

Homogeneity Study– Effect on Reproducibility

Introduction

It is common to see poor reproducibility due to sample concentration stratification. when sampling either very concentrated samples or large molecules such as lambda and genomic DNA. Making the dilutions required for sample measurement on conventional cuvette-based spectrophotometers often masks the fact that the sample may not truly be in solution and homogeneous.

A study was done on the Thermo Scientific NanoDrop™1000 Spectrophotometer comparing the reproducibility when measuring lambda DNA heated to 37°C without vortexing and then again after heating to 63°C with vortexing. The standard deviations in the first data set ranged from 3.2-11.1% (% CV's,) while the after-mixing and heating to 63°C standard deviations were all <2.5%.

Lambda DNA, Worthington Biochemicals, labeled as 754ug/ml

Thawed, heated @ 37°C -15 minutes, stored as 0.1ml aliquots & measured at 260 nm

Sample	Original	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5
(ng/ul)	601.50	1110.75	638.29	637.49	581.30	644.39
	609.87	1021.34	647.95	598.91	619.93	756.65
	567.53	927.78	584.34	576.63	622.86	680.40
	566.16	913.84	558.53	654.87	664.66	655.85
	575.33	960.61	585.29	762.54	685.34	839.94
	572.48					743.60
						800.79
						763.67
Mean	582.15	986.86	602.88	646.09	634.82	735.66
SD	18.72	80.70	38.42	72.05	40.84	69.85
% CV	3.2%	8.2%	6.4%	11.2%	6.4%	9.5%

(2 ul)
(2 ul)

Lambda DNA, Worthington Biochemicals, labeled as 754ug/ml

The 0.1 ul aliquots were heated @ 63°C -15 minutes, vortexed & measured at 260 nm

Sample	Original Vial	Vial 1	Vial 2	Vial 3	Vial 4
(ng/ul)	761.00	755.20	754.66	770.90	761.14
	737.99	768.29	784.96	803.15	760.93
	753.26	760.81	753.33	769.09	765.19
Mean	750.75	761.43	764.32	781.05	762.42
SD	11.71	6.57	17.89	19.16	2.40
% CV	1.6%	0.9%	2.3%	2.5%	0.3%