

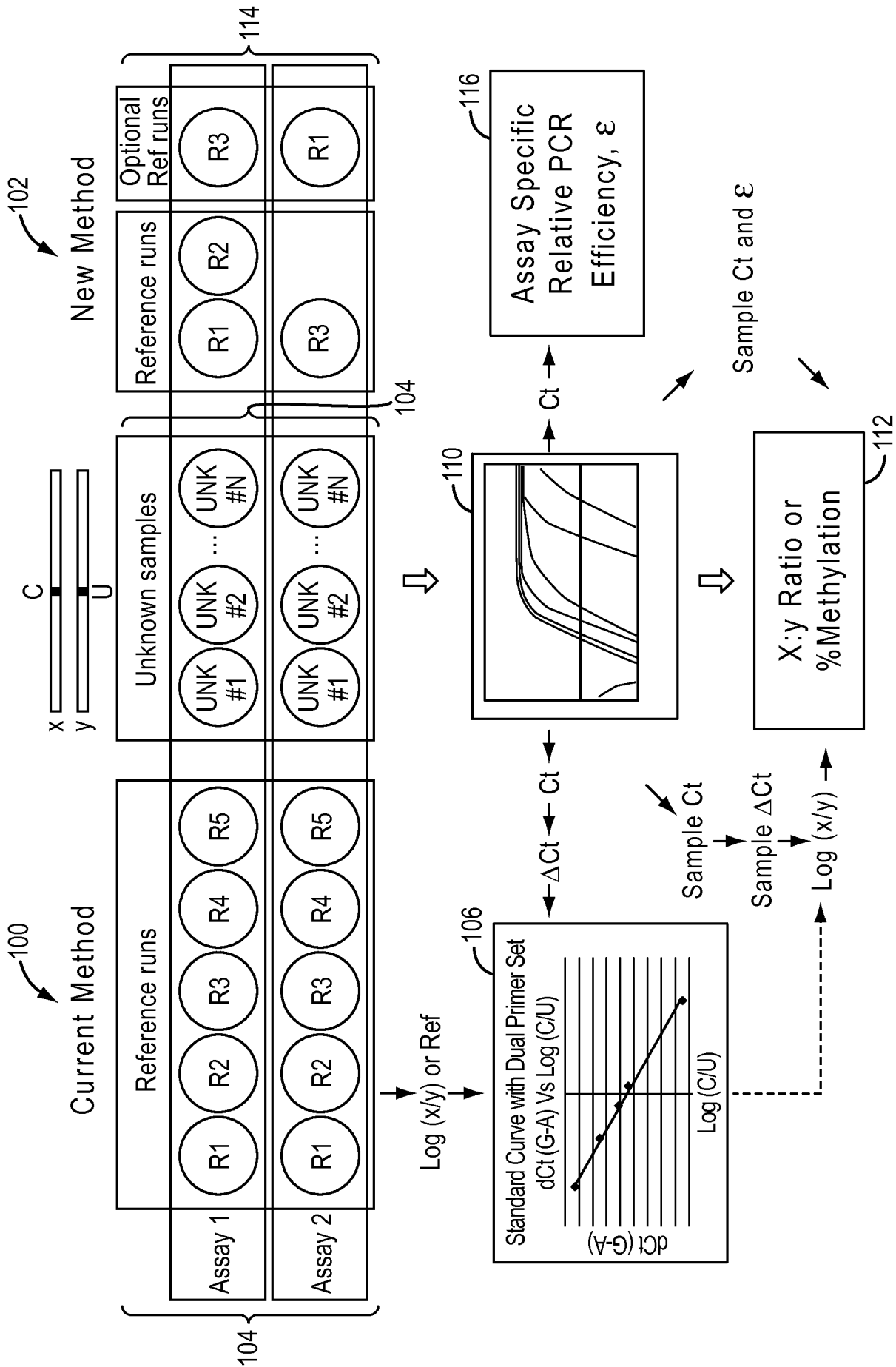
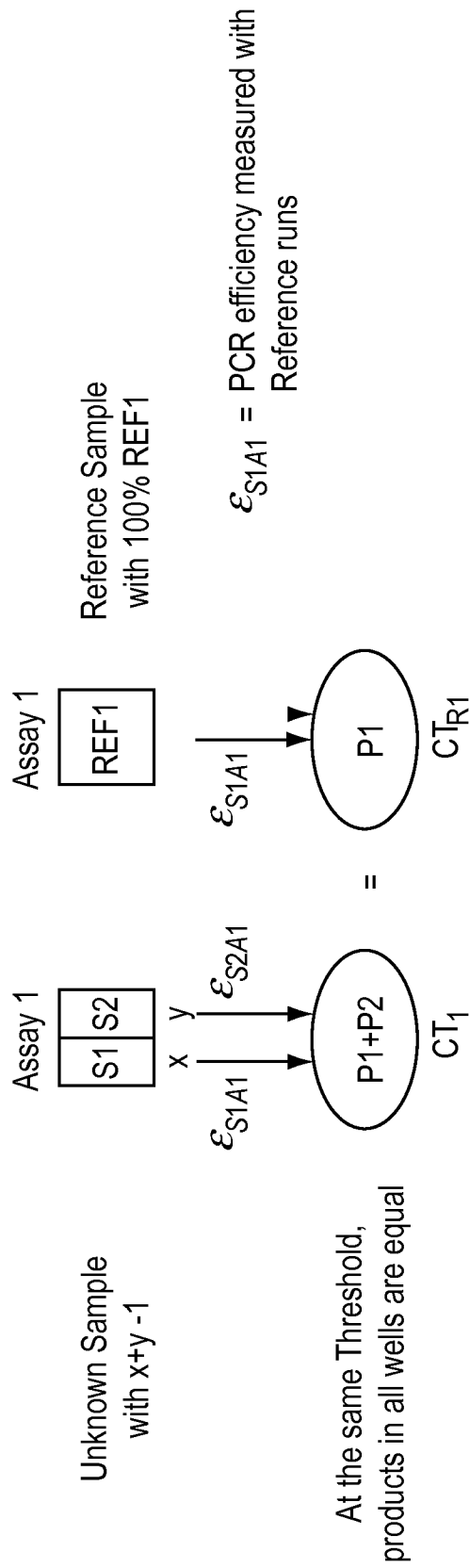


FIG. 1

Principle of calculating % of Seq1 when $x+y=1$ with 1 assay



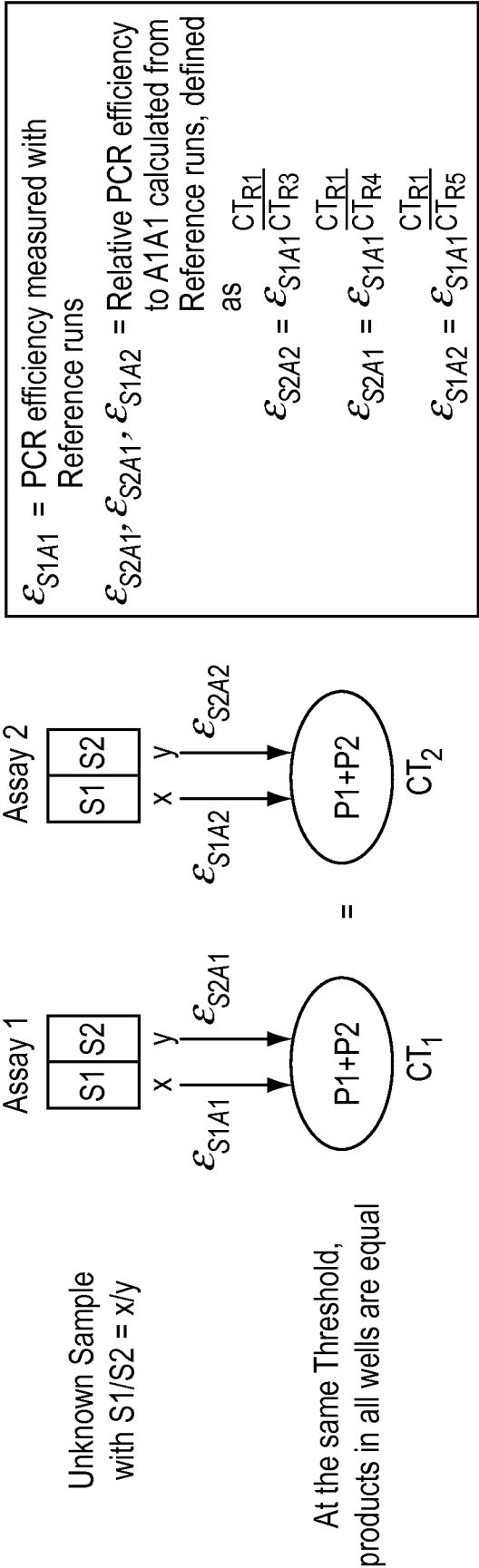
$$x \mathcal{E}_{S1A1}^{CT_1} + (1-x) \mathcal{E}_{S2A1}^{CT_1} = \mathcal{E}_{S1A1}^{CT_{R1}}$$

$$x\% = \left(\frac{\mathcal{E}_{S1A1}^{CT_{R1}} - \mathcal{E}_{S2A1}^{CT_1}}{\mathcal{E}_{S1A1}^{CT_1} - \mathcal{E}_{S2A1}^{CT_1}} \right) \times 100\%$$

$$x\% \equiv \varepsilon_{S1A1}^{(CT_{R1}-CT_1)} \times 100\%$$

FIG. 2

Principle of calculating Seq1/Seq2 ratio with 2 assays



$$x \mathcal{E}_{S1A1}^{CT_1} + y \mathcal{E}_{S2A1}^{CT_1} = x \mathcal{E}_{S1A2}^{CT_2} + y \mathcal{E}_{S2A2}^{CT_2}$$
$$\frac{x}{y} = \frac{\mathcal{E}_{S2A2}^{CT_2} - \mathcal{E}_{S2A1}^{CT_1}}{\mathcal{E}_{S1A1}^{CT_1} - \mathcal{E}_{S1A2}^{CT_2}} \quad (4 \text{ terms})$$

When $\mathcal{E}_{S2A1} \ll \mathcal{E}_{S2A2}$ and $\mathcal{E}_{S1A2} \ll \mathcal{E}_{S1A1}$, the equation can be simplified to

$$\frac{x}{y} \approx \frac{\mathcal{E}_{S2A2}^{CT_2}}{\mathcal{E}_{S1A1}^{CT_1}} \quad (2 \text{ terms})$$

FIG. 3

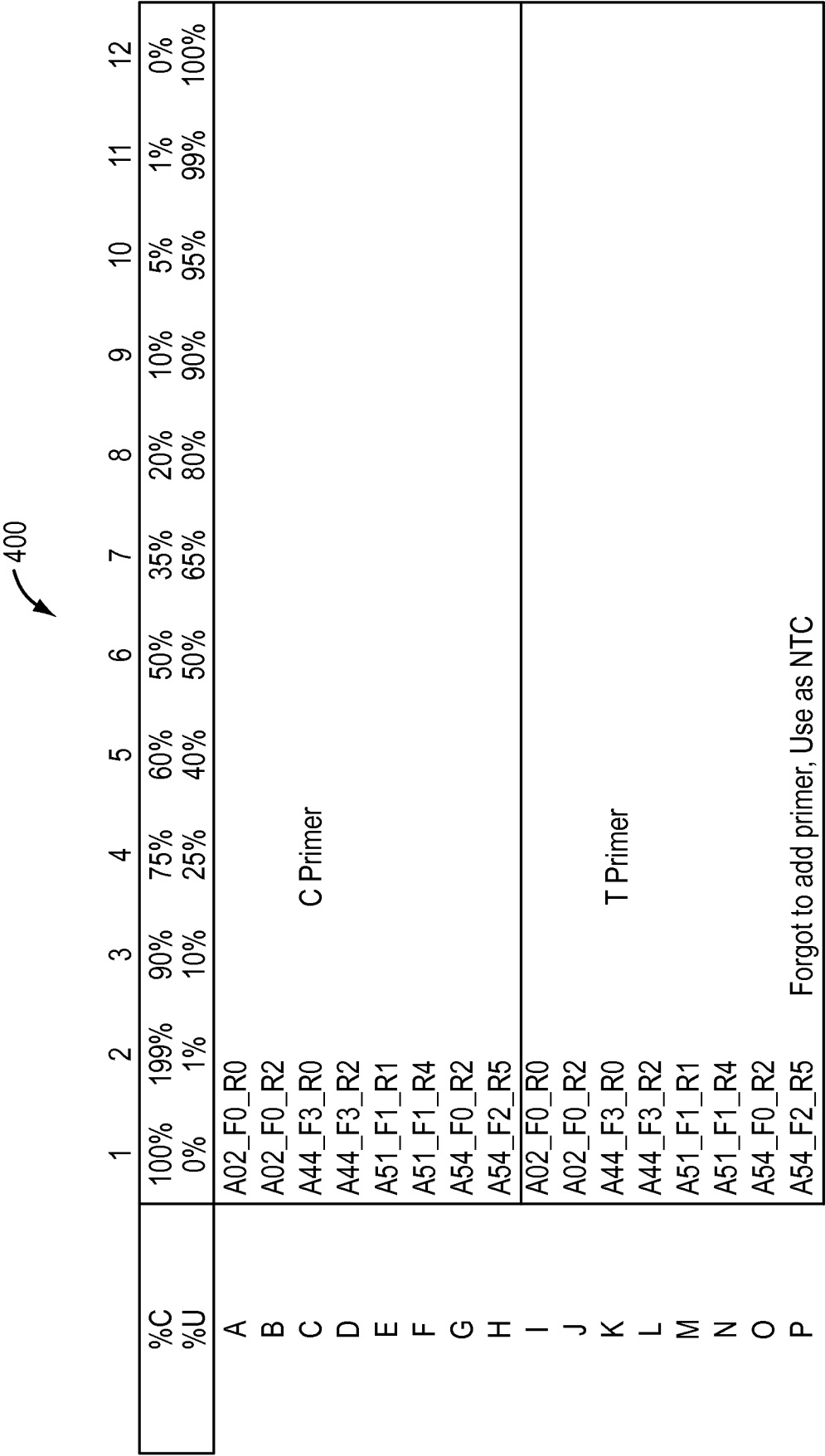


FIG. 4

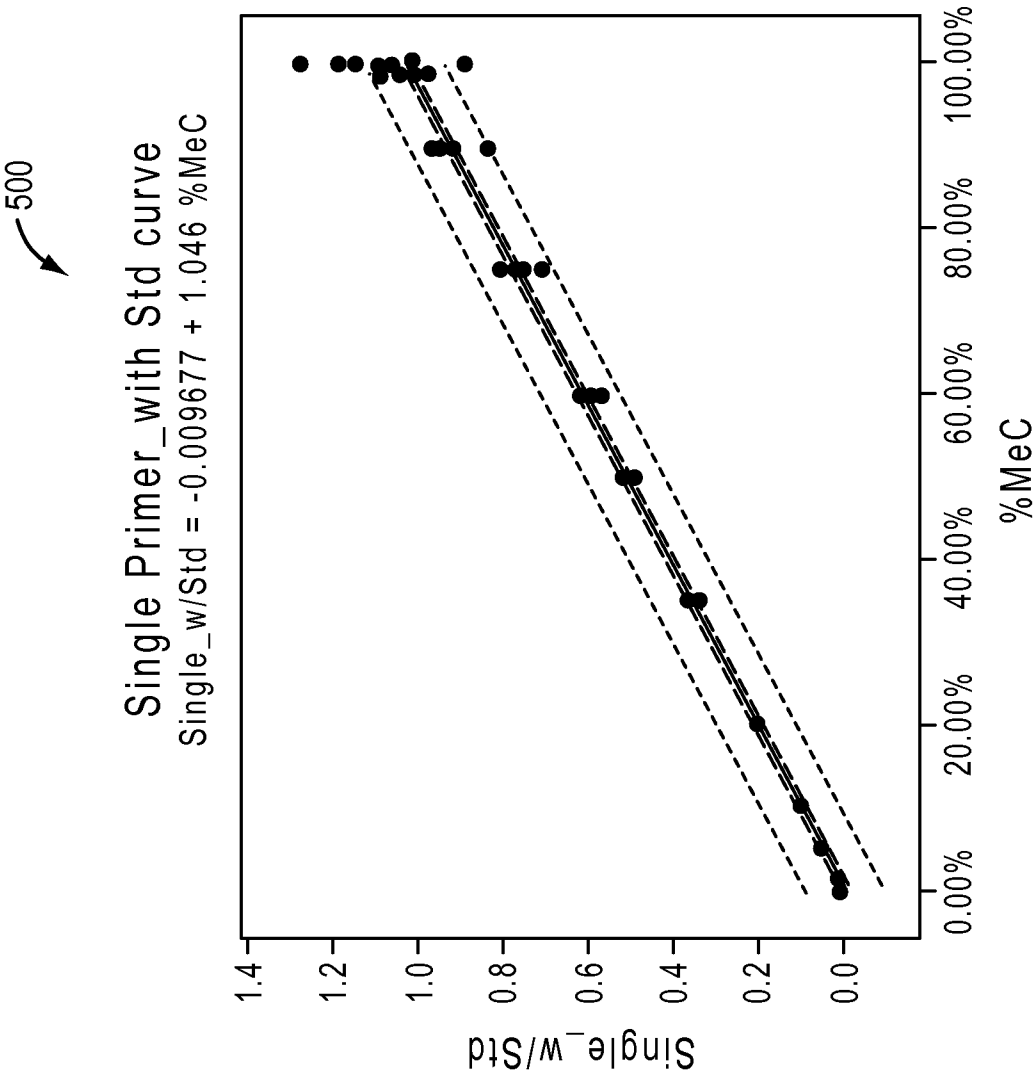


FIG. 5

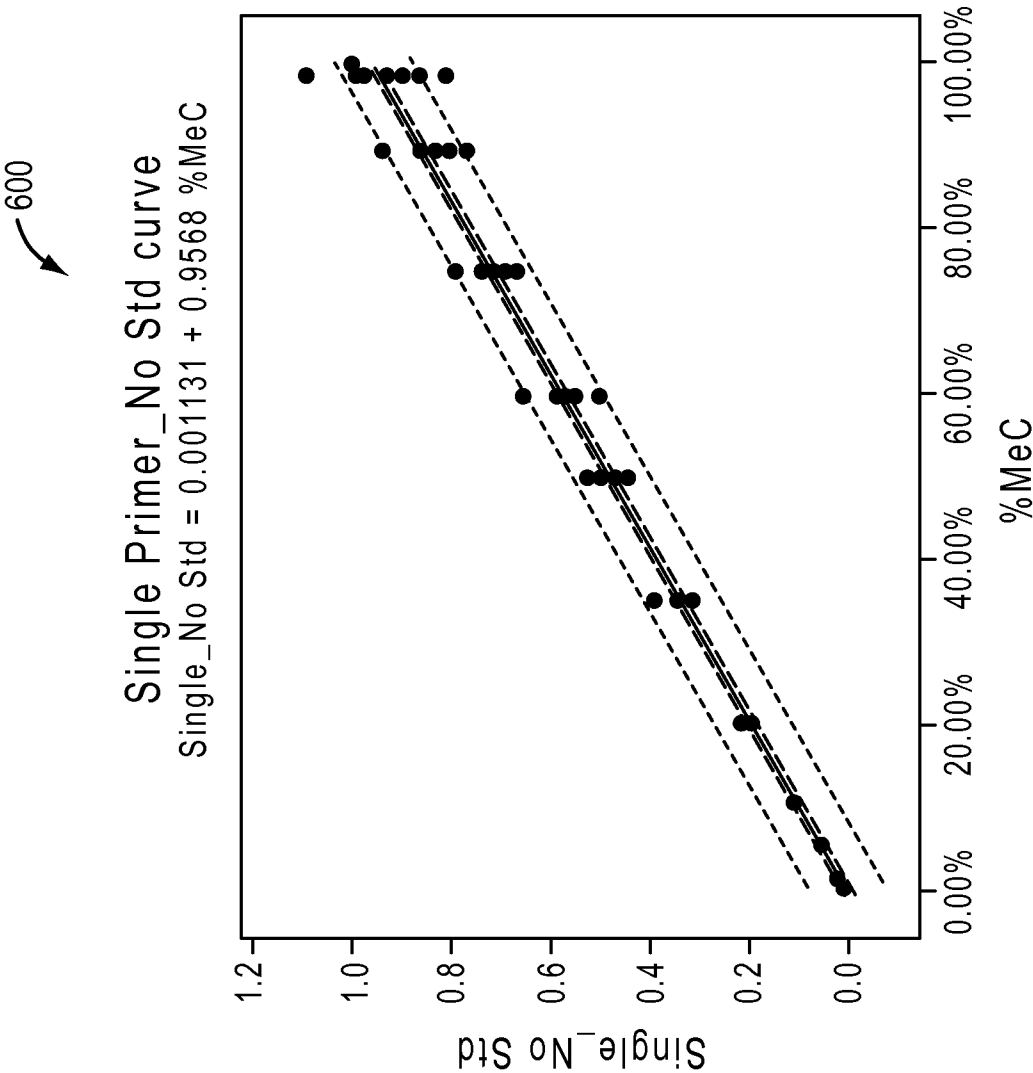


FIG. 6

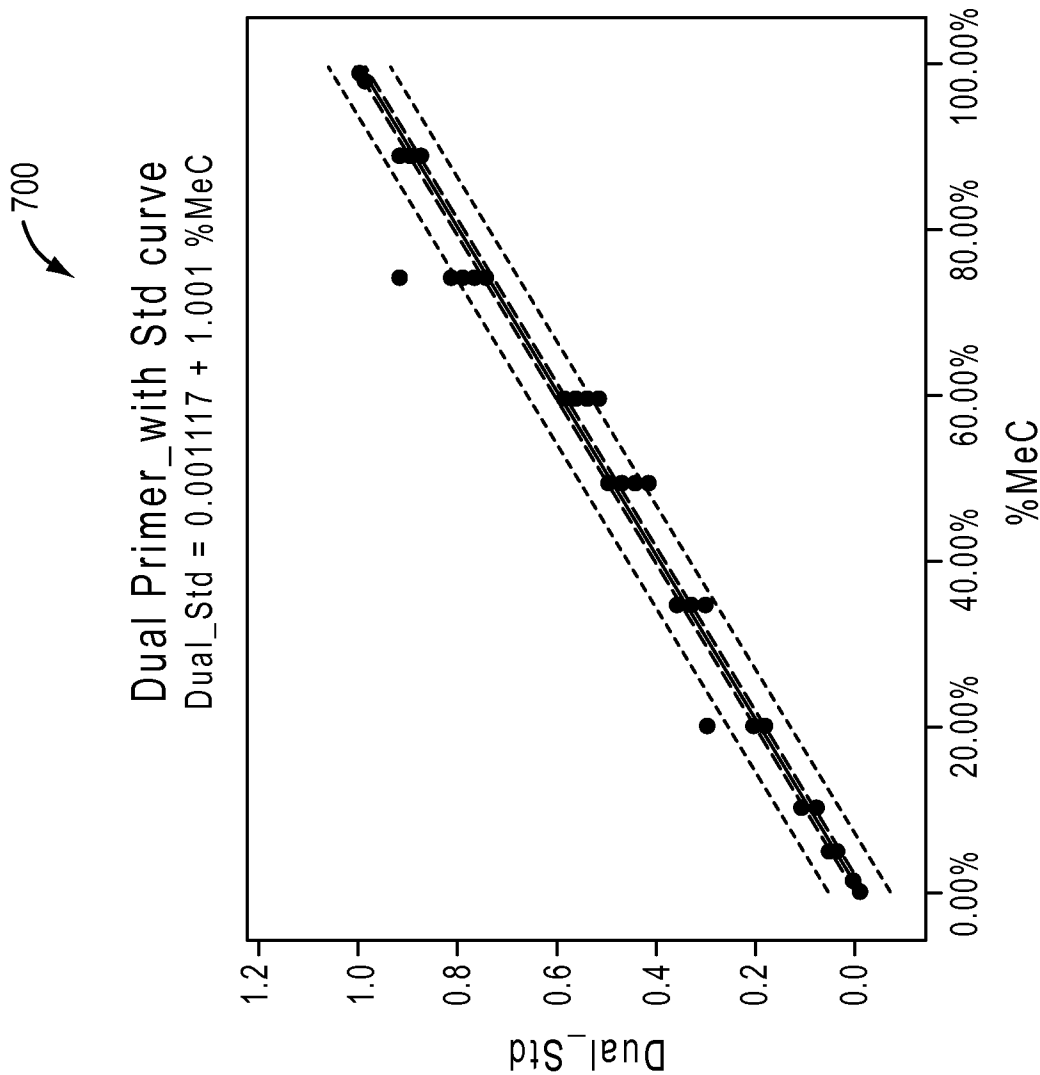


FIG. 7

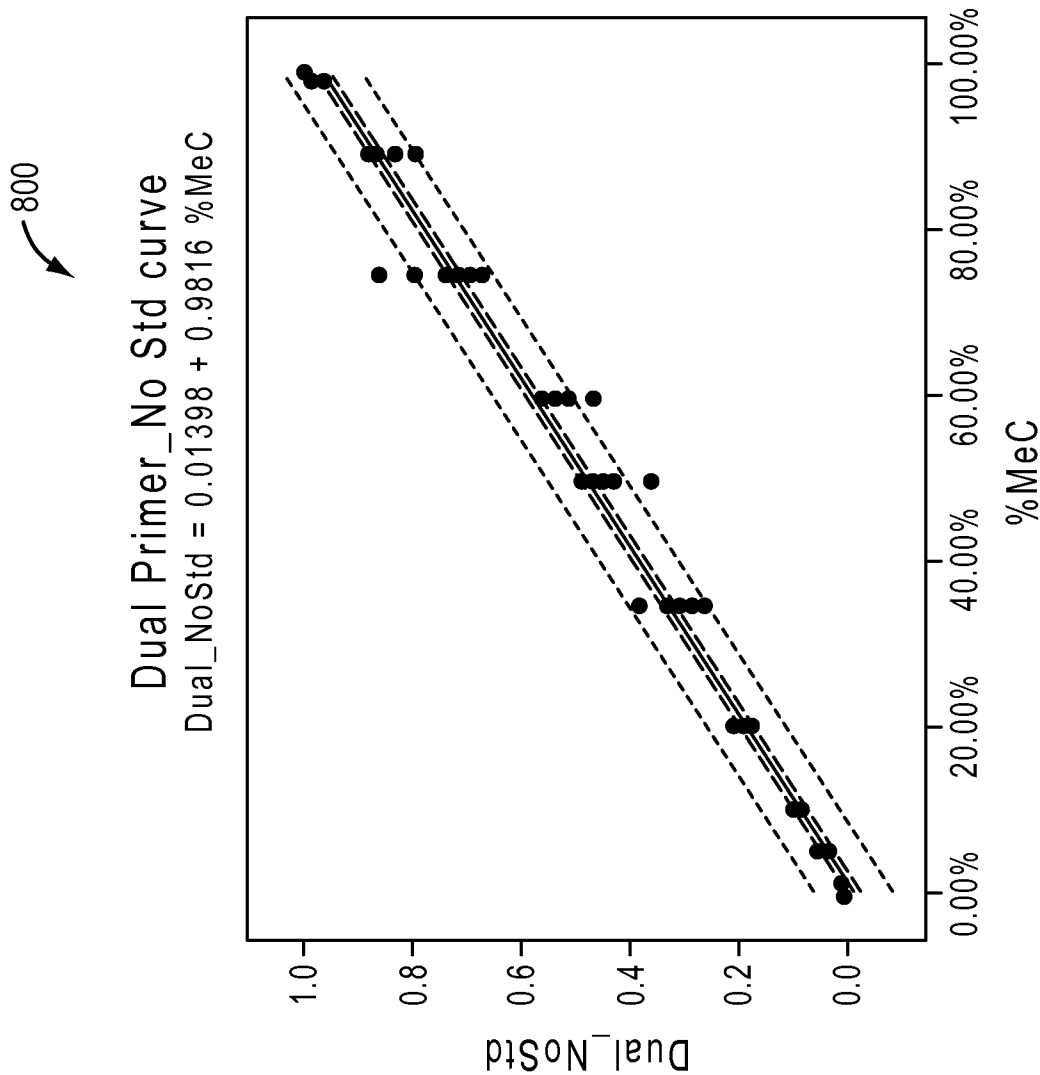


FIG. 8

Measured methylation level Vs Input level
Assays with good performance

900

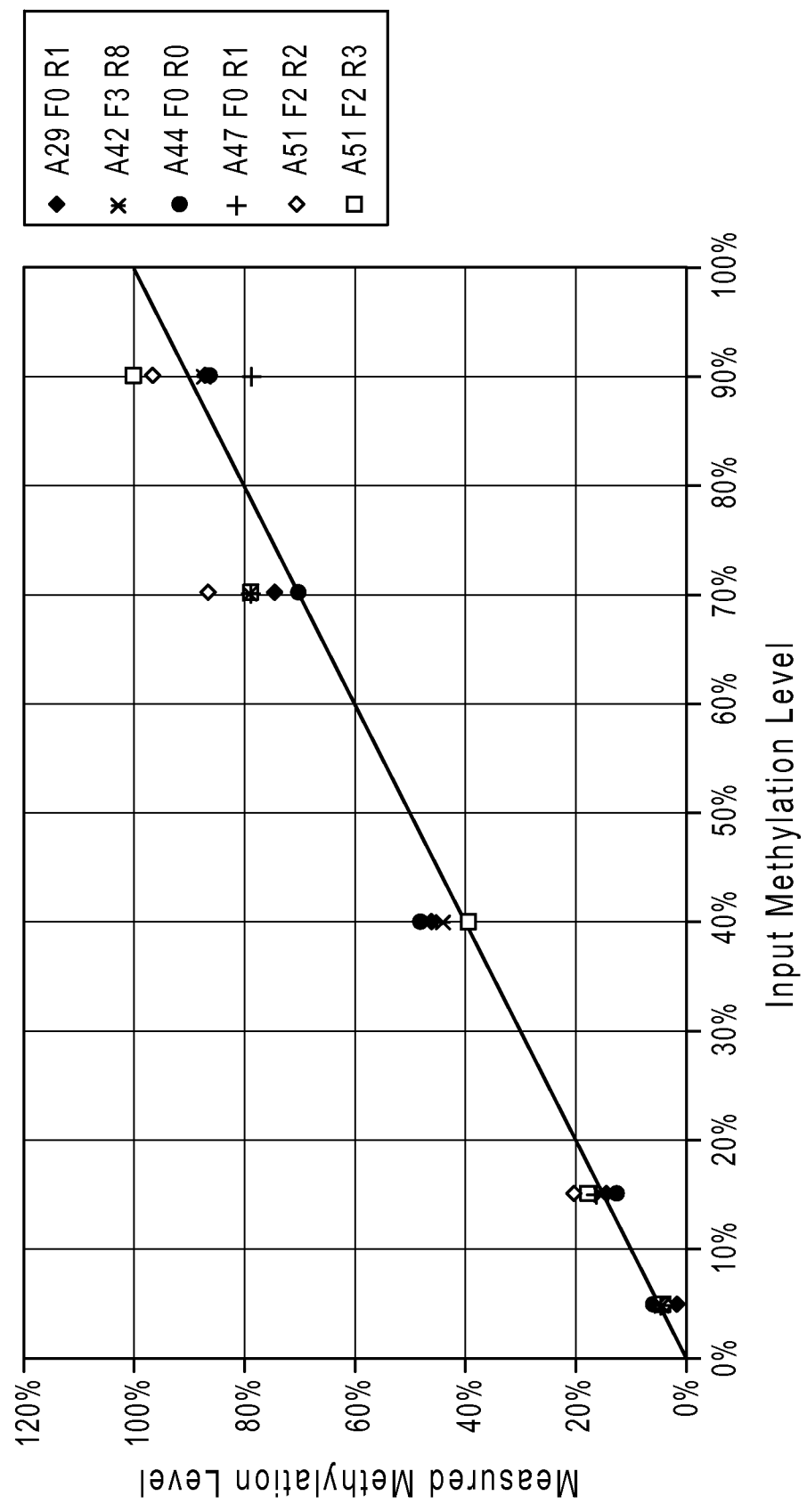


FIG. 9

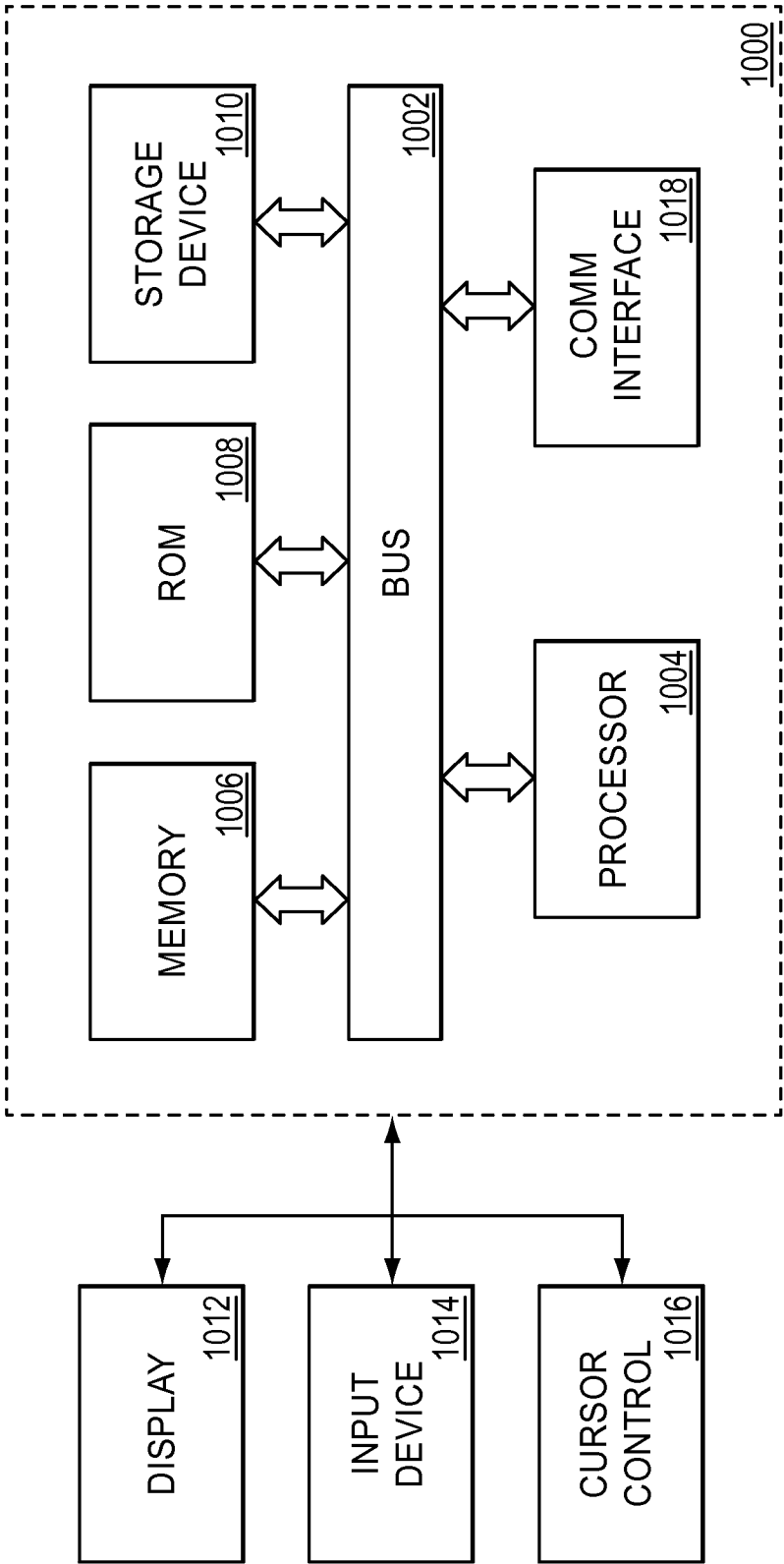


FIG. 10

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1100
↙

Table 1. Targets, Assay design, Sample Type and Experiments

Assay ID	Gene Symbol	Refseq	Chr	Strand	SYBR® Select Evaluation	Quantification Method	No. of assays	GC content range	Tm range (°C)	Primer Length range	Sample Type	
											gDNA	Synthetic
A02	APC	NM_001127511	5	+	X	X	12	43-65%	56.2-60.8	16-23		X
A29	ICAM1	NM_000201	19	+	X	X	16	26-100%	58.6-62.3	10-31	X	
A44	RPRM	NM_019845	2	-	X	X	15	31-61%	57.9-60.9	18-26	X	X
A51	SORBS3	NM_005775	8	+	X	X	15	38-61%	53.5-61.8	16-23	X	X
A54	SUB1	NM_006713	5	+	X	X	16	56-75%	58-60.7	13-18		X
A16	DPH1	NM_001383	17	+	X		6	43-63%	57.9-60.1	16-21	X	
A37	PTEN	NM_000314	10	+	X		11	32-67%	59.5-61.7	15-25	X	
A42	RPP14	NM_001098783	3	+	X		16	50-79%	57.9-62.1	13-22	X	
A45	SCGB3A1	NM_052863	5	-	X		15	41-85%	54.2-61.5	13-23	X	
A49	SYK	NM_003177	9	+	X		16	16-92%	57.8-60.9	12-32	X	
A47	SCGB3A1	NM_052863	5	-		X	1				X	

FIG. 11

1200

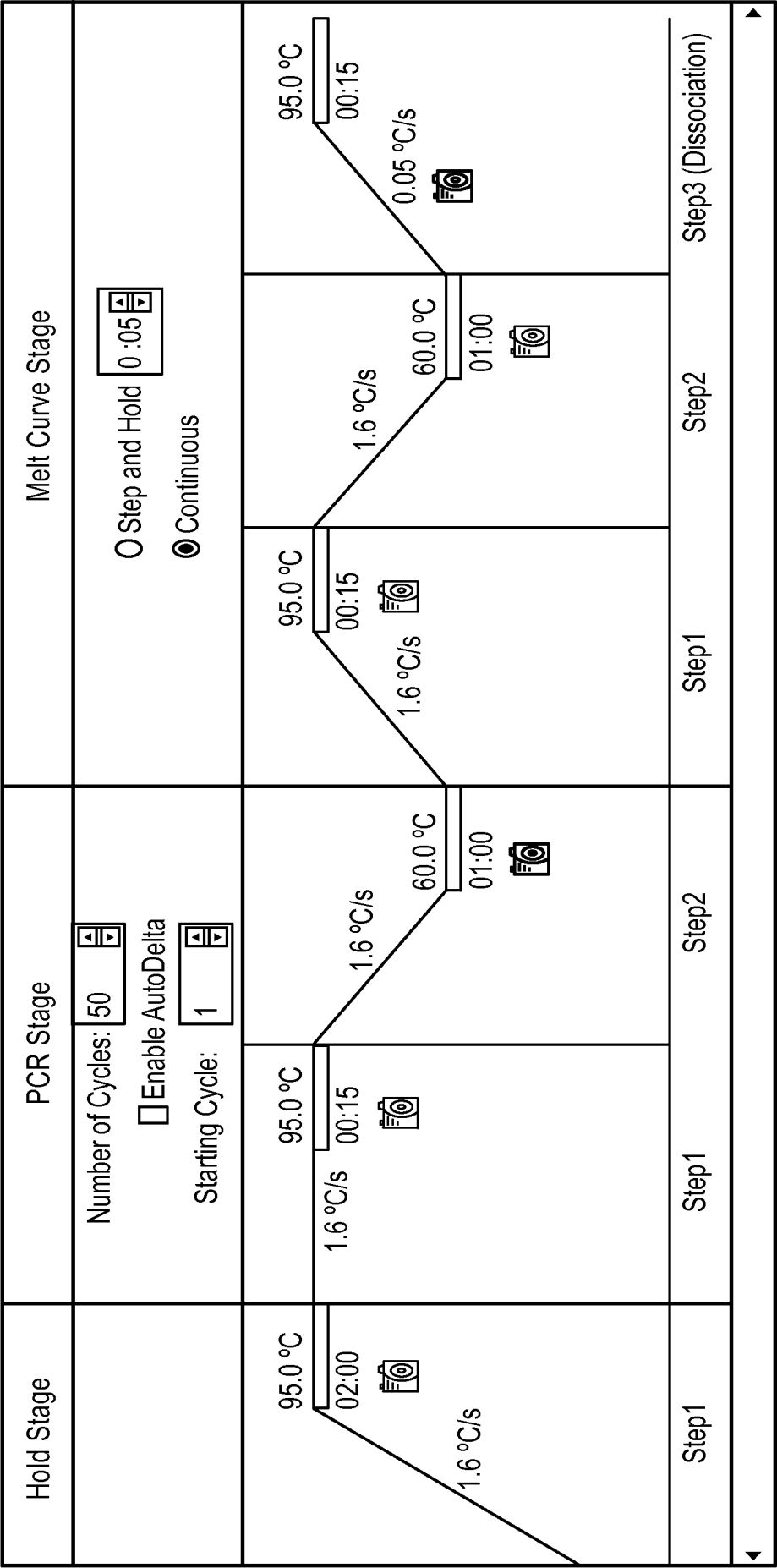


FIG. 12

1300

Schematic Workflow for Quantitation Measurement

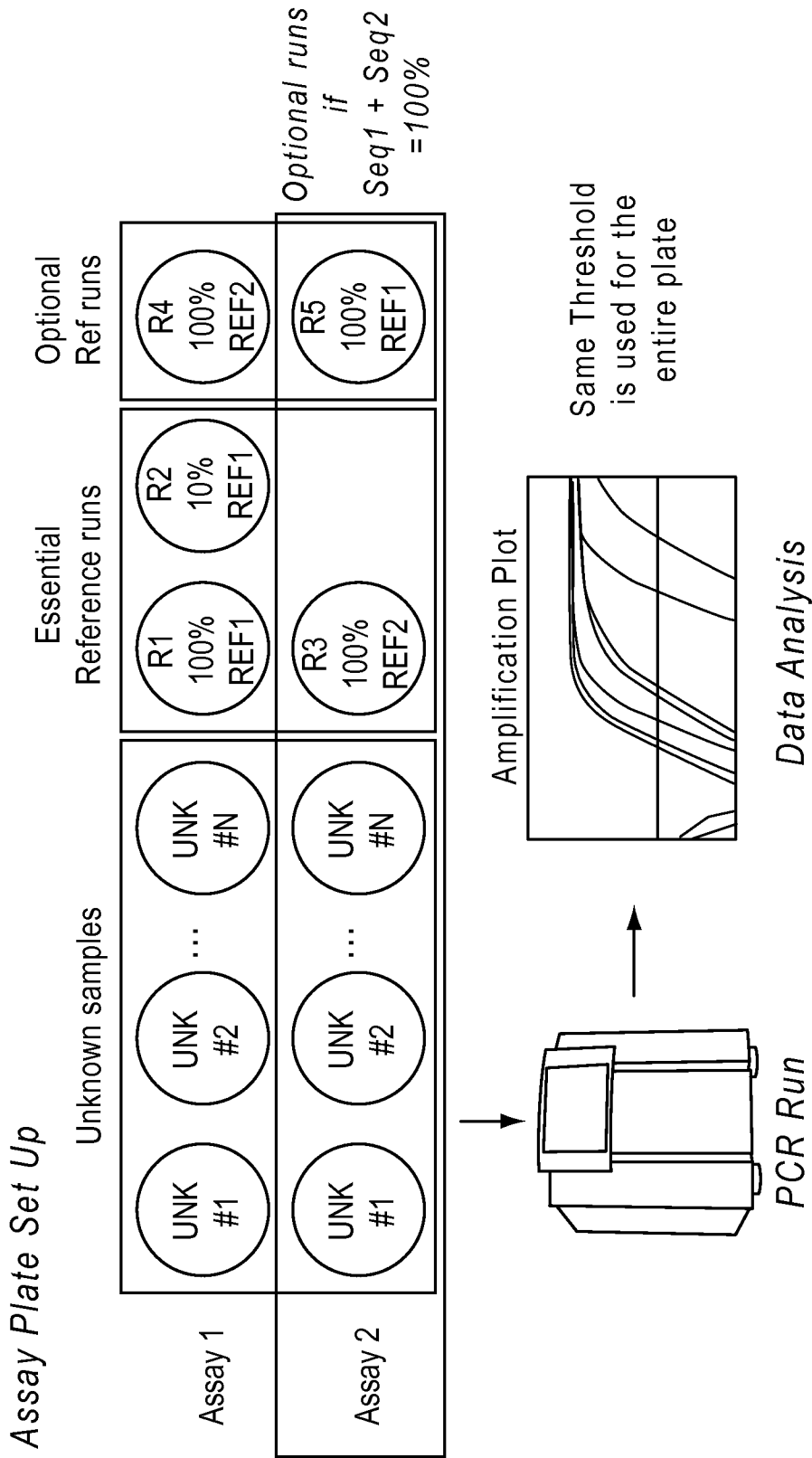


FIG. 13

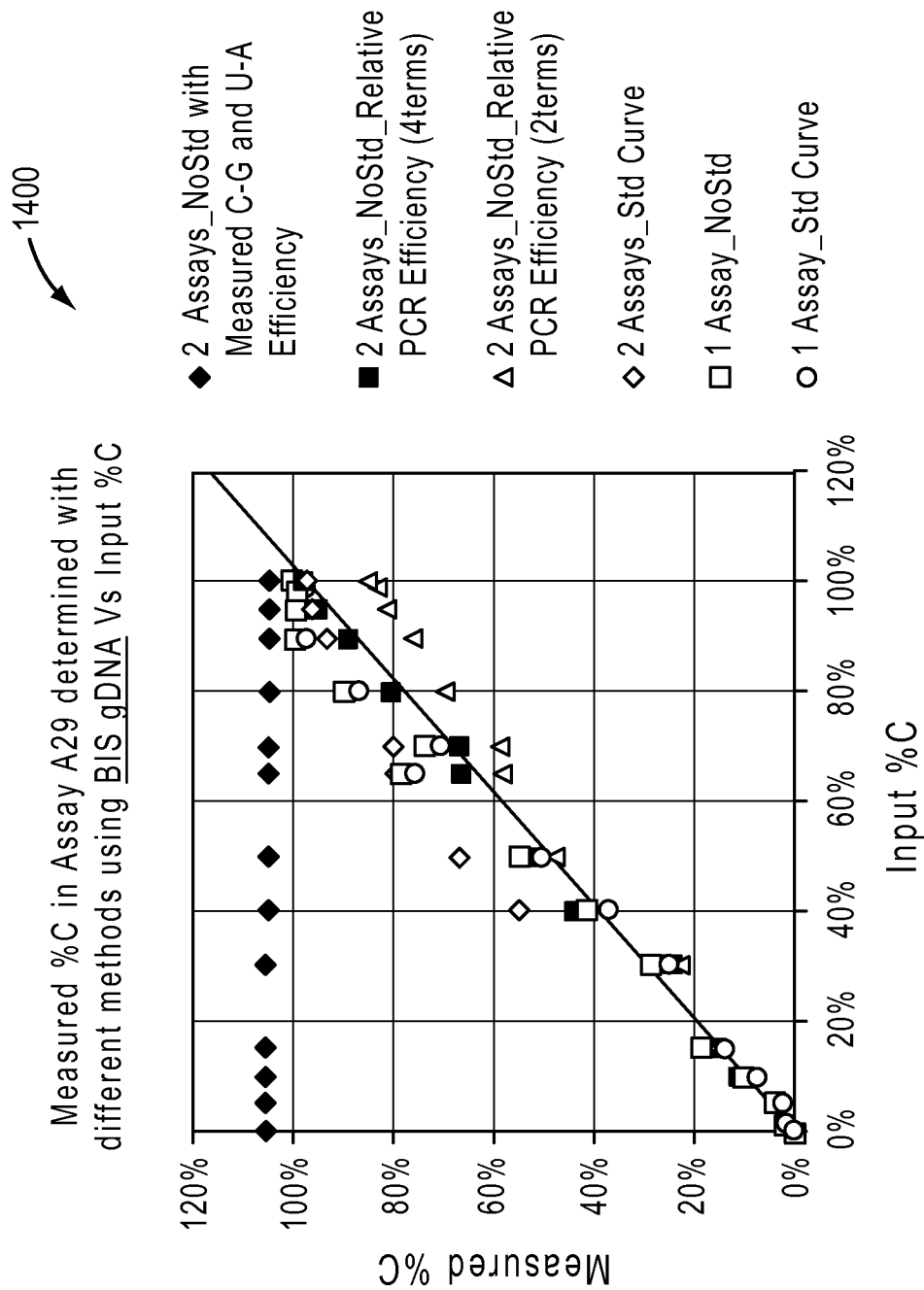


FIG. 14A

1402

Measured %C in Assay A44 F3R2 with different
methods using Synthetic Templates Vs Input %C

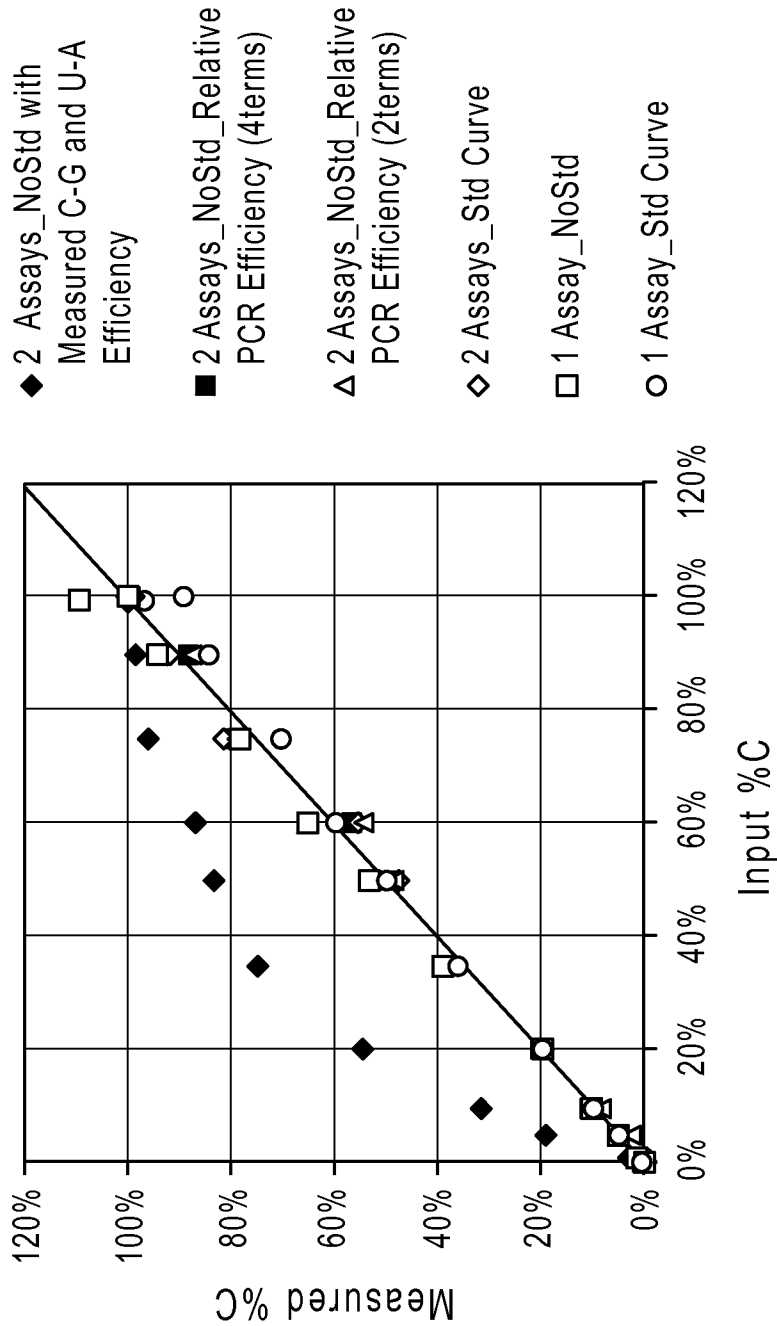


FIG. 14B

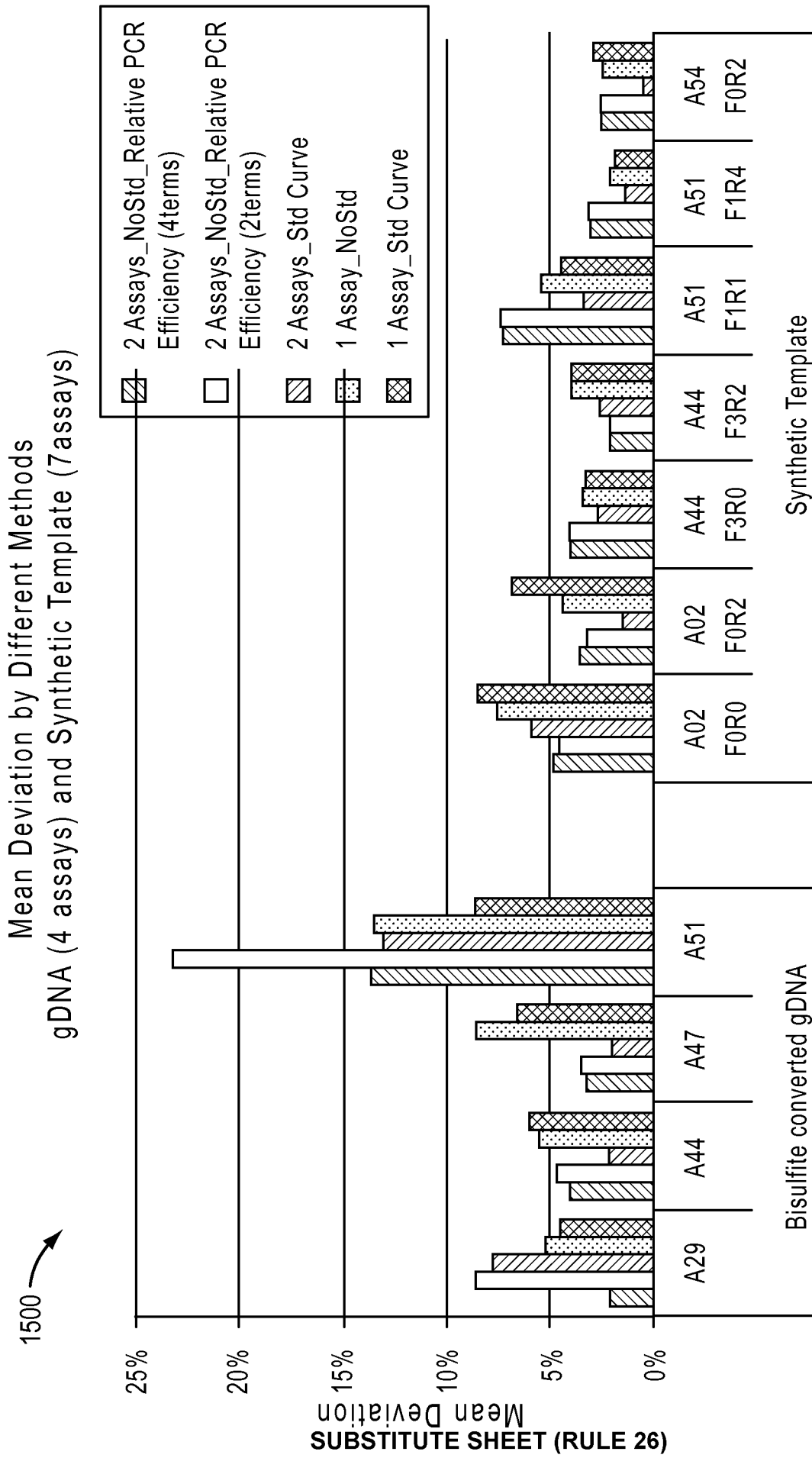


FIG. 15

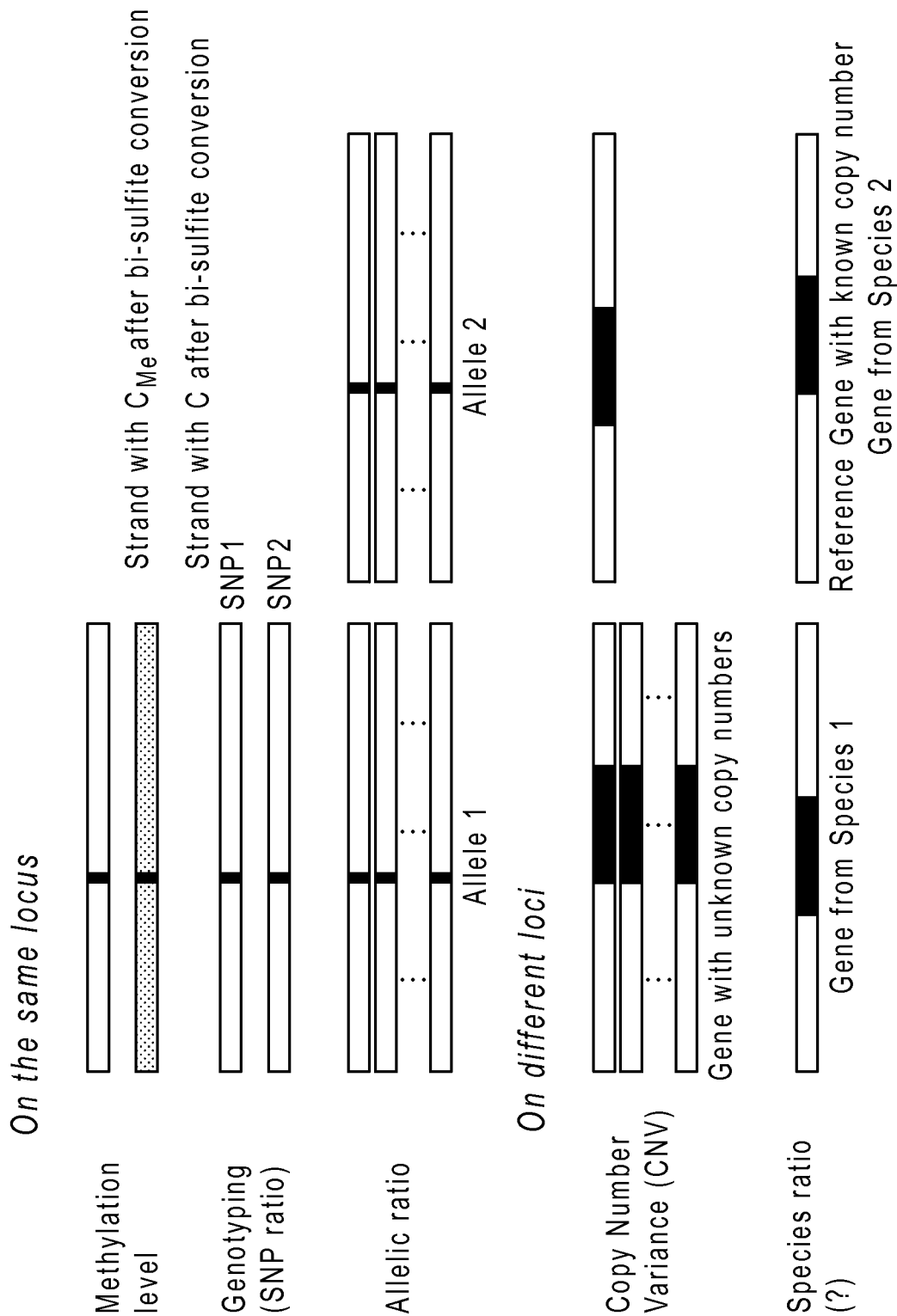


FIG. 16

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1700

Application	Assay 1	Assay 2		Ref1	Ref2	Ref3	Ref4	Ref5
Methylation Level	"C" assay	"U" assay	Sample	100% MeC	10% MeC + 90% UnM	100% UnM	100% MeC (optional)	100% UnM (optional)
			Assay	"C" assay	"C" assay	"U" assay	"U" assay	"U" assay
CNV	Reference assay	Target assay	Sample	Known X and Y	Not required	Known X and Y	Not required	Not required
			Assay	Reference assay		Target assay		
Genotyping	SNP1 assay	SNP2 assay	Sample	100% SNP1	50% SNP1 + 50% SNP2	100% SNP2	100% SNP1	100% SNP2
			Assay	SNP1 assay	SNP1 assay	SNP2 assay	SNP2 assay	SNP1 assay

FIG. 17

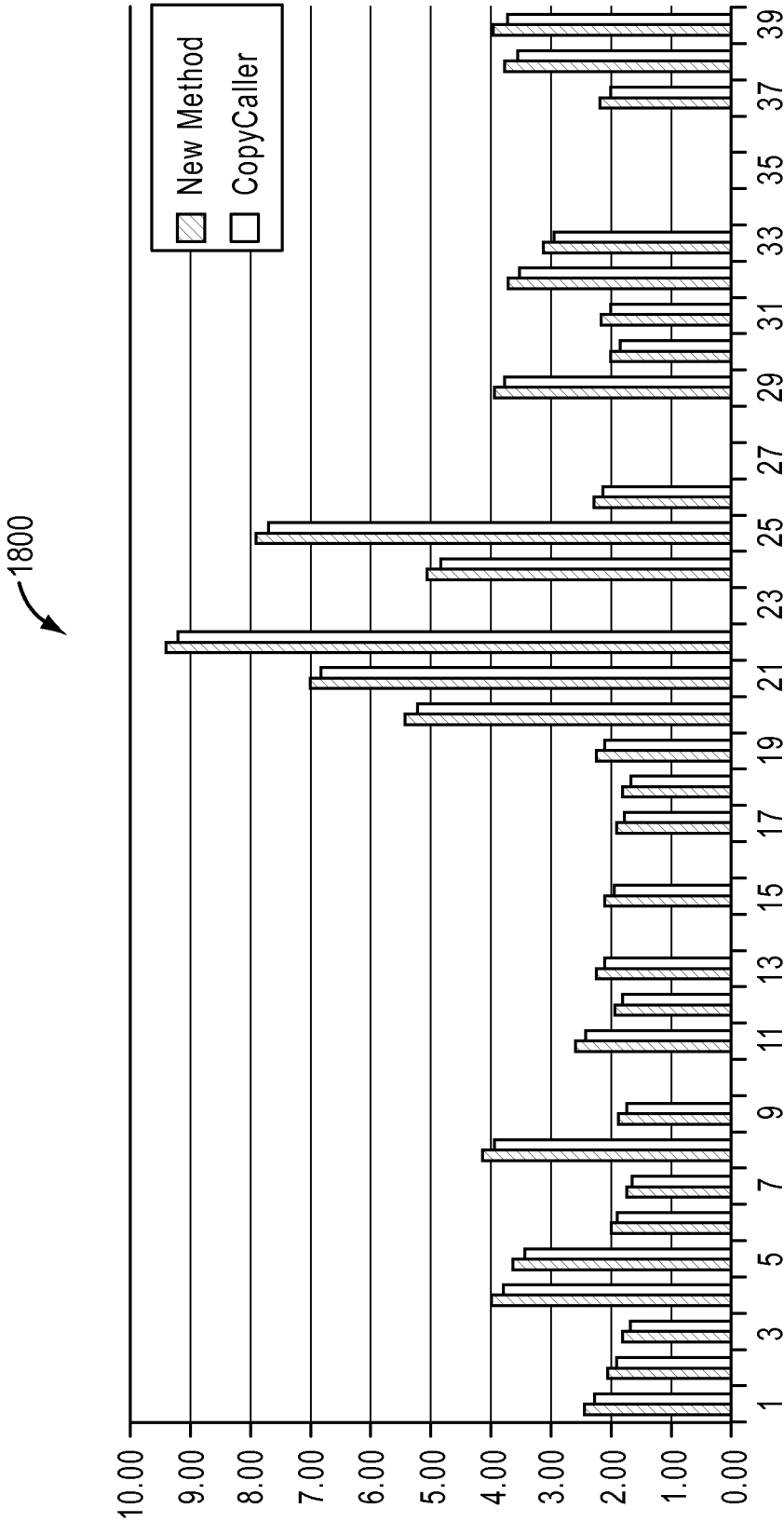


FIG. 18

Assay	A44 F3R0	A51 F1R4
Measured ϵ S1A1	1.93	1.97
Measured ϵ S2A2	1.84	2.02
Relative ϵ S2A2	1.58	1.85
CT(S1A1)	12.05	12.37
CT(S2A2)	17.21	13.61

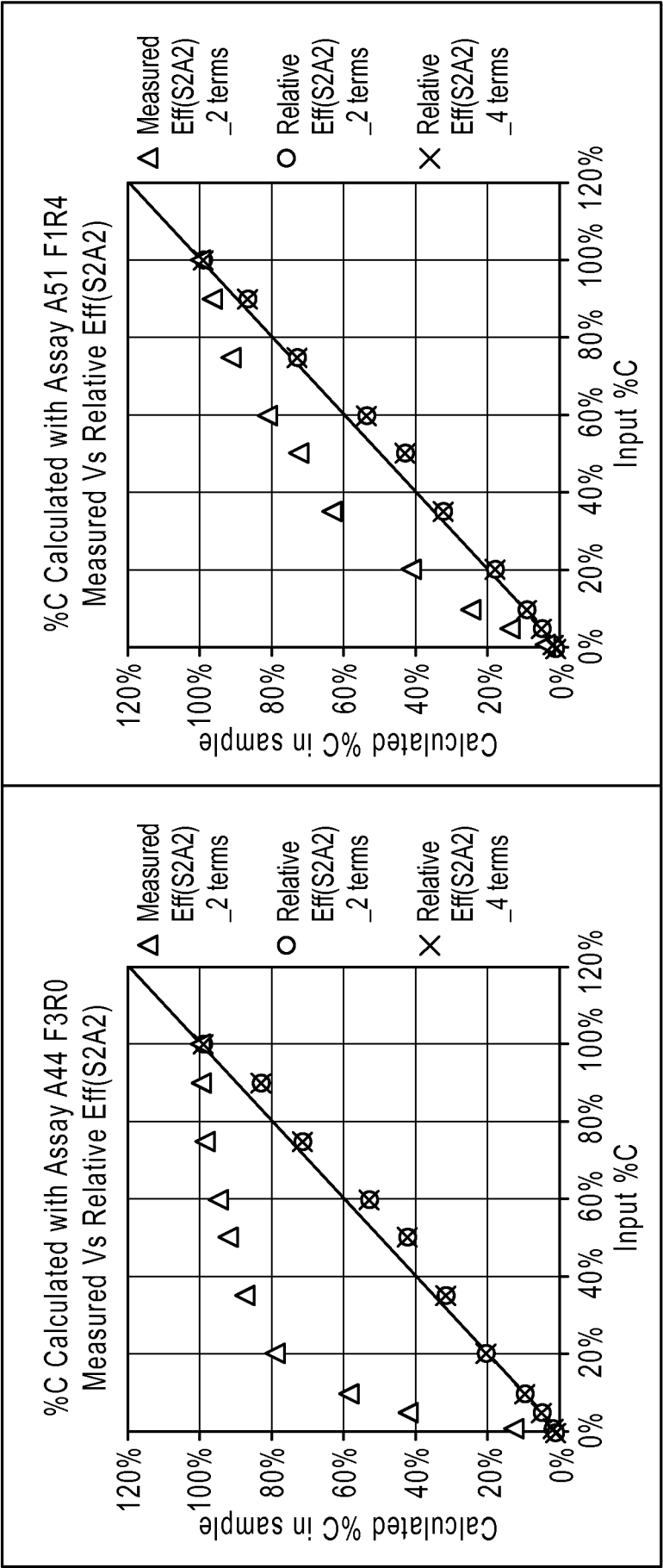


FIG. 19