,11.58,11.84,09.90,10.27,10.37,10.49,10.45,10.72,
11.57,11.30,11.36,11.47,10.25,09.92,11.11,10.57,10.57,10.76,
11.69,11.43,11.64,11.36,10.60,10.72,10.67,10.79,10.58,10.79,
11.84,11.81,11.59,11.23,10.64,11.77,11.52,11.20,11.13,10.71,
11.68,11.38,11.75,10.86,10.75,10.55,10.88,10.47,10.35,10.38,
11.53,11.55,11.66,11.05,10.30,10.53,10.60,10.72,10.80,10.84,
11.50,12.35,11.17,11.40,10.83,10.89,10.51,10.78,10.68,10.50,
11.49,10.97,10.87,10.57,10.77,10.89,10.38,10.37,10.77,10.48,
11.38,12.16,11.11,10.98,10.58,11.52,10.92,10.87,10.02,10.23),.Dim=c(3,10,5,2)),
lamb2=structure(.Data=c(
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12.16,12.38,11.66,12.51,12.32,12.04,13.73,13.34,11.27,10.32,
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12.16,12.37,12.77,12.12,11.82,12.14,14.14,15.61,11.30,11.66,
11.97,12.11,12.25,12.33,11.84,11.68,13.68,13.13,10.96,11.14,
12.16,12.14,12.46,12.15,11.15,11.81,13.40,13.55,11.23,11.30,
12.08,11.84,12.91,12.86,11.44,11.63,13.30,13.22,11.38,11.62,
12.09,12.55,13.01,12.97,11.79,11.94,13.70,13.65,11.12,11.13,
11.35,11.78,12.95,12.92,12.31,11.88,14.18,14.27,11.48, NA ,
13.73,14.40,15.98,16.40,13.30,13.27,13.11,13.38,12.63,12.99,
13.59,13.09,16.18,16.85,13.70,13.23,12.75,13.32,12.88,12.48,
12.56,12.54,15.96,16.29,13.27,13.13,13.07,13.33,13.03,13.17,
13.64,13.13, NA ,15.27,13.80,12.99,13.48,12.86,13.06,12.79,
13.58,13.44,16.39,16.11,13.40,13.15,13.18,13.58,13.25,13.19,
13.44,13.42,15.39,14.95,12.99,12.28,12.50,12.57,12.63,12.44,
13.68,13.66,14.32,15.51,13.31,12.86,13.22,13.07,13.24,12.81,
13.41,13.73,14.49,15.60,12.96,12.80,12.97,13.00,13.48,12.82,
```

13.75,13.03,15.61,15.18,13.71,13.09,12.89,12.68,12.72,12.60,
13.05,13.98,15.48,15.03,13.34,13.72,13.17,13.33,13.54, NA ,
11.98,12.53,11.02,11.00,10.67,10.46,10.80,10.61,10.92,10.71,
11.73,11.58,11.58,11.84,09.90,10.27,10.37,10.49,10.45,10.72,
11.57,11.30,11.36,11.47,10.25,09.92,11.11,10.57,10.57,10.76,
11.69,11.43,11.64,11.36,10.60,10.72,10.67,10.79,10.58,10.79,
11.84,11.81,11.59,11.23,10.64,11.77,11.52,11.20,11.13,10.71,
11.68,11.38,11.75,10.86,10.75,10.55,10.88,10.47,10.35,10.38,
11.53,11.55,11.66,11.05,10.30,10.53,10.60,10.72,10.80,10.84,
11.50,12.35,11.17,11.40,10.83,10.89,10.51,10.78,10.68,10.50,
11.49,10.97,10.87,10.57,10.77,10.89,10.38,10.37,10.77,10.48,
11.38,12.16,11.11,10.98,10.58,11.52,10.92,10.87,10.02,10.23),.Dim=c(3,10,5,2))))

Results

The results in Tables S2 through S6 were obtained using OpenBUGS Version 3.2.3 rev 1012. Initial burn-in used 10 000 samplings with 10-fold thinning. The posterior distributions were defined with 100 000 samplings with 2-fold thinning. For each parameter, the listed statistics summarize the resulting posterior distributions.

Mean..... arithmetic mean
SD..... standard deviation
2.5 %..... 0.025 percentile
25 %..... 0.25 percentile (1st quartile)
median ..... 0.50 percentile (2nd quartile)
75 %..... 0.75 percentile (3rd quartile)
97.5 %..... 0.975 percentile
Asym ..... right-hand/left-hand asymmetry, (97.5 % - 50 %)/(50 % - 2.5 %)

Note: when "Asym" is about 1, the mean and median results will be similar and the SD will provide a useful estimate of the standard deviation of that mean value. As the asymmetry increasingly deviates from 1, the mean and SD estimates become increasingly biased. For this reason, we base our estimated values on the median results. The results of interest to this study are in red font.

Table S2 Component Means, λ/F Copies per Nanoliter

Parameter	Mean	SD	2.5 %	25 %	median	75 %	97.5 %	Asym
m0[1]	12.29	0.59	11.14	11.98	12.29	12.60	13.46	1.02
m0[2]	13.64	0.69	12.31	13.28	13.64	14.00	15.00	1.02
m0[3]	10.99	0.29	10.41	10.83	10.99	11.14	11.56	0.98

Table S3 Component Relative Standard Uncertainties, %

Parameter	Mean	SD	2.5 %	25 %	median	75 %	97.5 %	Asym
c0[1]	9.59	5.43	4.42	6.49	8.29	11.03	22.6	3.70
c0[2]	9.86	10.42	4.38	6.67	8.54	11.39	23.06	3.49
c0[3]	5.14	2.93	2.29	3.46	4.46	5.96	12.06	3.49
c0A	8.2	4.04	4.88	6.46	7.64	9.25	14.70	2.55

Table S4 Between-Assay Relative Standard Uncertainties, %

Parameter	Mean	SD	2.5 %	25 %	median	75 %	97.5 %	Asym
c1aA[1]	2.20	0.57	1.21	1.81	2.16	2.54	3.42	1.33
c1aA[2]	2.19	0.63	1.18	1.78	2.14	2.53	3.51	1.44
c1aA[3]	2.19	0.53	1.25	1.82	2.16	2.52	3.33	1.30
c1aGA	2.19	0.33	1.60	1.97	2.17	2.39	2.89	1.25

Table S5 Relative Between-Assay Differences, %

Parameter	Mean	SD	2.5 %	25 %	median	75 %	97.5 %	Asym
d1[1,1]	2.43	5.98	-8.07	-1.01	2.19	5.49	14.17	1.17
d1[1,2]	-0.70	5.97	-11.34	-4.20	-0.94	2.43	11.17	1.16
d1[1,3]	0.05	6.27	-11.10	-3.64	-0.17	3.40	12.51	1.16
d1[1,4]	-1.41	5.83	-11.68	-4.79	-1.64	1.62	10.21	1.18
d1[1,5]	2.82	6.70	-9.21	-1.18	2.55	6.48	16.24	1.16
d1[1,6]	-1.24	5.75	-11.28	-4.55	-1.47	1.71	10.12	1.18
d1[1,7]	-1.04	5.76	-11.11	-4.37	-1.27	1.94	10.34	1.18
d1[1,8]	-0.27	5.69	-10.24	-3.53	-0.49	2.64	10.98	1.18
d1[1,9]	1.10	6.02	-9.52	-2.37	0.86	4.23	13.03	1.17
d1[1,10]	2.48	6.49	-9.18	-1.40	2.25	5.98	15.57	1.17
d1[2,1]	2.30	6.48	-9.34	-1.61	2.03	5.79	15.42	1.18
d1[2,2]	1.45	6.81	-10.89	-2.64	1.19	5.21	15.16	1.16
d1[2,3]	0.22	6.52	-11.64	-3.67	-0.01	3.79	13.40	1.15
d1[2,4]	-1.18	5.73	-11.32	-4.45	-1.42	1.74	10.32	1.19
d1[2,5]	2.36	6.45	-9.19	-1.47	2.09	5.83	15.35	1.18
d1[2,6]	-2.54	6.00	-13.22	-6.08	-2.79	0.64	9.58	1.19
d1[2,7]	-0.27	5.73	-10.51	-3.55	-0.50	2.67	11.25	1.17
d1[2,8]	-0.60	5.83	-10.94	-3.97	-0.83	2.40	11.06	1.18
d1[2,9]	-0.59	6.06	-11.46	-4.16	-0.82	2.59	11.63	1.17
d1[2,10]	1.78	5.99	-8.84	-1.68	1.53	4.87	13.78	1.18
d1[3,1]	0.81	3.57	-6.00	-1.37	0.75	2.90	7.96	1.07
d1[3,2]	-0.79	3.67	-7.84	-3.05	-0.85	1.36	6.54	1.06
d1[3,3]	-0.83	3.33	-7.13	-2.82	-0.89	1.08	5.86	1.08
d1[3,4]	0.42	3.14	-5.51	-1.43	0.35	2.18	6.66	1.08
d1[3,5]	3.31	3.20	-2.73	1.45	3.23	5.08	9.68	1.08
d1[3,6]	-0.69	3.25	-6.87	-2.63	-0.75	1.15	5.74	1.06
d1[3,7]	-0.20	3.18	-6.24	-2.08	-0.27	1.57	6.09	1.06
d1[3,8]	0.74	3.38	-5.66	-1.30	0.67	2.68	7.44	1.07
d1[3,9]	-2.05	2.91	-7.50	-3.71	-2.12	-0.48	3.69	1.08
d1[3,10]	-0.02	3.50	-6.68	-2.14	-0.08	2.01	6.89	1.06
d1GA	0.28	3.44	-4.50	-1.33	0.16	1.71	5.61	1.17

Table S6 Relative Standard Measurement Repeatability, %

Parameter	Mean	SD	2.5 %	25 %	median	75 %	97.5 %	Asym
c1bA[1]	2.65	0.31	2.11	2.43	2.63	2.84	3.32	1.36
c1bA[2]	2.63	0.37	2.08	2.41	2.60	2.82	3.32	1.39
c1bA[3]	2.62	0.28	2.13	2.42	2.60	2.80	3.22	1.32
c1bGA	2.63	0.19	2.32	2.51	2.62	2.74	3.00	1.21

POISSON SAMPLING UNCERTAINTY AND CHOICE OF COPIES PER DROPLET

The relative standard uncertainty associated with Poisson processes involved in ddPCR is a function of both the mean number of copies per droplet, λ, and the number of droplets counted, N_{tot} [4]:

$$\mathrm{CV_{Poisson}} = f(\lambda, N_{\mathrm{tot}}) = 100\sqrt{\frac{1-e^{-\lambda}}{N_{\mathrm{tot}}\lambda^2 e^{-\lambda}}}\ \%.$$

For λ = 0.3 copies per droplet and N_{tot} = 17 000 droplets, the relative standard deviation due to Poisson sampling is

$$\mathrm{CV_{Poisson}} = 100\sqrt{\frac{1-e^{-0.3}}{(17\,000)(0.3^2)(e^{-0.3})}} = 1.51\ \%.$$

From Table S6, we estimate the measurement repeatability for the processes used in this study as 2.62 %.

The "extra" variability of the processes can be estimated from the difference between the variances associated with the mean relative repeatability, $\mathrm{CV_{observed}}$ (the "c1bGA" term in the OpenBUGS code and Table S6) and $\mathrm{CV_{Poisson}}$:

$$\mathrm{CV_{extra}} = \sqrt{\mathrm{CV_{observed}^2} - \mathrm{CV_{Poisson}^2}} = \sqrt{2.62^2 - 1.51^2} \cong 2.14\ \%.$$

Assuming that the source(s) of $\mathrm{CV_{extra}}$ are independent of the DNA content in the sample, the expected relative standard uncertainty for the total measurement process becomes:

$$\mathrm{CV_{expected}} = f(\lambda, N_{\mathrm{tot}}, \mathrm{CV_{extra}}) = 100\sqrt{\frac{1-e^{-\lambda}}{N_{\mathrm{tot}}\lambda^2 e^{-\lambda}} + \left(\frac{\mathrm{CV_{extra}}}{100}\right)^2}\ \%.$$

Fig. S2 displays $\mathrm{CV_{Poisson}}$ and $\mathrm{CV_{expected}}$ as functions of λ for N_{tot} = 17 000.

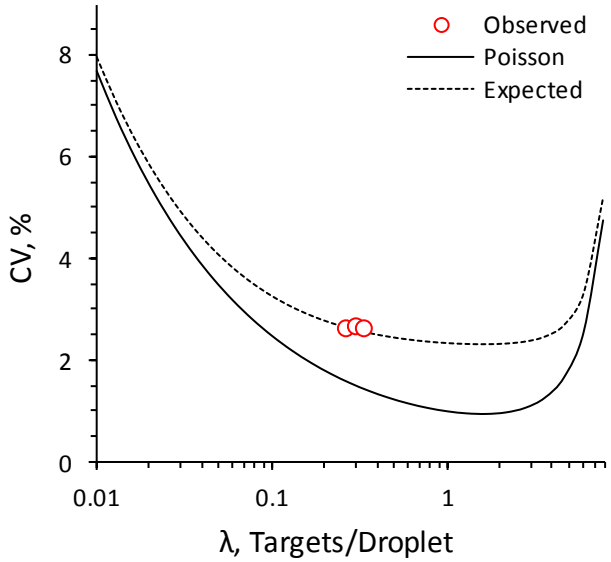

Fig. S2 Relative Standard Deviations as Functions of λ for N_{tot} = 17 000

One implication of a non-zero $\mathrm{CV_{extra}}$ is the effective "flattening" of the $\mathrm{CV_{Poisson}}$ curve. While the minimum value for both $\mathrm{CV_{Poisson}}$ and the $\mathrm{CV_{expected}}$ remains the same ≈1.6 copies per droplet, the interval of "almost minimum" variability is much wider for $\mathrm{CV_{expected}}$. In our experience, ddPCR results for targets on minimally fragmented human nDNA can become biased-low for λ > ≈0.8 (data not shown). We therefore chose to make our certification studies with λ ≈0.3 copies per droplet, accepting some increase in variability to minimize the potential for bias.

dMIQE CHECKLIST

Table S7 follows the "Minimum Information for Publication of Quantitative Digital PCR Experiments" guidelines [5]. Note that much of the requested information is intended for studies focused on biological issues and is "Not Applicable" to studies characterizing dPCR platform performance.

Table S7 dMIQE Checklist

EXPERIMENTAL DESIGN	Comments
Definition of experimental and control groups	Studies on the metrological traceability of dPCR platforms using human nuclear DNA (nDNA)
Number within each group	Variable
Assay carried out by core lab or investigator's lab?	Investigator's lab
SAMPLE	**Comments**
Description	nDNA extracted from Buffy coat cells using a modified salt-out method [6]
Volume or mass of sample processed	Multiple 5 mL aliquots
Microdissection or macrodissection	Not applicable
Processing procedure	Not applicable
If frozen - how and how quickly?	Not applicable
If fixed - with what, how quickly?	Not applicable
Sample storage conditions and duration	Samples stored at 4 °C.
NUCLEIC ACID EXTRACTION	**Comments**
Quantification - instrument/method	denatured to ssDNA, spectrophotometry (Cary 100 Bio)
Storage conditions: temperature, concentration, duration, buffer	4 °C in TE^{-4} pH 8.0 buffer (10 mmol/L tris(hydroxymethyl)aminomethane HCl, 0.1 mmol/L ethylenediaminetetraacetic acid).
DNA or RNA quantification	DNA
Template structural information	Very large fragment (> 48 502 bp) dsDNA, gel electrophoresis and UV absorption spectroscopy
Template modification (digestion, sonication, pre-amplification etc.)	none
Template treatment (initial heating or chemical denaturation)	none
Inhibition dilution or spike;	Not applicable
DNA contamination assessment of RNA sample	Not applicable
Details of DNase treatment where performed	Not applicable
Manufacturer of reagents used and catalogue number	
Storage of nucleic acid: temperature, concentration, duration, buffer	nDNA: < 1 year at 4 °C
dPCR TARGET INFORMATION	**Comments**
Sequence accession number	See Table 1
Location of amplicon	See Table 1
Amplicon length	See Table 1
In silico specificity screen (BLAST, etc.)	BLAST
Location of each primer by exon or intron	Not applicable
dPCR OLIGONUCLEOTIDES	**Comments**
Primer sequences and/or amplicon context sequence	See Table 1
Probe sequences	See Table 1
Location and identity of any modifications	Not applicable
Manufacturer of oligonucleotides	Eurofins MWG Operon (Huntsville, AL)
Purification method	salt-free purification.

Table S7 (Continued). dMIQE Checklist

dPCR PROTOCOL	Comments
Complete reaction conditions	See text
Reaction volume and amount of RNA/cDNA/DNA	See text
Primer, (probe), Mg++ and dNTP concentrations	See text
Polymerase identity and concentration	See text
Buffer/kit Catalogue No and manufacturer	See text
Exact chemical constitution of the buffer	Proprietary
Additives (SYBR Green I, DMSO, etc.)	Not applicable
Plates/tubes Catalogue No and manufacturer	Various
Complete thermocycling parameters	See text
Reaction setup	See text
Gravimetric or volumetric dilutions (manual/robotic)	Gravimetric and manual volumetric
Total PCR reaction volume prepared	See text
Partition number	10 000 to 20 000
Individual partition volume	ddPCR: 0.7349 nL ± 1.2 %, see [7]
Total volume of the partitions measured	Variable
Partition volume variance/standard deviation	See [4]
Comprehensive details and appropriate use of controls	See text
Manufacturer of dPCR instrument	ddPCR: Bio-Rad
dPCR VALIDATION	**Comments**
Optimisation data for the assay	See [8,9]
Specificity (when measuring rare mutations, pathogen sequences etc.)	Not applicable
Limit of detection of calibration control	Not applicable
If multiplexing, comparison with singleplex assays	Not applicable
DATA ANALYSIS	**Comments**
Average copies per partition	λ =0.3
dPCR analysis program (source, version)	Bio-Rad QuantaSoft, Ver 1.7.4.0917 In-house spreadsheet systems
Outlier identification and disposition	three technical failures (of 400 measurements) identified by manufacture's diagnostics
Results of NTCs	Zero to few
Examples of positive(s) and negative experimental results as supplemental data	Not applicable
Where appropriate, justification of number and choice of reference genes	See text
Where appropriate, description of normalisation method	Not applicable
Number and concordance of biological replicates	Not applicable
Number and stage (RT or qPCR) of technical replicates	Not applicable
Repeatability (intra-assay variation)	See Table S4
Reproducibility (inter-assay variation)	See Table S3
Experimental variance or confidence interval	Variable
Statistical methods used for analysis	Not applicable
Data submission using RDML	Not applicable

DISCLAIMER

Certain commercial equipment, instruments, or materials are identified in this report to specify adequately experimental conditions or reported results. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment, instruments, or materials identified are necessarily the best available for the purpose.

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