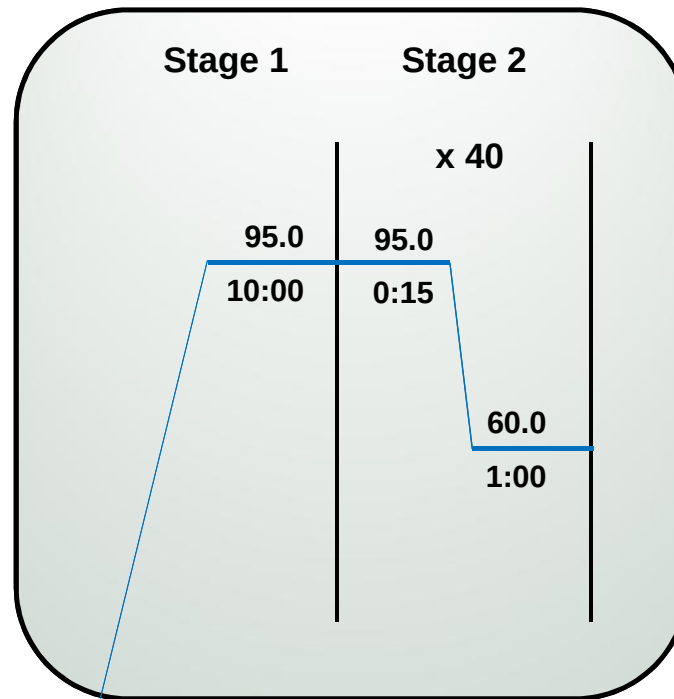


Step 1: DNA Functional Quantification and Quality Control

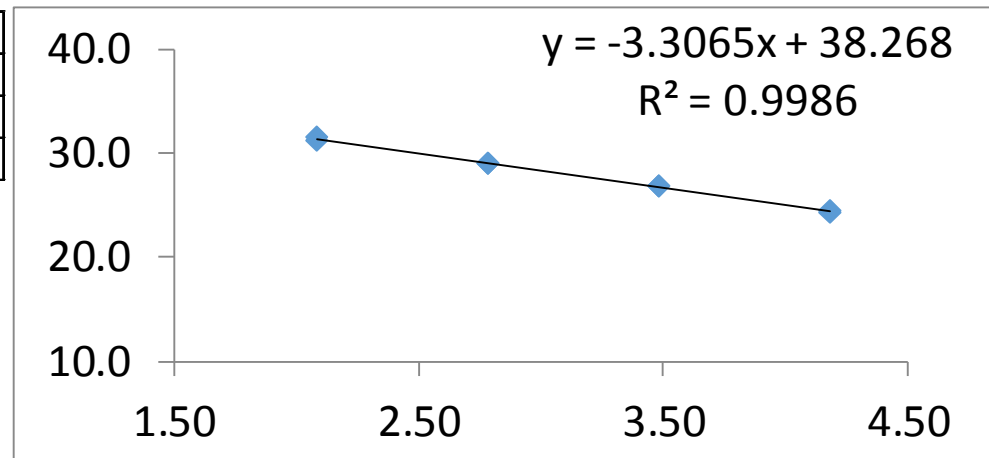


Step 1: DNA Functional Quantification and Quality Control

Standard Curve

ng	Cp#	Log10 (Cp#)	Endogenous Reference	Inhibition Reporter
			Ct Gene-1	Ct Gene-2
50	15150	4.18	24.3	31.0
50	15150	4.18	24.5	30.7
10	3030	3.48	26.8	30.0
10	3030	3.48	26.8	30.2
2	606	2.78	29.1	30.3
2	606	2.78	29.1	30.2
0.4	121	2.08	31.2	30.4
0.4	121	2.08	31.5	30.3

Slope	-3.31
Intercept	38.27
R2	1.00
PCR eff%	100.6

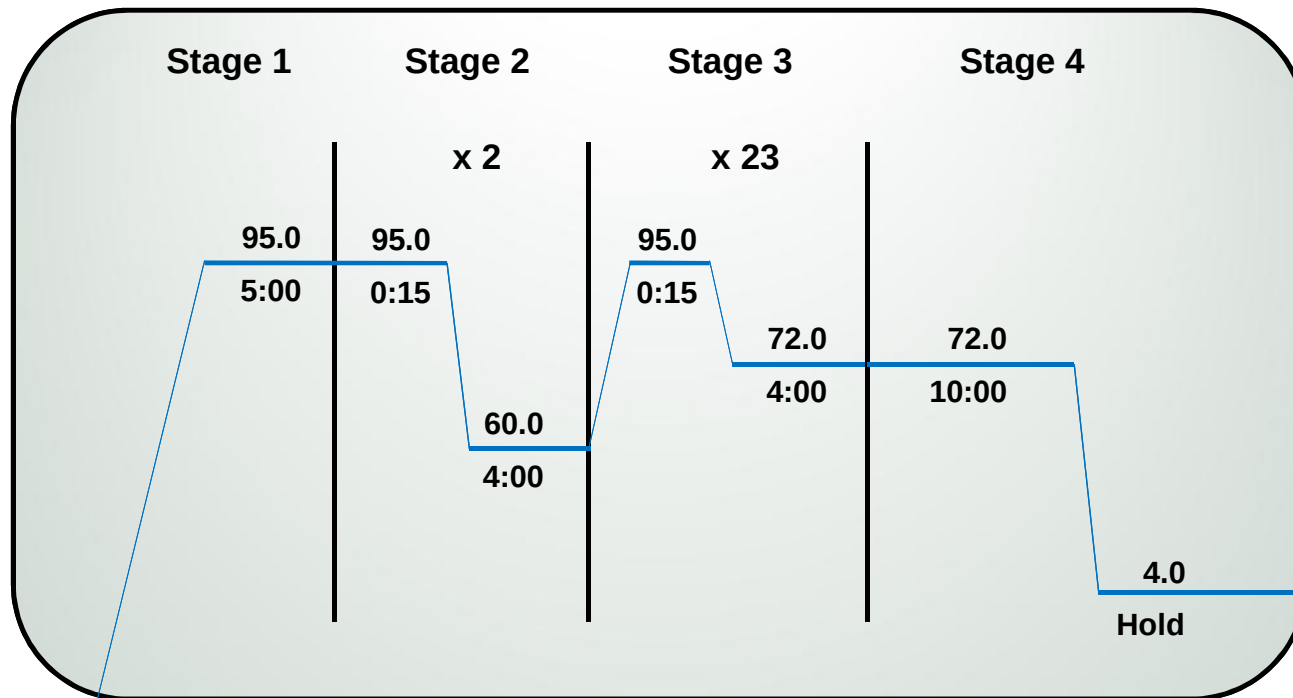


Step 1: DNA Functional Quantification and Quality Control

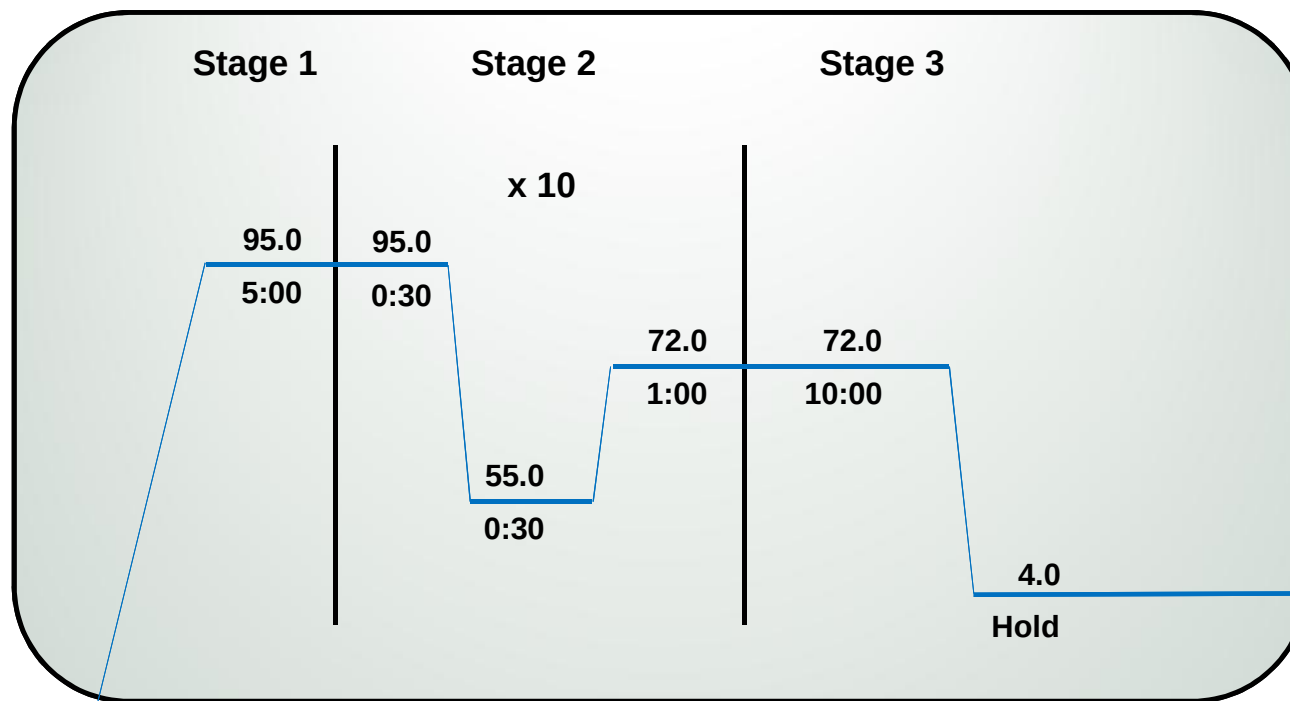
Sample DNA Results in Functional Copy Number from Standard Curve

Sample Name	Gene1 Cq (FAM) Endogenous Reference	Gene2 Cq (VIC/HEX) Inhibition Reporter	Functional Conc [cp/ul]
NTC	Undetermined	30.4	0
MVCtrl	28.8	30.1	708
Sample 1	30.35	30.25	248
Sample 2	30.38	30.33	243
Sample 4	30.73	30.23	190
Sample 5	30.43	30.27	235
Sample 6	27.98	29.97	1289

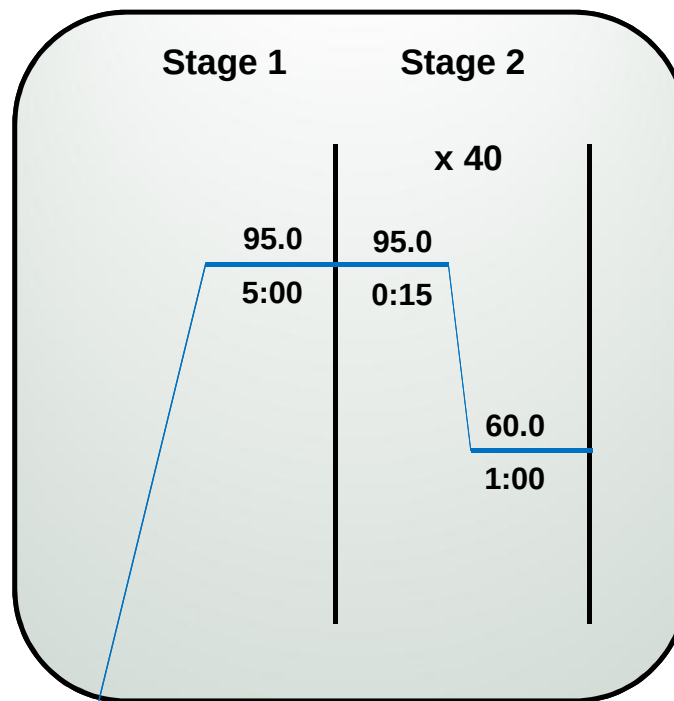
Step 2: Library Preparation – Gene-specific PCR



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
A3	FFPECtrl	16.8	18.6	1.8	10000	86.0
C1	MVCtrl	17.3	18.8	1.5	10000	71.3
C3	MVCtrl	16.9	18.5	1.7	10000	79.0
E1	NTC	22.1	18.8	-3.3	10000	2.5
E3	NTC	21.0	18.7	-2.2	10000	5.3
G1	EPC01	17.4	18.7	1.2	10000	58.1
G3	EPC01	16.7	18.5	1.8	10000	88.5
I1	EPC02	17.5	18.7	1.2	10000	58.9
I3	EPC02	16.8	18.6	1.8	10000	85.3
K1	EPC03	17.5	18.6	1.1	10000	54.6
K3	EPC03	17.1	18.6	1.4	10000	67.0
M1	EPC04	18.0	18.7	0.7	10000	41.5
M3	EPC04	17.0	18.7	1.8	10000	84.9
O1	EPC05	18.2	18.6	0.5	10000	34.2
O3	EPC05	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
A3	FFPECtrl		86.0	3	3.4
C1	MVCtrl		71.3	4	3.8
C3	MVCtrl		79.0	4	4.2
E1	NTC	NTC	2.5	4	0.1
E3	NTC	NTC	5.3	4	0.3
G1	EPC01		58.1	5	3.8
G3	EPC01		88.5	3	3.5
I1	EPC02		58.9	5	3.9
I3	EPC02		85.3	3	3.4
K1	EPC03		54.6	5	3.6
K3	EPC03		67.0	4	3.5
M1	EPC04		41.5	7	3.8
M3	EPC04		84.9	3	3.4
O1	EPC05		34.2	9	4.0
O3	EPC05		41.4	7	3.8
			58.5	76	52.3

		1	2	3	4
Sample ID	A	FFPECtrl		FFPECtrl	
	B				
	C	MVCtrl		MVCtrl	
	D				
	E	NTC		NTC	
	F				
	G	EPC01		EPC01	
	H				
	I	EPC02		EPC02	
	J				
	K	EPC03		EPC03	
	L				
Volume (uL) Stock	M	EPC04		EPC04	
	N				
	O	EPC05		EPC05	
	P				
	A	6		3	
	B				
	C	4		4	
	D				
	E	4		4	
	F				
	G	5		3	
	H				
	I	5		3	
	J				
	K	5		4	
	L				
	M	7		3	
	N				
	O	9		7	
	P				