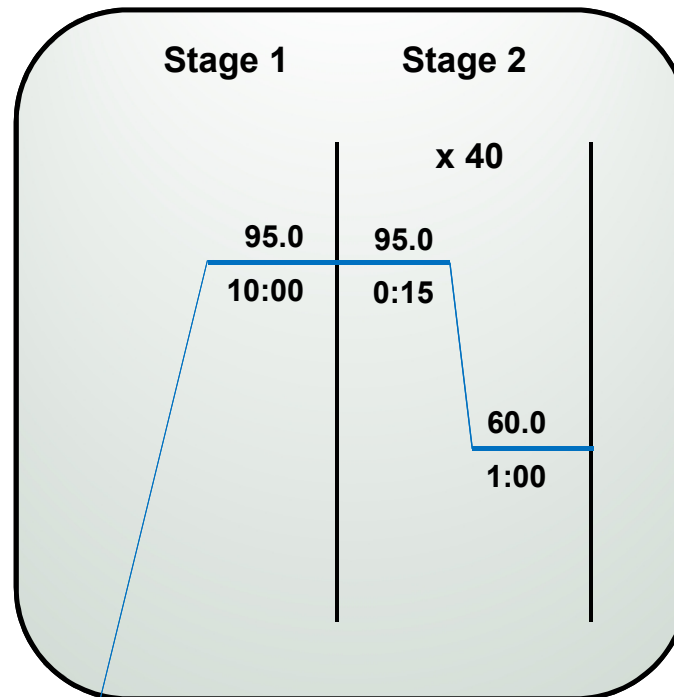


Step 1: DNA Functional Quantification and Quality Control

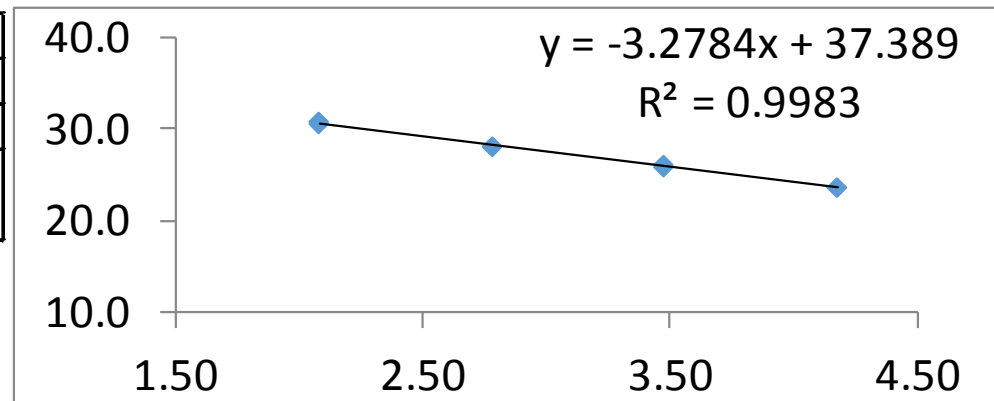


Step 1: DNA Functional Quantification and Quality Control

Standard Curve

			Endogenous Reference	Inhibition Reporter
ng	Cp#	Log10 (Cp#)	Ct Gene-1	Ct Gene-2
50	15150	4.18	23.7	31.0
50	15150	4.18	23.7	30.7
10	3030	3.48	26.0	30.0
10	3030	3.48	26.1	30.2
2	606	2.78	28.2	30.3
2	606	2.78	28.1	30.2
0.4	121	2.08	30.7	30.4
0.4	121	2.08	30.5	30.3

Slope	-3.28
Intercept	37.39
PCR eff%	101.8
Inhibition Factor	30.4

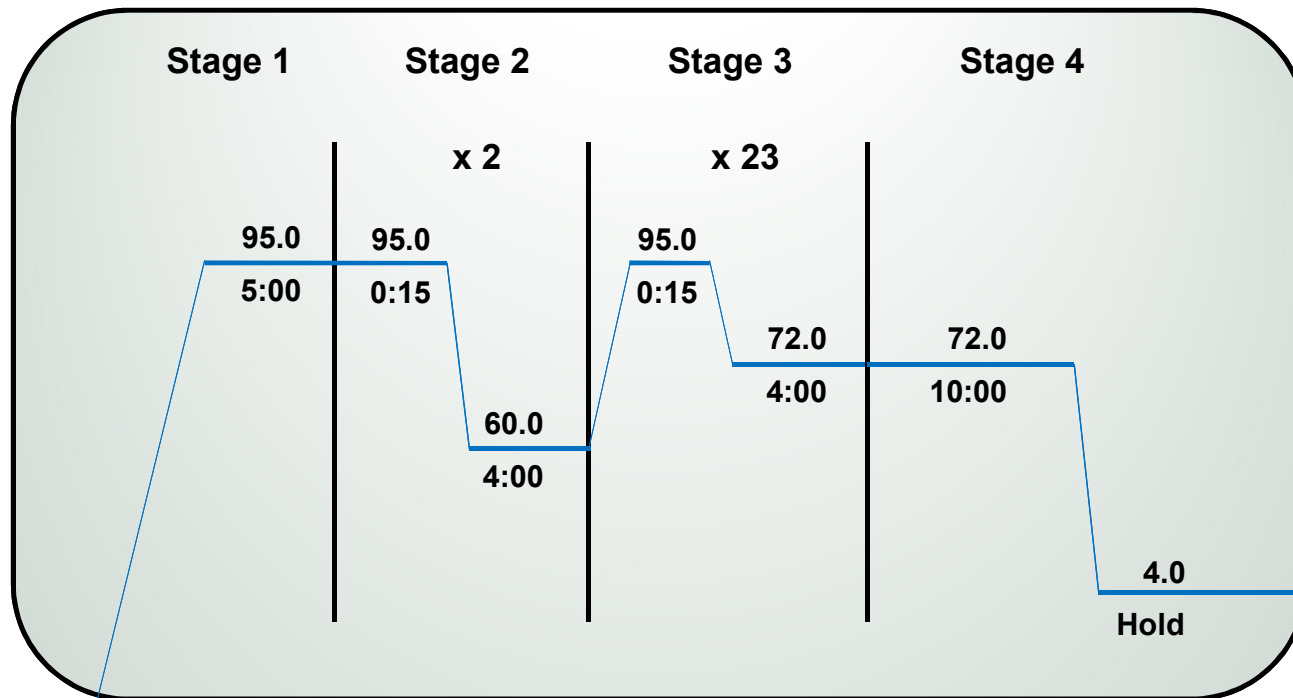


Step 1: DNA Functional Quantification and Quality Control

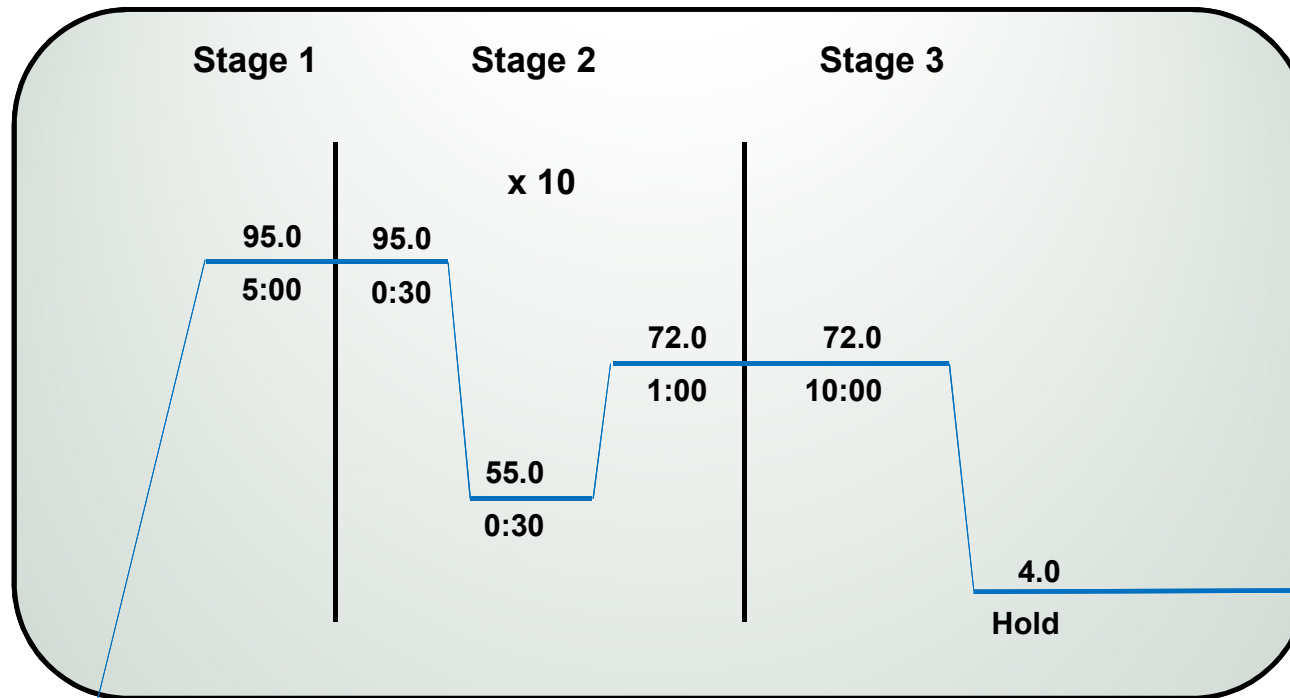
Sample DNA Results in Functional Copy Number from Standard Curve

Sample Name	Gene1 Cq (FAM) Endogenous Ref	Gene2 Cq (VIC/HEX) Inhibition Reporter	Functional Conc [cp/ul]
Sample1	26.37	30.1	2297
Sample2	27.2	30.1	1282
Sample3	27.3	30.2	1179
Sample4	27.8	30.3	853
Sample5	28.98	30.25	367
Sample6	28.19	30.30	640
Sample7	29.10	30.21	338
Sample8	29.27	30.25	300
Sample9	28.05	30.26	706
Sample10	28.02	30.23	721
Sample11	27.82	30.28	830
Sample12	27.98	30.10	741
Sample13	28.17	30.13	649
NTC	Undetermined	30.3	0

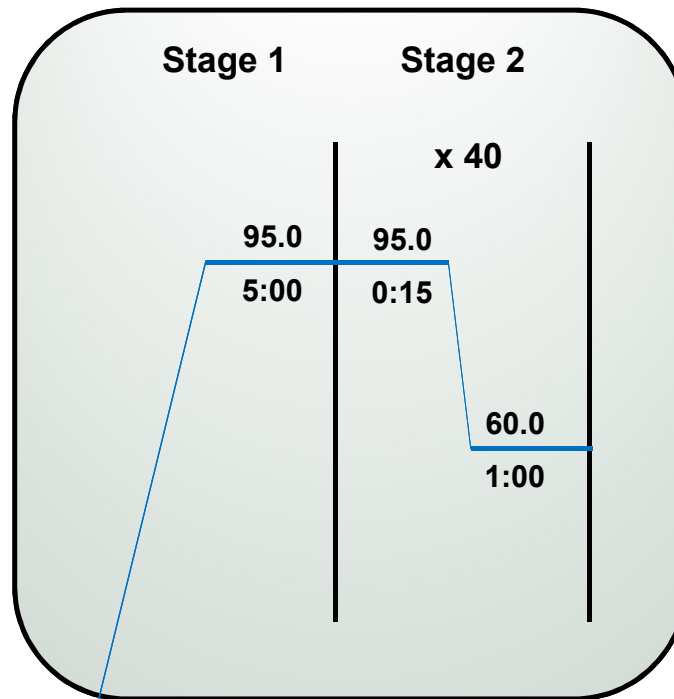
Step 2: Library Preparation – Gene-specific PCR



Step 3: Library Preparation – Tag PCR



Step 5: Library Quantification



Step 6: Library Normalization and Sample Pooling

$$[Library]^{nM} = 12.5 \times 2^{\Delta C_q}$$

Dilution Factor	10000
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Well	Sample Name	FAM Ct (Library)	VIC Ct (Standard)	Delta Ct [VIC Ct - Fam Ct]	Dilution Factor	Library Stock Conc. (nM)
A7	LQ-PositiveControl	18.3	18.7	0.4	10000	32.3
C7	LQ-PositiveControl	18.4	18.7	0.3	10000	31.3
E7	LQ-NTC	Undetermined	18.5			
A1	FFPECtrl	17.8	18.7	1.0	10000	48.6
C1	MVCtrl	17.3	18.8	1.5	10000	71.3
E1	NTC	22.1	18.8	-3.3	10000	2.5
G1	Sample 1	17.4	18.7	1.2	10000	58.1
I1	Sample 2	17.5	18.7	1.2	10000	58.9
K1	Sample 3	17.5	18.6	1.1	10000	54.6
M1	Sample 4	18.0	18.7	0.7	10000	41.5
O1	Sample 5	18.2	18.6	0.5	10000	34.2
A3	Sample 6	16.8	18.6	1.8	10000	86.0
C3	Sample 7	16.9	18.5	1.7	10000	79.0
E3	Sample 8	17.3	18.7	1.4	10000	66.9
G3	Sample 9	16.7	18.5	1.8	10000	88.5
I3	Sample 10	16.8	18.6	1.8	10000	85.3
K3	Sample 11	17.1	18.6	1.4	10000	67.0
M3	Sample 12	17.0	18.7	1.8	10000	84.9
O3	Sample 13	18.1	18.8	0.7	10000	41.4

Step 6: Library Normalization and Sample Pooling

Normalization and Pooling					
Well	Sample Name	Notes	Library Stock Conc. (nM)	Library Volume to Pool [uL]	Conc of Library in Pool (nM)
A7	LQ-PositiveControl	LQ-PC			
C7	LQ-PositiveControl	LQ-PC			
E7	LQ-NTC	LQ-NTC			
A1	FFPECtrl		48.6	6	3.8
C1	MVCtrl		71.3	4	3.8
E1	NTC	NTC	2.5	4	0.1
G1	Sample 1		58.1	5	3.8
I1	Sample 2		58.9	5	3.9
K1	Sample 3		54.6	5	3.6
M1	Sample 4		41.5	7	3.8
O1	Sample 5		34.2	9	4.0
A3	Sample 6		86.0	3	3.4
C3	Sample 7		79.0	4	4.2
E3	Sample 8		66.9	5	3.9
G3	Sample 9		88.5	3	3.5
I3	Sample 10		85.3	3	3.4
K3	Sample 11		67.0	4	3.5
M3	Sample 12		84.9	3	3.4
O3	Sample 13		41.4	7	3.8
			66.9	77	56

		1	2	3	4
Sample ID	A	FFPECtrl		Sample 6	
	B				
	C	MVCtrl		Sample 7	
	D				
	E	NTC		Sample 8	
	F				
	G	Sample 1		Sample 9	
	H				
	I	Sample 2		Sample 10	
	J				
	K	Sample 3		Sample 11	
	L				
	M	Sample 4		Sample 12	
	N				
	O	Sample 5		Sample 13	
	P				
		1	2	3	4
Volume (uL) Stock	A	6		3	
	B				
	C	4		4	
	D				
	E	4		5	
	F				
	G	5		3	
	H				
	I	5		3	
	J				
	K	5		4	
	L				
	M	7		3	
	N				
	O	9		7	
	P				