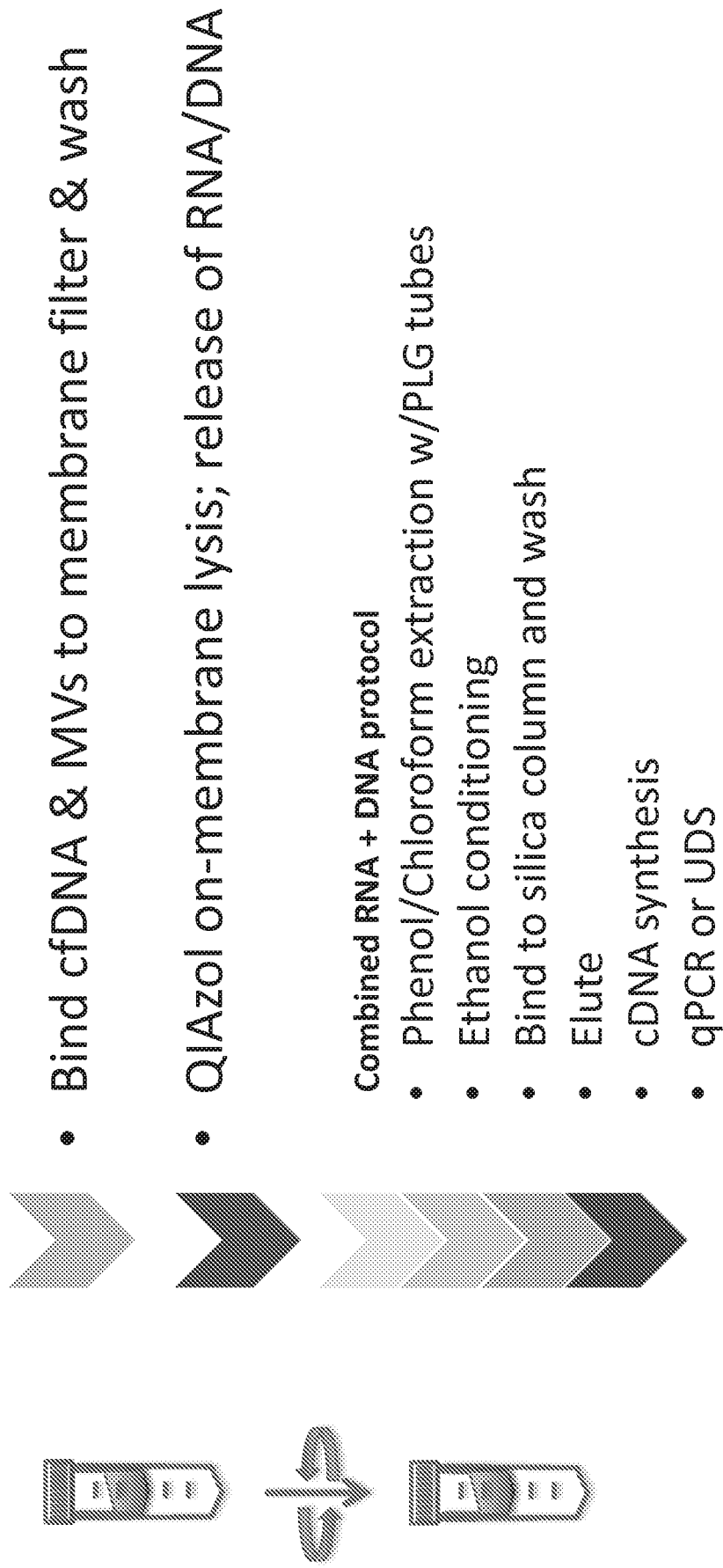


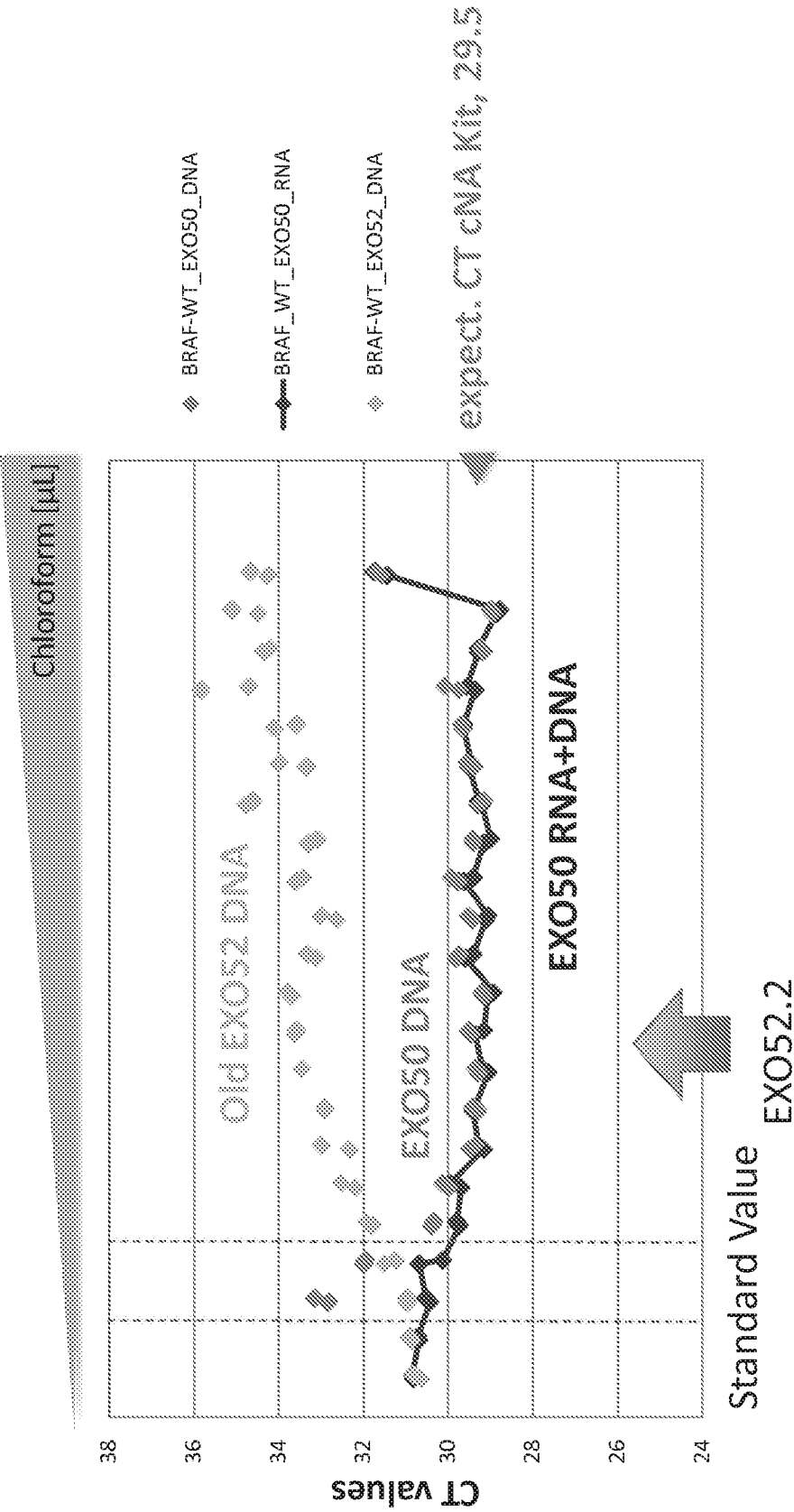


Figure

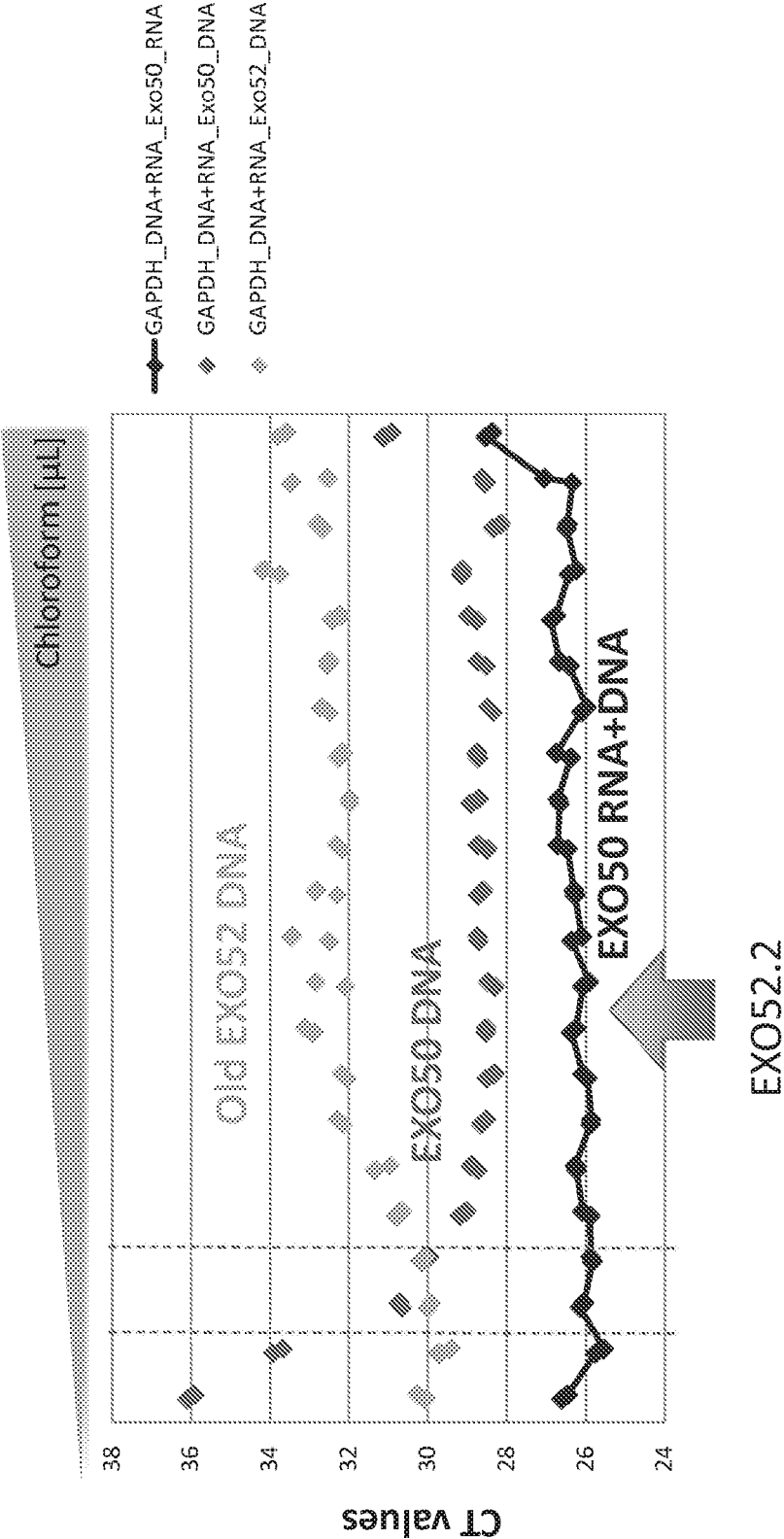
One combined workflow instead of two separate ones



Figure

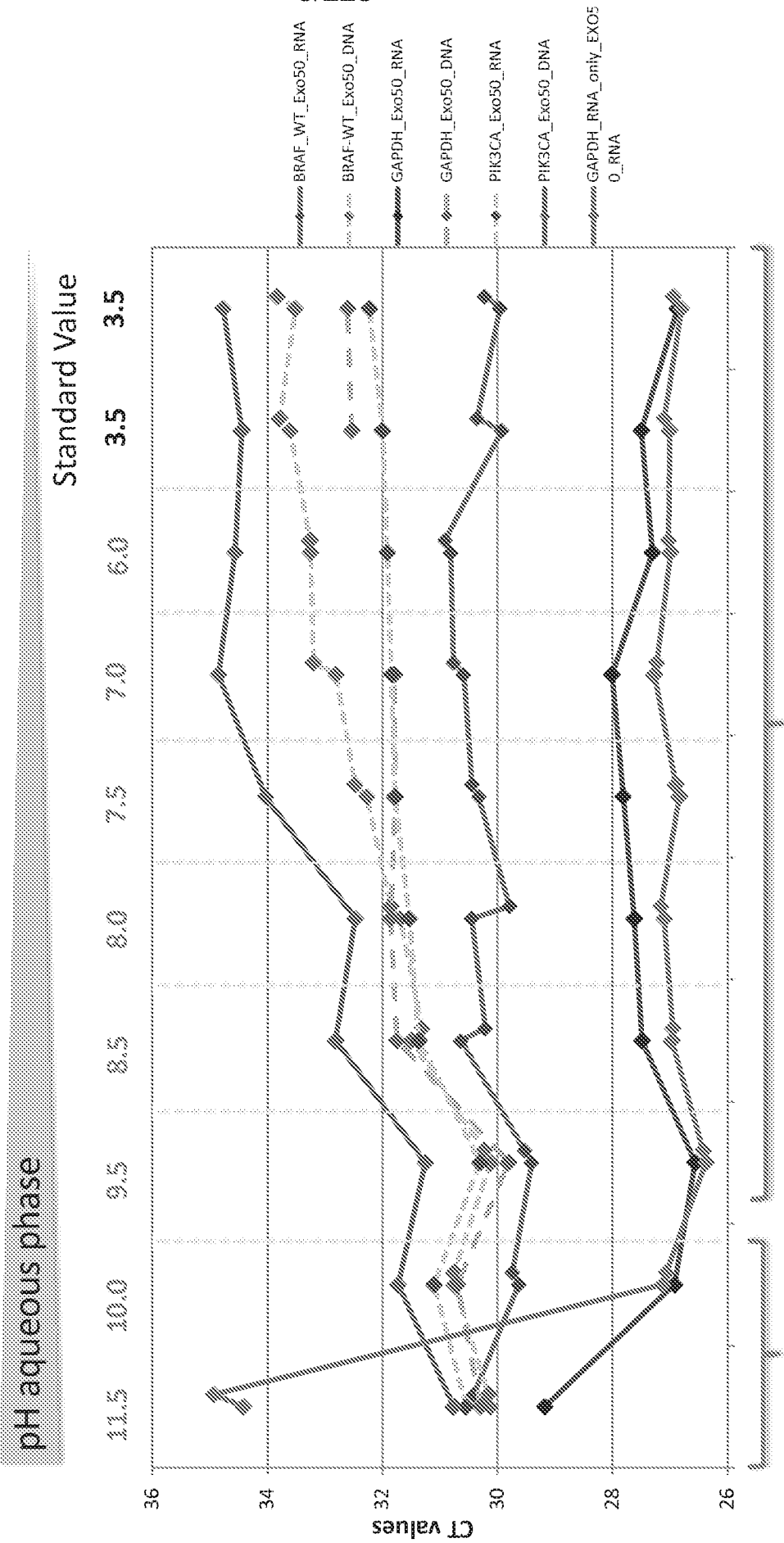


Figure



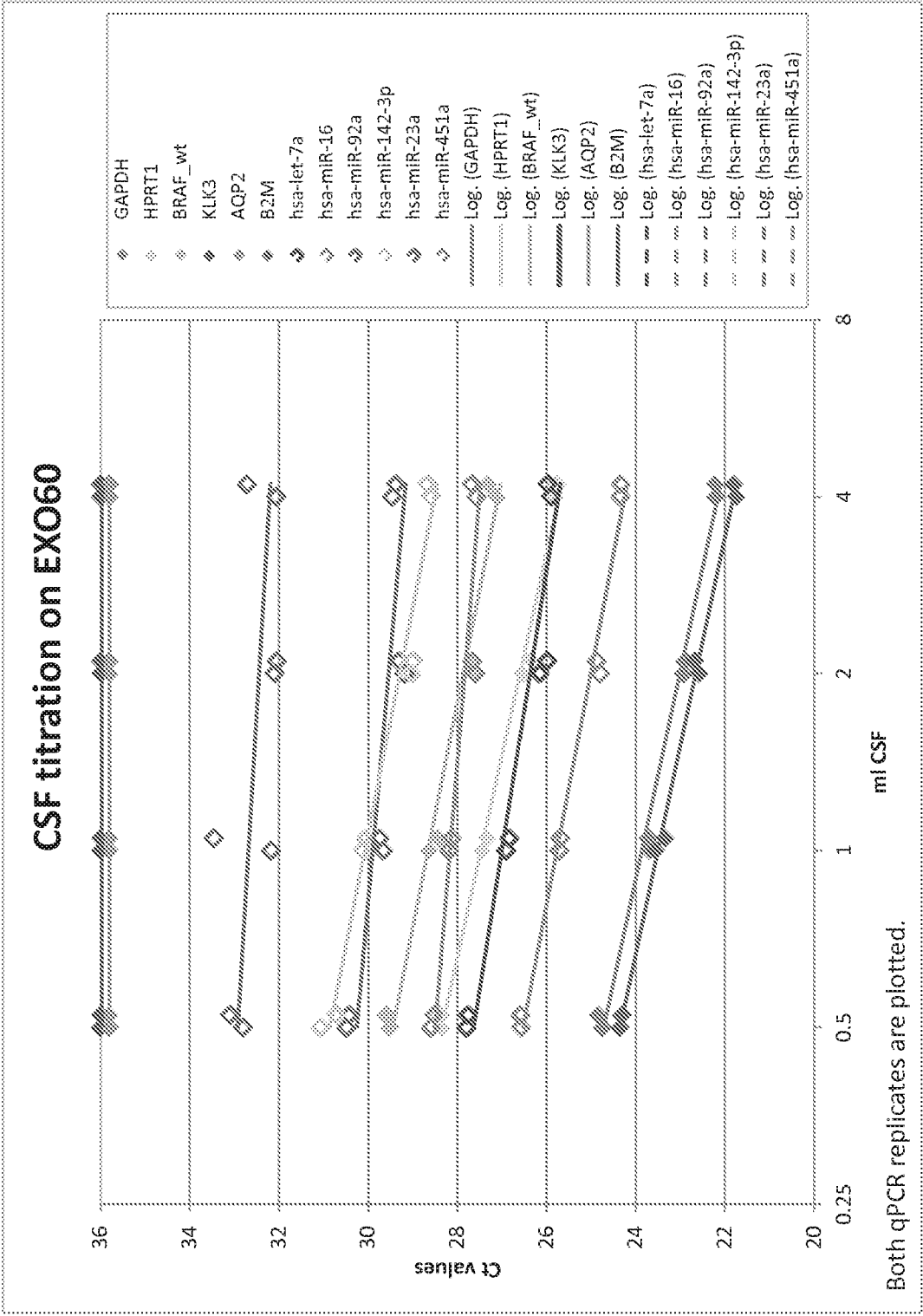
Figure

Adjusting pH in phase separation adds the DNA to the EXO52.2 prep

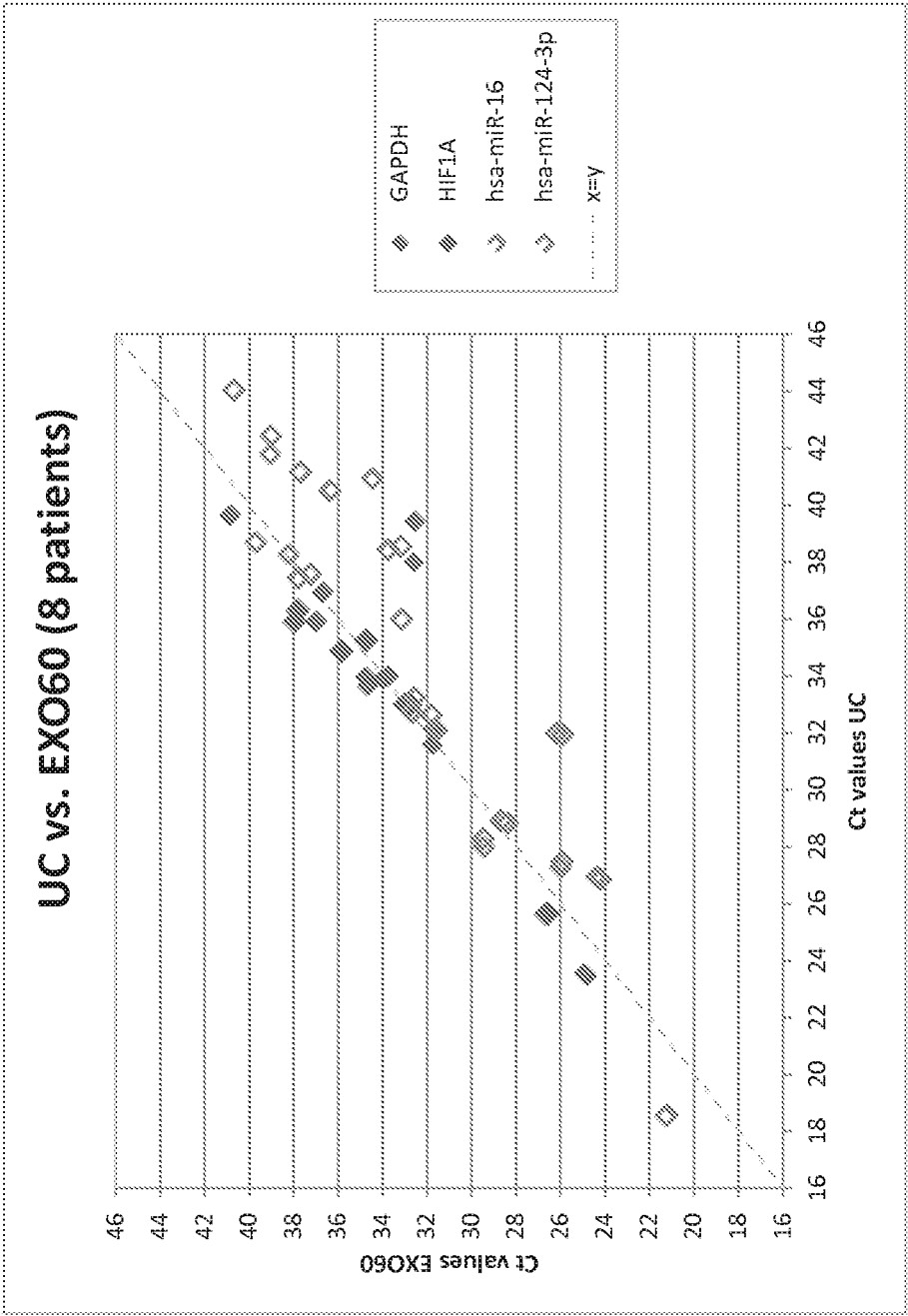


Negative Effect on RNA 3-4 drop in CT b/c addition of DNA (dotted lines; EXO50 w/o RT)

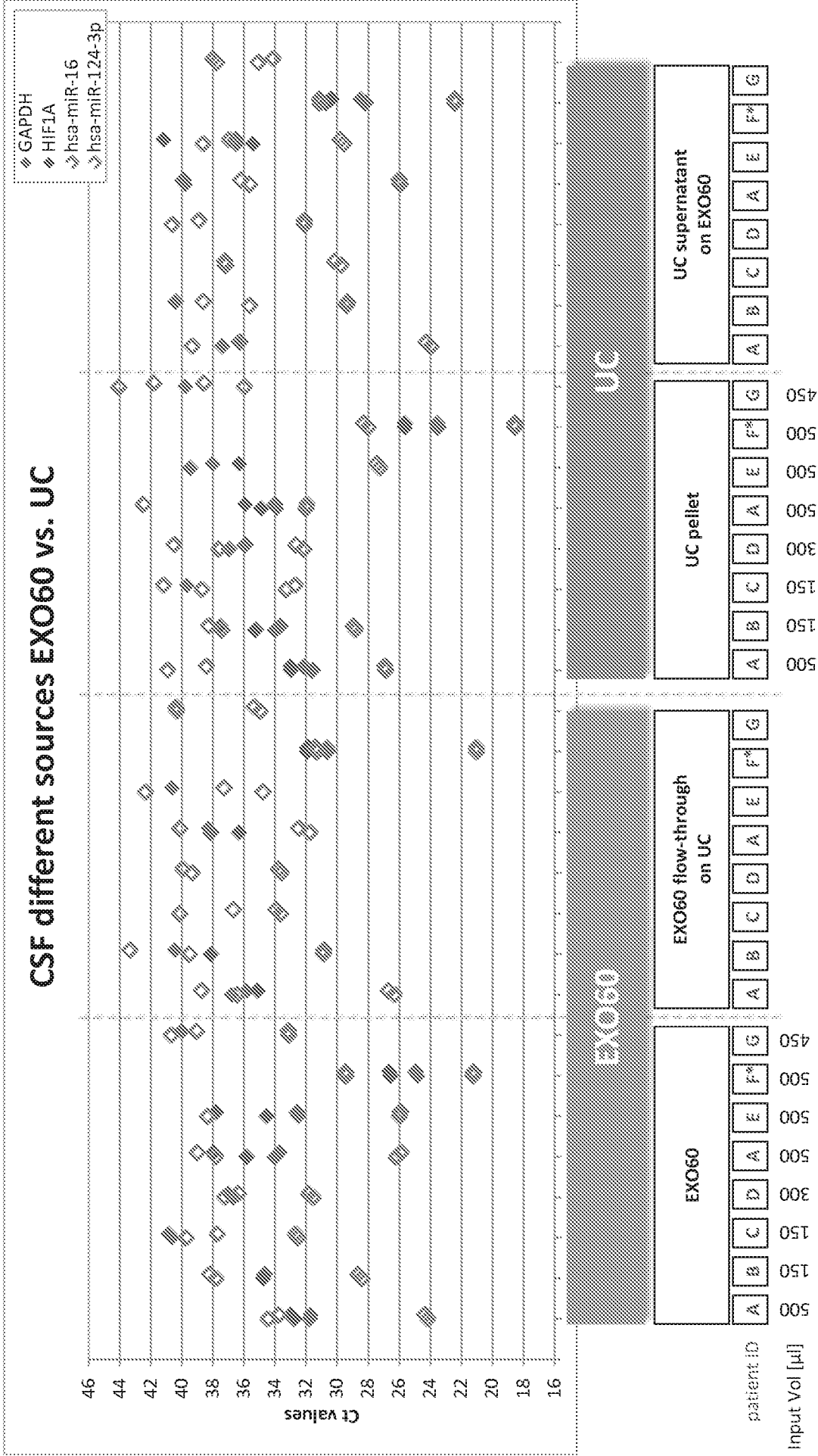
Figure



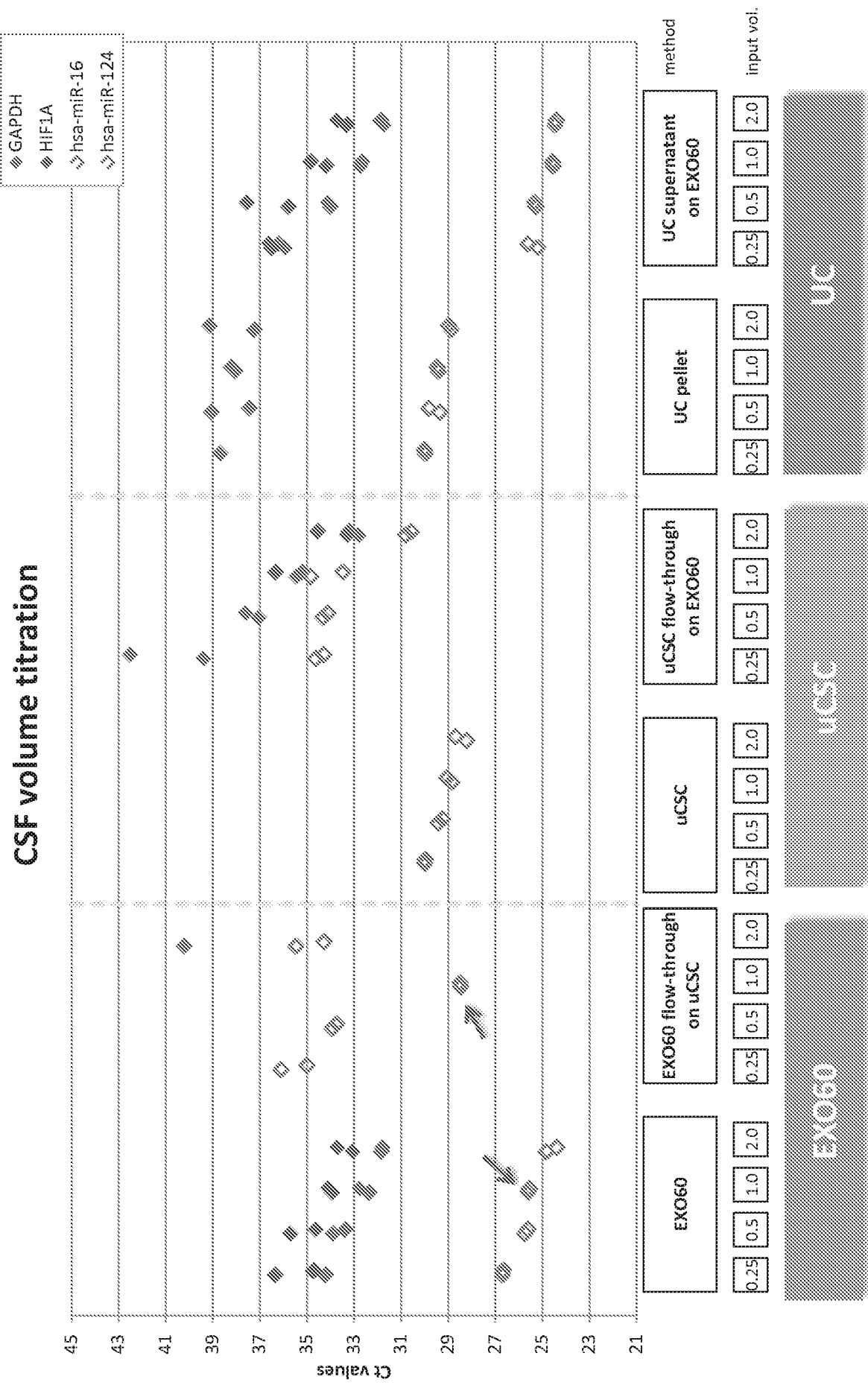
Figure



Figure

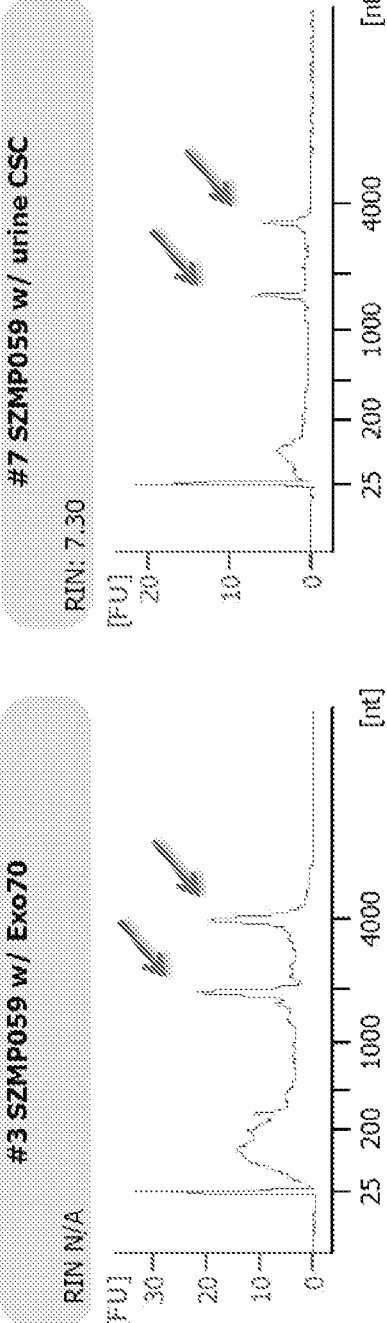
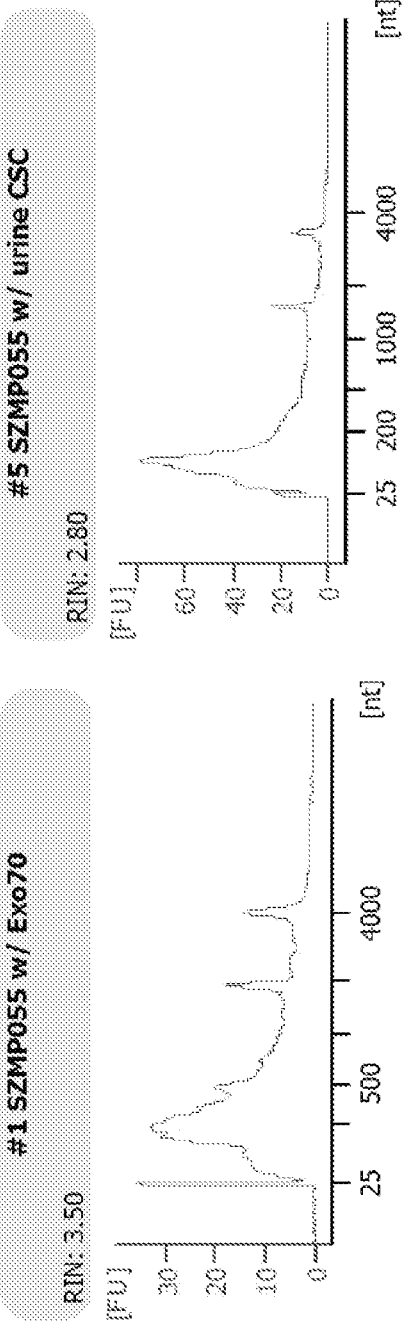


Figure

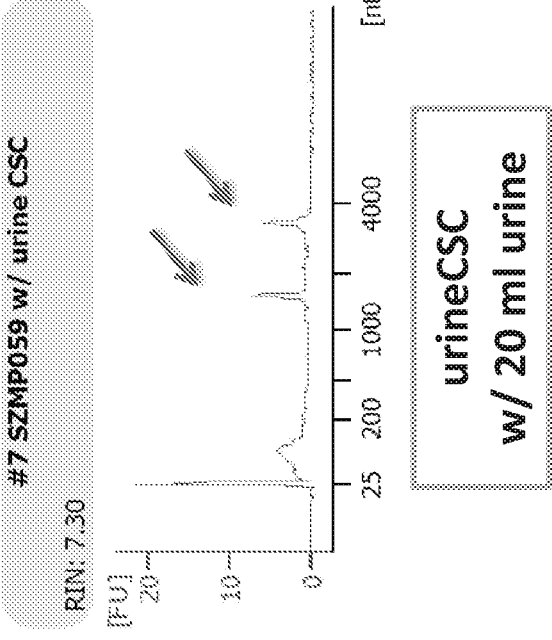
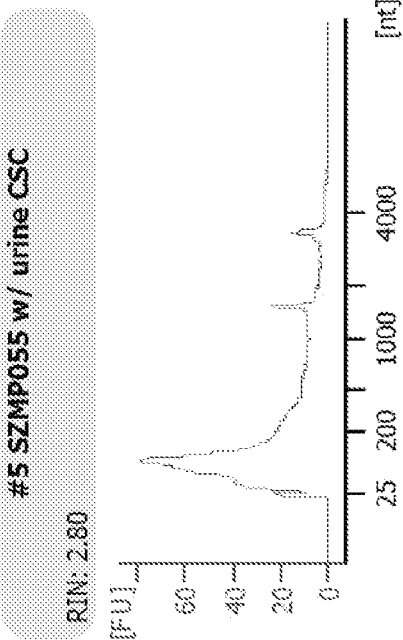


Figure

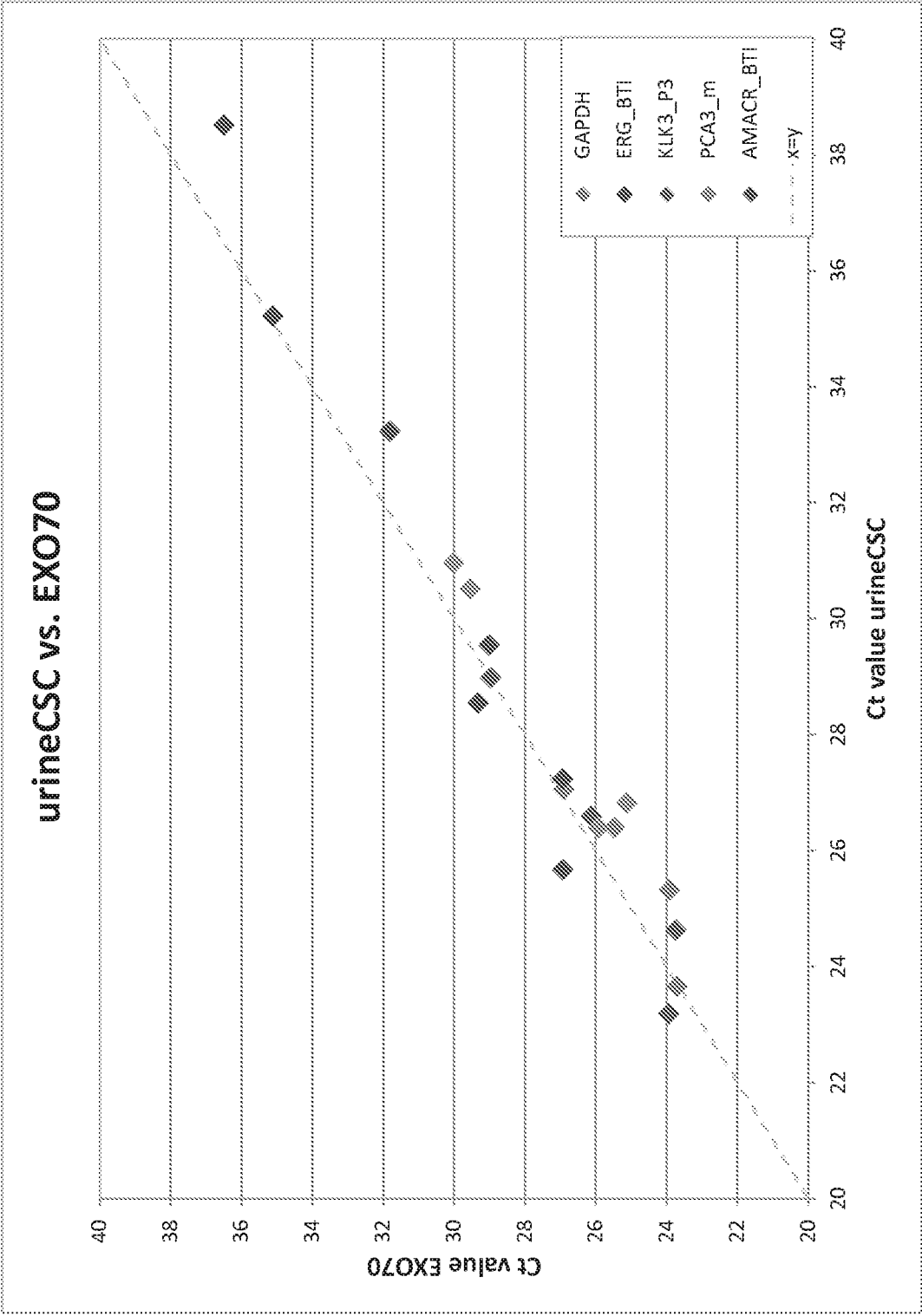
Patient #1



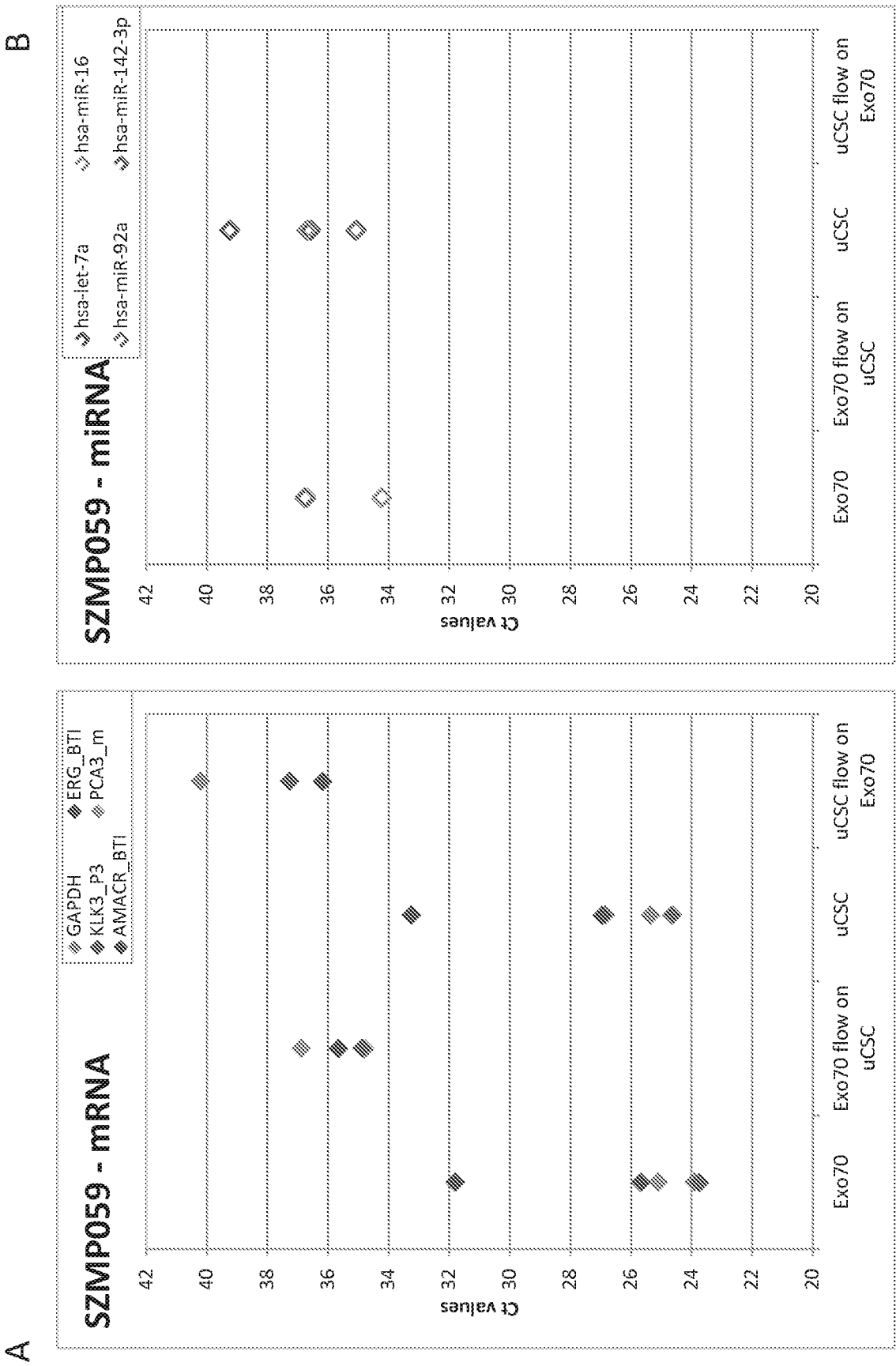
Patient #2



Figure

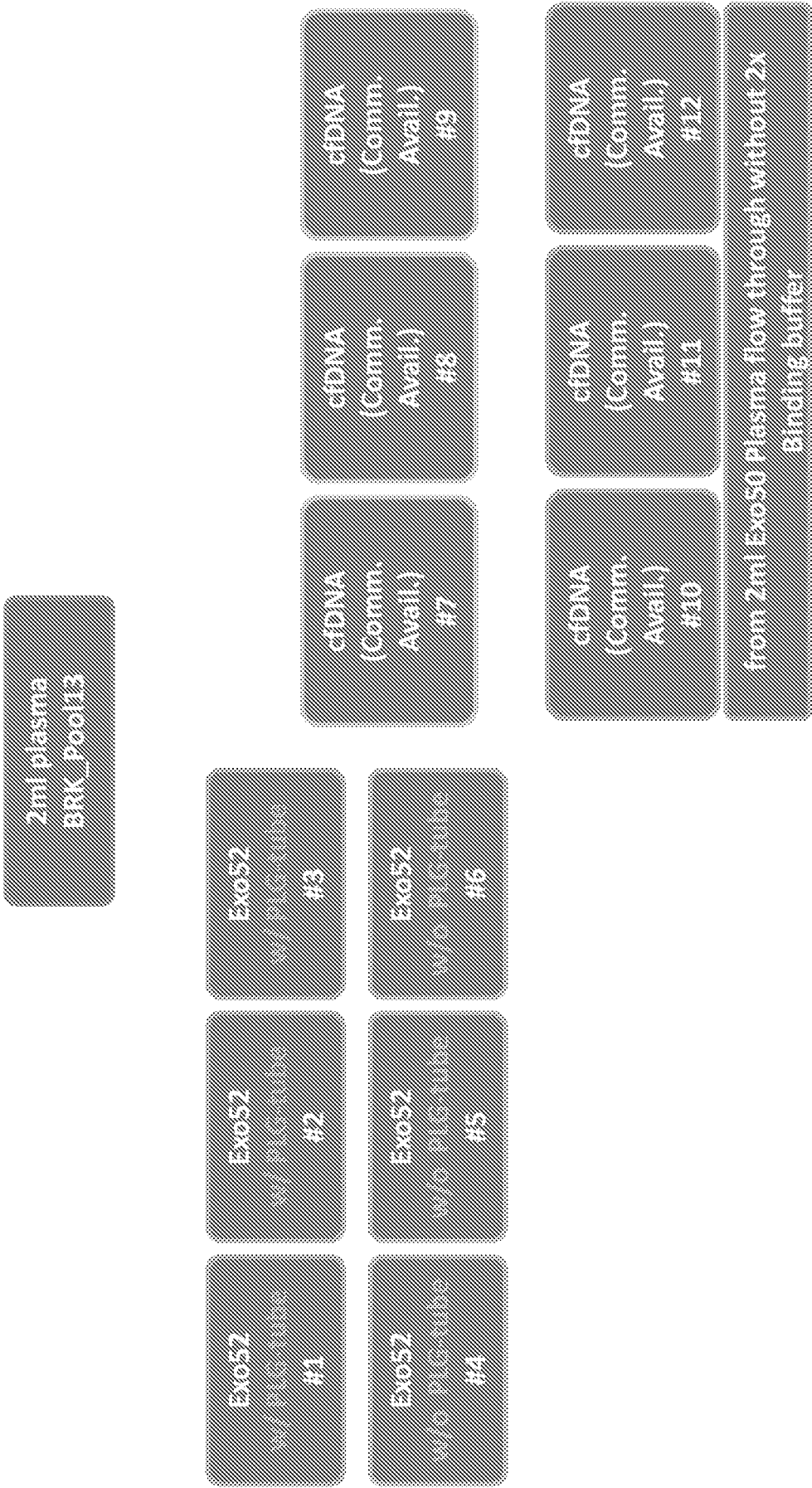


Figure

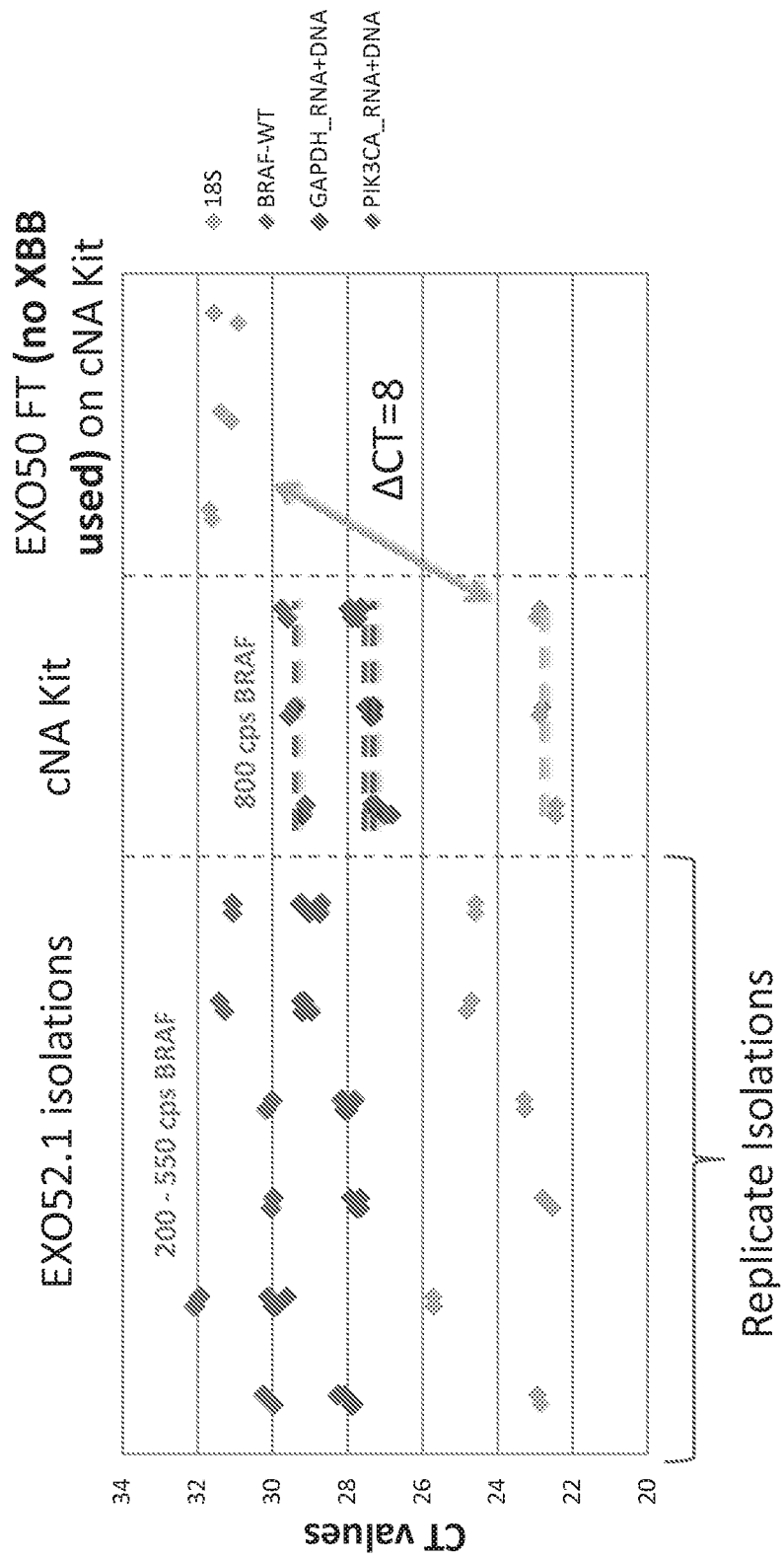


Figure

Exo52 protocol optimization: DNA extraction with and without PLG-tube

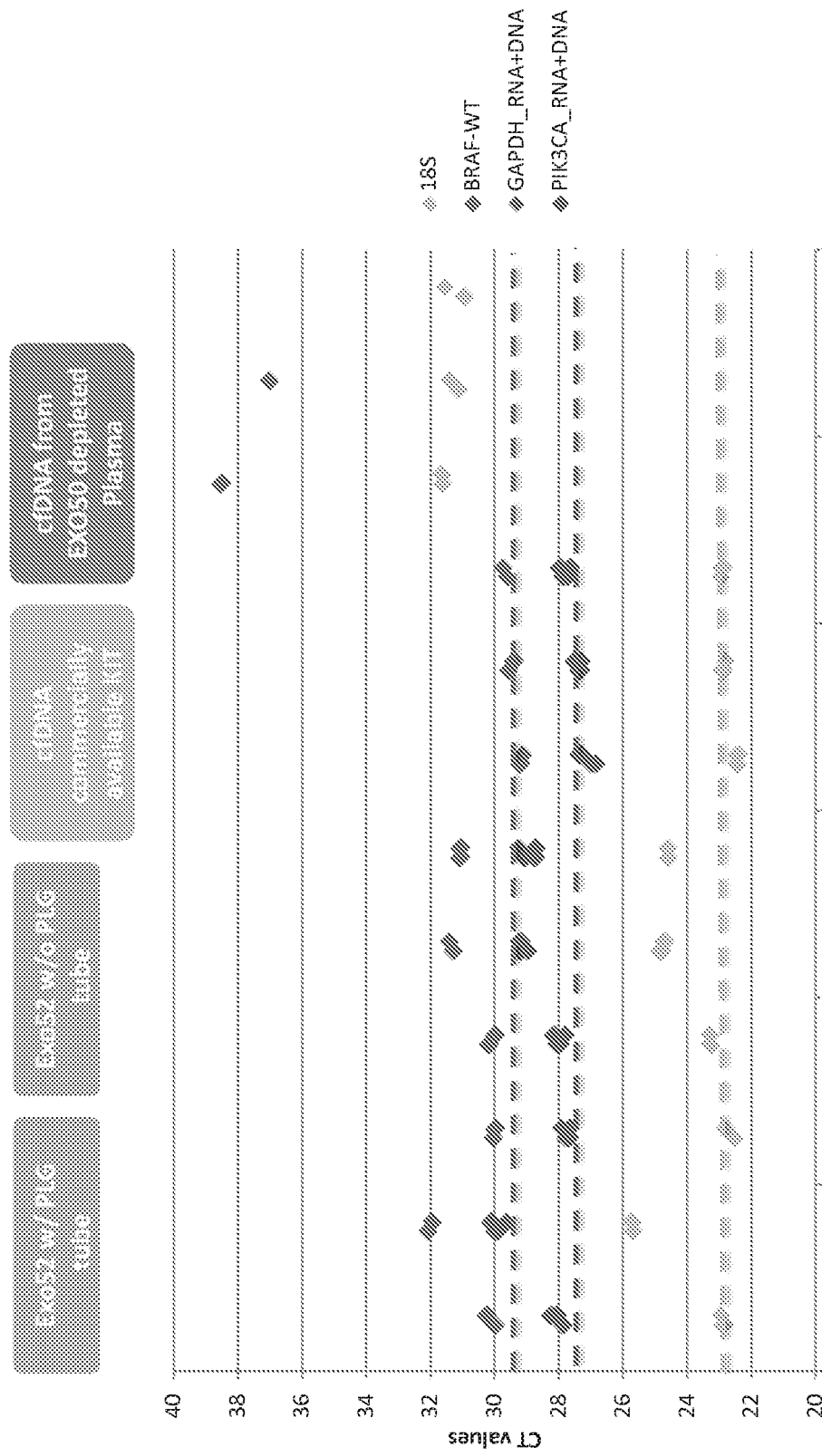


Figure



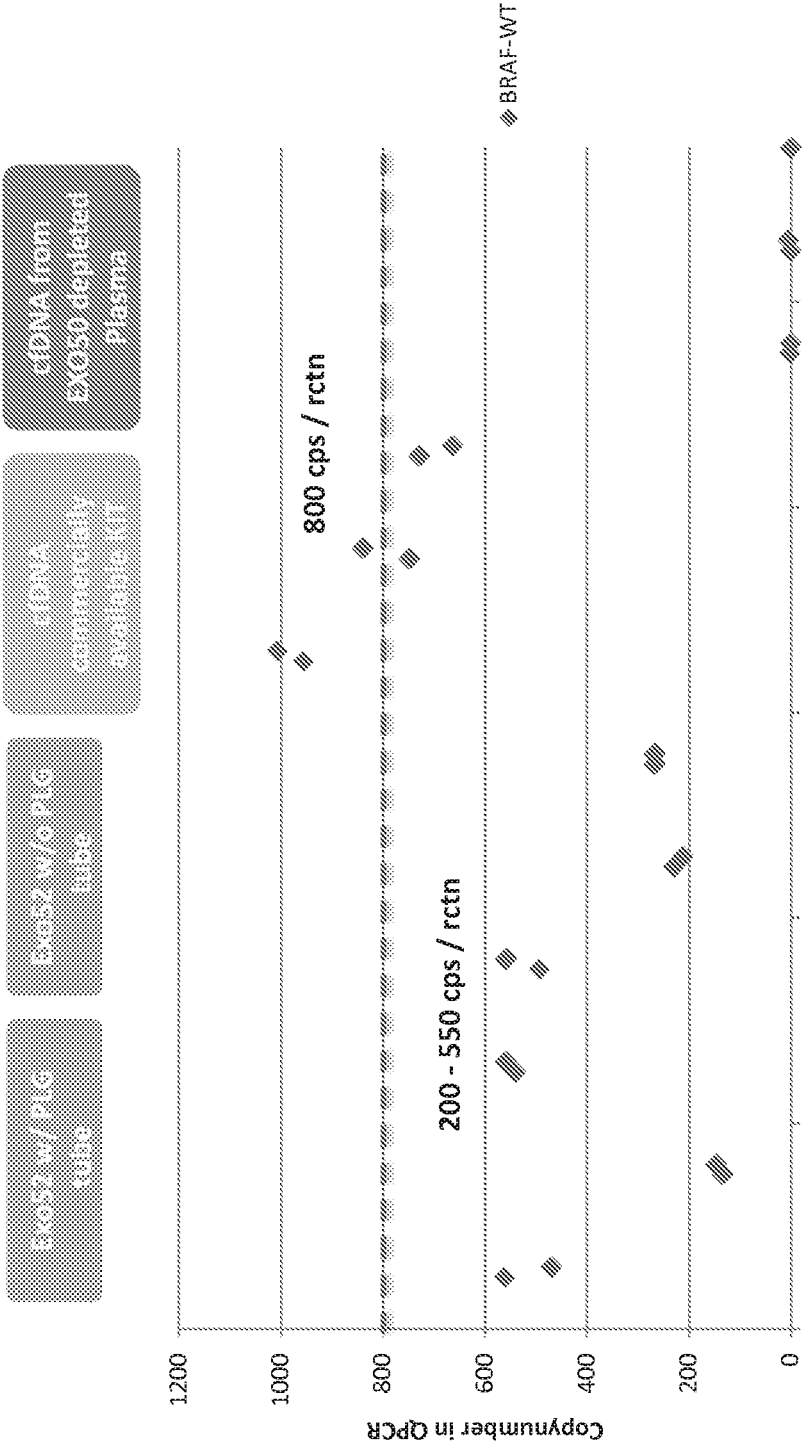
Figure

Exo52 protocol optimization: DNA extraction with and without PLG-tube



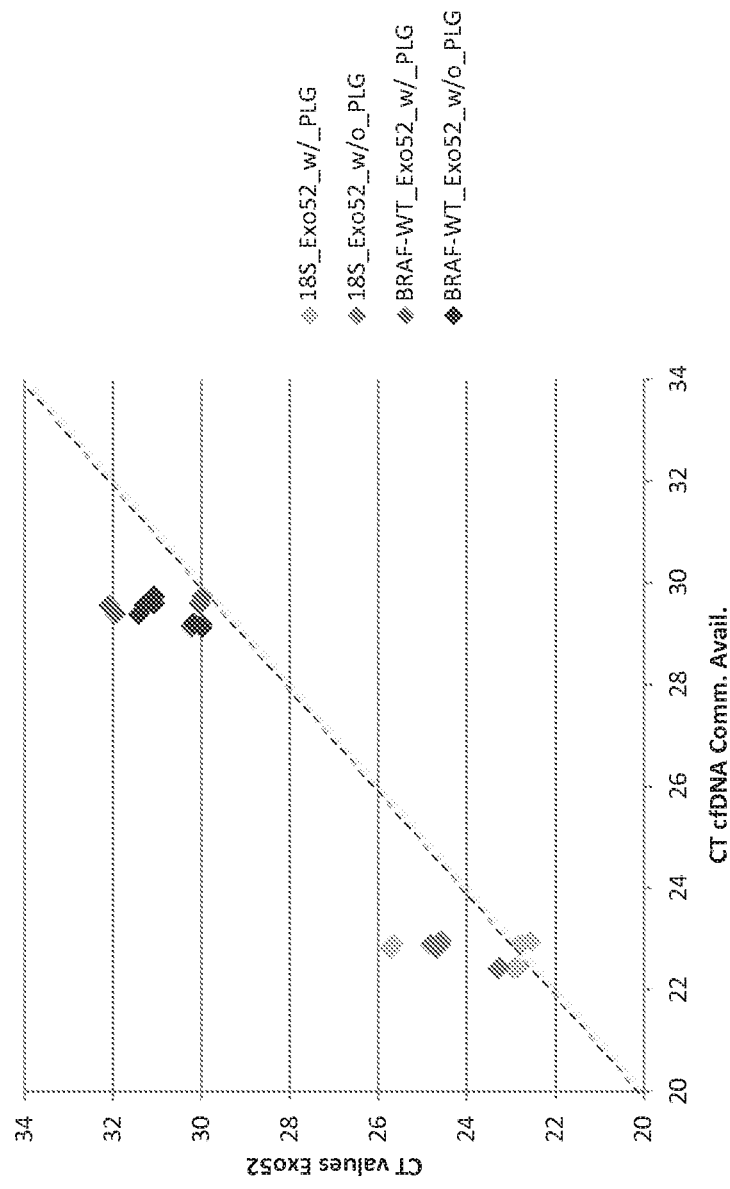
Figure

Exo52 protocol optimization: DNA extraction with and without
PLG-tube



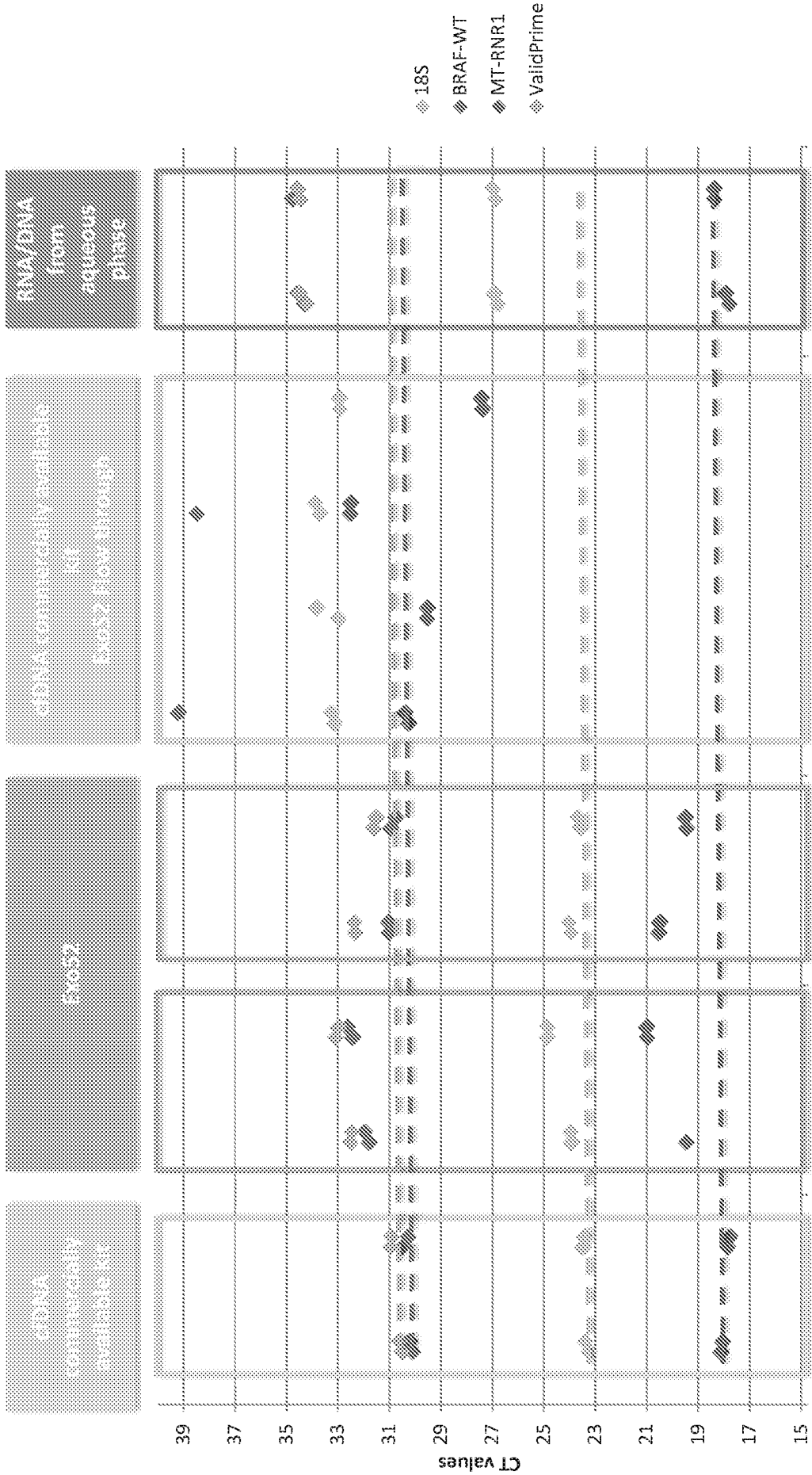
Figure

Exo52 protocol optimization: DNA extraction with and without PLG-tube



Figure

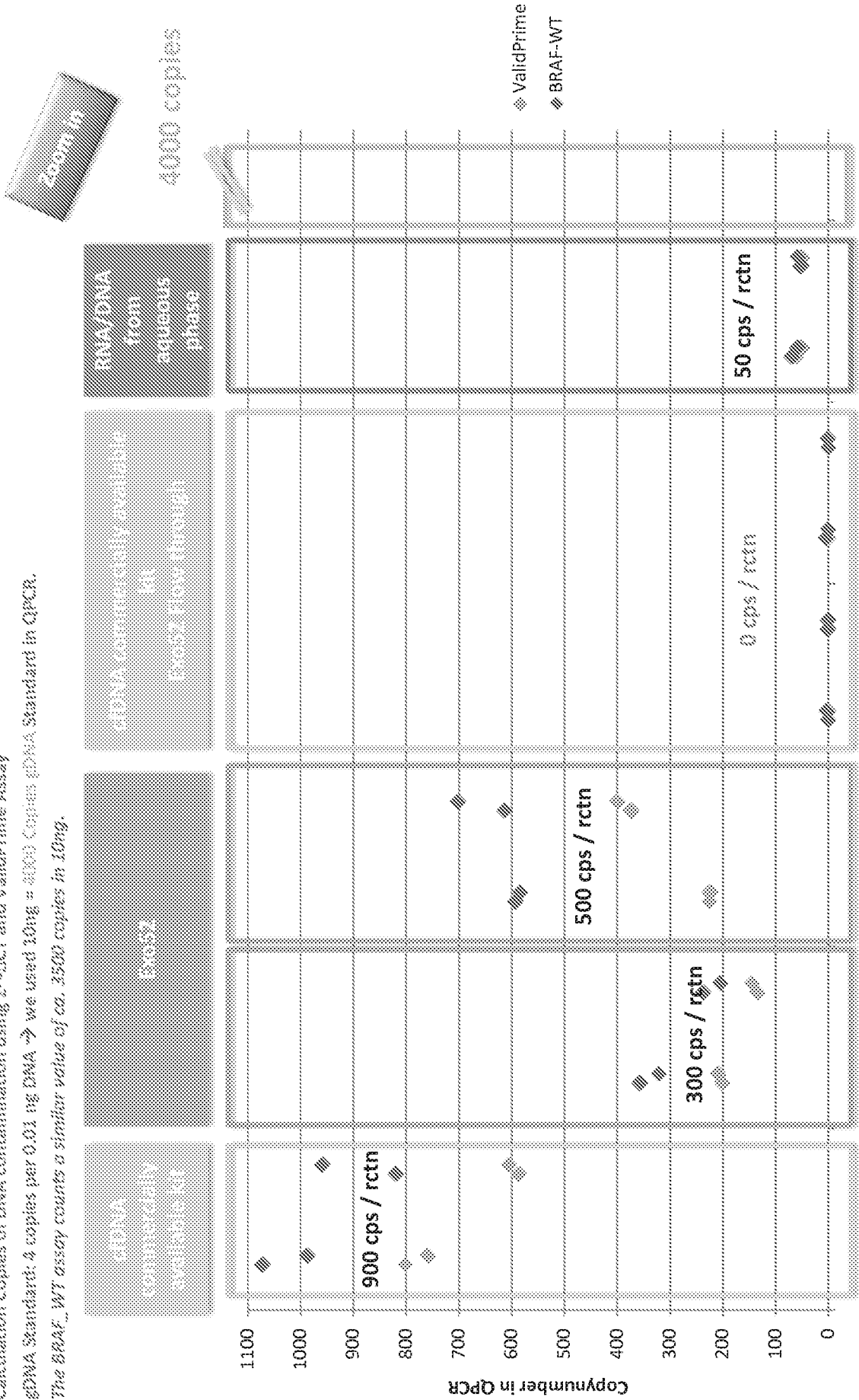
EXO52 (ExDx) vs. circNA Kit - CTs



Figure

EXO52 (ExDx) vs. circNA Kit- Abs.Quant.

Calculation Copies of DNA contamination using $2^{-\Delta\Delta CT}$ and ValidPrime Assay
gDNA Standard: 4 copies per 0.01 ng DNA → we used 10ng = 4000 Copies gDNA Standard in qPCR.
The BRAF_{WT} assay counts a similar value of ca. 3500 copies in 10ng.



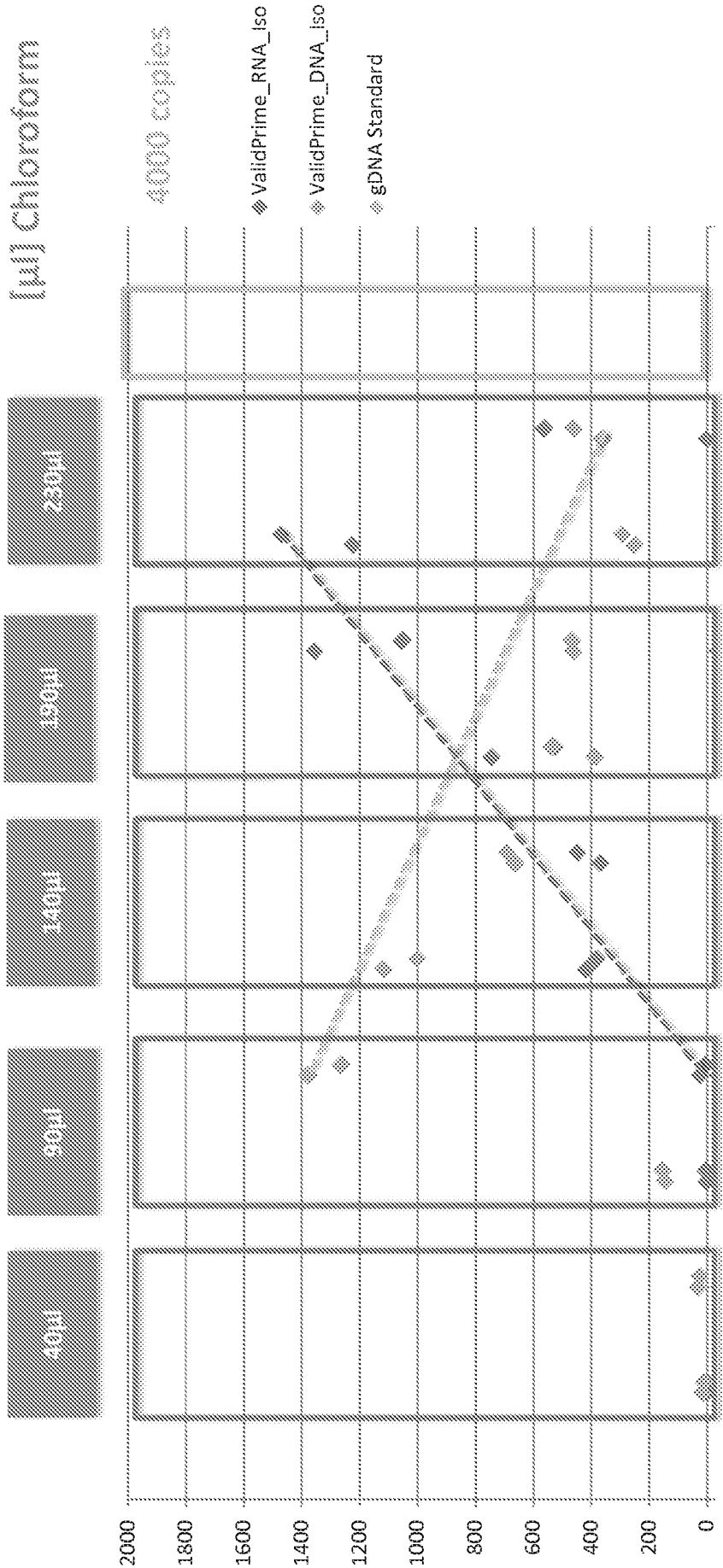
w/o carrier RNA

with carrier RNA into QIAzol

Figure

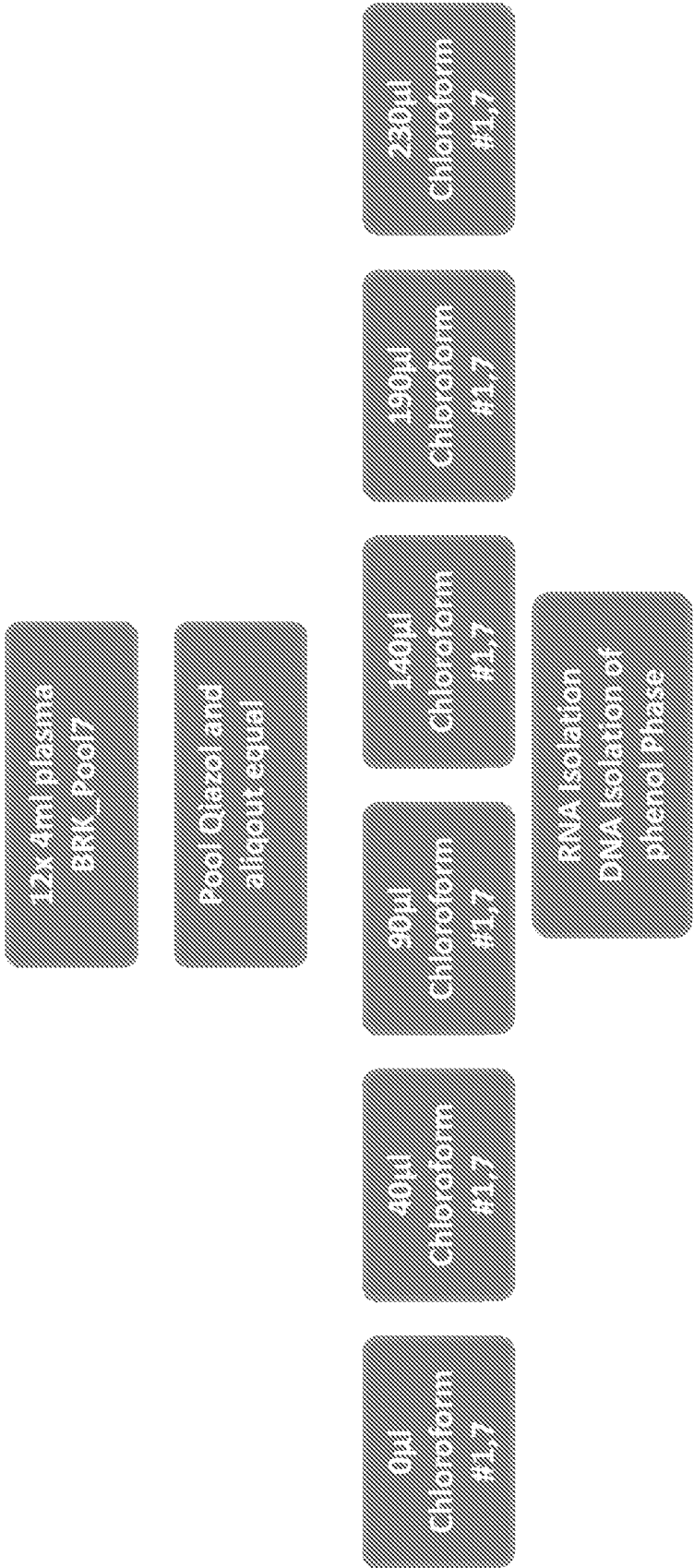
Ad3 Chloroform Titration: RNA Isolation + DNA Isolation of phenol phase (PLG-tube) Zoom in

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Figure

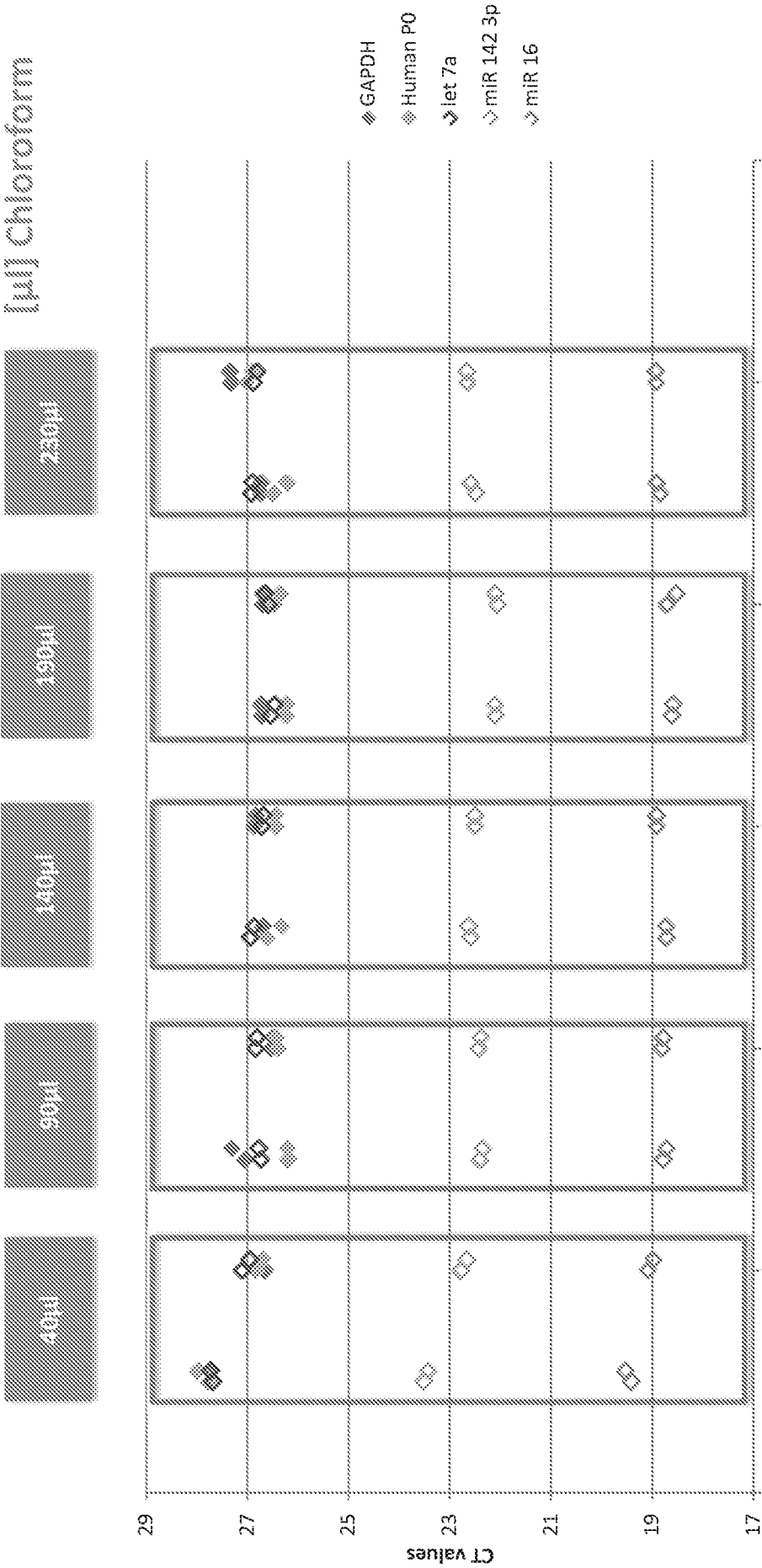
Ad3 Chloroform Titration: RNA Isolation + DNA Isolation of phenol phase (PLG-tube)



- Filter:
- homemade 3 layer
- Buffer
- Loading buffer: 2x buffer 100mM Phosphate buffer 370mM NaCl pH 6.5
 - Wash buffer: 1x buffer 50mM Phosphate buffer 185 mM NaCl pH 6.5
- Isolation of DNA from Chloroform Phenol Phase

Figure

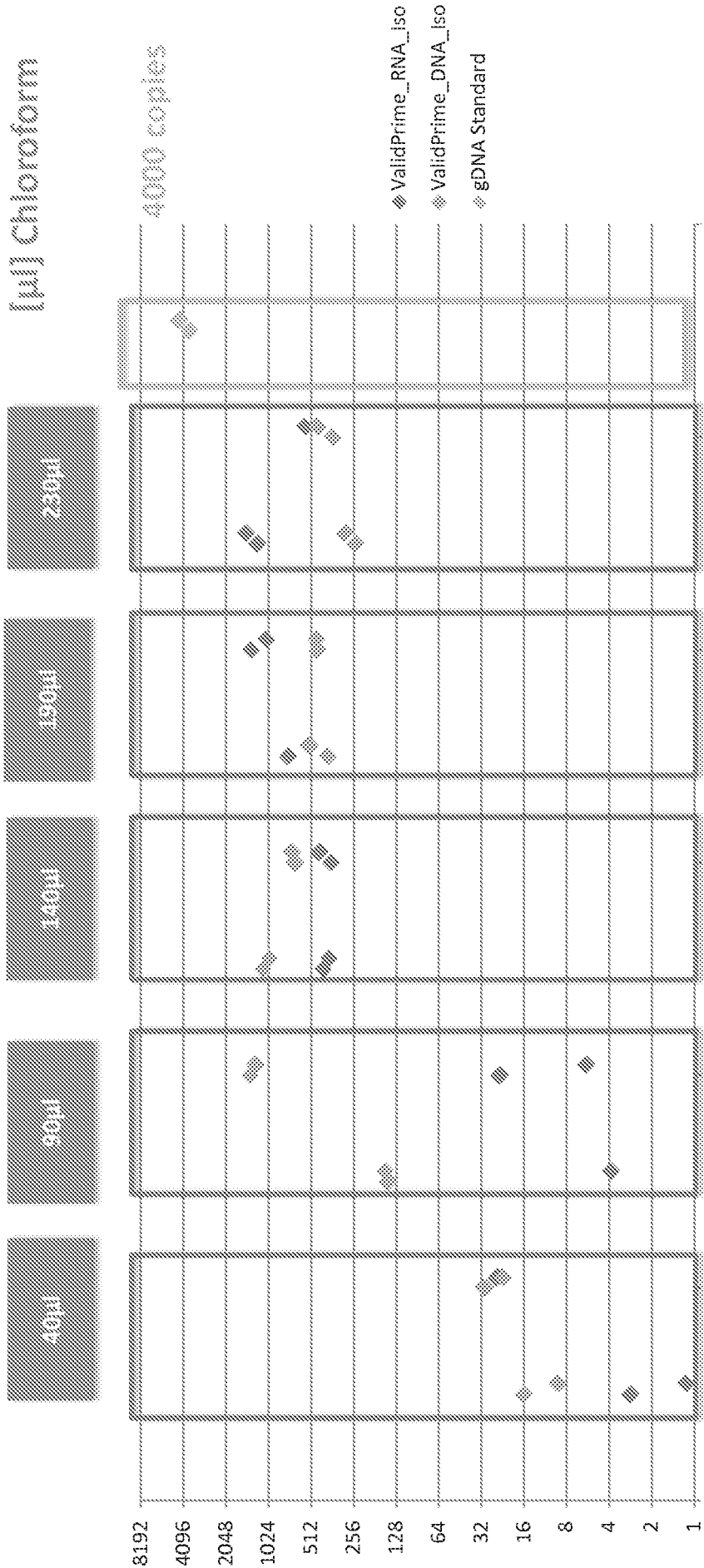
Ad3 Chloroform Titration: RNA Isolation



Figure

Ad3 Chloroform Titration: RNA Isolation + DNA Isolation of phenol phase (PLG-tube)

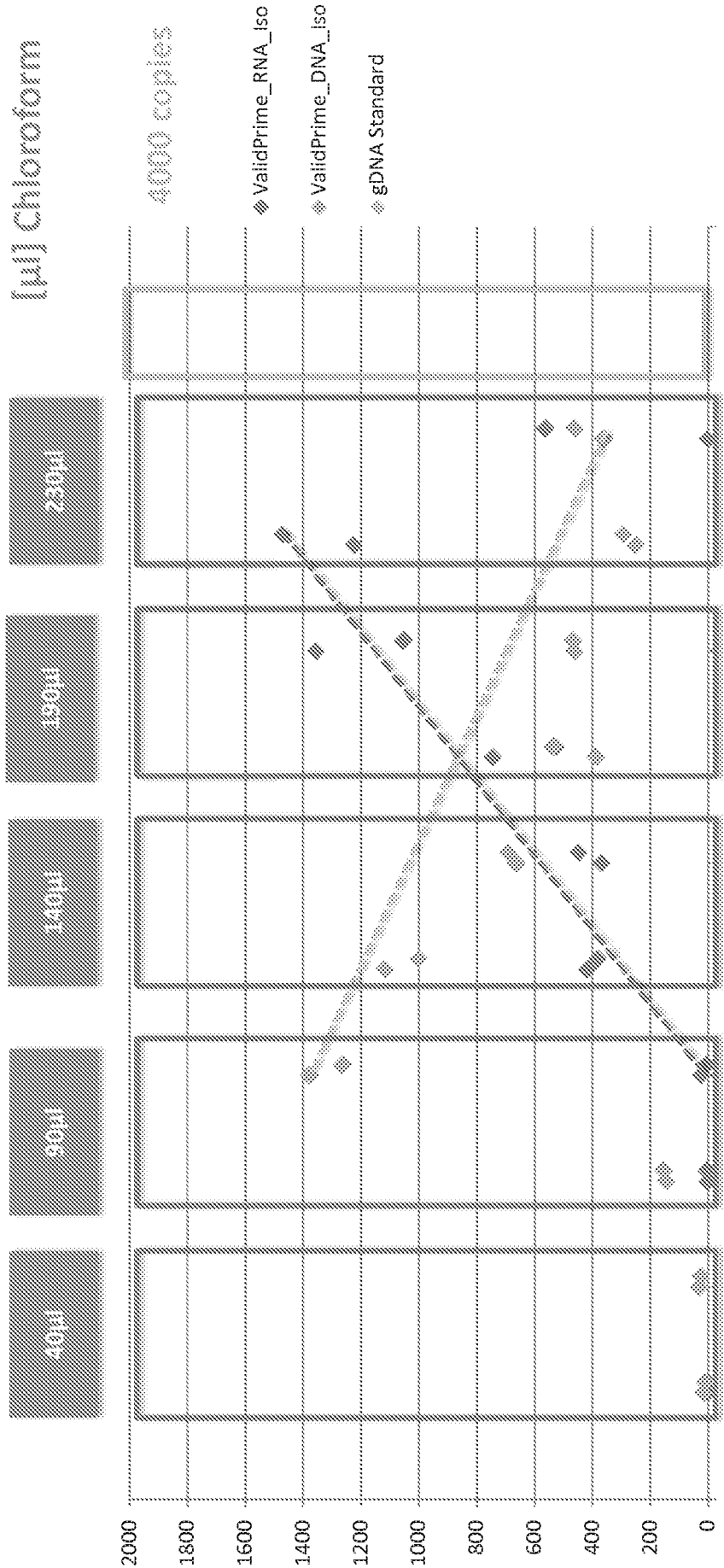
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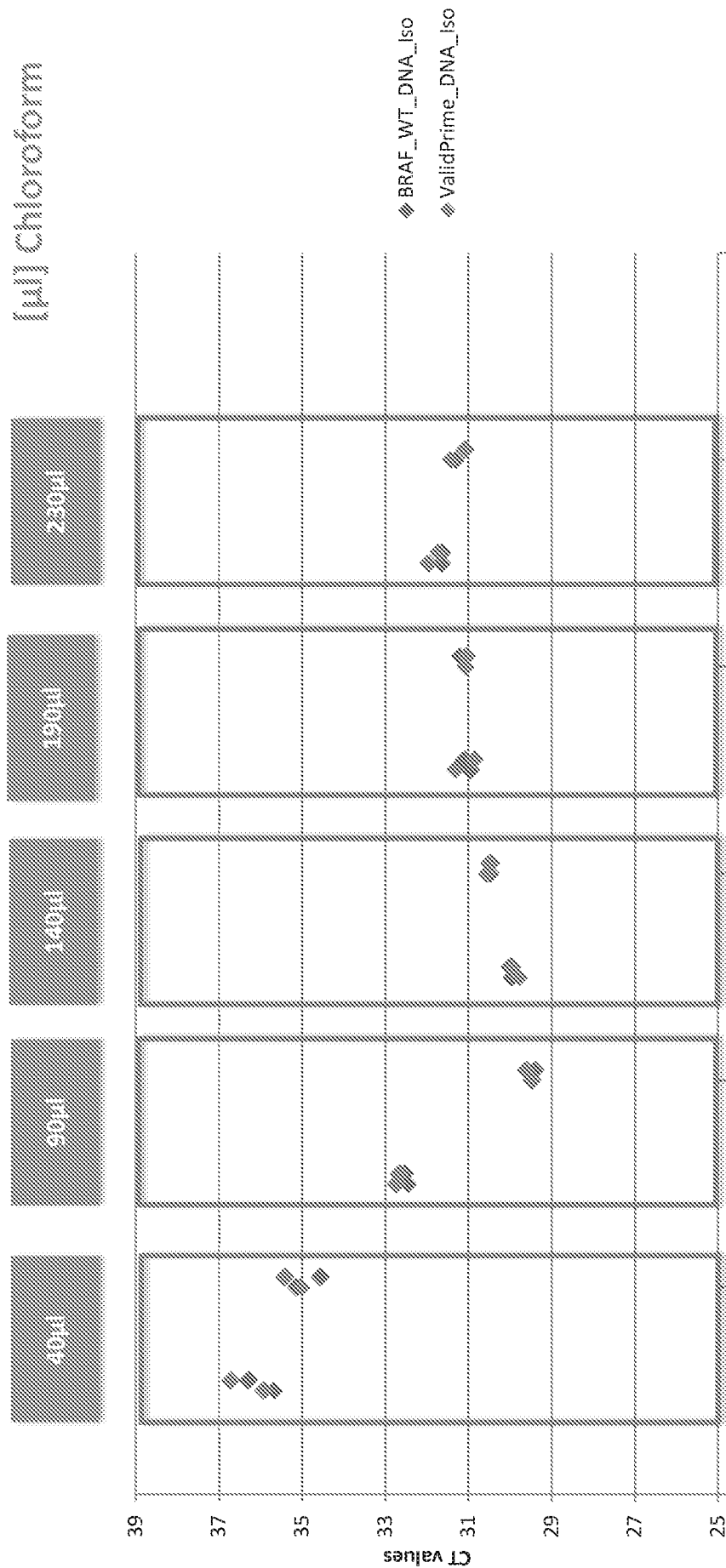
Ad3 Chloroform Titration: RNA Isolation + DNA Isolation of phenol phase (PLG-tube) Zoom in

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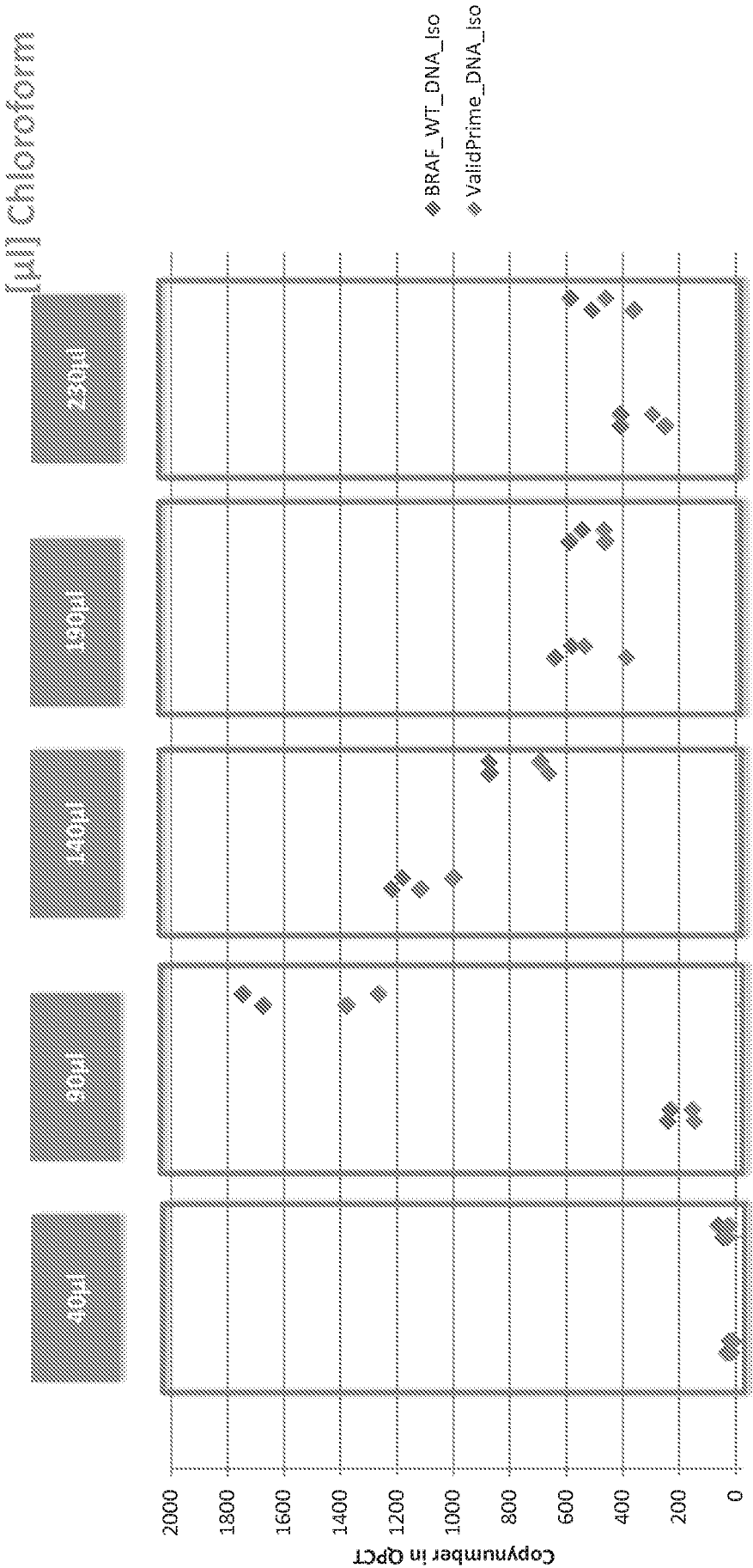
Figure

Ad3 Chloroform Titration: DNA Isolation of phenol phase (PLG-tube)



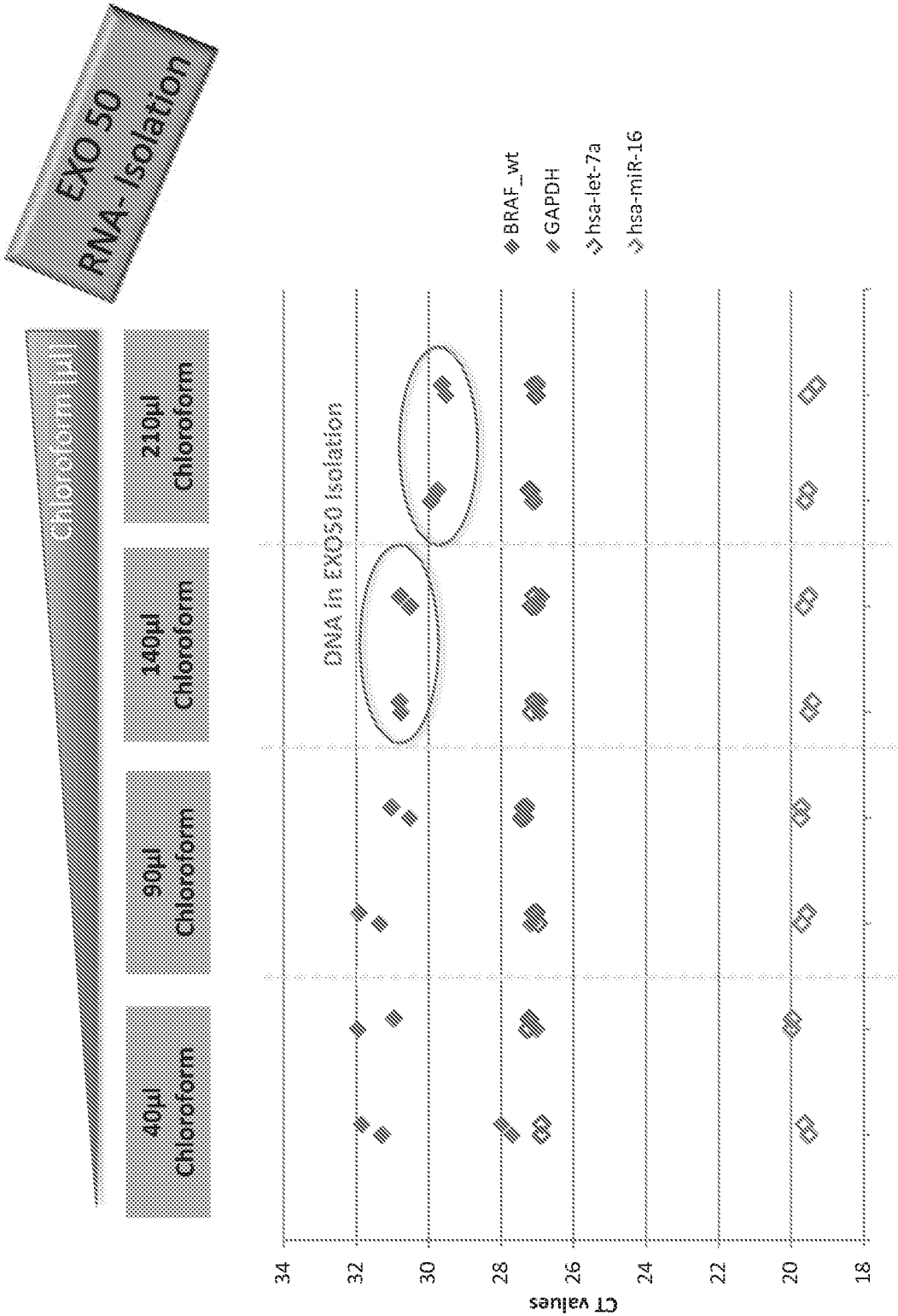
Figure

Ad3 Chloroform Titration: DNA Isolation of phenol phase (PLG-tube)



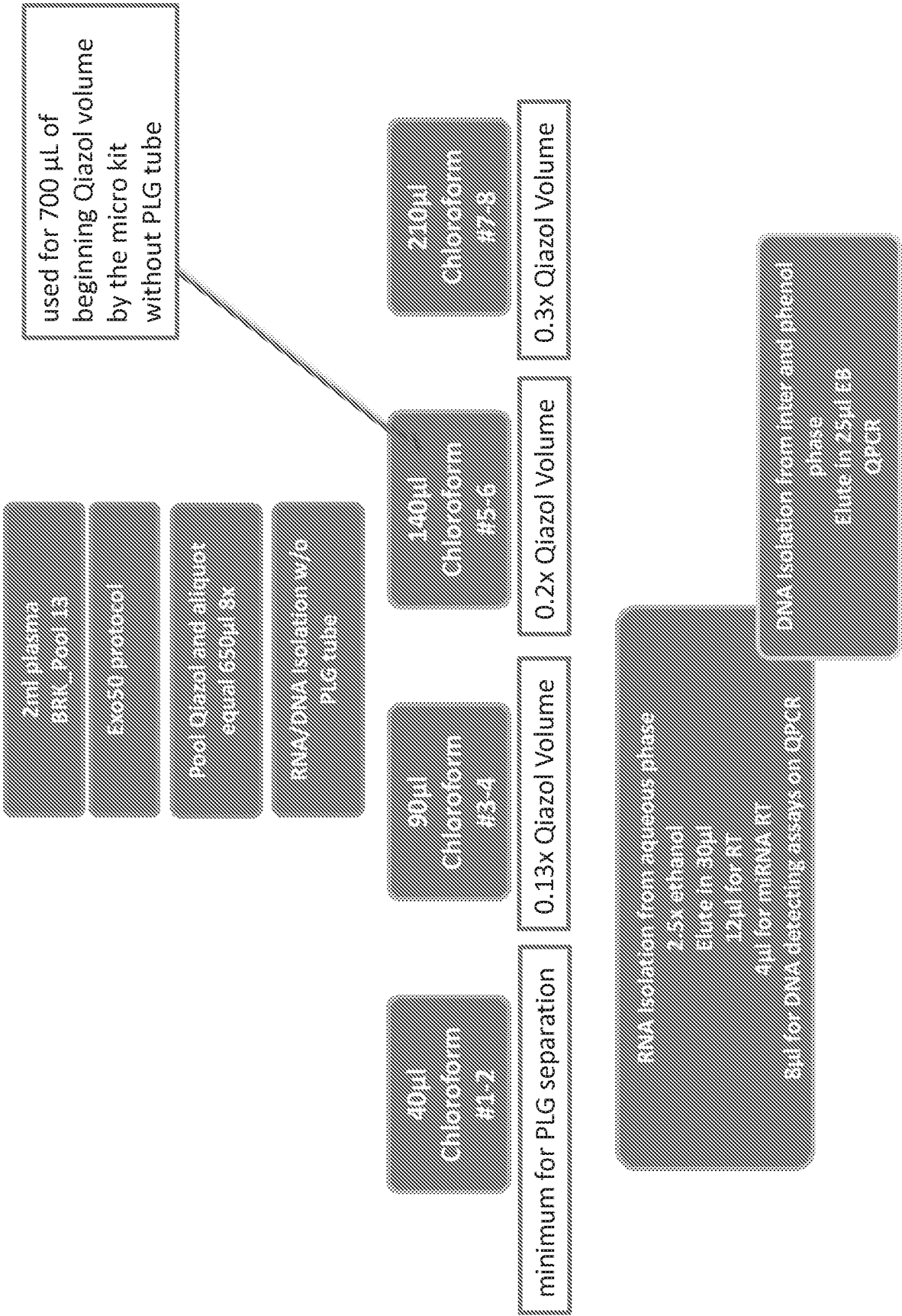
Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and
chloroform titration



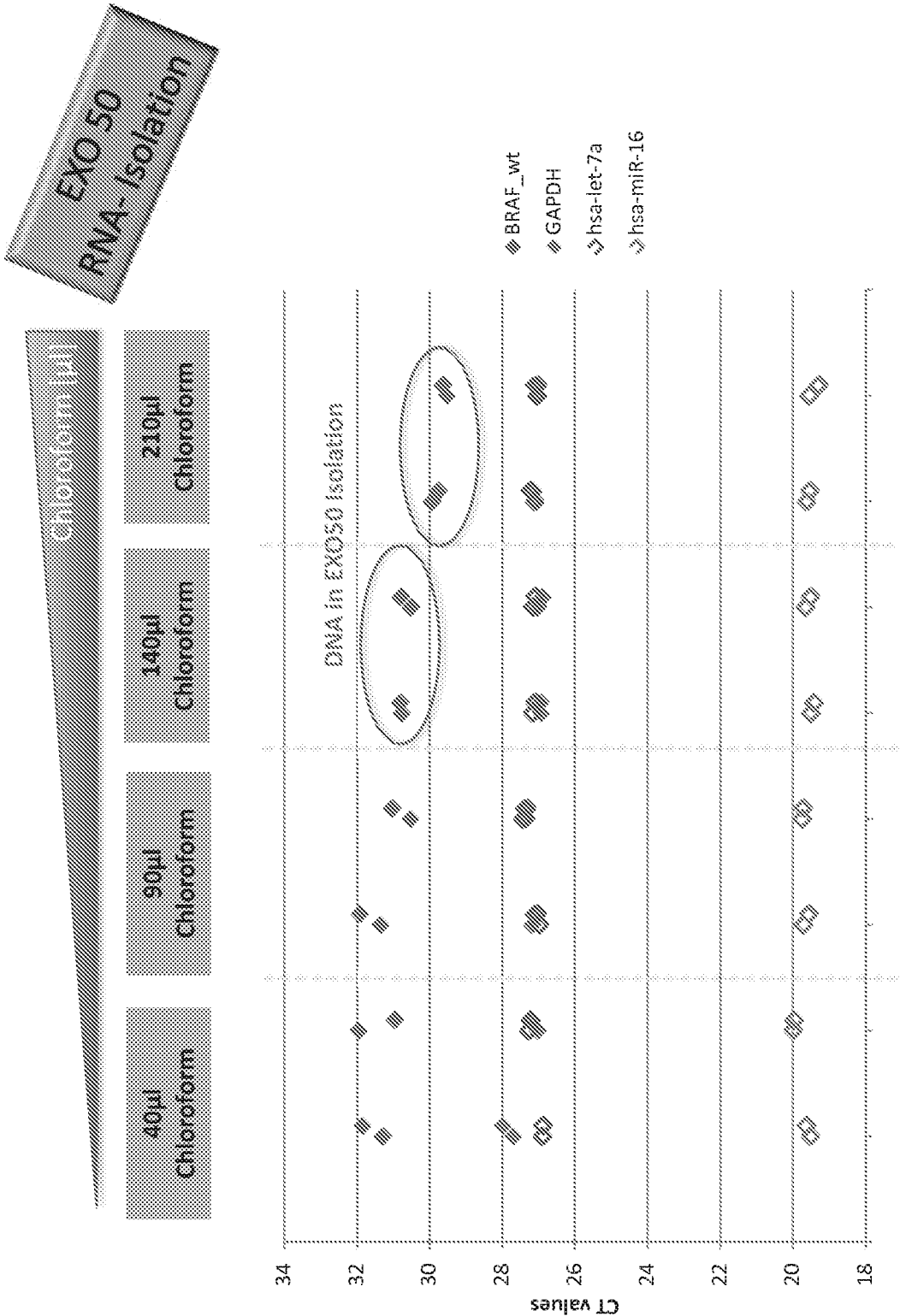
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DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration



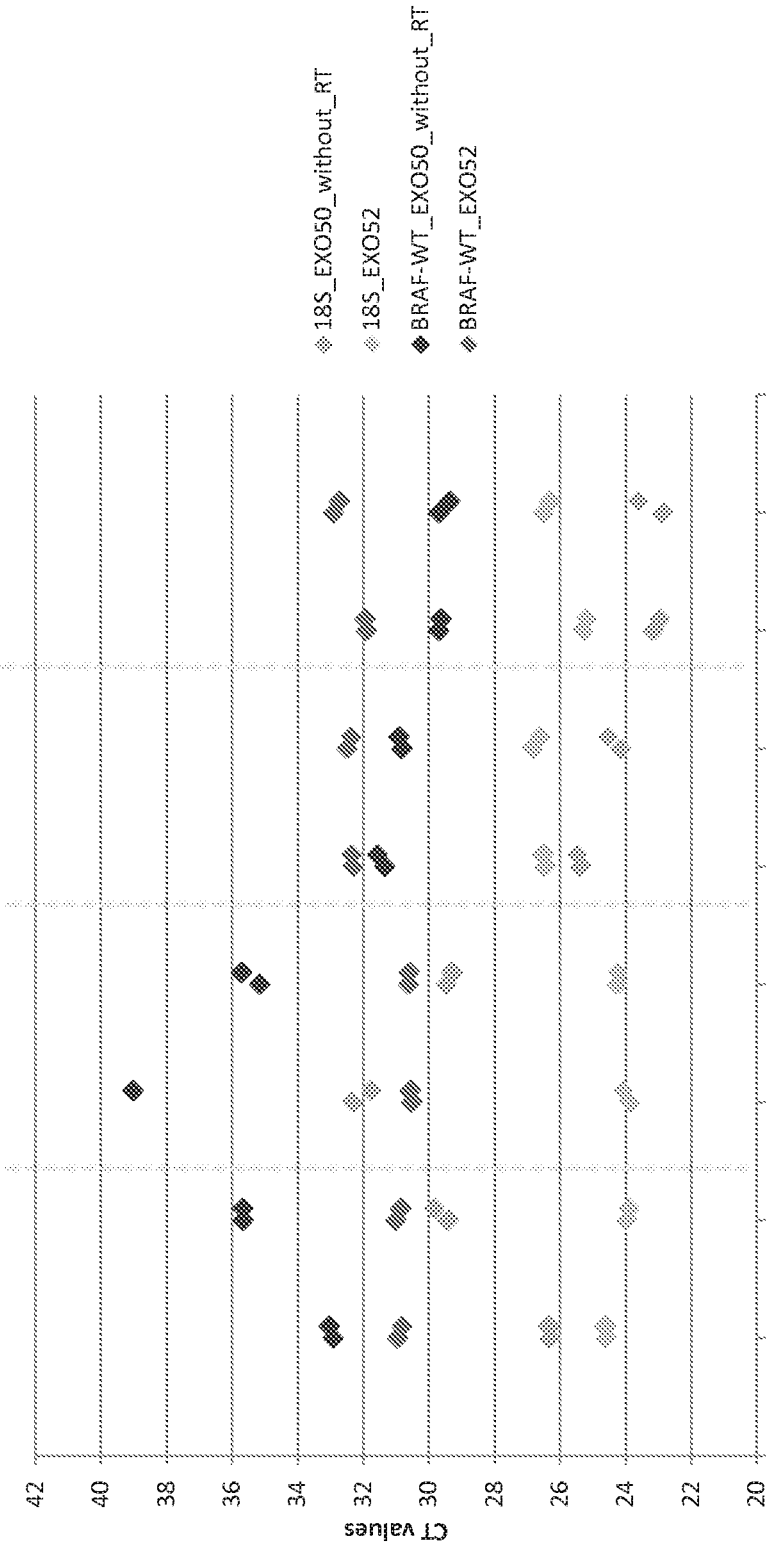
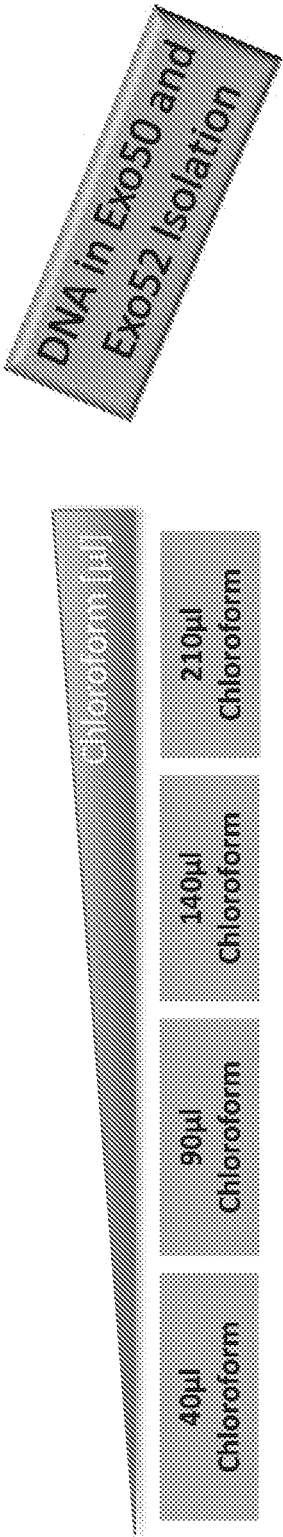
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DNA isolation using RNeasy protocol (w/o PLG tube) and
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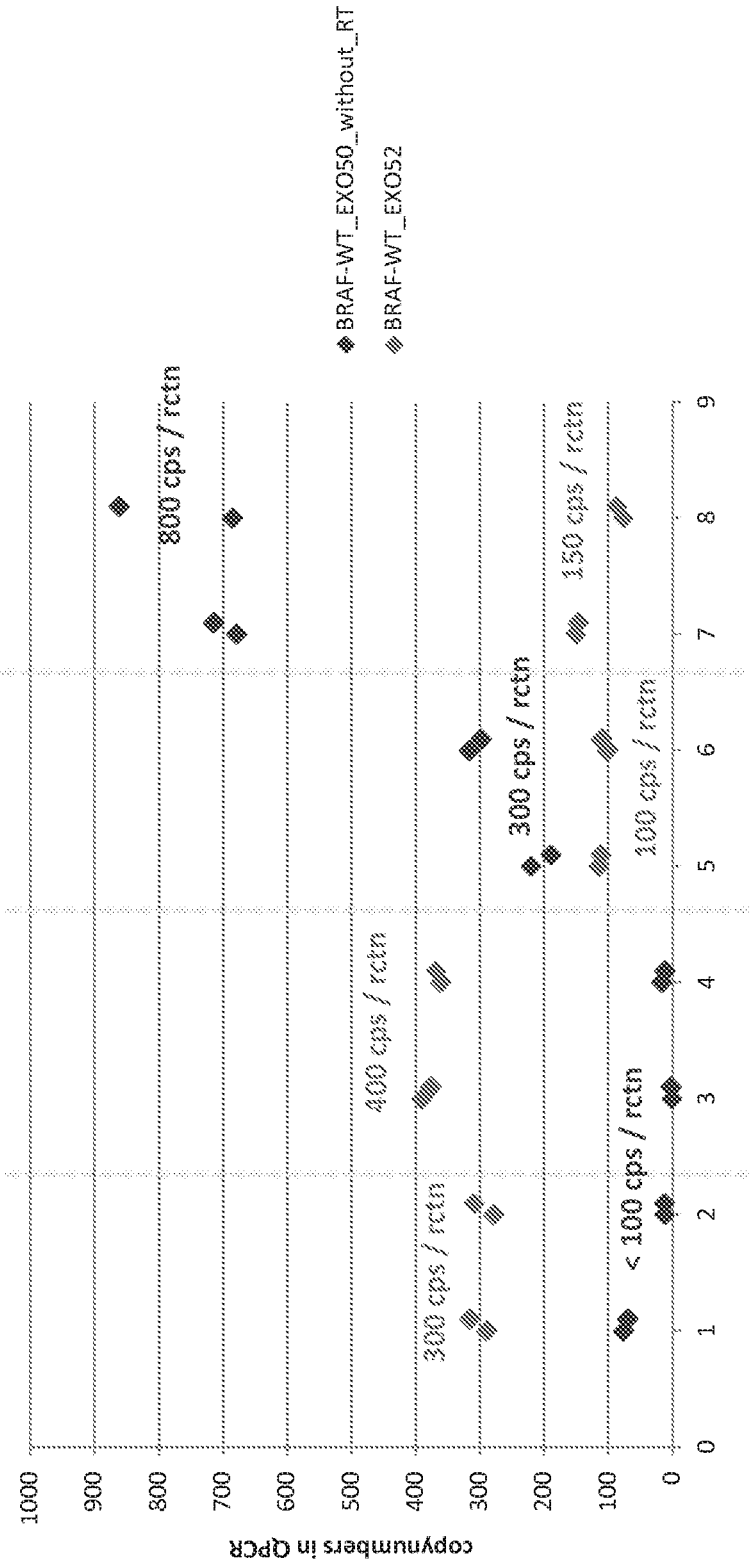
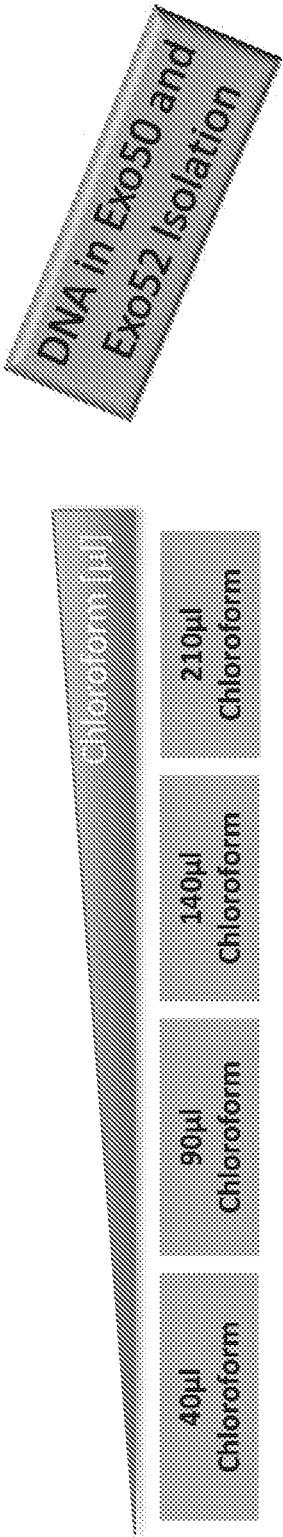
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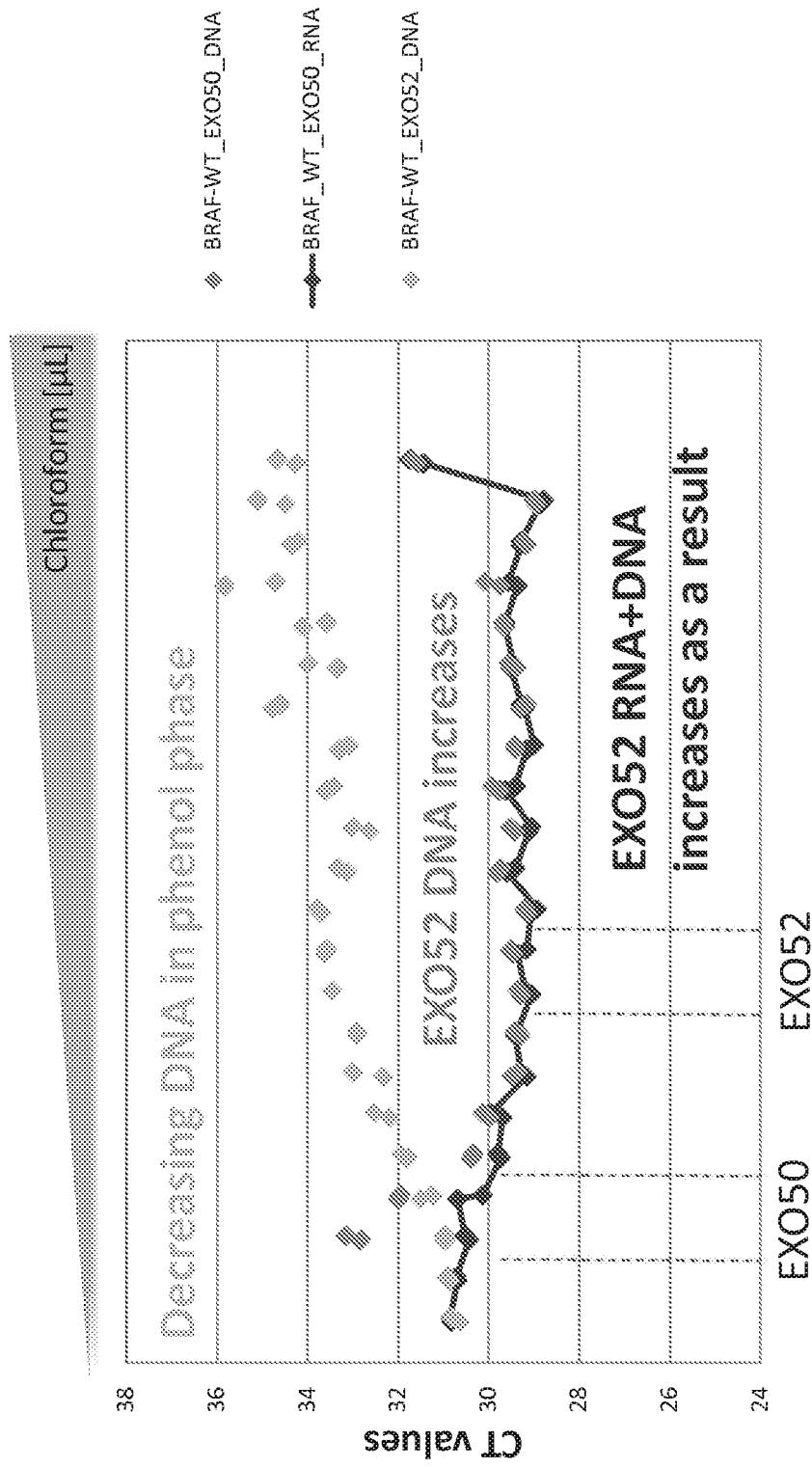
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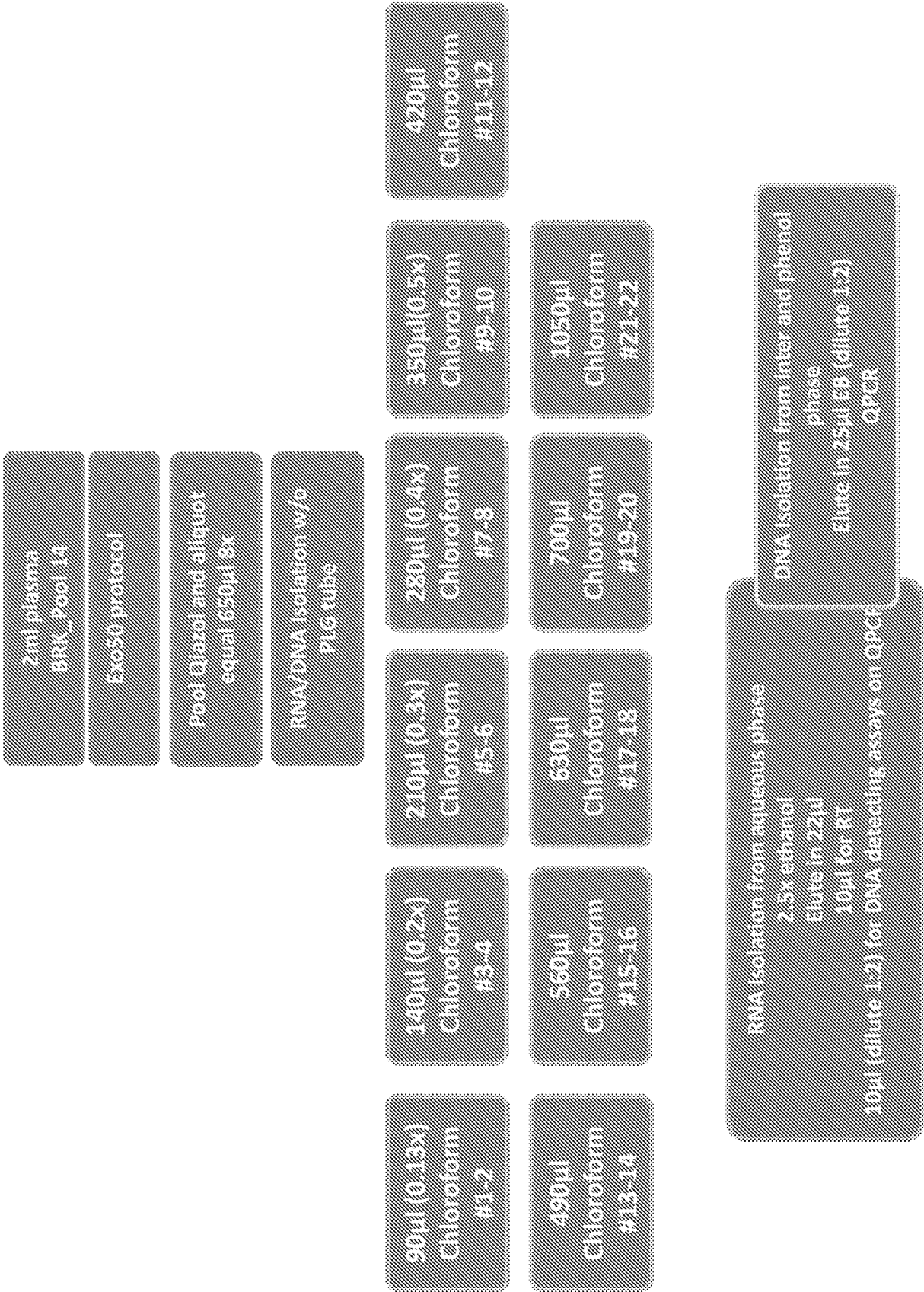
Figure

Adjusted chloroform addition co-isolates DNA/RNA



Figure

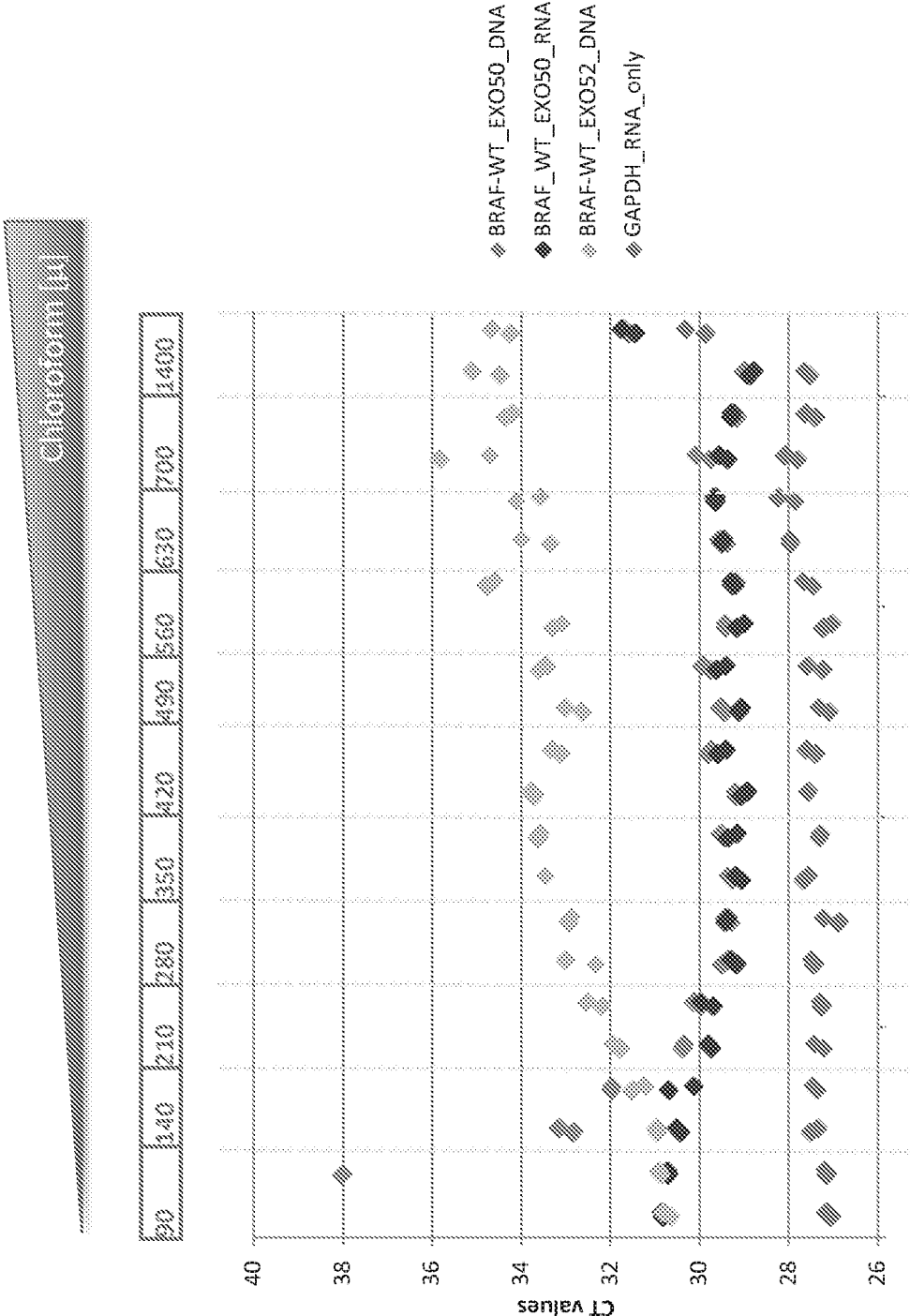
DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

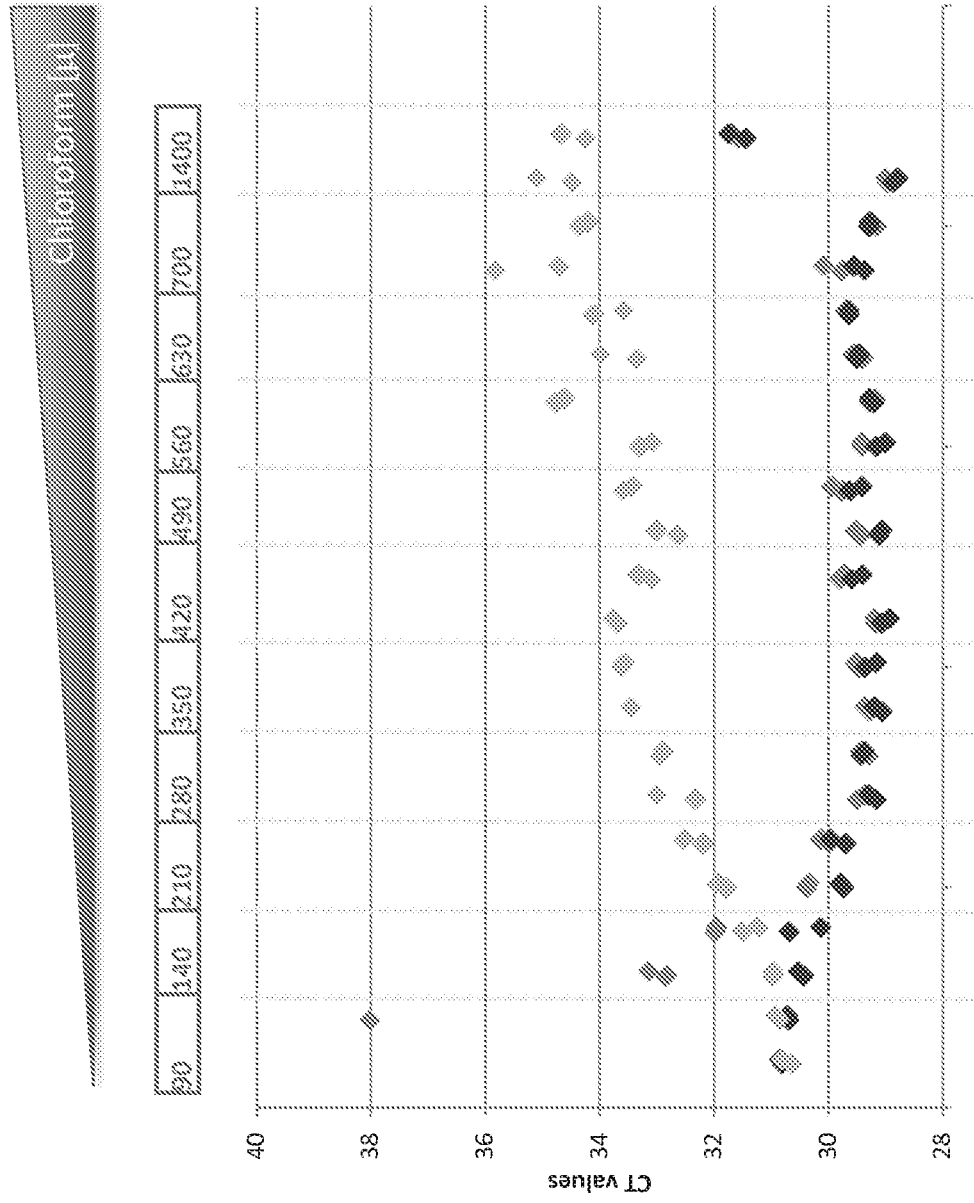
EXO50 RNA: 10µl RNA in 20µl RT Mix
EXO50 DNA: 1:2 diluted
EXO52 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

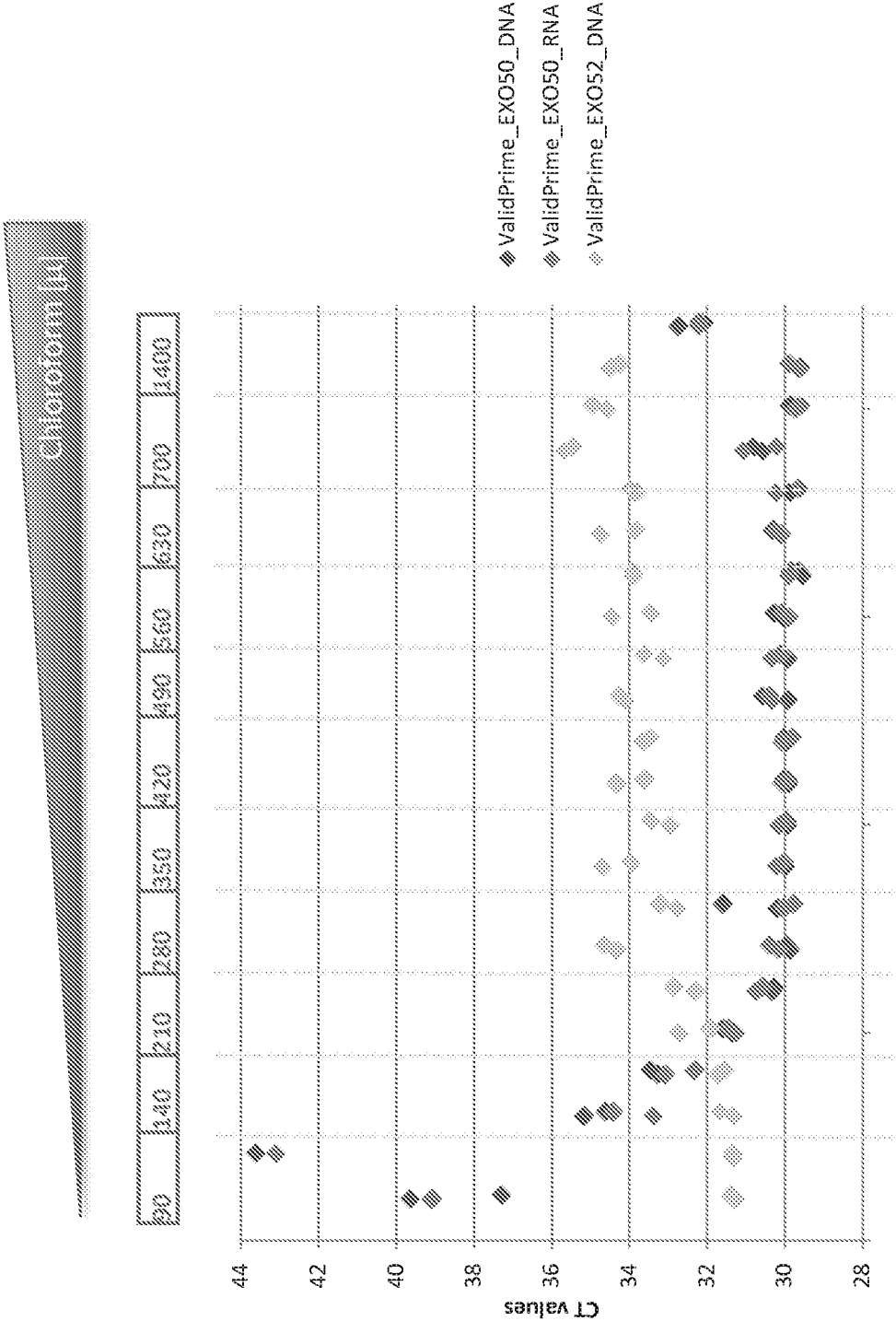
EXOS0 RNA: 10µl RNA in 20µl RT Mix
 EXOS0 DNA: 1:2 diluted
 EXOS2 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

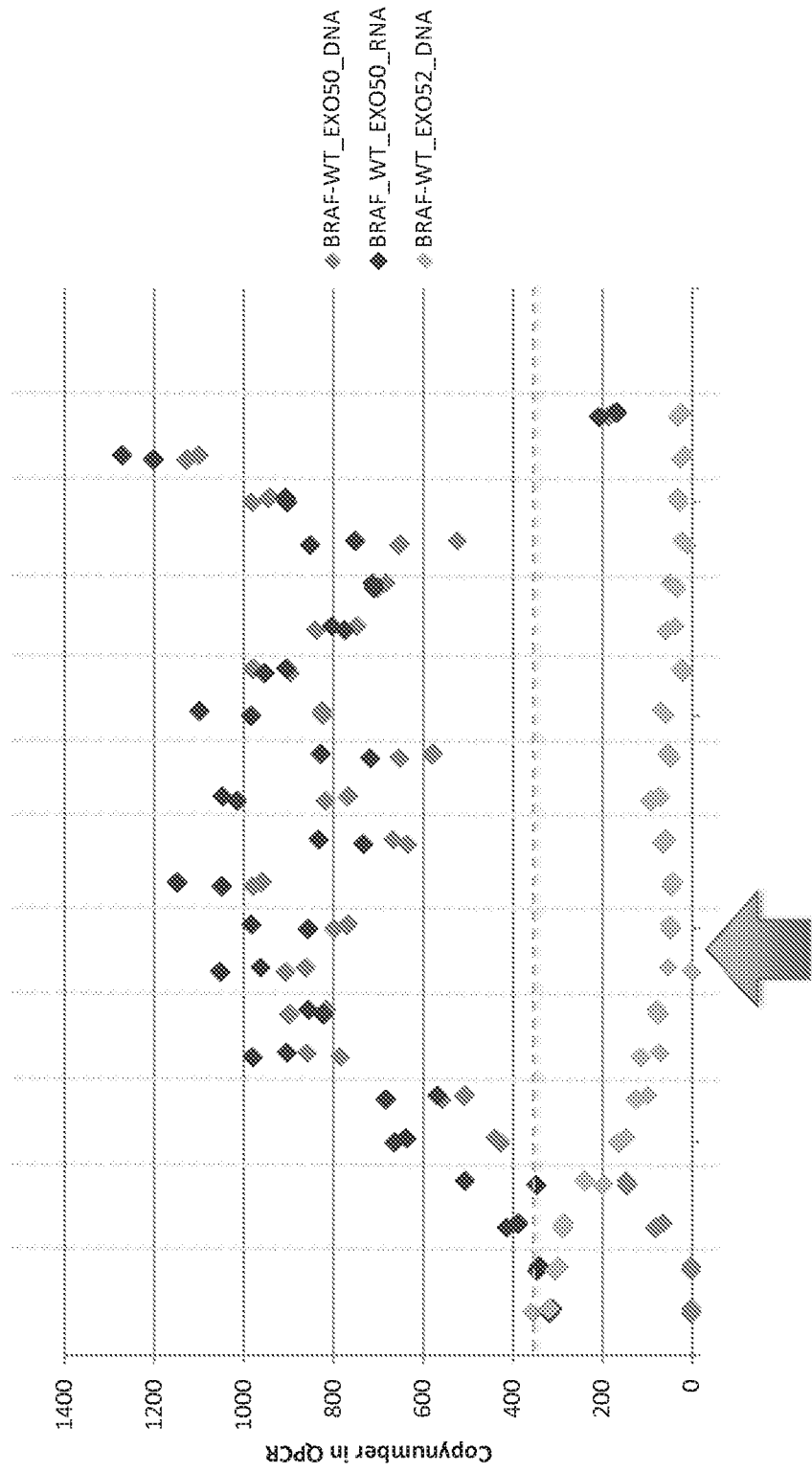
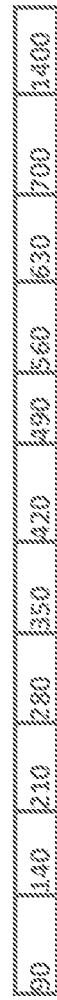
EXO50 RNA: 10µl RNA in 20µl RT Mix
EXO50 DNA: 1:2 diluted
EXO52 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

EXO50 RNA: 10 μ l RNA in 20 μ l RT Mix
EXO50 DNA: 1:2 diluted
EXO52 DNA 1:2 diluted



Figure

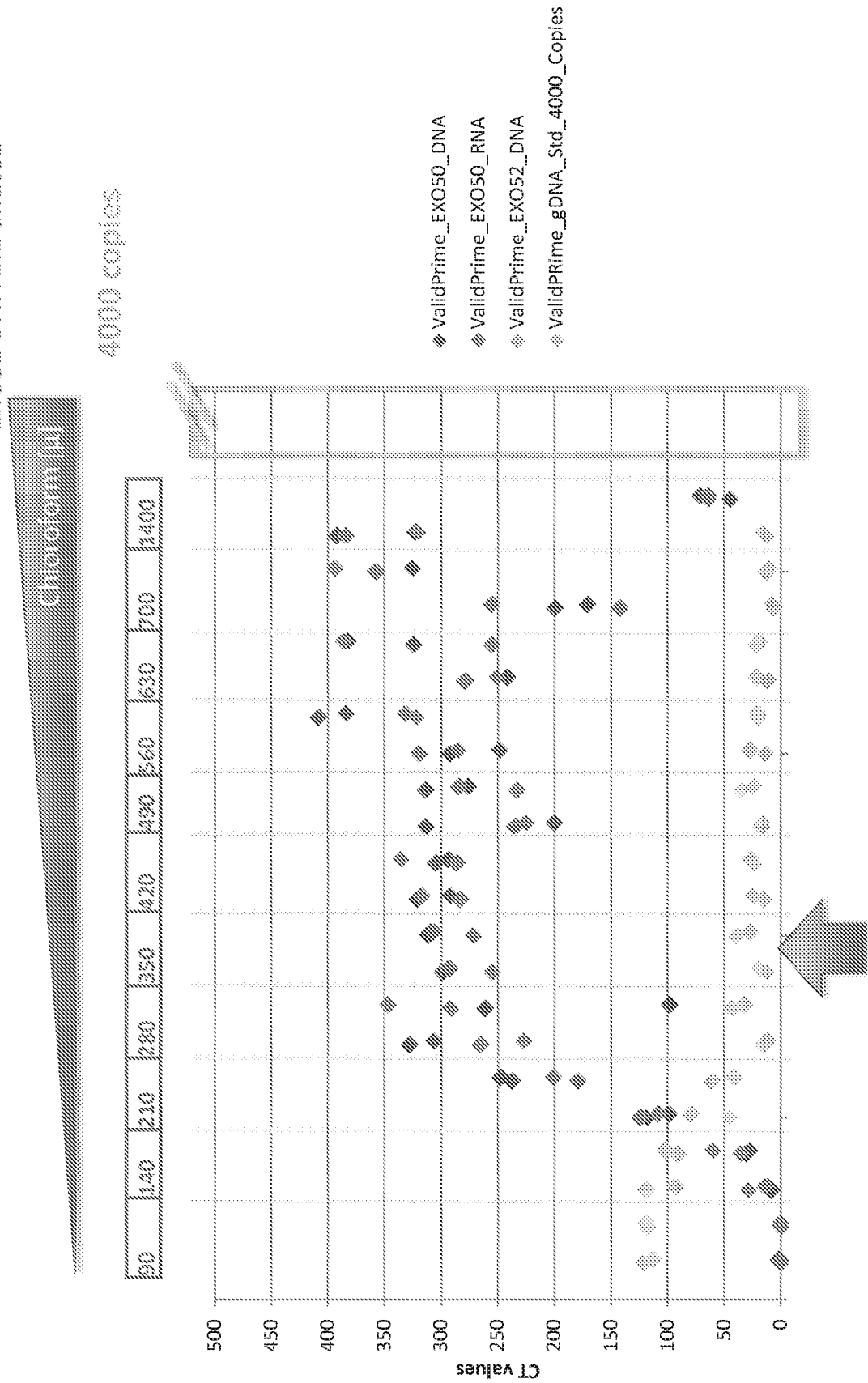
DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

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PCT/US2015/039760

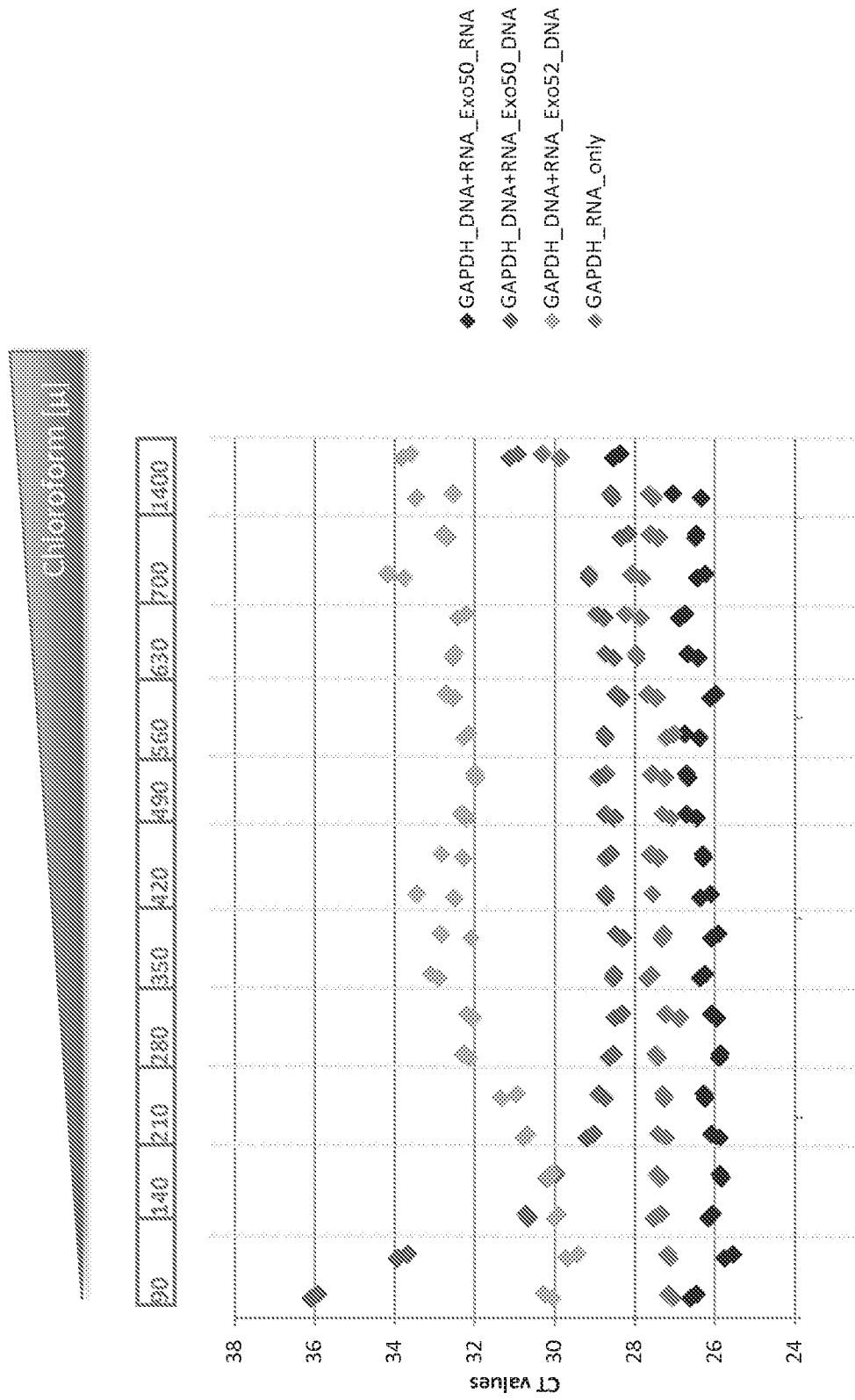
EXO50 RNA: 10µl RNA in 20µl RT Mix
 EXO50 DNA: 1:2 diluted
 EXO52 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

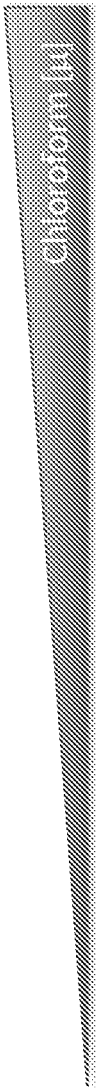
EXOS0 RNA: 10µl RNA in 20µl RT Mix
EXOS0 DNA: 1:2 diluted
EXOS2 DNA 1:2 diluted



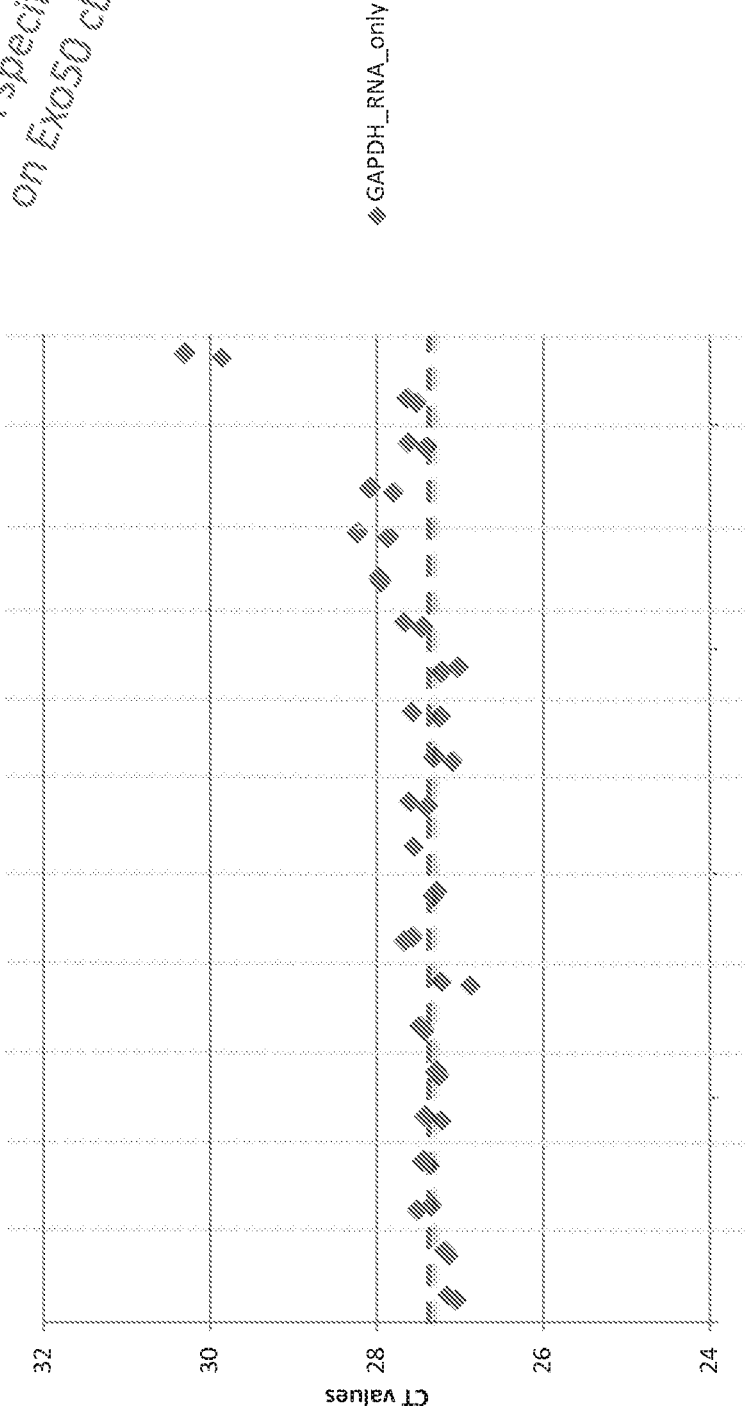
Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

EXOS0 RNA: 10µl RNA in 20µl RT Mix
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EXOS2 DNA 1:2 diluted



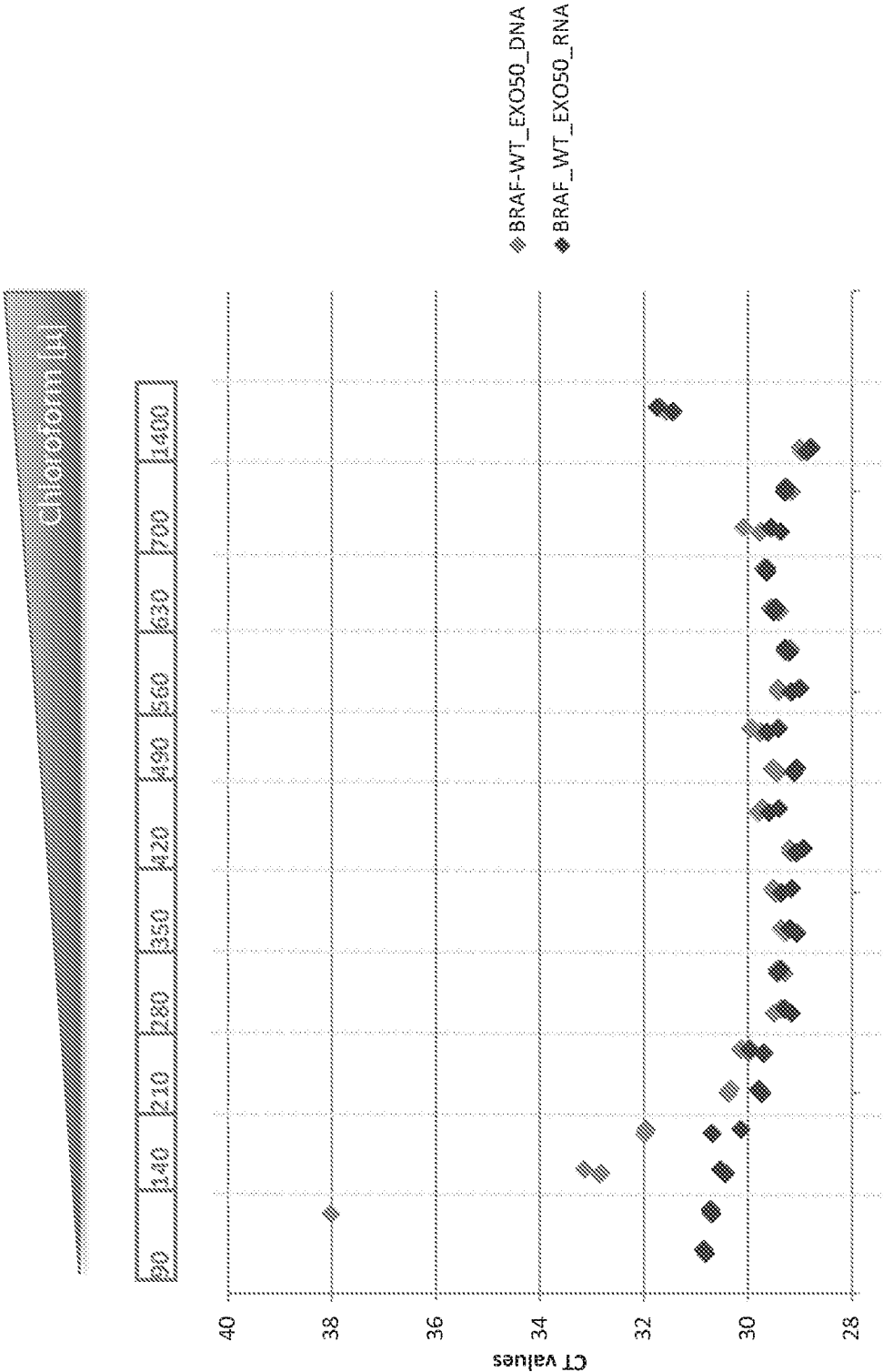
*RNA specific assay
on EXOS0 cDNA*



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

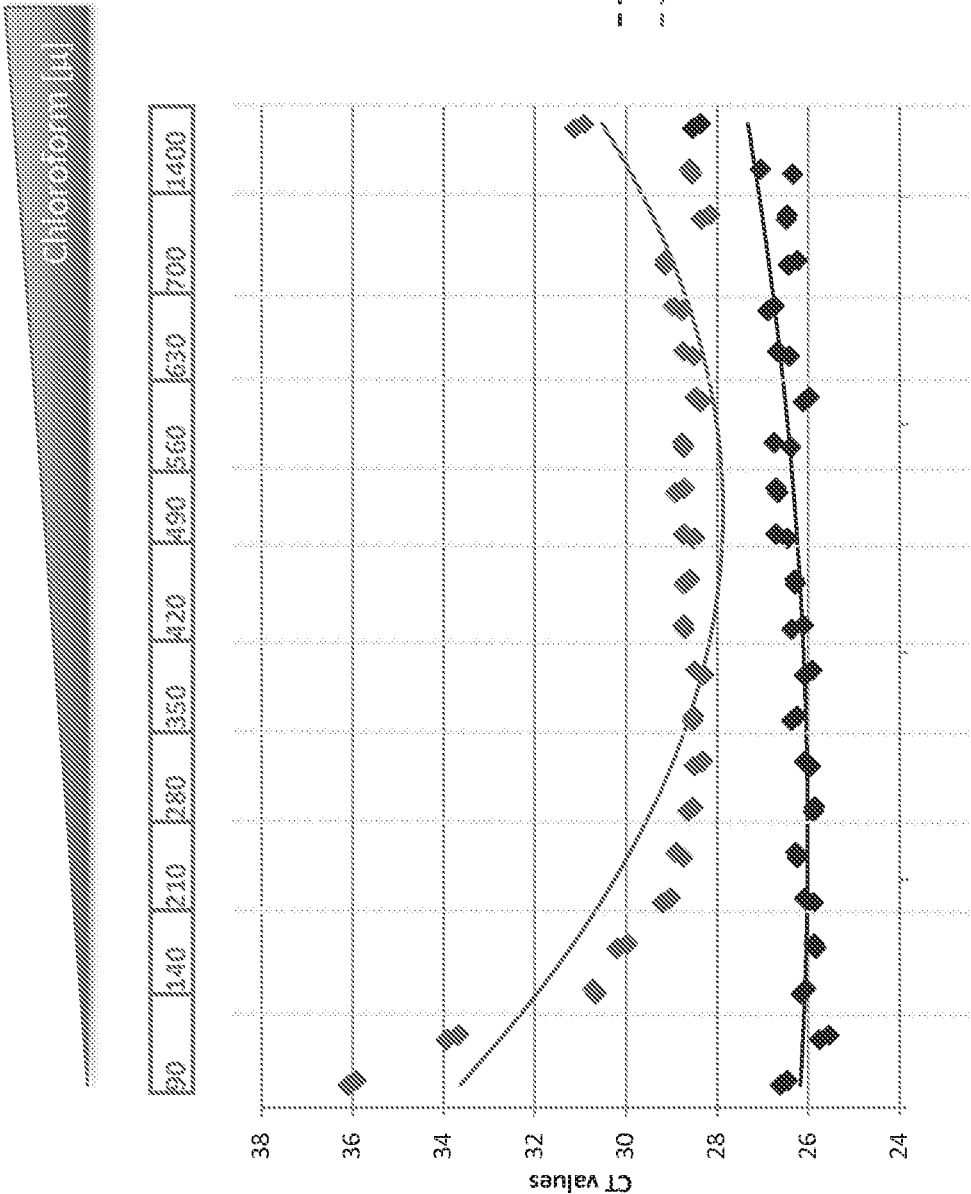
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EXO52 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

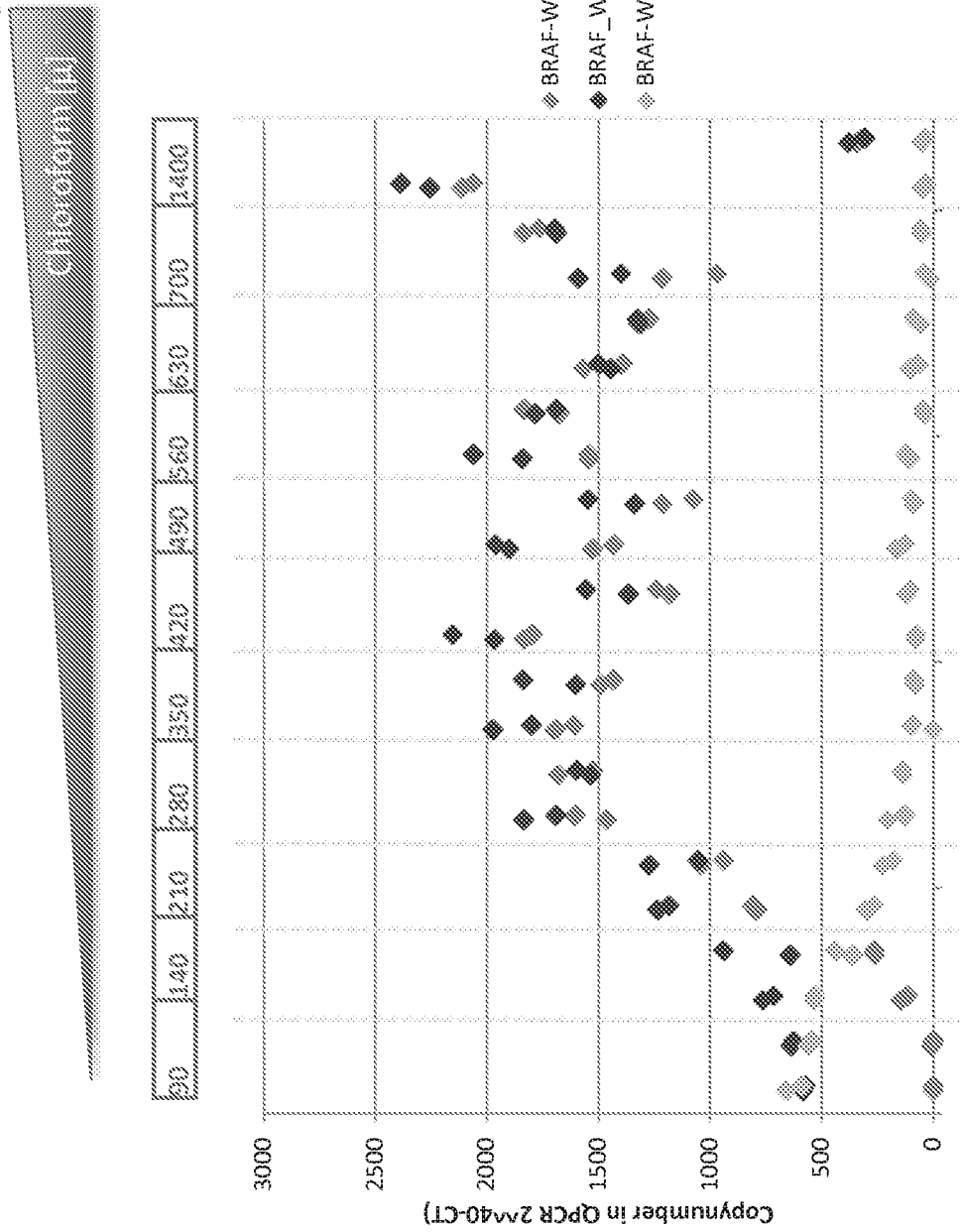
EXOS0 RNA: 10µl RNA in 20µl RT Mix
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EXOS2 DNA 1:2 diluted



Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

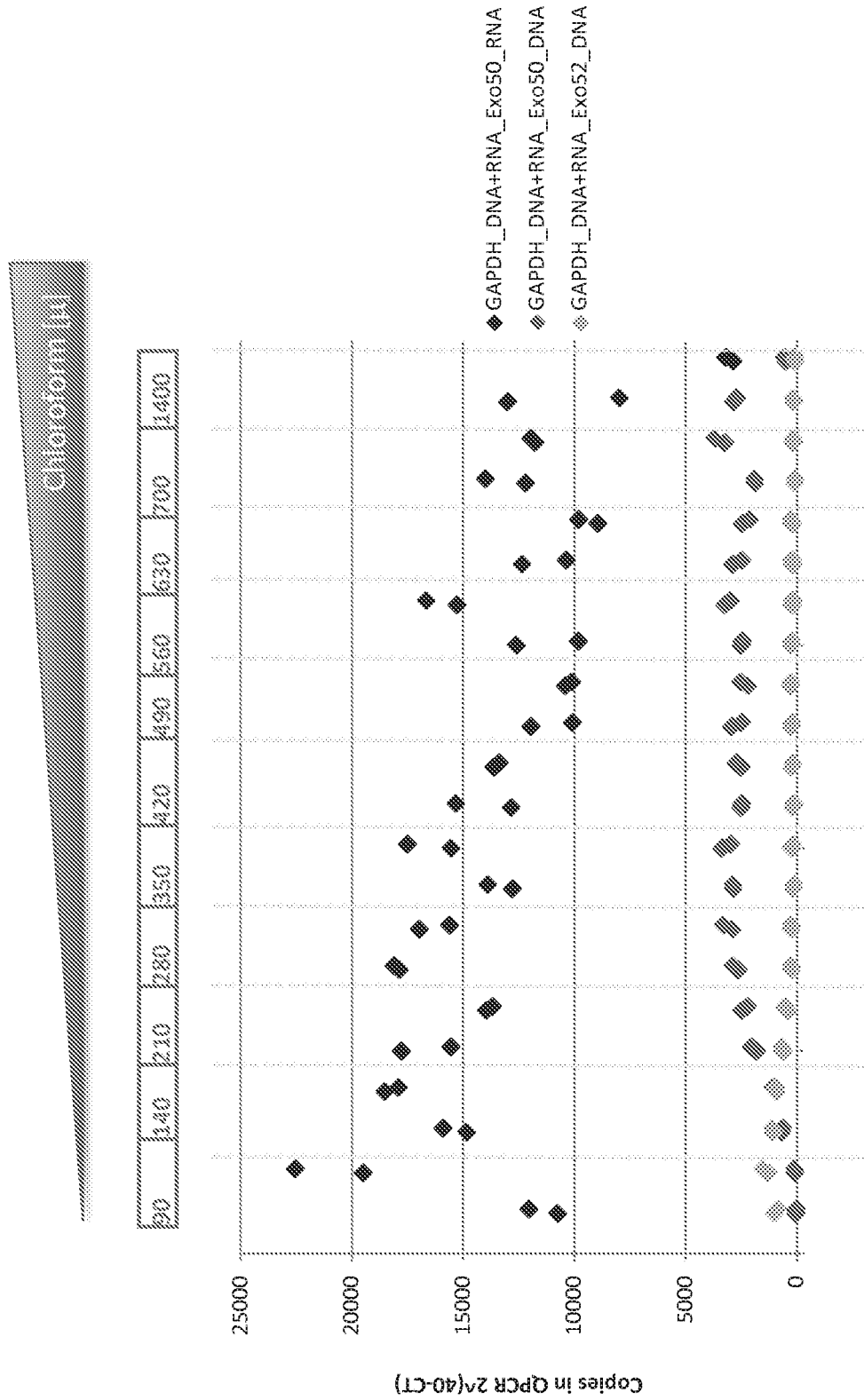
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Figure

DNA isolation using RNeasy protocol (w/o PLG tube) and chloroform titration

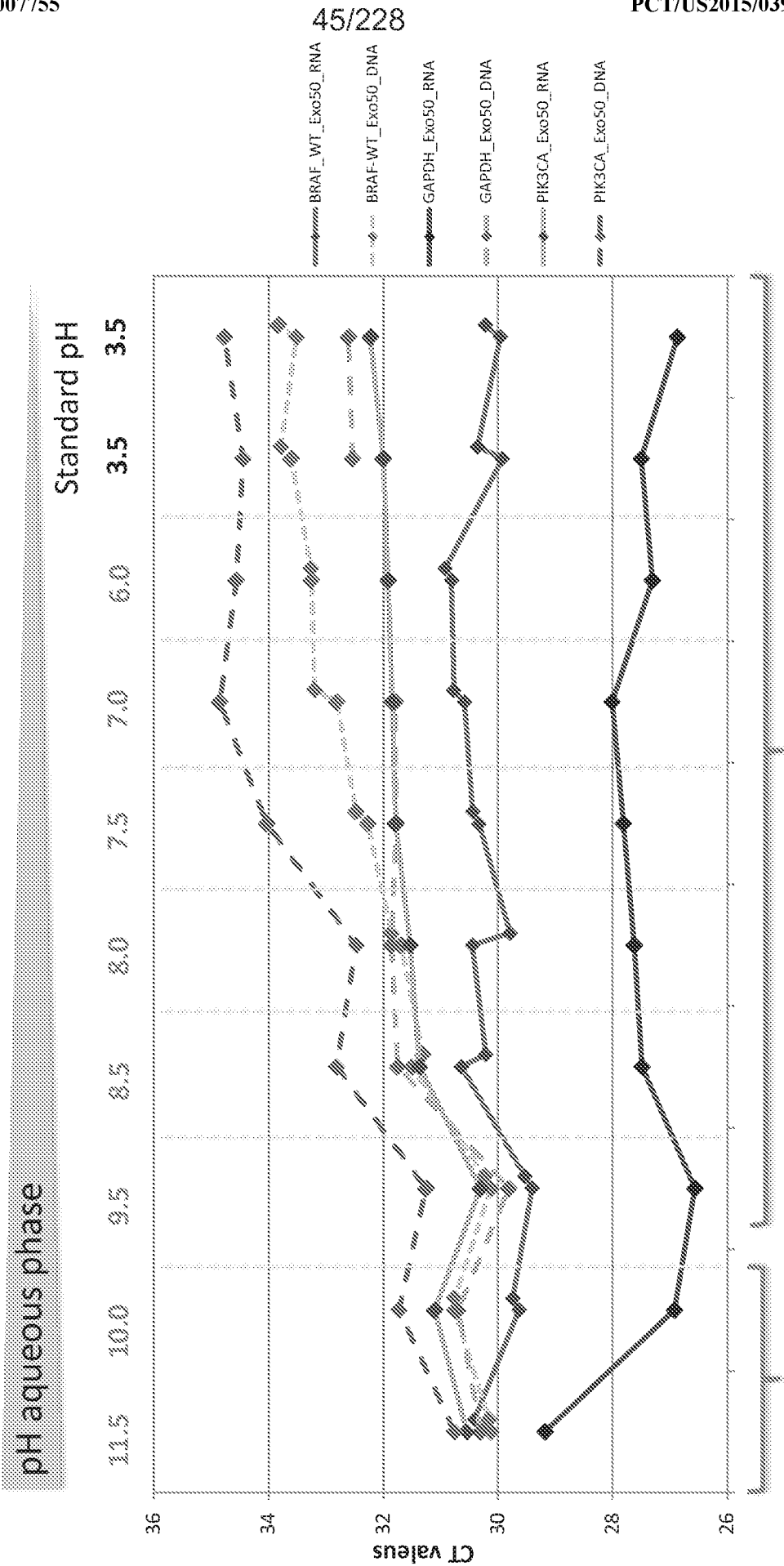
EXO50 RNA: 10 μ l RNA in 20 μ l RT Mix
EXO50 DNA: 1:2 diluted
EXO52 DNA 1:2 diluted



Figure

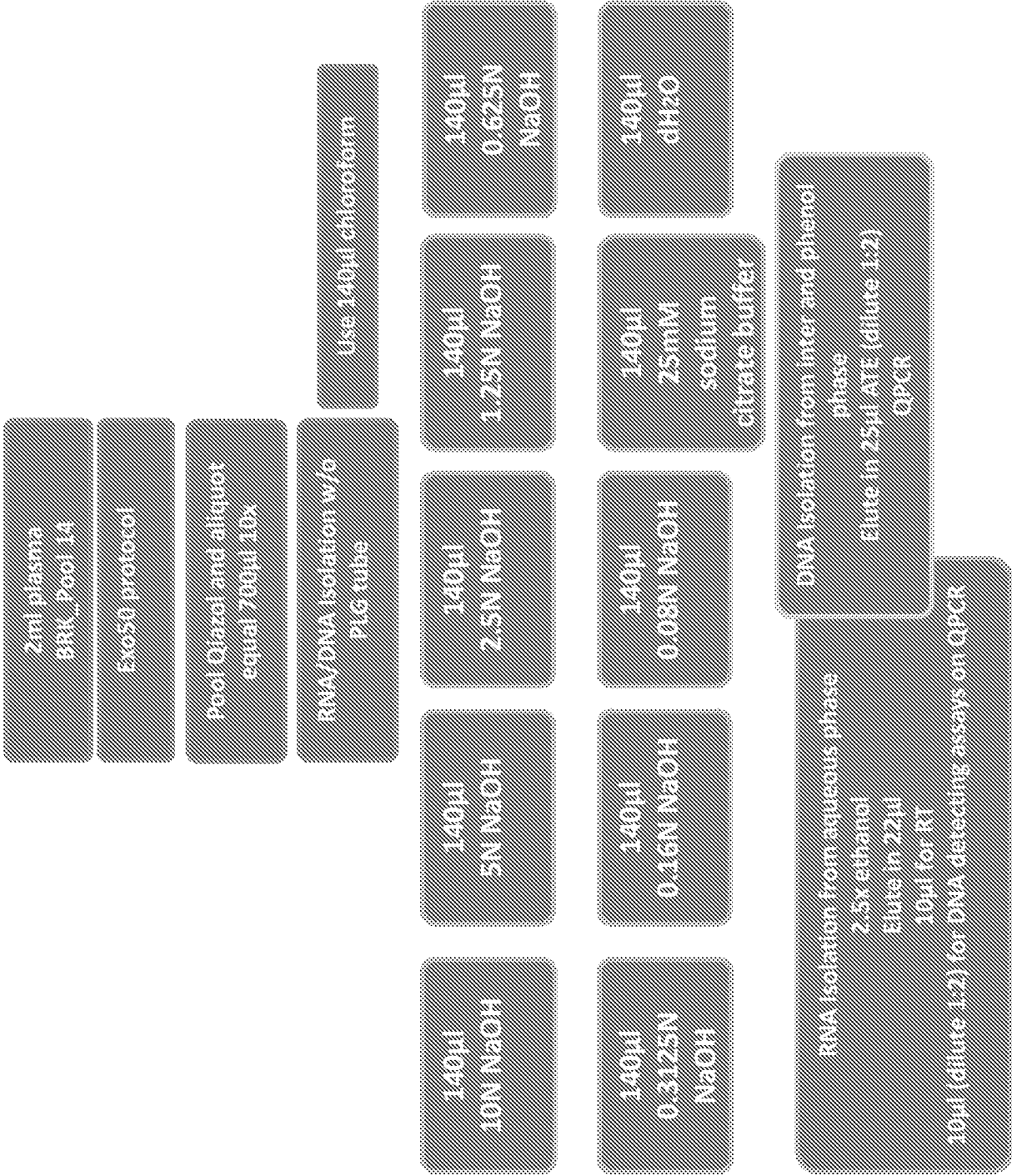
EX052.2: pH changes in phase separation

Adjusting pH in phase separation adds the DNA to the EX052.2 prep



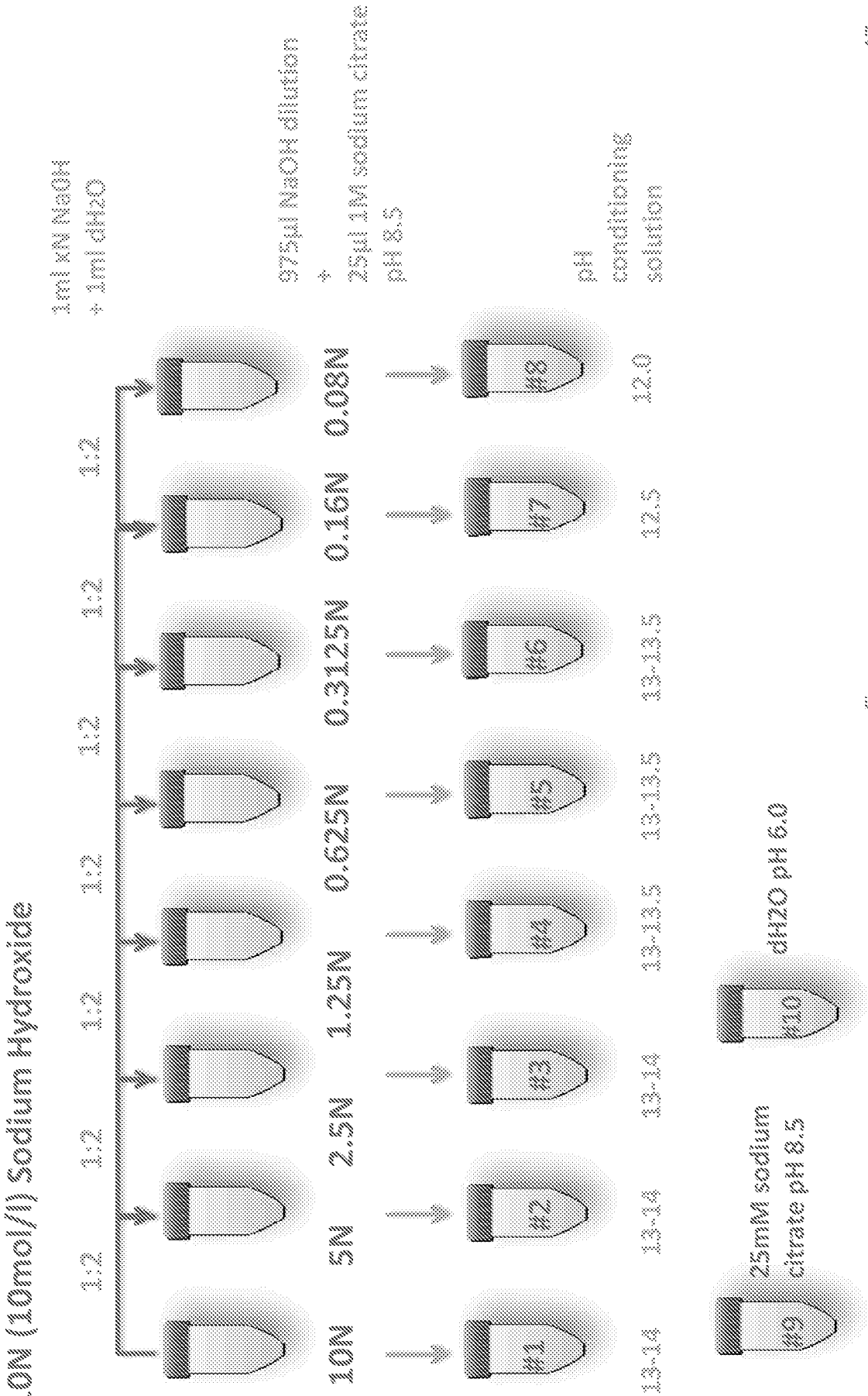
Negative Effect on RNA Dotted lines = w/o RT (i.e. DNA only): 3-4 drop in CT b/c addition of DNA
Solid lines = with RT (i.e. DNA + RNA): 0-2 drop in CT (different expression levels of RNA)

DNA isolation from aqueous phase: pH titration



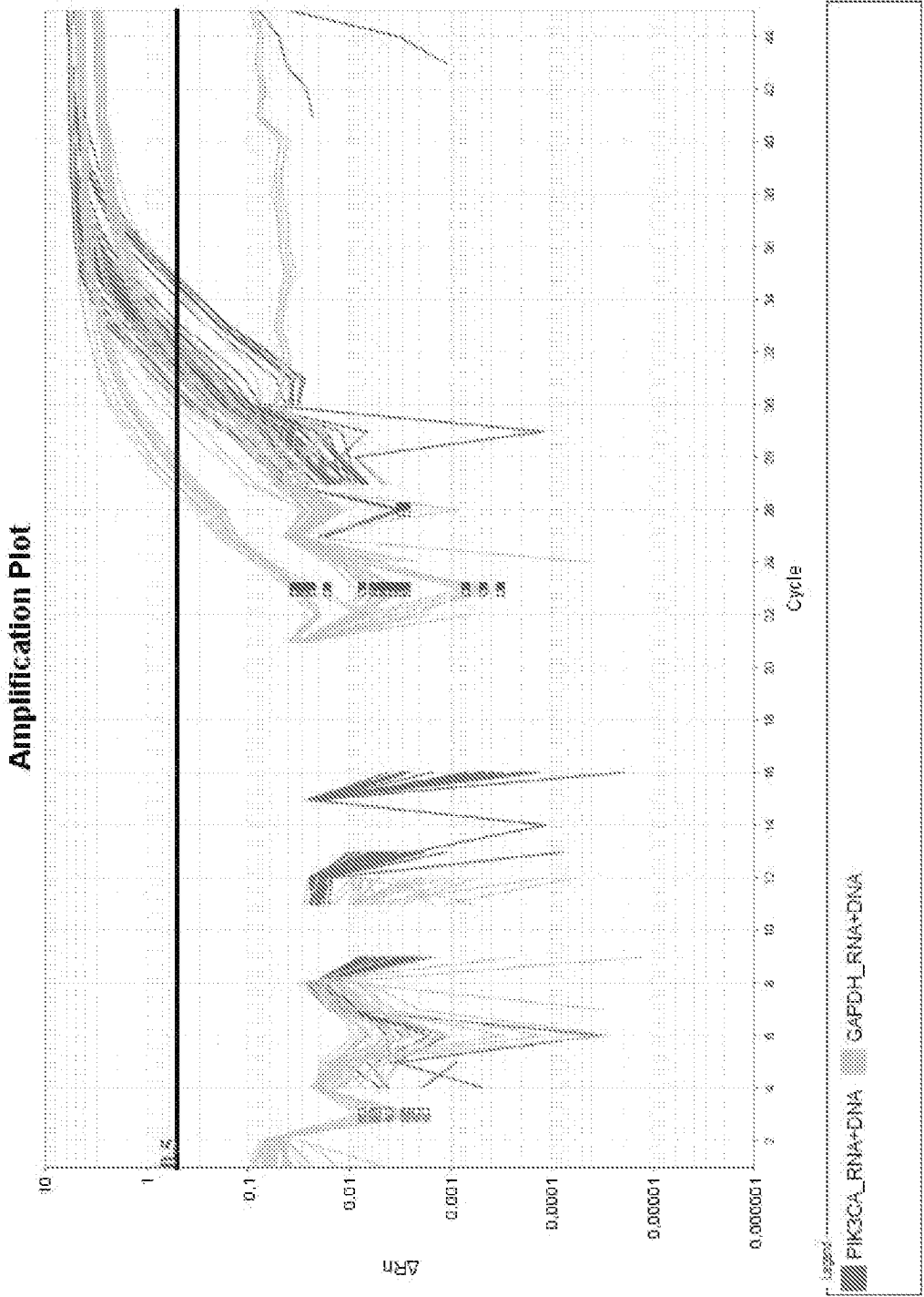
Figure

Preparing pH conditioning solution



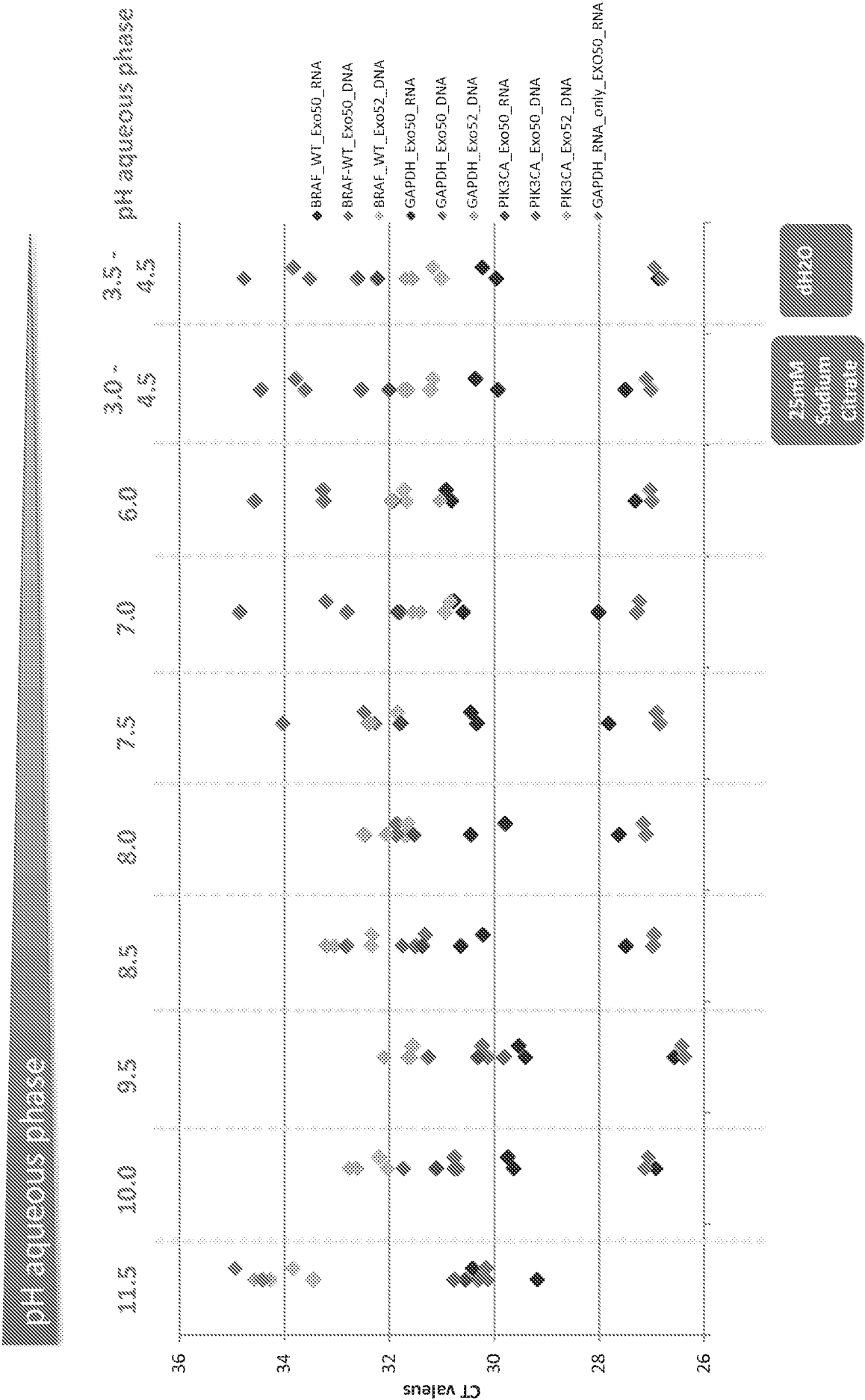
Figure

Nala amplification curves



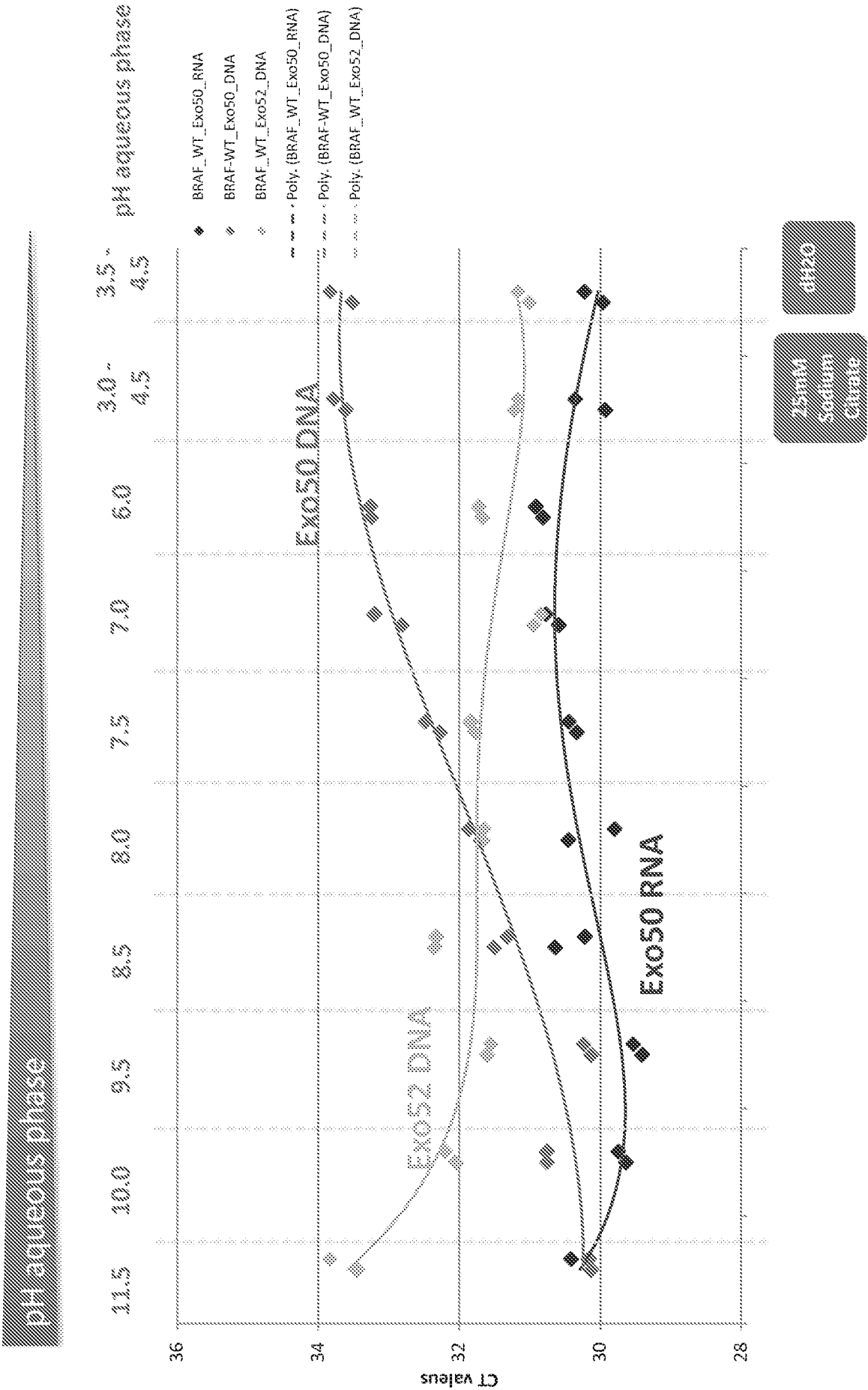
Figure

DNA isolation from aqueous phase: pH titration



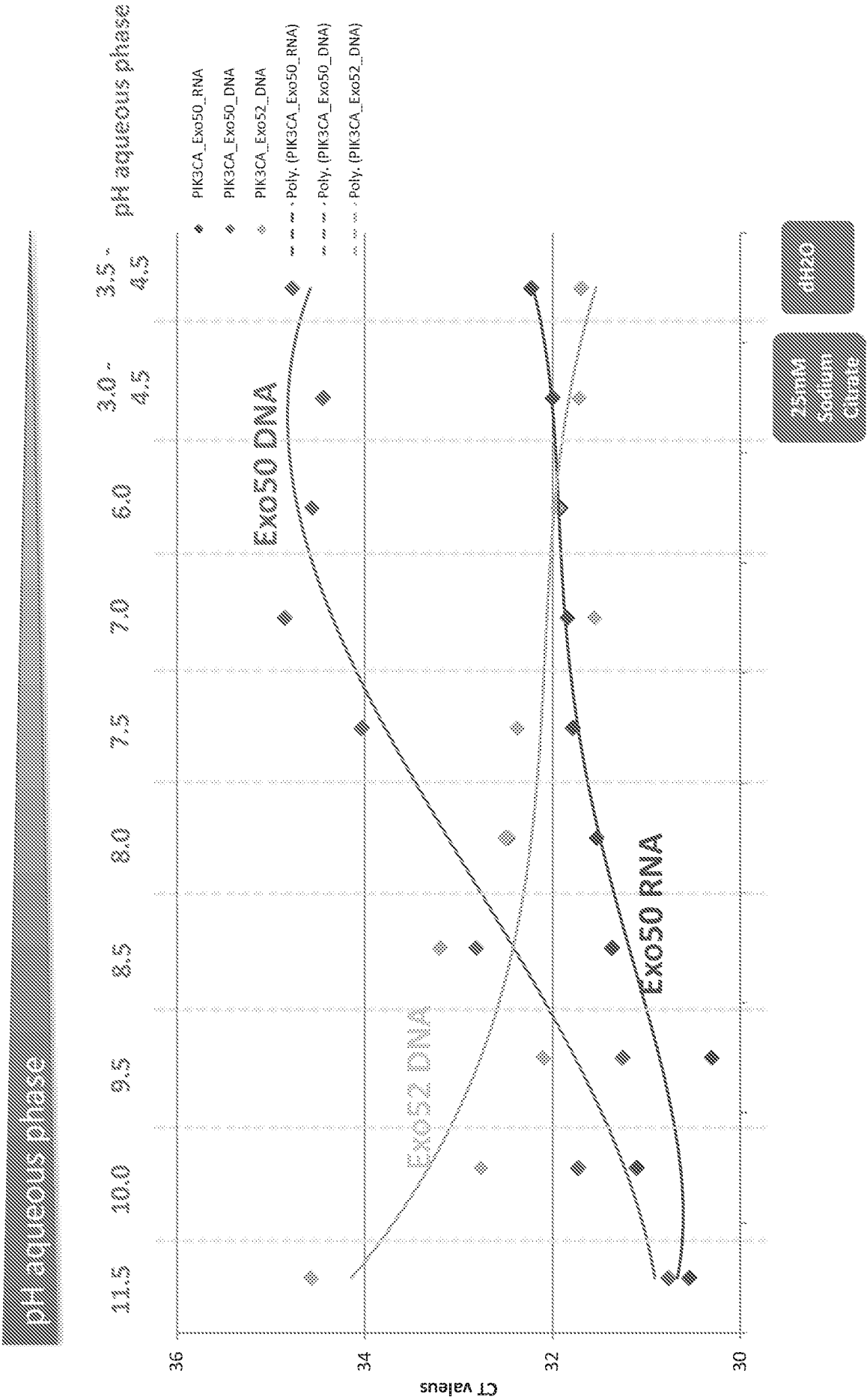
Figure

DNA isolation from aqueous phase: pH titration



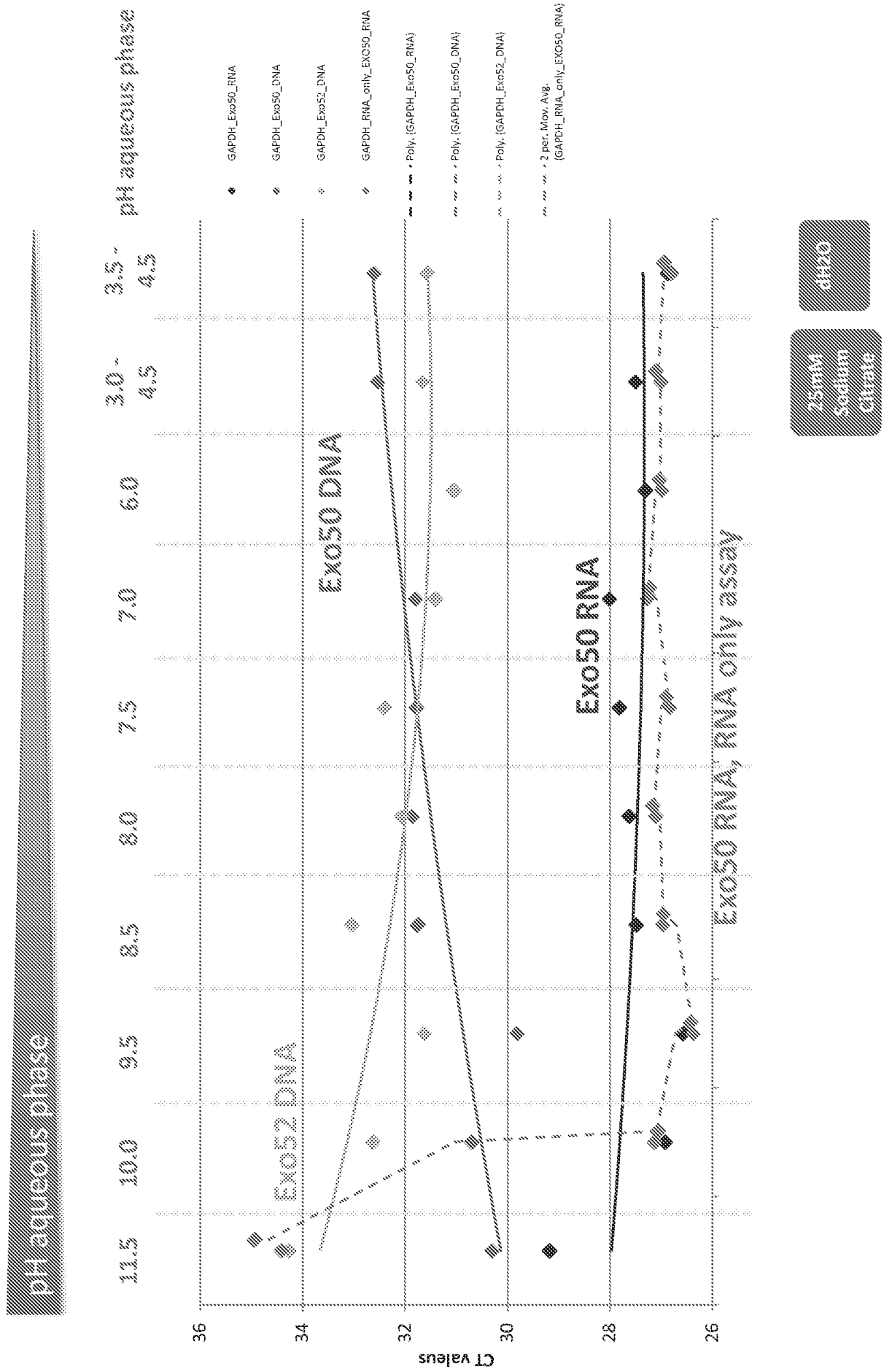
Figure

DNA isolation from aqueous phase: pH titration



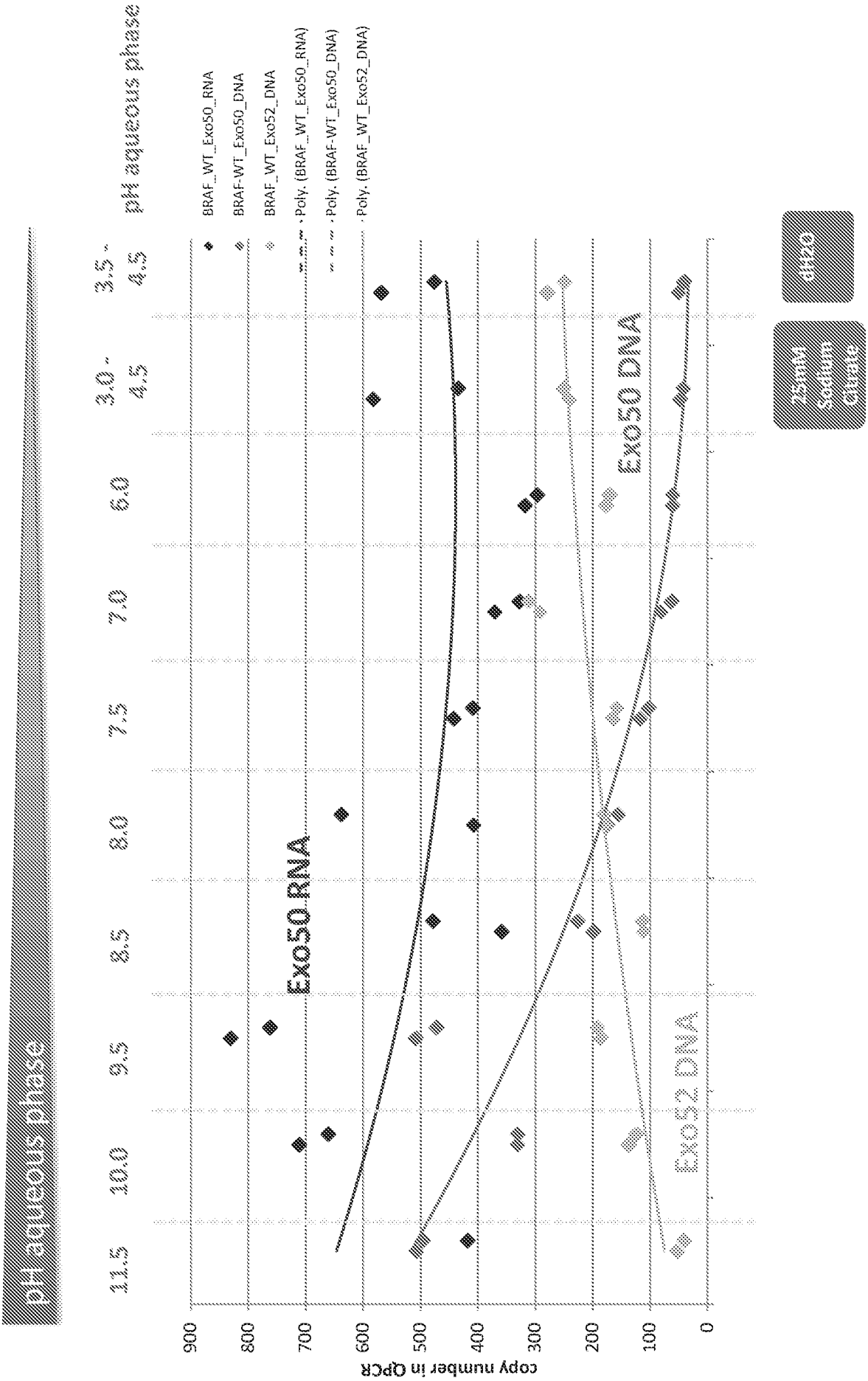
Figure

DNA isolation from aqueous phase: pH titration



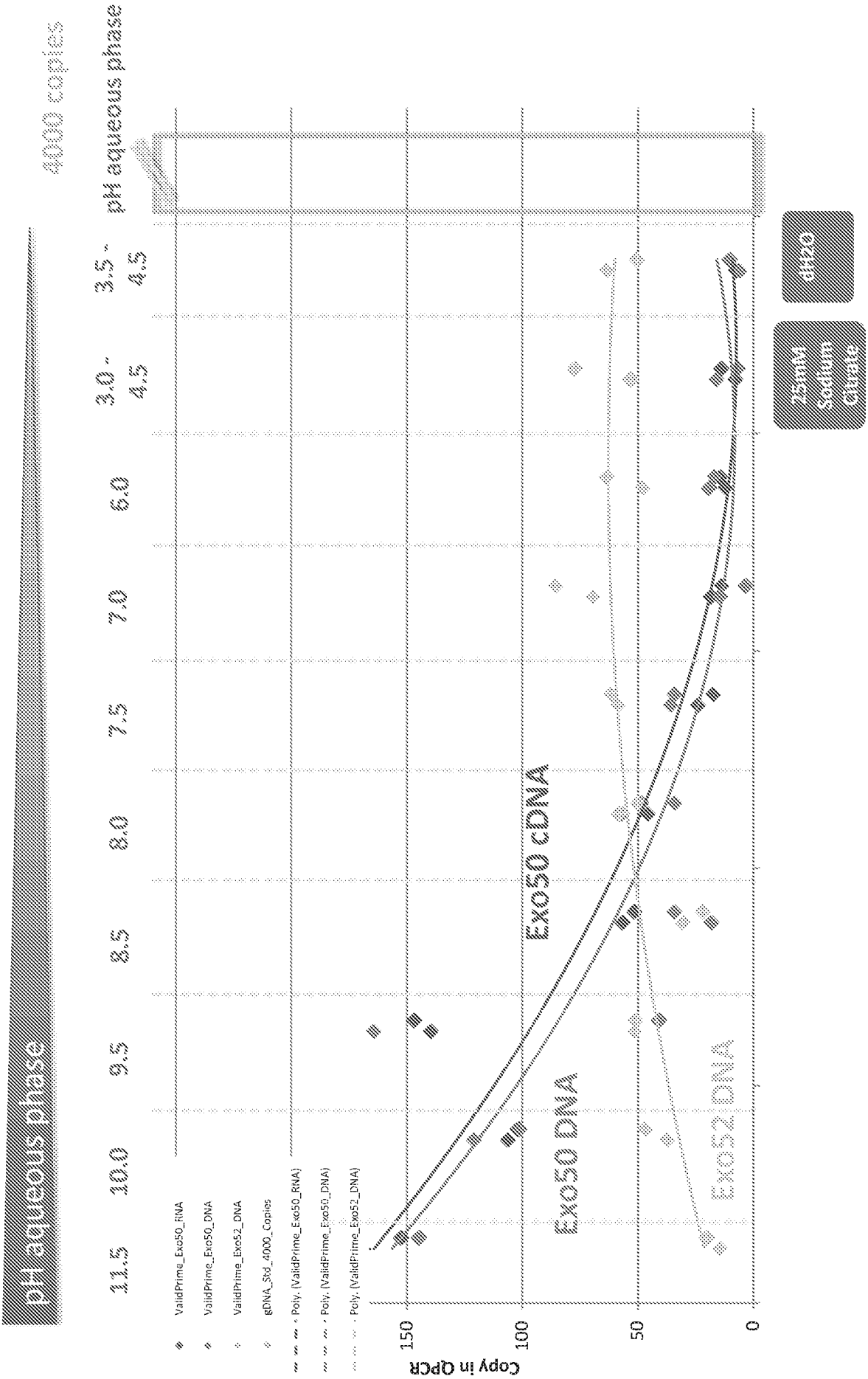
20

DNA isolation from aqueous phase: pH titration



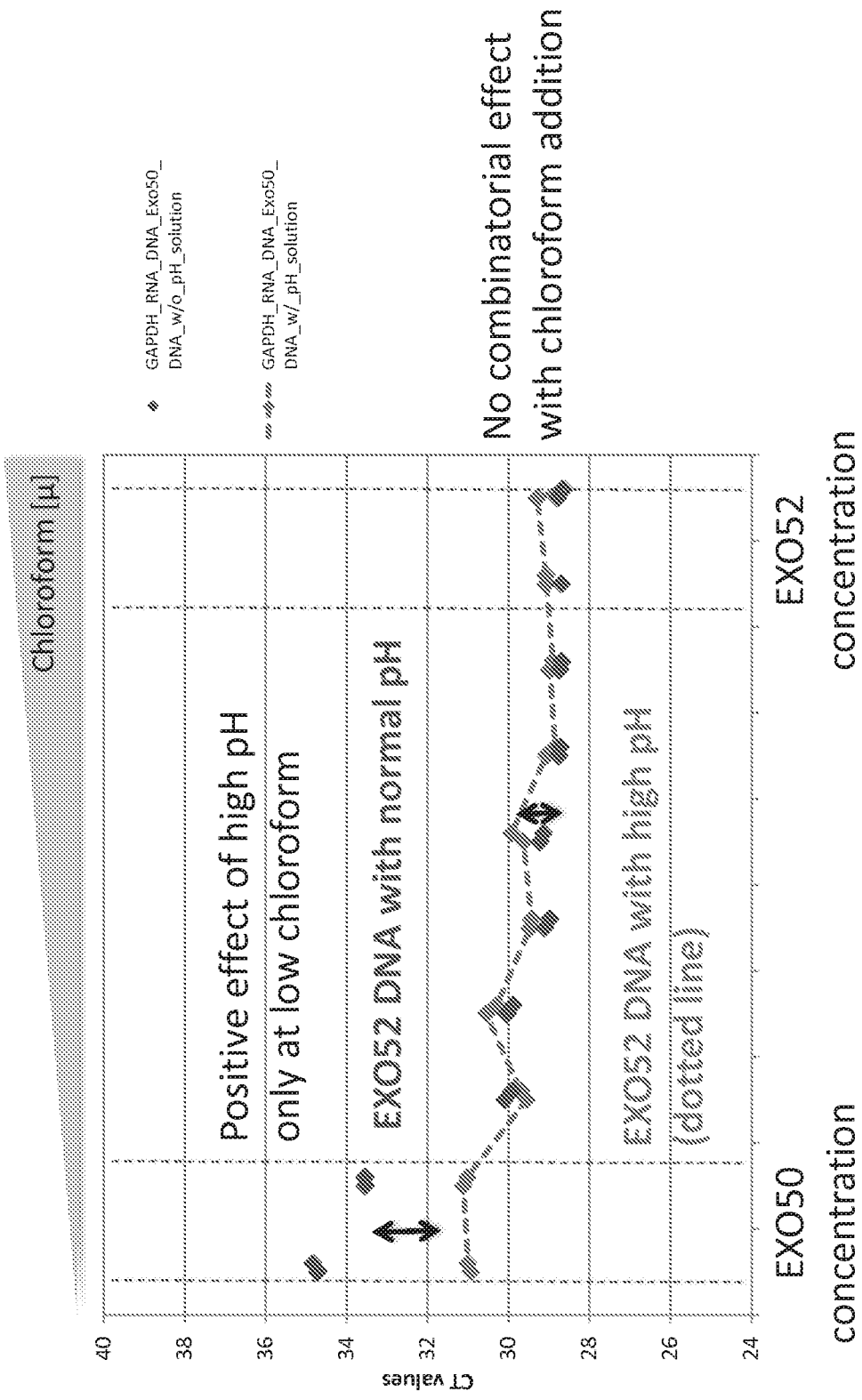
Figure

DNA isolation from aqueous phase: pH titration



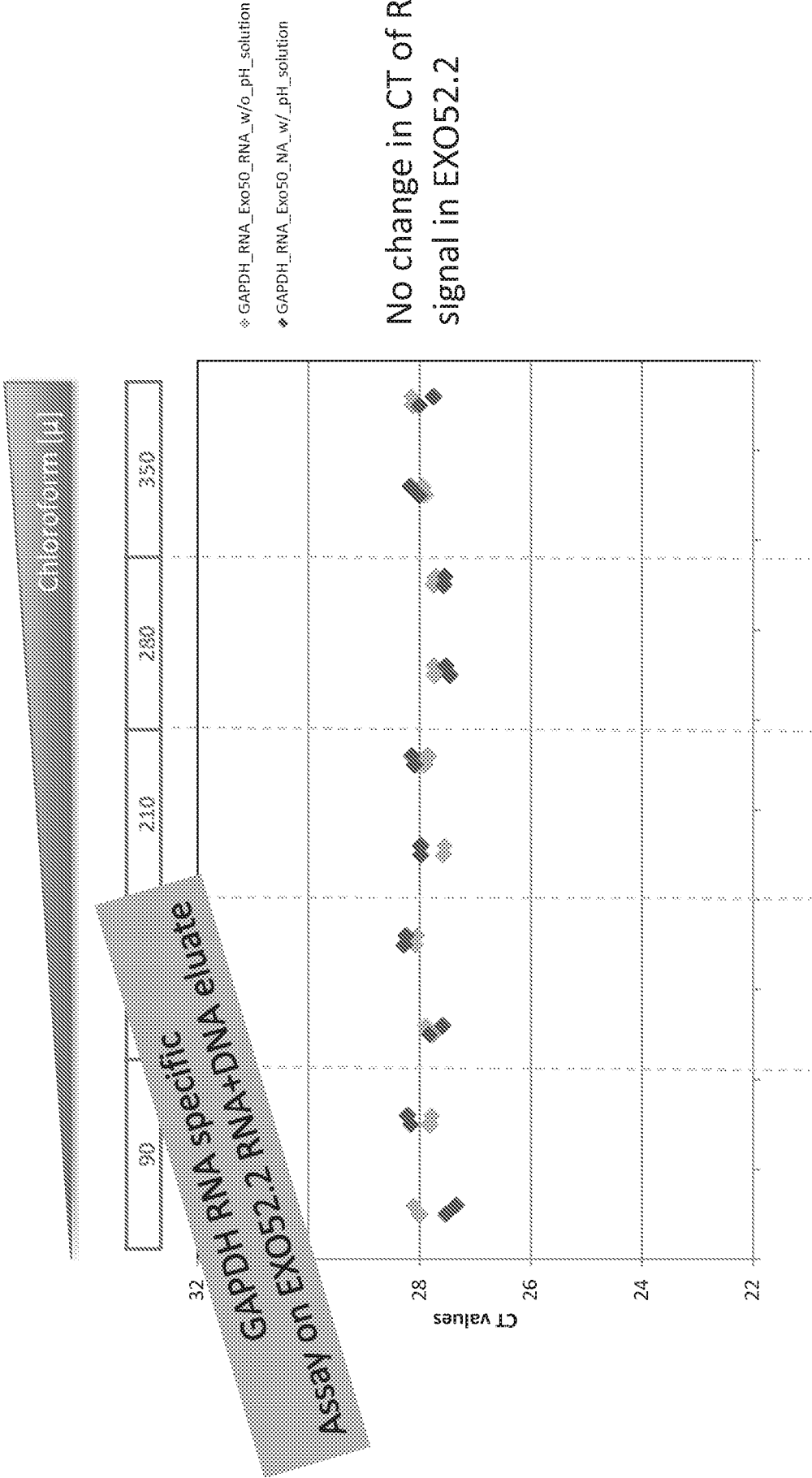
Figure

Chloroform addition is the predominant factor in determining the DNA content of the aqueous phase



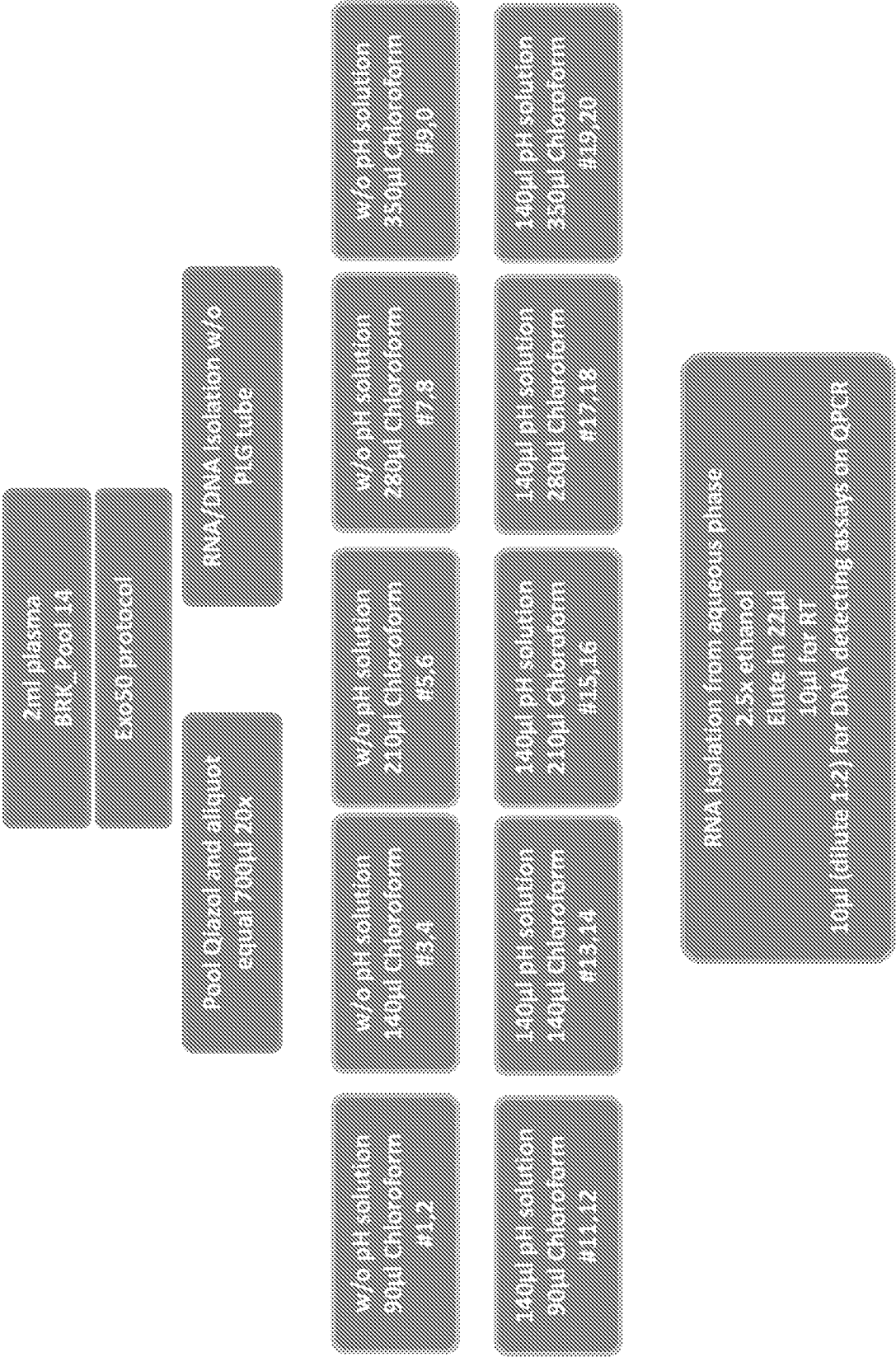
Figure

The RNA signal is not affected through addition of DNA



Figure

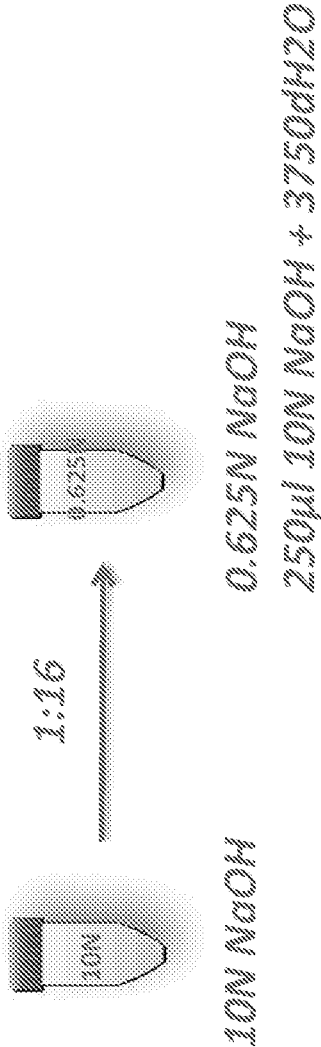
DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



Figure

Preparing pH conditioning solution

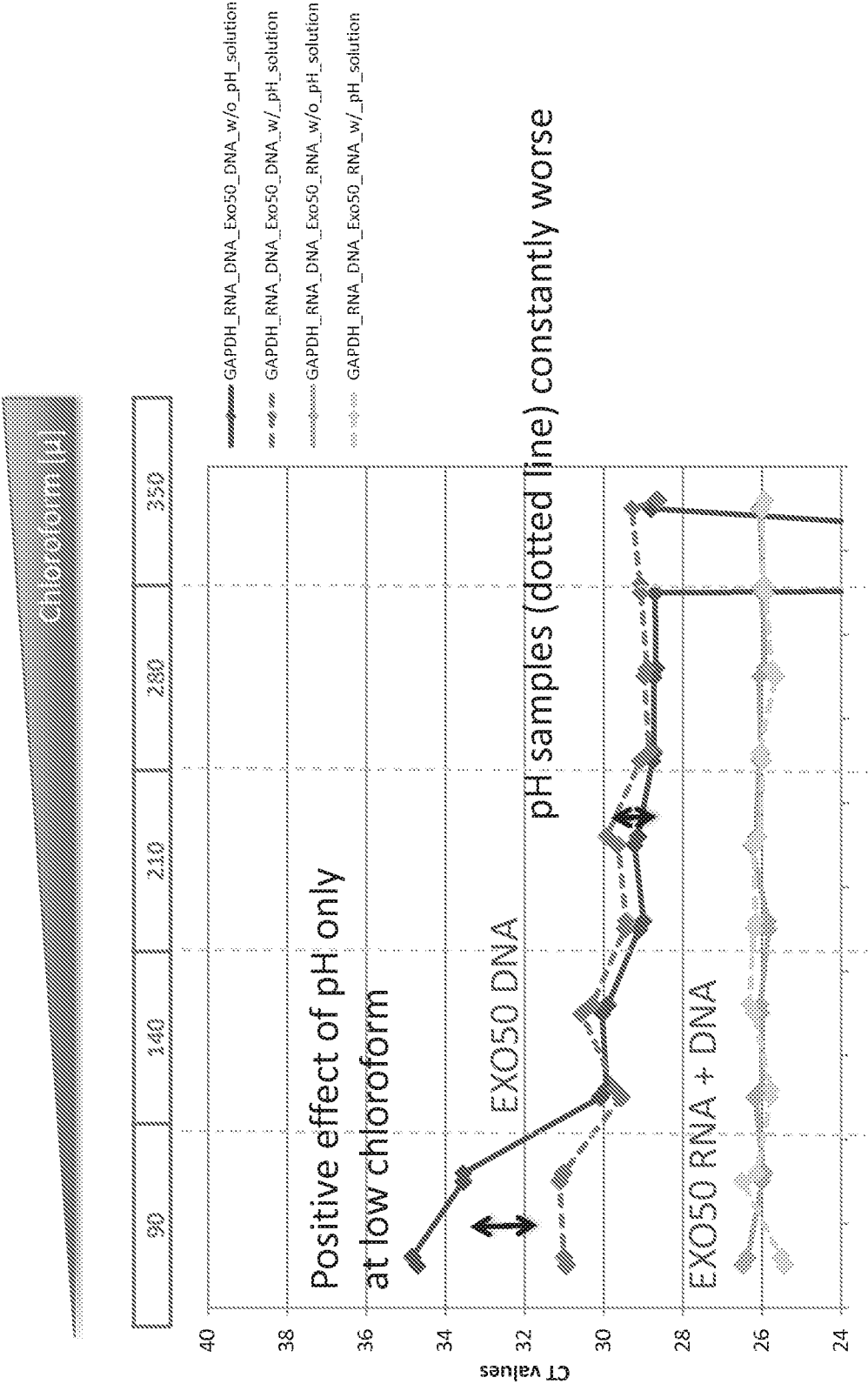
10N (10mol/l) Sodium Hydroxide
1M sodium citrate pH 8.5 (selfmade)



0.625N NaOH + 1M sodium citrate pH 8.5
2340µl + 60µl (=25mM)

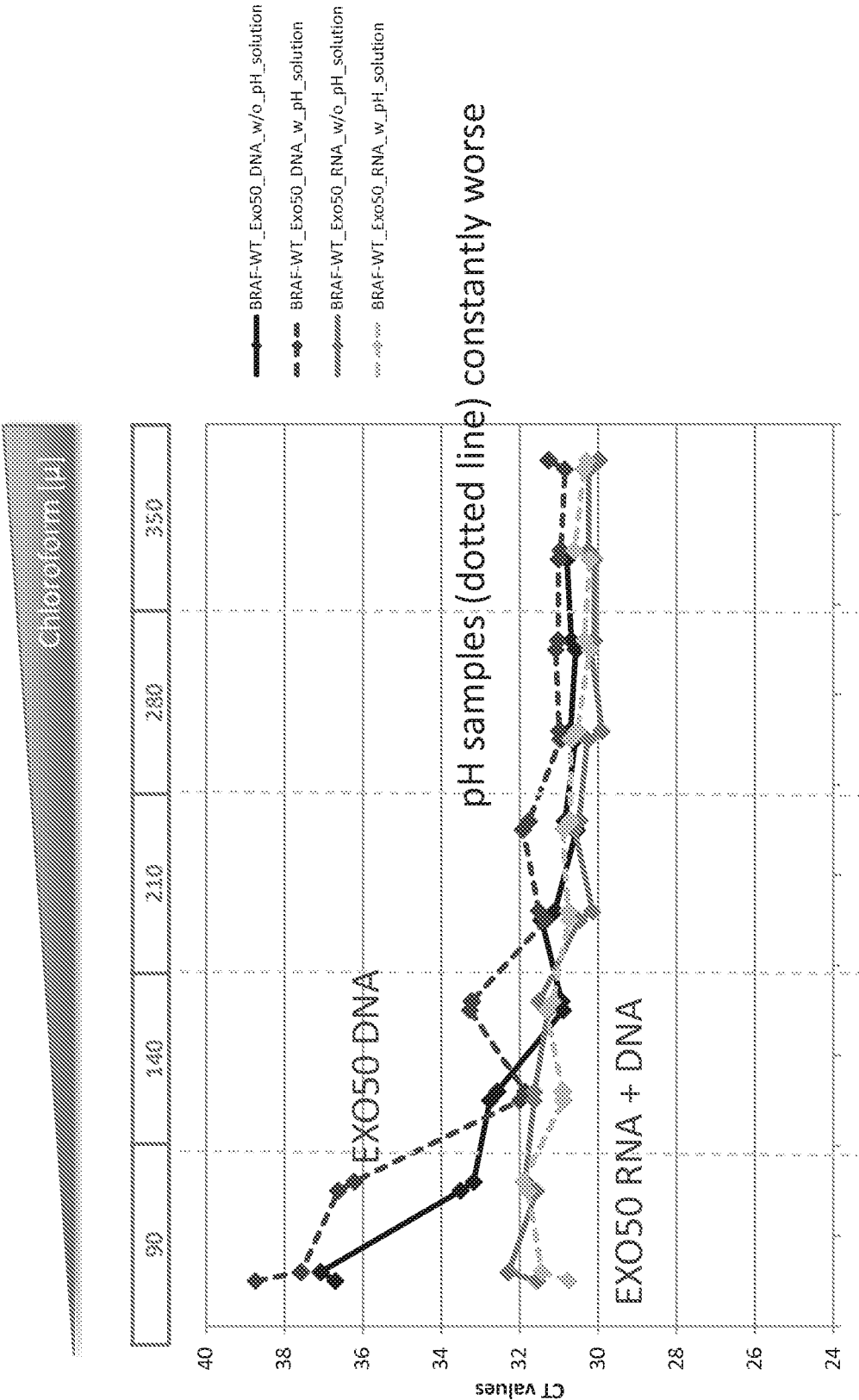
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



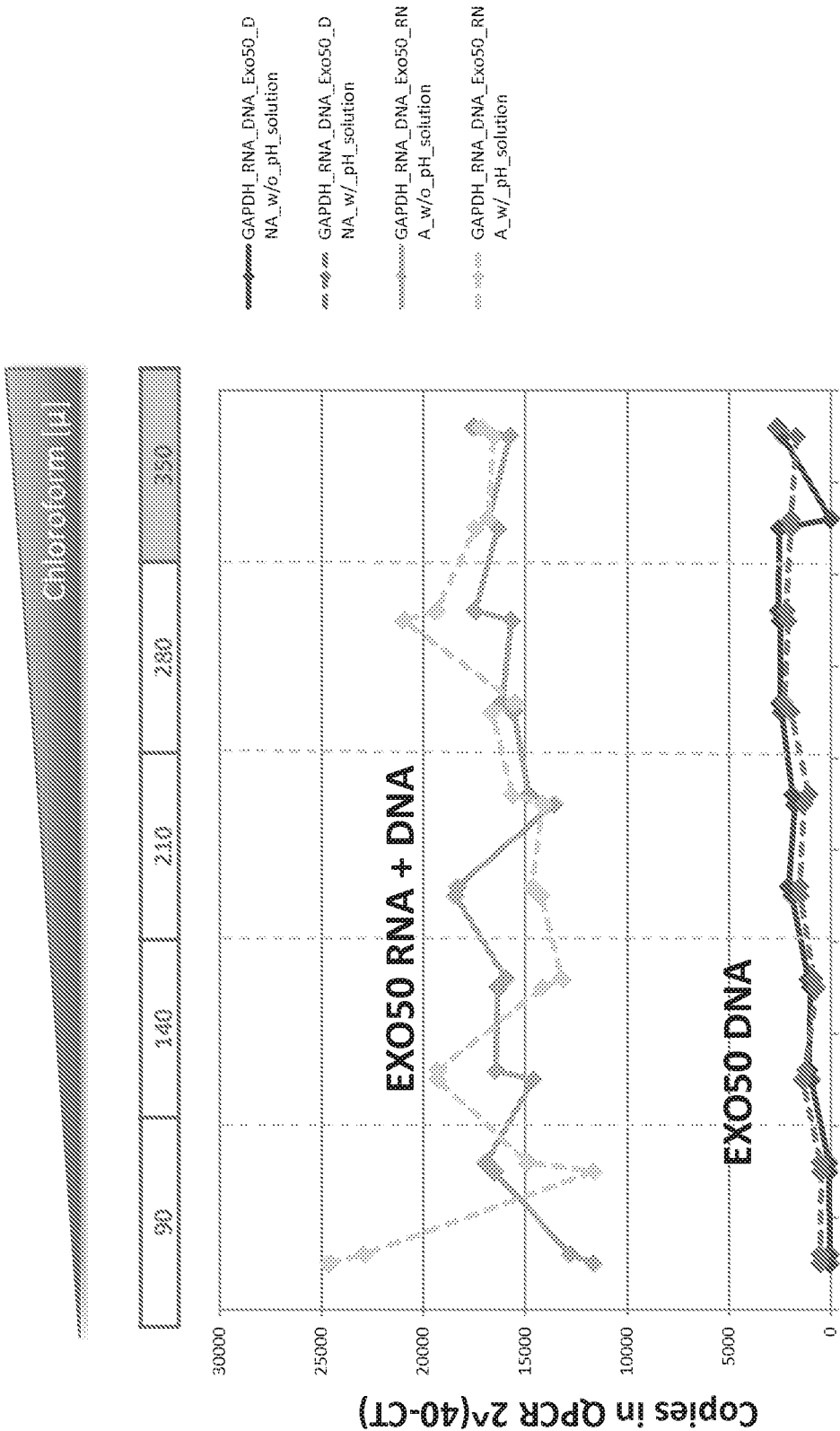
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



Figure

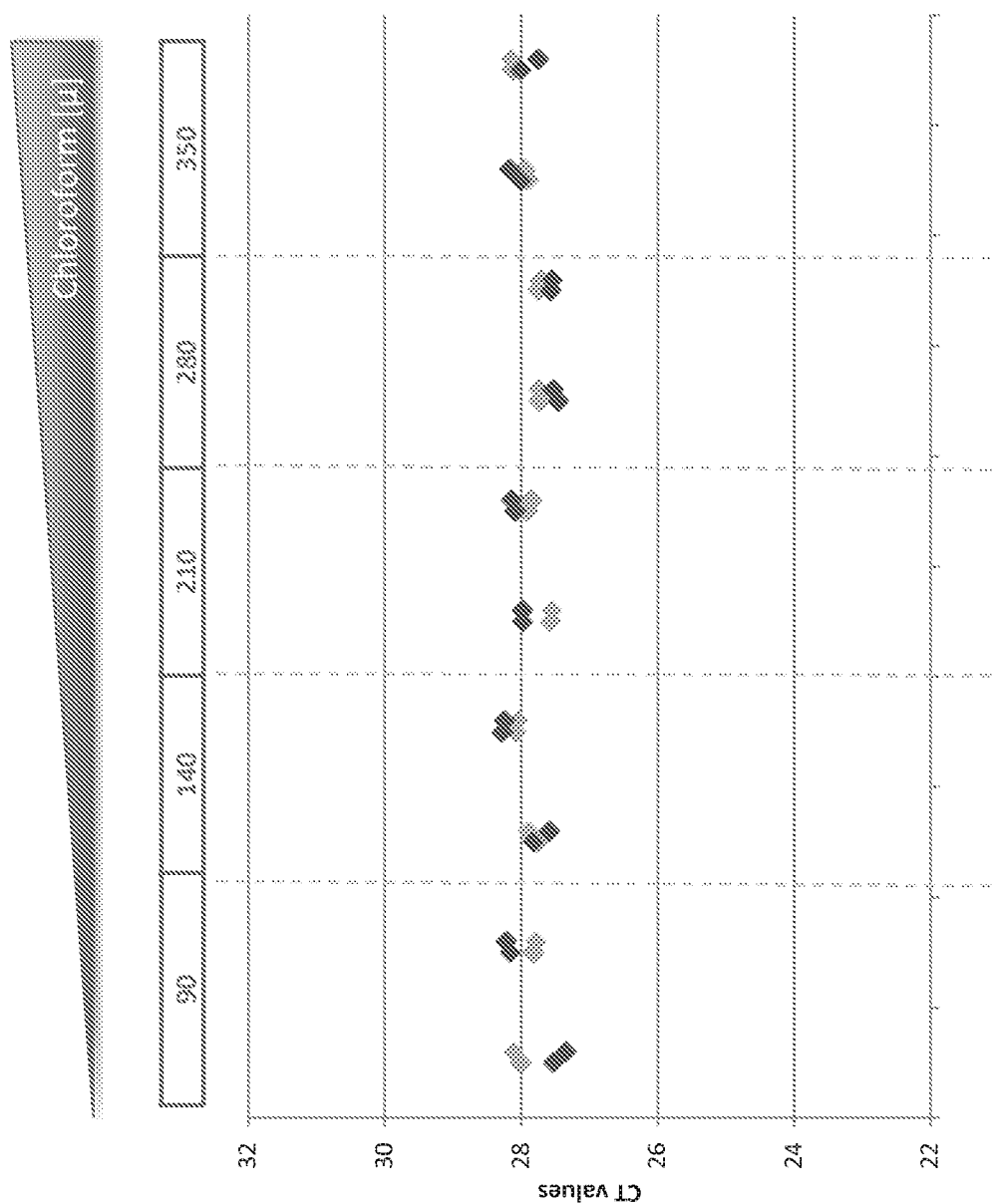
DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



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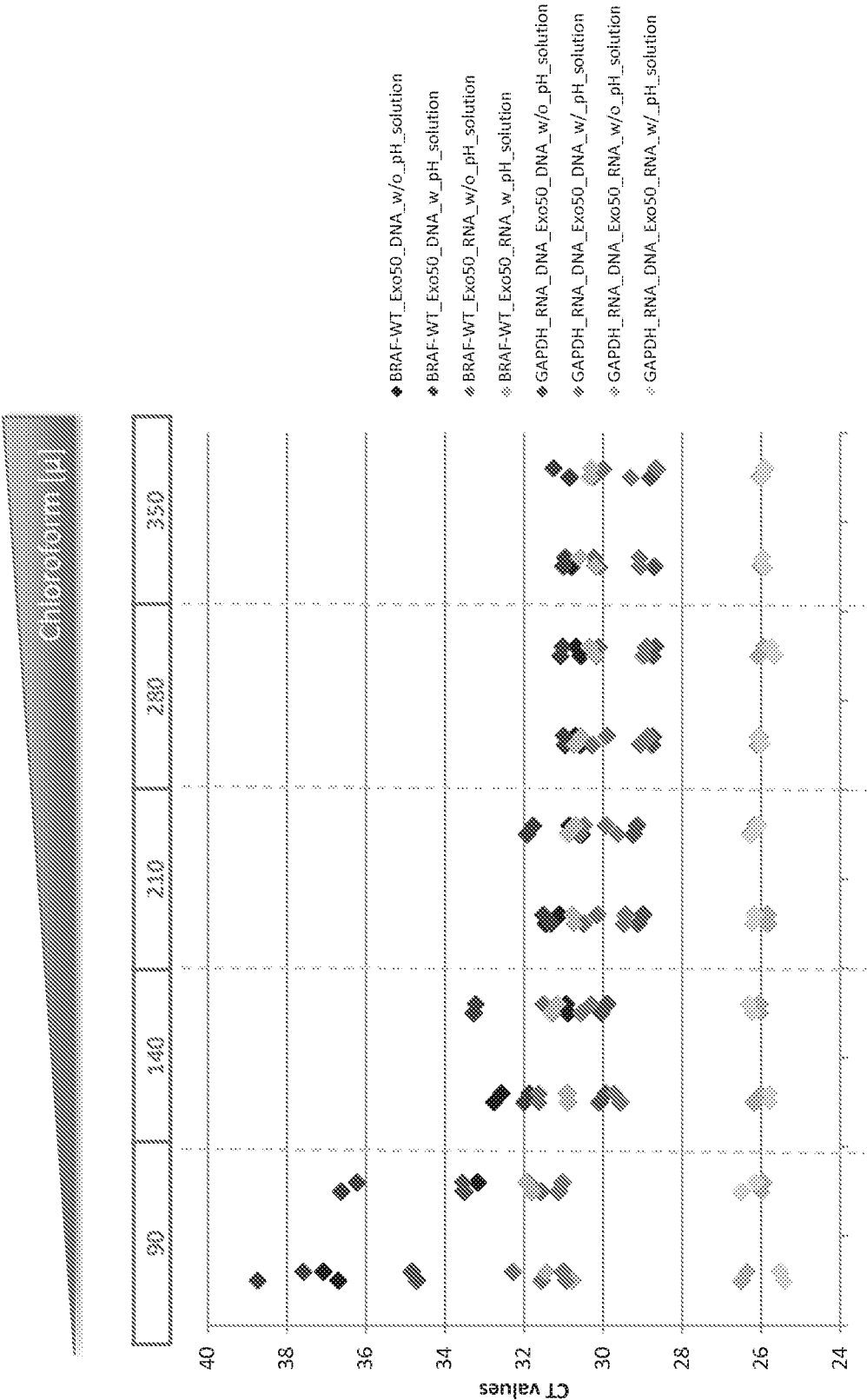
DNA isolation from aqueous phase: chloroform titration with and without adding pH solution

GAPDH RNA specific
Assay on EXO50 RNA



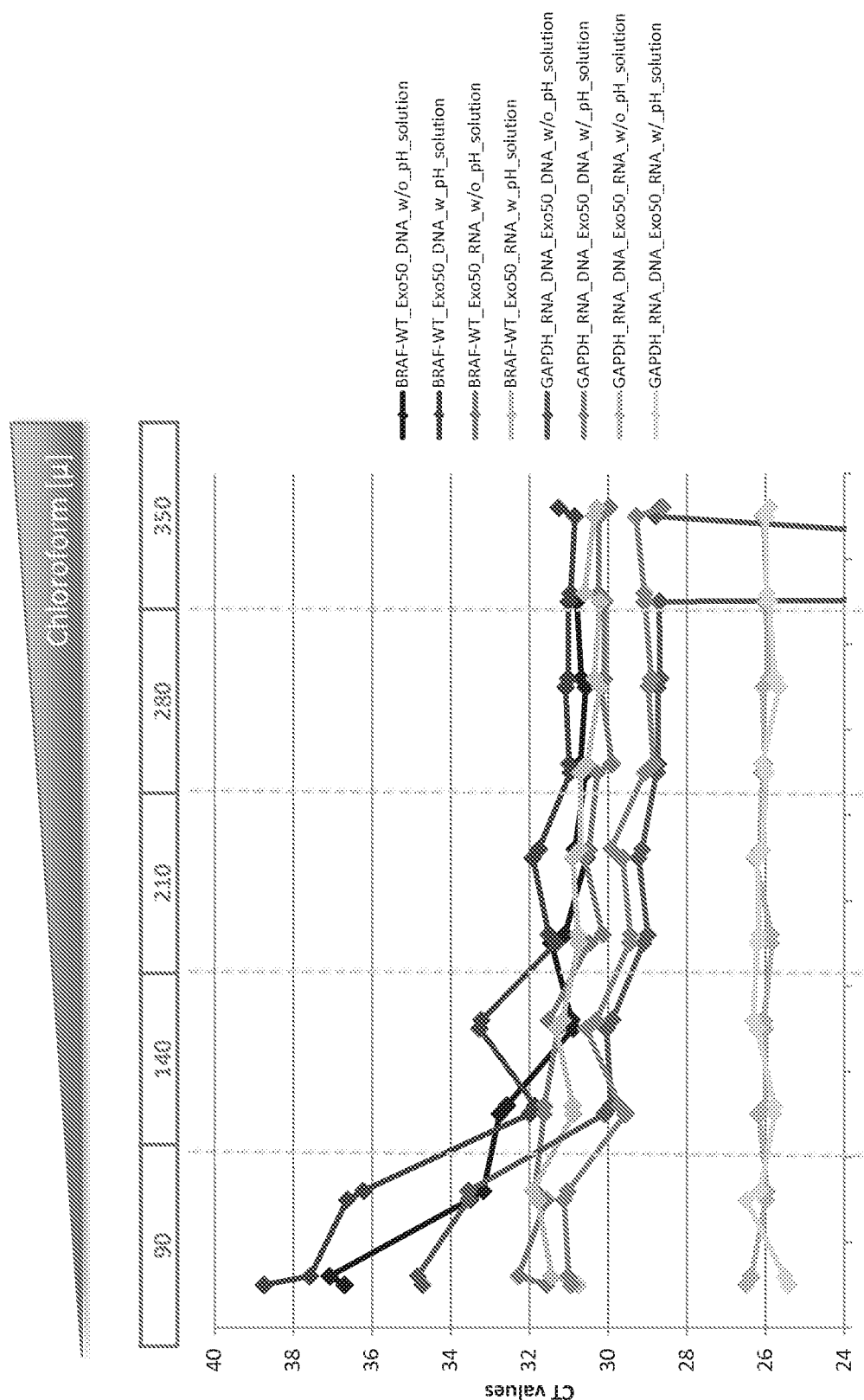
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



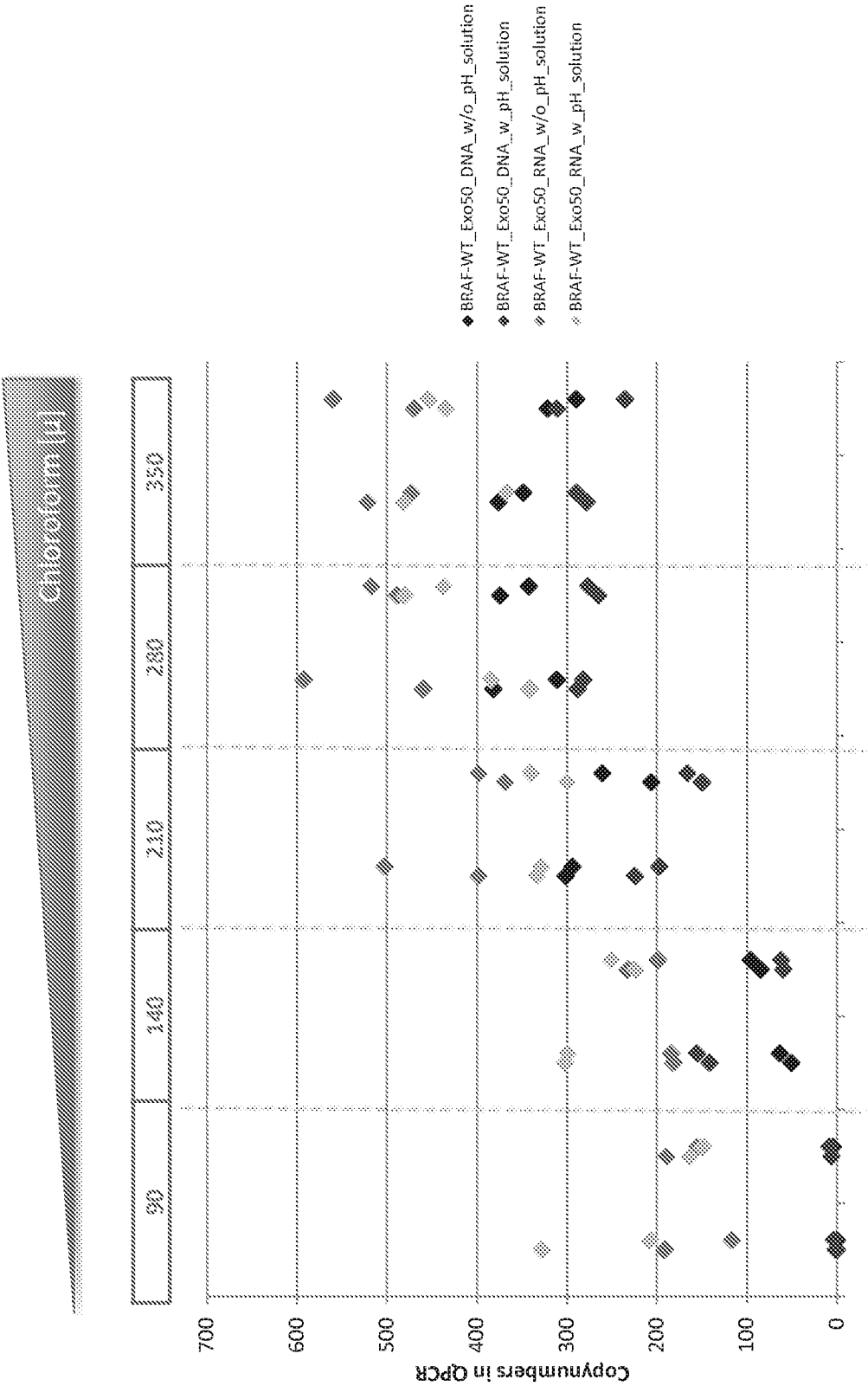
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



Figure

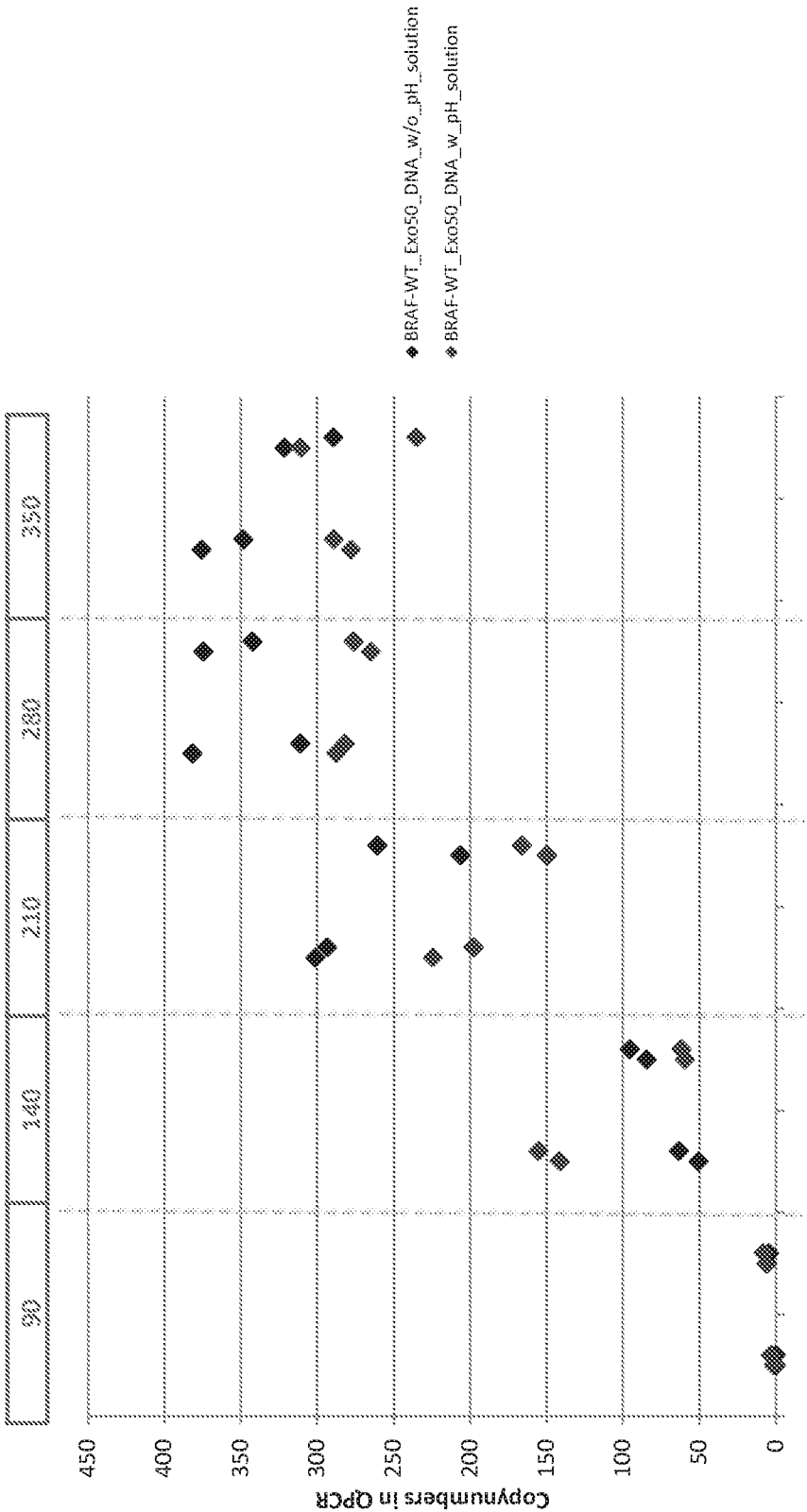
DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



Figure

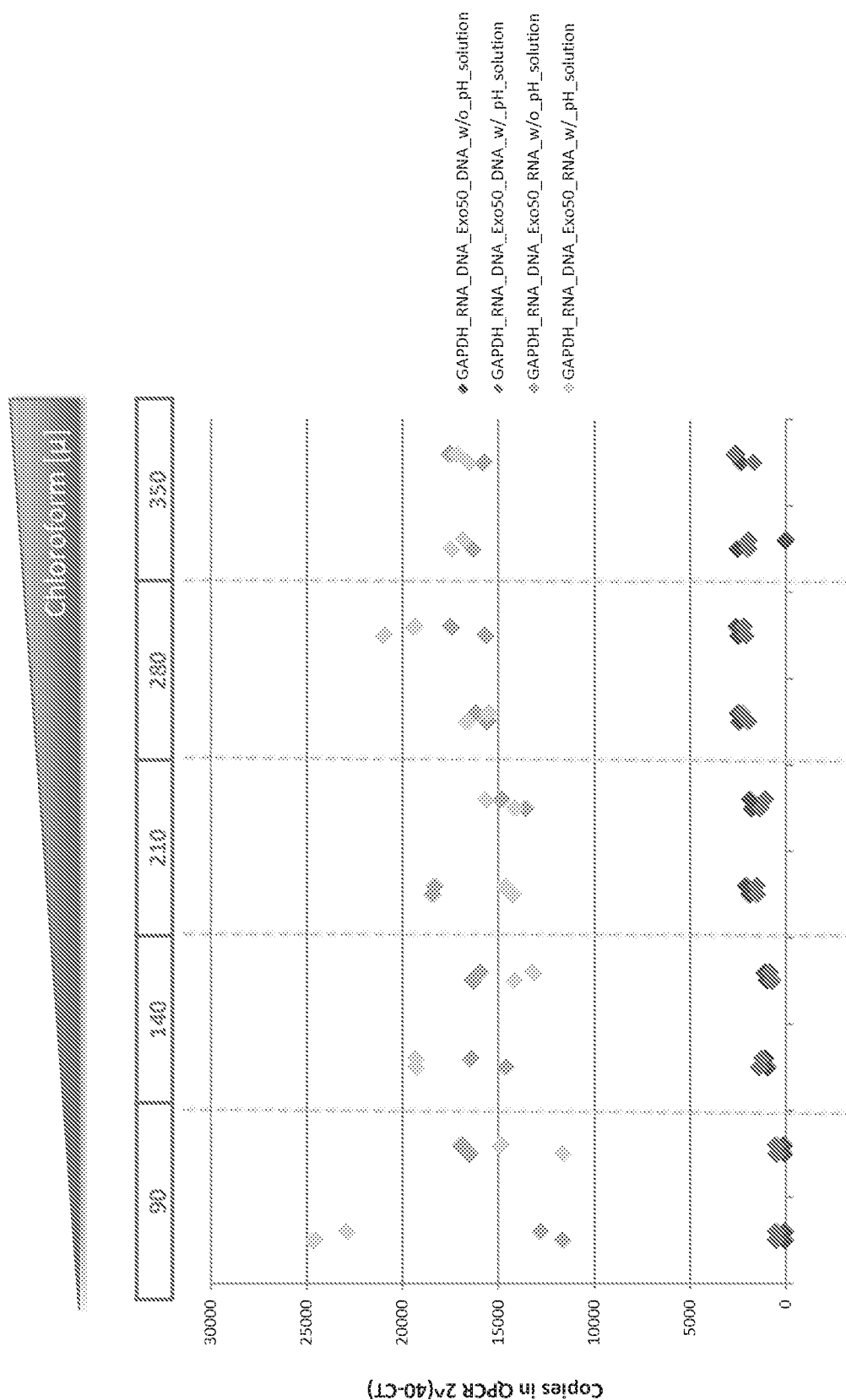
DNA isolation from aqueous phase: chloroform titration with and without adding pH solution

Exo50 DNA



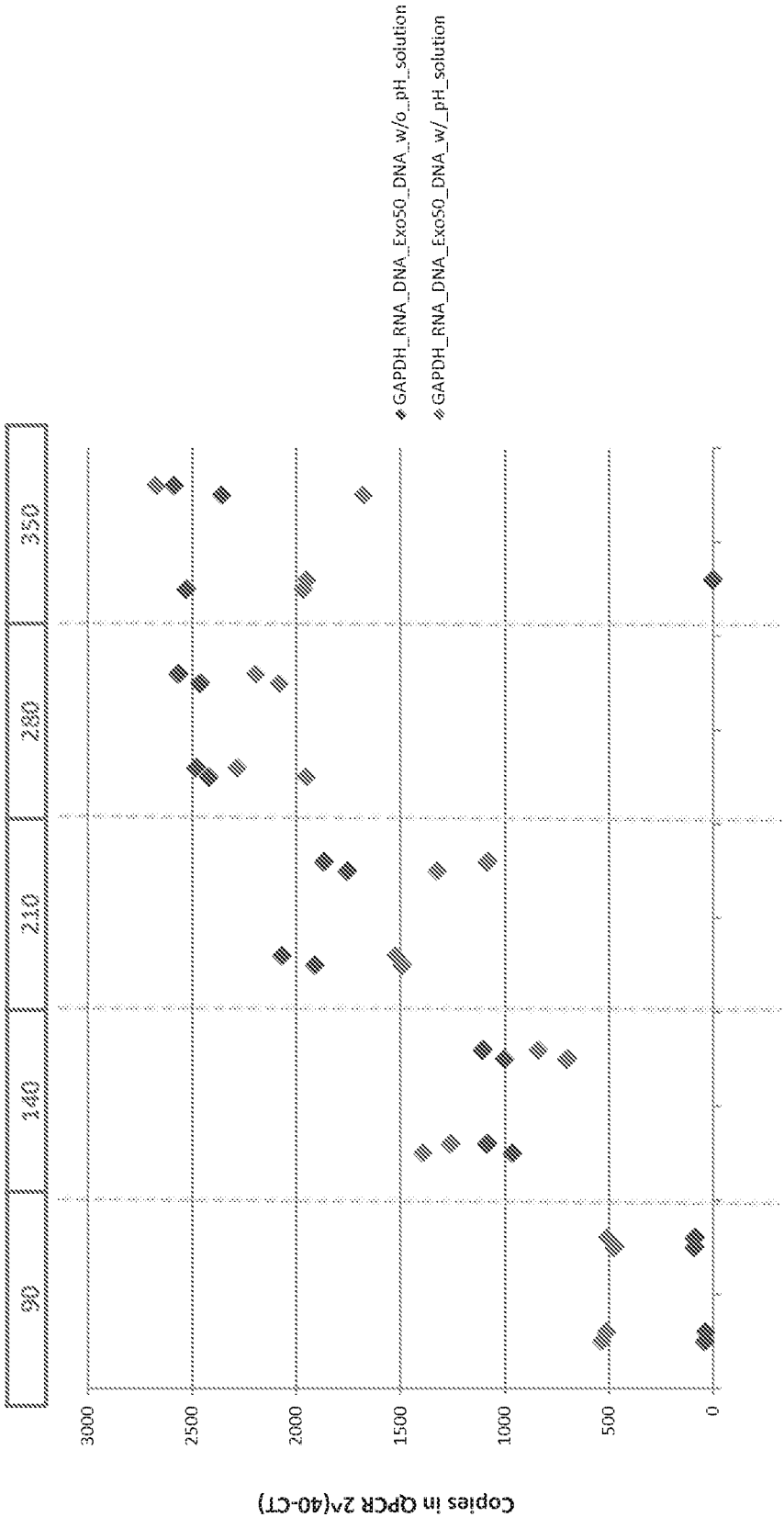
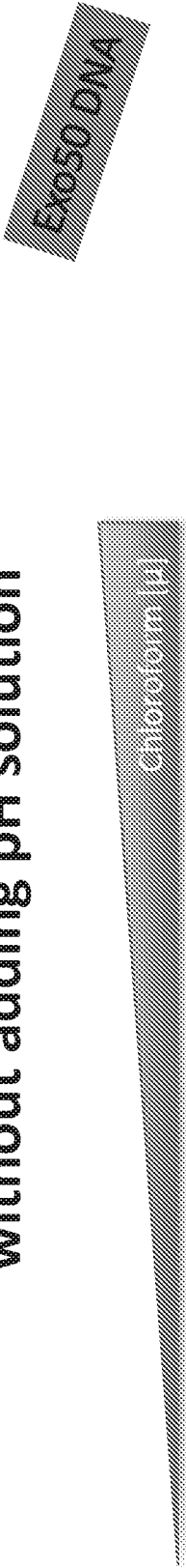
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



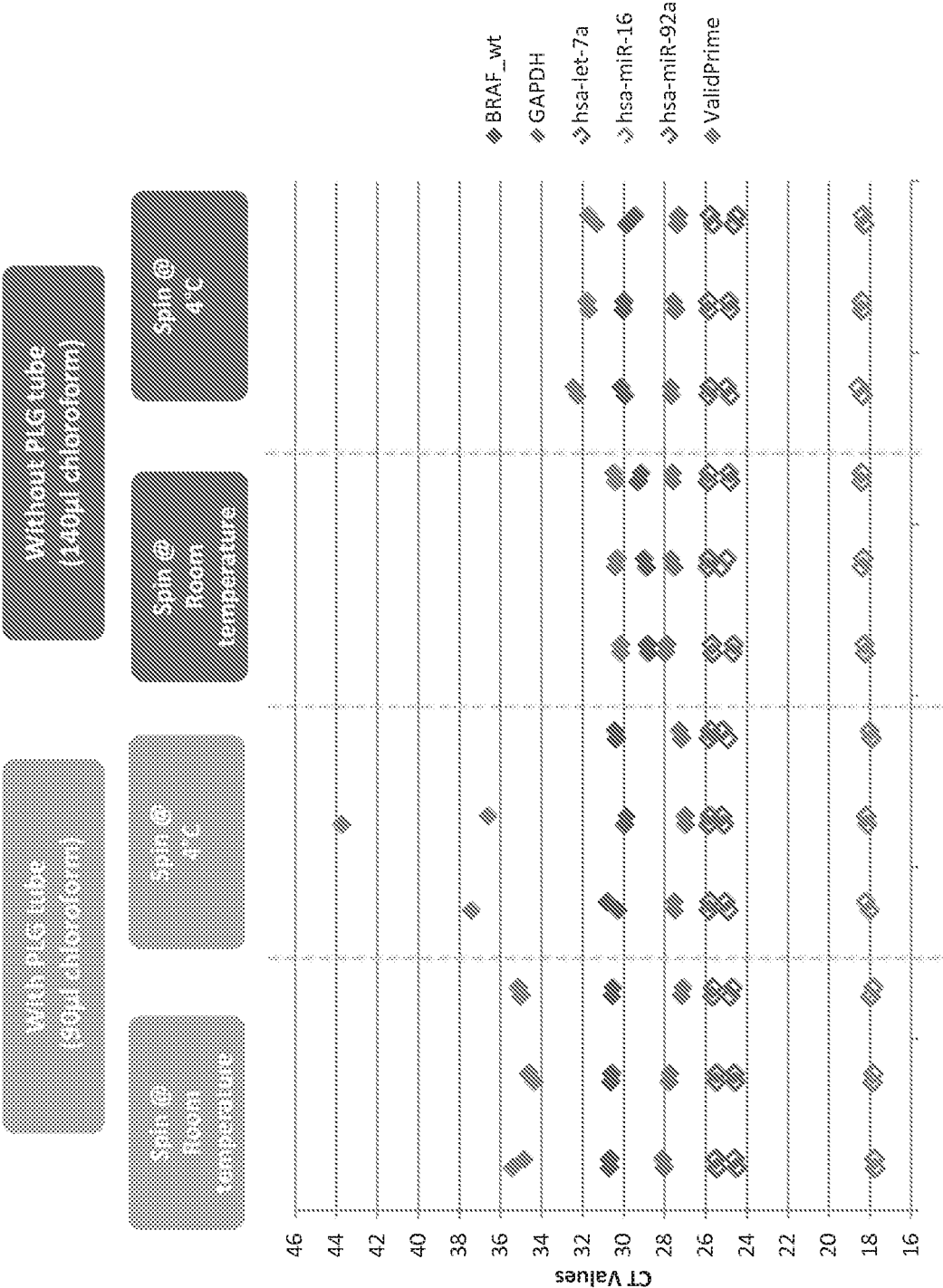
Figure

DNA isolation from aqueous phase: chloroform titration with and without adding pH solution



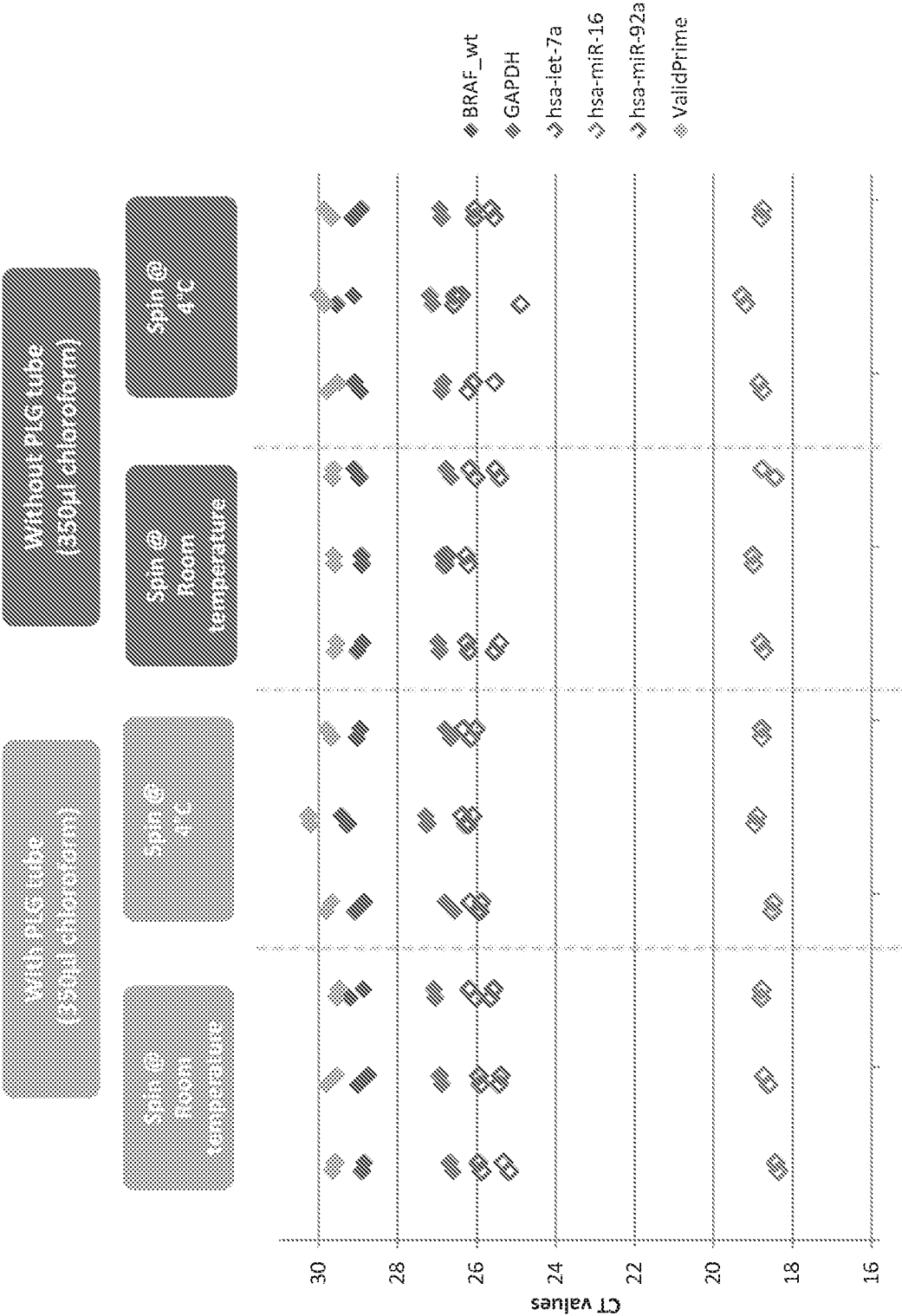
Figure

Comm. Avail. RNA-Isolation: 4°C vs room temperature for Qiazol spin step



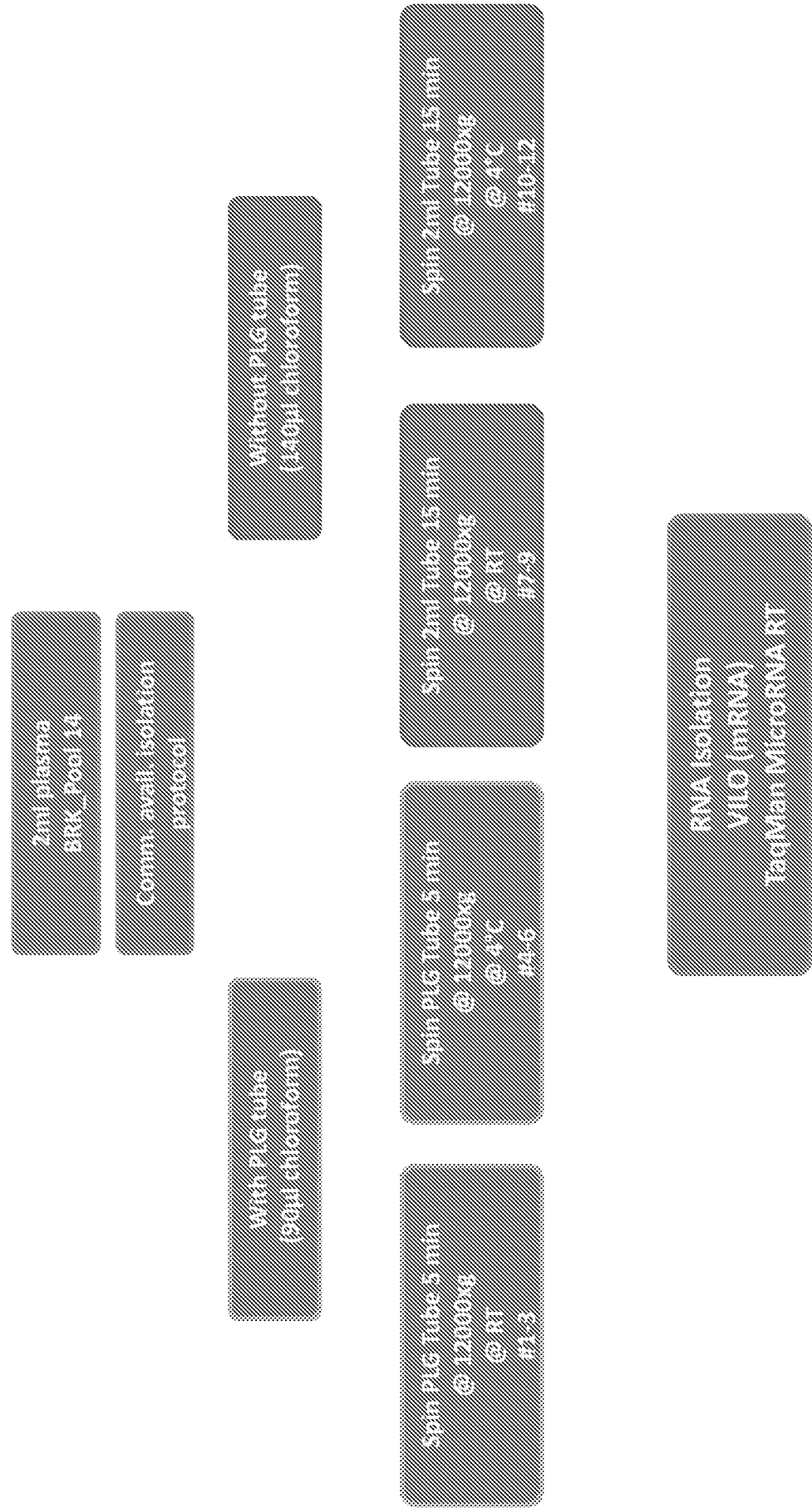
Figure

EXO502.2 DNA-Isolation: 4°C vs room temperature for Qiazol spin step



Figure

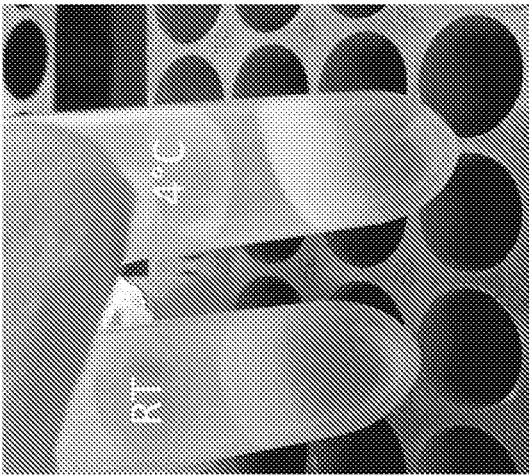
Comm. Avail. RNA-Isolation: 4°C vs room temperature for Qiazol spin step



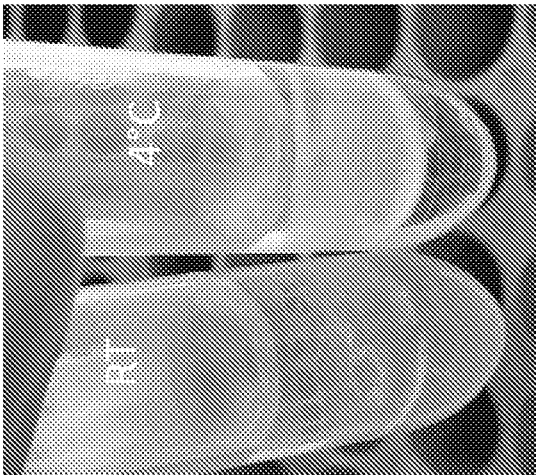
Figure

Measure aqueous phase: exoRNeasy v1 (2.0x ethanol)

sample	PLG Tube	aqueous phase [μl]	Volume 100% Ethanol for RNA Isolation
1	yes	450	900
2	yes	450	900
3	yes	450	900
4	yes	450	900
5	yes	450	900
6	yes	450	900
7	no	350	700
8	no	400	800
9	no	350	700
10	no	350	700
11	no	350	700
12	no	350	700



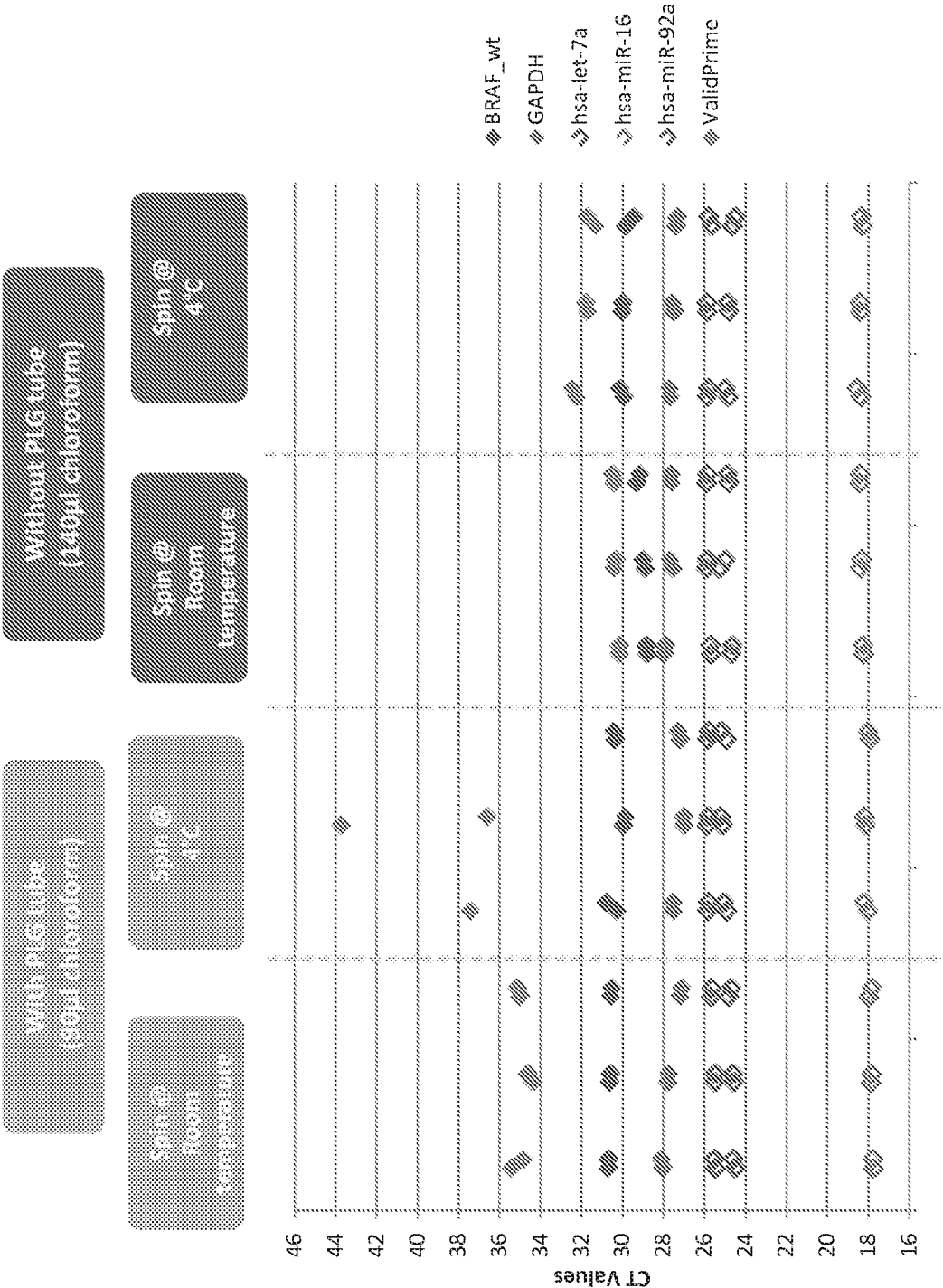
No difference in
Phase separation (PLG
Tube) using RT or 4°C
spin temperature



RT samples has less
Interphase (white ring)
and phenol phase is
More cloudy than 4°C
sample

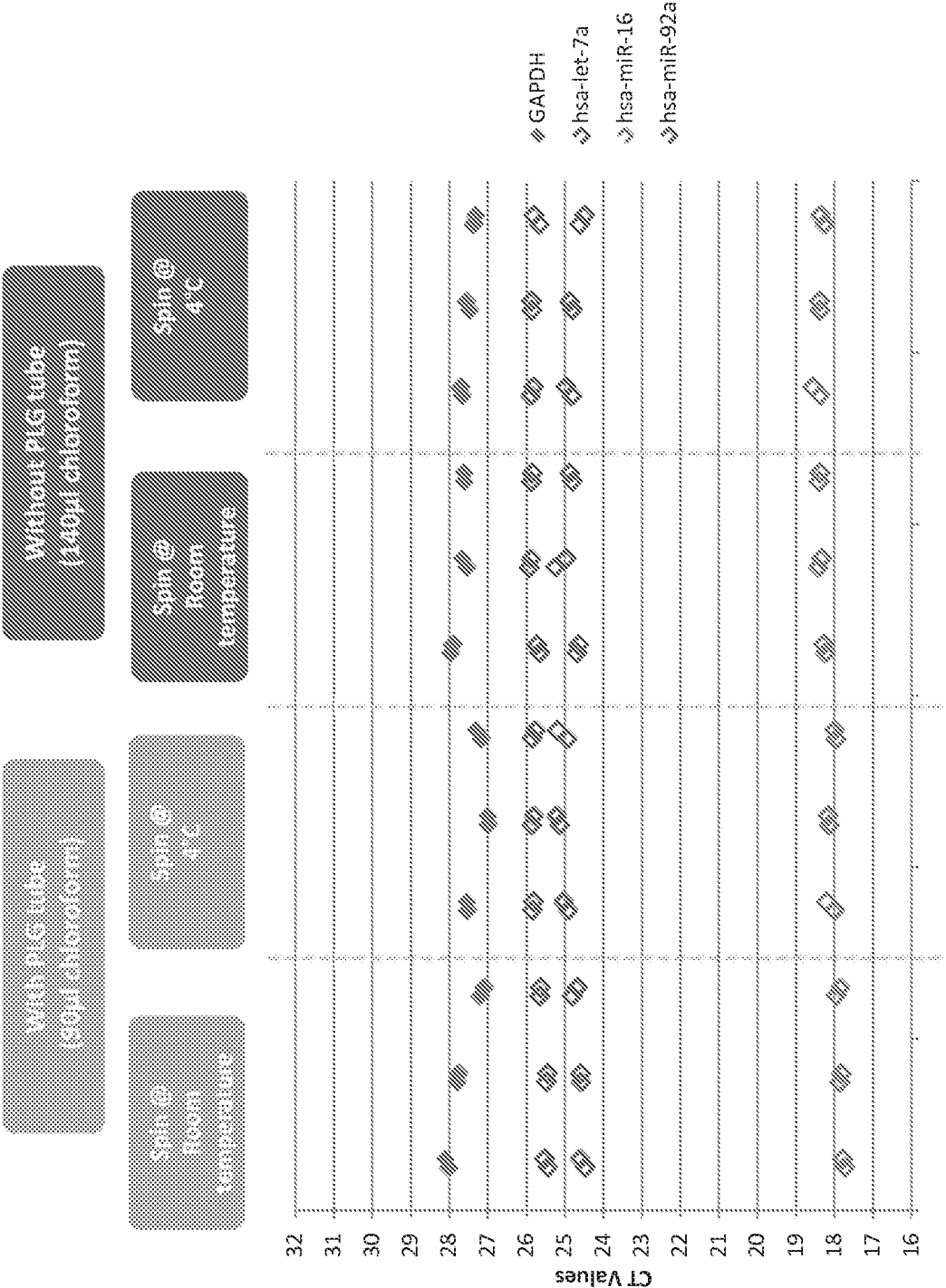
Figure

Comm. Avail. RNA-Isolation: 4°C vs room temperature for Qiazol spin step



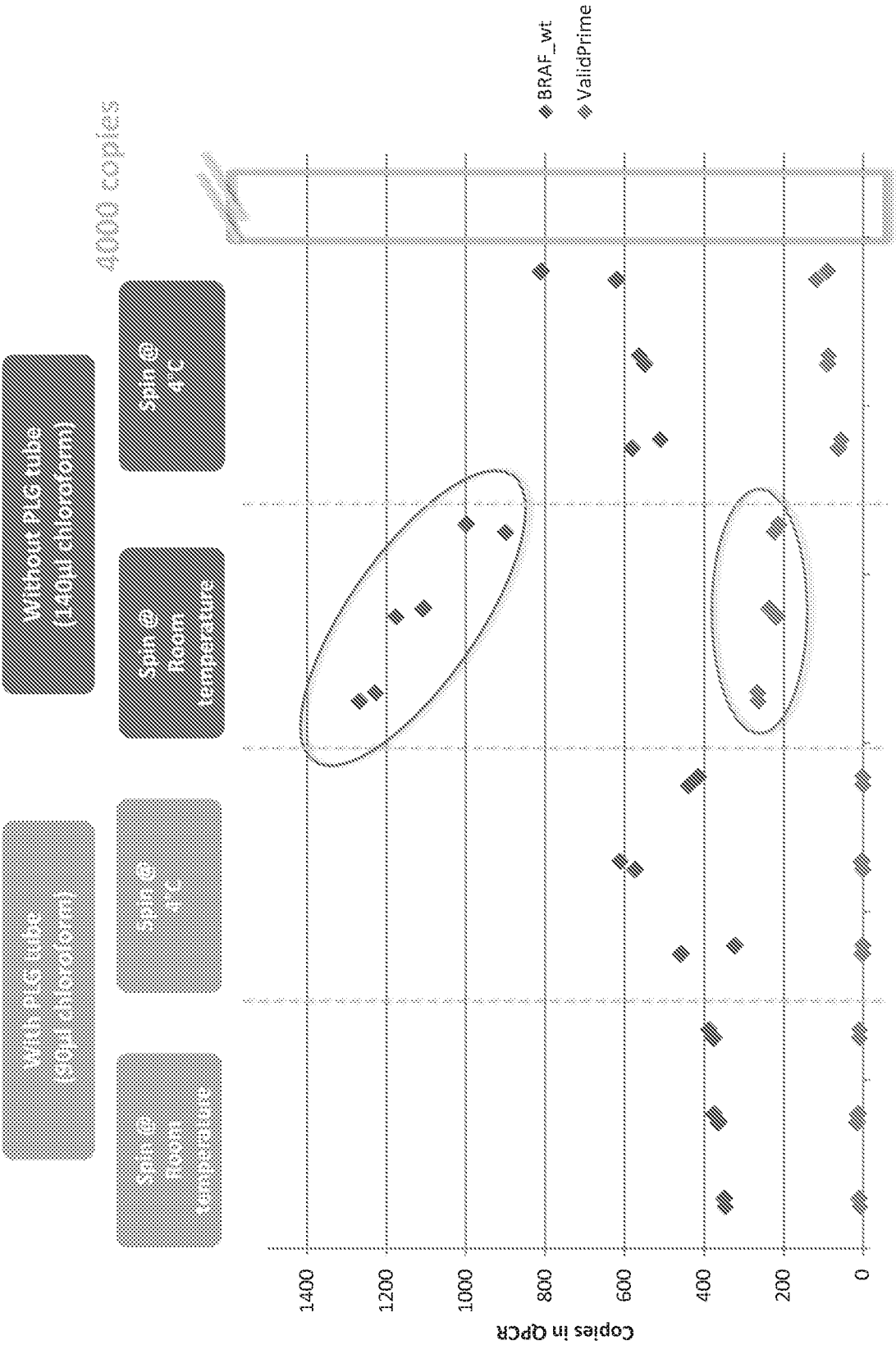
Figure

Comm. Avail. RNA-Isolation: 4°C vs room temperature for Qiazol spin step



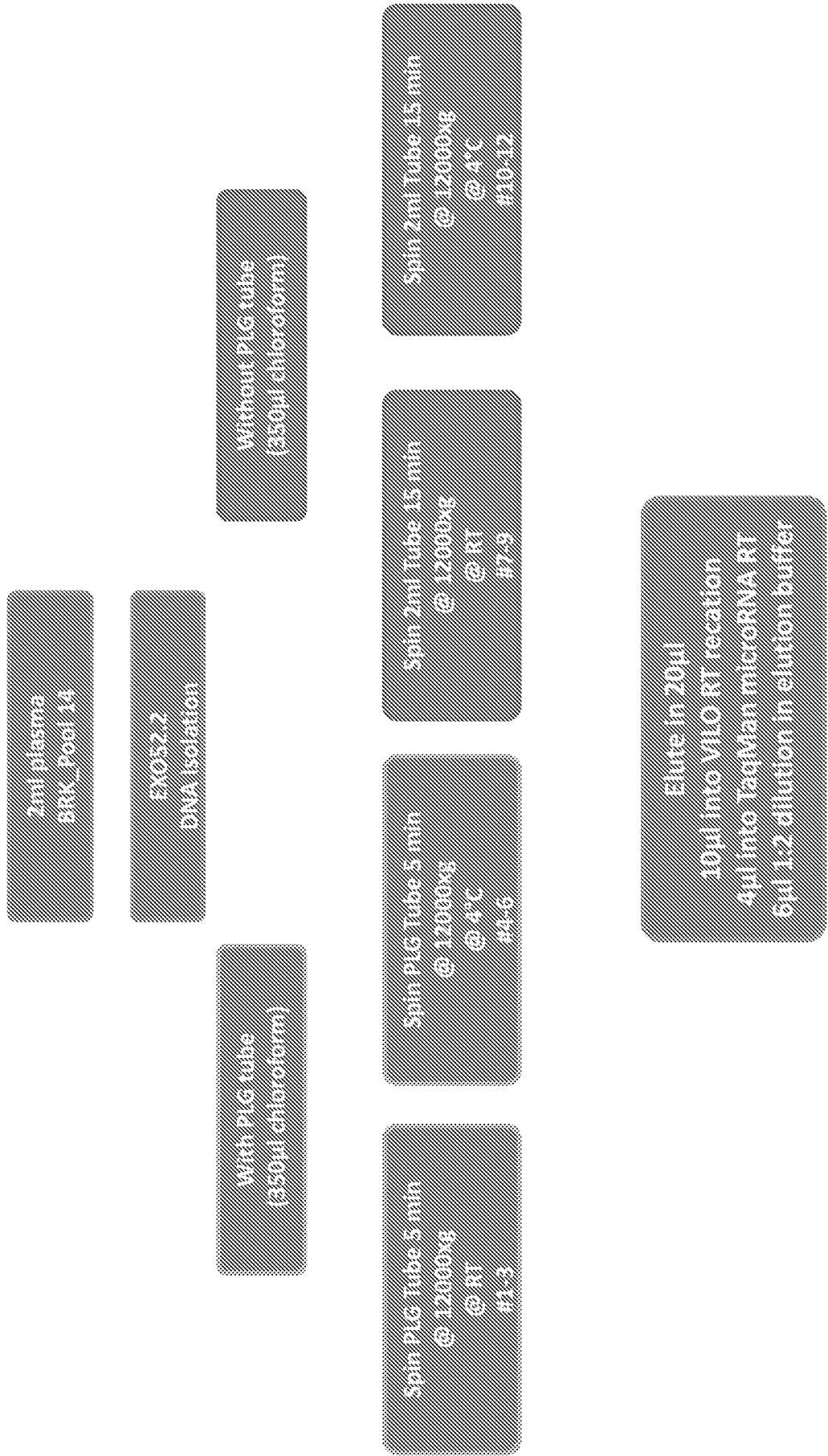
Figure

Comm. Avail. RNA-Isolation: 4° C vs room temperature for Qiazol
spin step



Figure

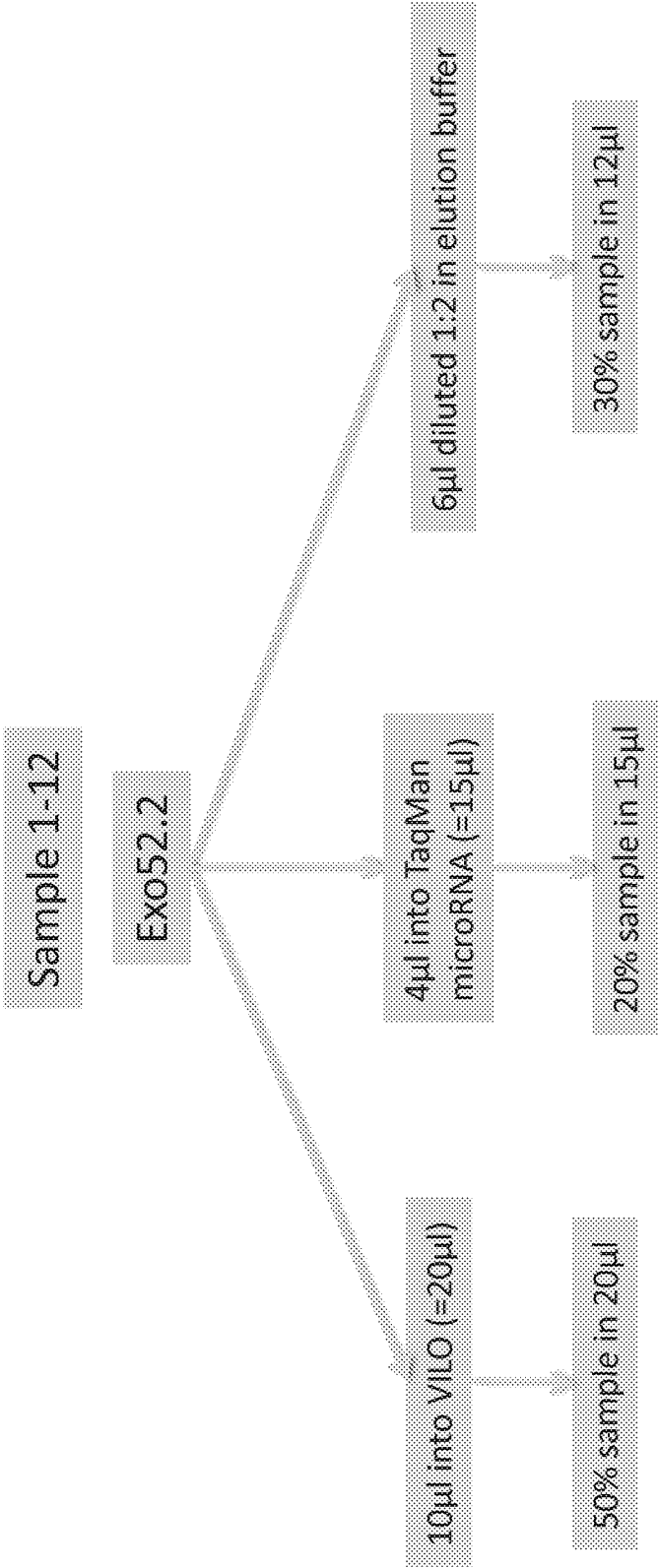
EXO502.2 DNA-Isolation: 4°C vs room temperature for Qiazol spin step



Figure

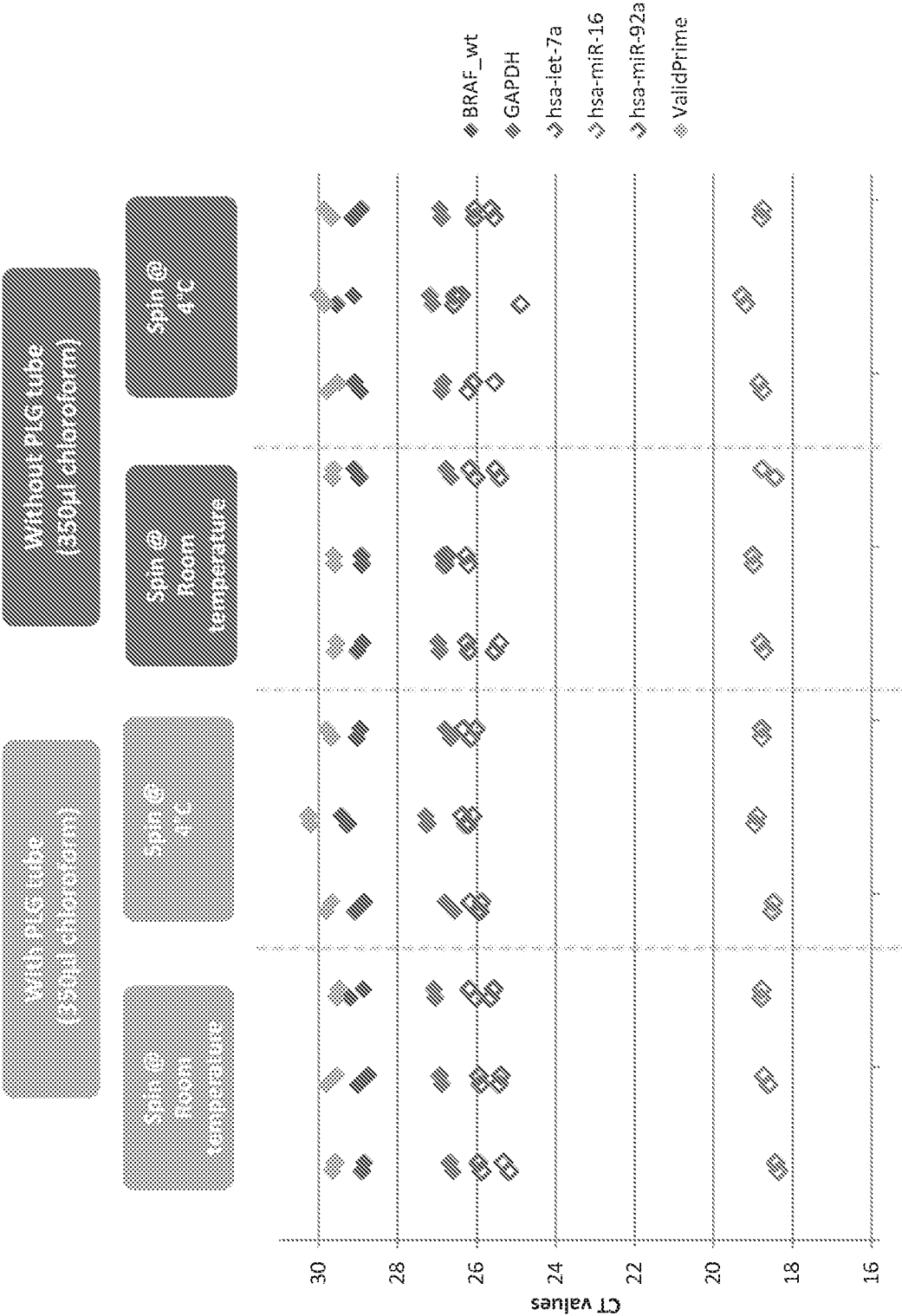
Dilution overview

All sample were eluted to yield 20µl
22µl for RNeasy column



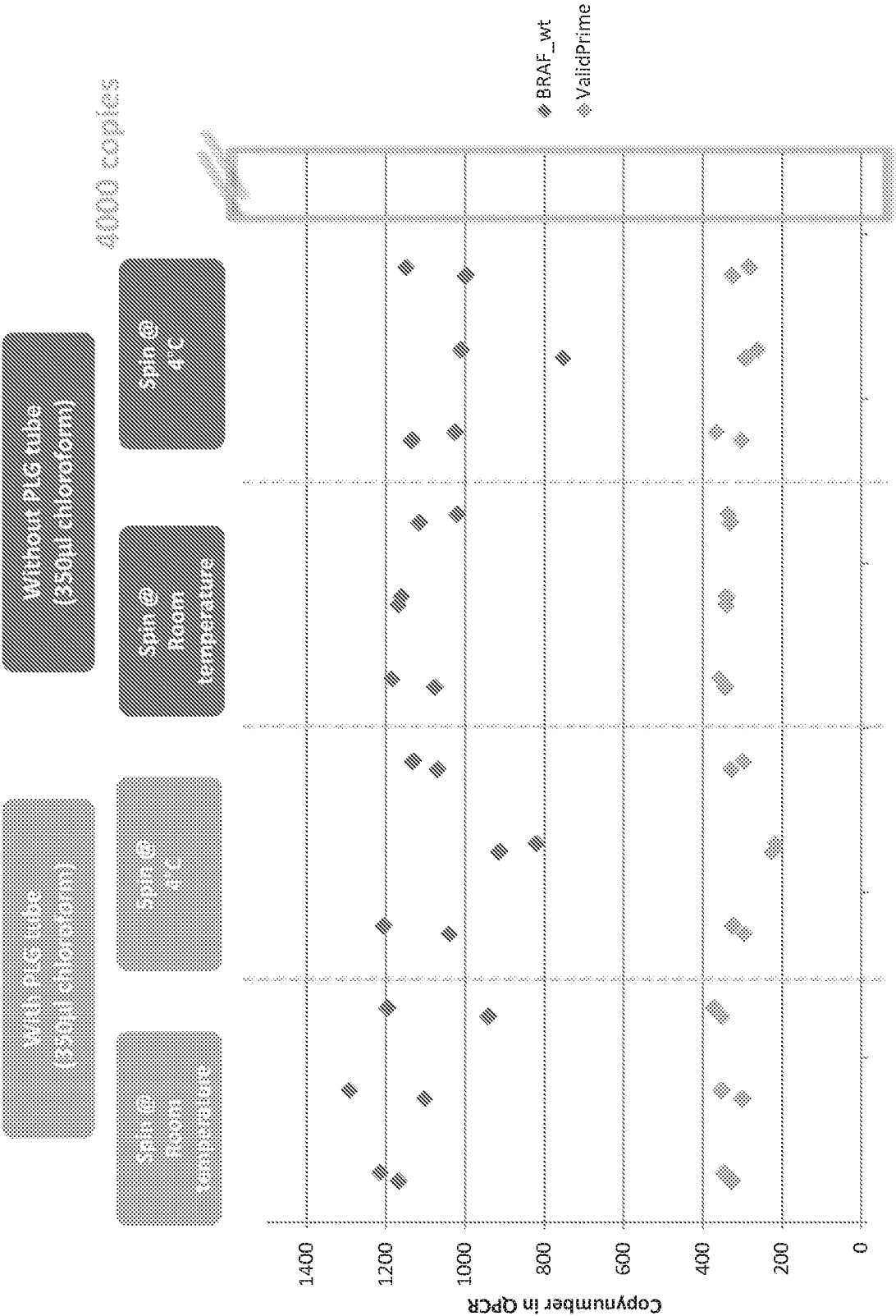
Figure

EXO502.2 DNA-Isolation: 4°C vs room temperature for Qiazol spin step



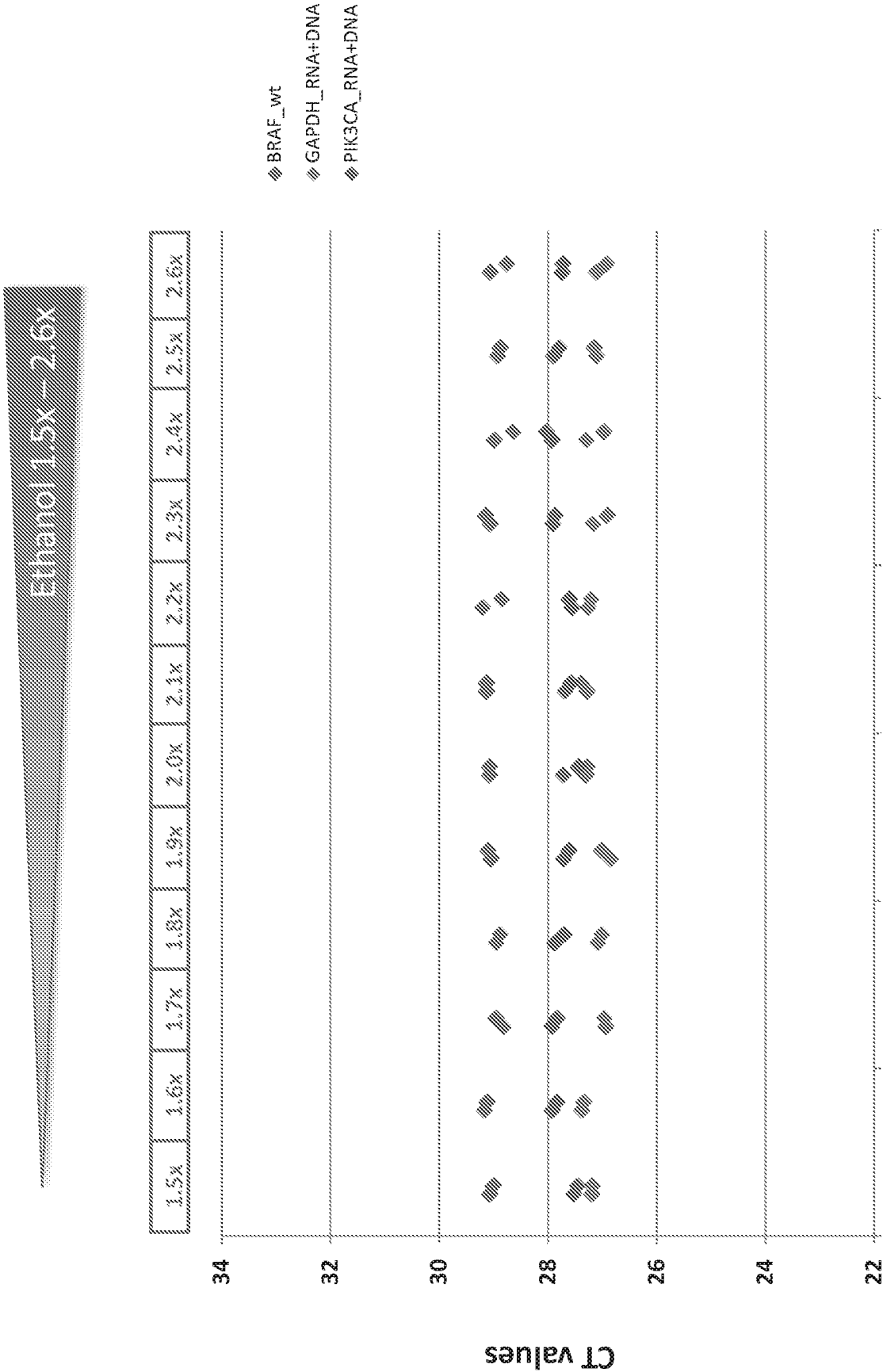
Figure

EXO502.2 DNA-Isolation: 4° C vs room temperature for Qiazol spin step



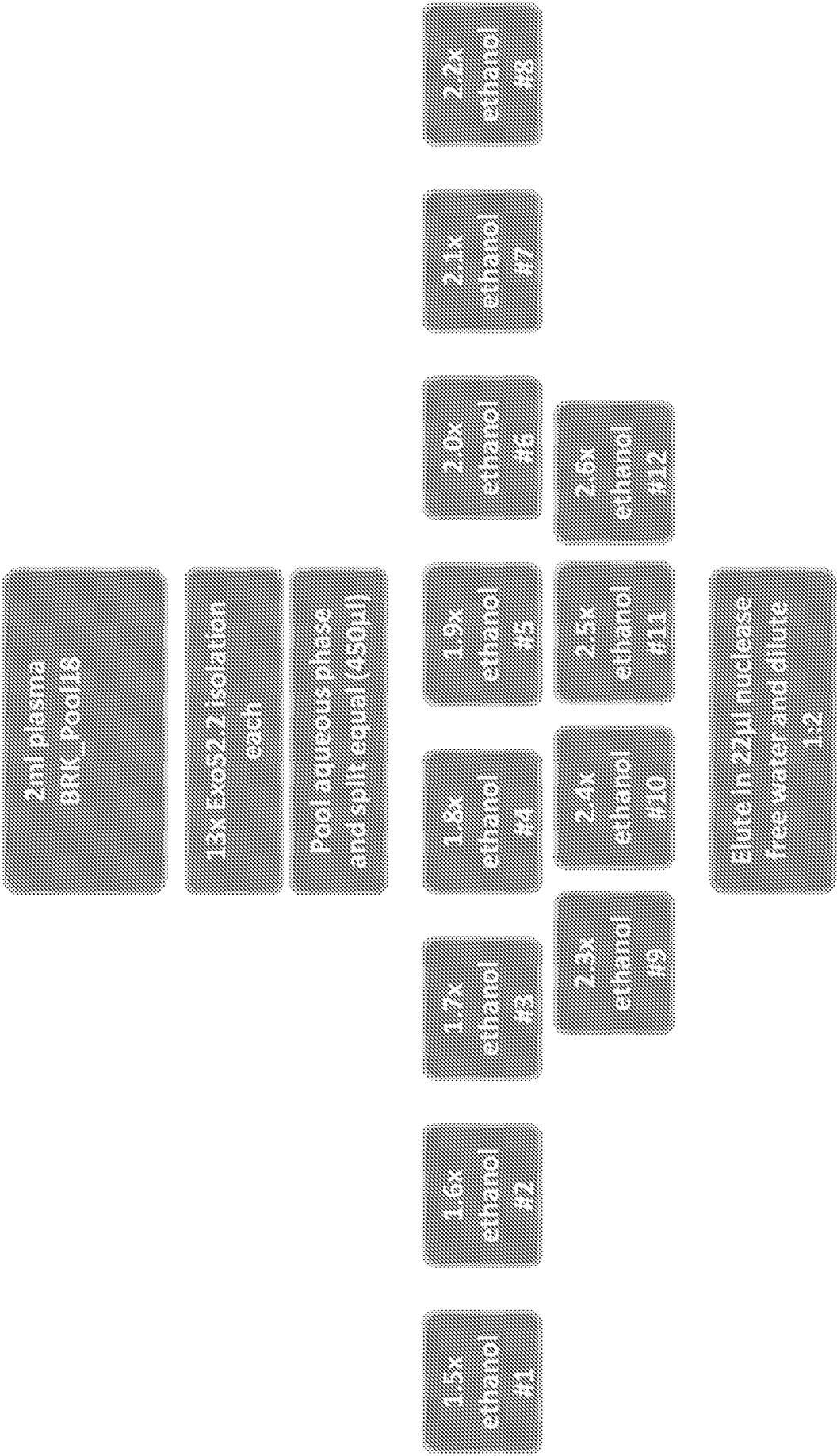
Figure

Exo52.2 : different ethanol volume 1.5x – 2.6x: No change in CT Value



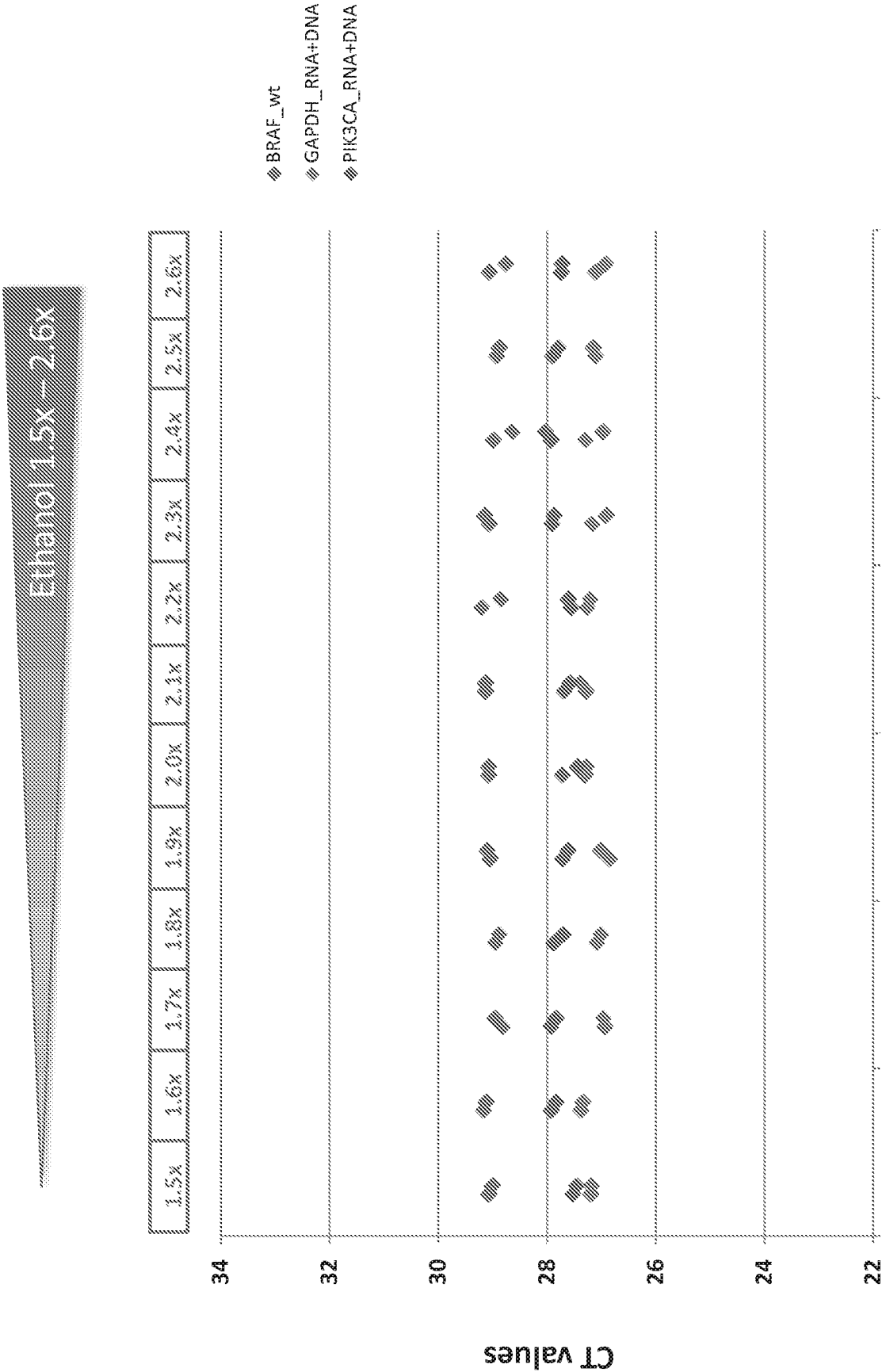
Figure

Exo52.2 : different ethanol volume 1.5x – 2.6x



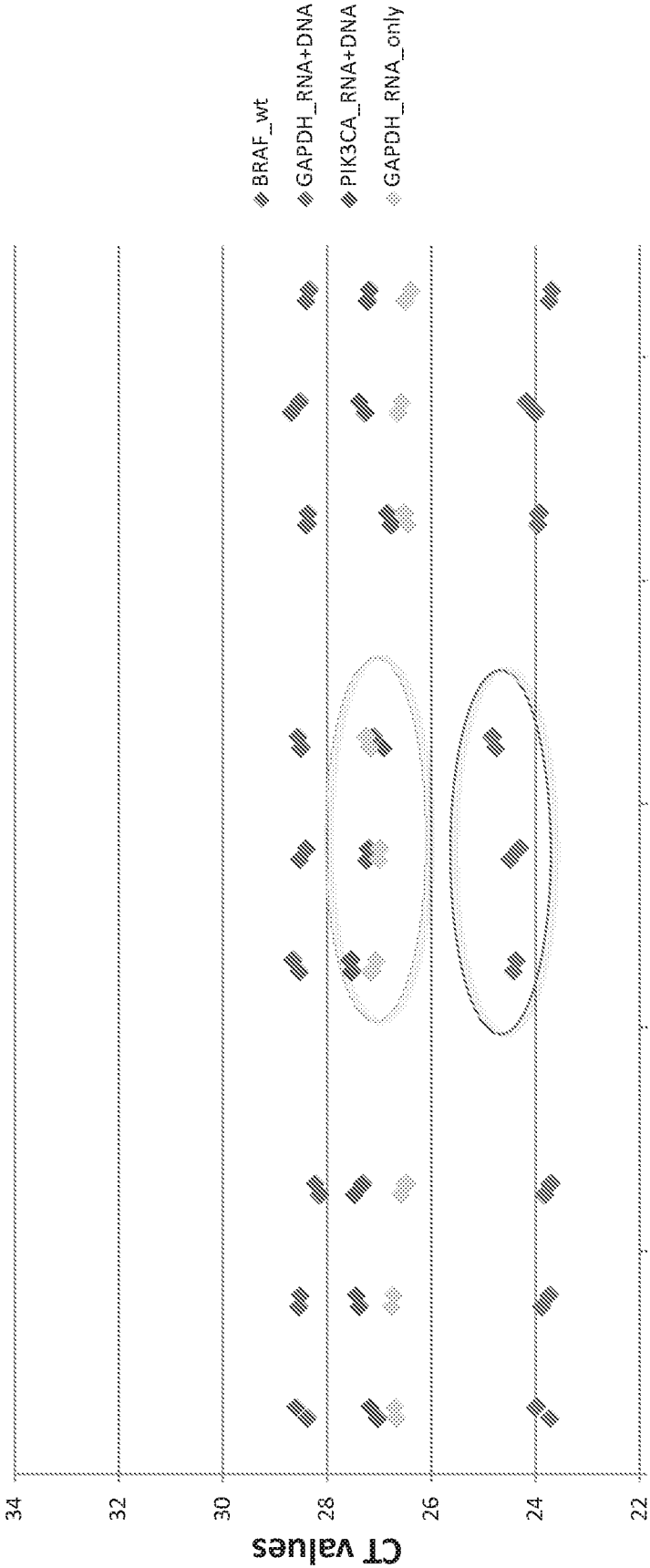
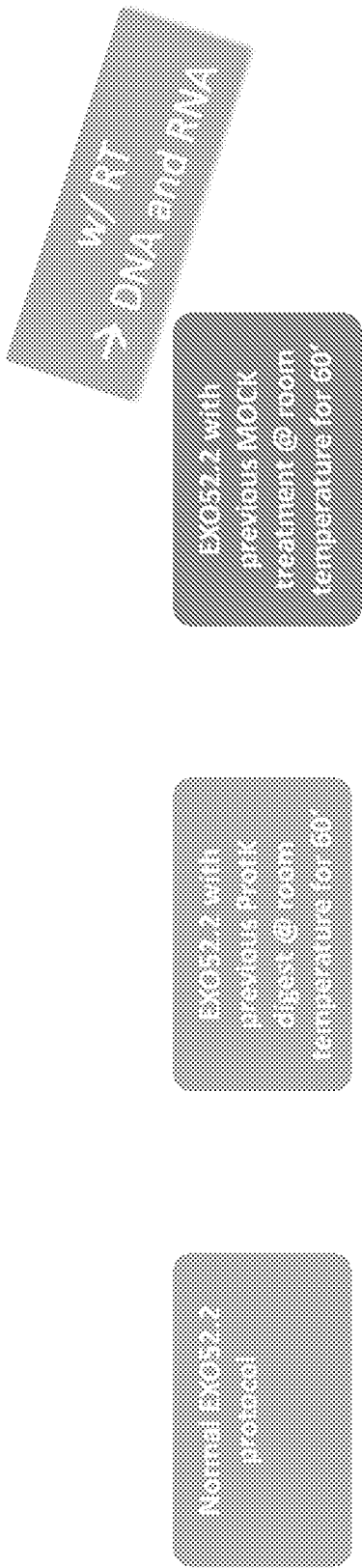
Figure

Exo52.2 : different ethanol volume 1.5x – 2.6x



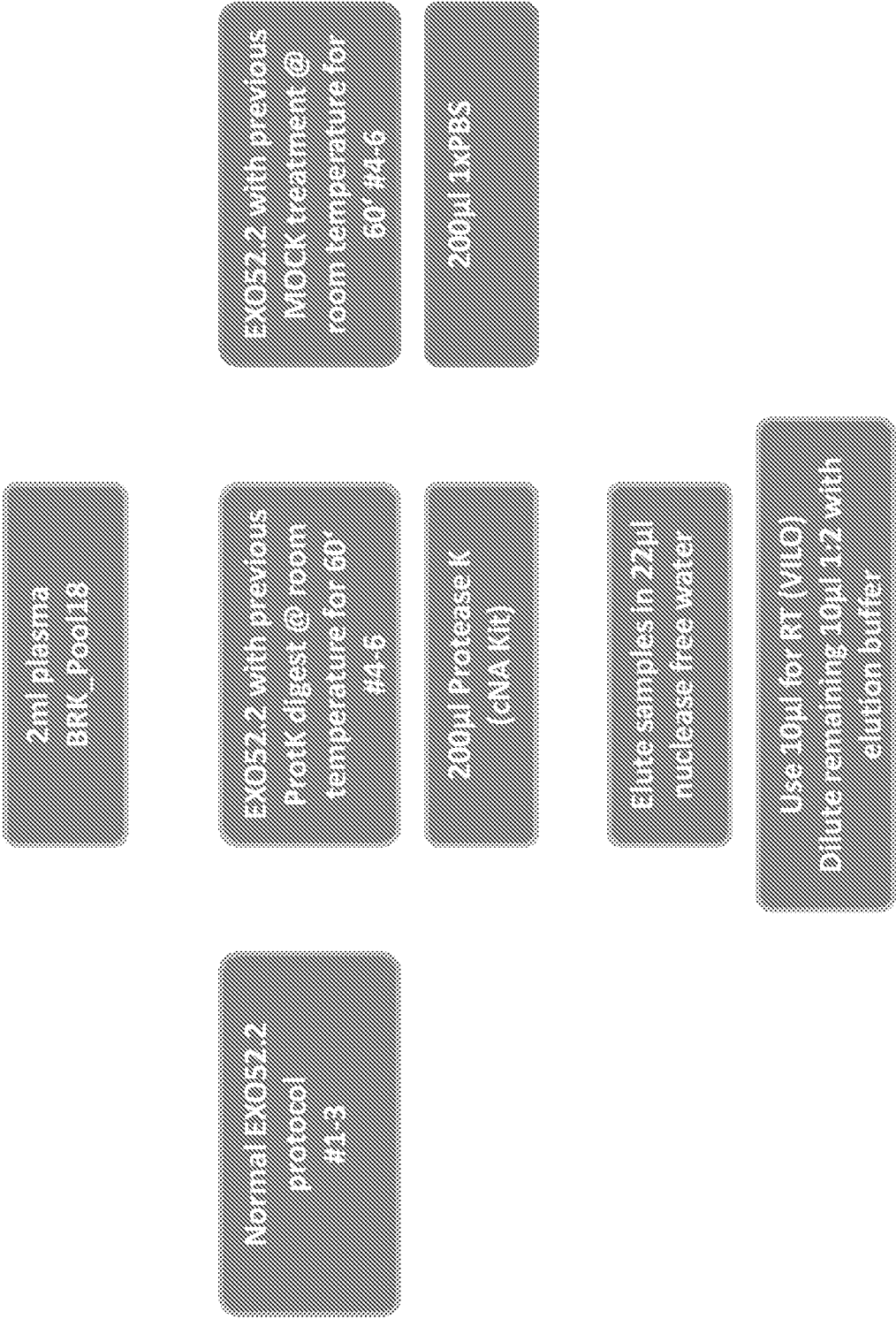
Figure

Exo52.2 : ProtK digest @ room temperature before EXO52.2 binding step



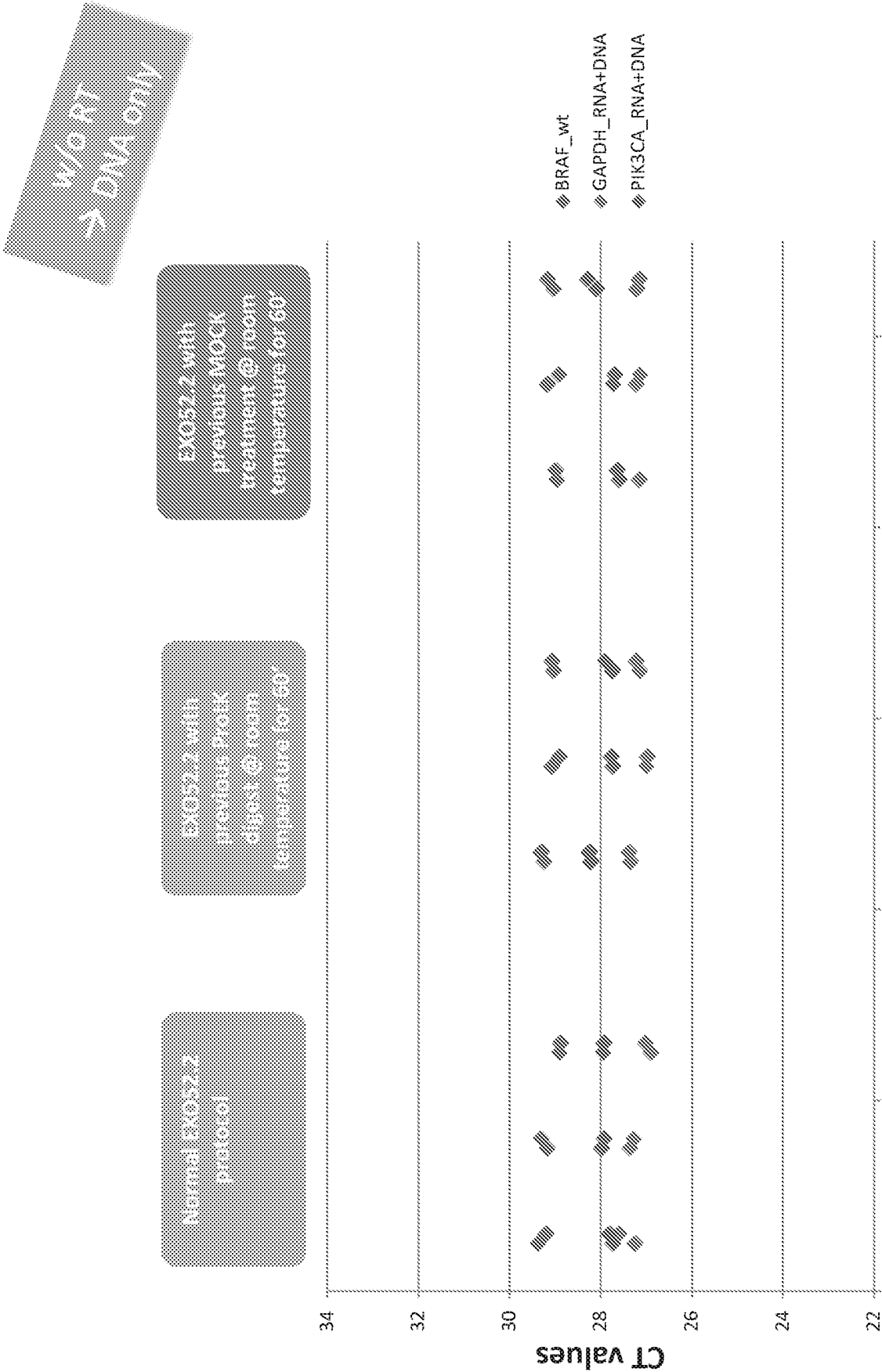
RNA only assay and GAPDH DNA+RNA assay perform worse with Prot K treated samples

Exo52.2 : ProtK digest @ room temperature before EXO52.2 binding step



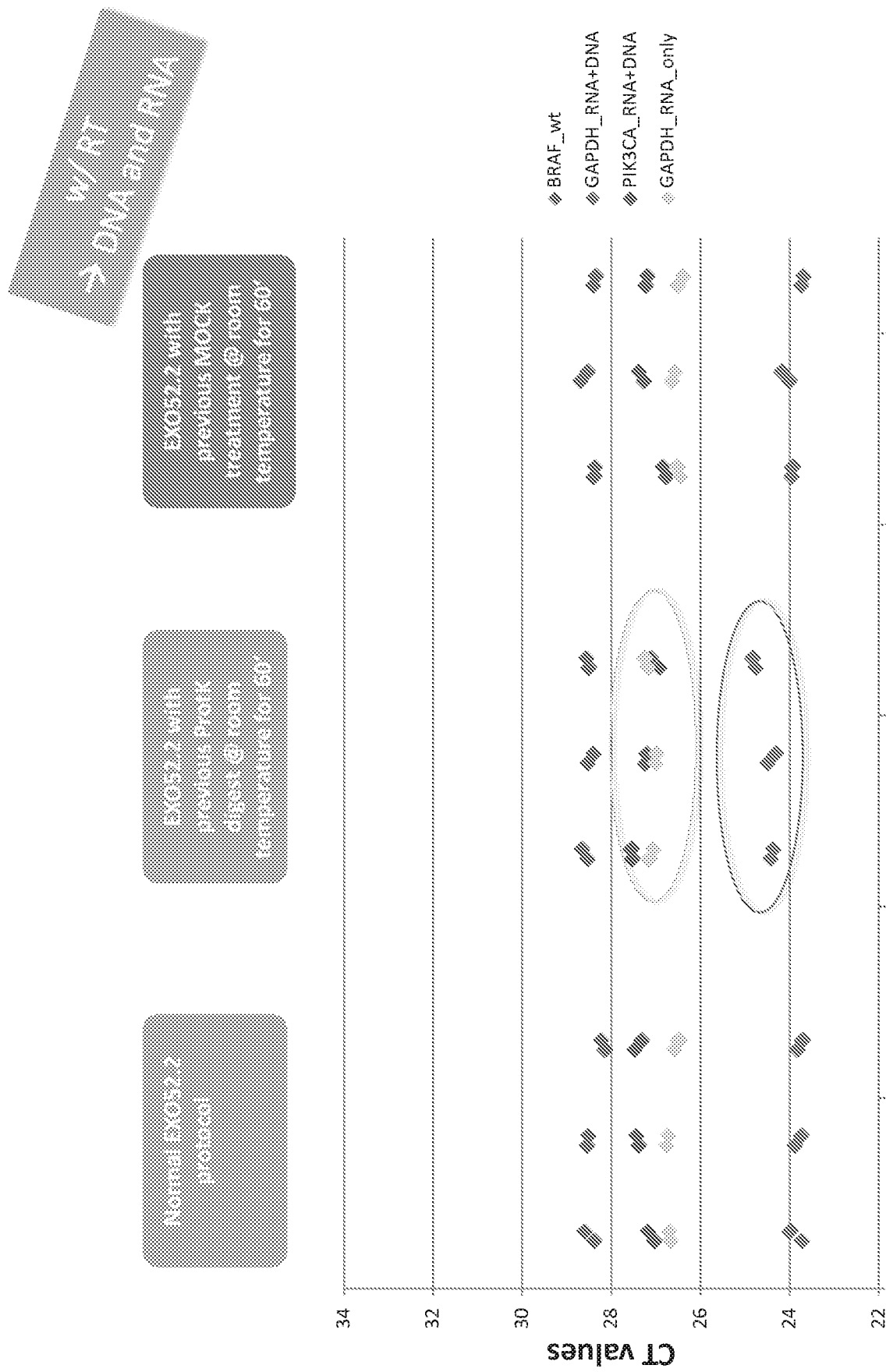
Figure

Exo52.2 : ProtK digest @ room temperature before EXO52.2 binding step



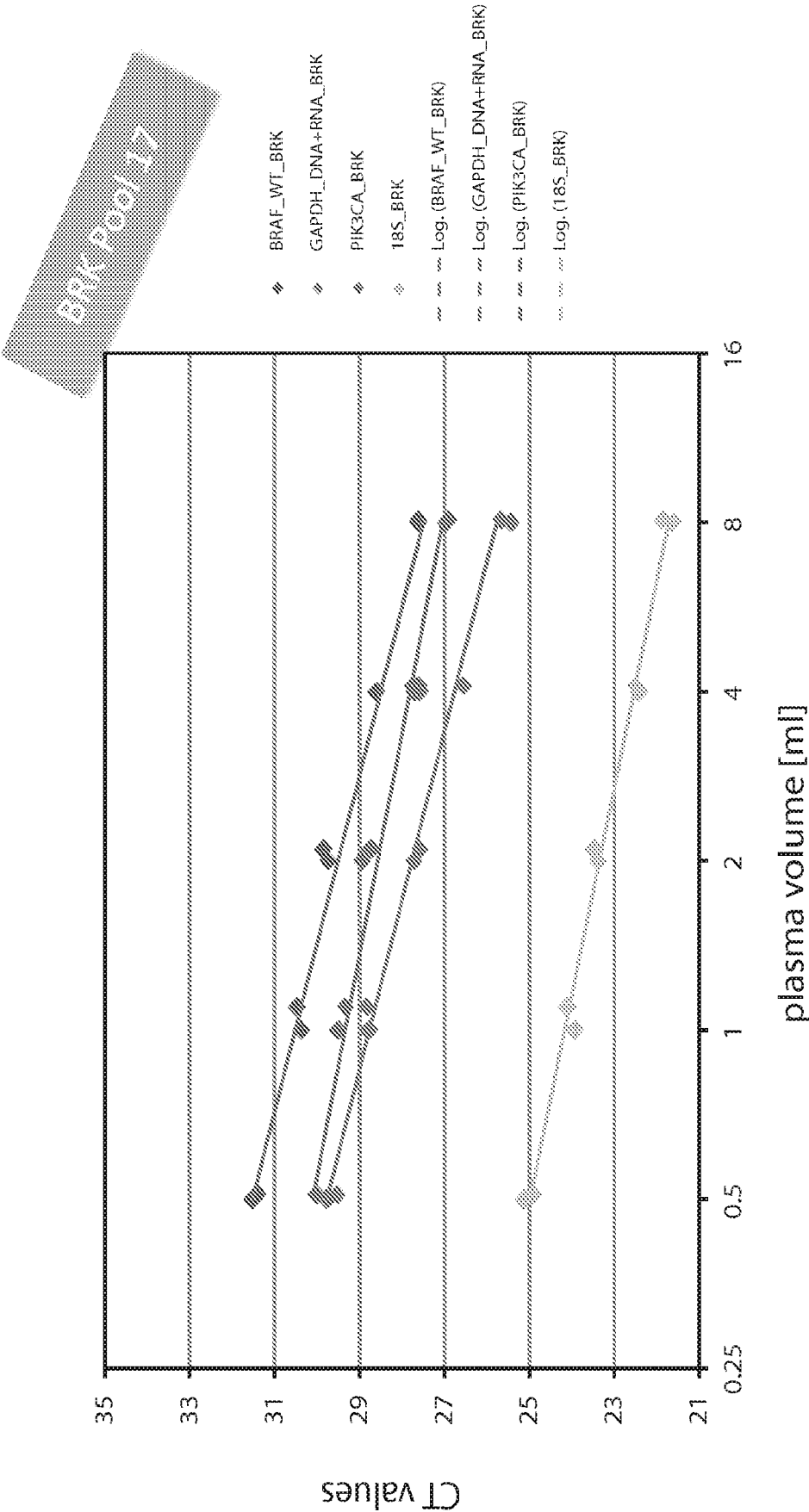
Figure

Exo5.2.2 : ProtK digest @ room temperature before EXO5.2.2 binding step



RNA only assay and GAPDH DNA+RNA assay perform worse with Prot K treated samples

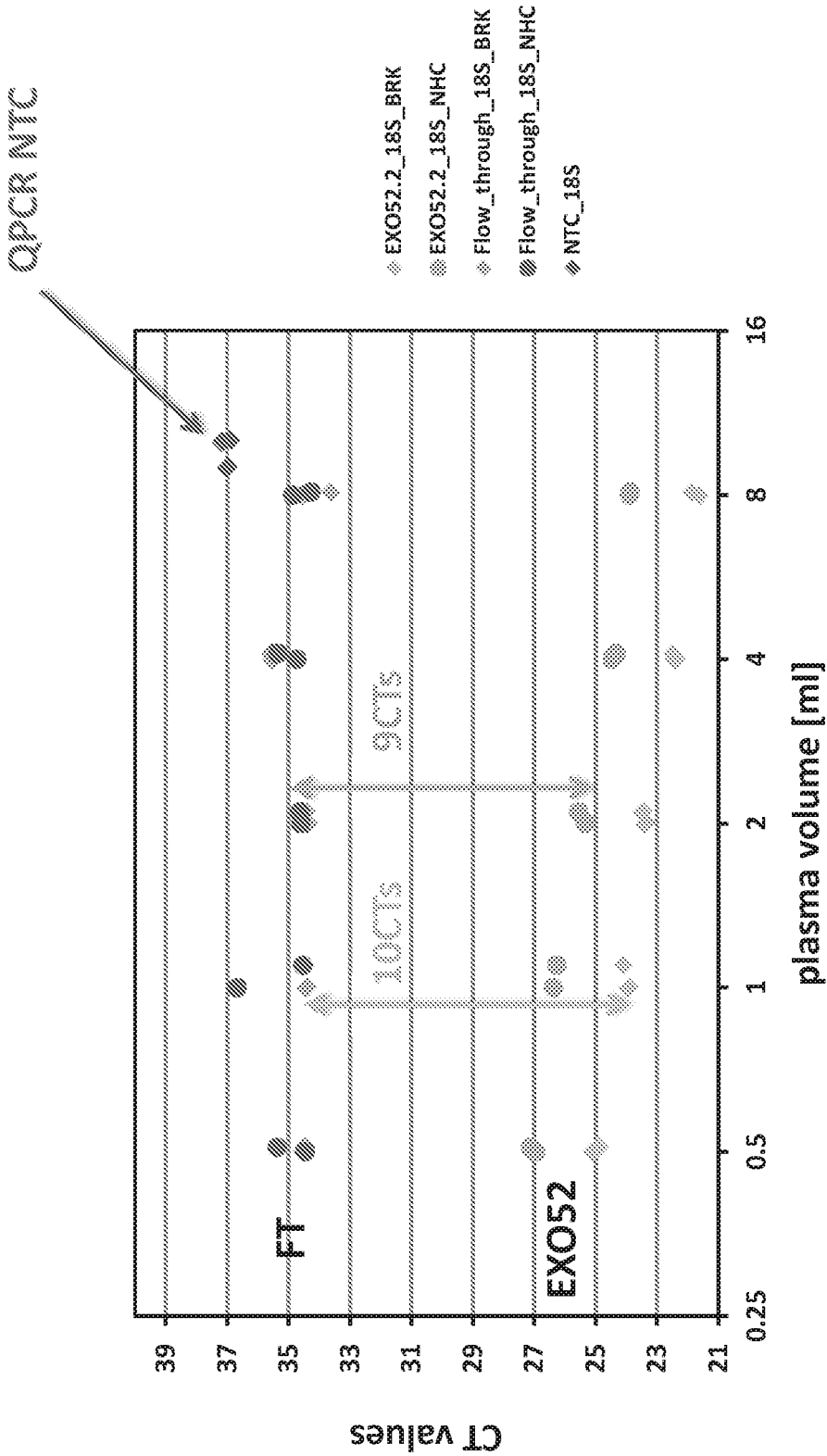
EX052.2 – loading capacity is over 8 mL of plasma



BRK Samples	Slope	Intercept
BRAF-WT	-3.32	30.48
GAPDH RNA+DNA	-3.30	28.73
PIK3CA RNA+DNA	-2.53	29.31
18S	-2.70	24.13

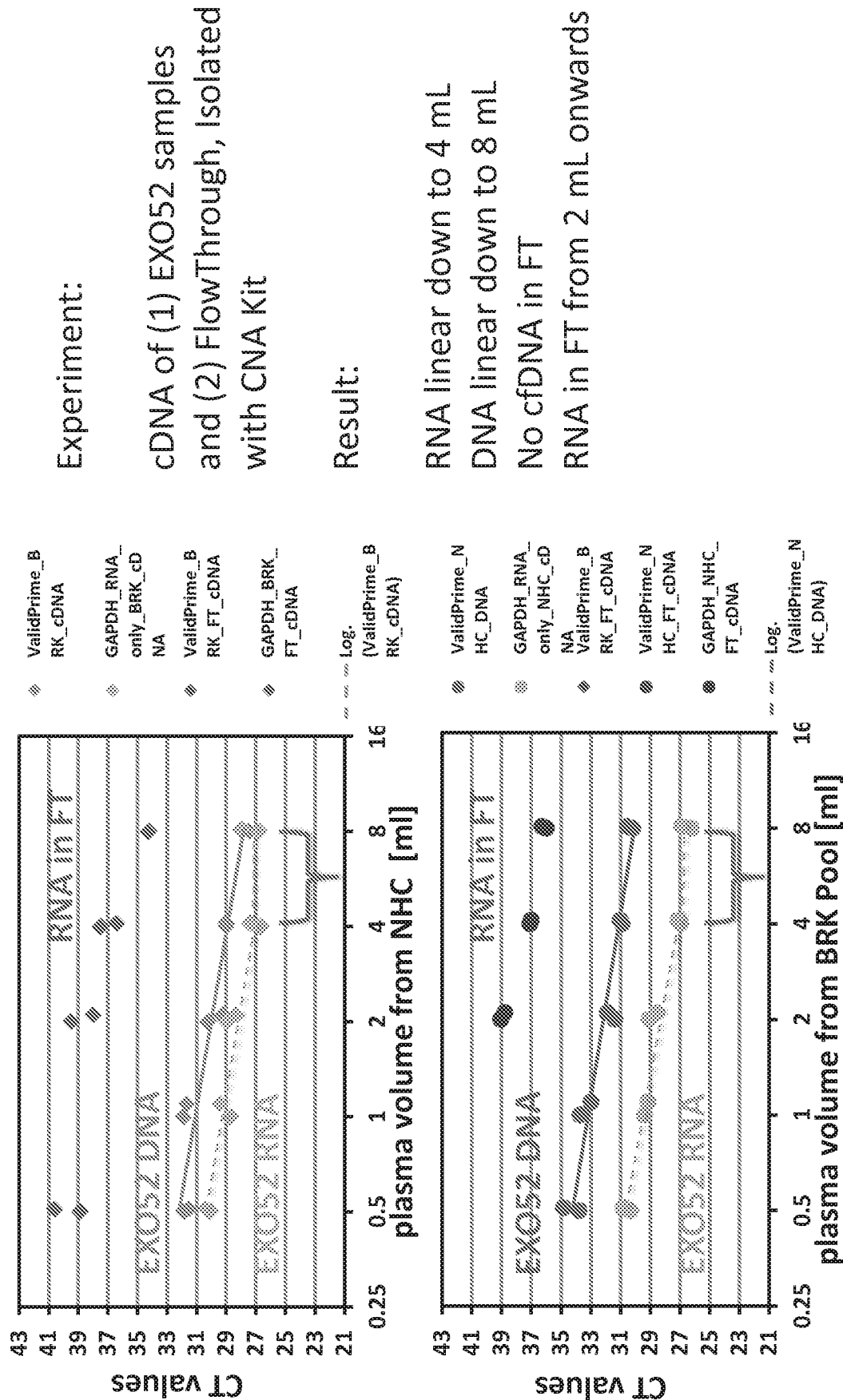
Figure 87

The Flow-through of EXO52.2 does not have a breakthrough point
up to 8 mL of plasma

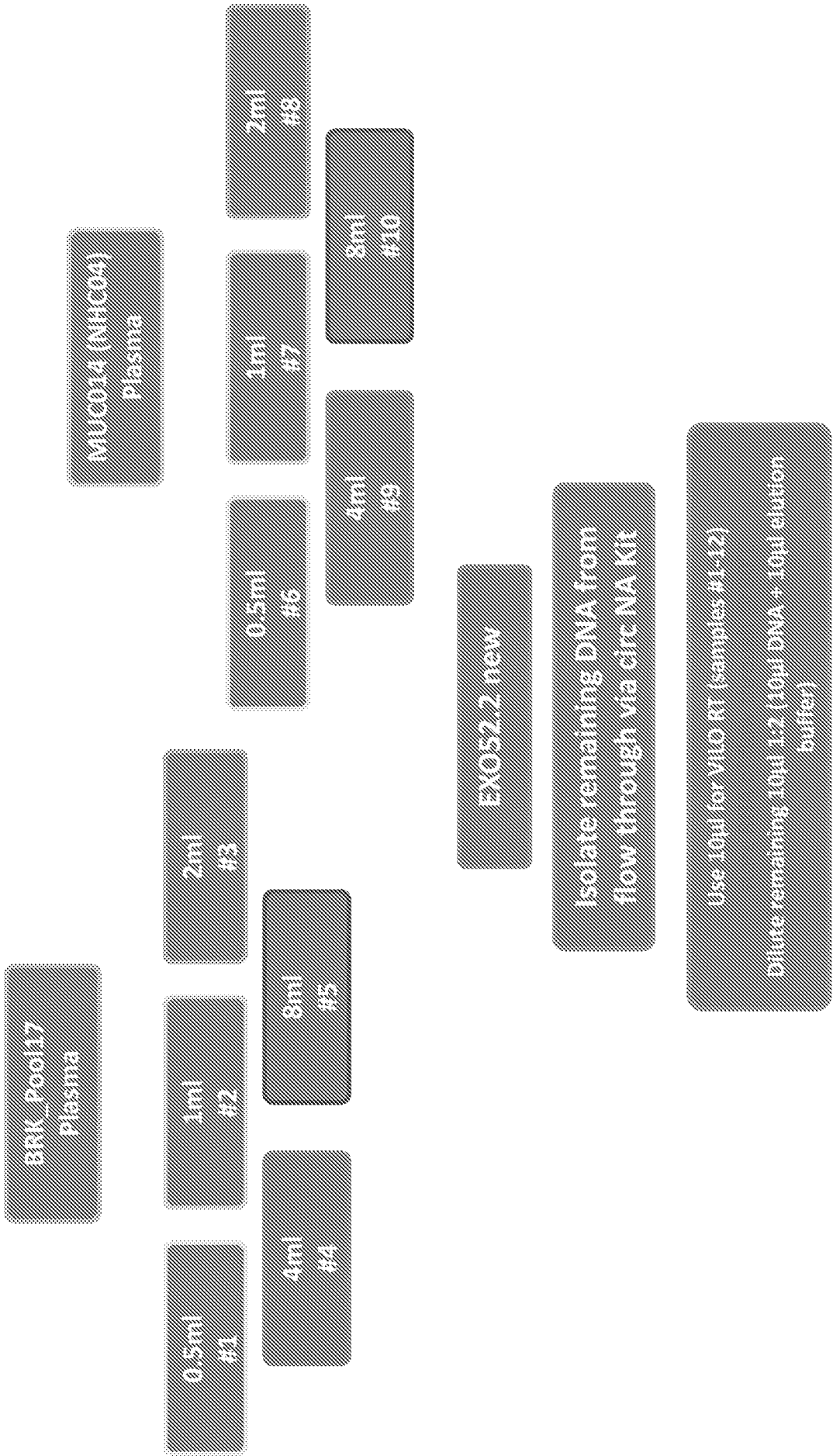


Figure

Different EXO52 binding capacity for exosomes and nucleosomes

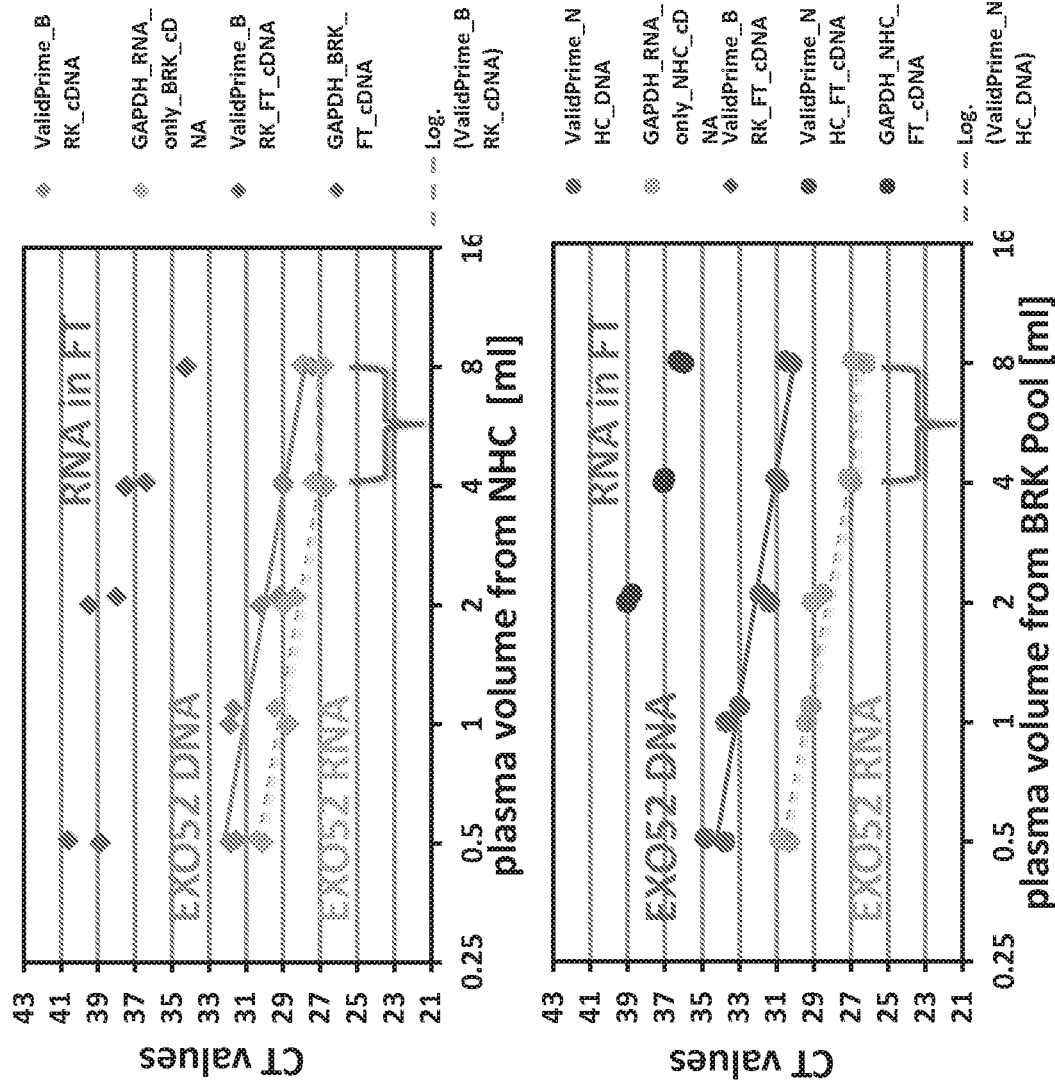


EXO52.2 – loading capacity



Figure

Different EXO52 binding capacity for exosomes and nucleosomes



Figure

Experiment:

cDNA of (1) EXO52 samples and (2) FlowThrough, Isolated with CNA Kit

Result:

RNA linear down to 4 mL
DNA linear down to 8 mL
No cfDNA in FT
RNA in FT from 2 mL onwards

EX052.2 – loading capacity is over 8 mL of plasma

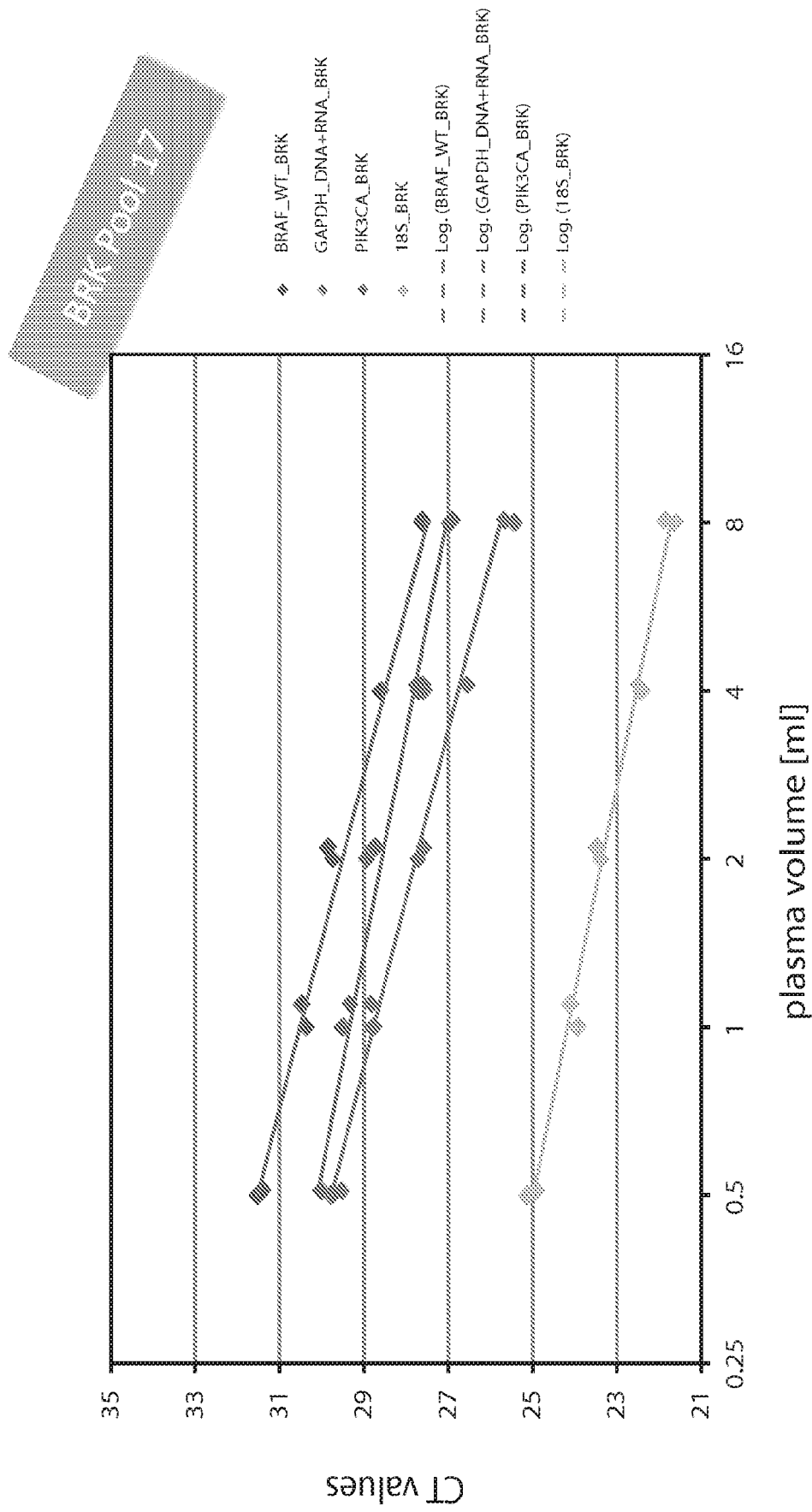
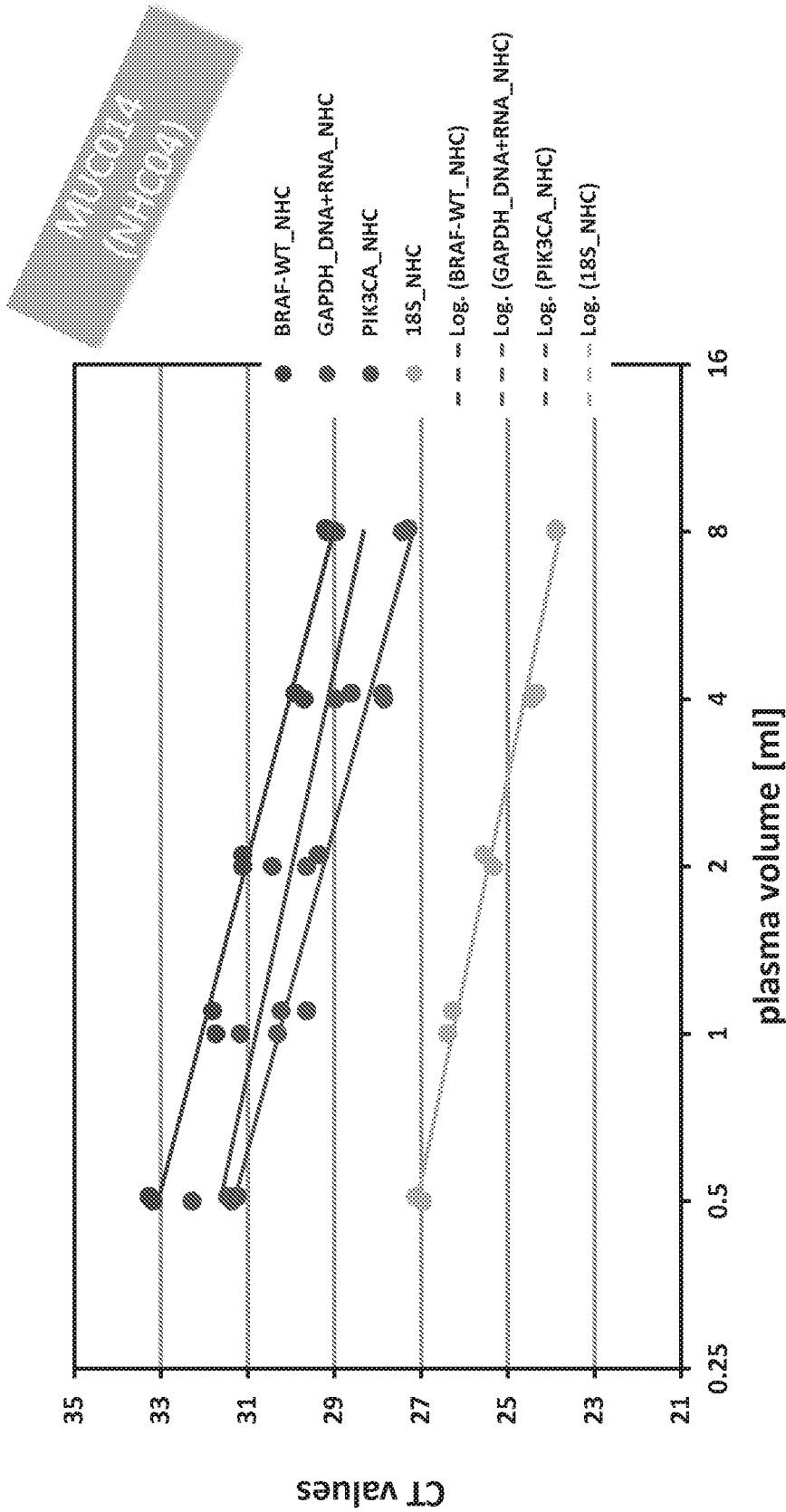
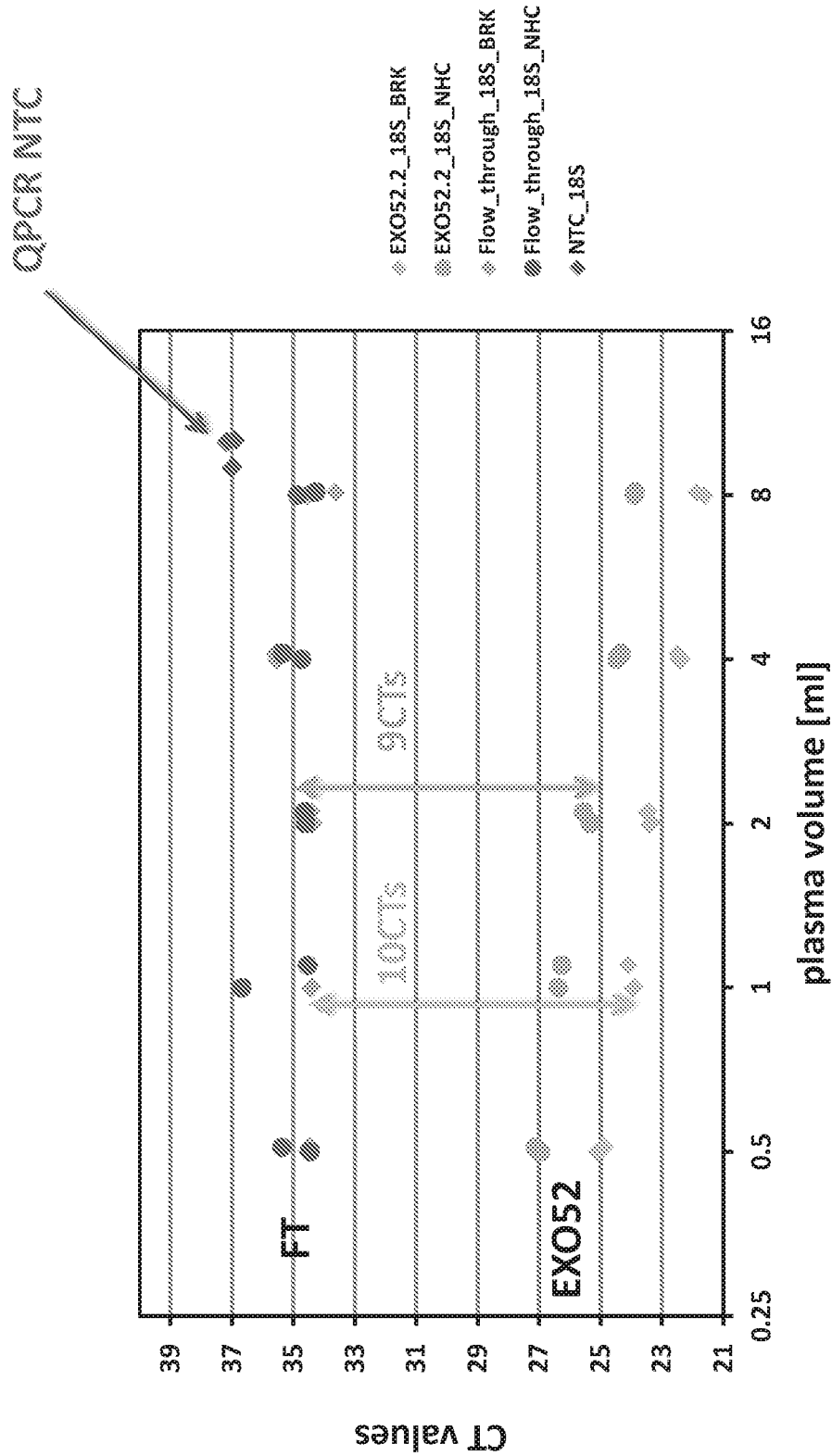


Figure 92

EX052.2 – loading capacity is over 8 mL of plasma

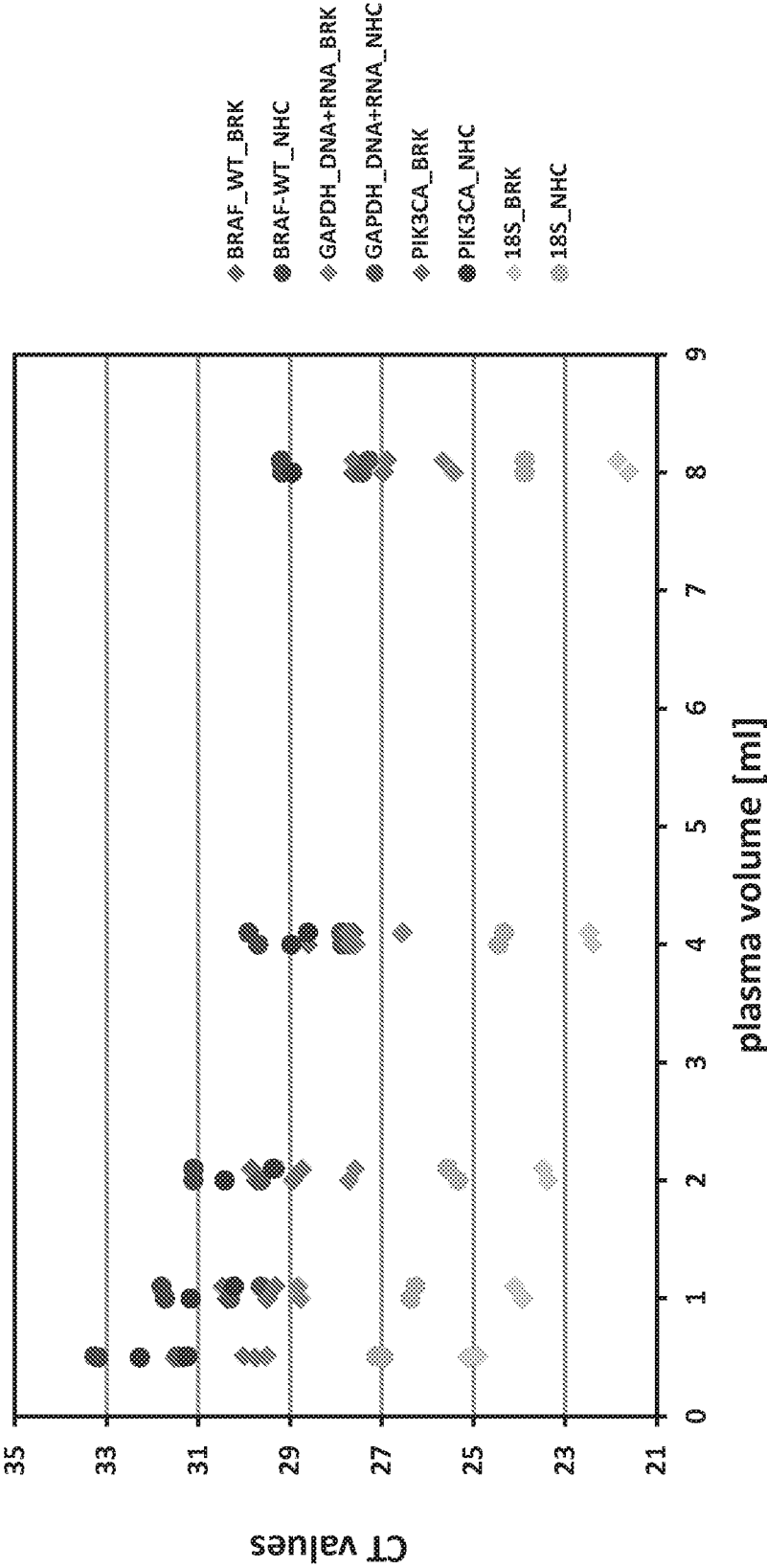


The Flow-through of EXO52.2 does not have a breakthrough point
up to 8 mL of plasma



Figure

EX052.2 – loading capacity



Figure

EX052.2 – loading capacity

BRK Pool 17

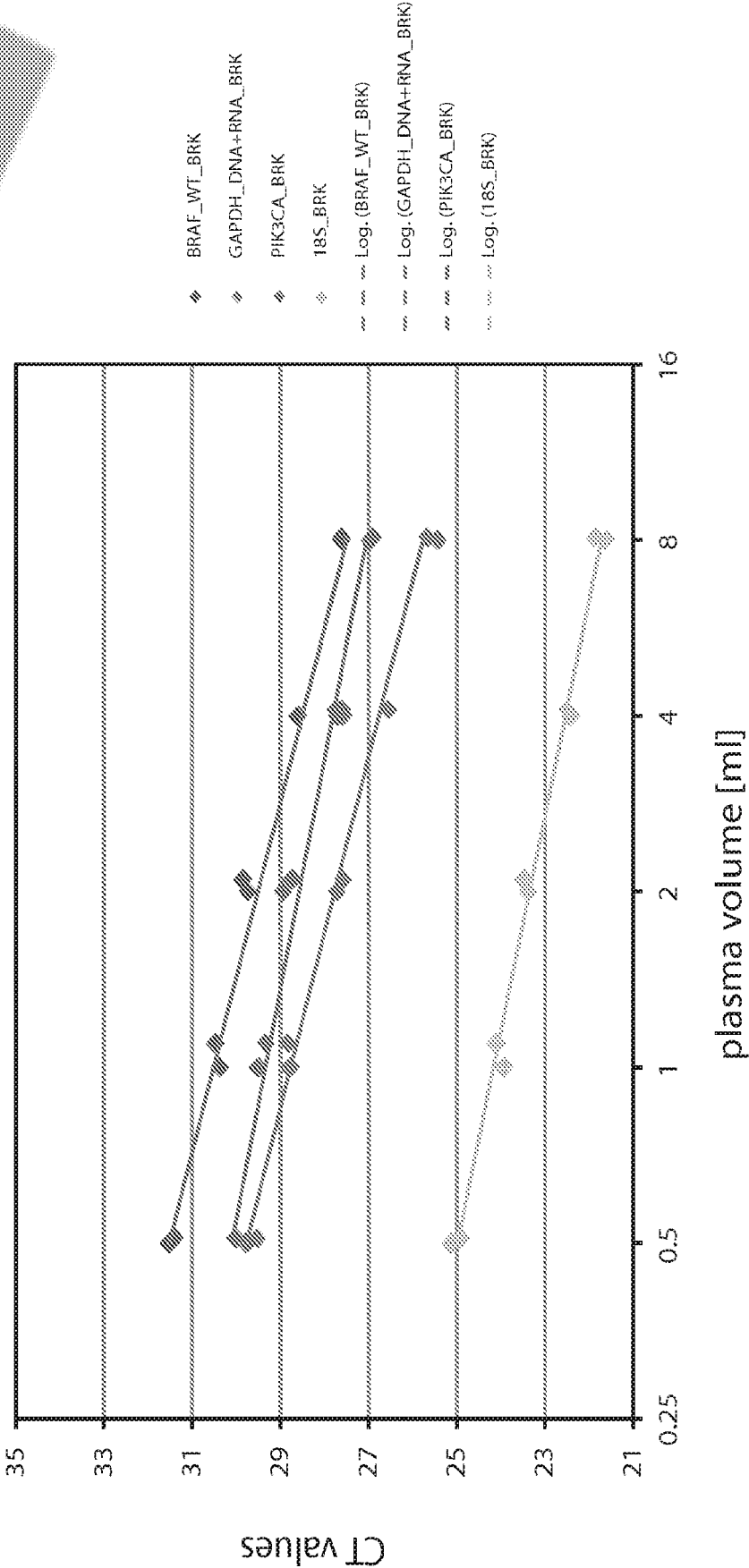
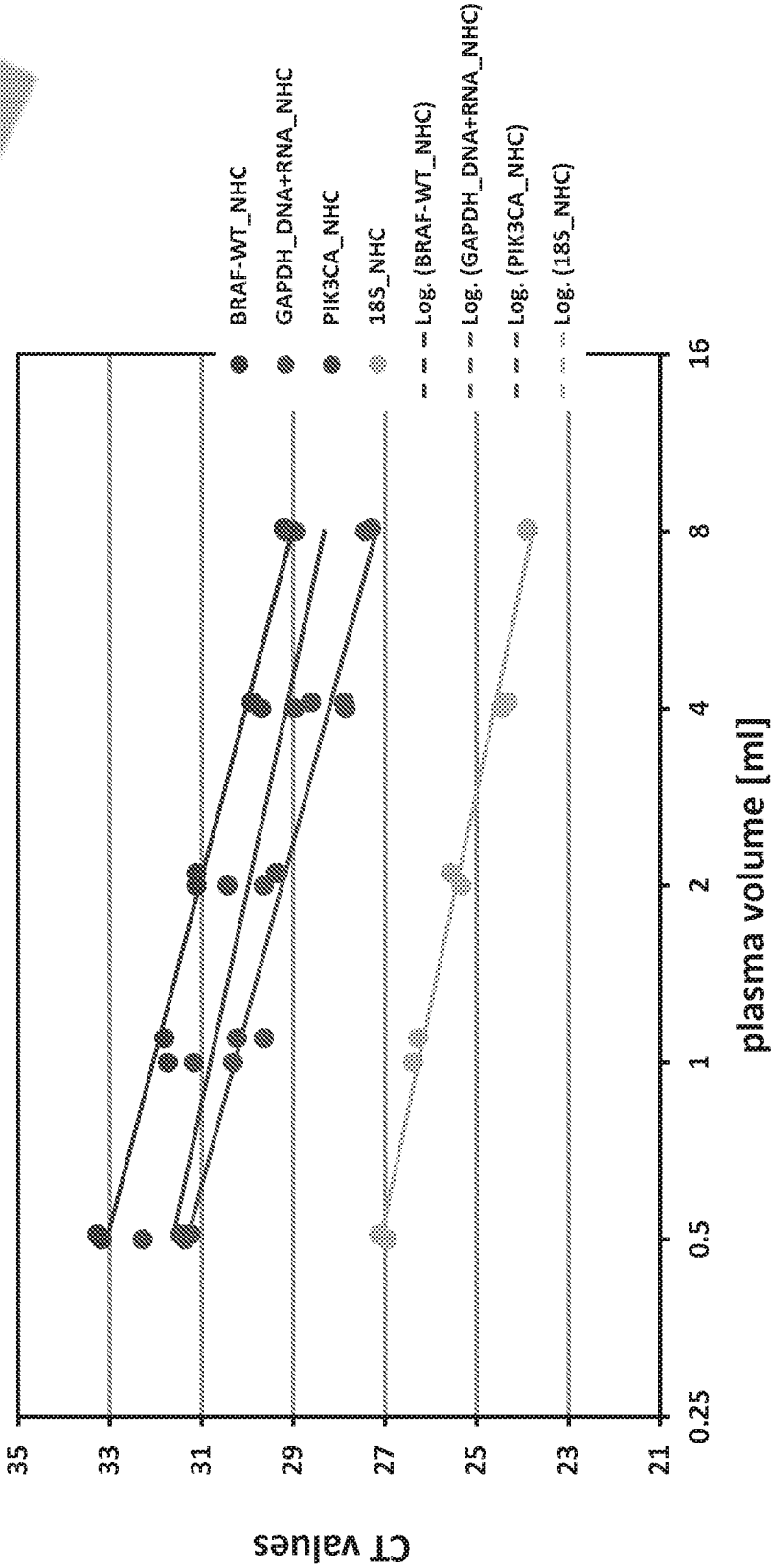


Figure 96

EX052.2 – loading capacity

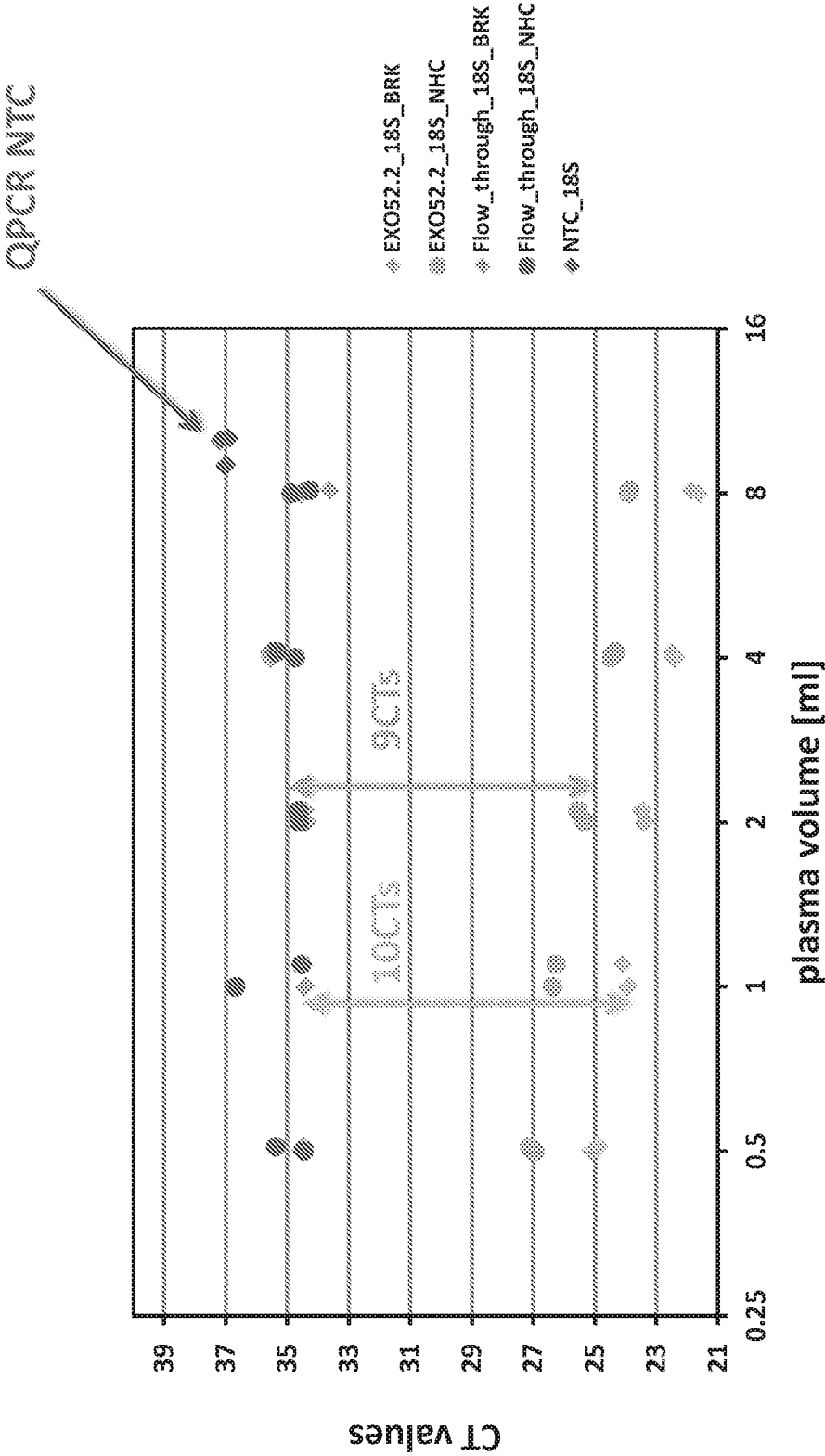
MUC014
(NHC04)



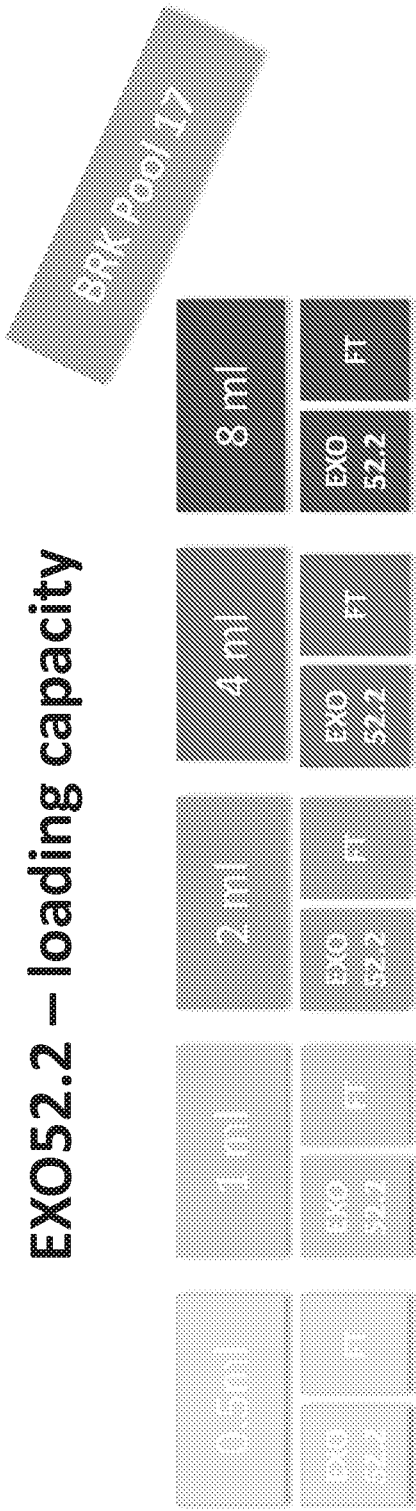
NHC Samples	Slope	Intercept
BRAF-WT	-3.34	32.02
GAPDH RNA+DNA	-3.39	30.24
PIK3CA RNA+DNA	-2.99	30.29
18S	-2.74	26.24

Figure

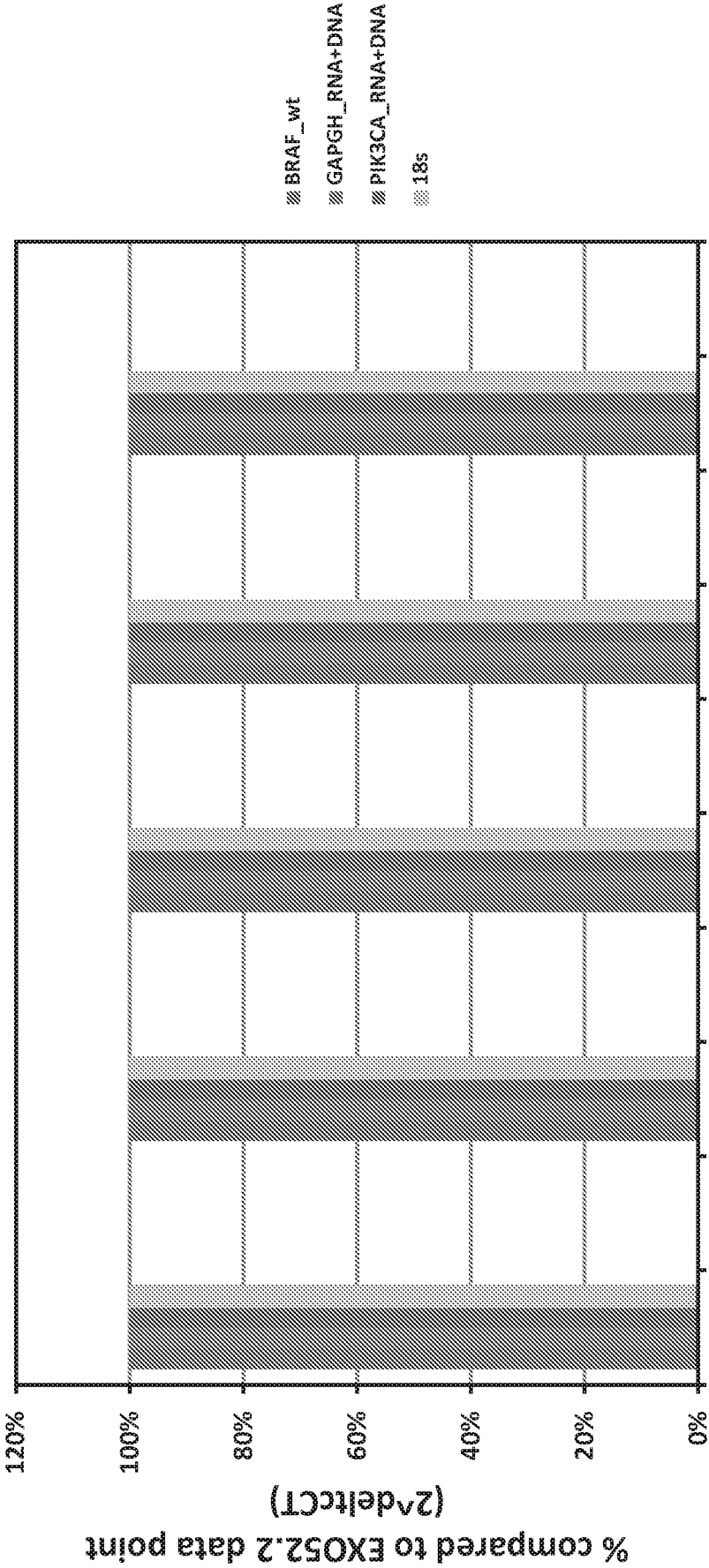
The Flowthrough of EXO52.2 does not have a breakthrough point
up to 8 mL of plasma



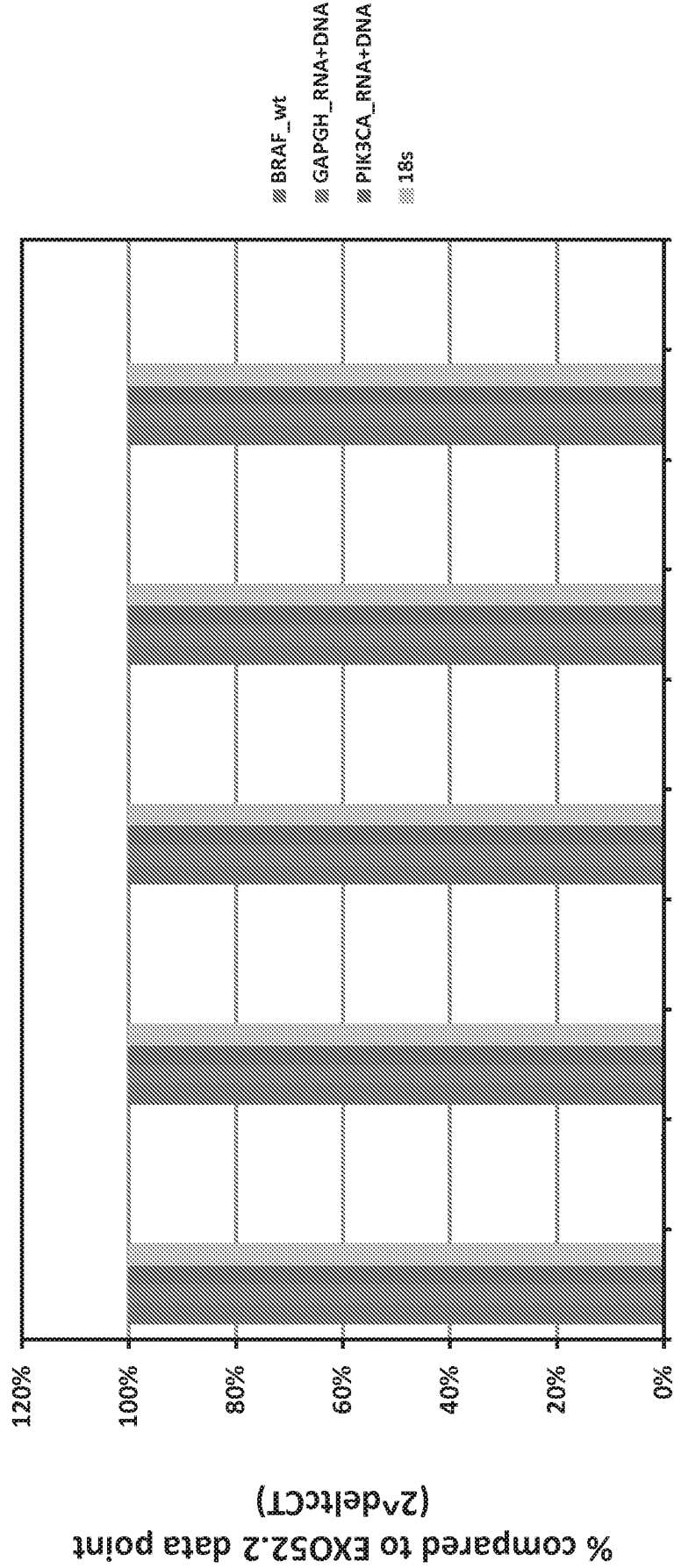
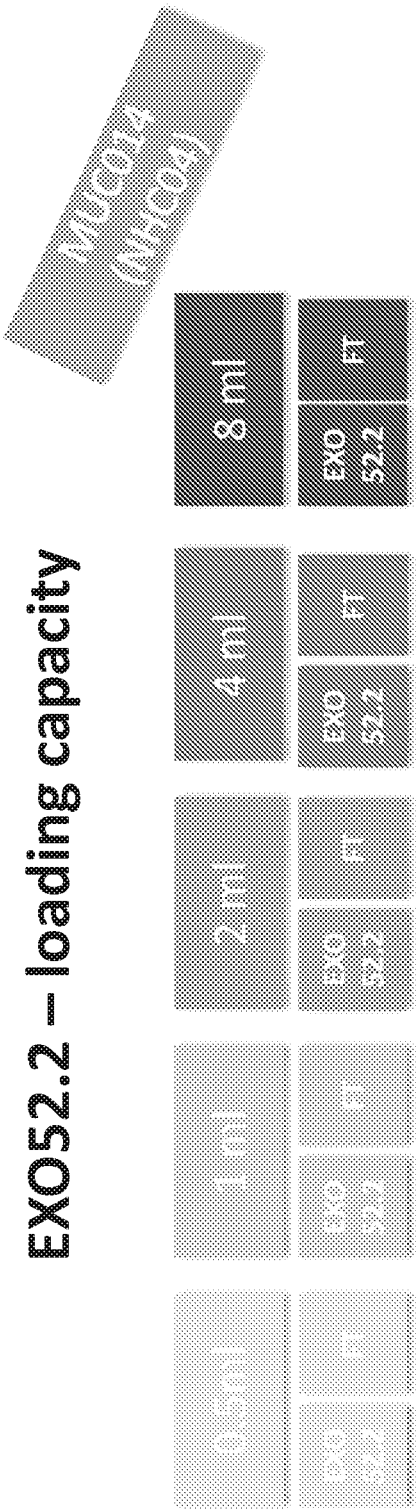
Figure



EXO52.2 – loading capacity



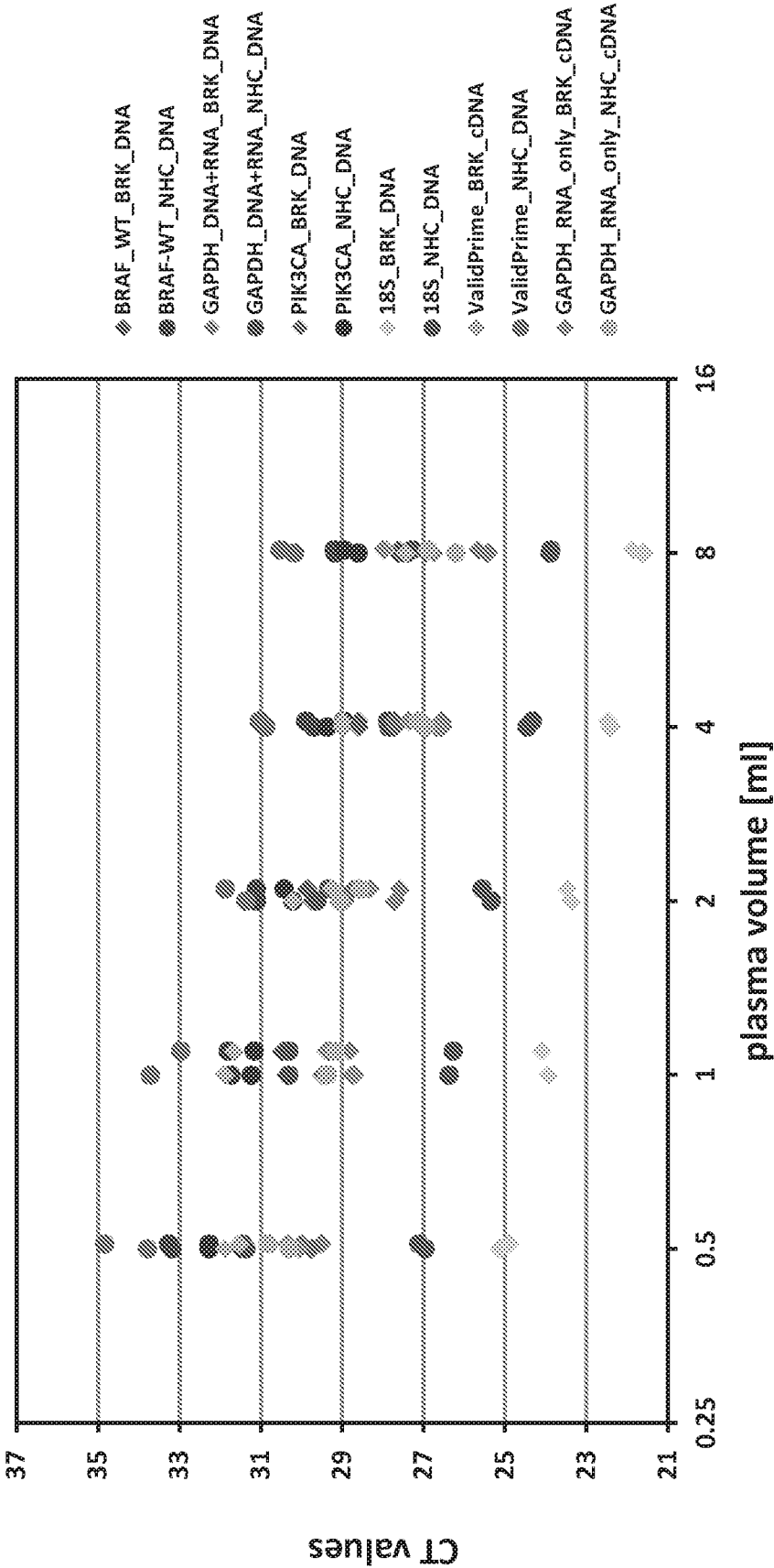
Figure



Figure

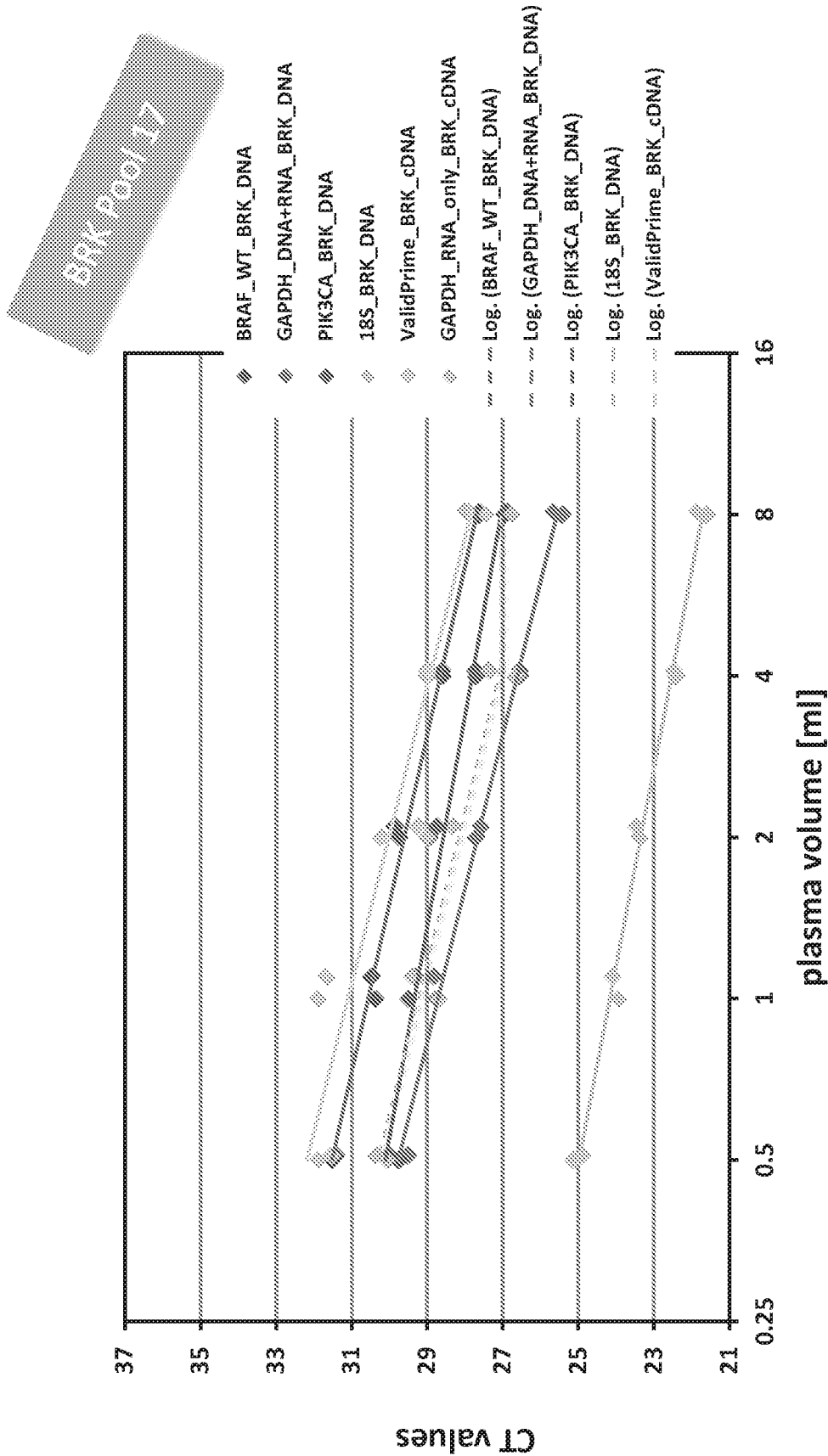
EX052.2 – loading capacity

Different binding capacity for exosomes and nucleosomes



Figure

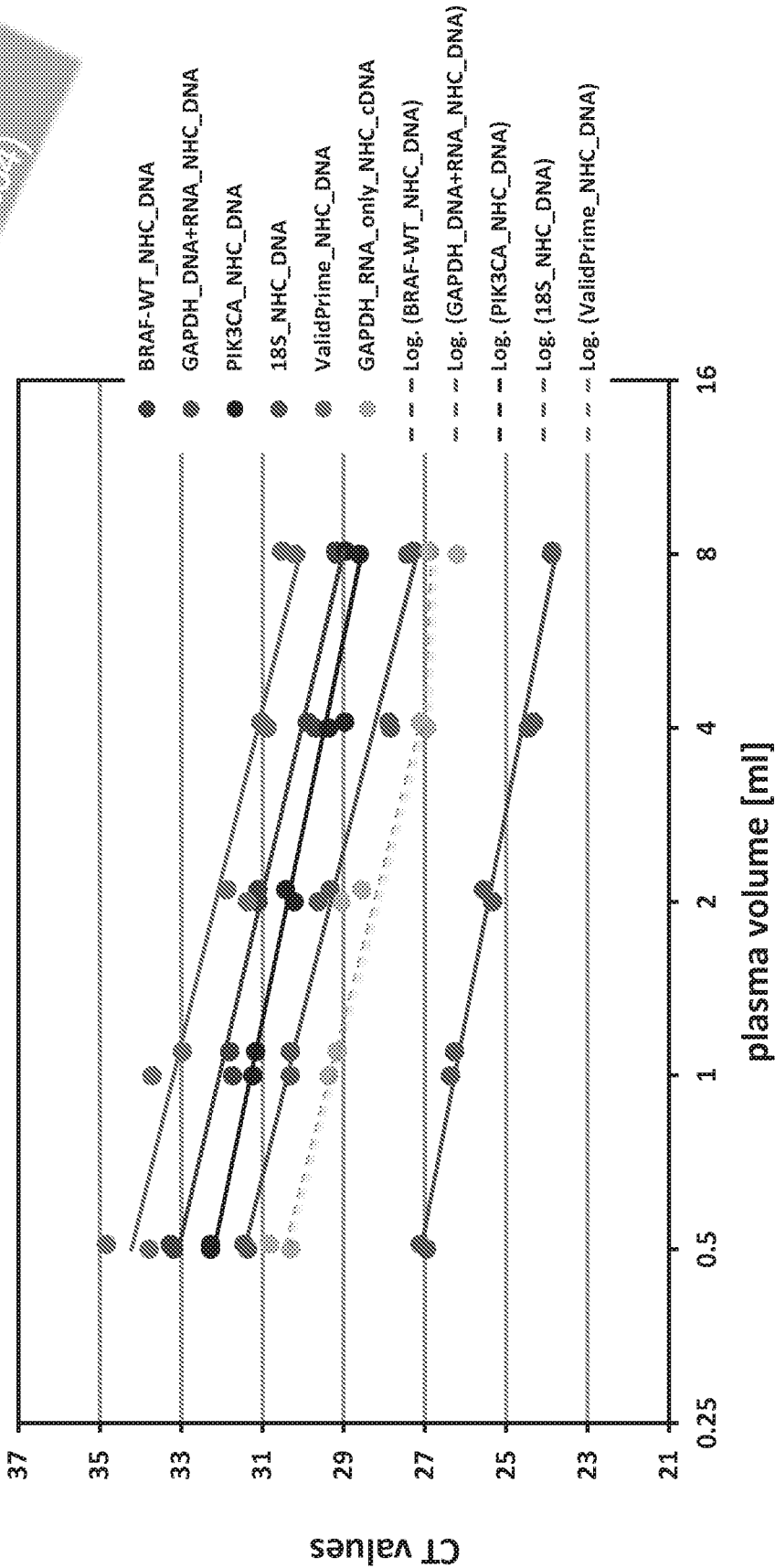
EX052.2 – loading capacity
Different binding capacity for exosomes and nucleosomes



Figure

EX052.2 – loading capacity
Different binding capacity for exosomes and nucleosomes

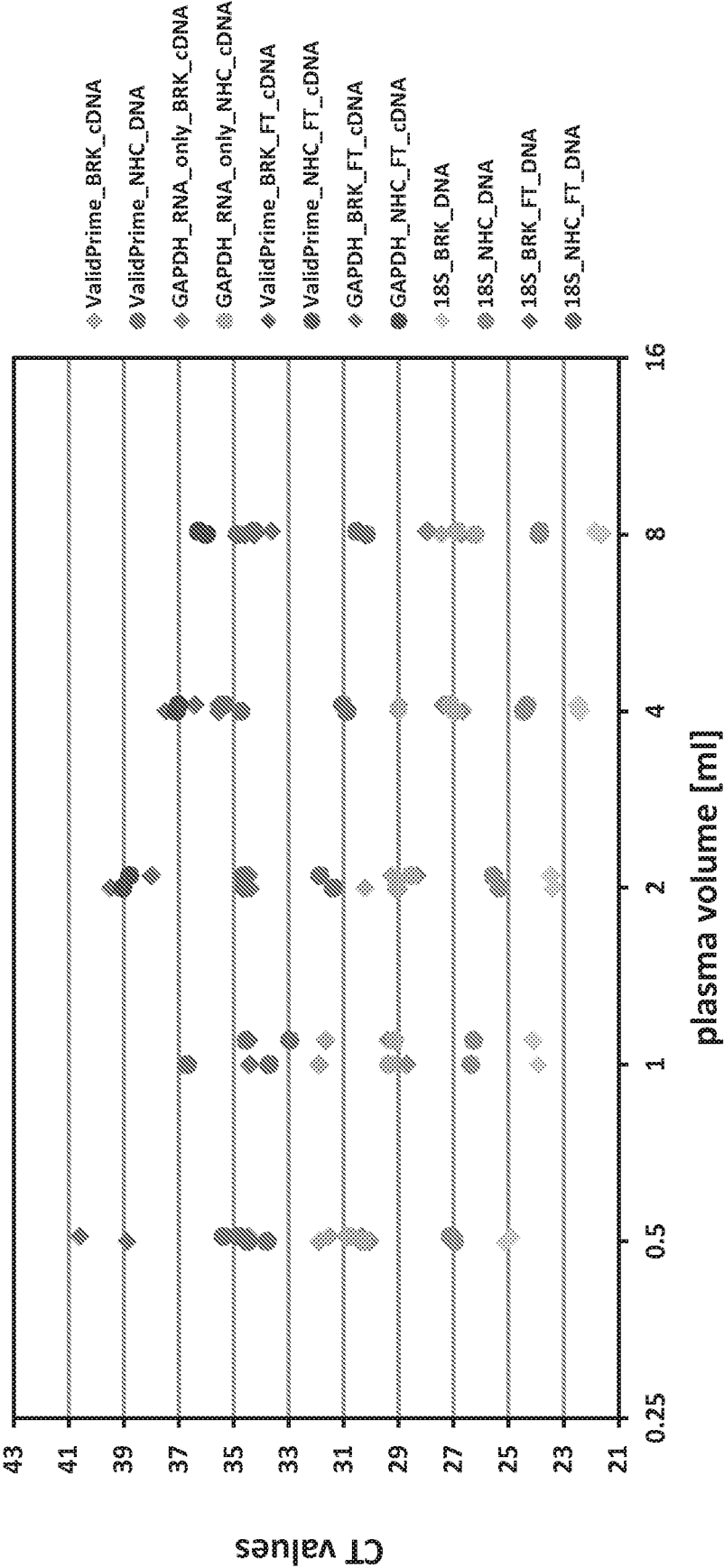
MUC014
(NHC04)



Figure

EX052.2 – loading capacity

Different binding capacity for exosomes and nucleosomes

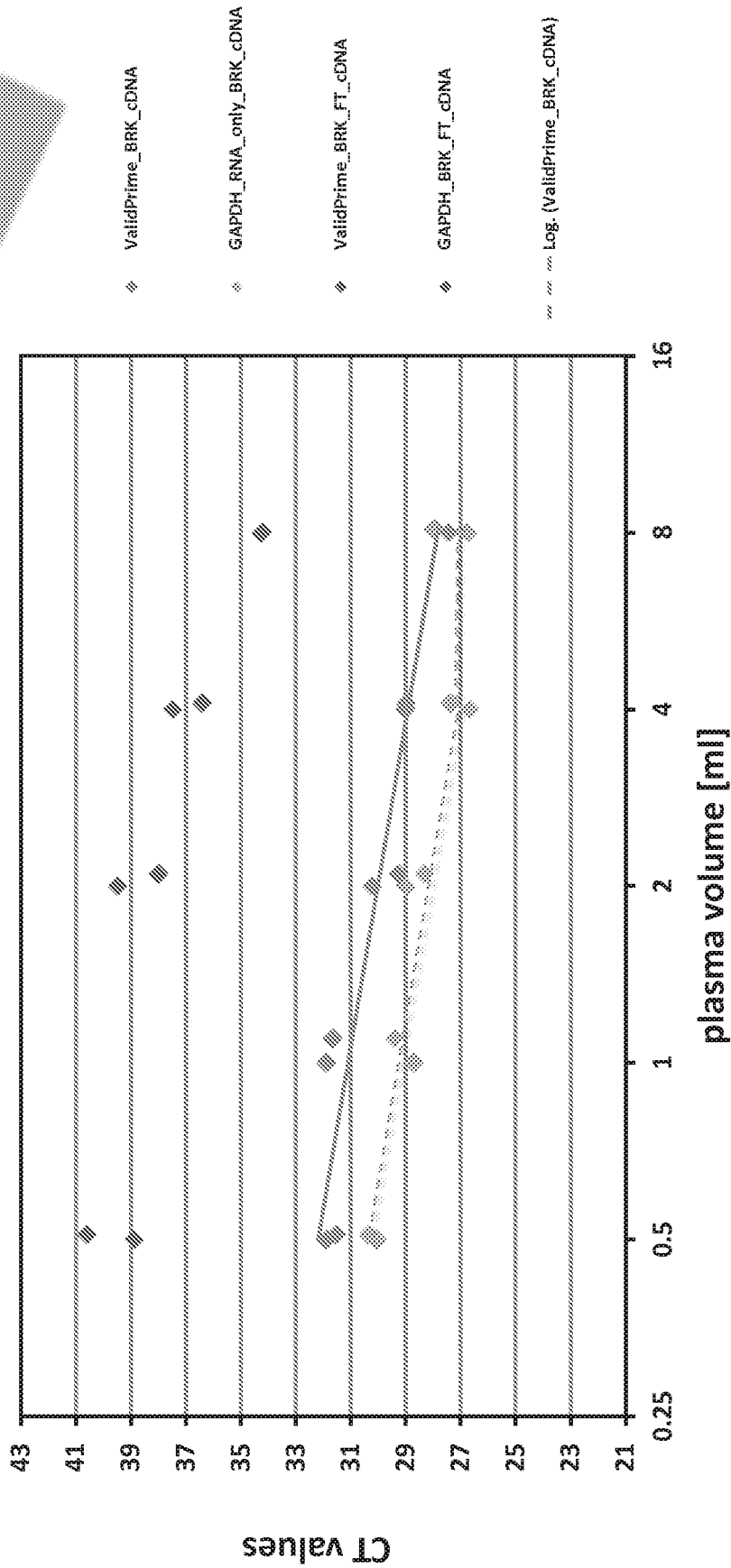


Figure

EX052.2 – loading capacity

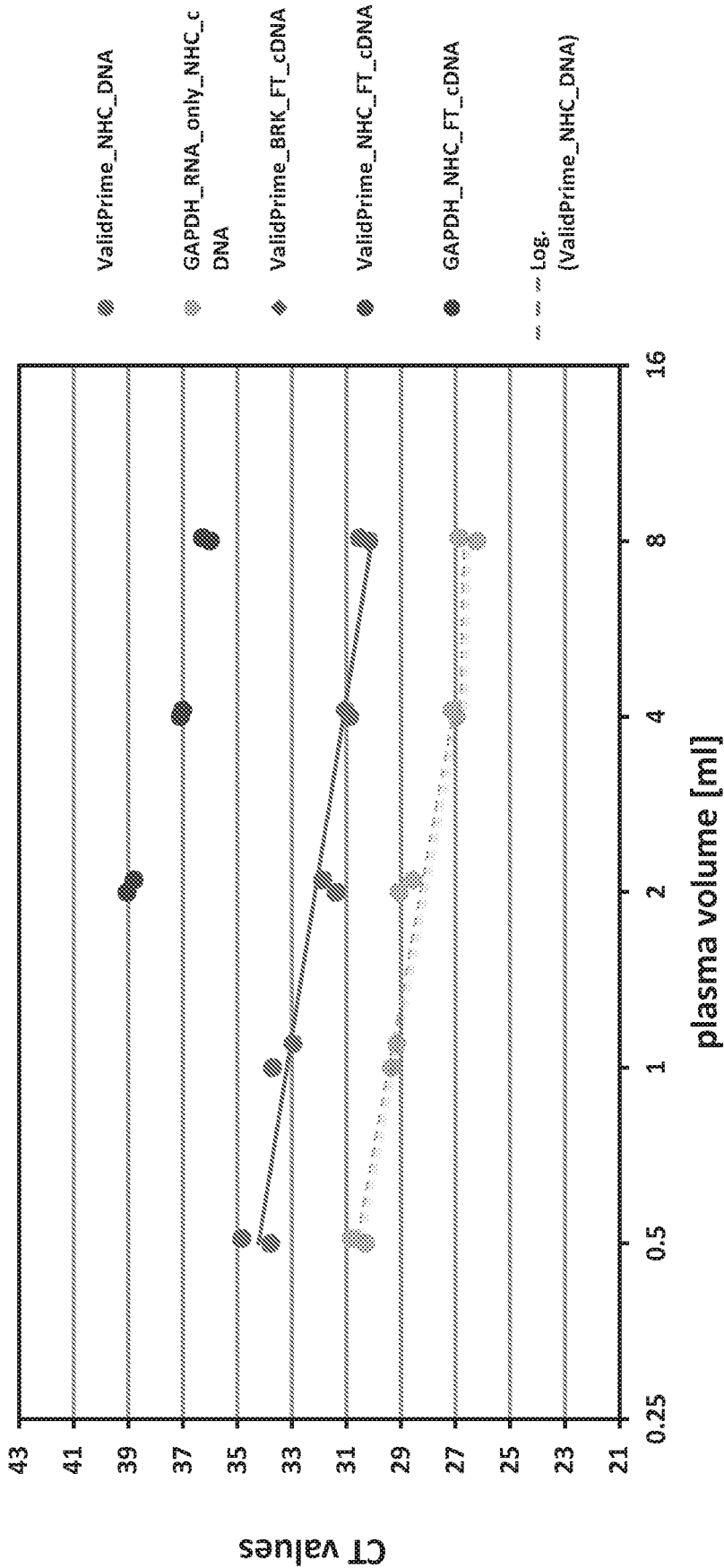
Different binding capacity for exosomes and nucleosomes

BRK Pool 17



Figure

EX052.2 – loading capacity
Different binding capacity for exosomes and nucleosomes



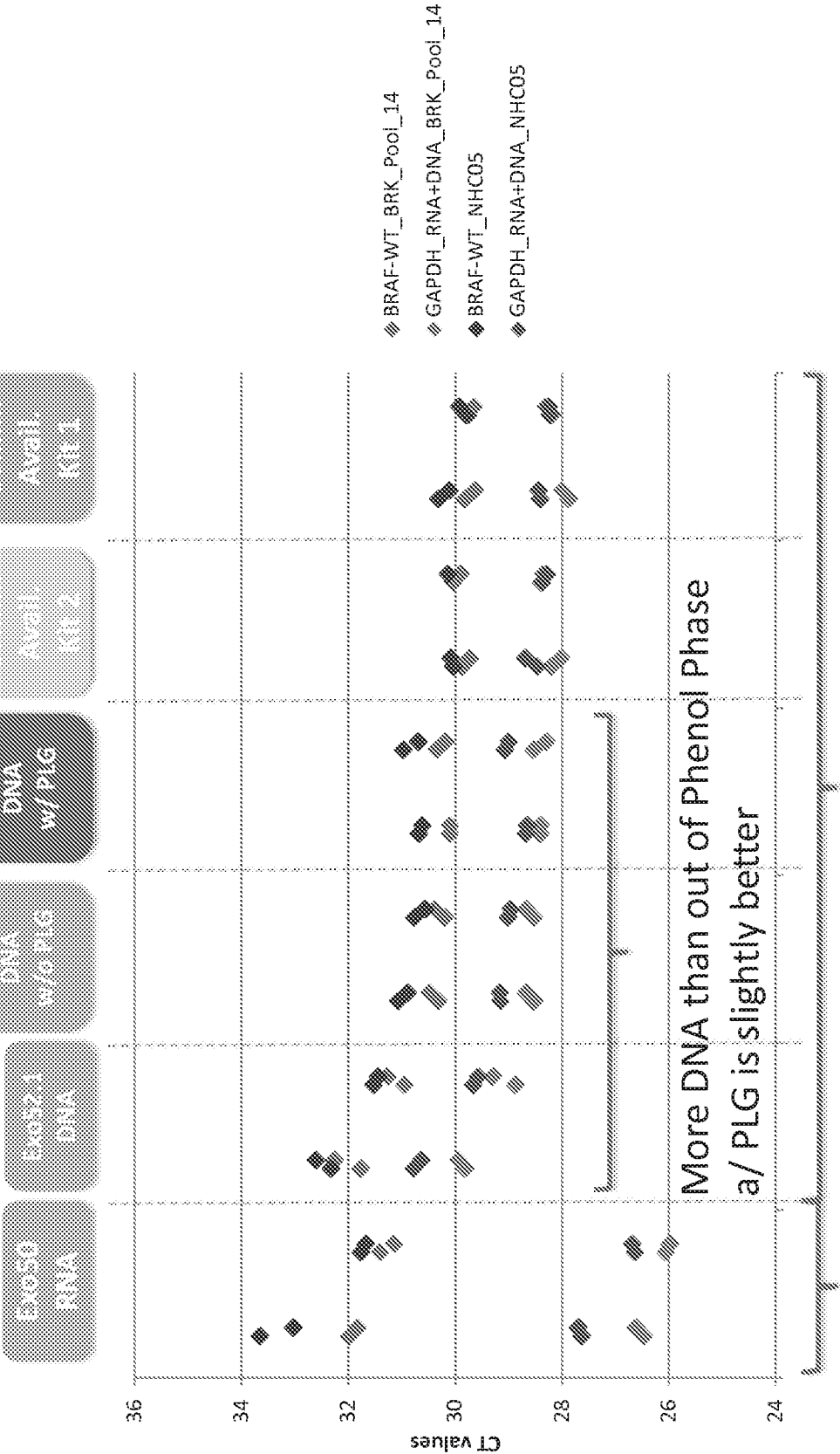
Figure

cfDNA Isolation using different Isolation techniques

Commercially Available Kit 1 – Commercially Available Kit 2– Exo52.1 –

Exo52.2(cfDNA+RNA)

Exo50 DNA n.a.



Figure

cfDNA Isolation using different Isolation techniques

Commercially Available Kit 1 – Commercially Available Kit 2 – Exo52.1 –

Exo52.2(cfDNA+RNA)

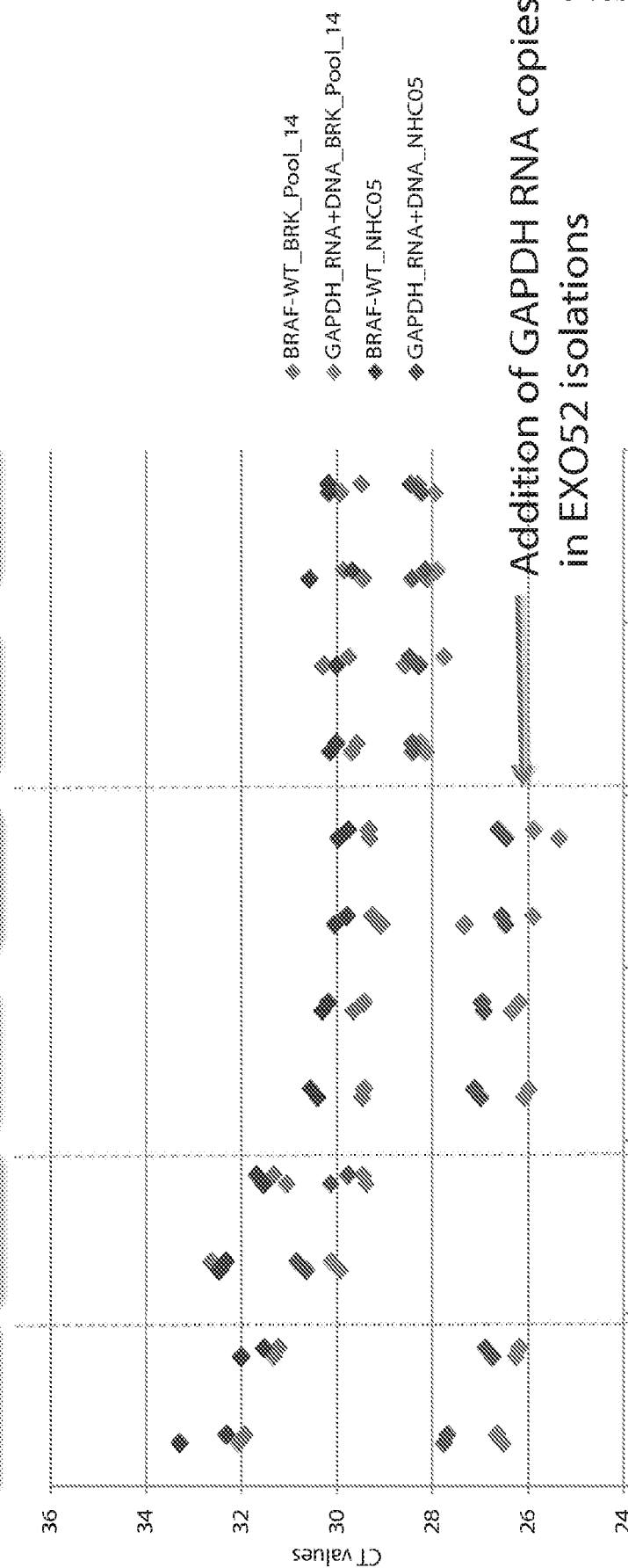
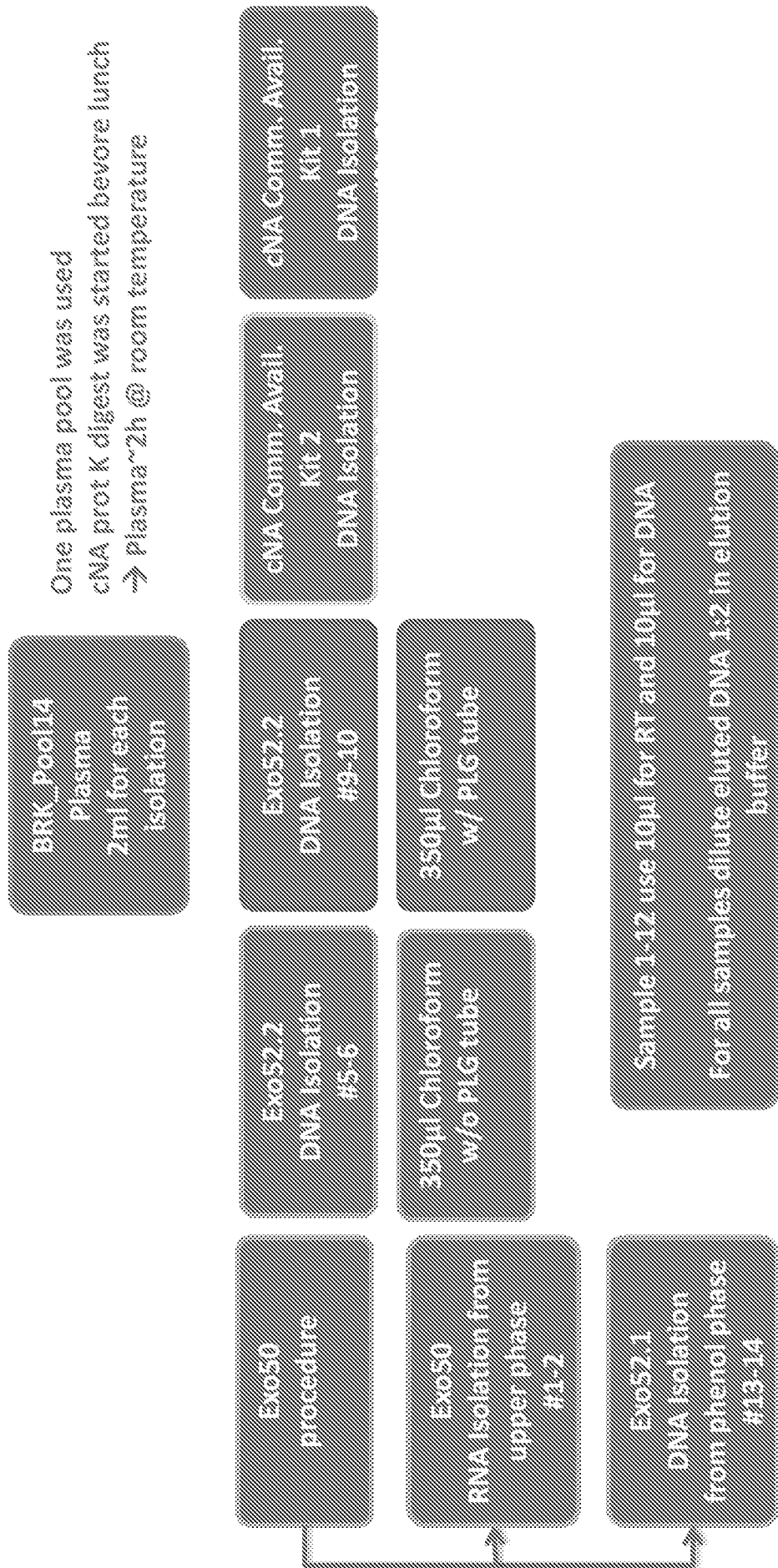


Figure 108

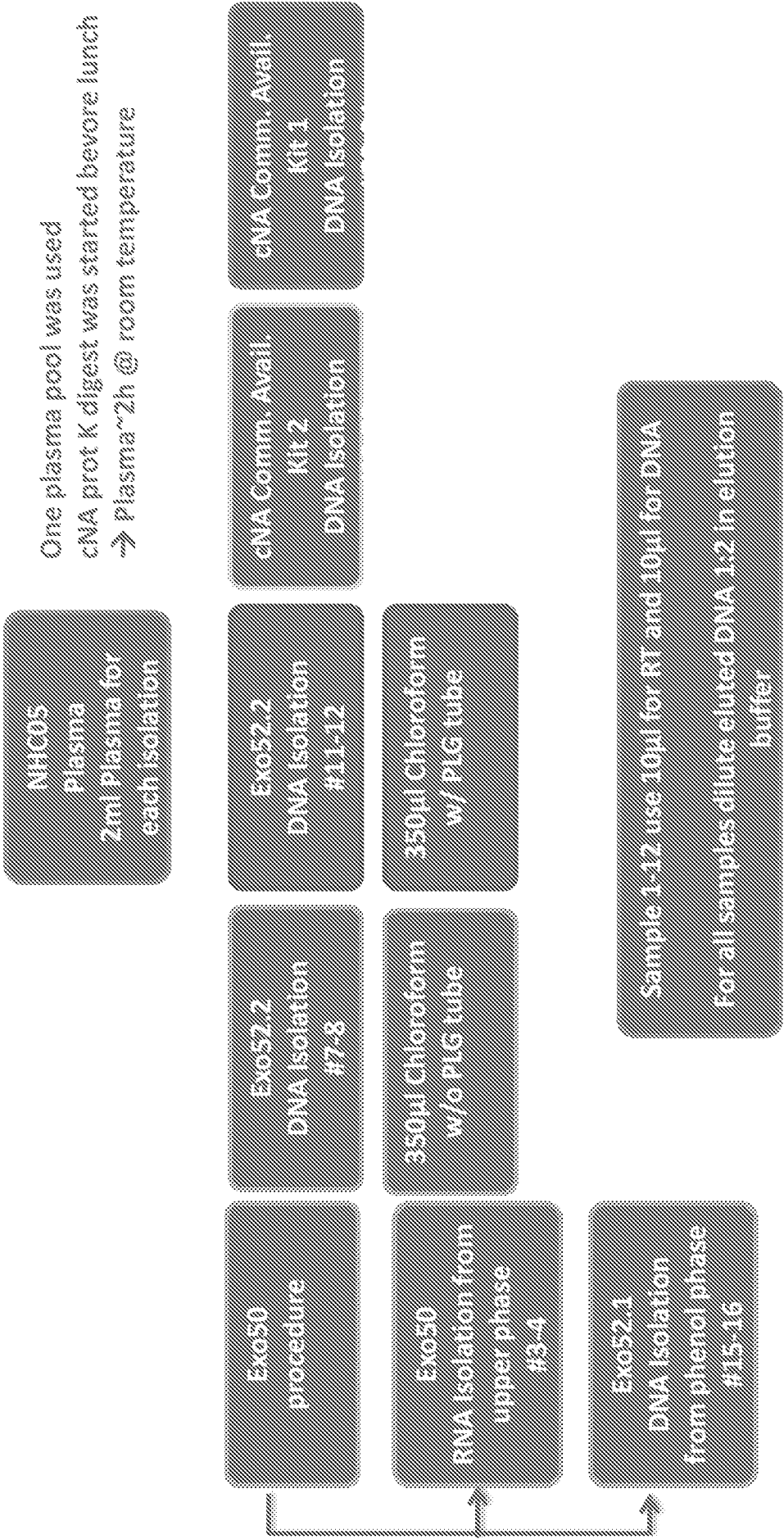
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52 – Exo50_DNA



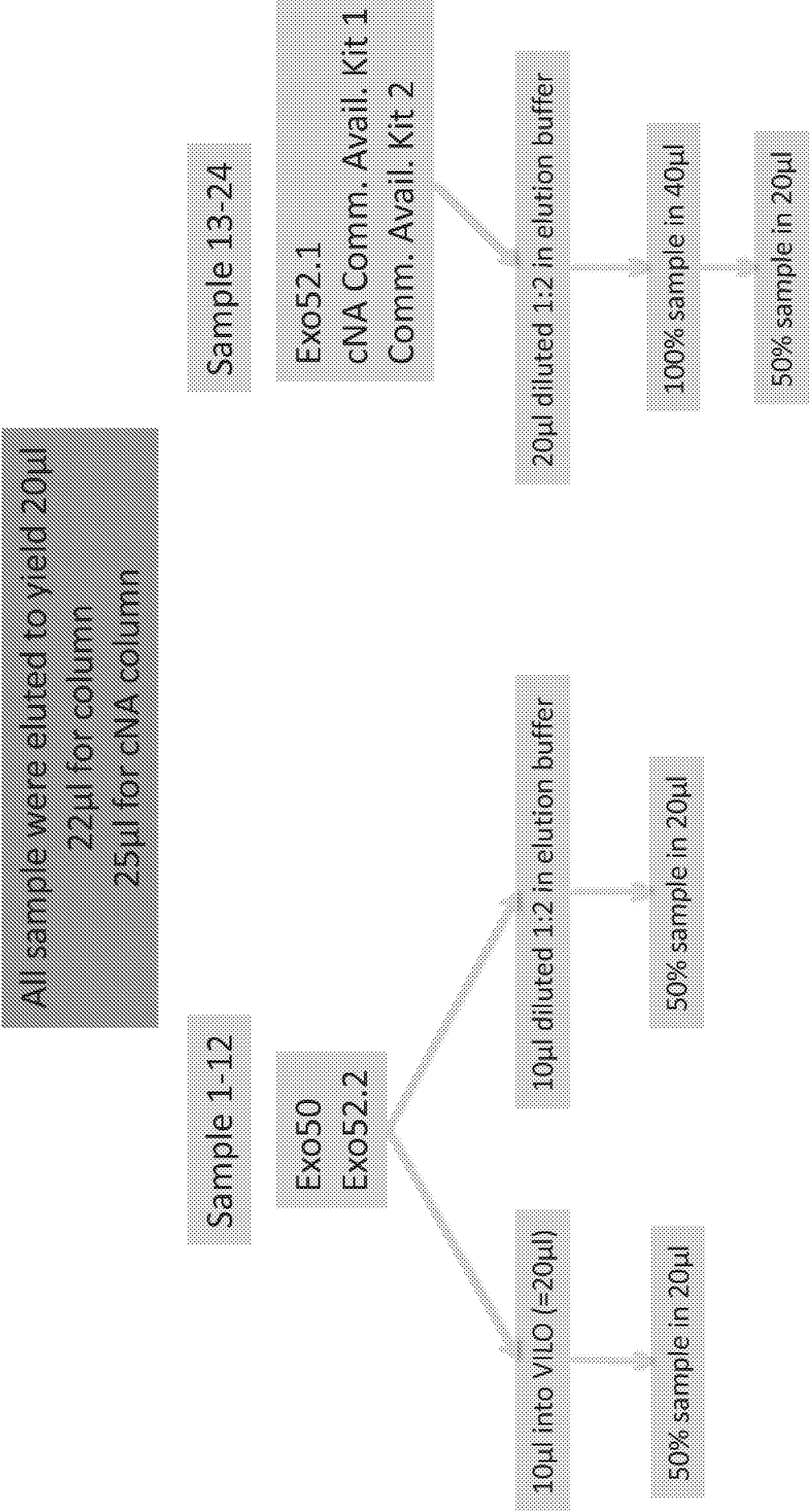
Figure

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52 – Exo50_DNA



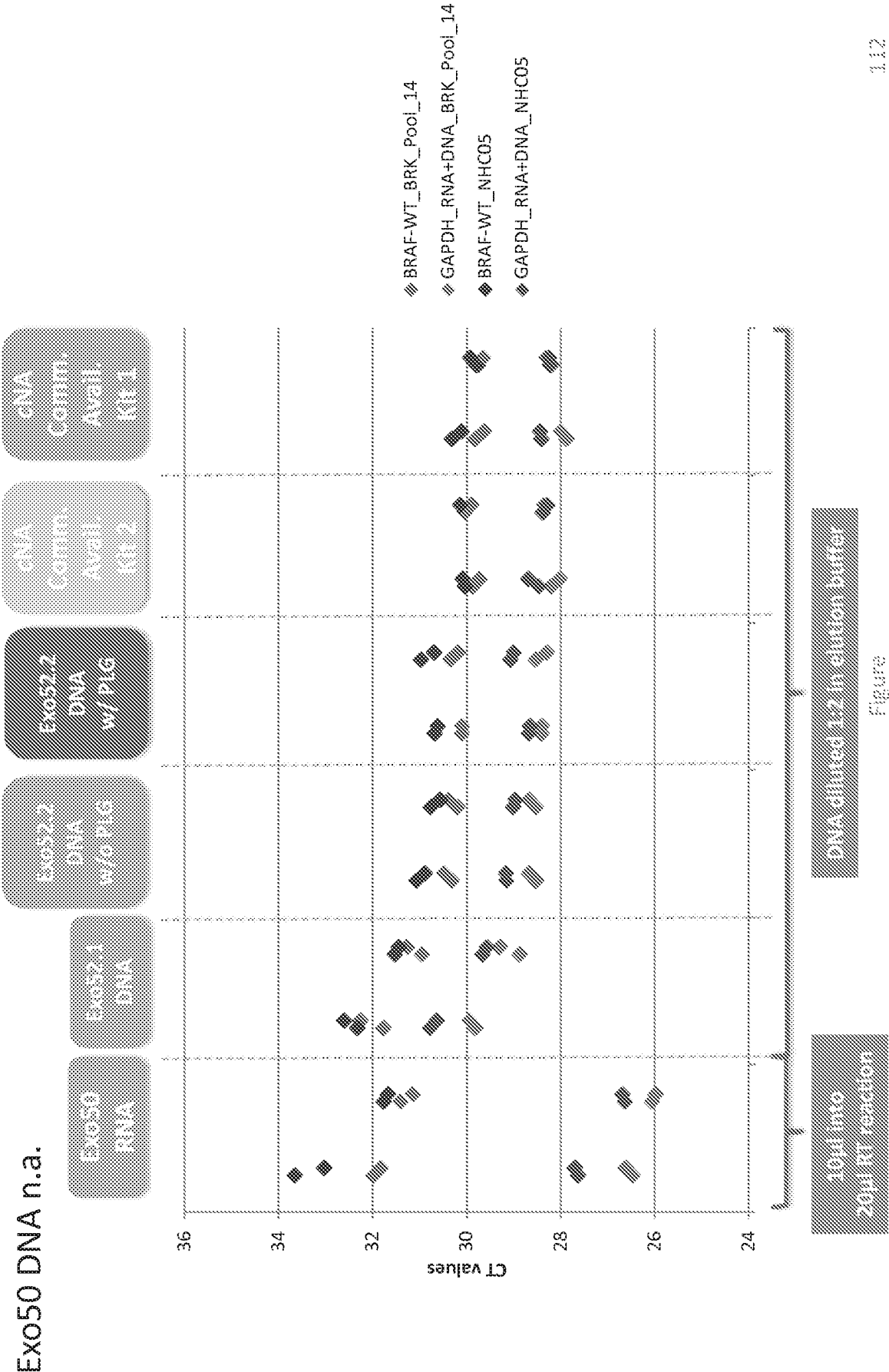
Figure

Dilution overview



Figure

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)



cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)

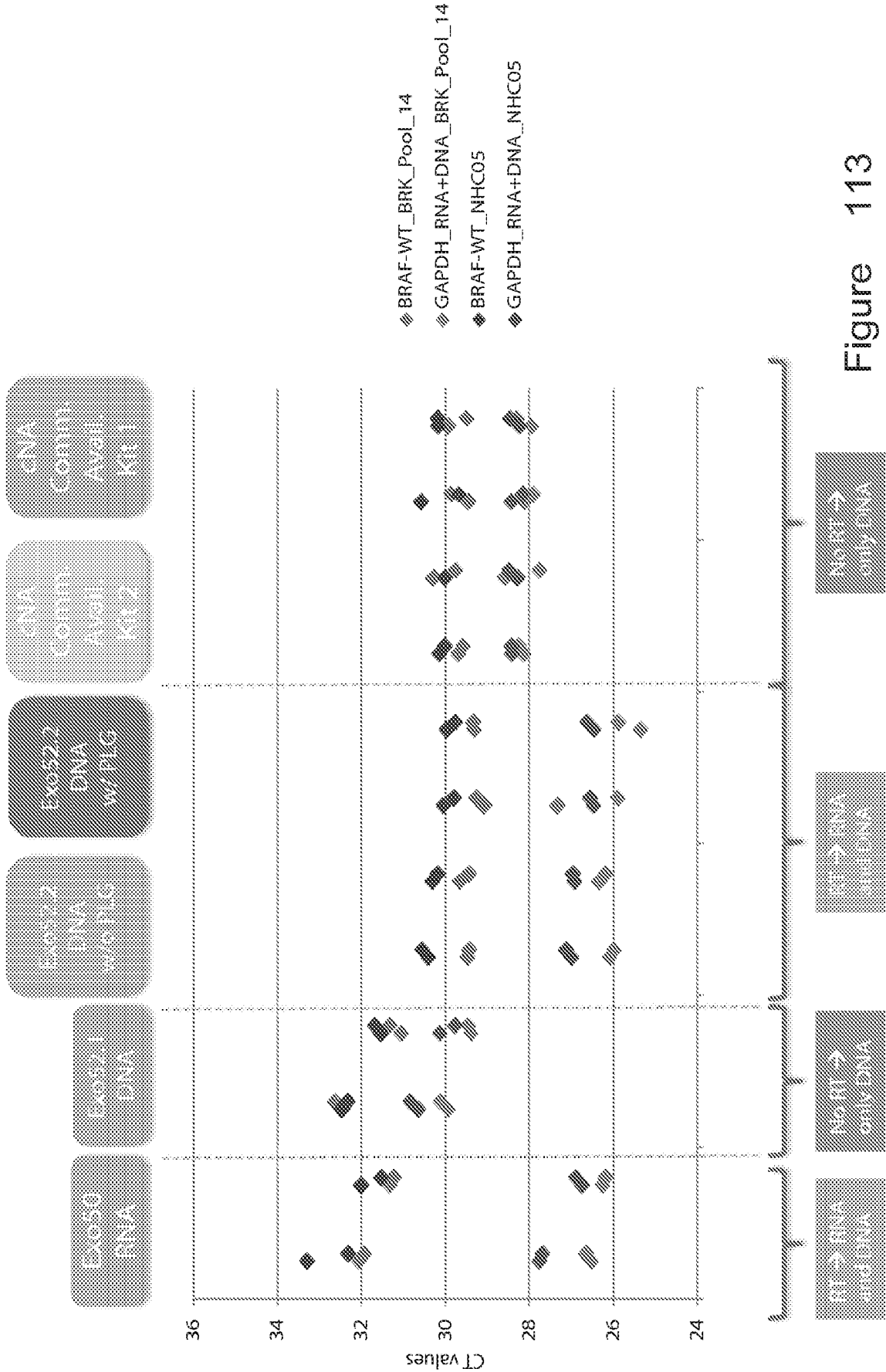
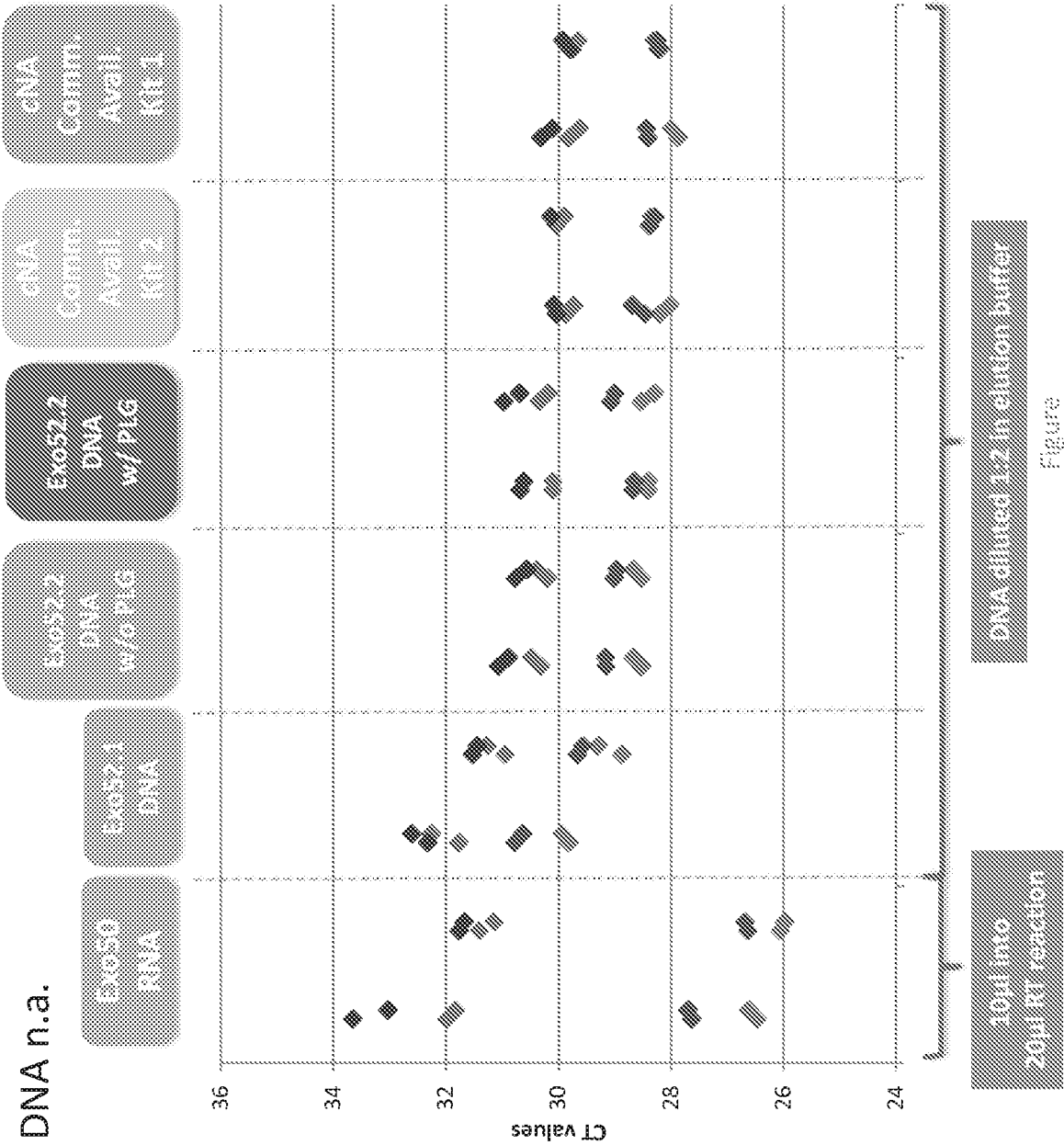


Figure 113

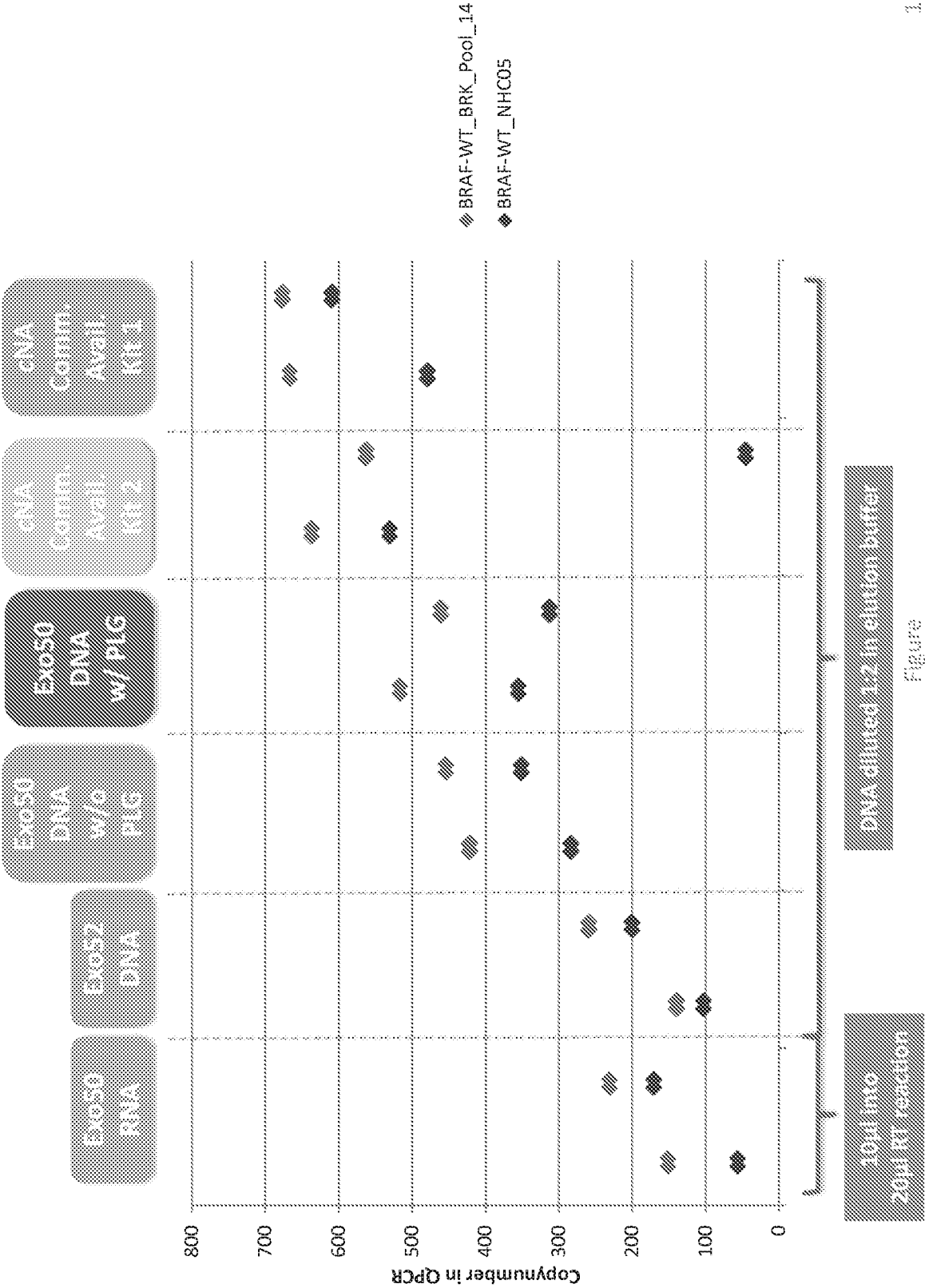
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)

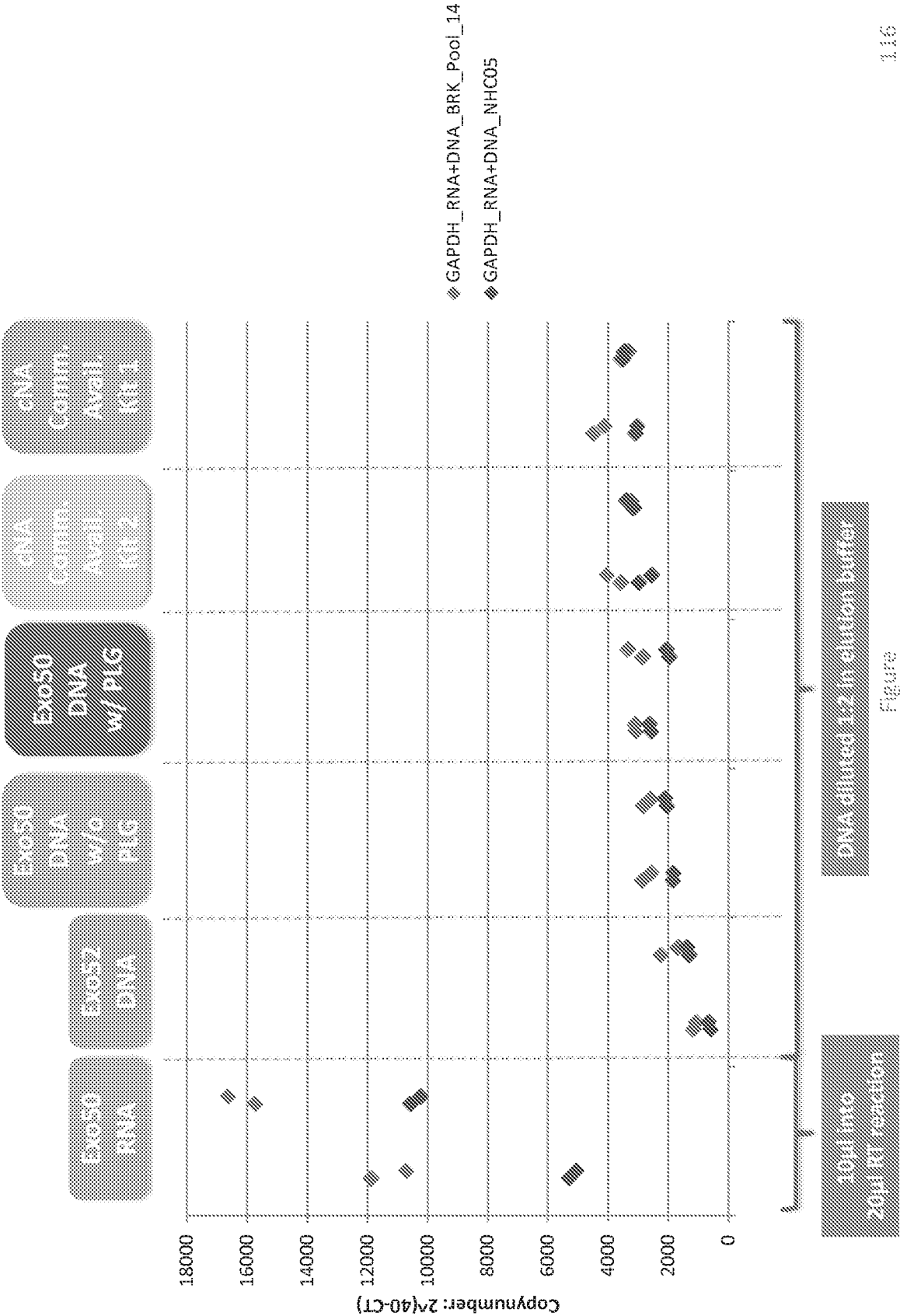
Exo50 DNA n.a.



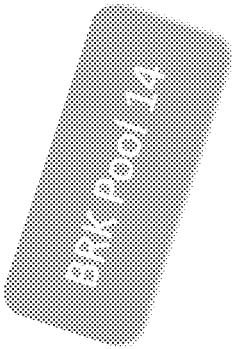
cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)



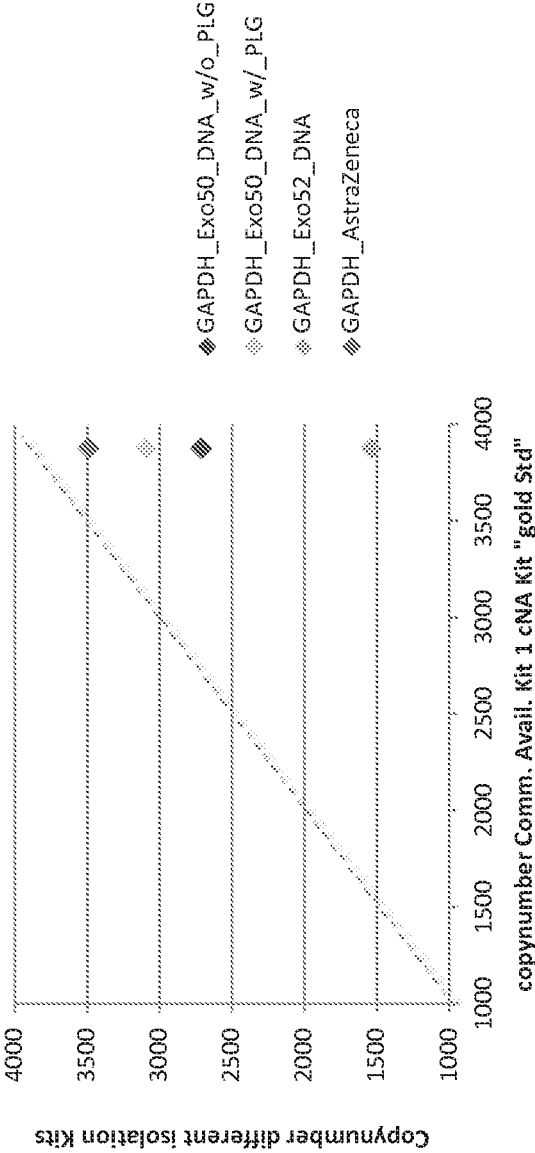
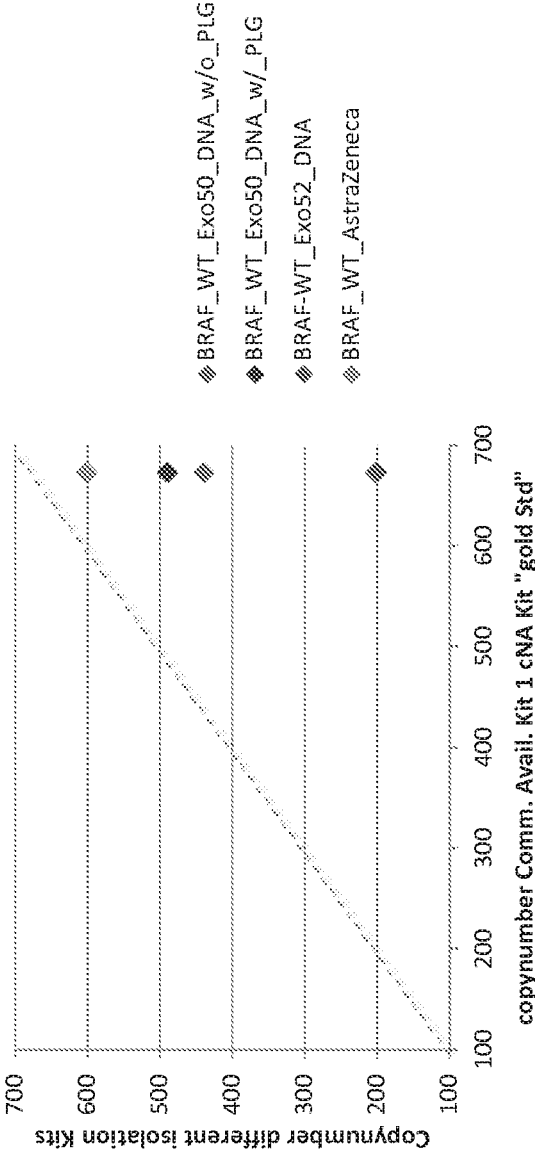
cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)



Compared to gold Standard

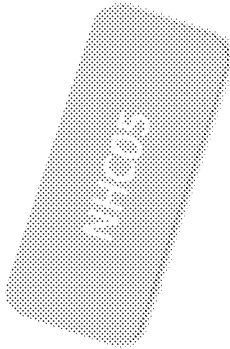
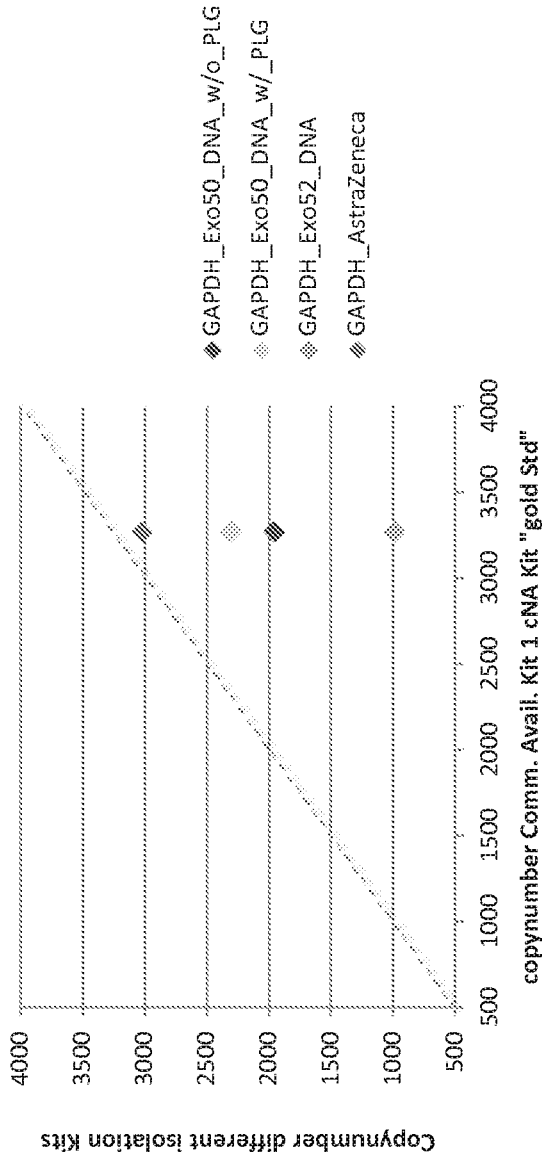
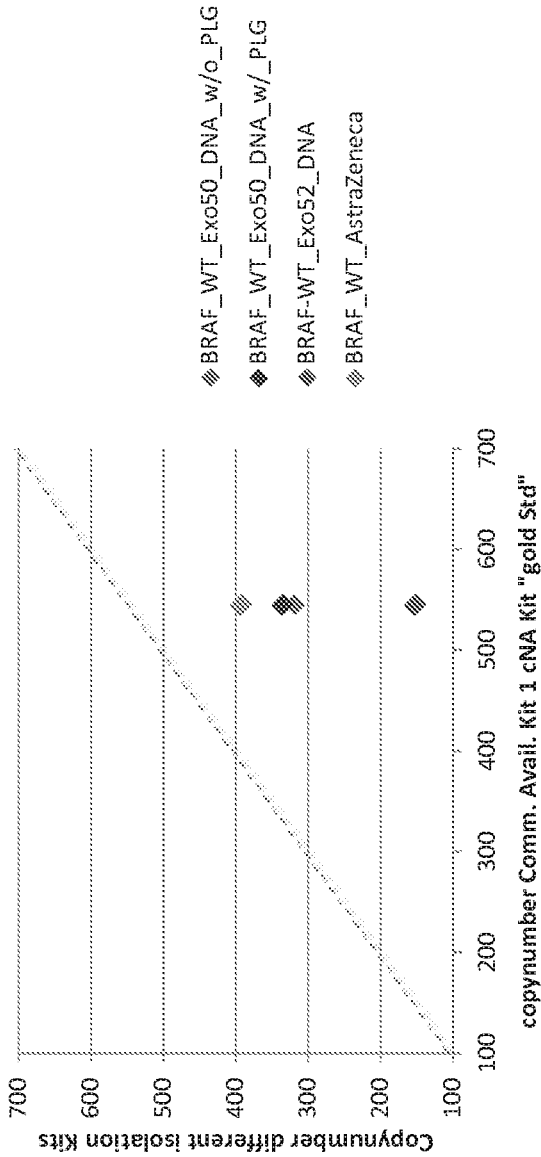


All samples w/o
RT → only DNA



Figure

Compared to gold Standard



All samples w/o
RT → only DNA

Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)

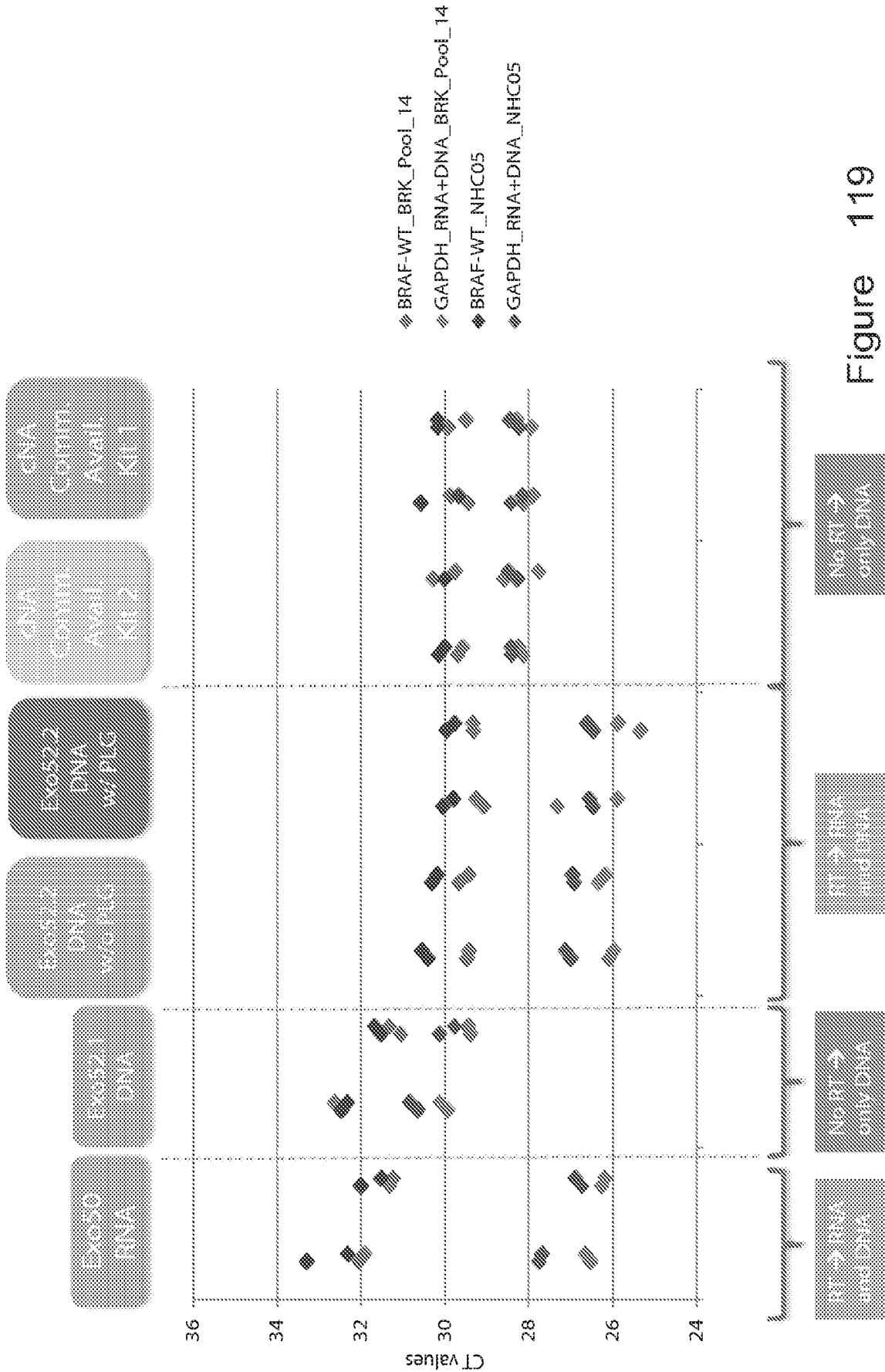


Figure 119

Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)

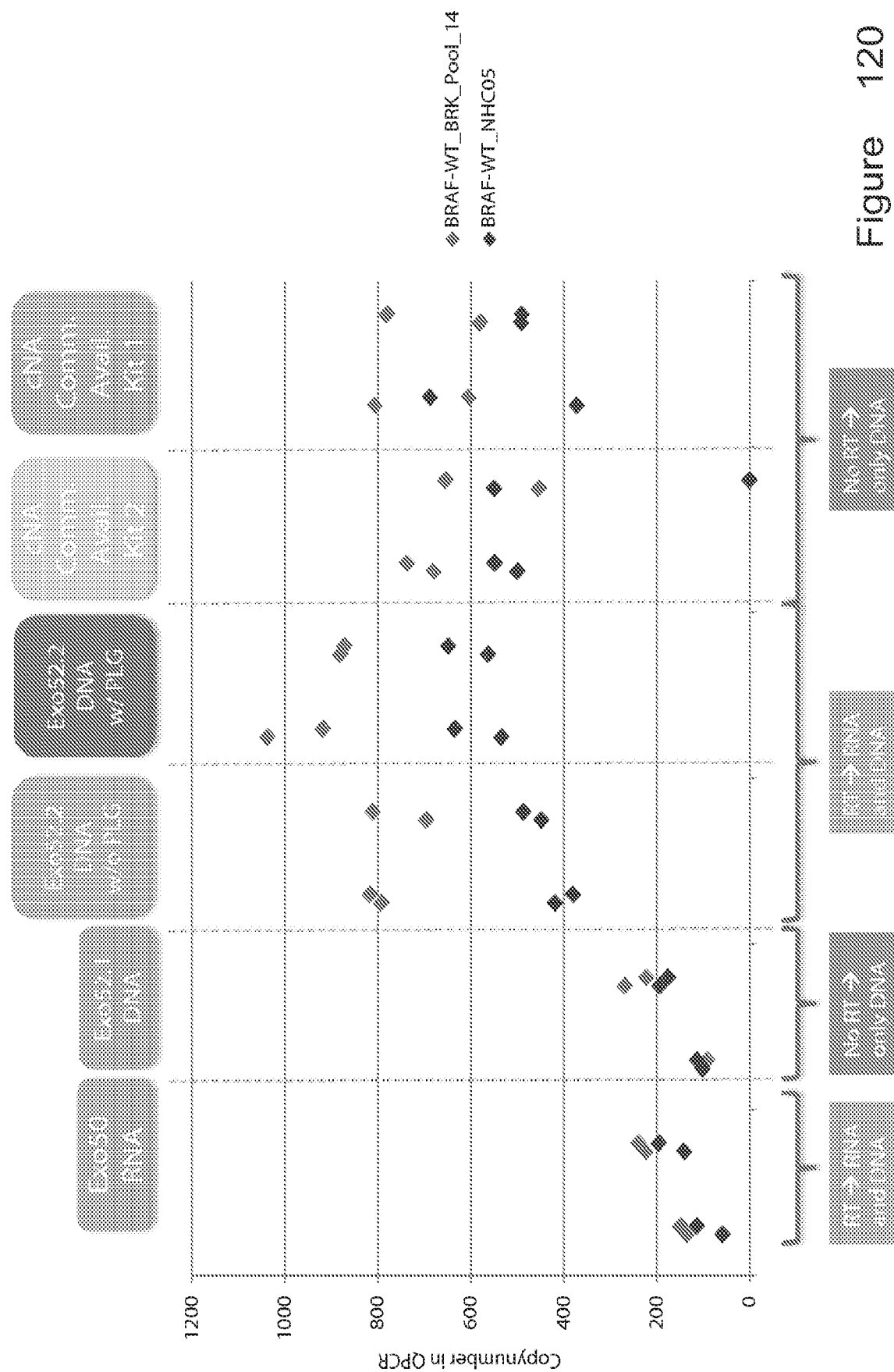


Figure 120

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 – Comm. Avail. Kit 2 – Exo52.1 – Exo52.2(cfDNA+RNA)

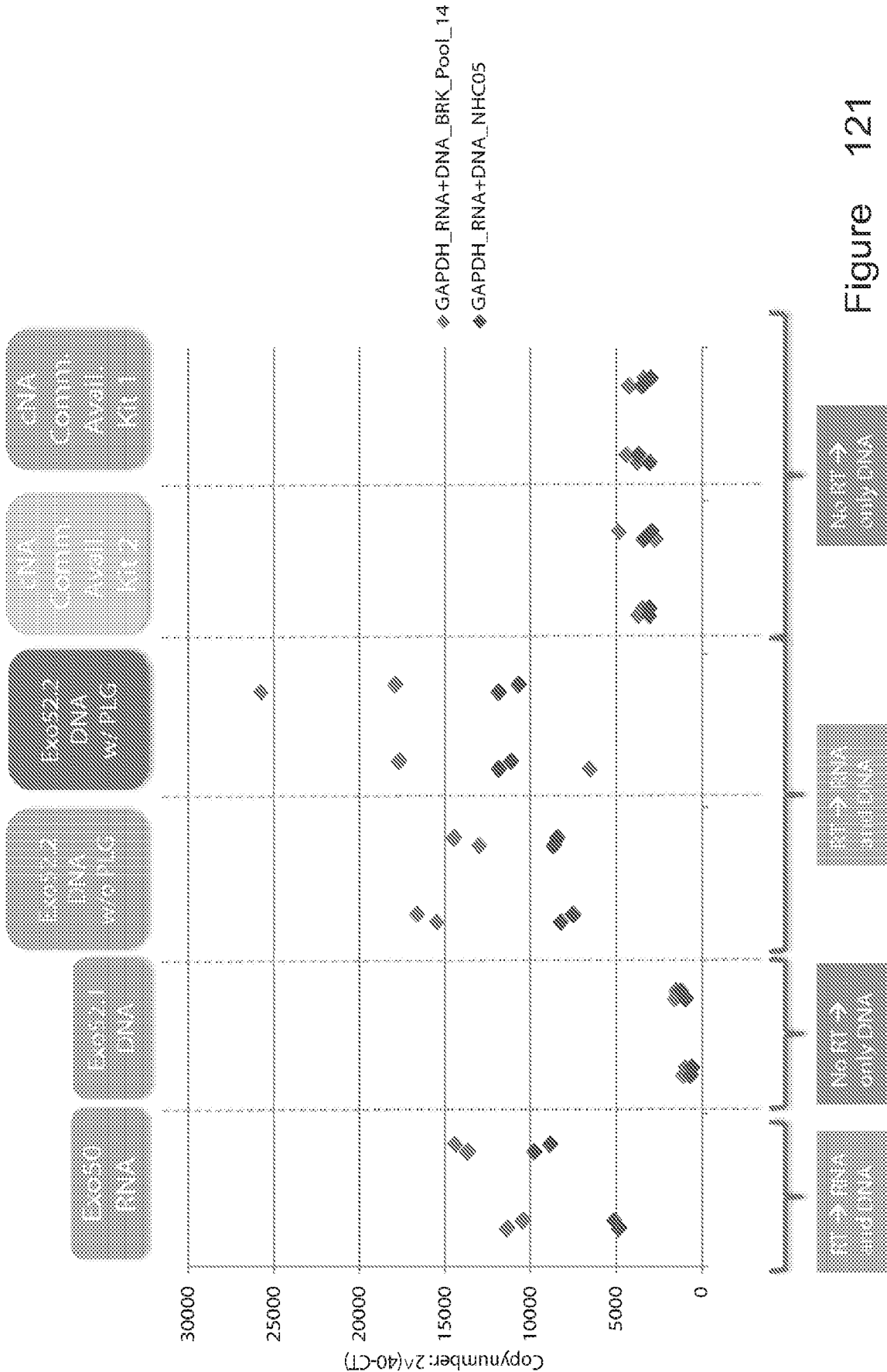
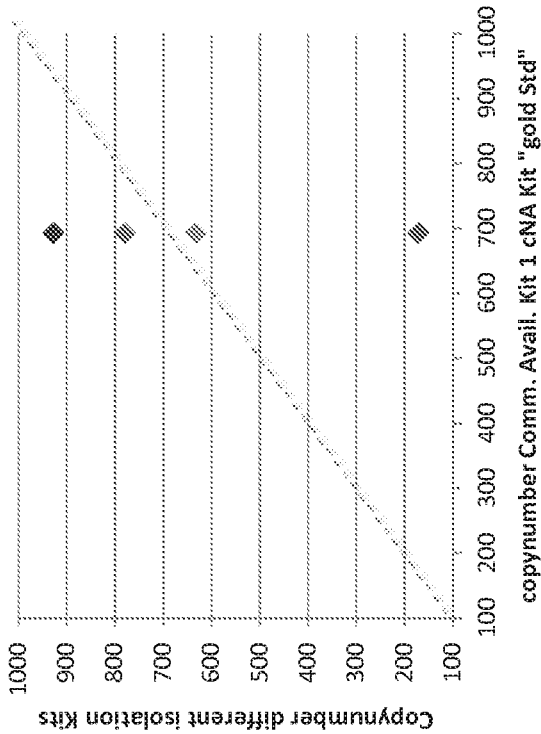
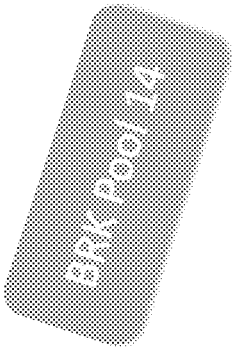


Figure 121

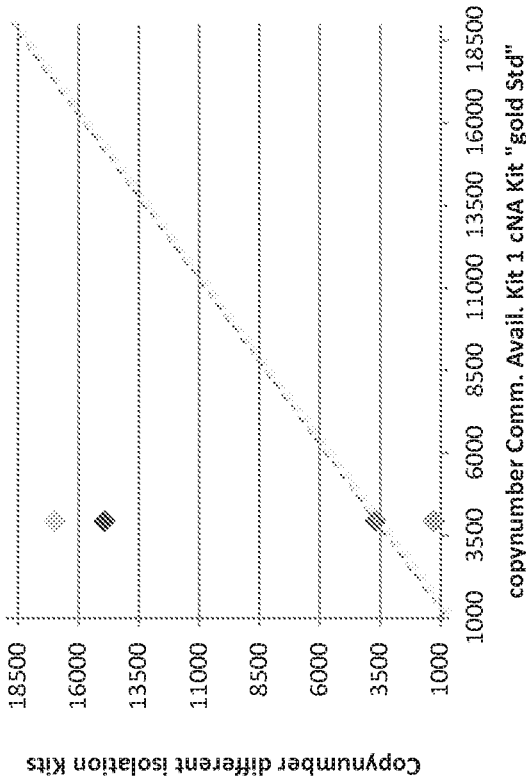
Compared to gold Standard



- ◆ BRAF_WT_Exo50_DNA_w/o_PLG
- ◆ BRAF_WT_Exo50_DNA_w/_PLG
- ◆ BRAF-WT_Exo52_DNA
- ◆ BRAF_WT_AstraZeneca

EXO50 DNA = EXO52.2
With and w/o PLG:
With RT → RNA + DNA

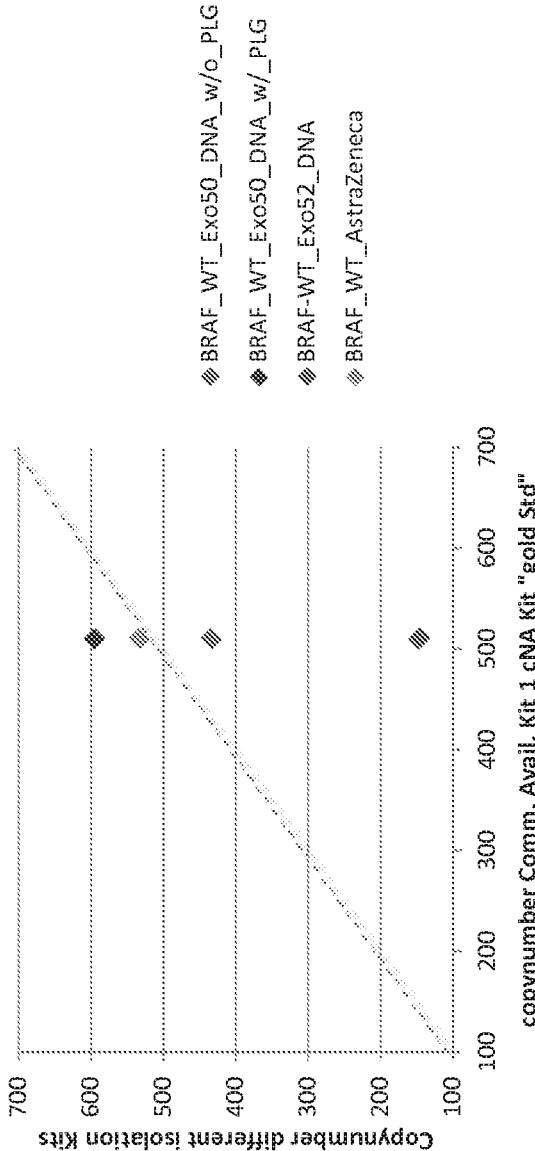
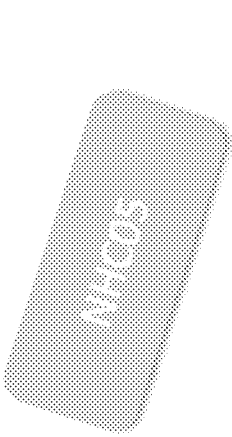
Comm. Avail. Kit 1 +
Comm. Avail. Kit 2 +
EXO52.1
w/o RT → only DNA



- ◆ GAPDH_Exo50_DNA_w/o_PLG
- ◆ GAPDH_Exo50_DNA_w/_PLG
- ◆ GAPDH_Exo52_DNA
- ◆ GAPDH_AstraZeneca

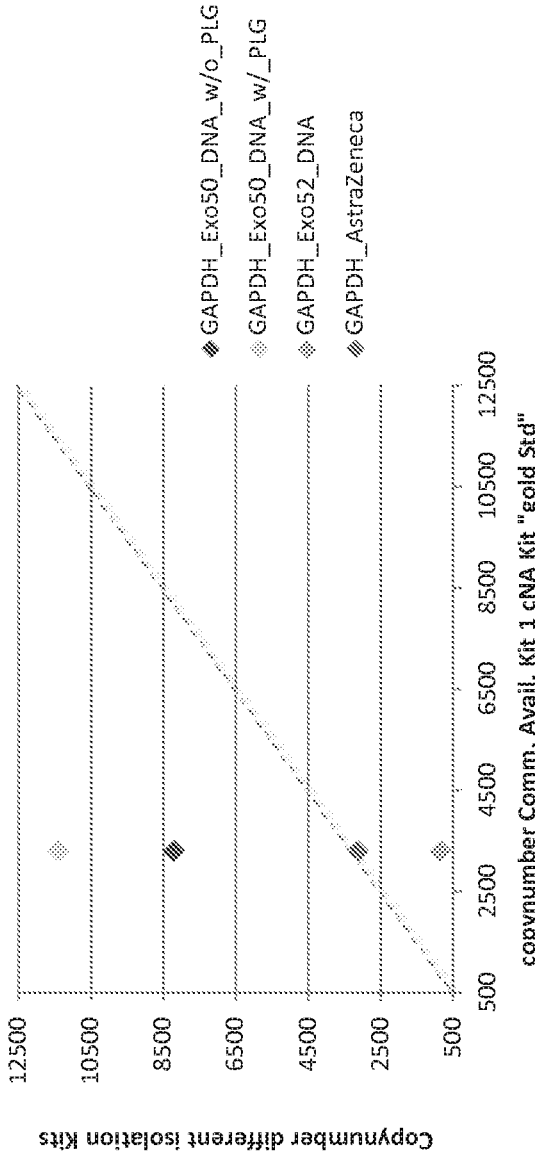
Figure

Compared to gold Standard



EXO50 DNA = EXO52.2
With and w/o PLG:
With RT → RNA + DNA

Comm. Avail. Kit 1 +
Comm. Avail. Kit 2 +
EXO52.1
w/o RT → only DNA



Figure

Result: Comparison of cfDNA copy number within different KITS

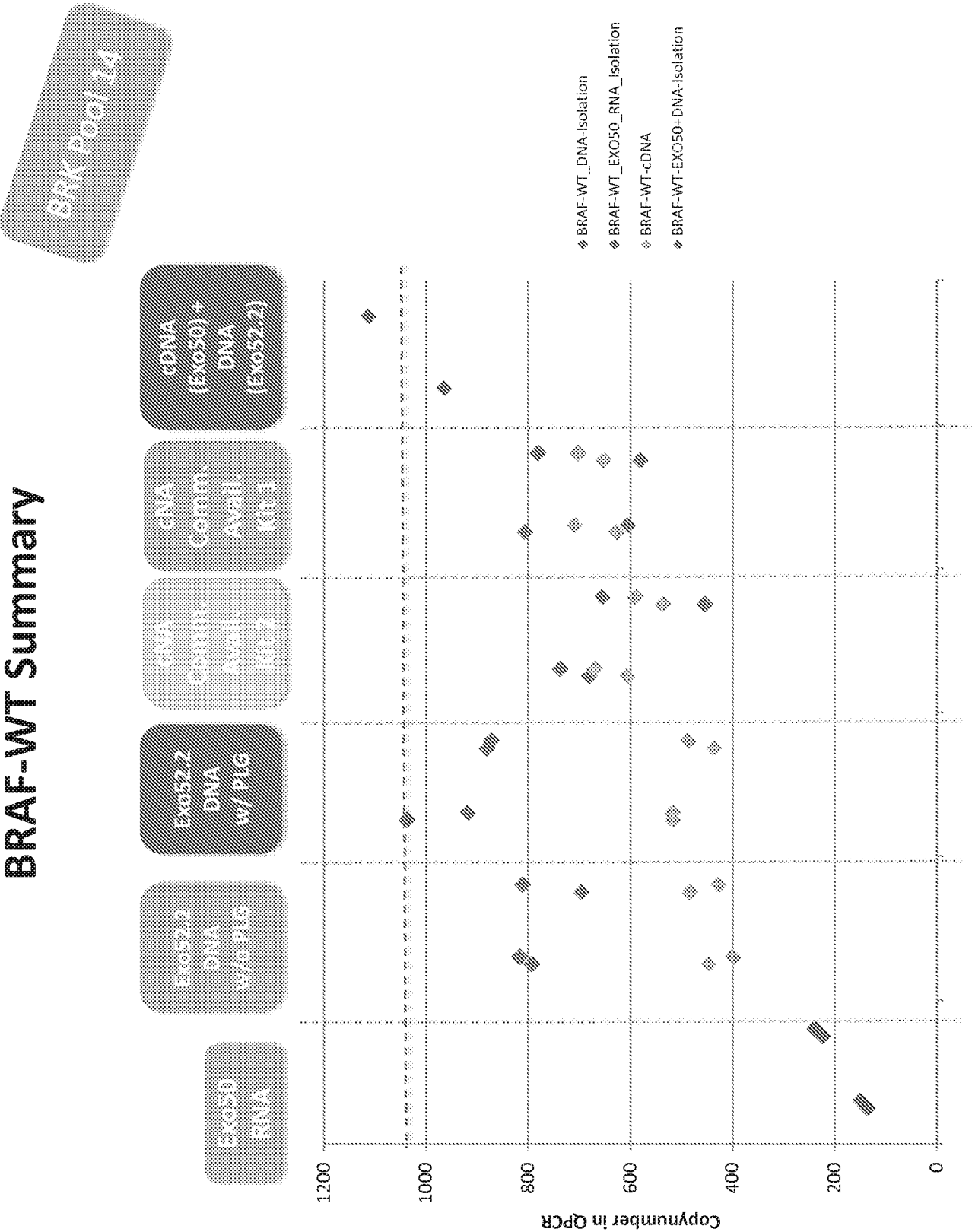
Copynumber in QPCR	BRAF-WT		GAPDH_RNA+DNA	
	Min	Max	Min	Max
Qiagen QIAamp circulating nucleic acid Kit *	500	700	3000	4000
AstraZeneca *	400	700	3000	3500
Exo50 DNA Isolation w/o PLG *	350	450	2000	2500
Exo50 DNA Isolation with PLG *	350	500	2500	3000
Exo52 DNA Isolation *	150	200	1000	1500

Copynumber in QPCR	BRAF-WT		GAPDH_RNA+DNA	
	MIN	MAX	MIN	MAX
Qiagen QIAamp circulating nucleic acid kit*	500	800	3000	4000
AstraZeneca*	500	750	3000	4000
EXO50 DNA Isolation w/o PLG {Exo52.2}**	400	800	7500	16500
EXO50 DNA Isolation w/ PLG {Exo52.2}**	500	900	10000	17000
EXO52.1 DNA Isolation*	100	250	700	1000
EXO50 RNA Isolation**	100	250	10000	15000

* samples without RT step → only DNA
** samples with RT step → contain transcript RNA and DNA

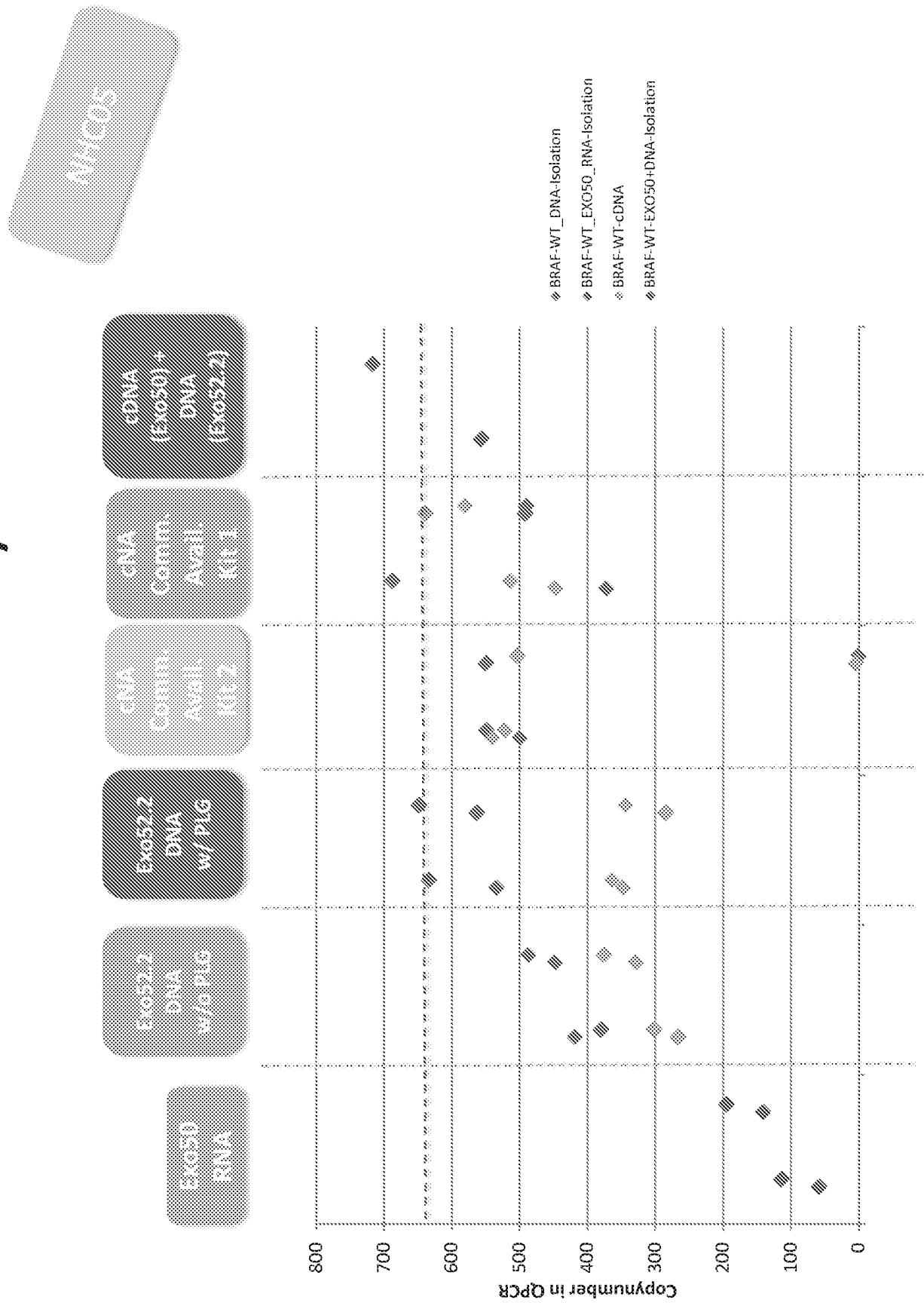
Figure

BRAF-WT Summary



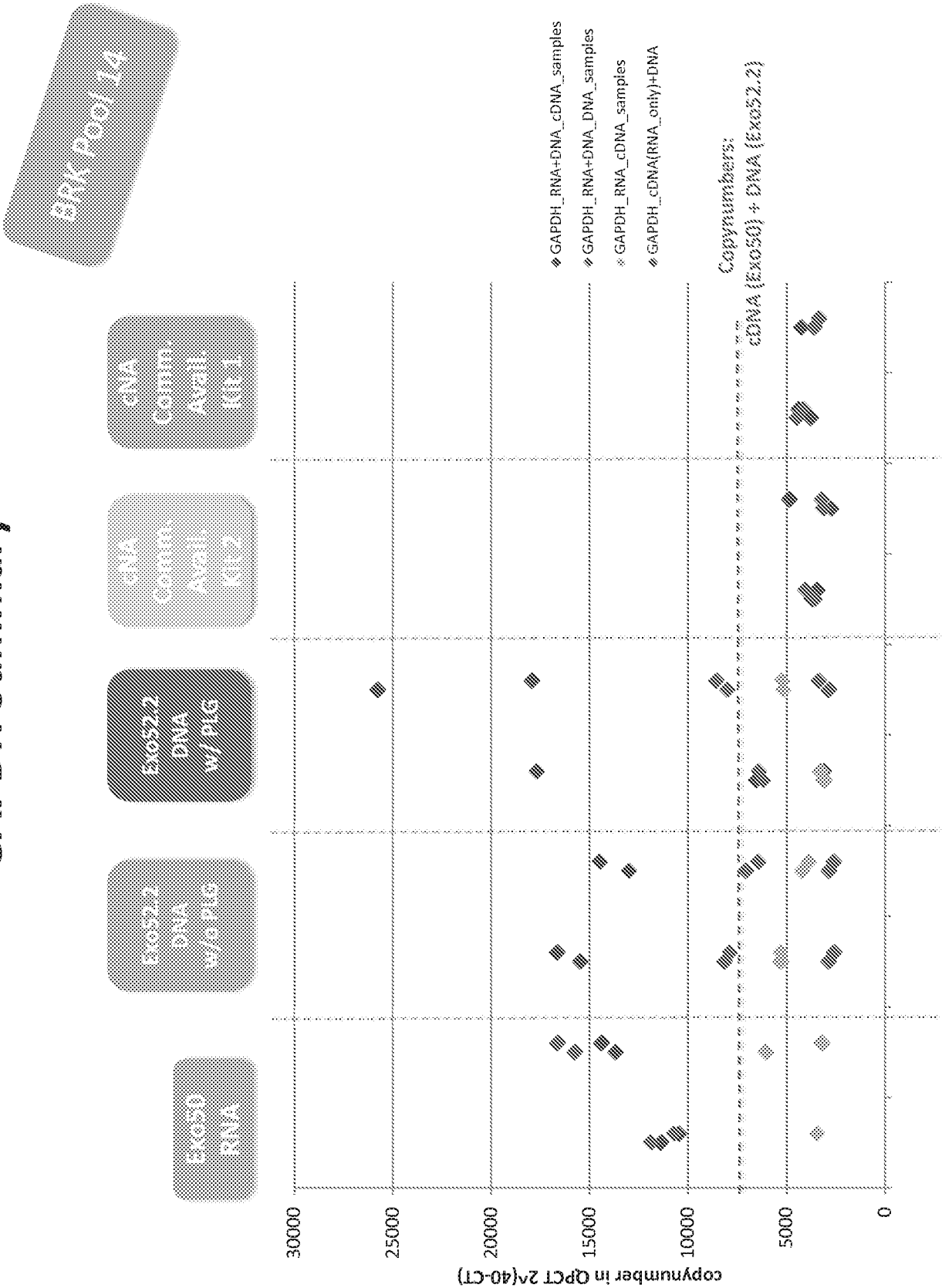
Figure

BRAF-WT Summary



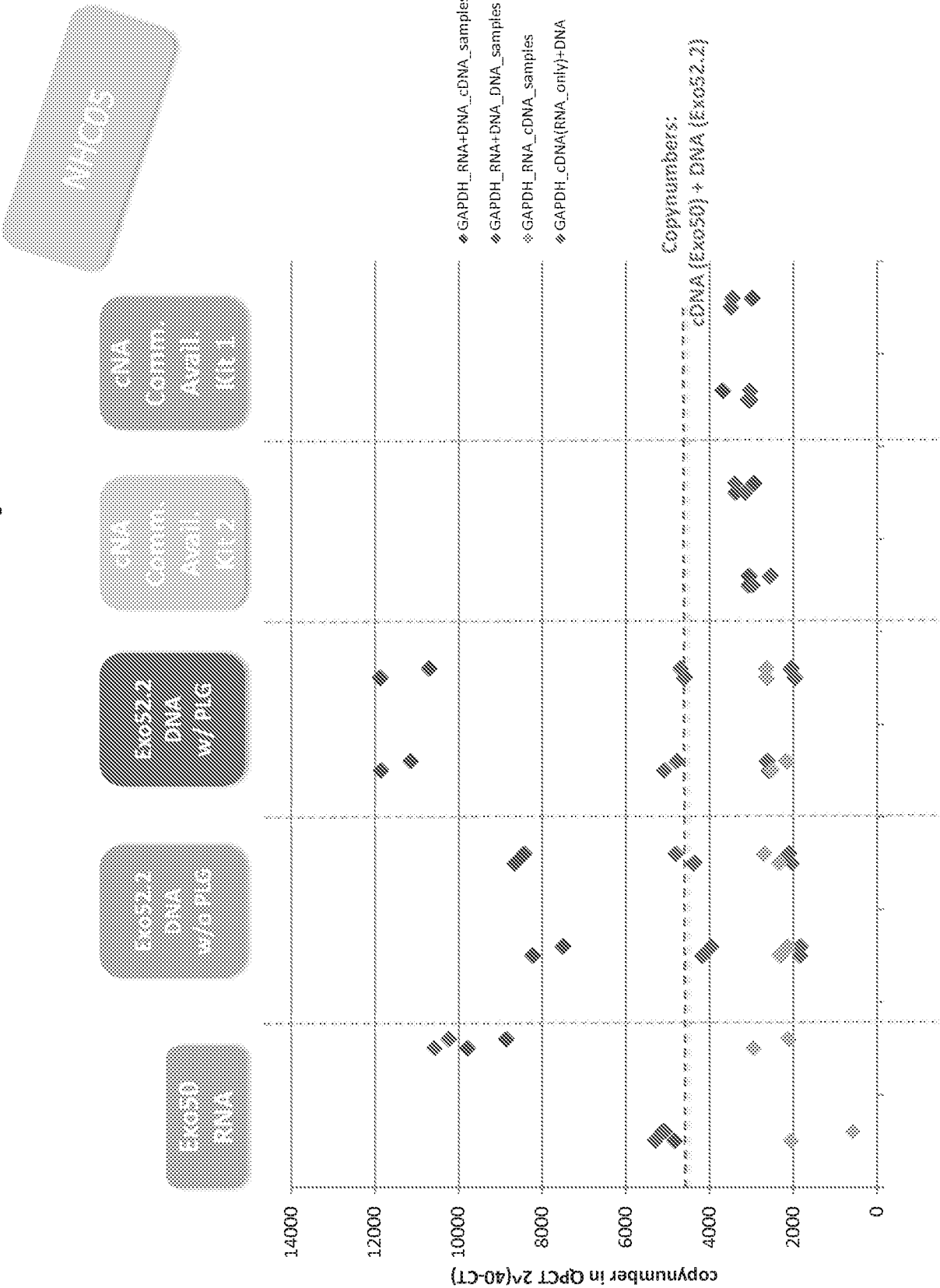
29

GAPDH Summary



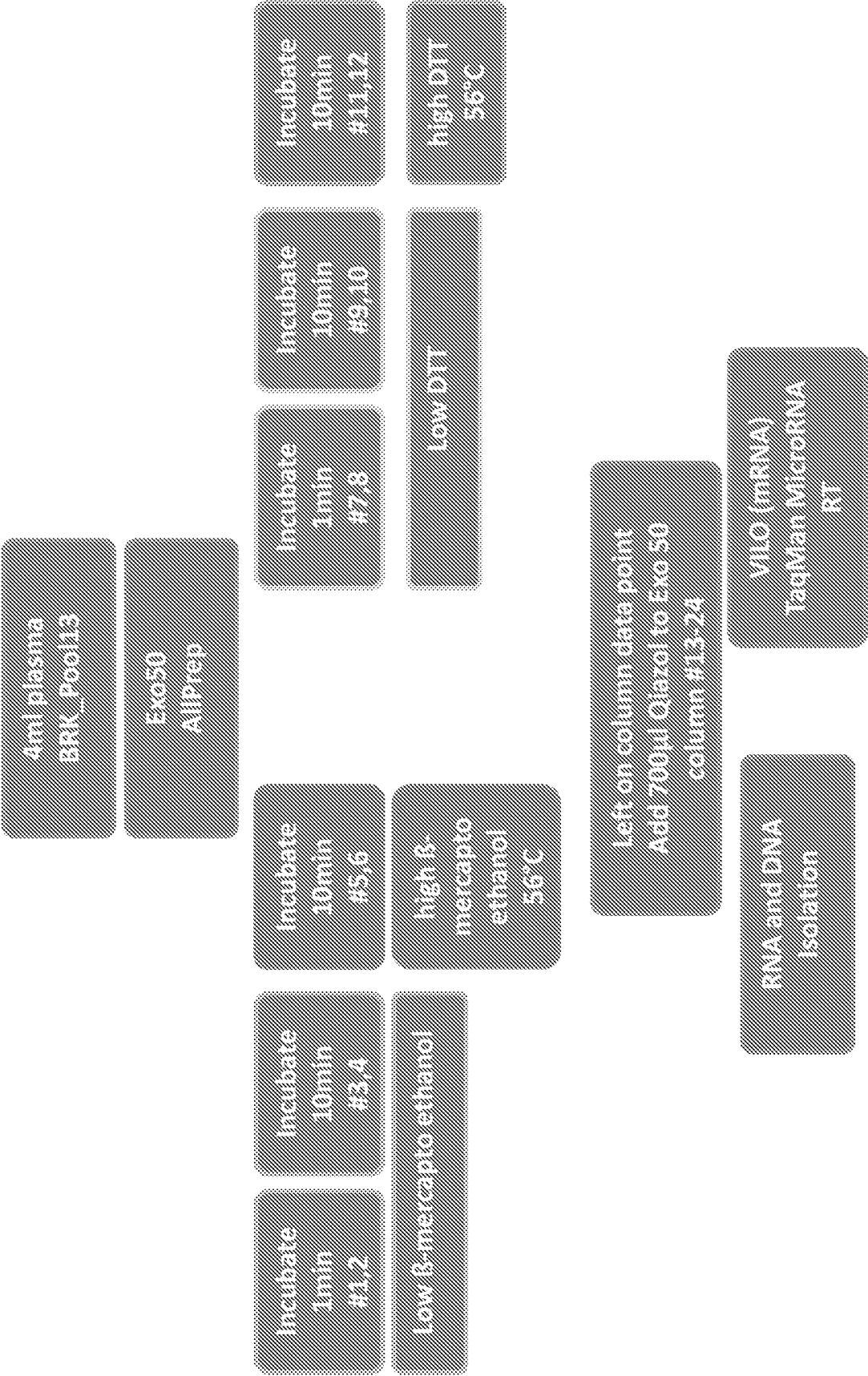
Figure

GAPDH Summary



Figure

Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure



Figure

Notes:

Carrier RNA: polyA from commercially available kit 0.2µg/µl = 200ng/µl → Dilute to 4ng/µl (1:50) and use 5µl per sample

β-mercaptoethanol 14.3 M

Low concentration: 10µl per 1ml RLT buffer → 143M

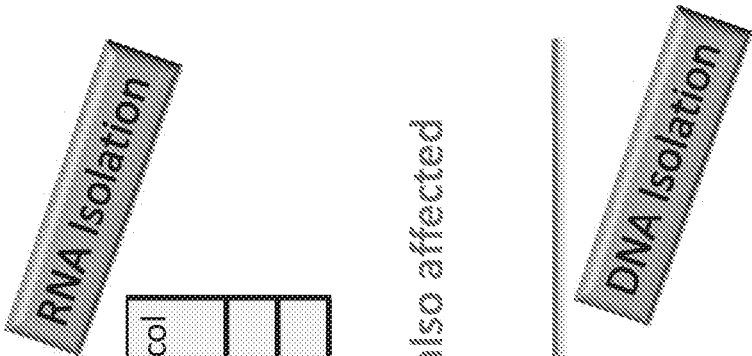
High concentration: 50µl per 1ml RLT buffer → 715M

DTT 1M

Kit recommendation 20µl of 2M DTT per 1ml RLT buffer

→ 40µl of 1M DTT per 1ml RLT (=low concentration) → 40M

→ 200µl of 1M DTT per 1ml RLT (=high concentration) → 200M



	recommendation	homemade protocol
Isolation mRNA	AllPrep MicroKit	Exo50
	1x 70% Ethanol	1.5x 100% Ethanol
Isolation total RNA (containing small RNAs)	1.5x 100% Ethanol	2x 100% Ethanol

RNeasy Mini Elute Columns clogging during binding step; further steps are also affected

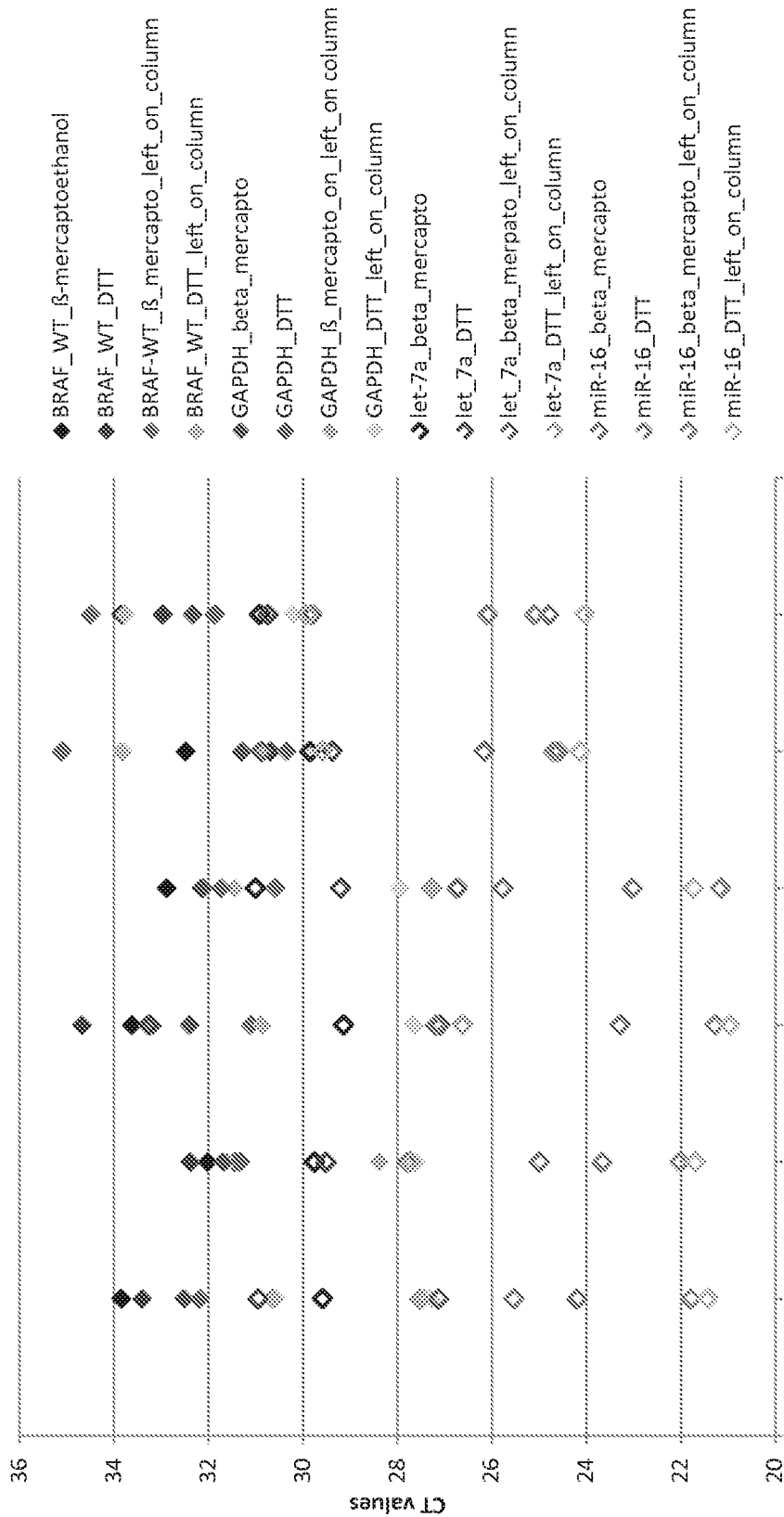
Specification	AllPrep DNA spin column	RNeasy MiniElute spin column
Maximum binding capacity	100 µg DNA*	45 µg RNA
Maximum loading volume	700 µl	700 µl
Nucleic acid size distribution	DNA of 15–30 kb†	RNA >200 nucleotides‡
Minimum elution volume	30 µl → 25µl	10 µl → 20µl
Maximum amount of starting material		
Animal and human cells	5 x 10 ⁵ cells	Entire flow-through from AllPrep DNA spin column
Animal and human tissues	5 mg	Entire flow-through from AllPrep DNA spin column

Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure

Concentration β -mercapto/DTT



RNA Isolation



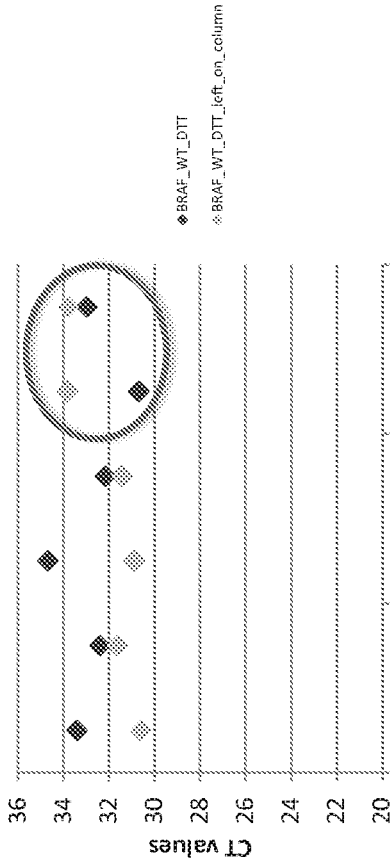
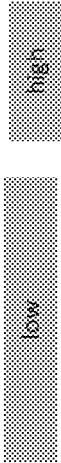
Incubation time on Exo50 column

Figure

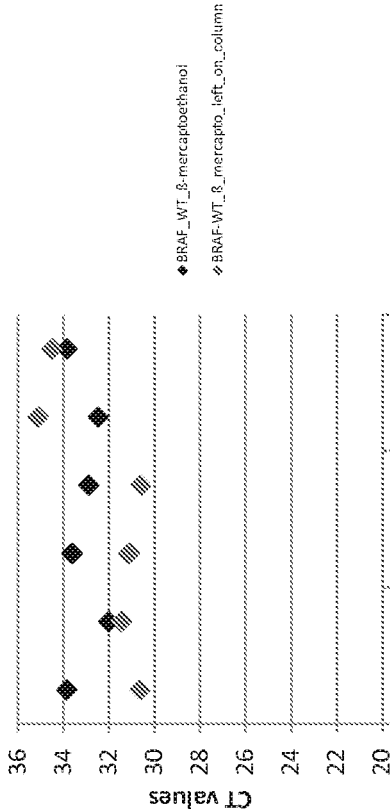
Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure

RNA Isolation
mRNA

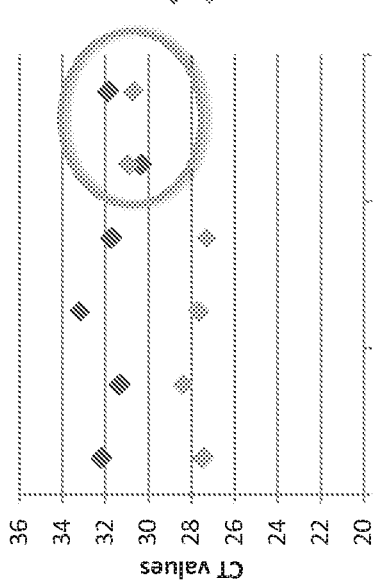
Concentration DTT



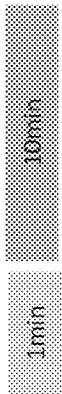
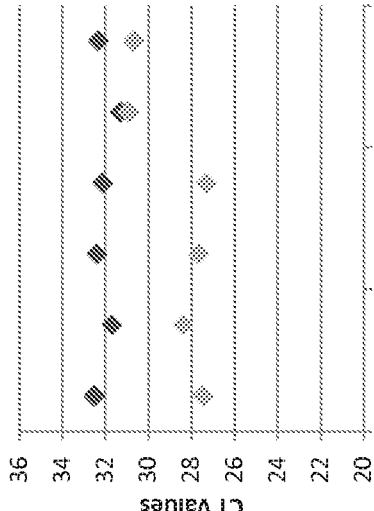
Concentration β -mercapto



◆ GAPDH_DTT
◇ GAPDH_β-mercapto_left_on_column



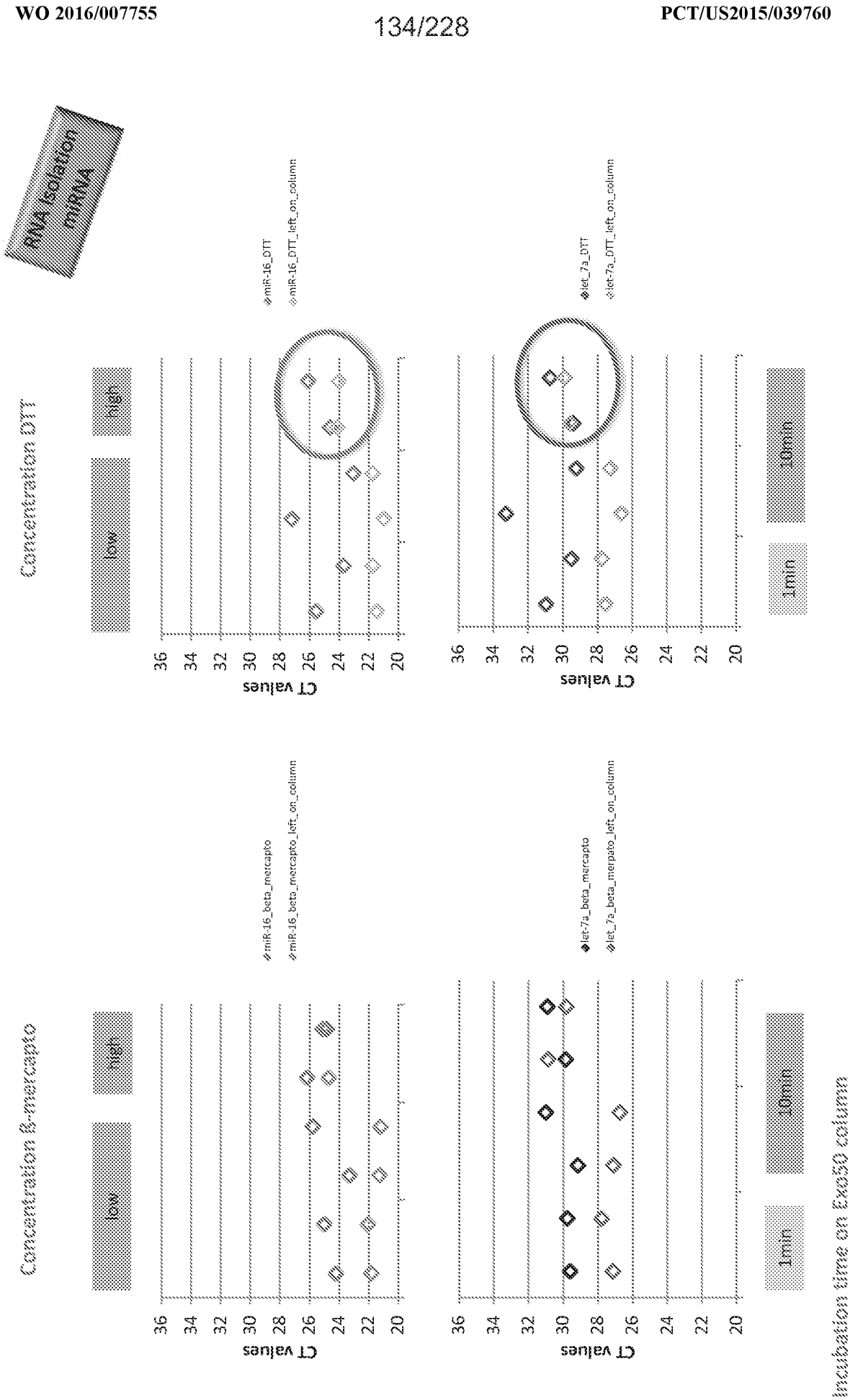
◆ GAPDH_beta_mercapto
◇ GAPDH_β-mercapto_left_on_column



Incubation time on Exo50 column

Figure

Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure



Figure

Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure

WO 2016/007755

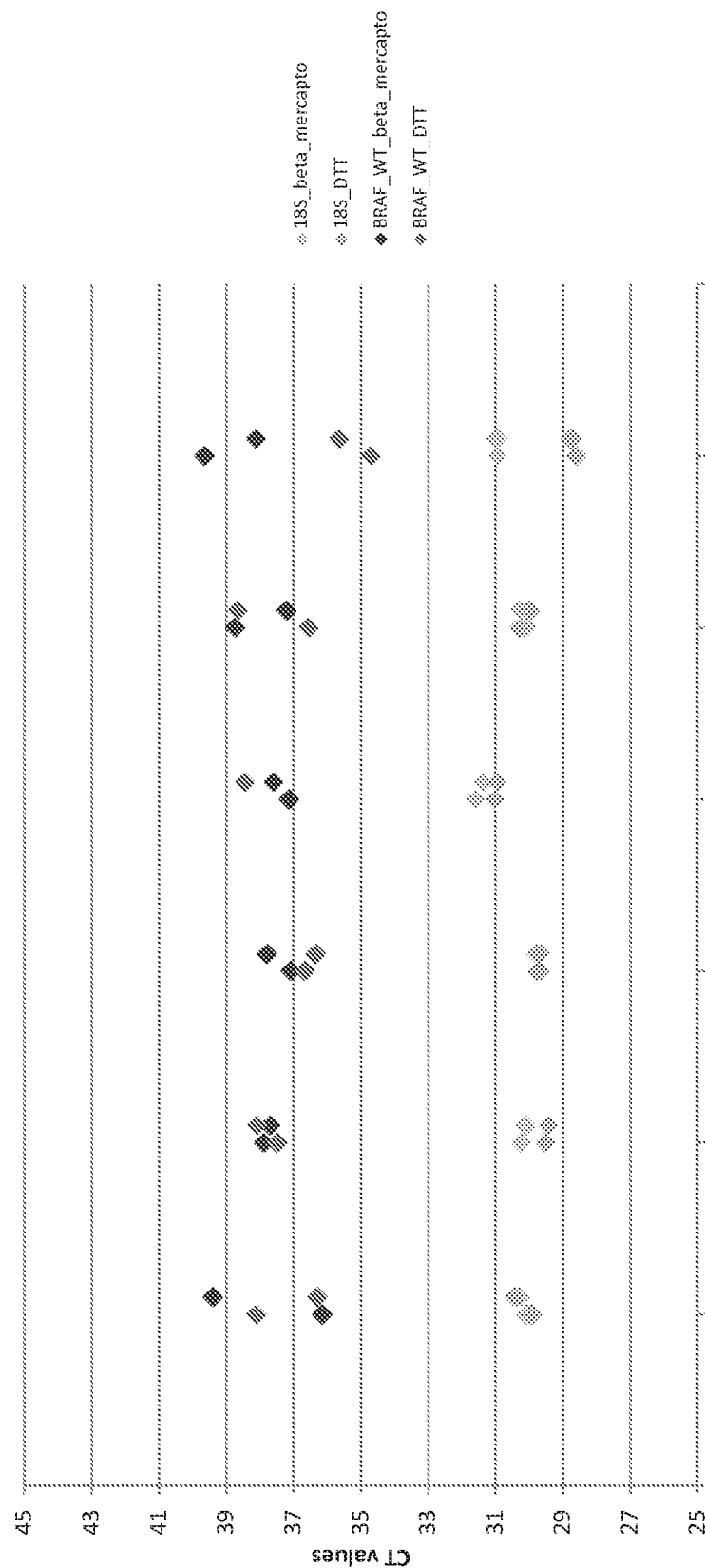
135/228

PCT/US2015/039760

DNA Isolation

Concentration β -mercapto/DTT

low high



1min 10min

Incubation time on Exo50 column

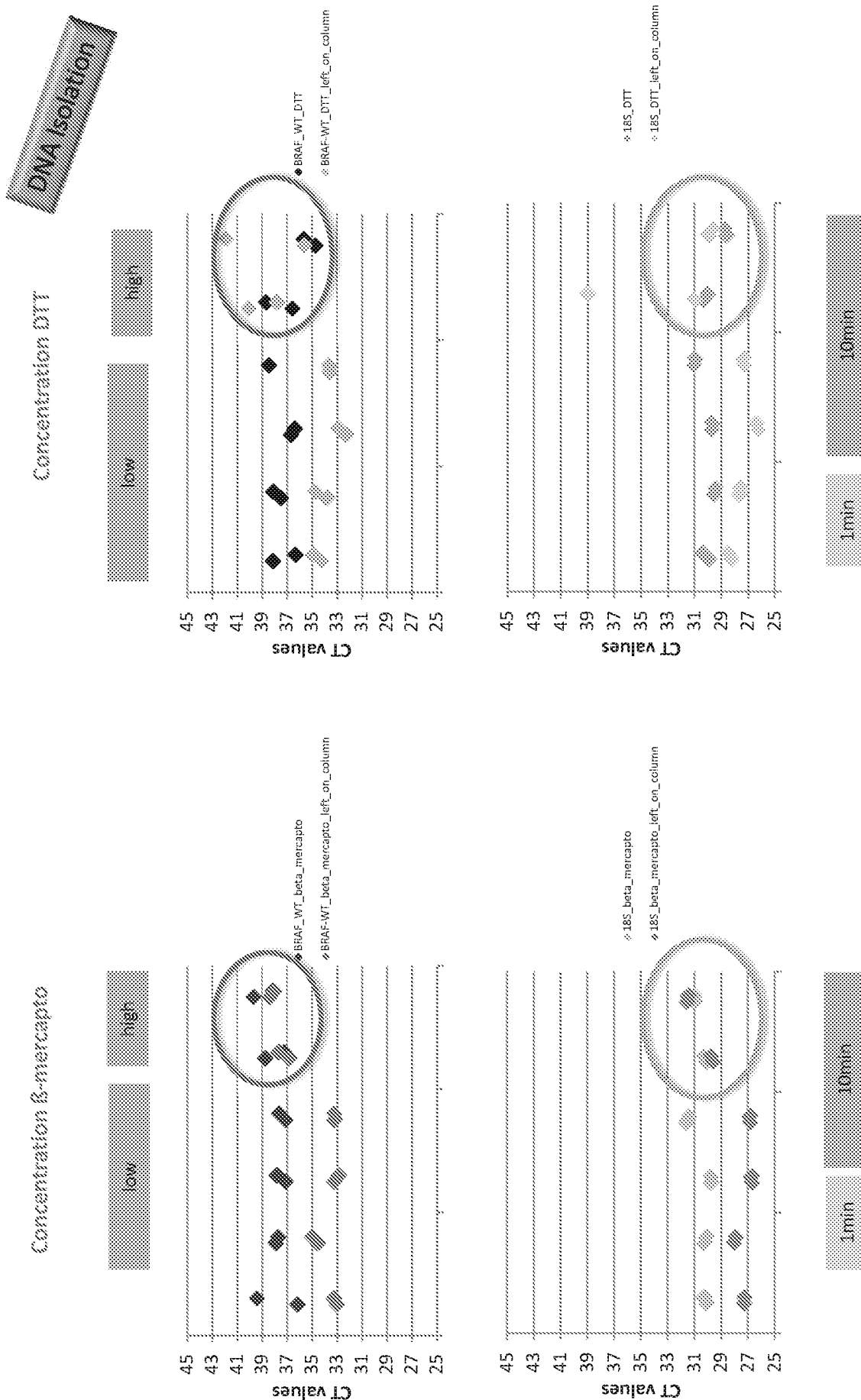
Figure

Exo50: AllPrep DNA/RNA Micro Kit for downstream procedure

WO 2016/007755

136/228

PCT/US2015/039760



Figure

cfDNA Isolation using different Isolations

Comm. Avail. Kit 1 circNA – cirmiNA – Exo52.2 old –

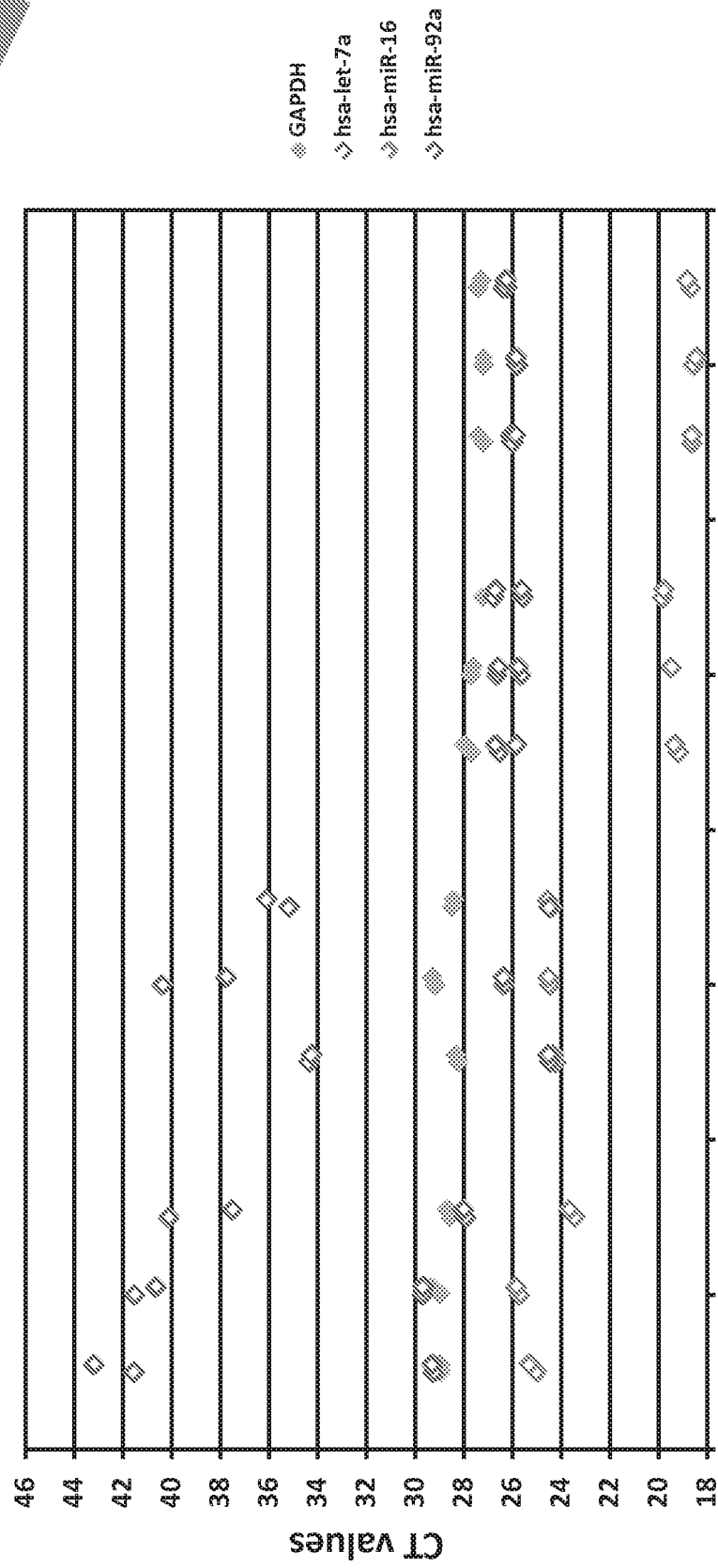
Exo52.2 new

4200

ANNOUNCING

BOOKS

RNA specific assays



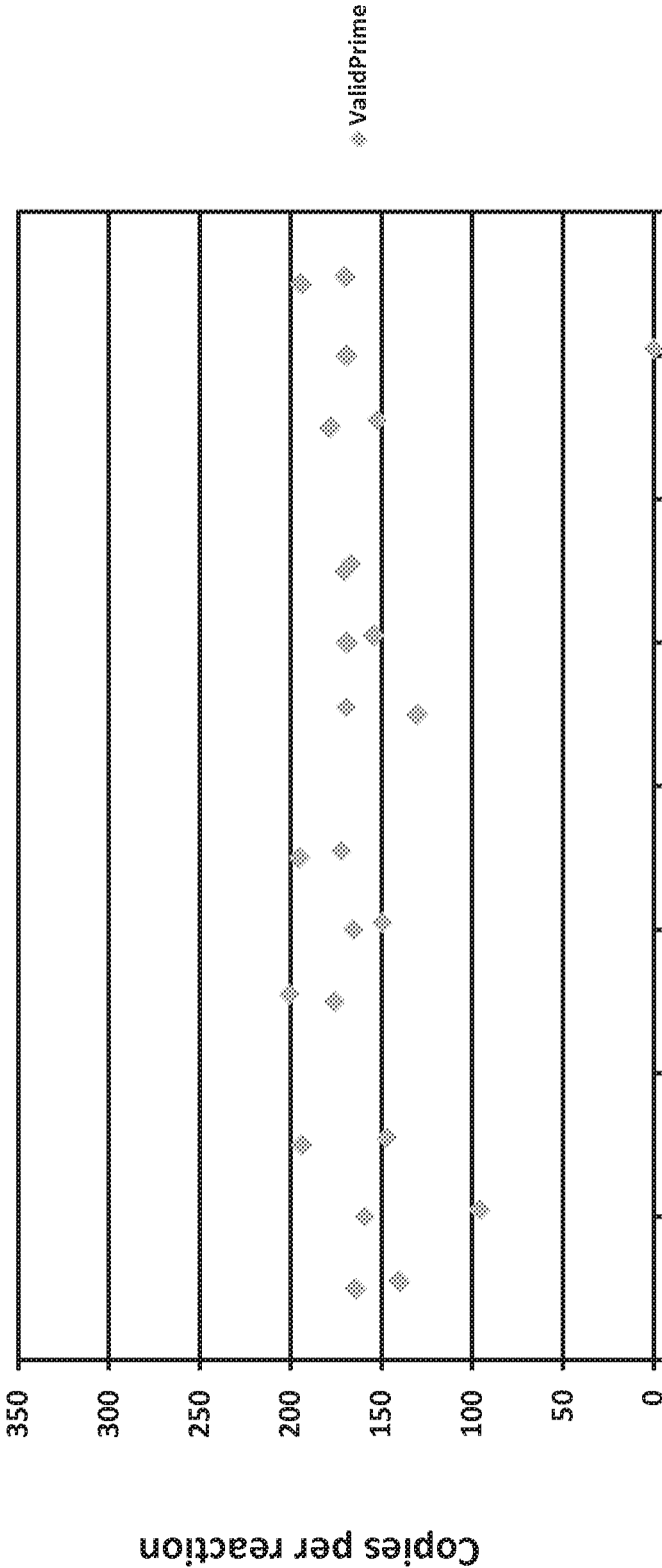
333

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirimiNA – Exo52.2 old –

Exo52.2 new

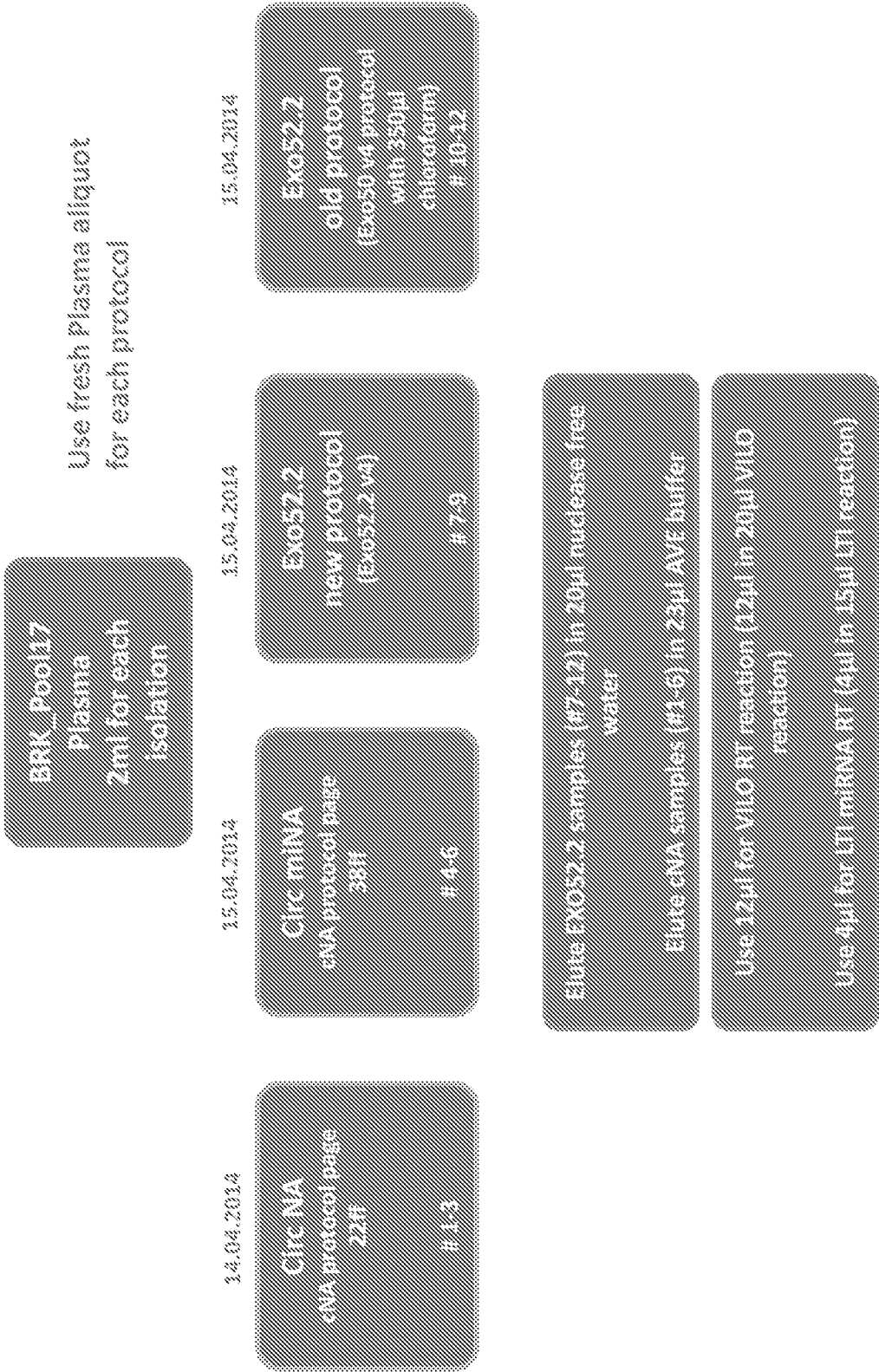
DNA specific assays



Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirRNA – Exo52.2 old – Exo52.2 new



Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirmiNA – Exo52.2 old –

Exo52.2 new

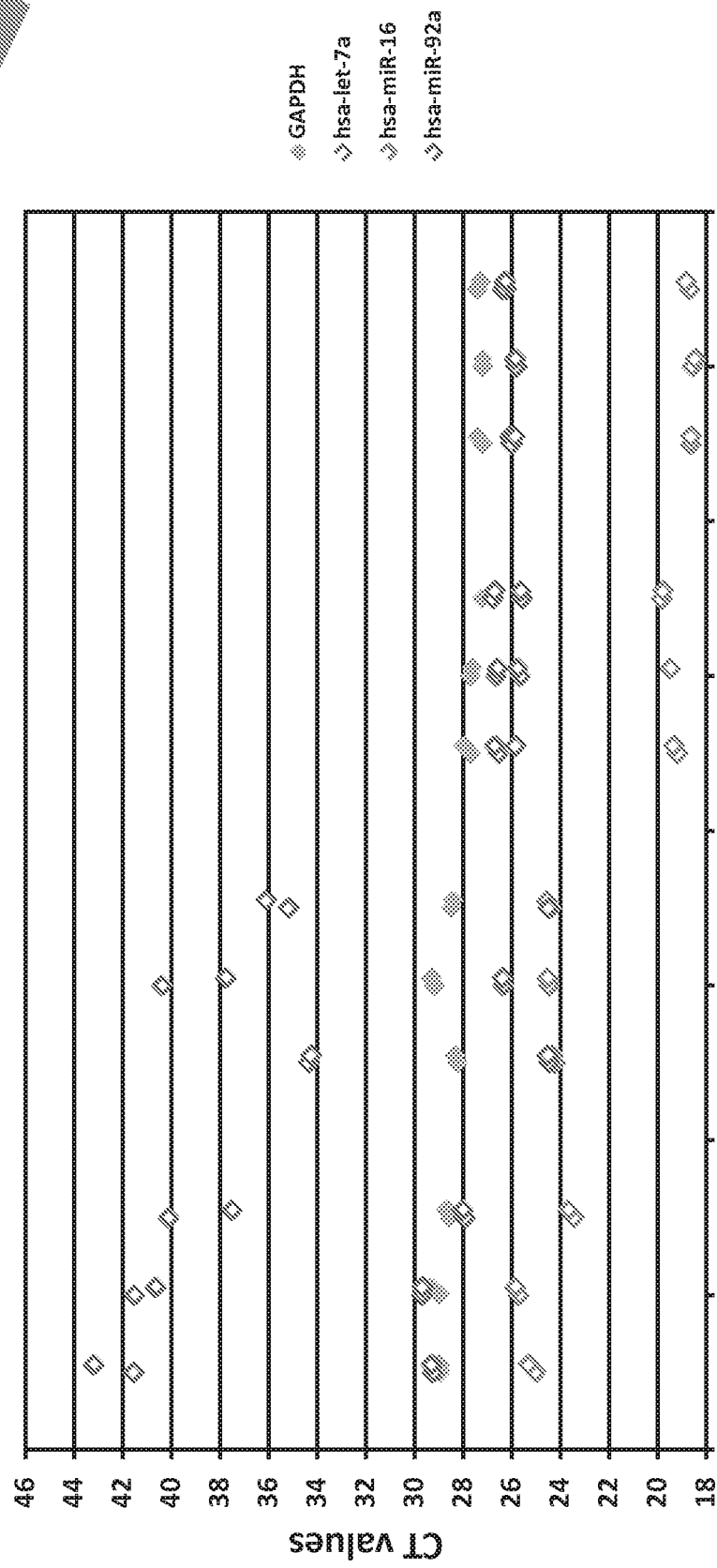
4700

42 ECHO

2000

Don't

RNA specific assays



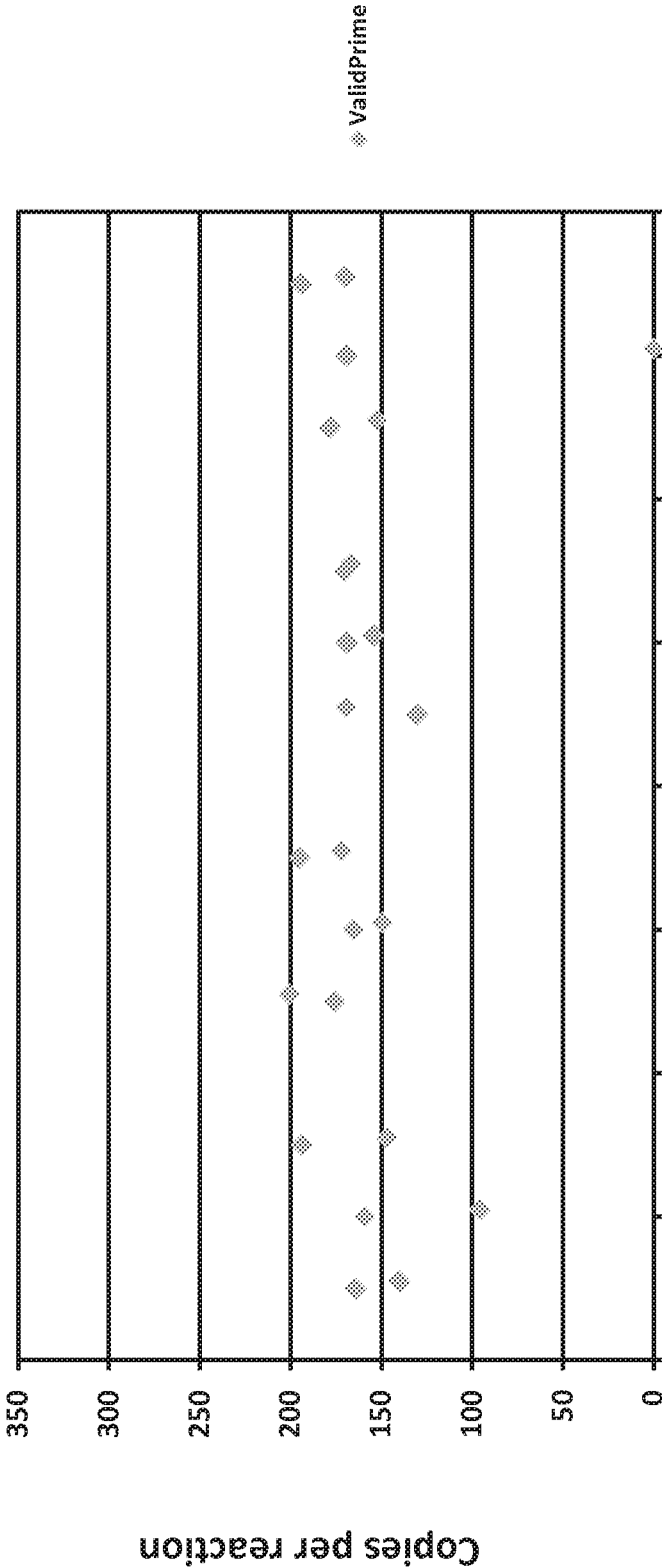
322

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirimiNA – Exo52.2 old –

Exo52.2 new

DNA specific assays

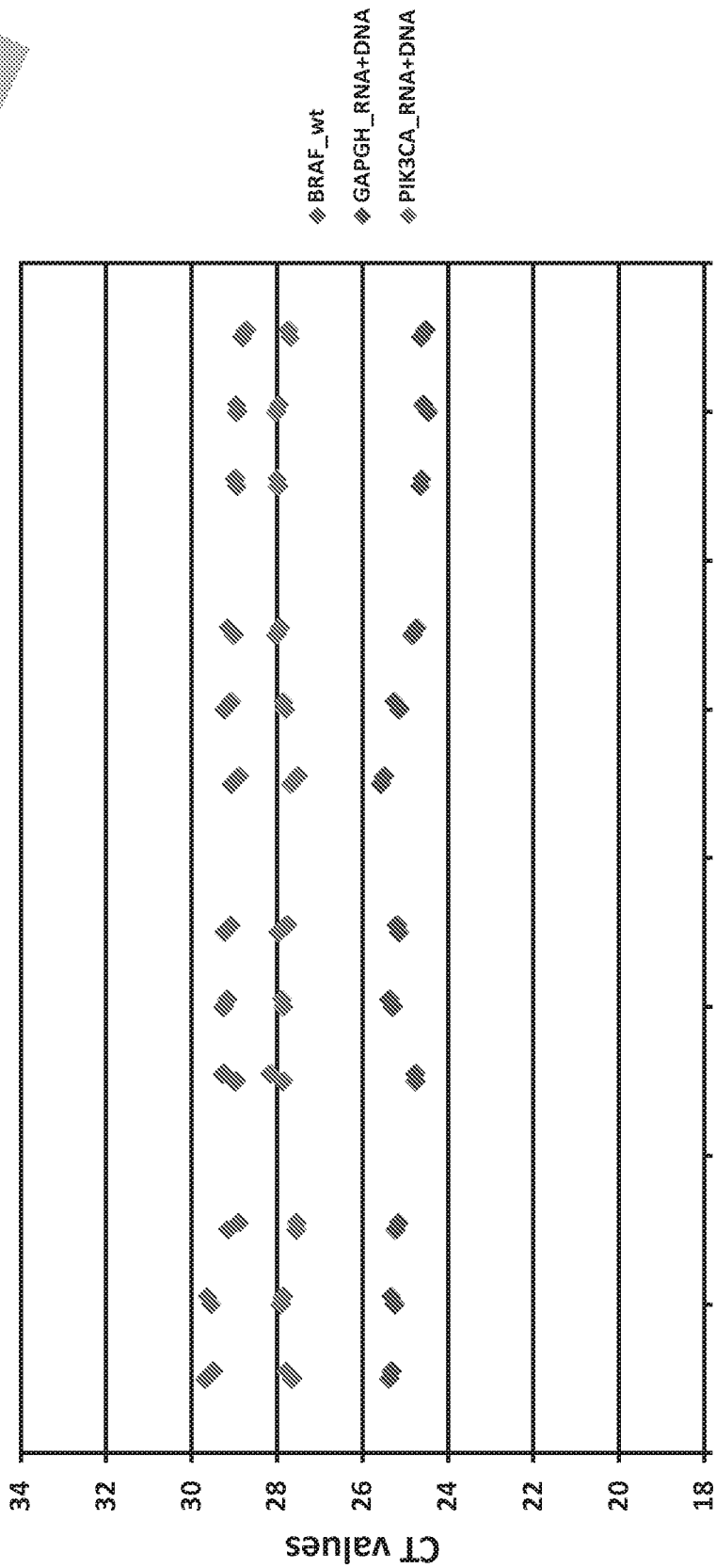
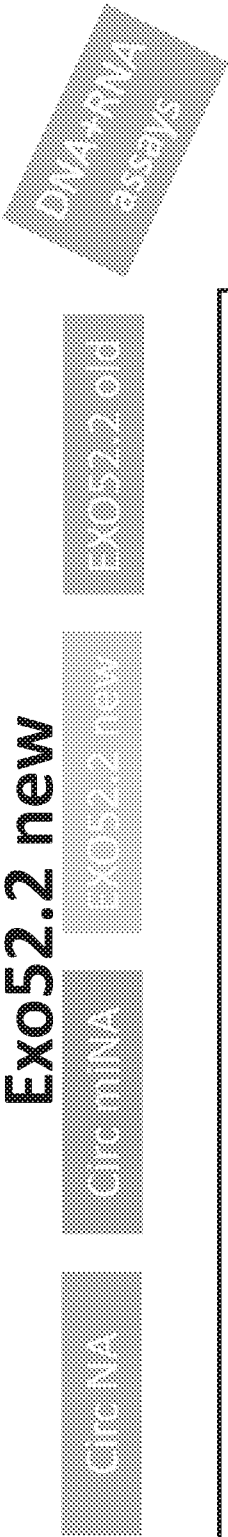


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirNA – Exo52.2 old –

Exo52.2 new

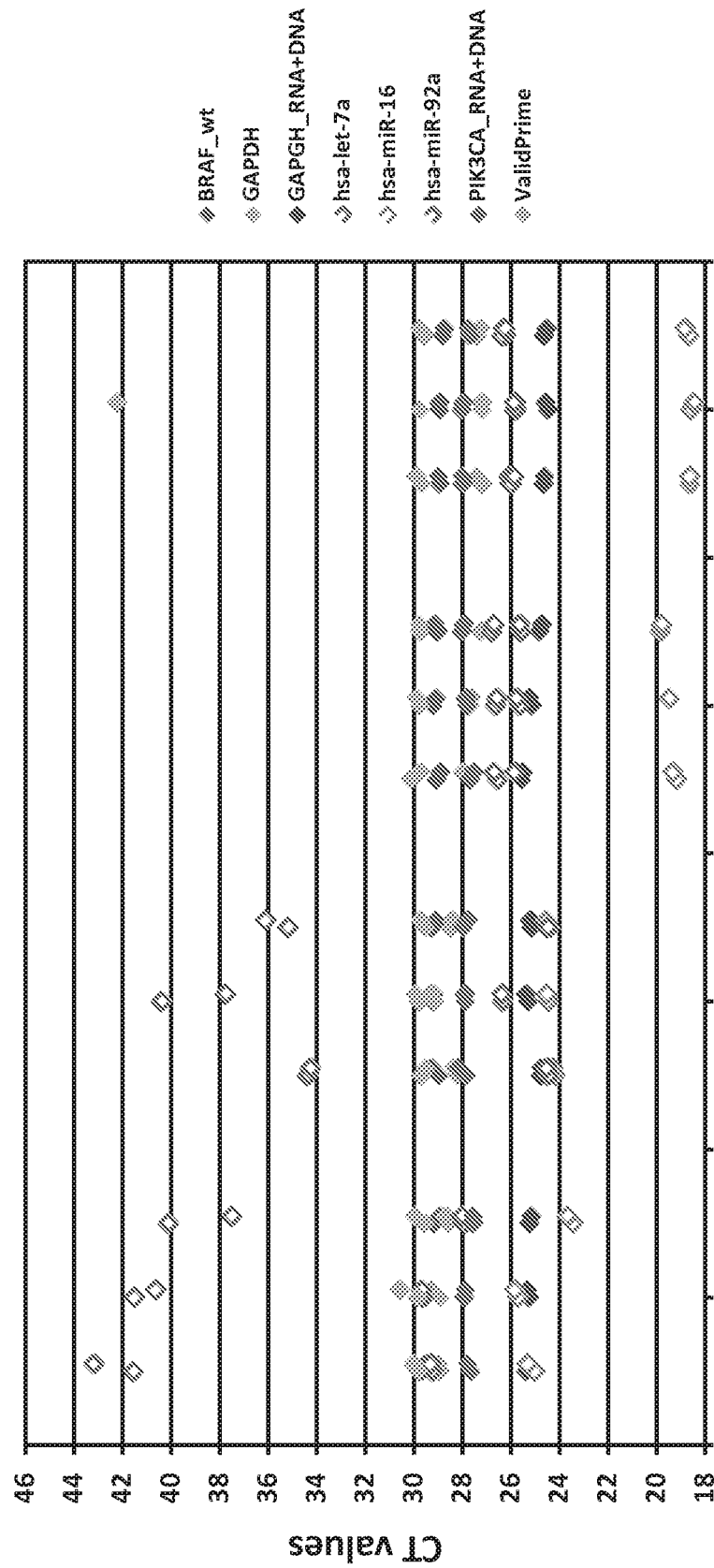


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirimiNA – Exo52.2 old –

Exo52.2 new

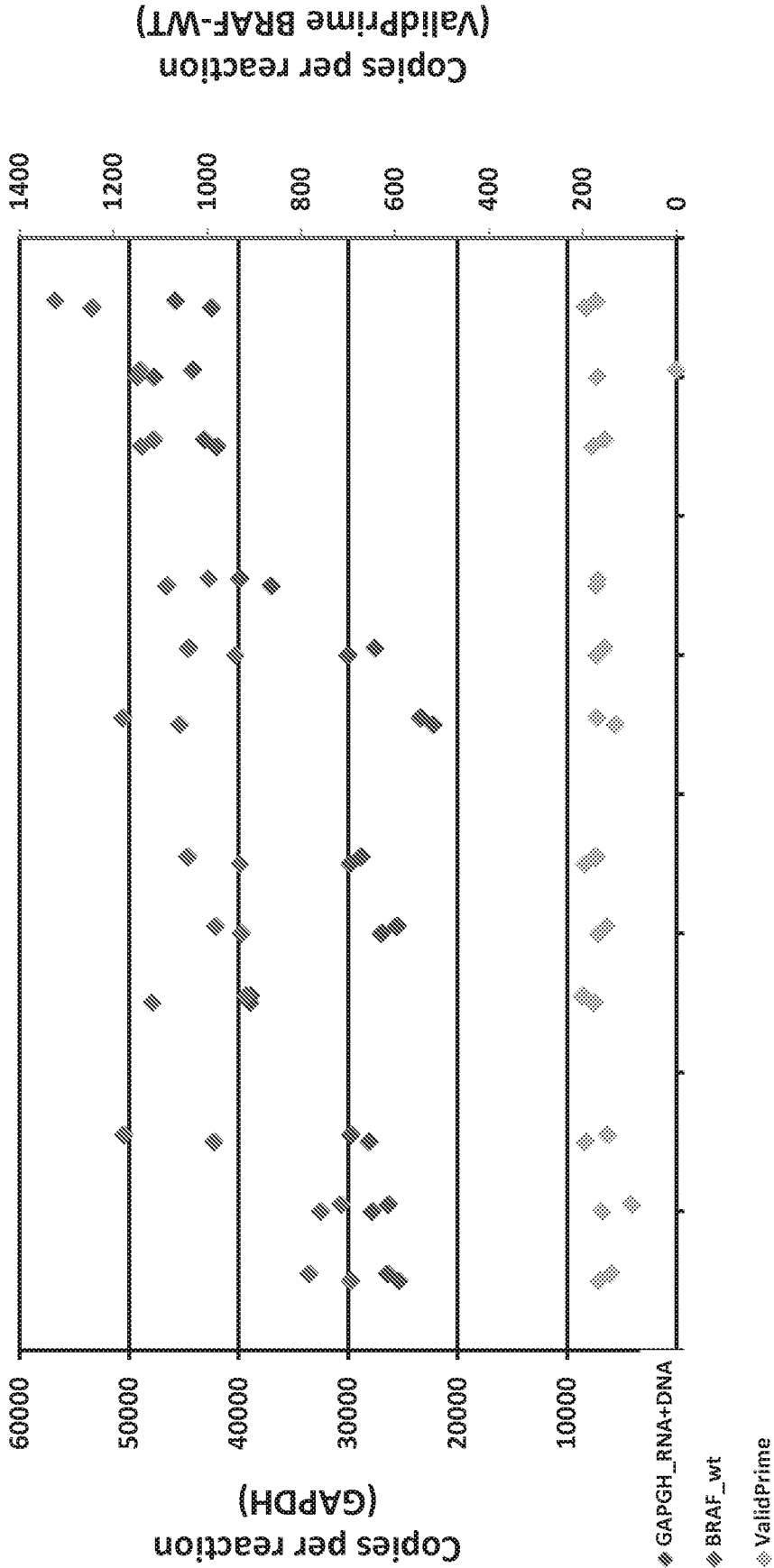


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirimiNA – Exo52.2 old –

Exo52.2 new

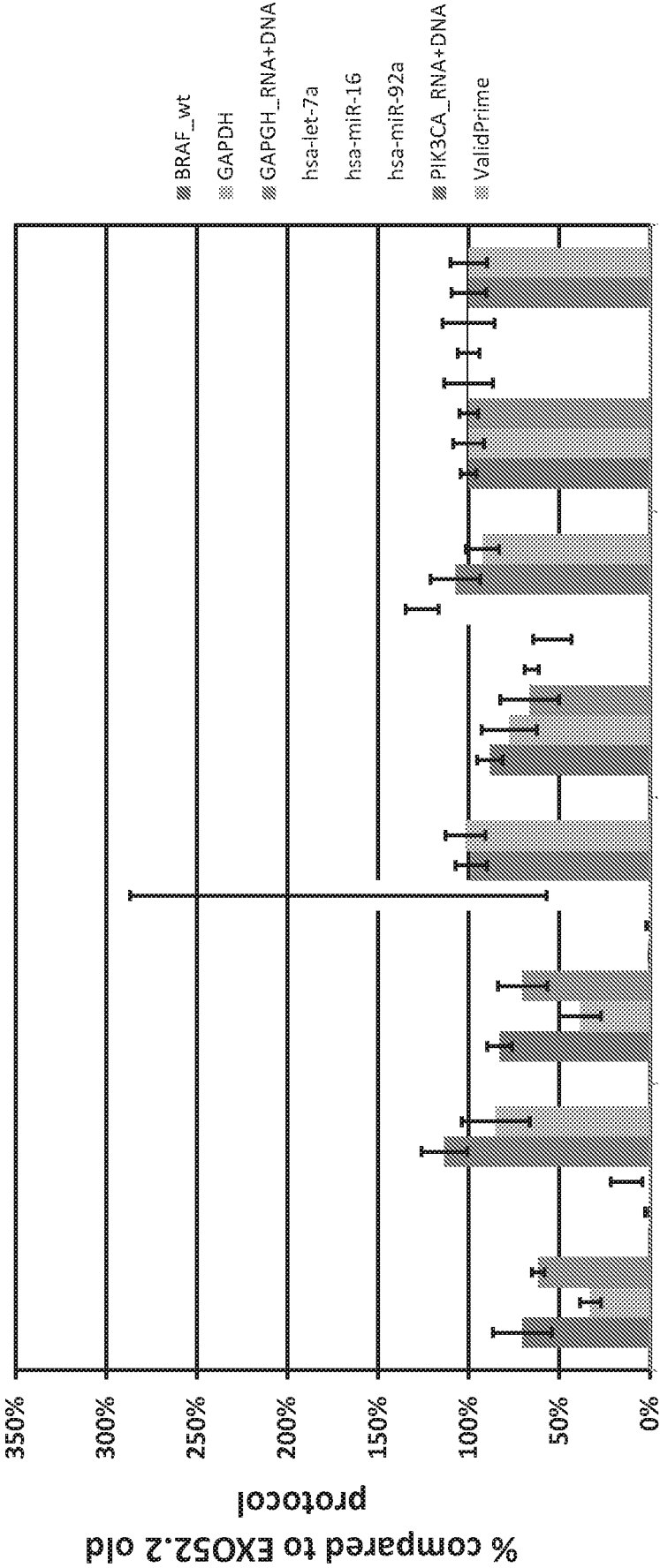


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – cirimiNA – Exo52.2 old –

Exo52.2 new

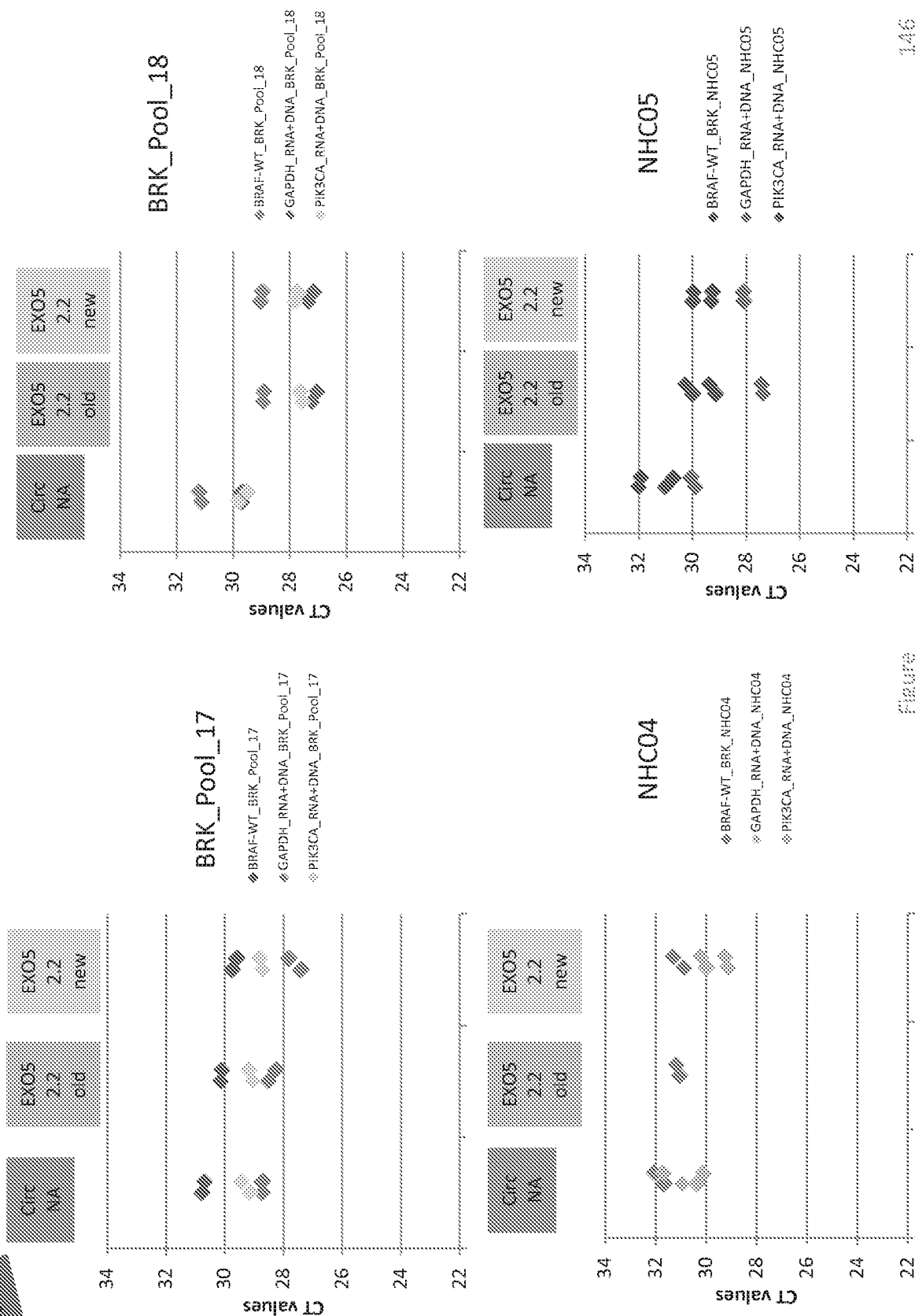


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

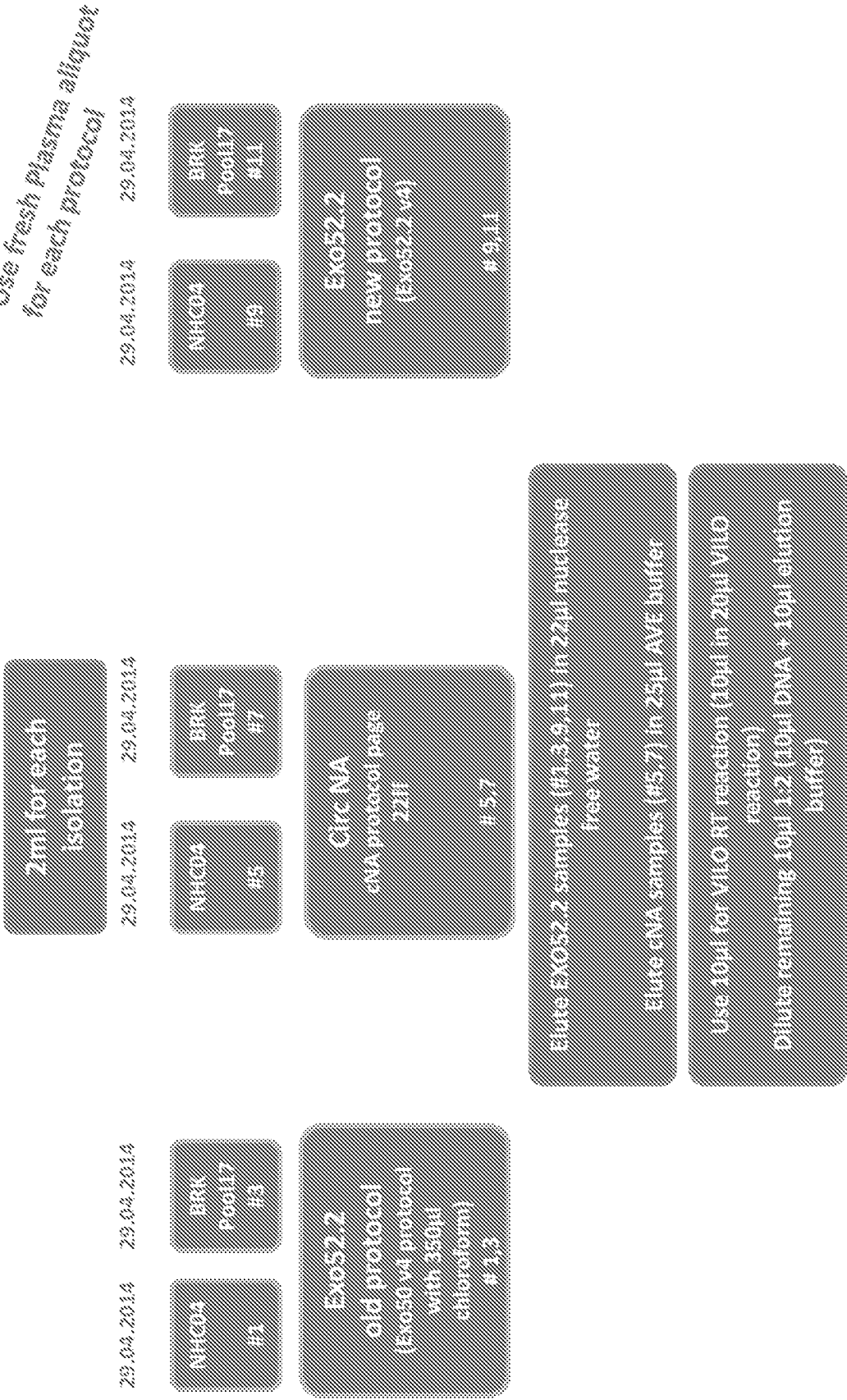
w/o RT



Figure

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

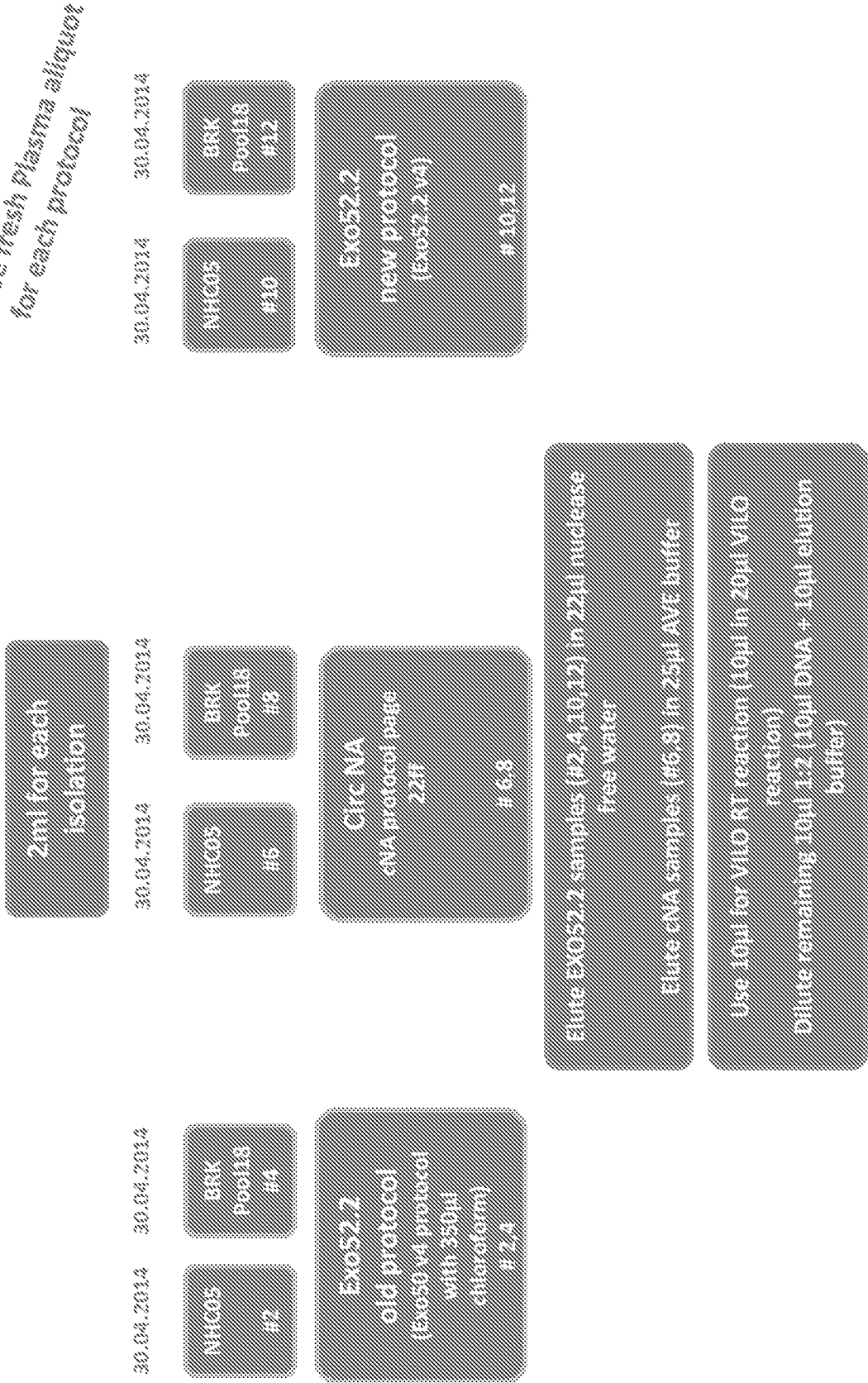
*Use fresh plasma aliquot
for each protocol*



Figure

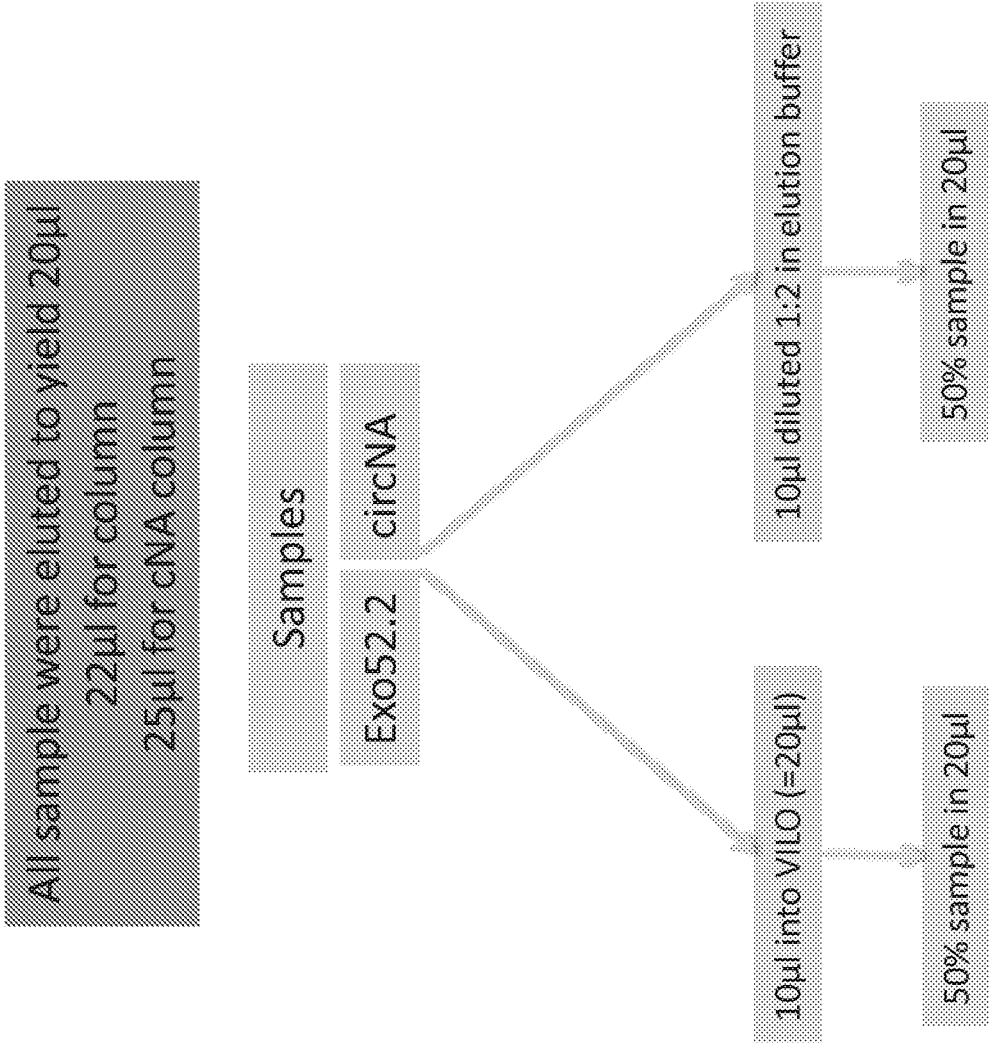
cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

*Use fresh plasma aliquot
for each protocol*



Figure

Dilution overview

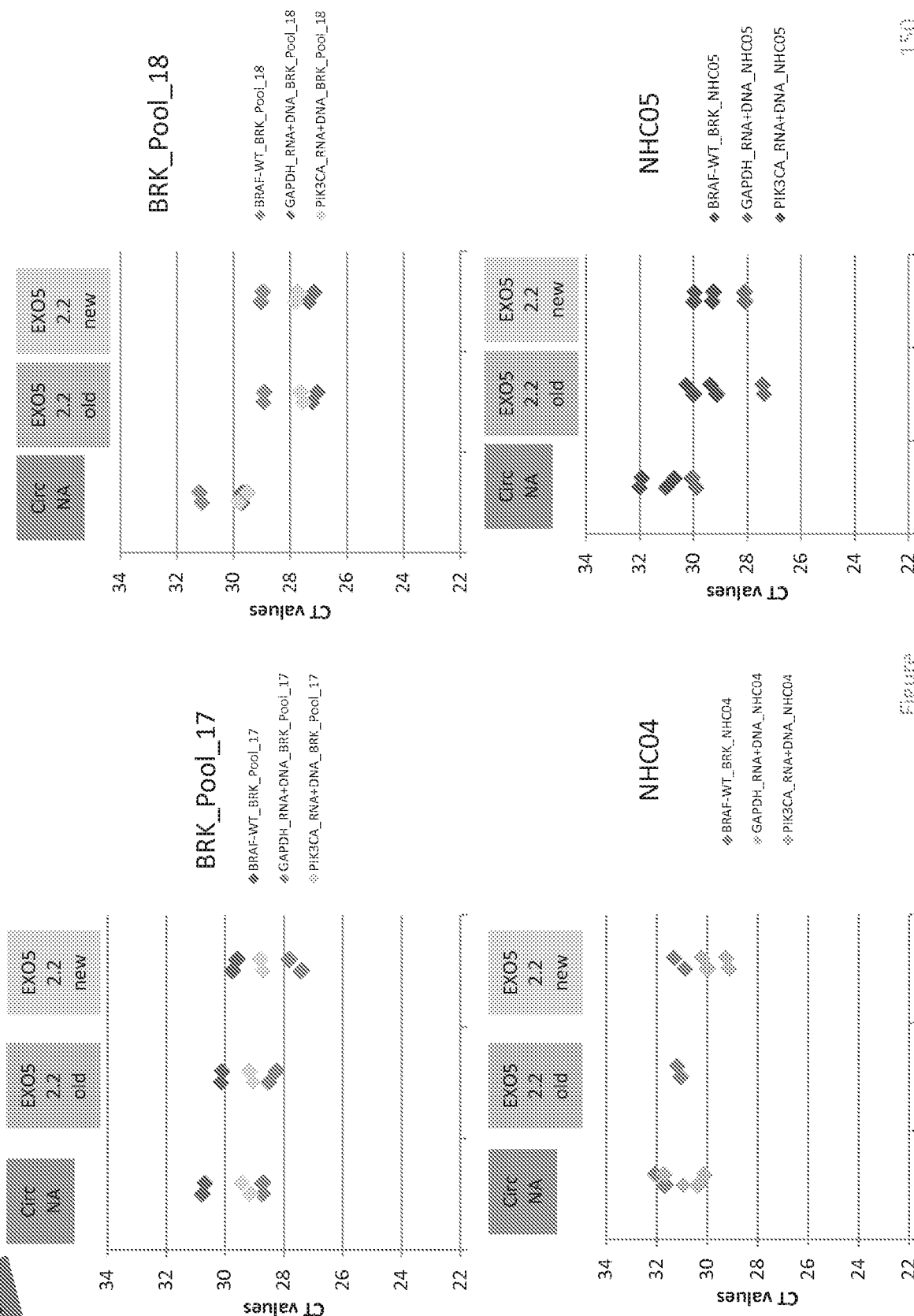


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

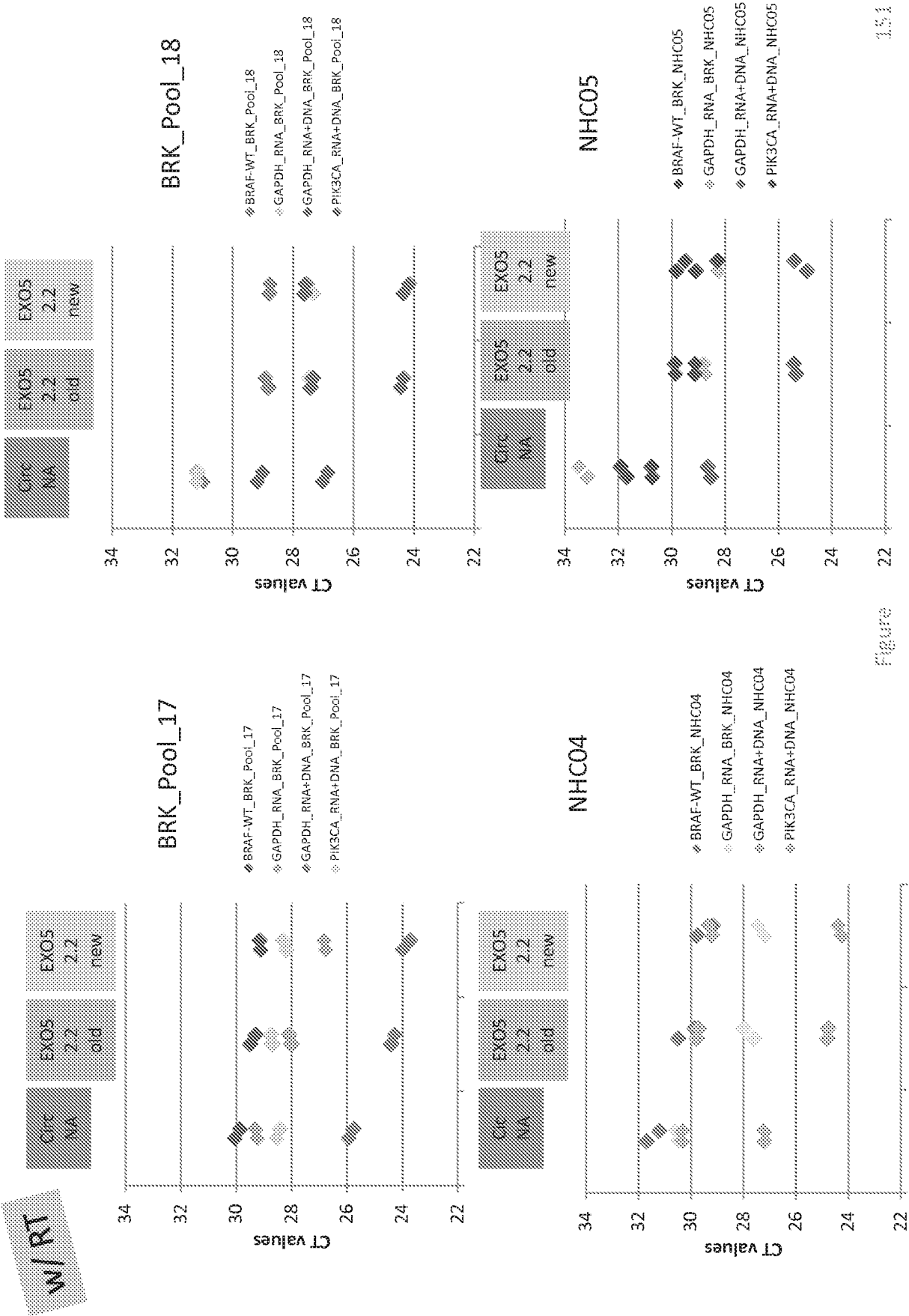
w/o RT



Figure

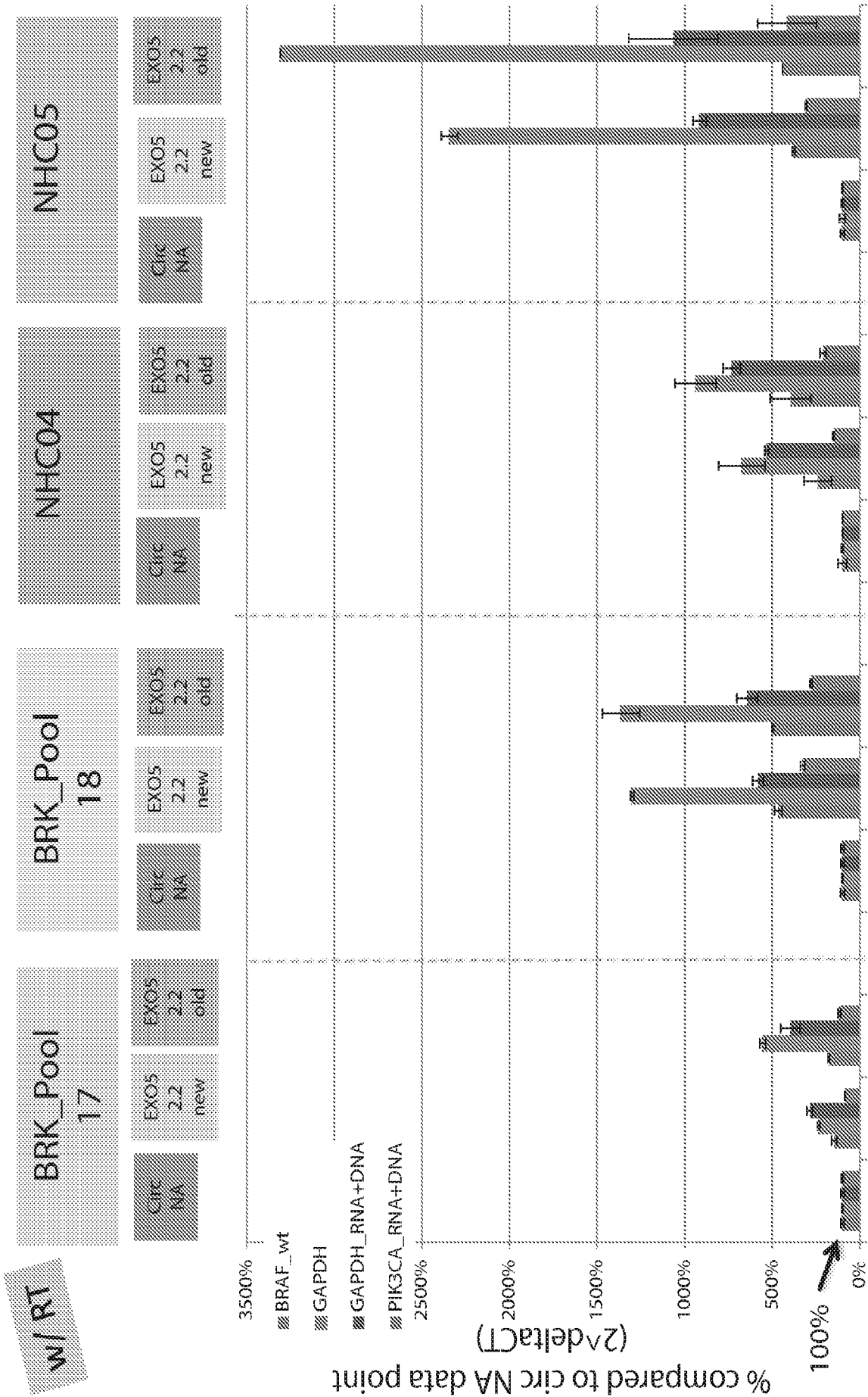
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



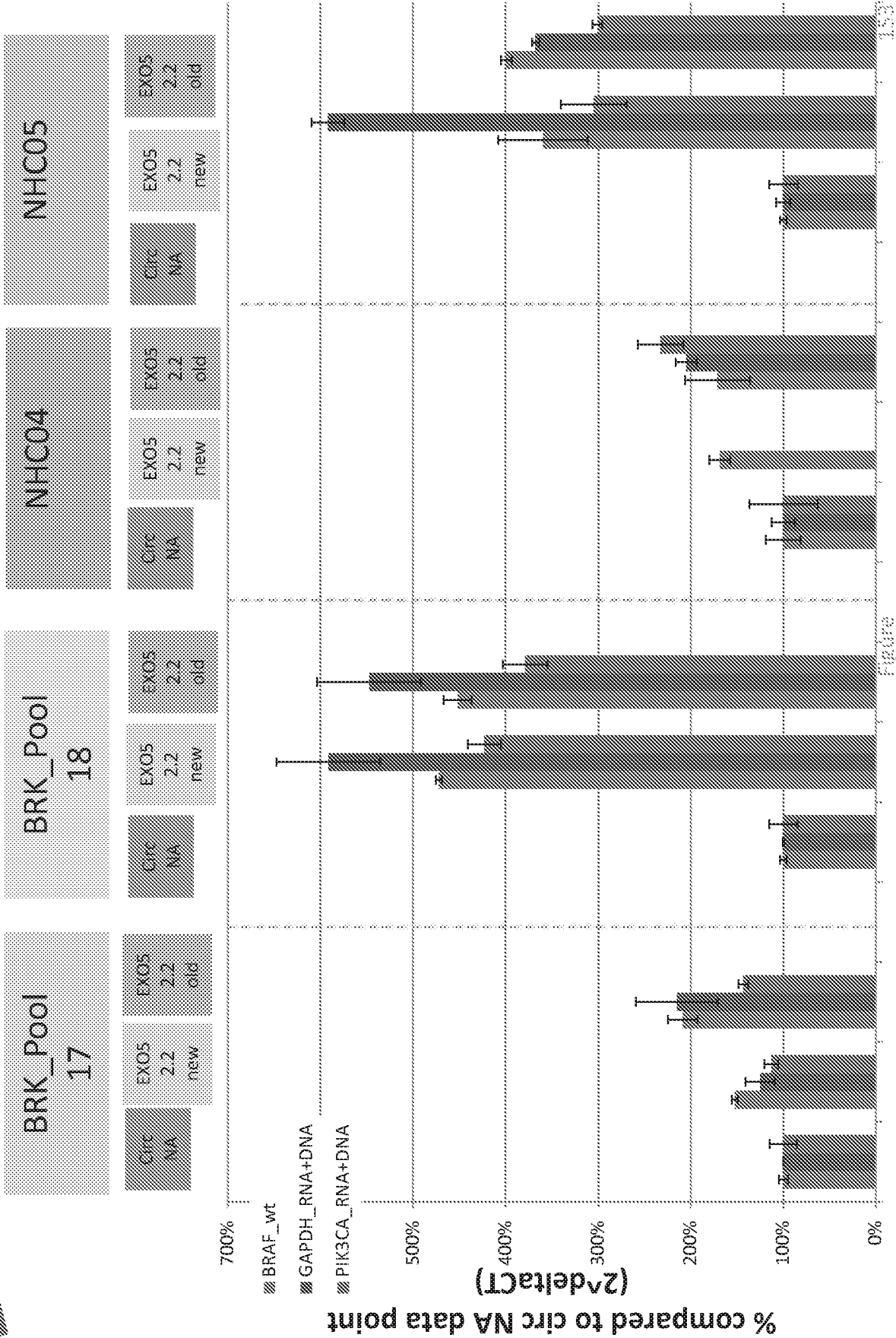
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



cfDNA Isolation using different Isolation techniques

w/o RT
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

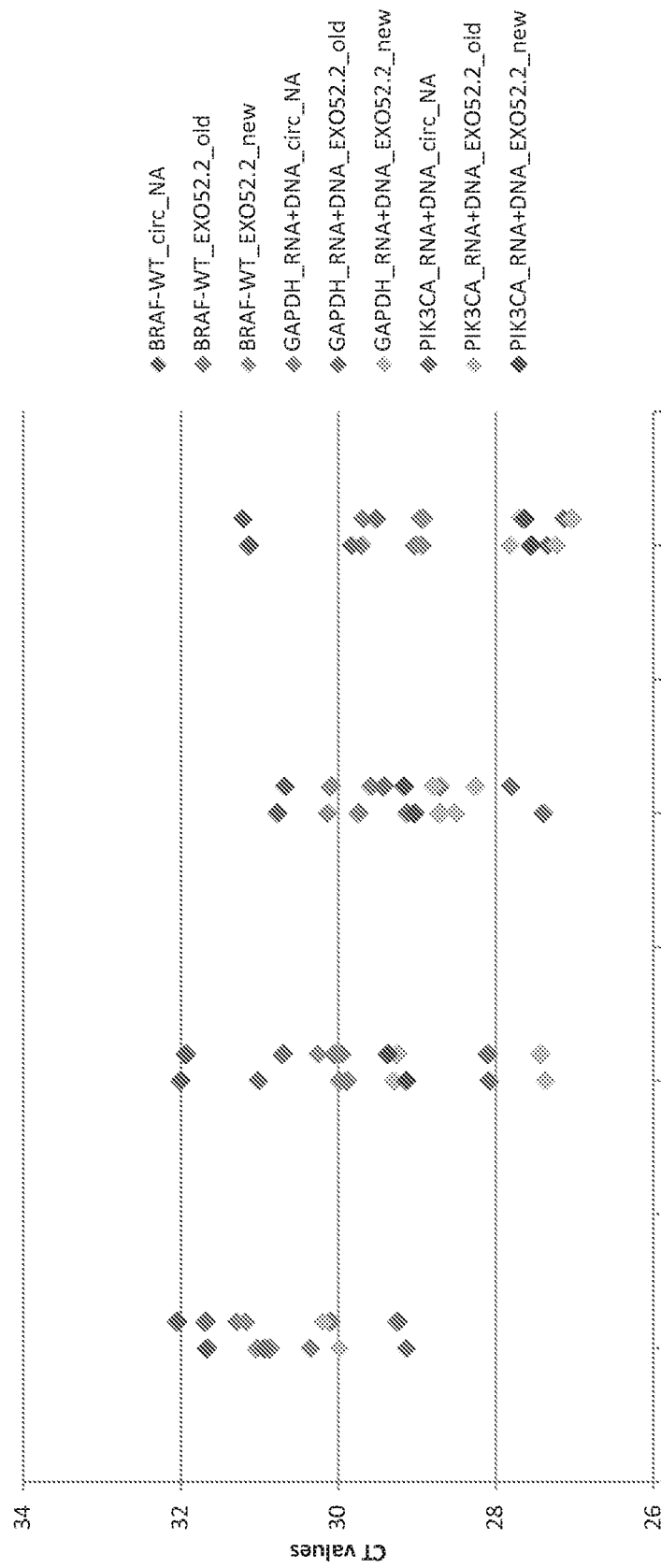


cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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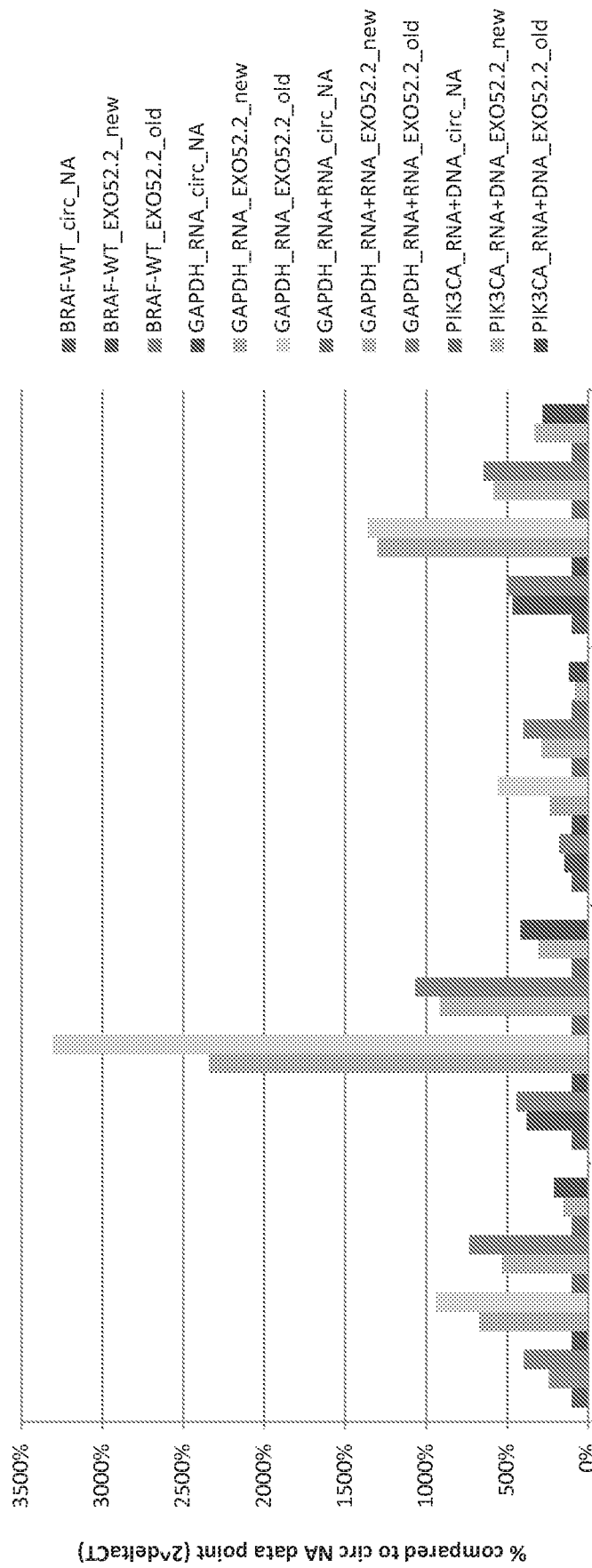
Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
-------------------	-------------------	----------------	----------------



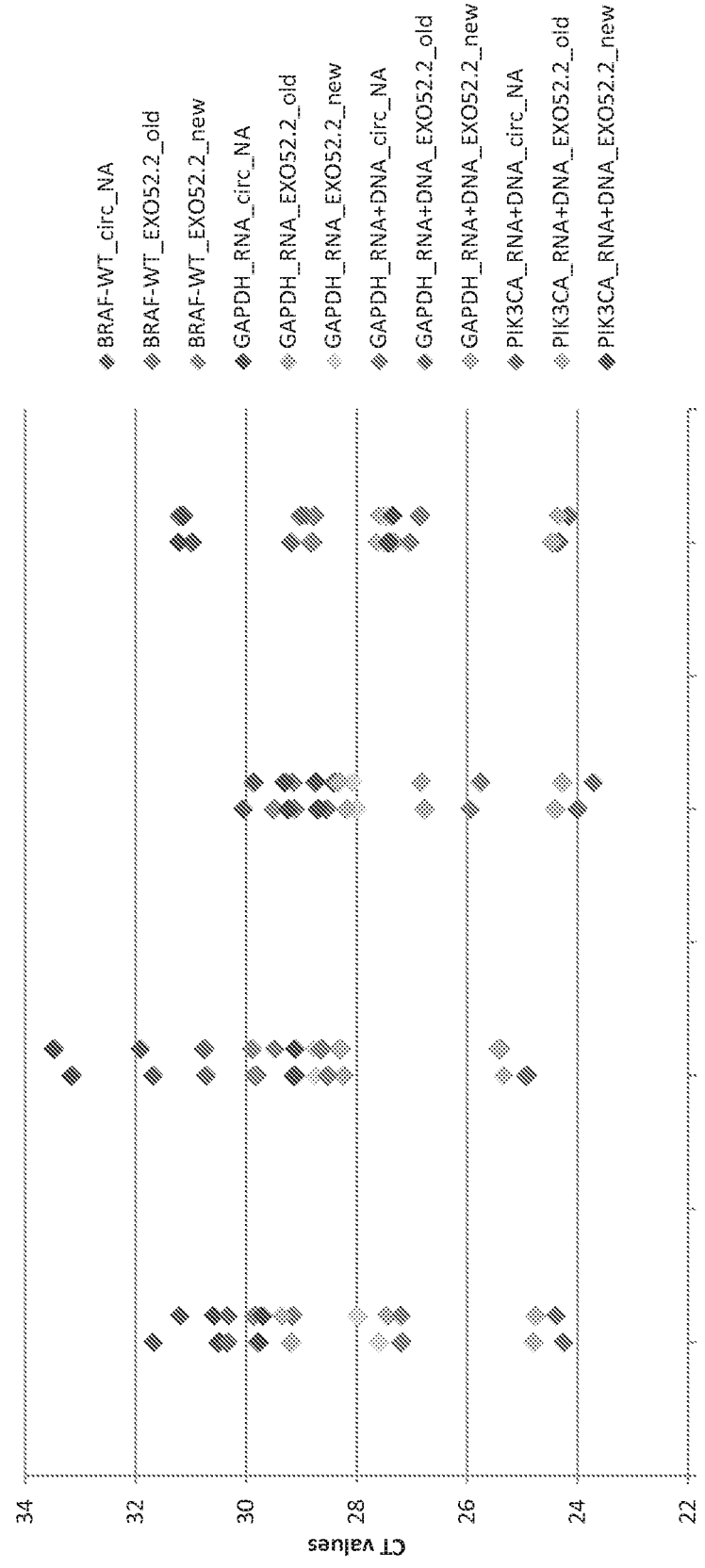
Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/ RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/ RT

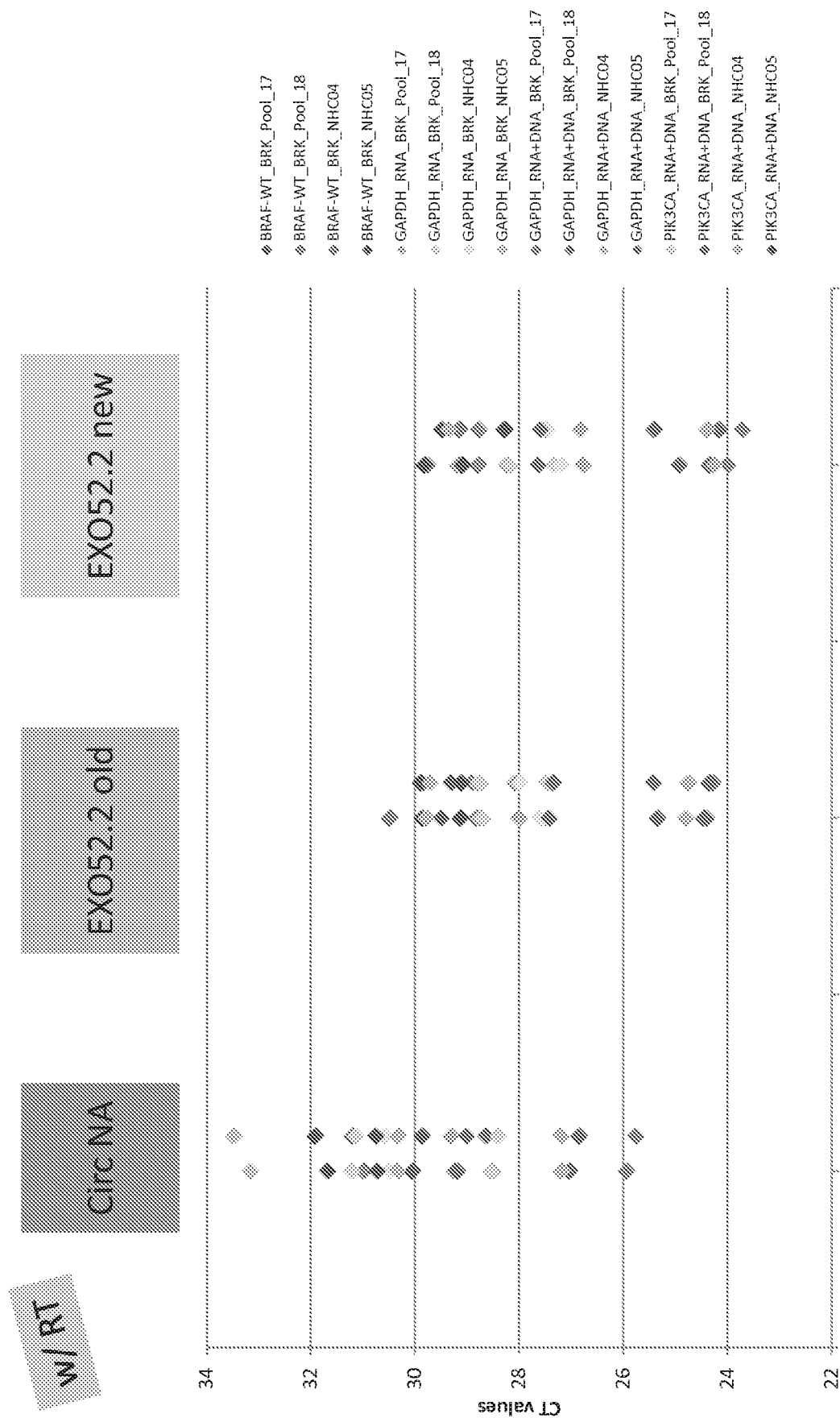
NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



Figure

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

cfDNA Isolation using different Isolation techniques

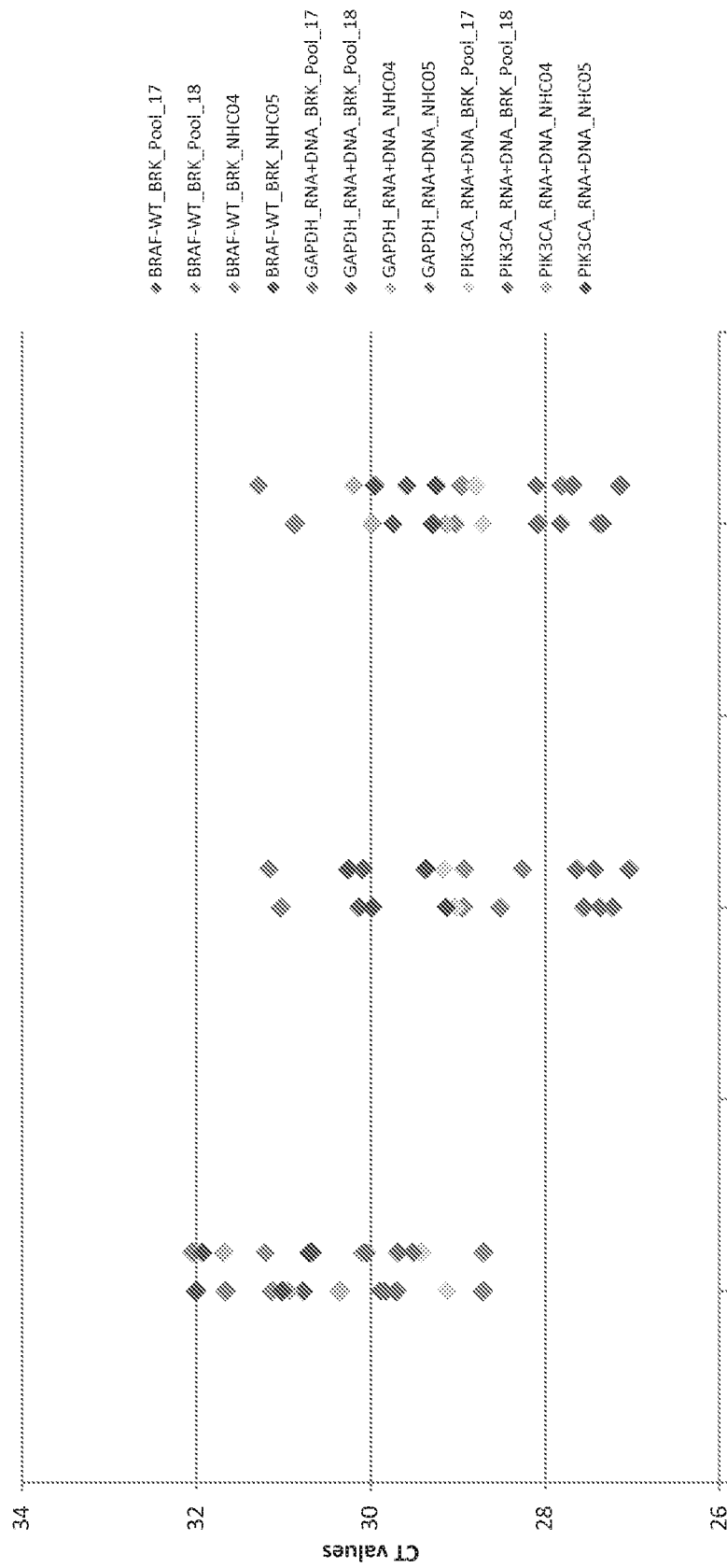
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT

Circ NA

Exo52.2 old

Exo52.2 new

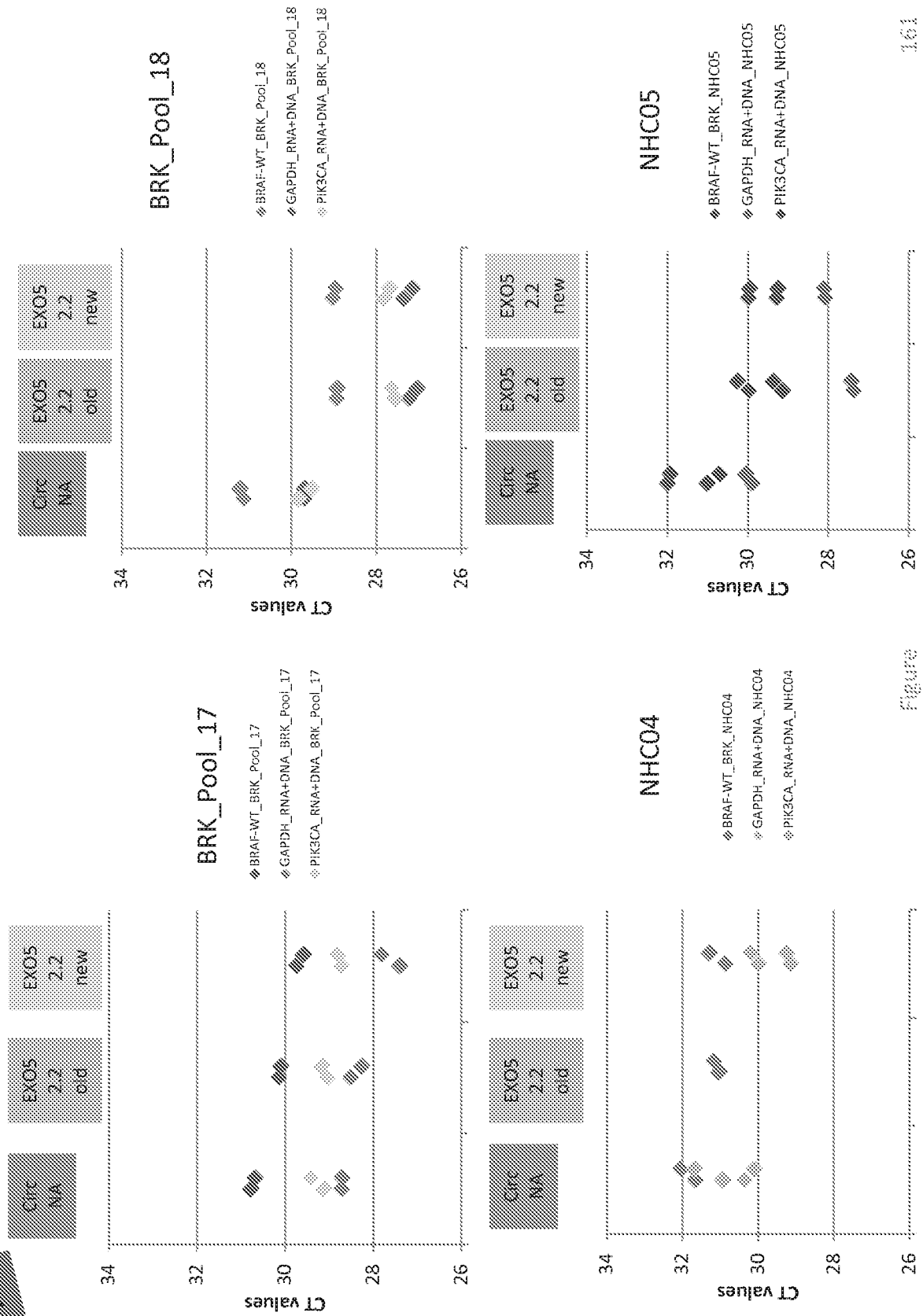


Figure

cfDNA Isolation using different Isolation techniques

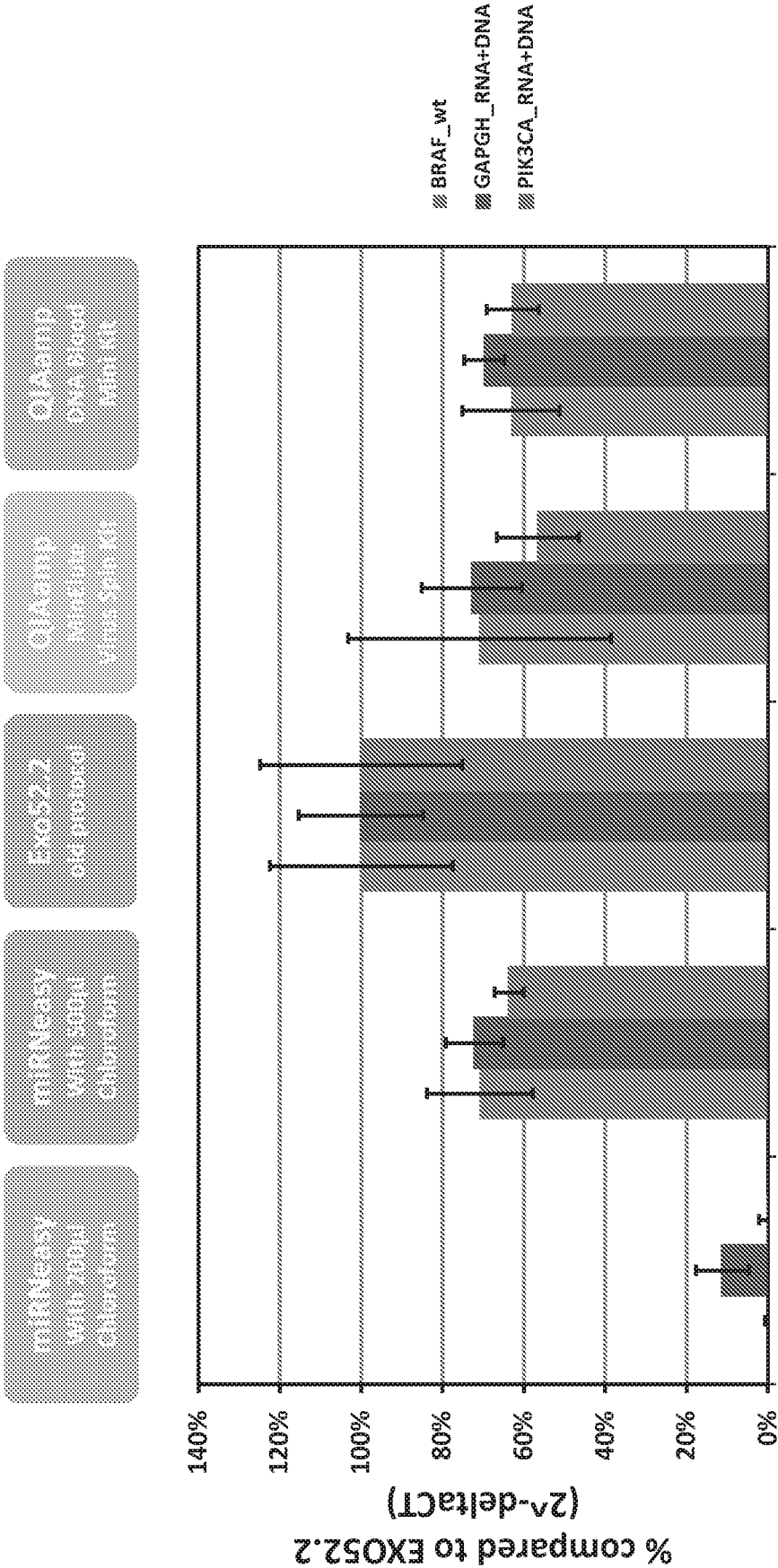
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT



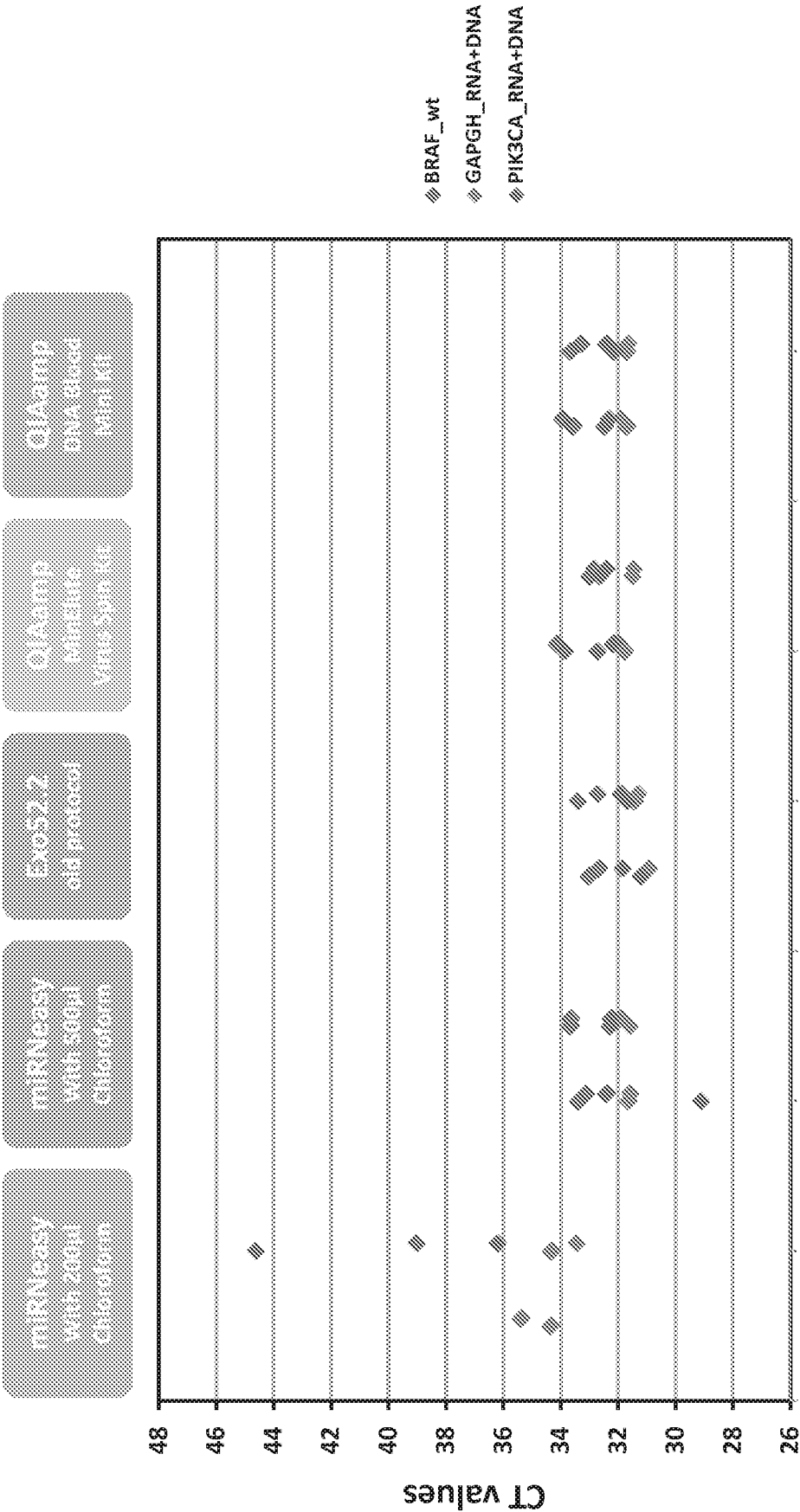
Figure

Comparison of DNA isolation methods on 200 μL of BRK plasma



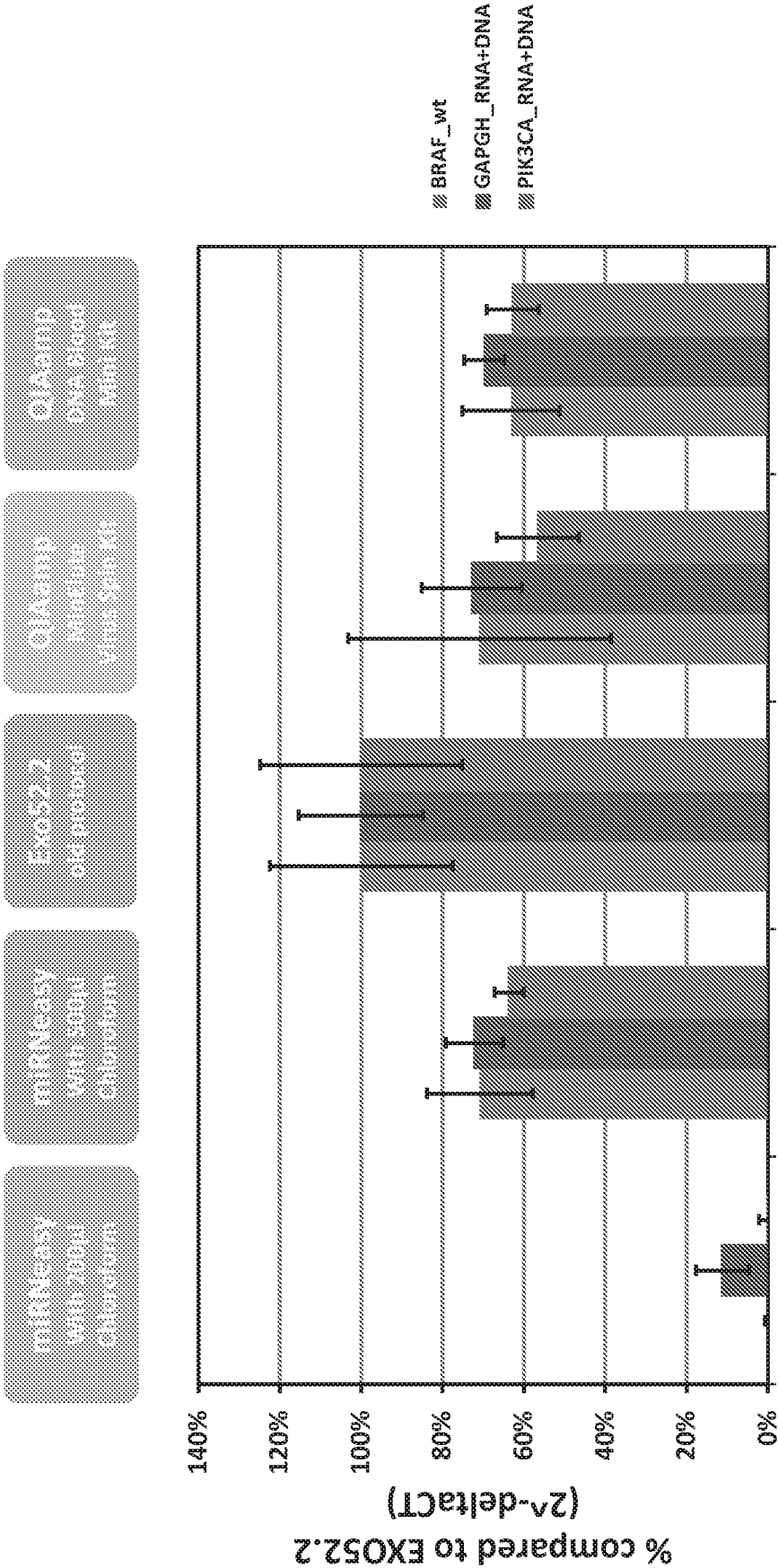
Figure

Comparison of DNA isolation methods on 200 μL of BRK plasma



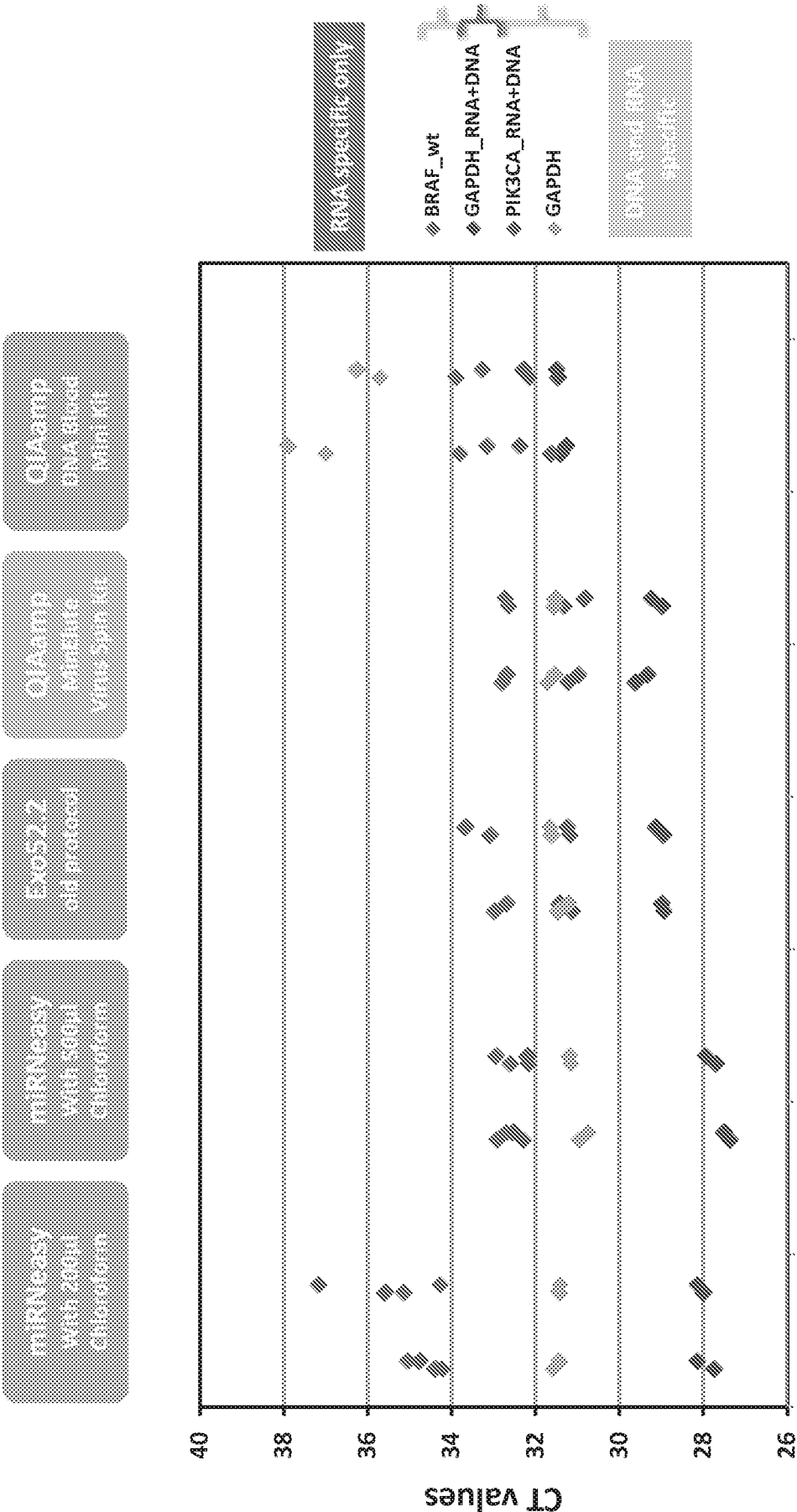
Figure

Comparison of DNA isolation methods on 200 μL of BRK plasma



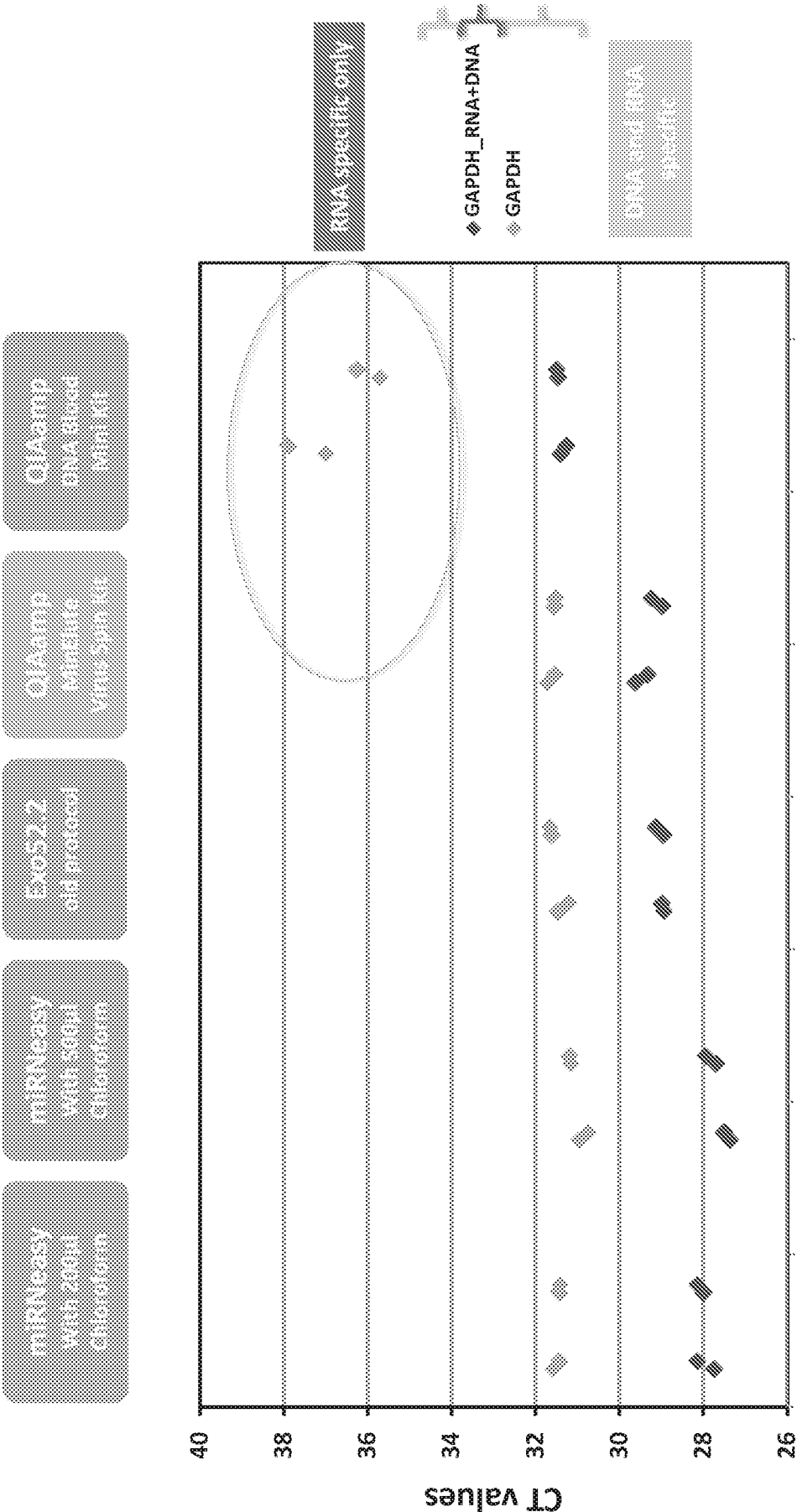
Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.



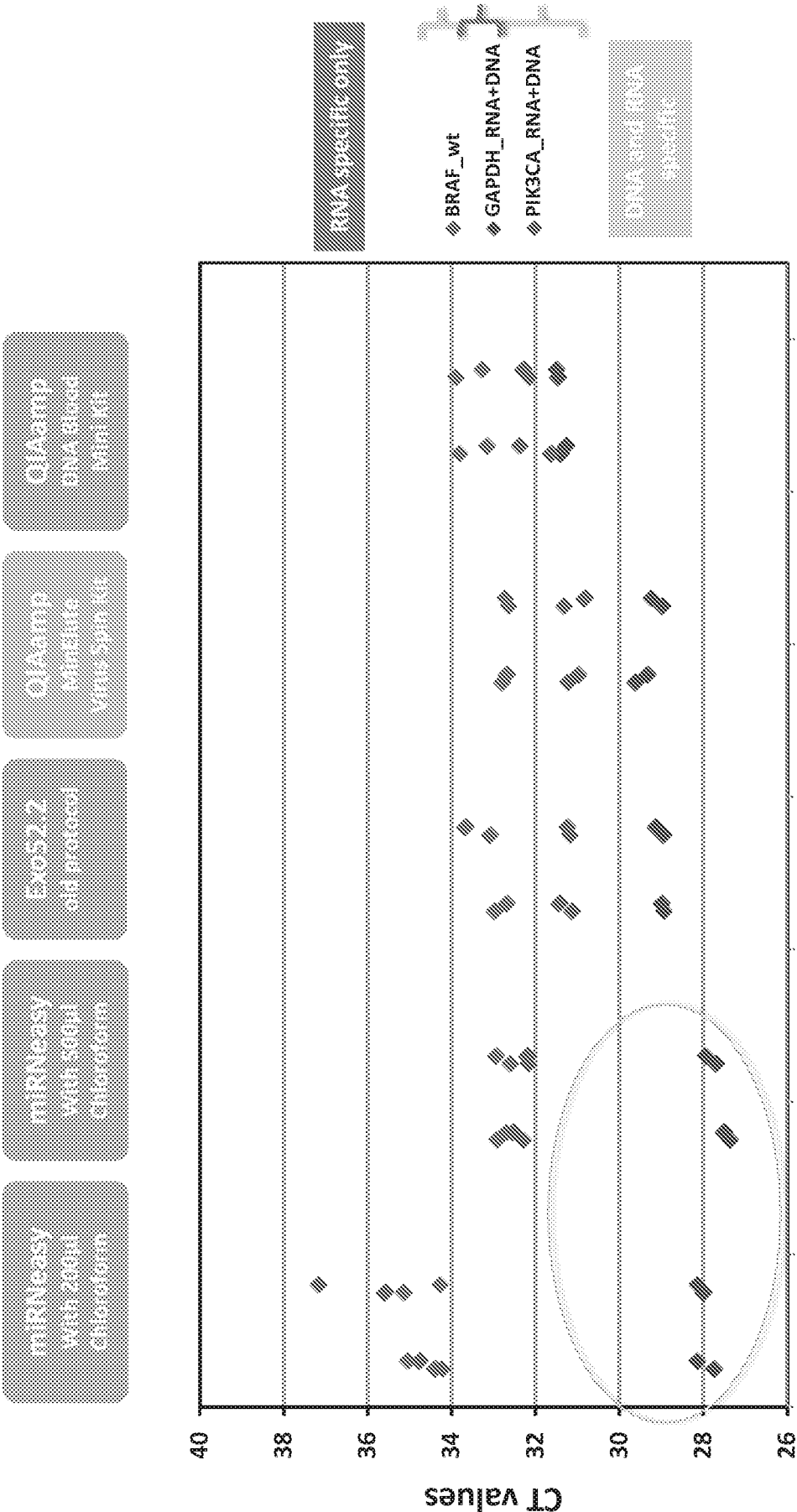
Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.



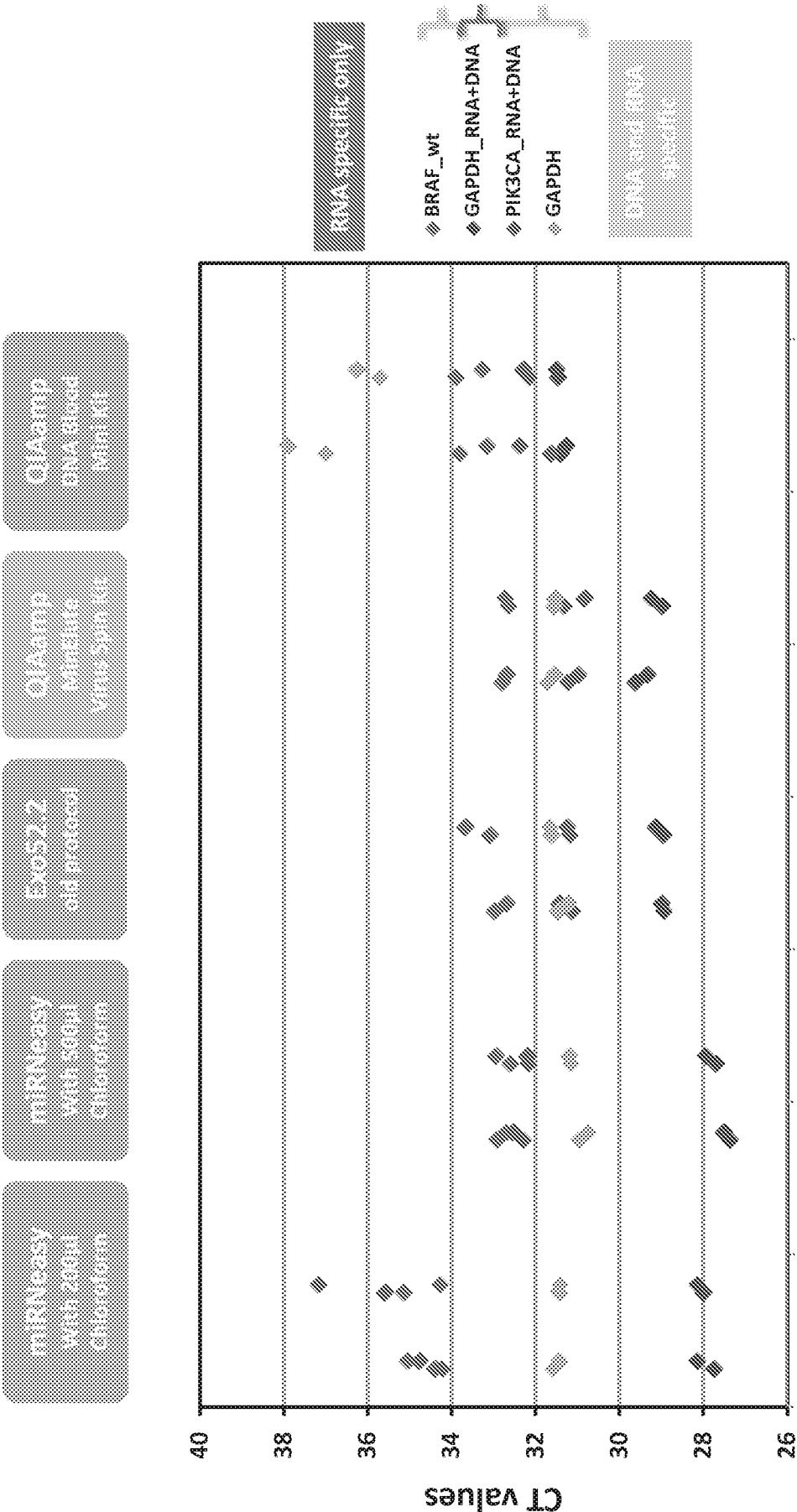
Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.



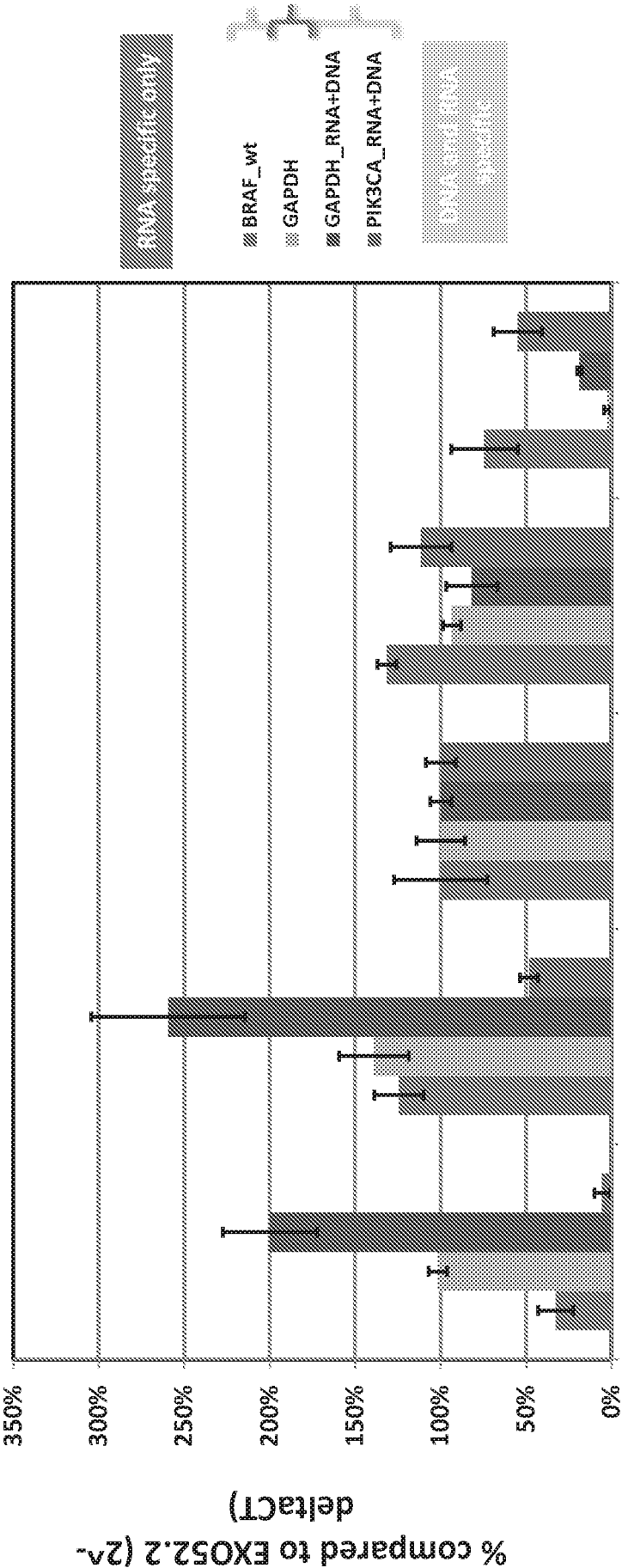
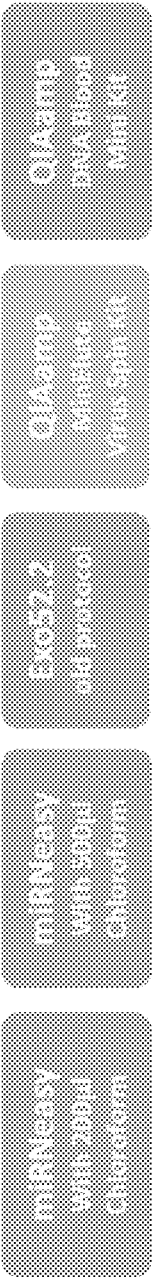
Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.



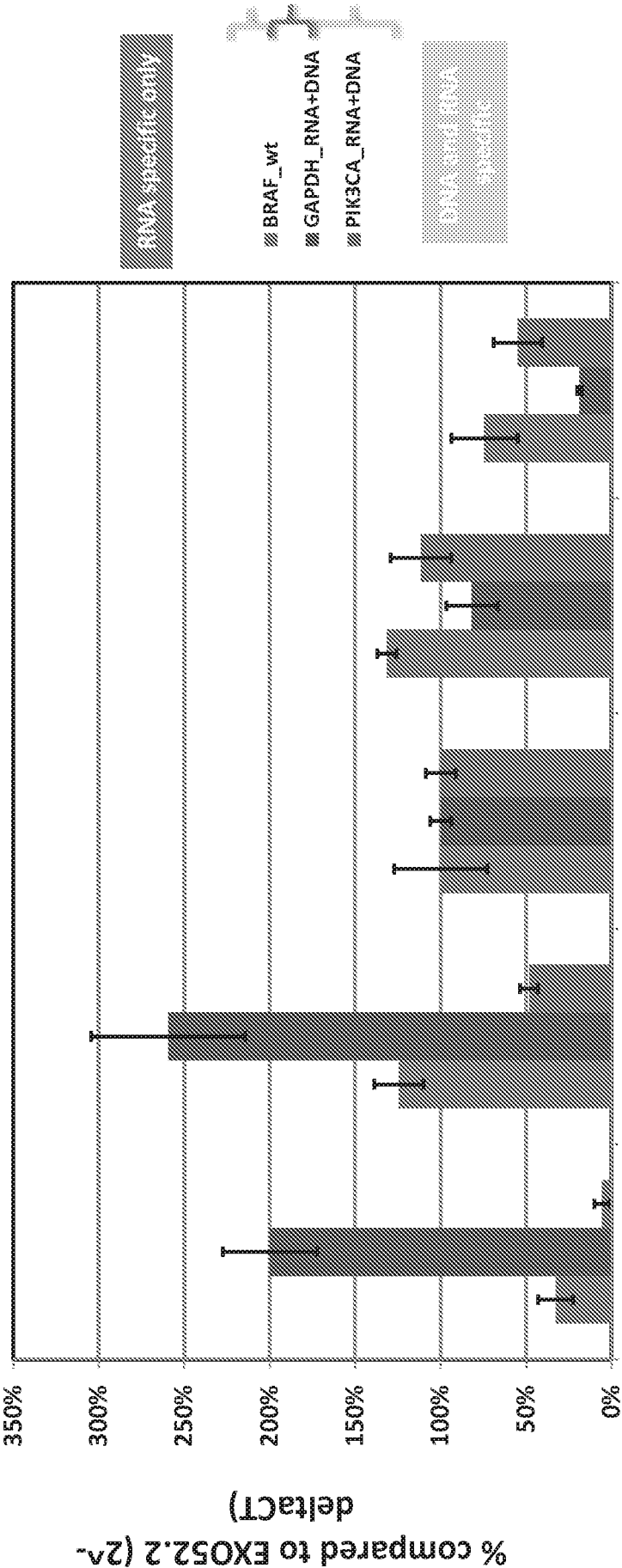
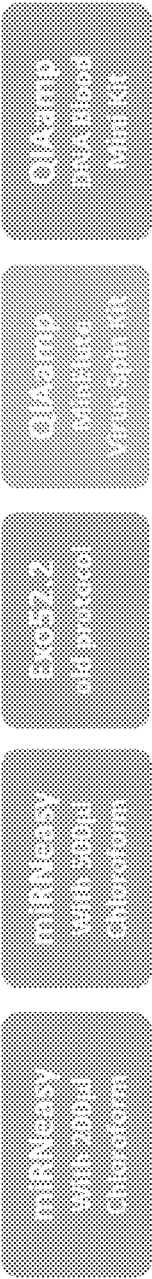
Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.

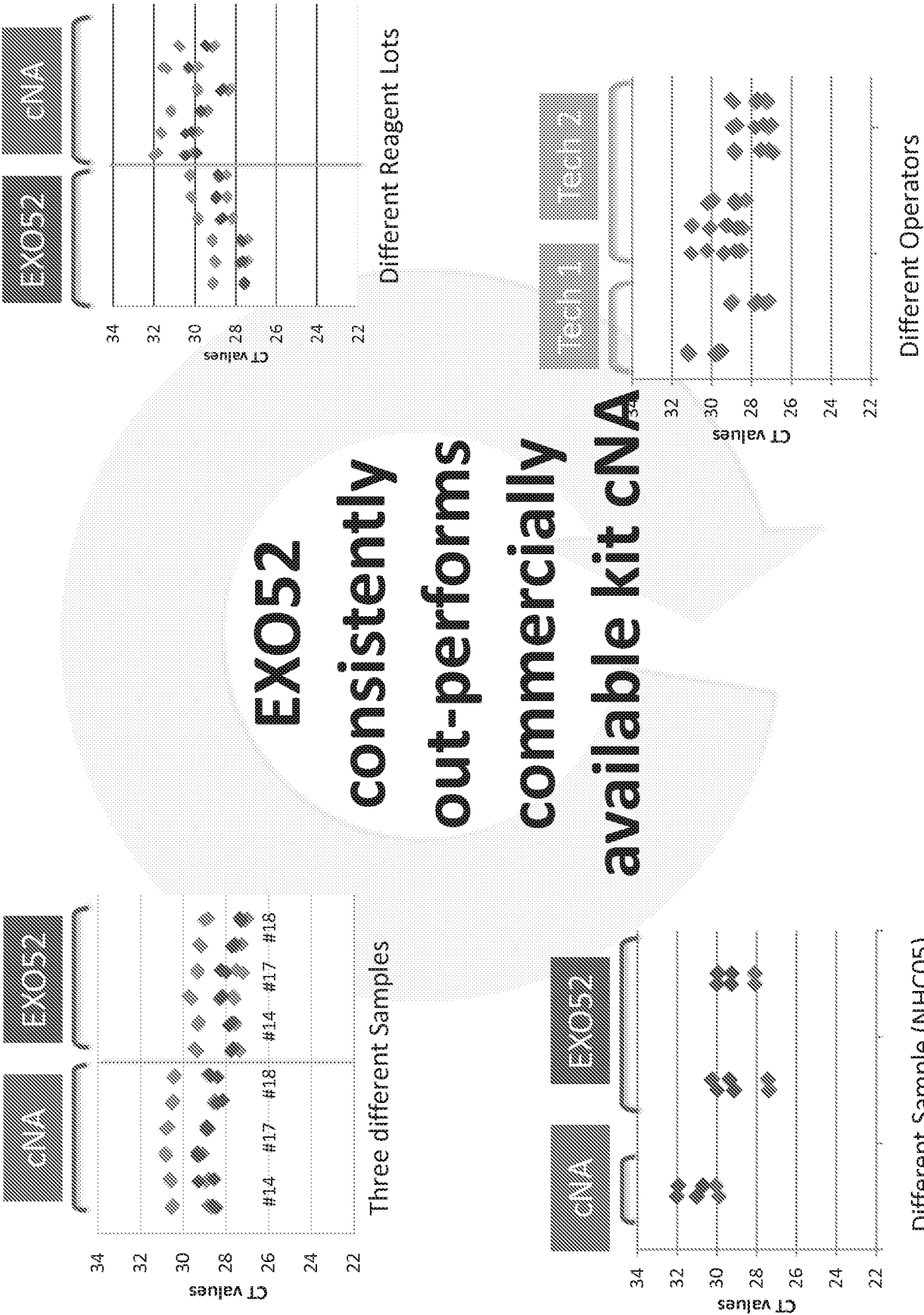


Figure

Comparison of DNA/RNA isolation methods on 200 µL of BRK plasma - with RT step.



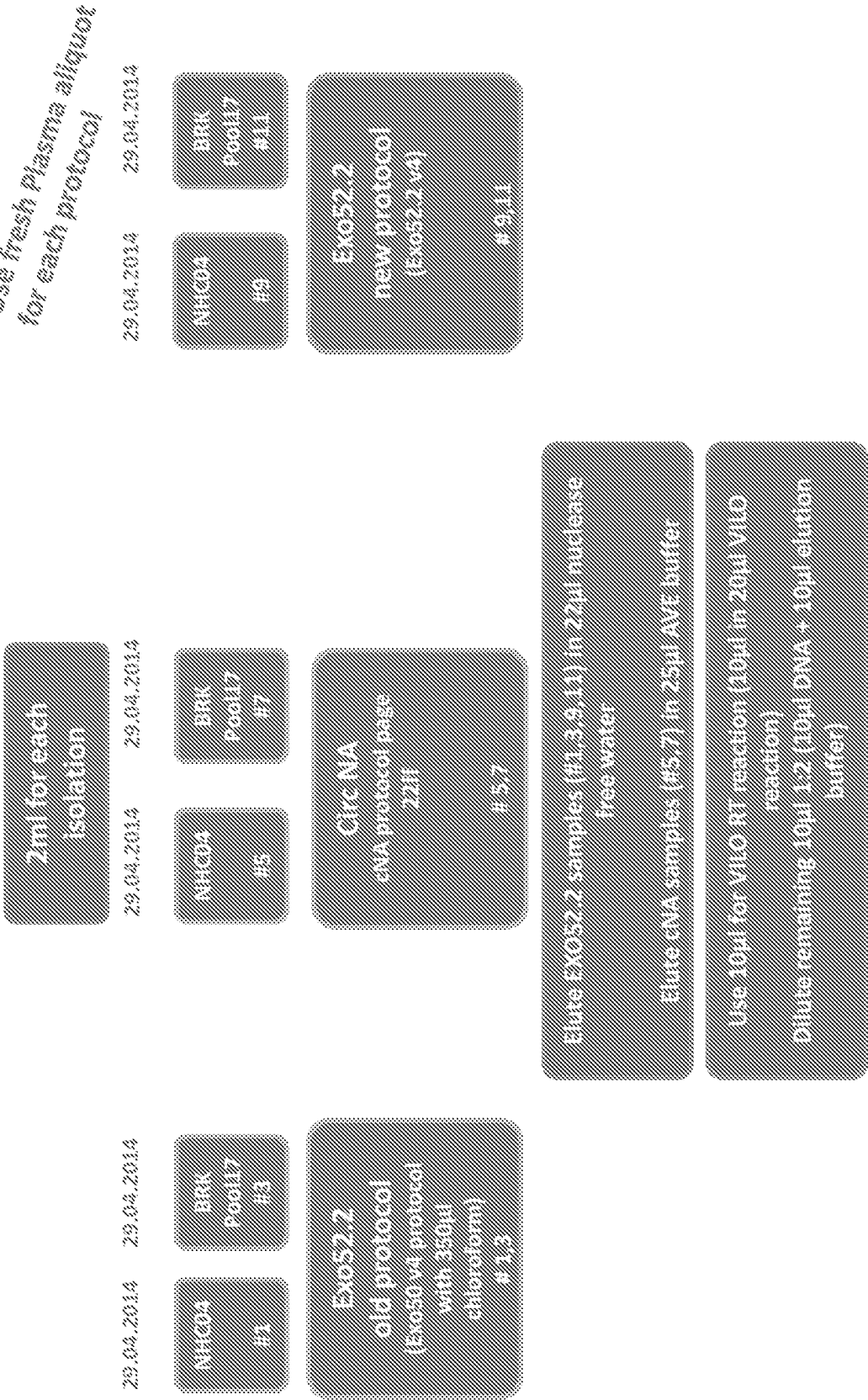
Figure



Figure

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

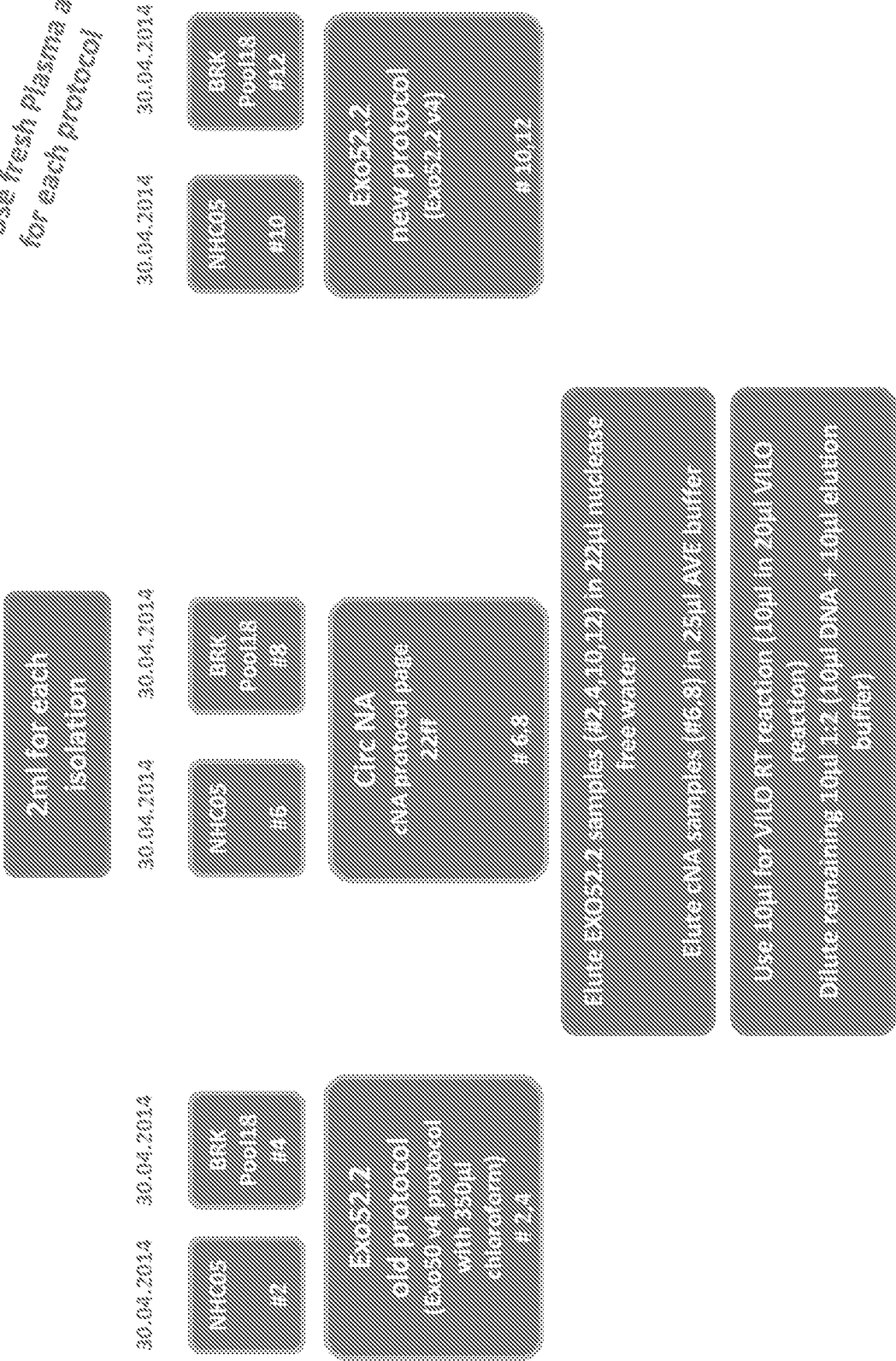
*Use fresh plasma aliquot
for each protocol*



Figure

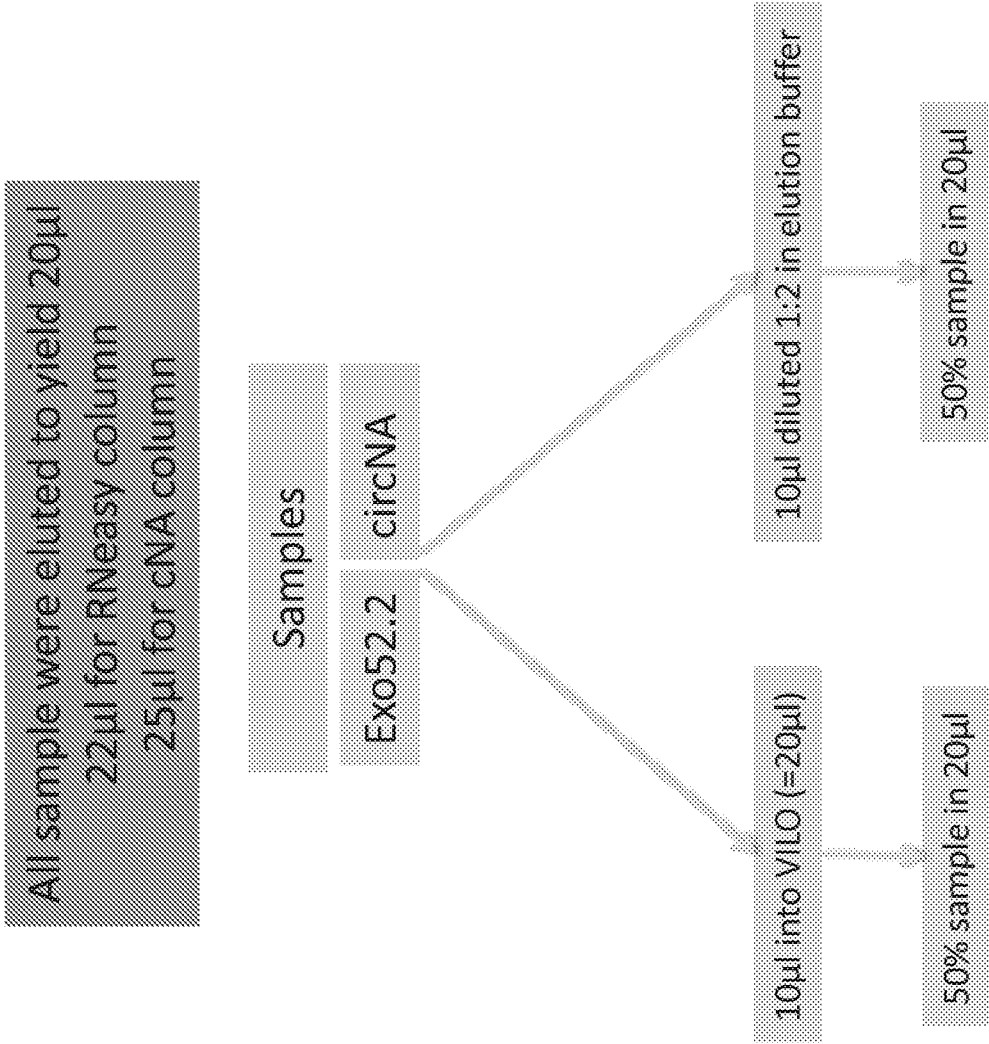
cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

*Use fresh Plasma aliquot
for each protocol*



Figure

Dilution overview

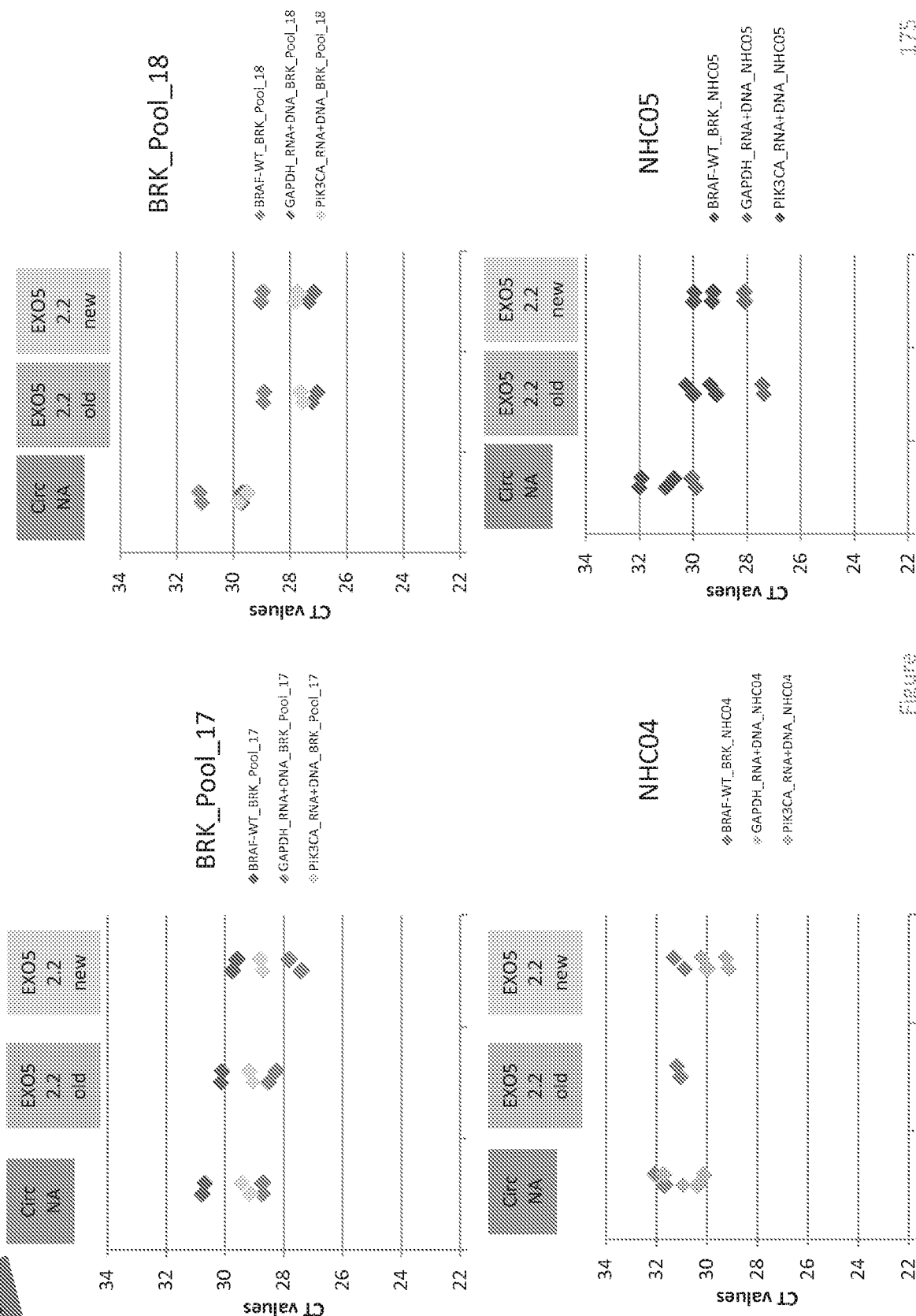


Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

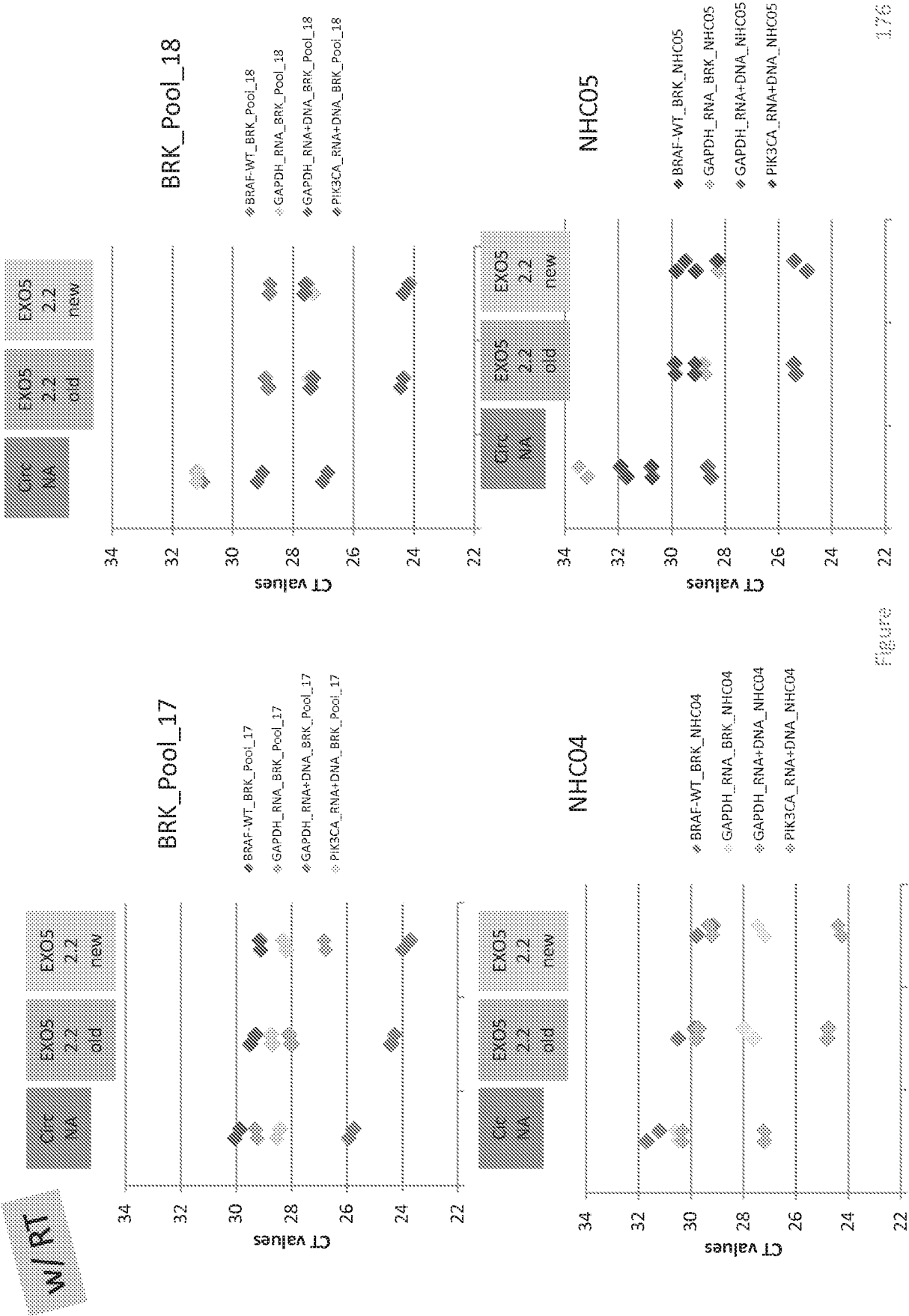
w/o RT



Figure

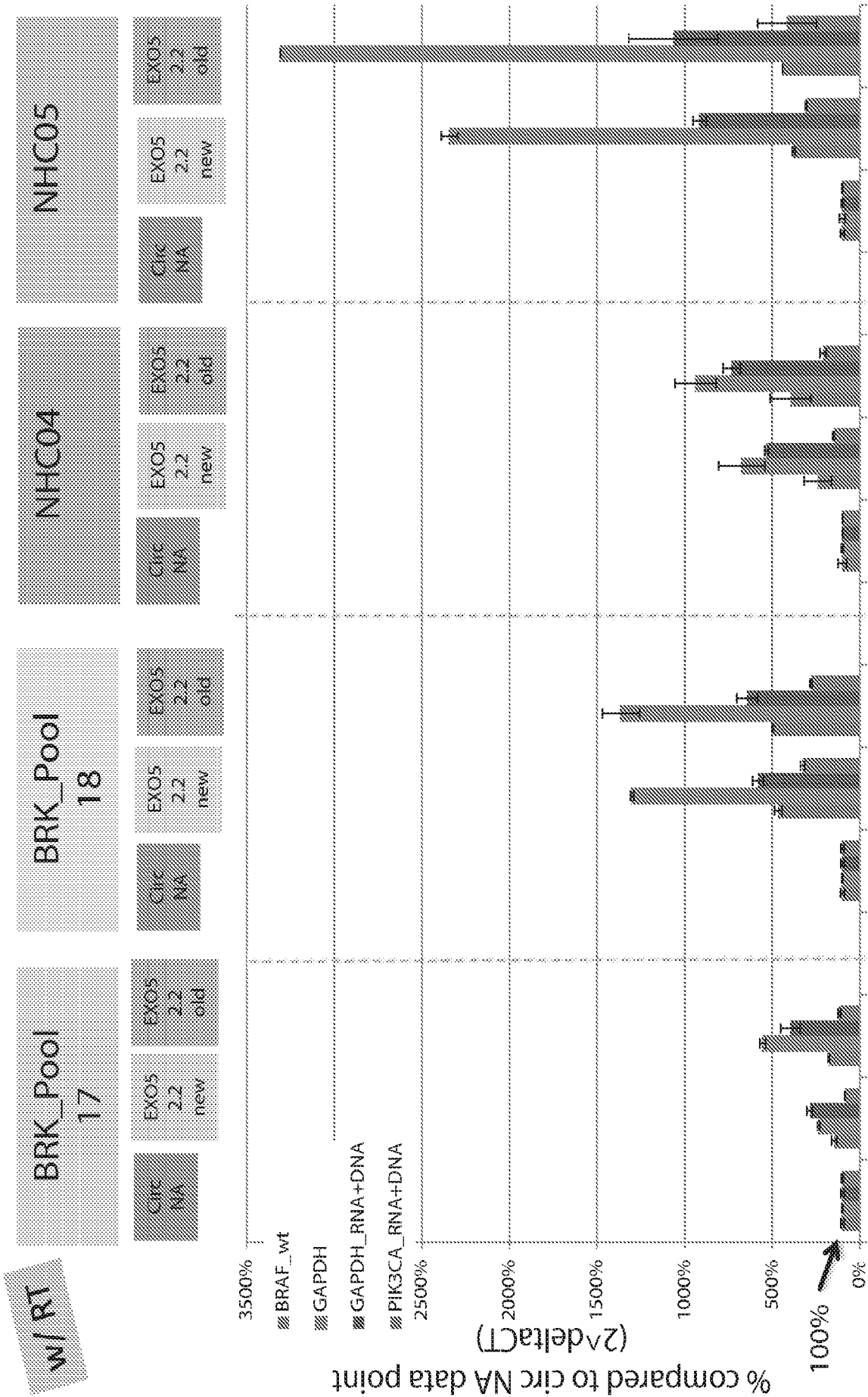
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



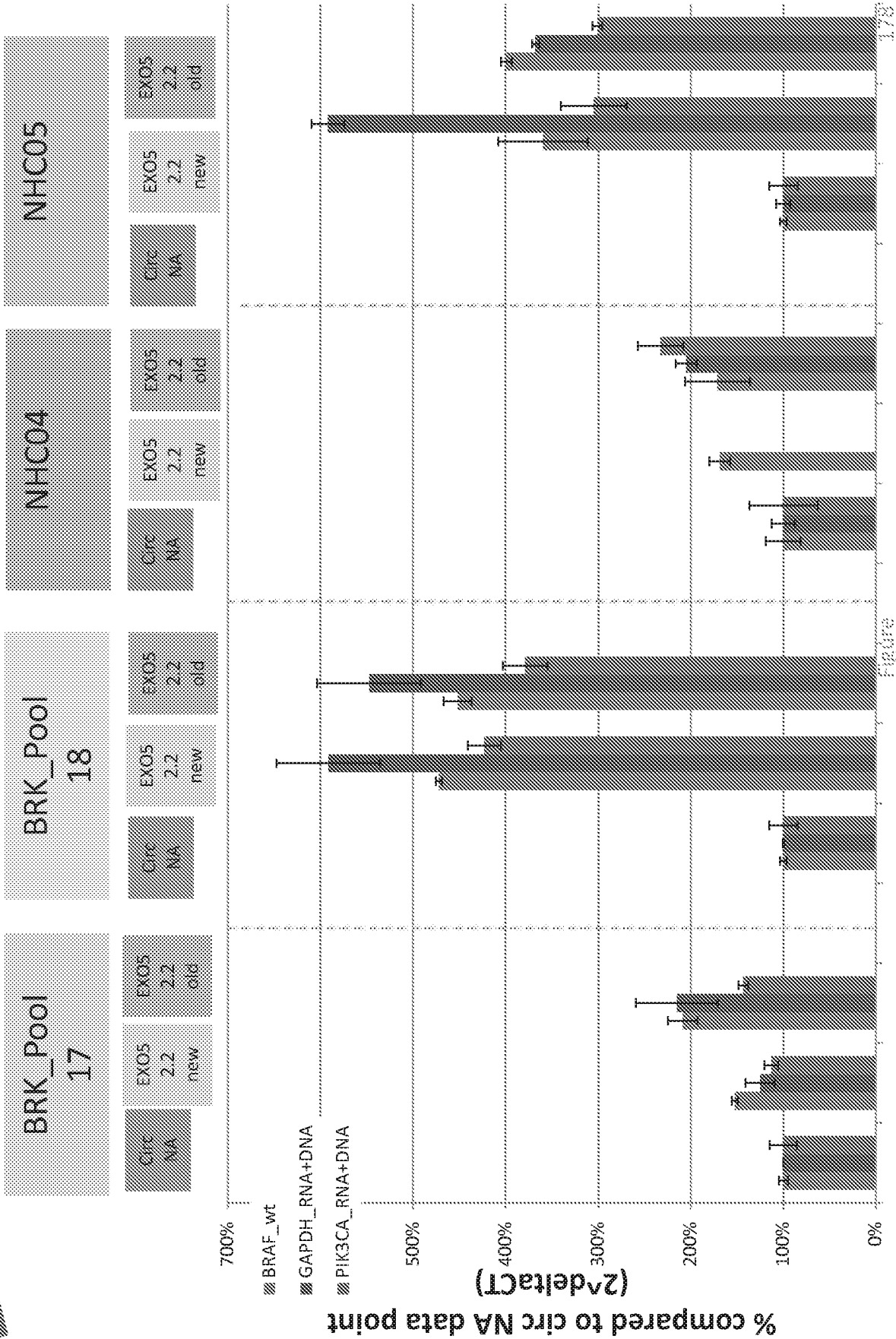
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



cfDNA Isolation using different Isolation techniques

w/o RT
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

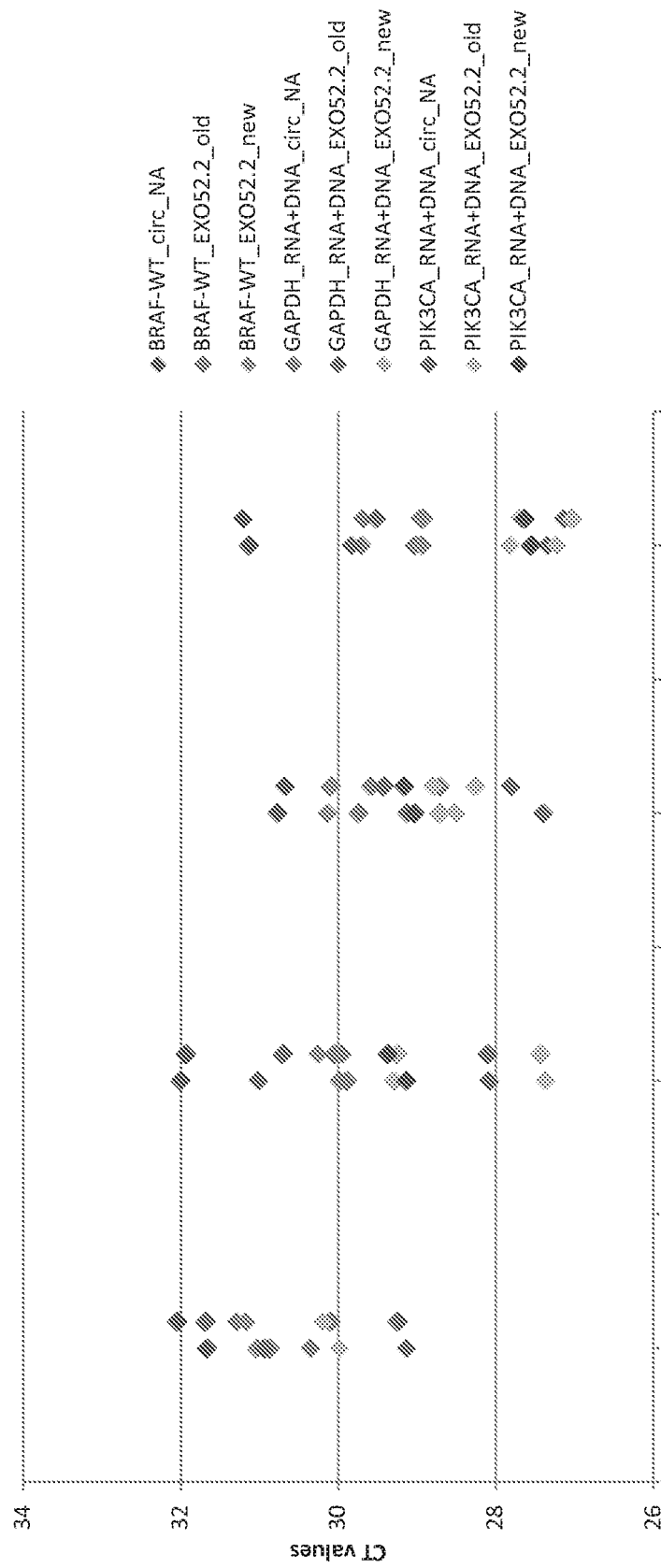


cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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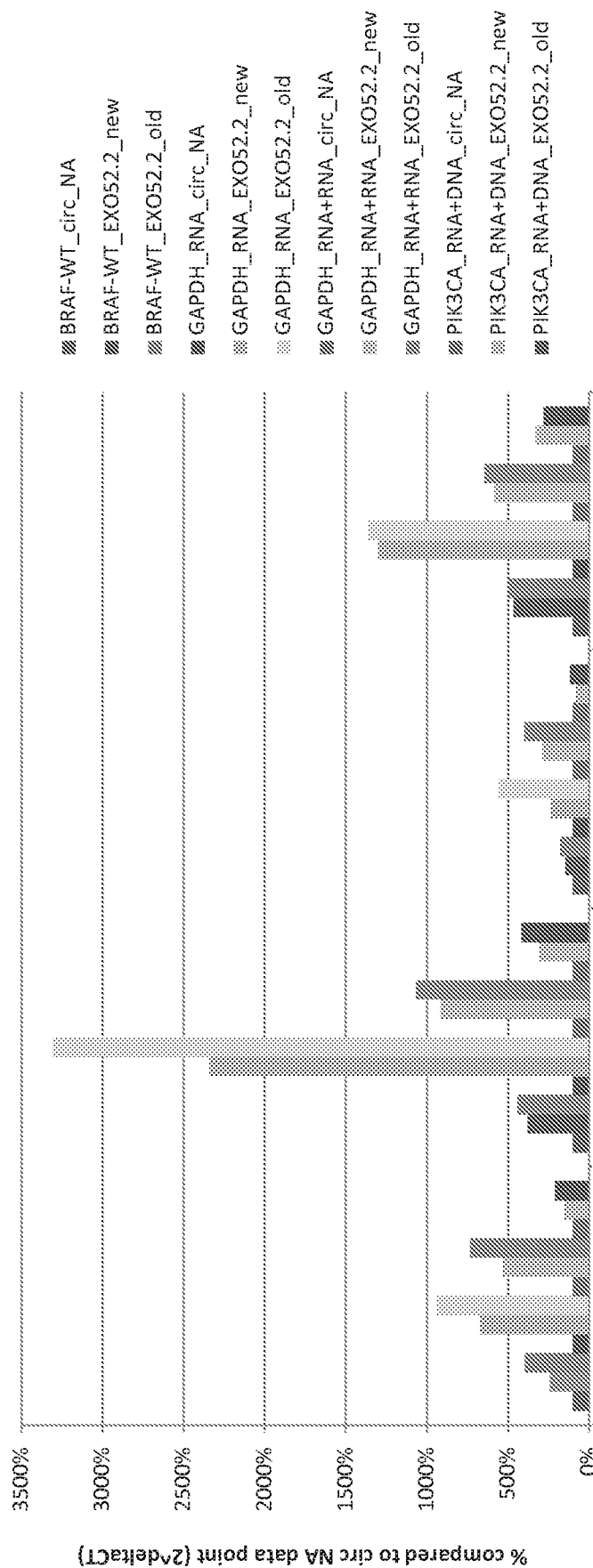
Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
-------------------	-------------------	----------------	----------------



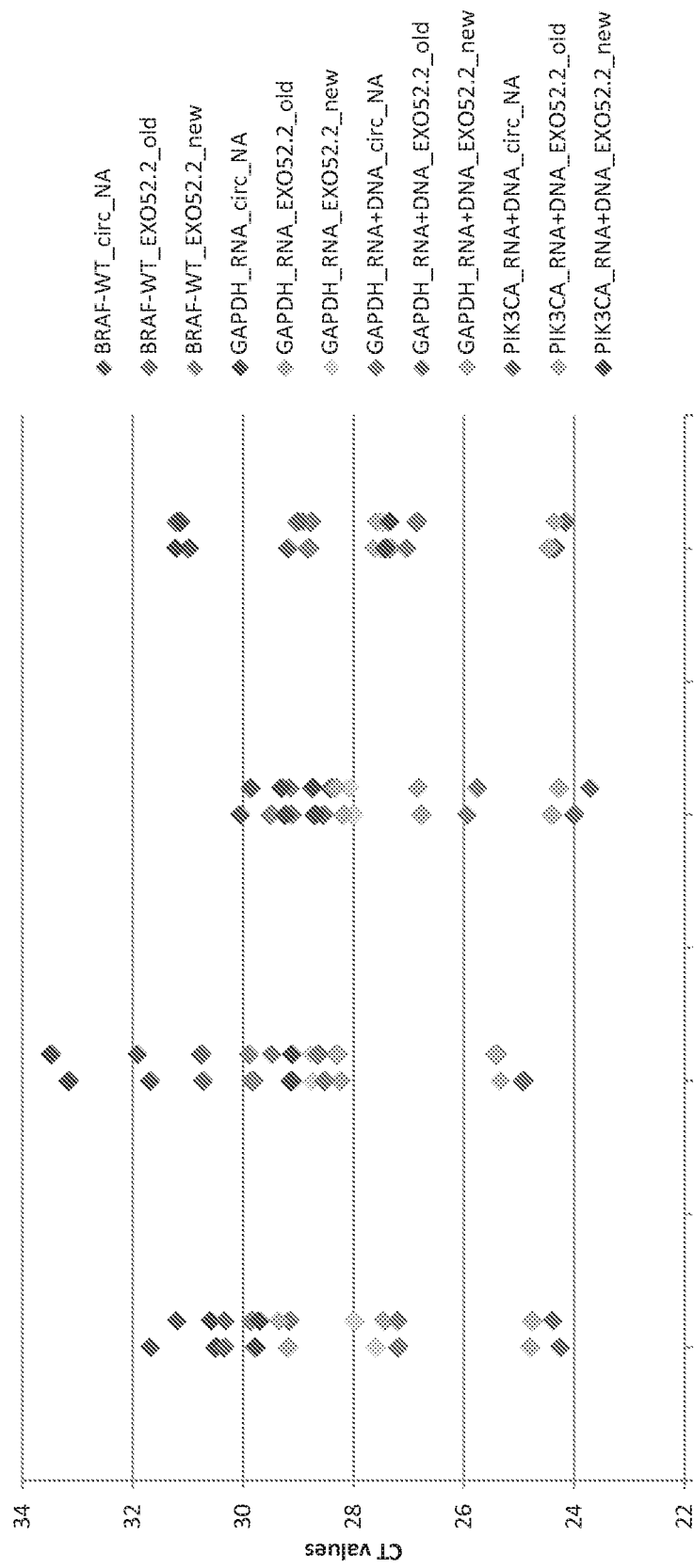
Figure

cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/ RT

NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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Figure

cfDNA Isolation using different Isolation techniques
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/ RT

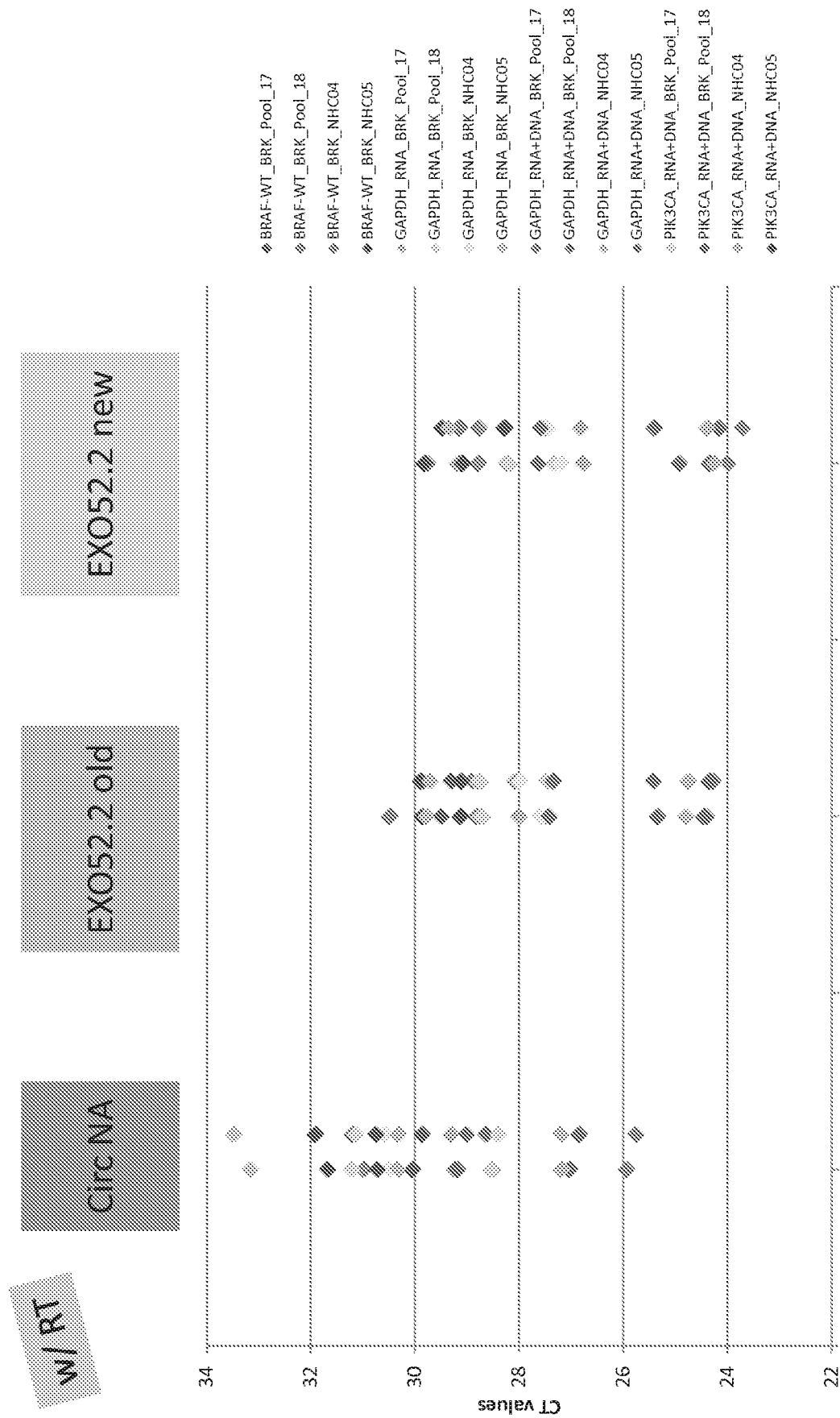
NHC04 (MUC014)	NHC05 (MUC011)	BRK_Pool 17	BRK_Pool 18
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Figure

cfDNA Isolation using different Isolation techniques

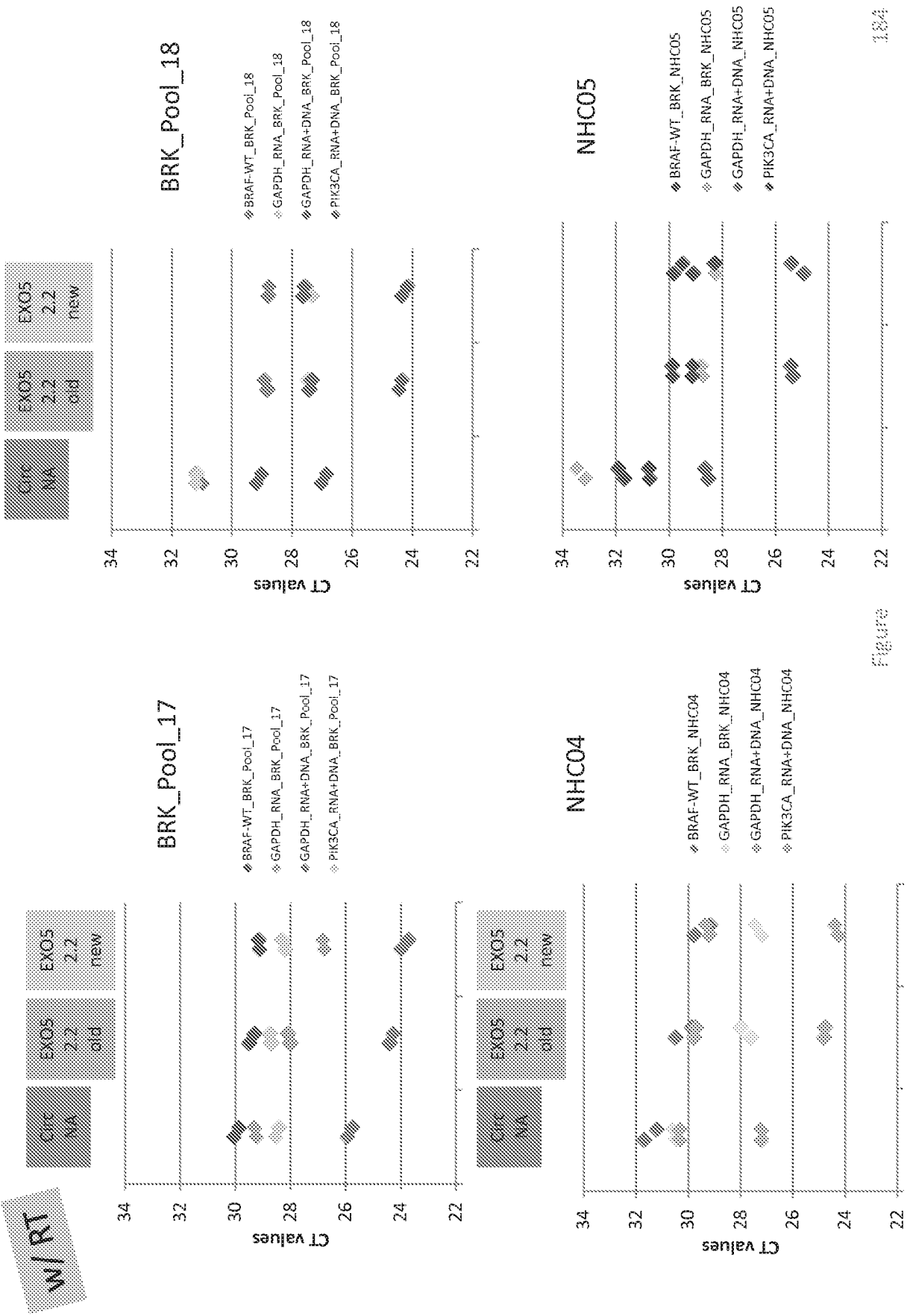
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



Figure

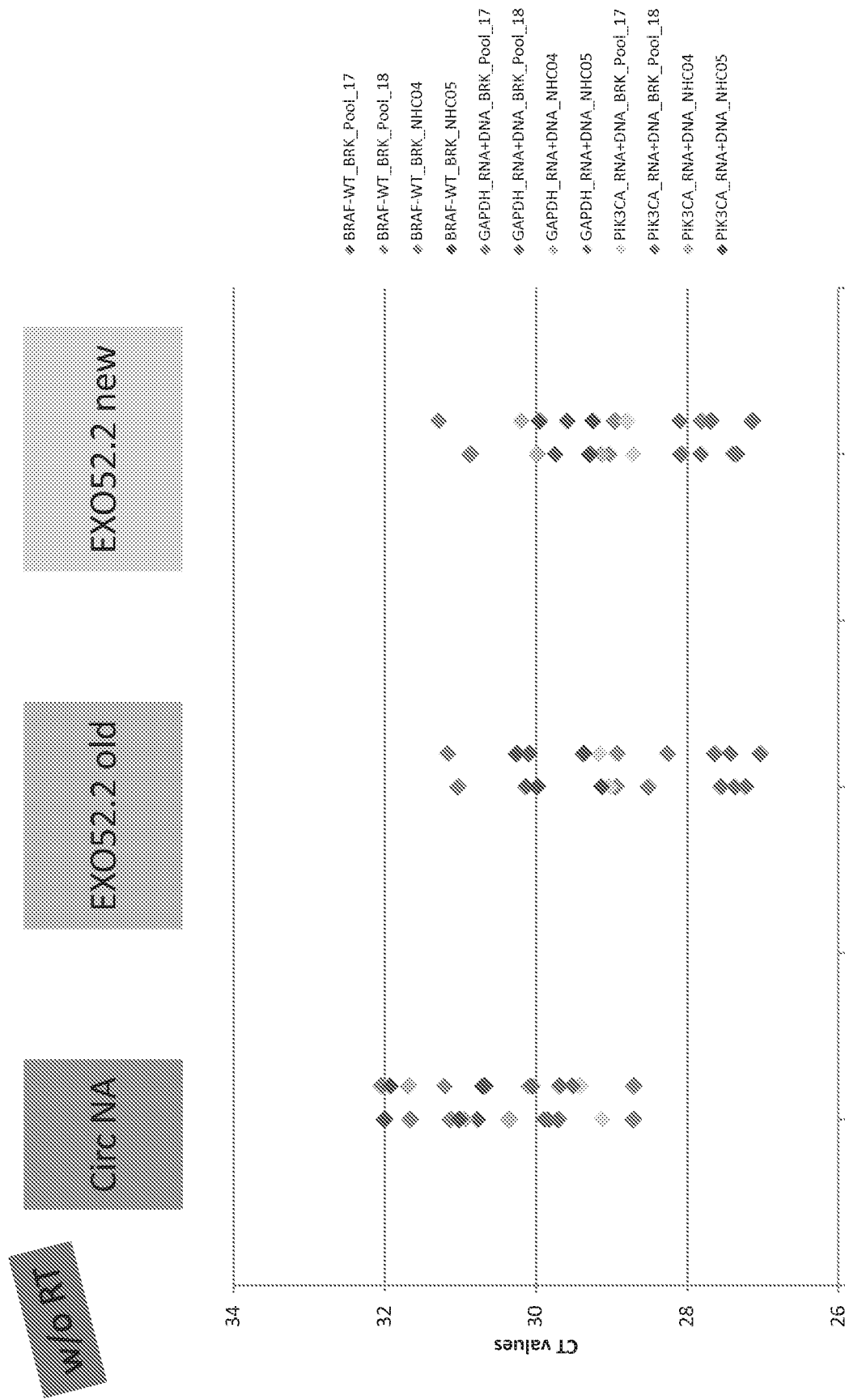
cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new



cfDNA Isolation using different Isolation techniques

Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

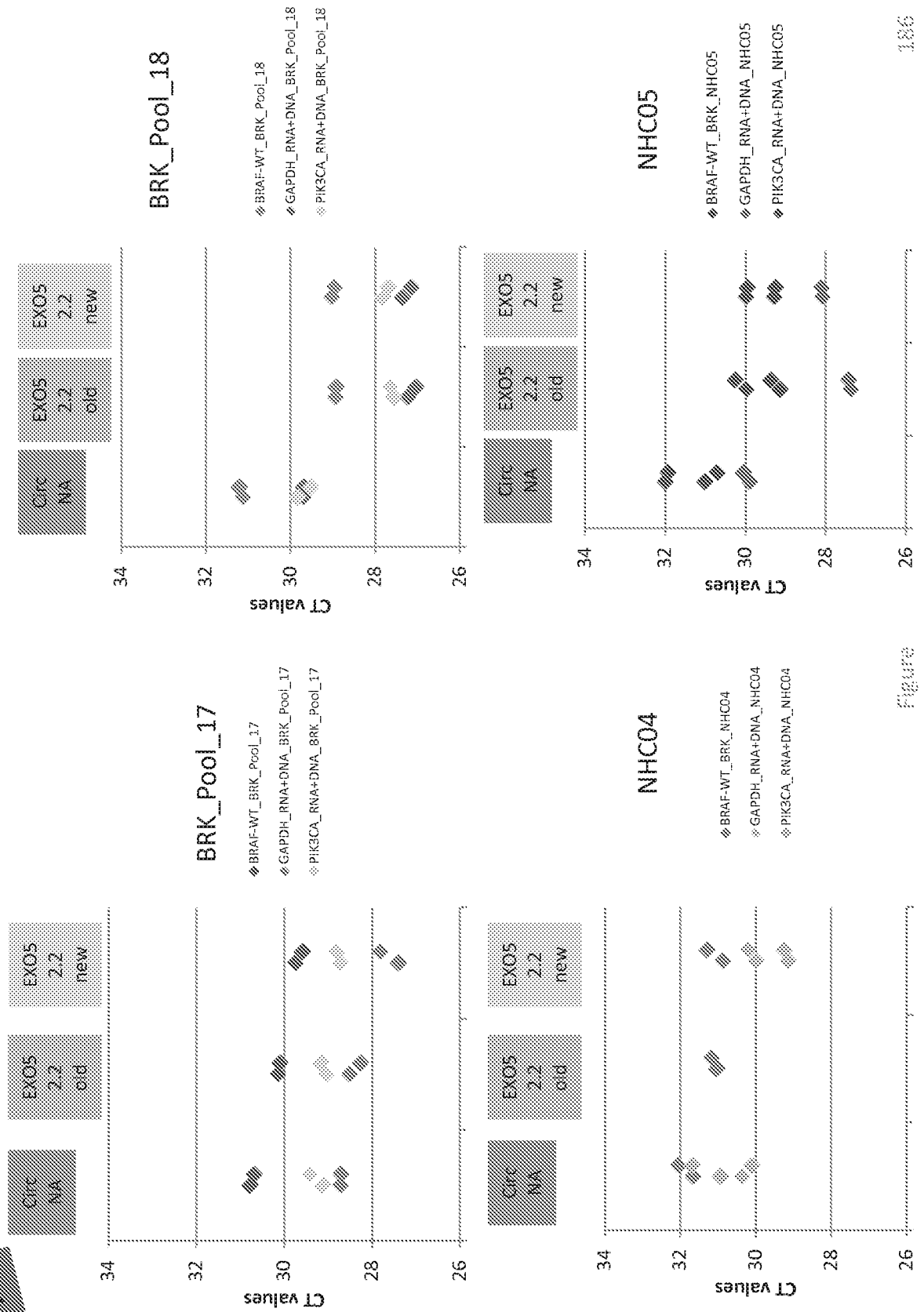


Figure

cfDNA Isolation using different Isolation techniques

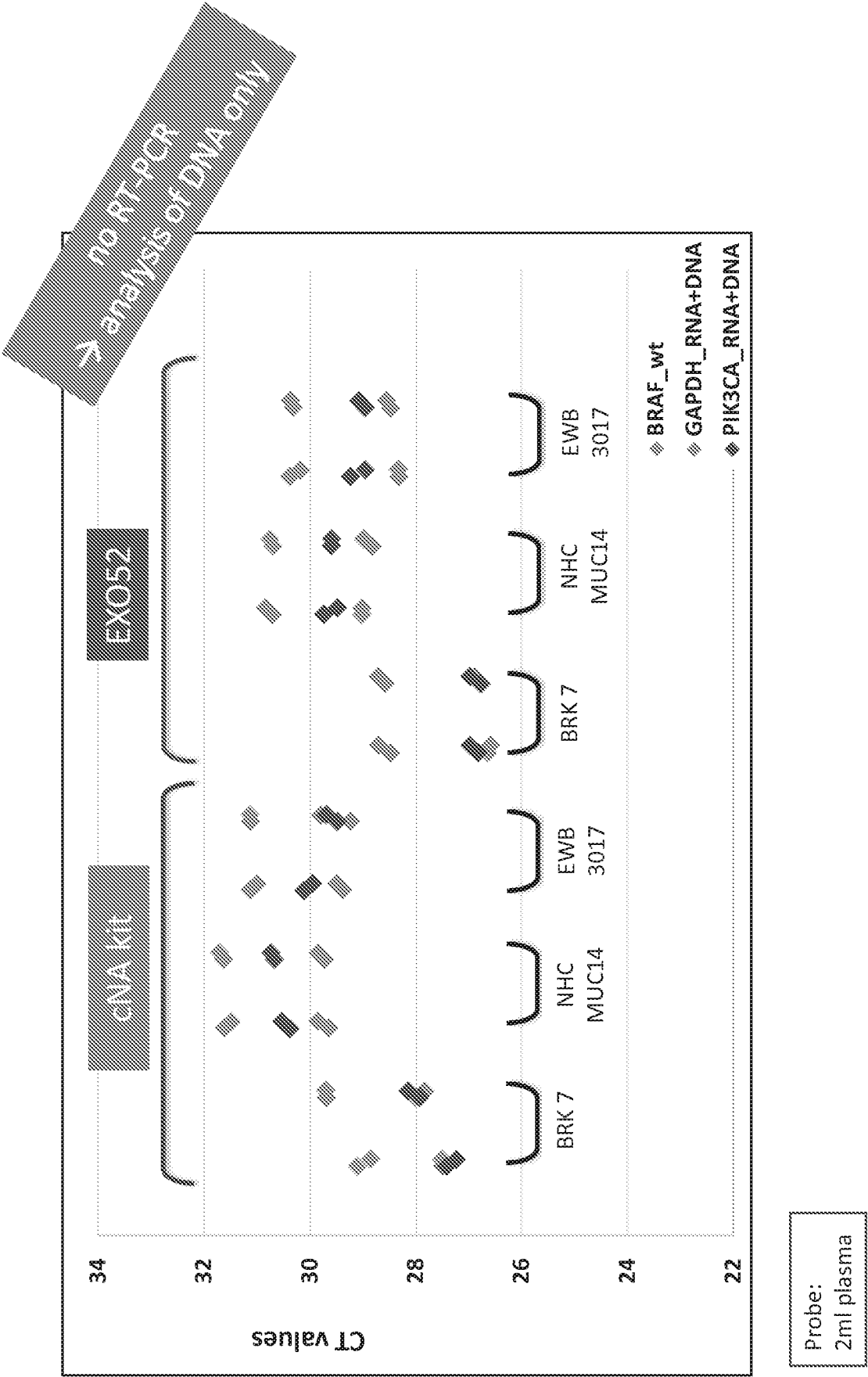
Comm. Avail. Kit 1 circNA – Exo52.2 old – Exo52.2 new

w/o RT



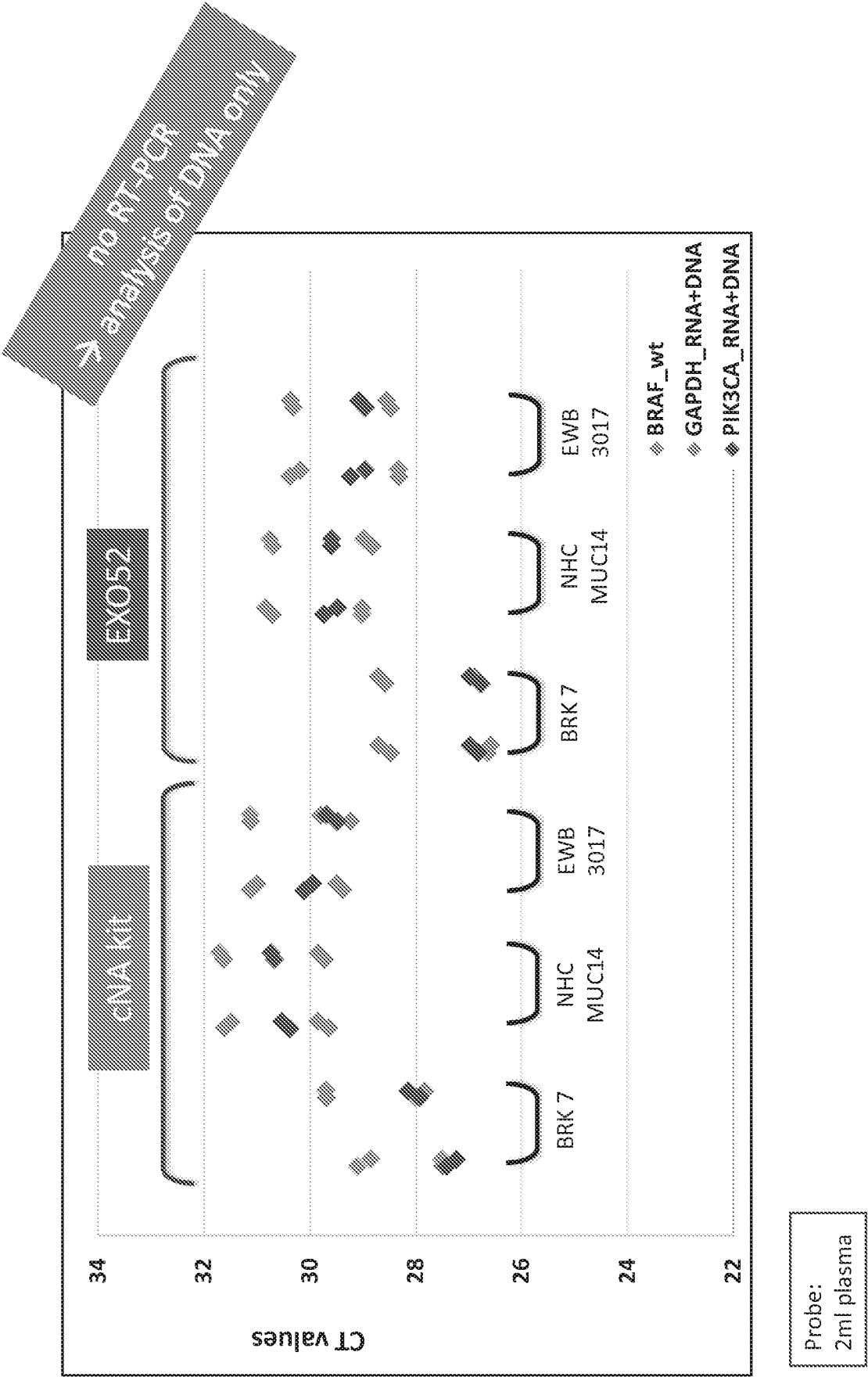
Figure

Comparison of
EXO52 / Comm. Avail. Kit 1 circNA on 3 plasma pools



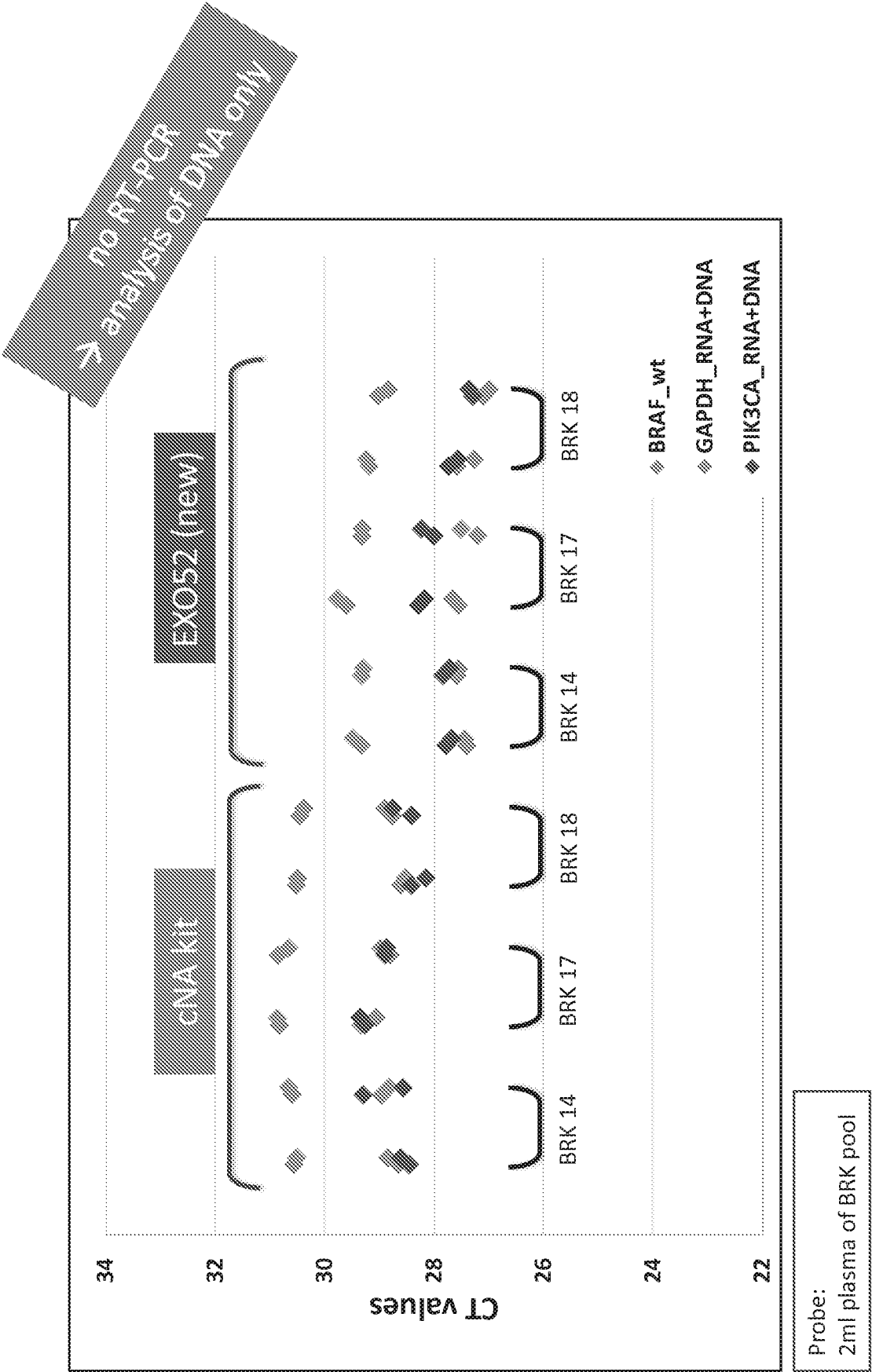
Figure

Comparison of
EXO52 / Comm. Avail. Kit 1 circNA on 3 plasma pools



Figure

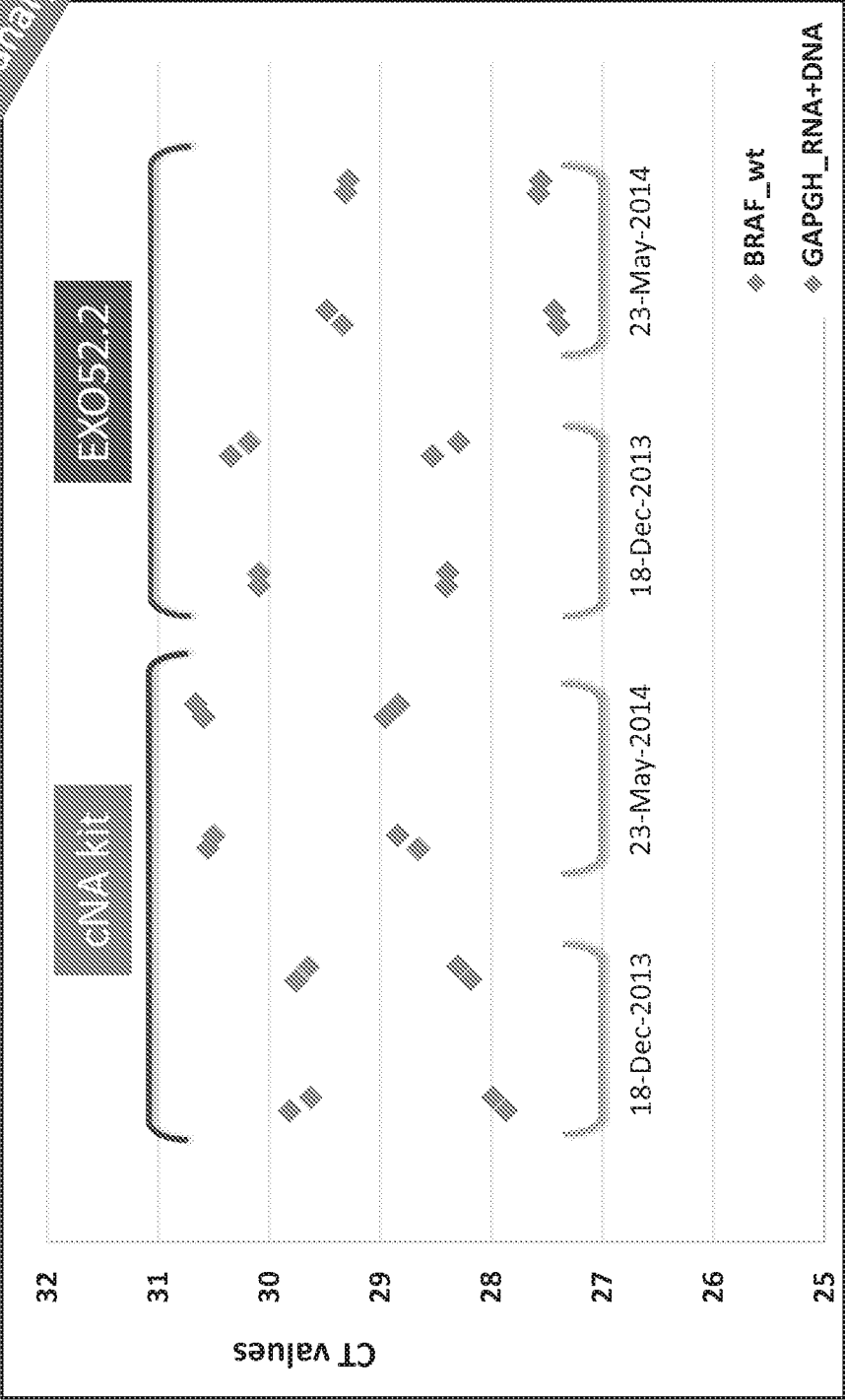
Comparison of
EXO52 / Comm. Avail. Kit 1 circNA on 3 plasma pools



Figure

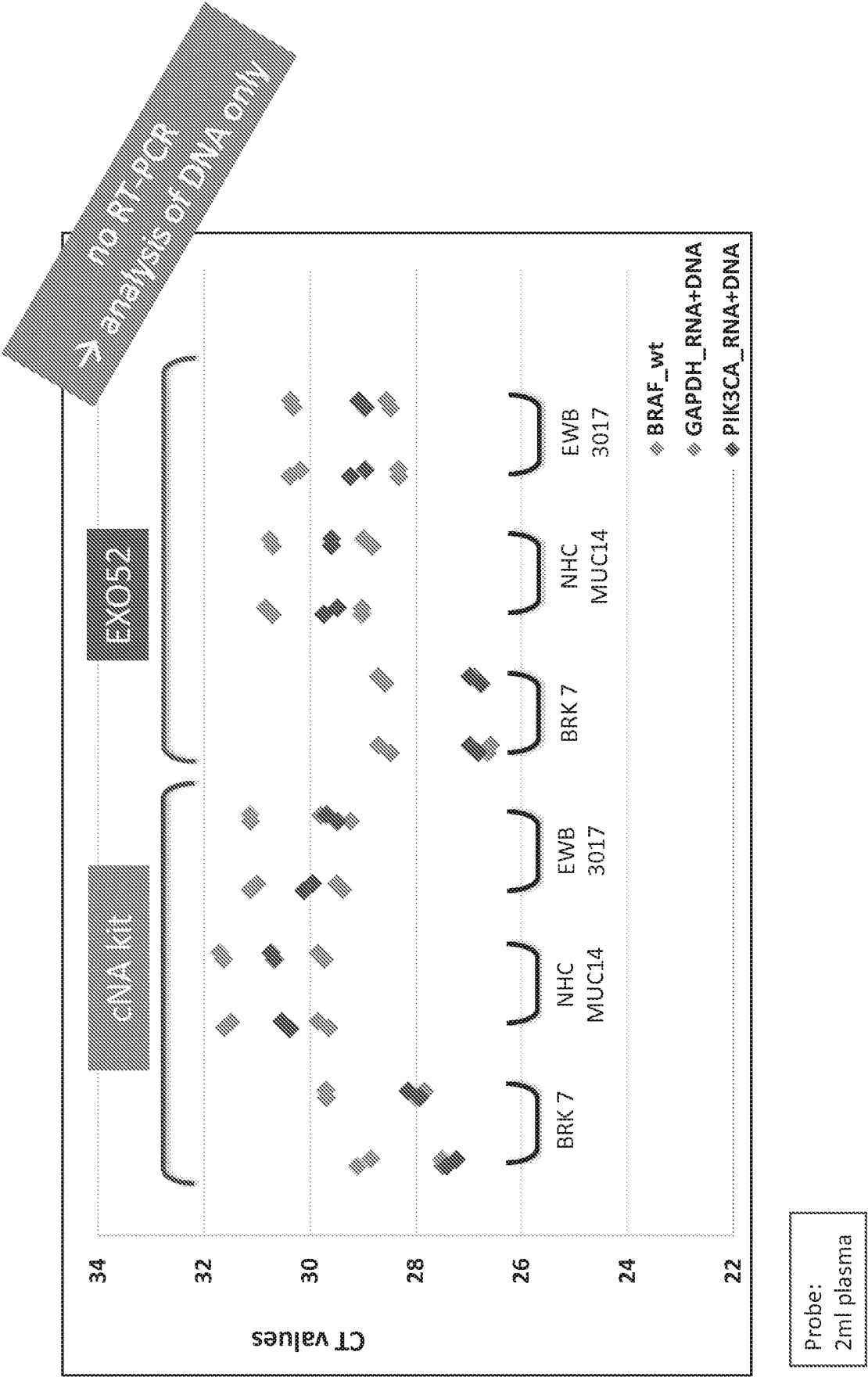
Comparison of EXO52 and Comm. Avail. Kit 1 circNA
old / new data

no RT-PCR
analysis of DNA only



Figure

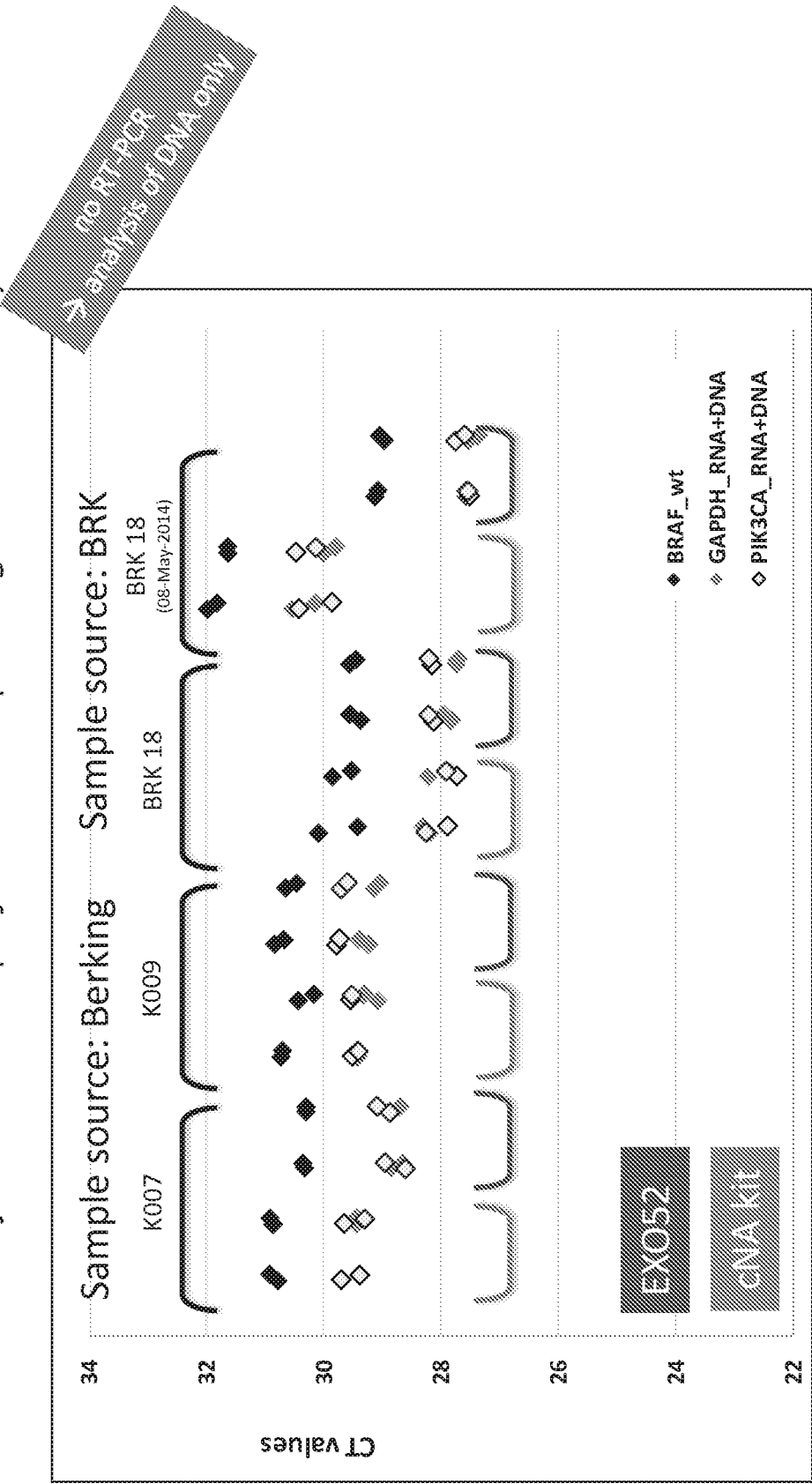
Comparison of
EXO52 / Comm. Avail. Kit 1 circNA on 3 plasma pools



Figure

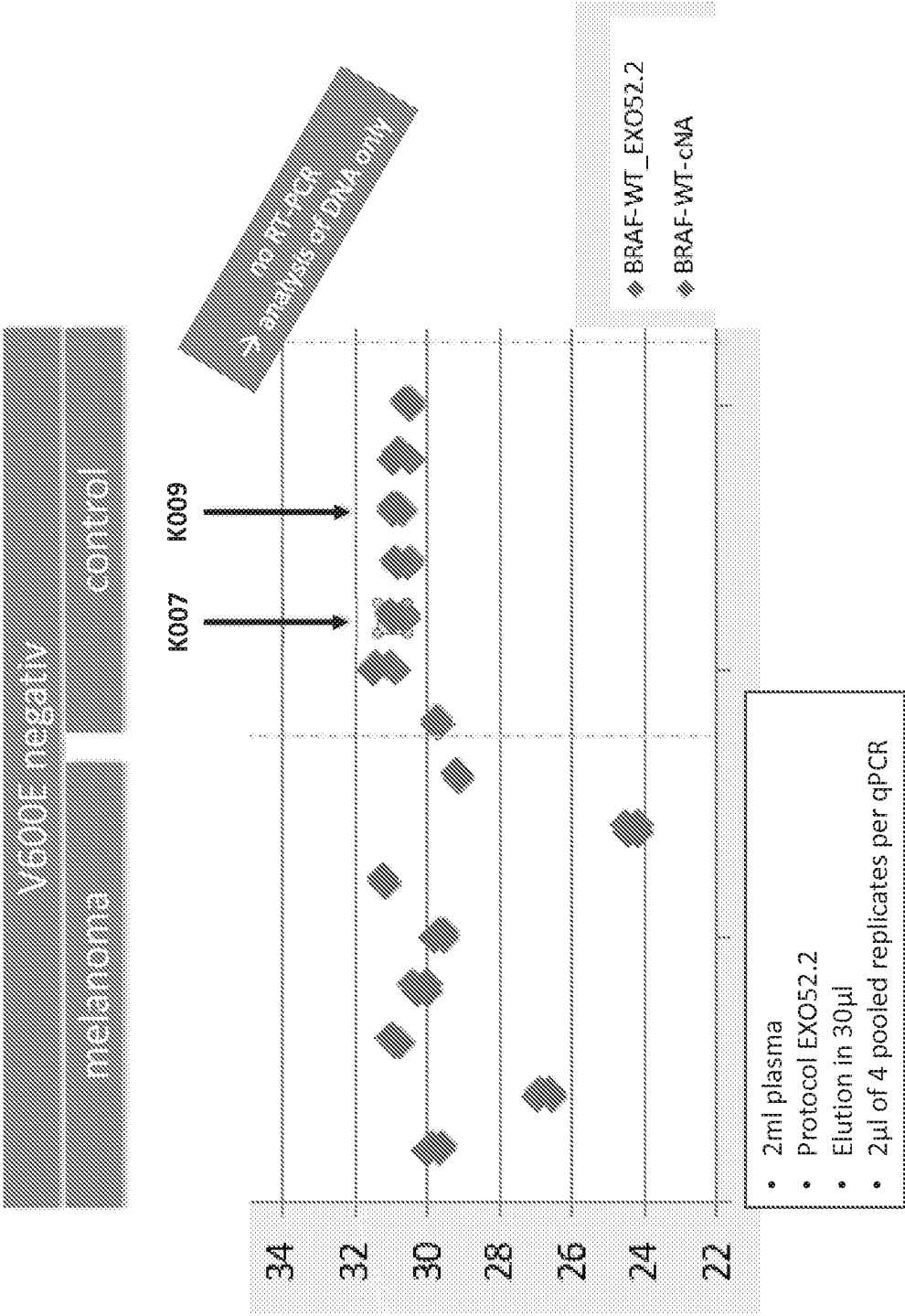
EXO52 is consistently better or equal than the CNA Kit

... but there is a variation depending on samples source and a variation of the CNA Kit's performance depending on isolation day!



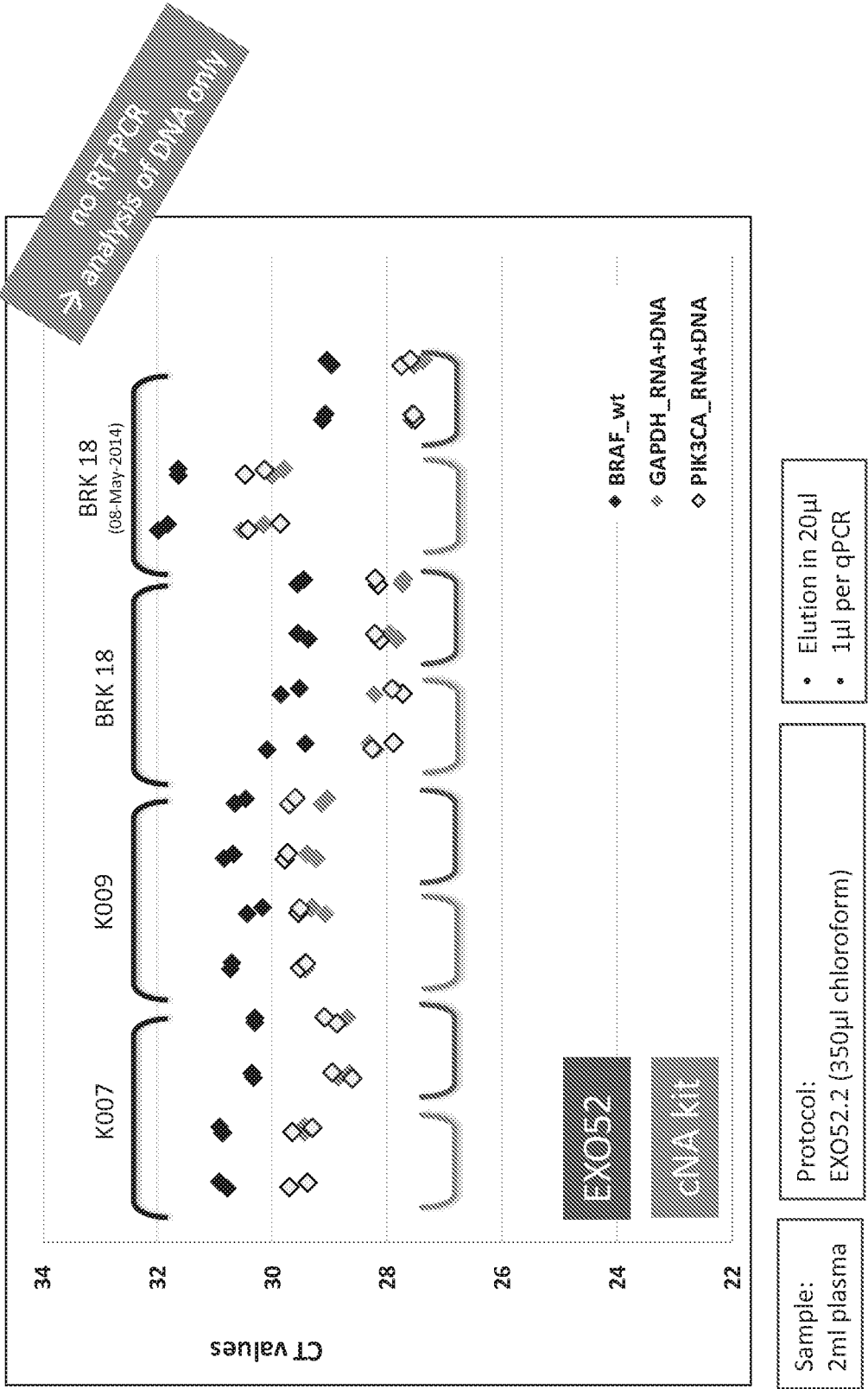
Figure

Origin BRAF-WT assay on cohort samples



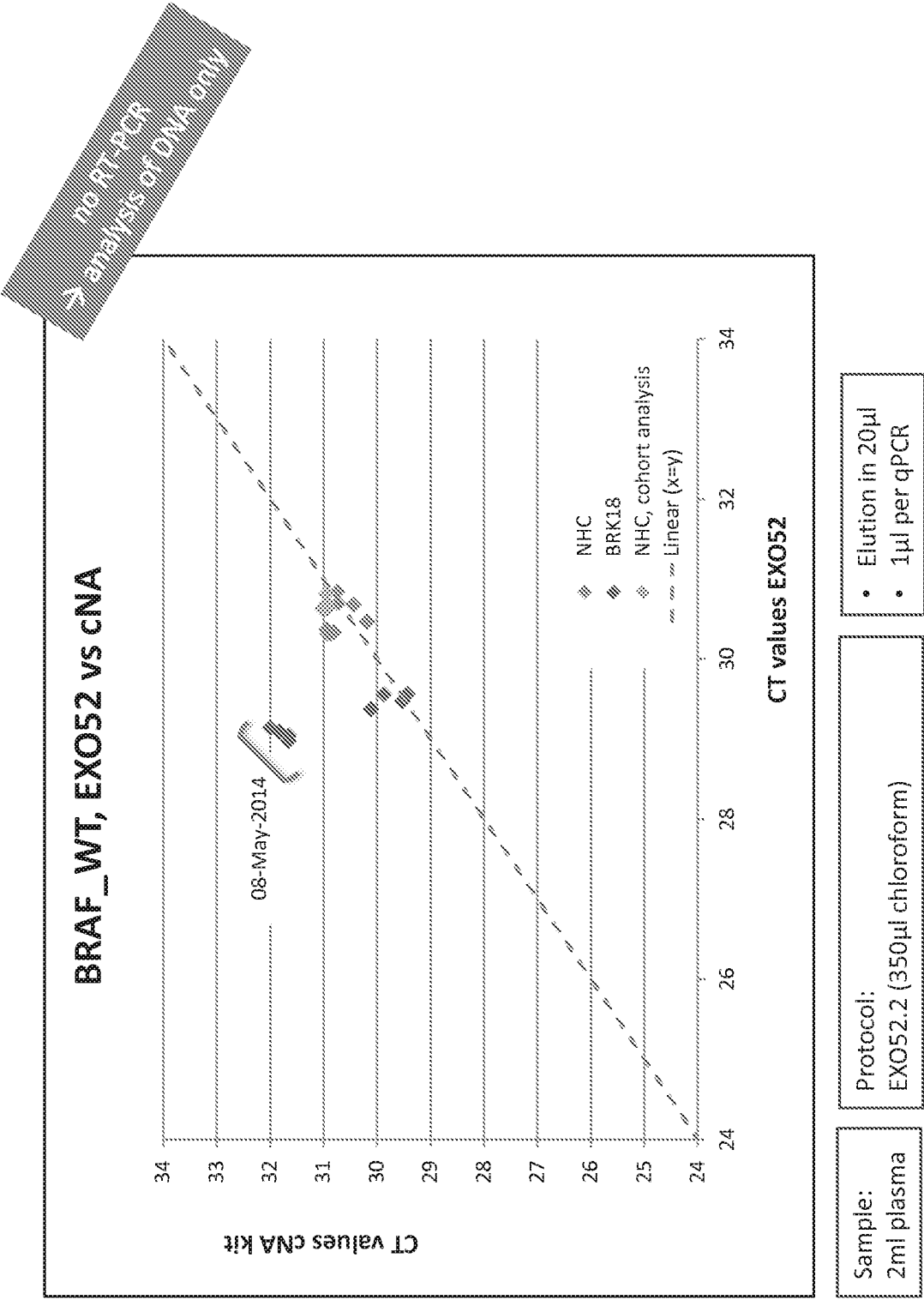
Figure

Comparison of EXO52 / Comm. Avail. Kit 1 circNA
on plasma derived from
melanoma cohort (normal healthy control)



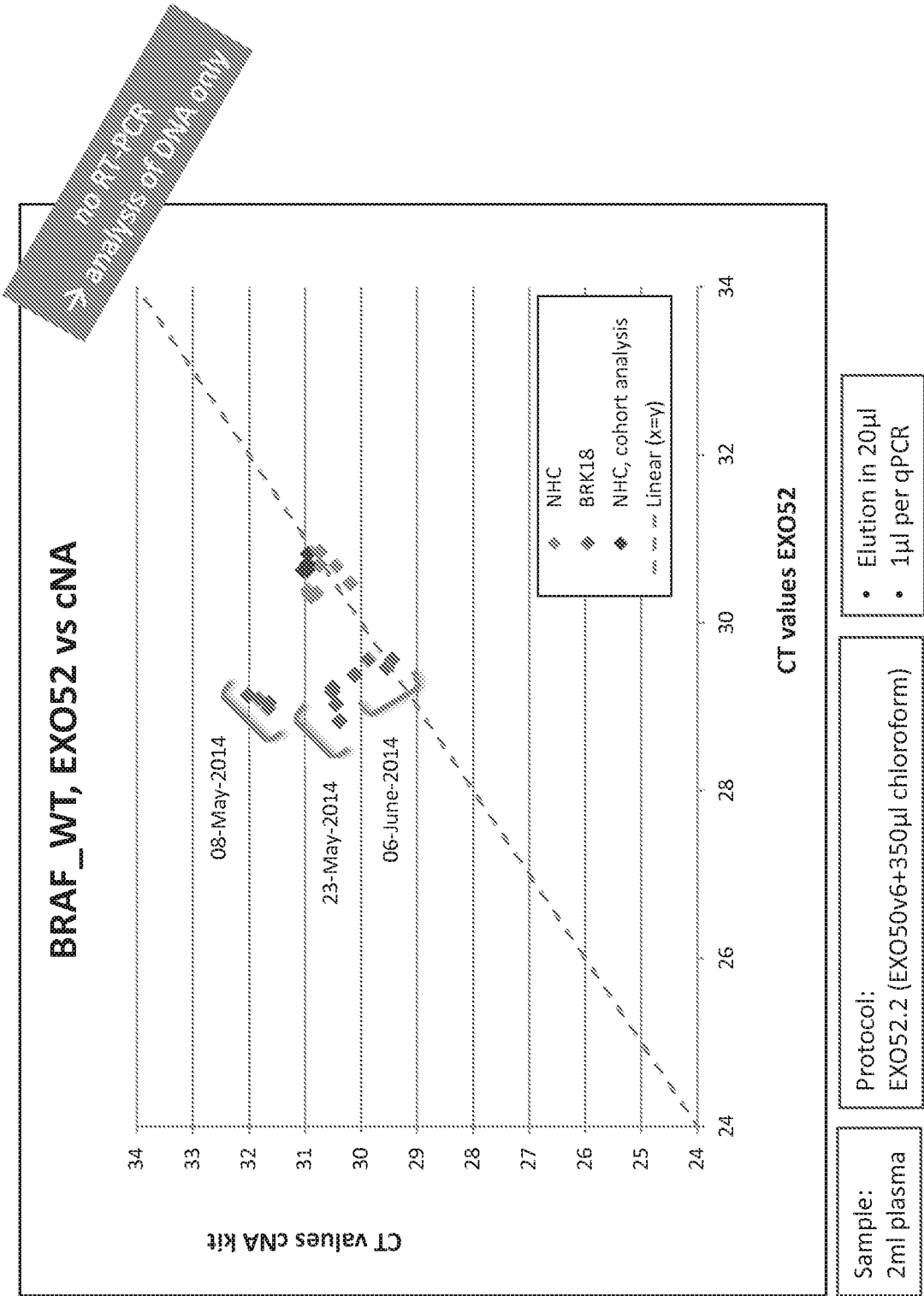
Figure

Comparison of EXO52 / Comm. Avail. Kit 1 circNA
on plasma derived from
melanoma cohort (normal healthy control)



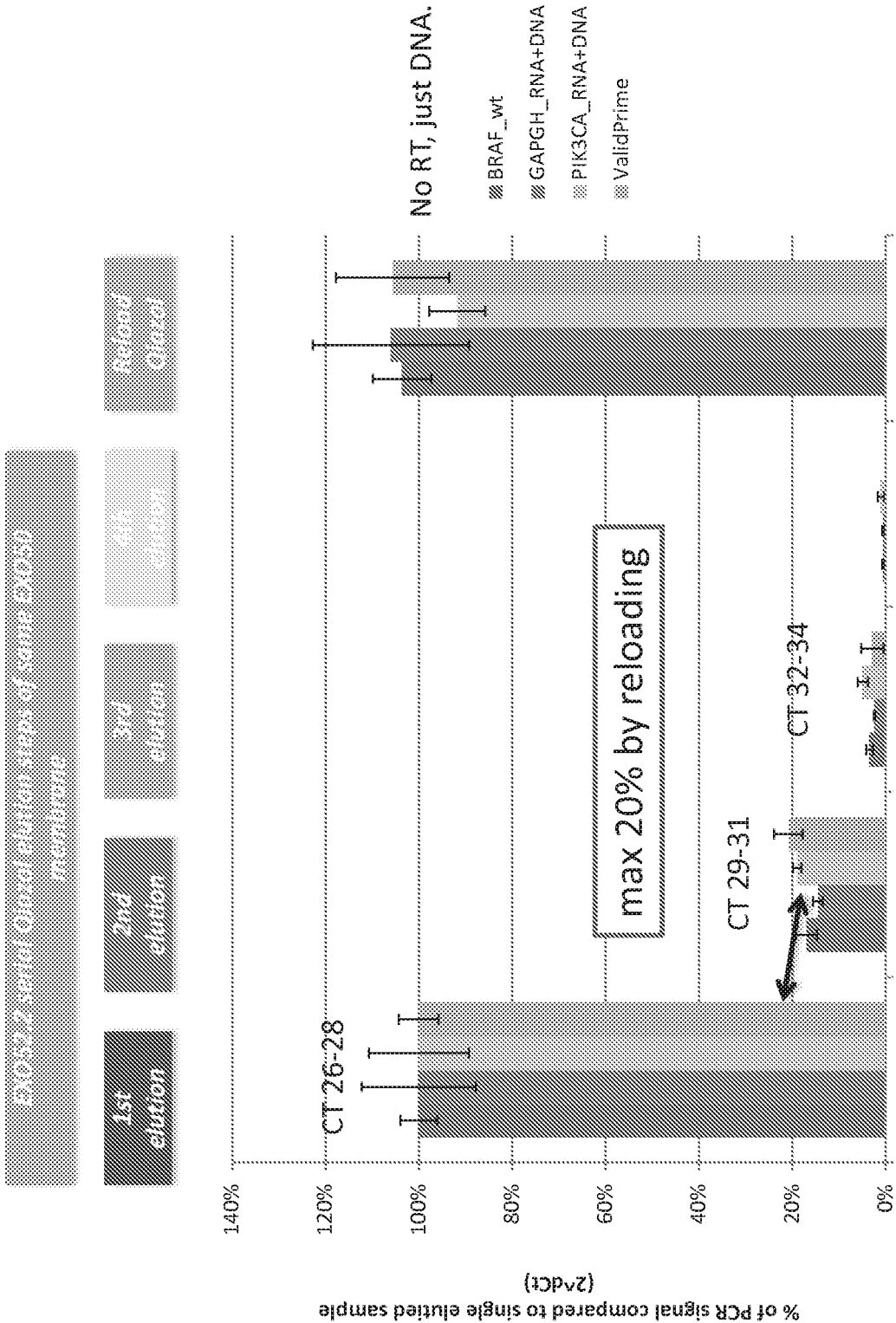
Figure

Comparison of EXO52 / Comm. Avail. Kit 1 circNA
on plasma derived from
melanoma cohort (normal healthy control)



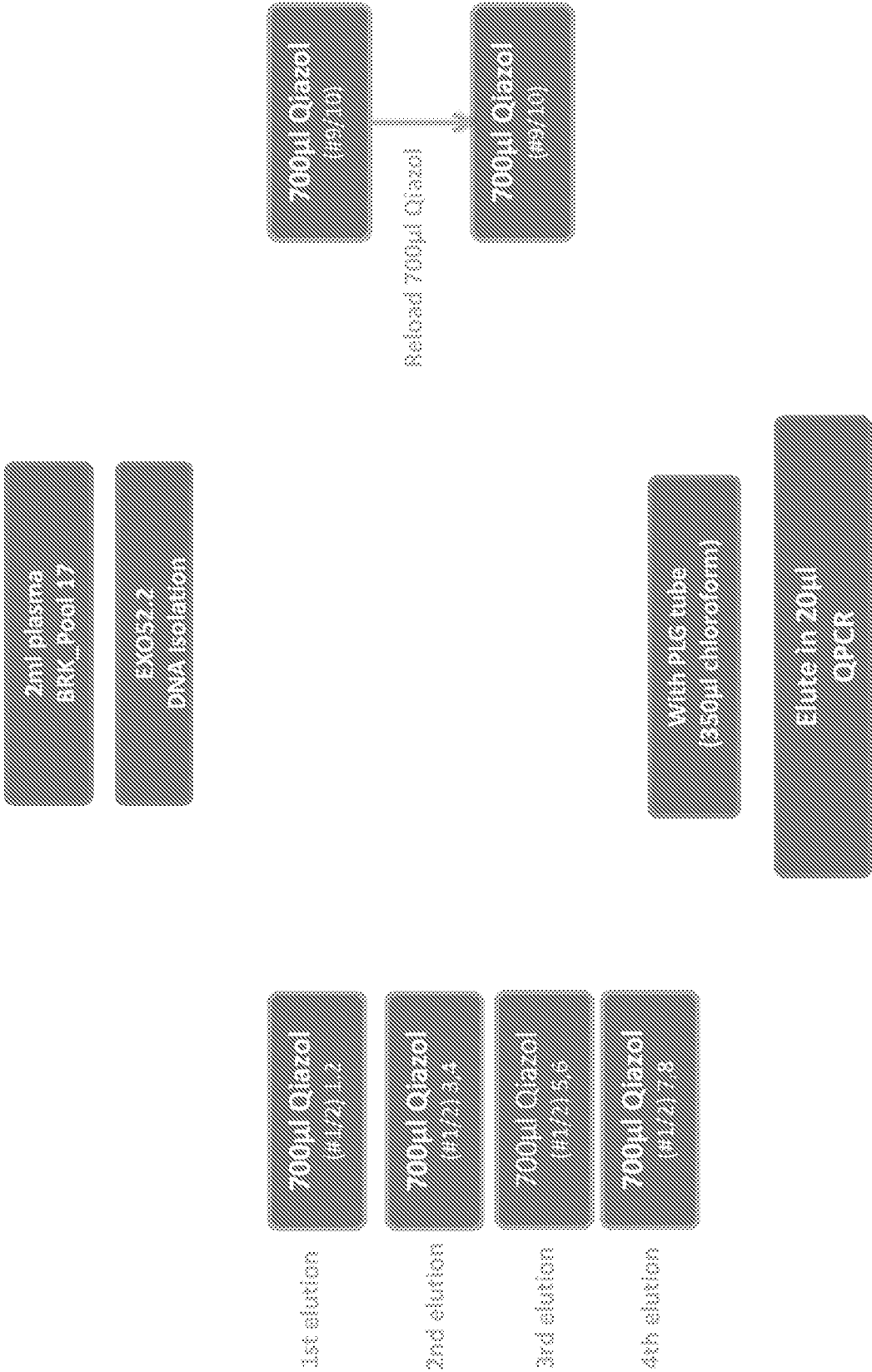
Figure

Multiple, separate QIAzol elution steps



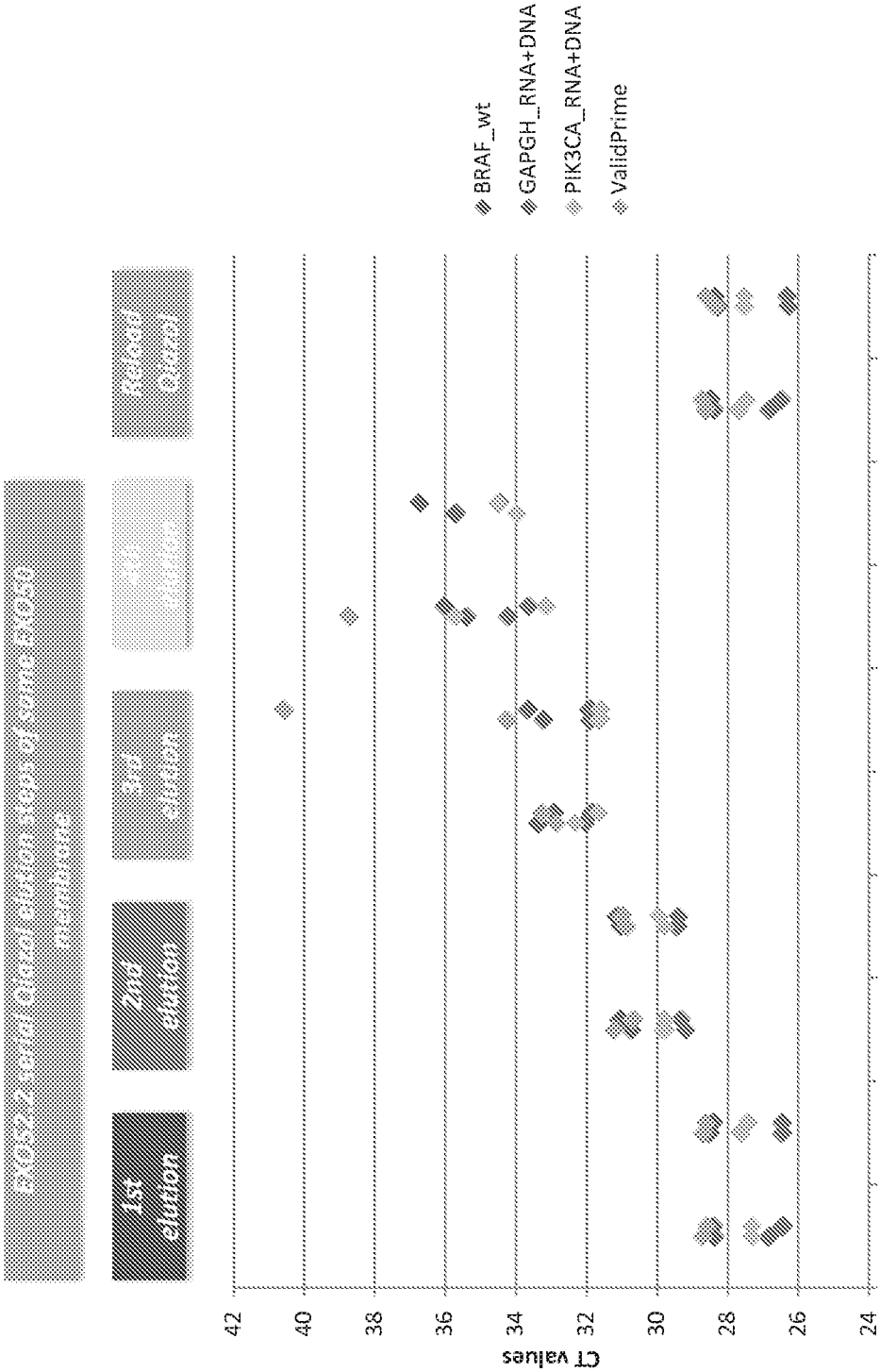
Figure

EXO502.2 DNA-Isolation: multiple Qiazol elution steps



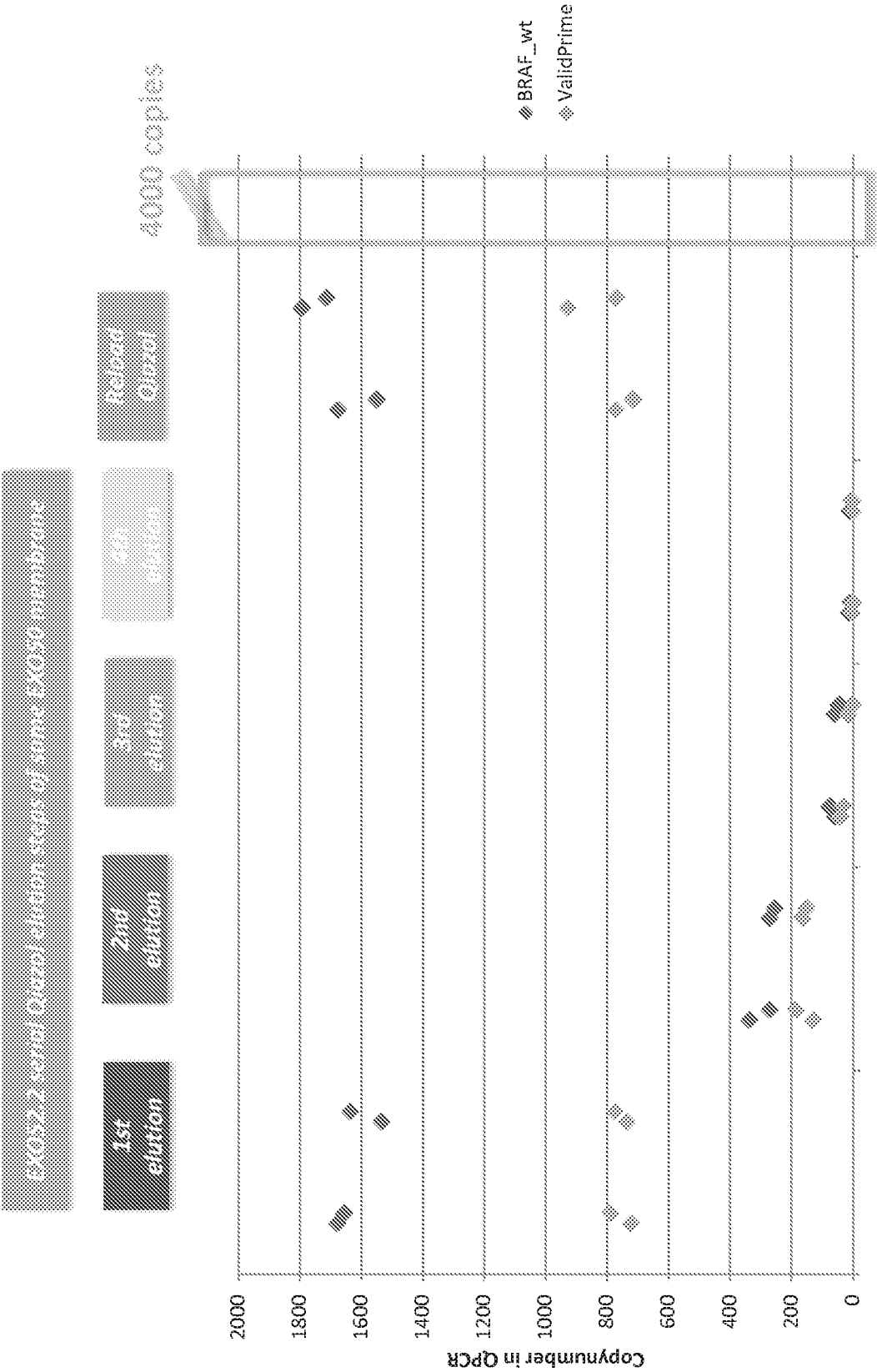
Figure

EXO502.2 DNA-Isolation: multiple Qiazol elution steps



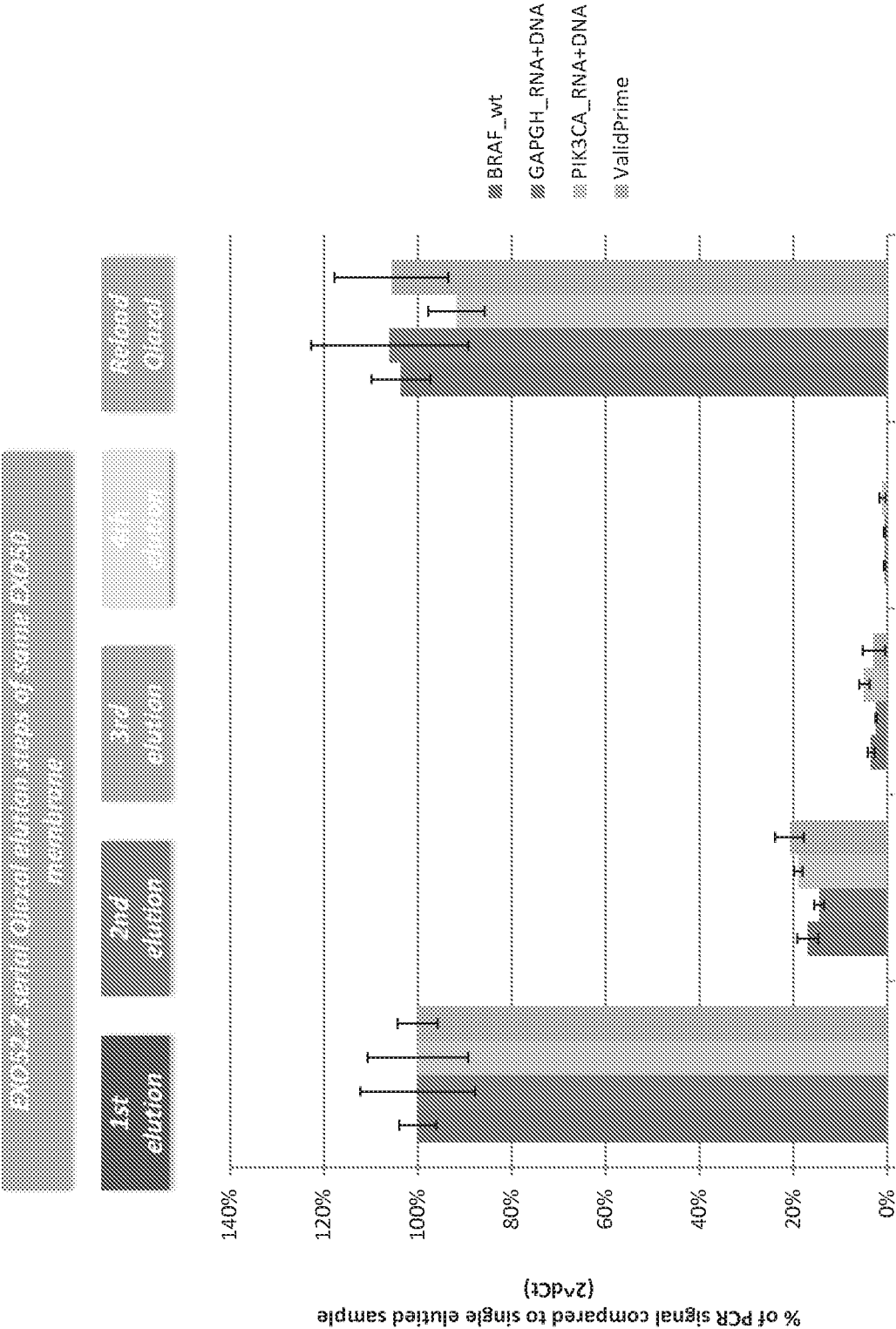
Figure

EXO502.2 DNA-Isolation: multiple Qiazol elution steps



Figure

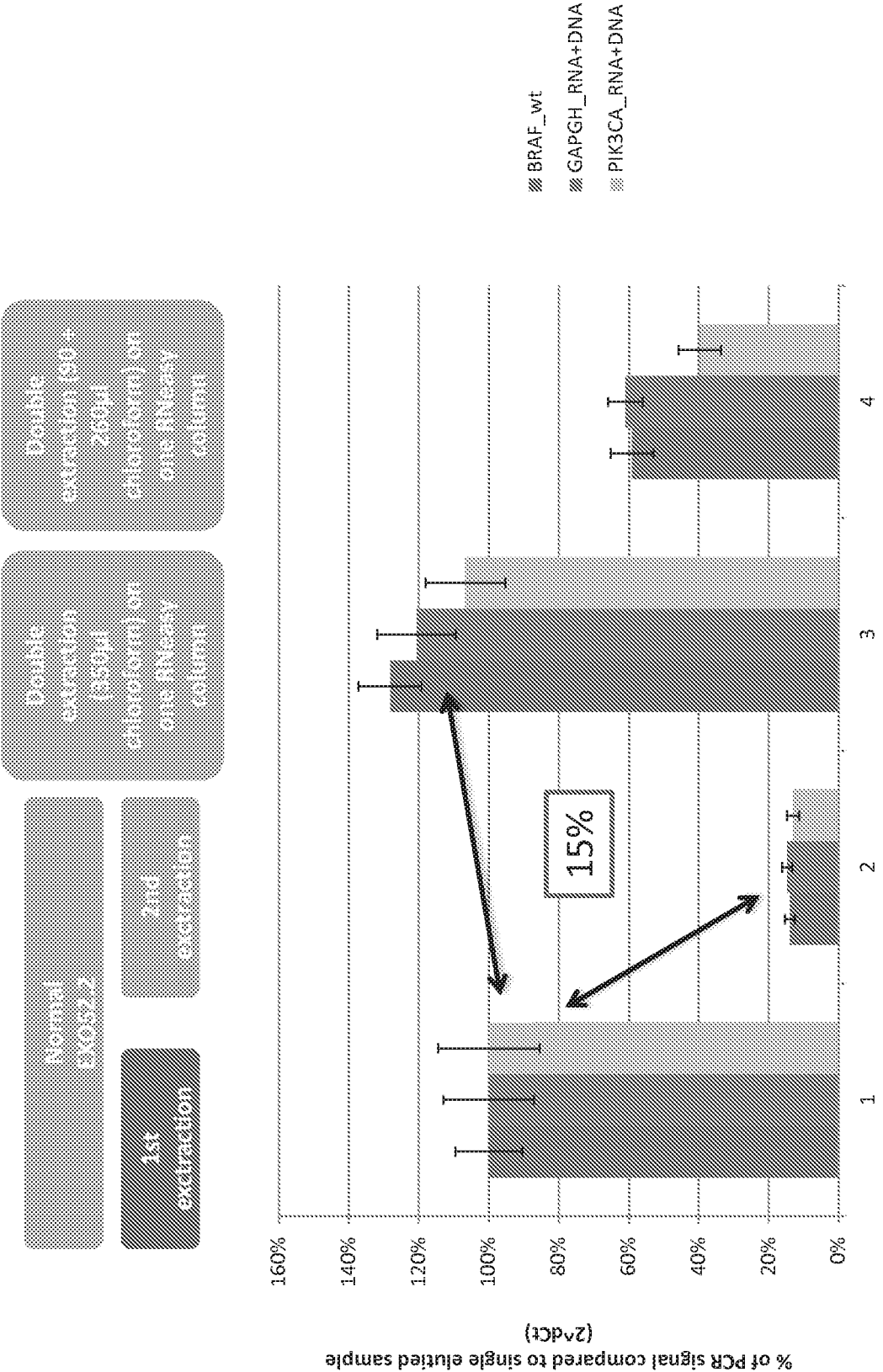
EXO502.2 DNA-Isolation: multiple Qiazol elution steps



Used average of all two isolation replicates and use Error Bars with standard deviation

Figure

EXO502.2 DNA-Isolation: multiple Qiazol extraction steps



Figure

EXO502.2 DNA-Isolation: multiple Qiazol extraction steps

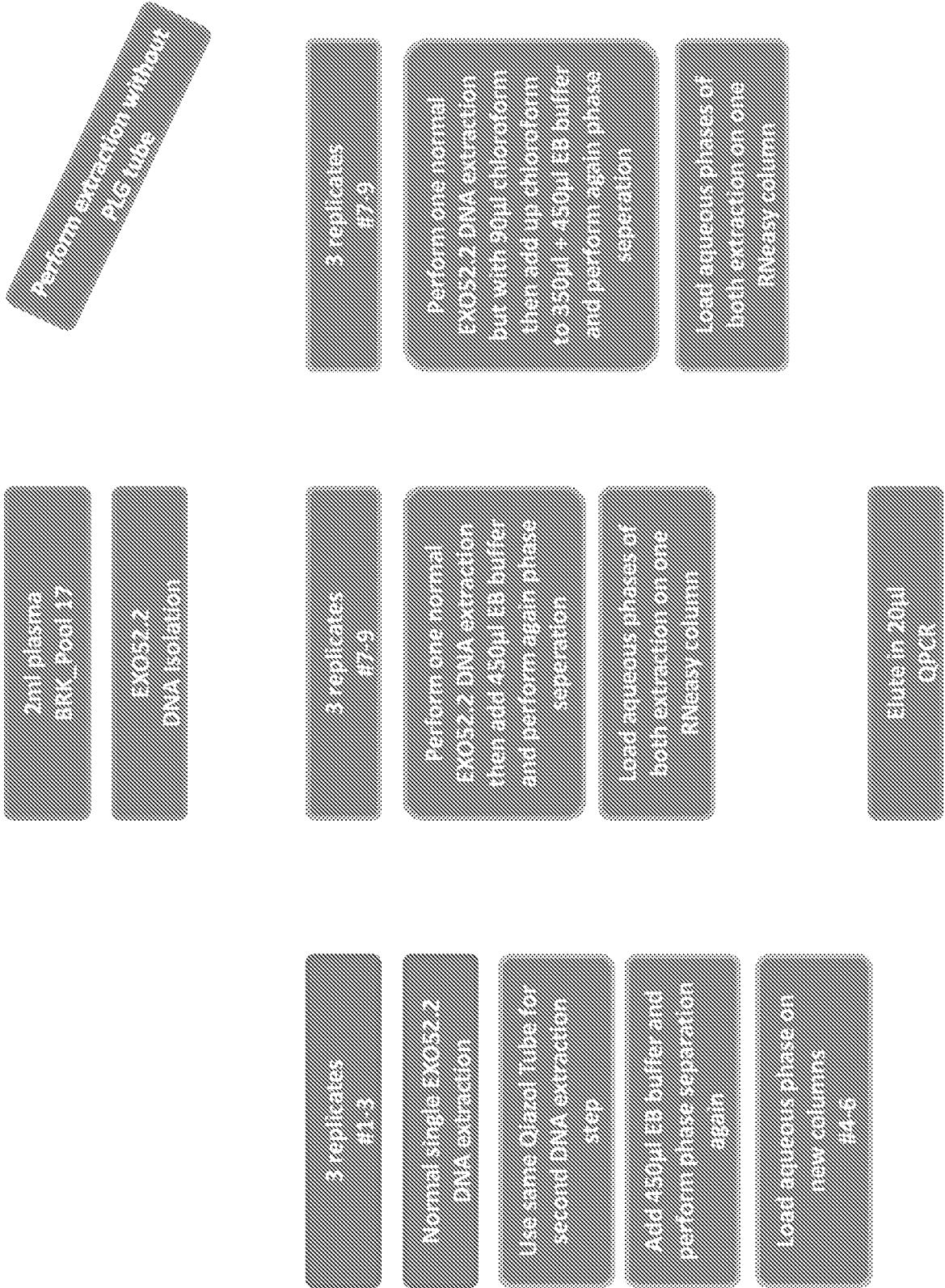
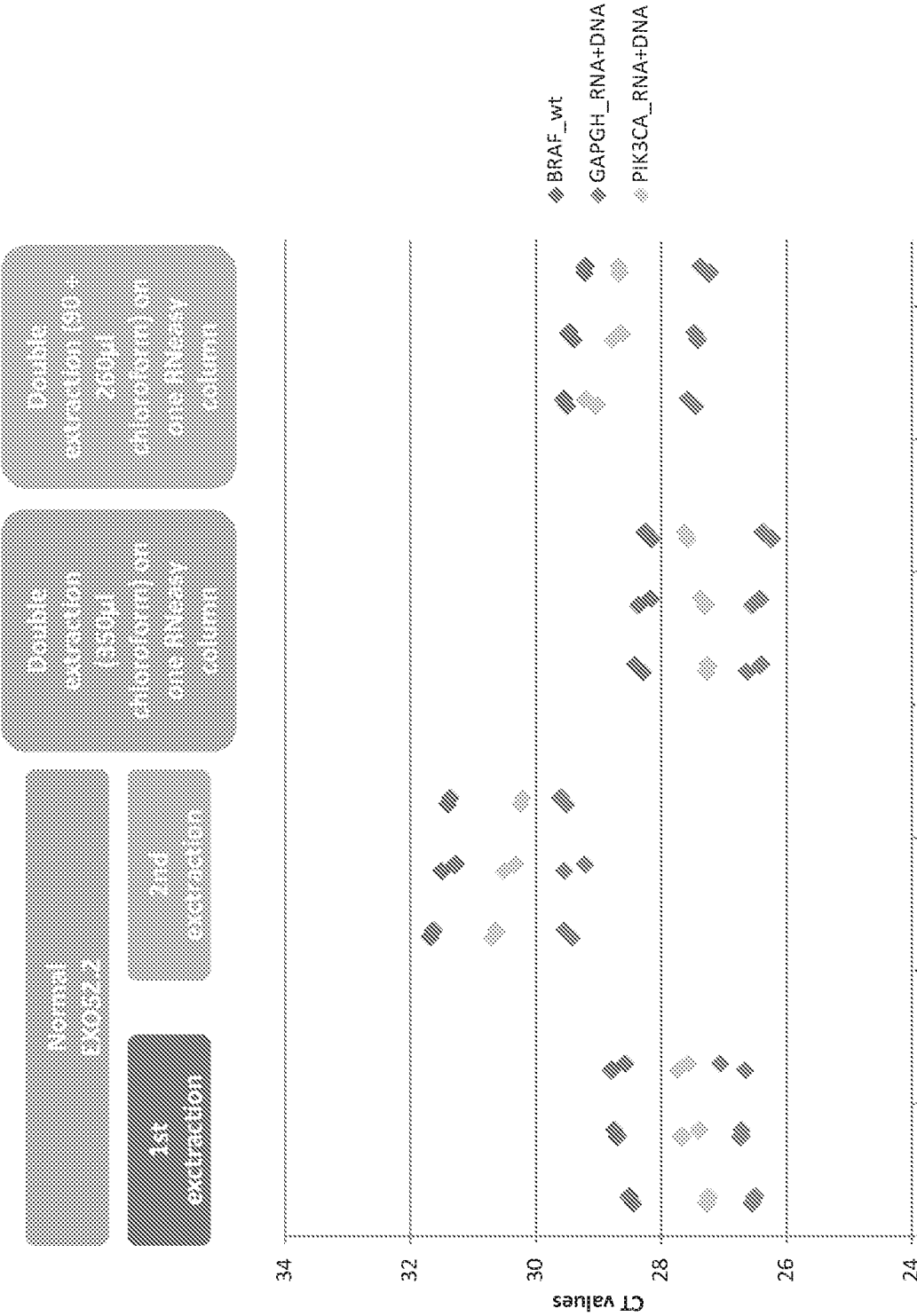


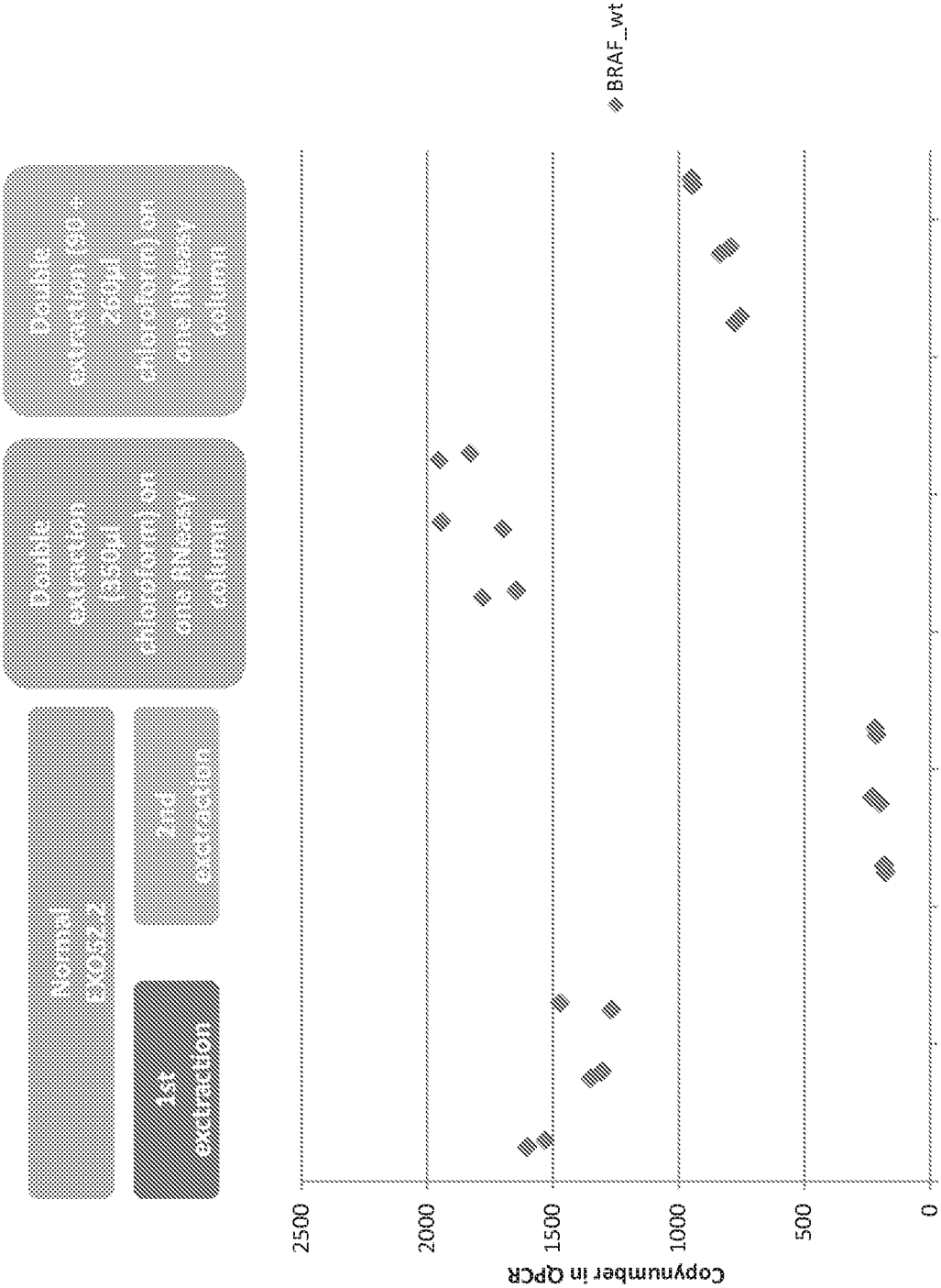
Figure Note : 350µl Chloroform /2.0x EtOH 203

EXO502.2 DNA-Isolation: multiple Qiazol extraction steps



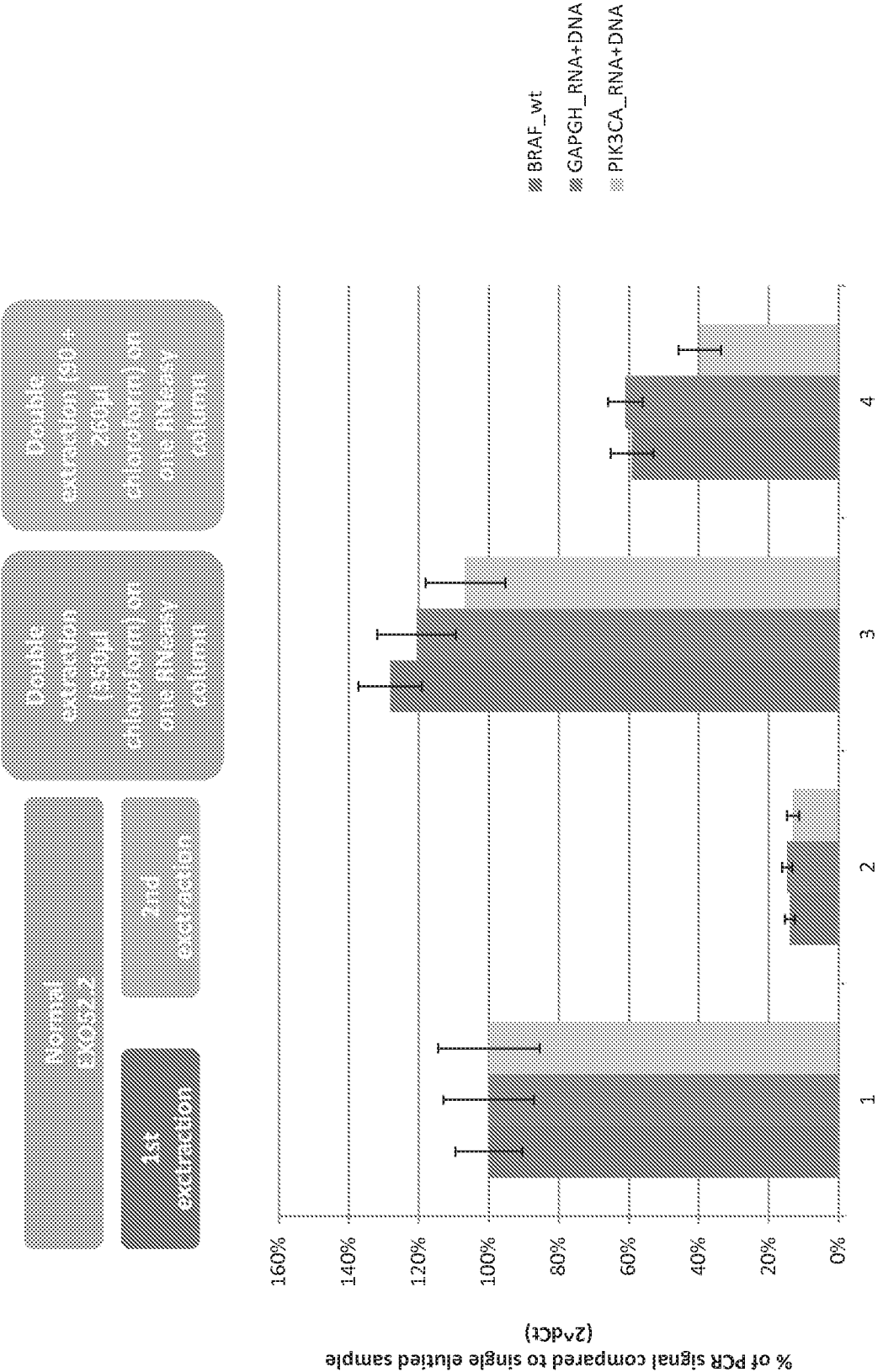
Figure

EXO502.2 DNA-Isolation: multiple Qiazol extraction steps



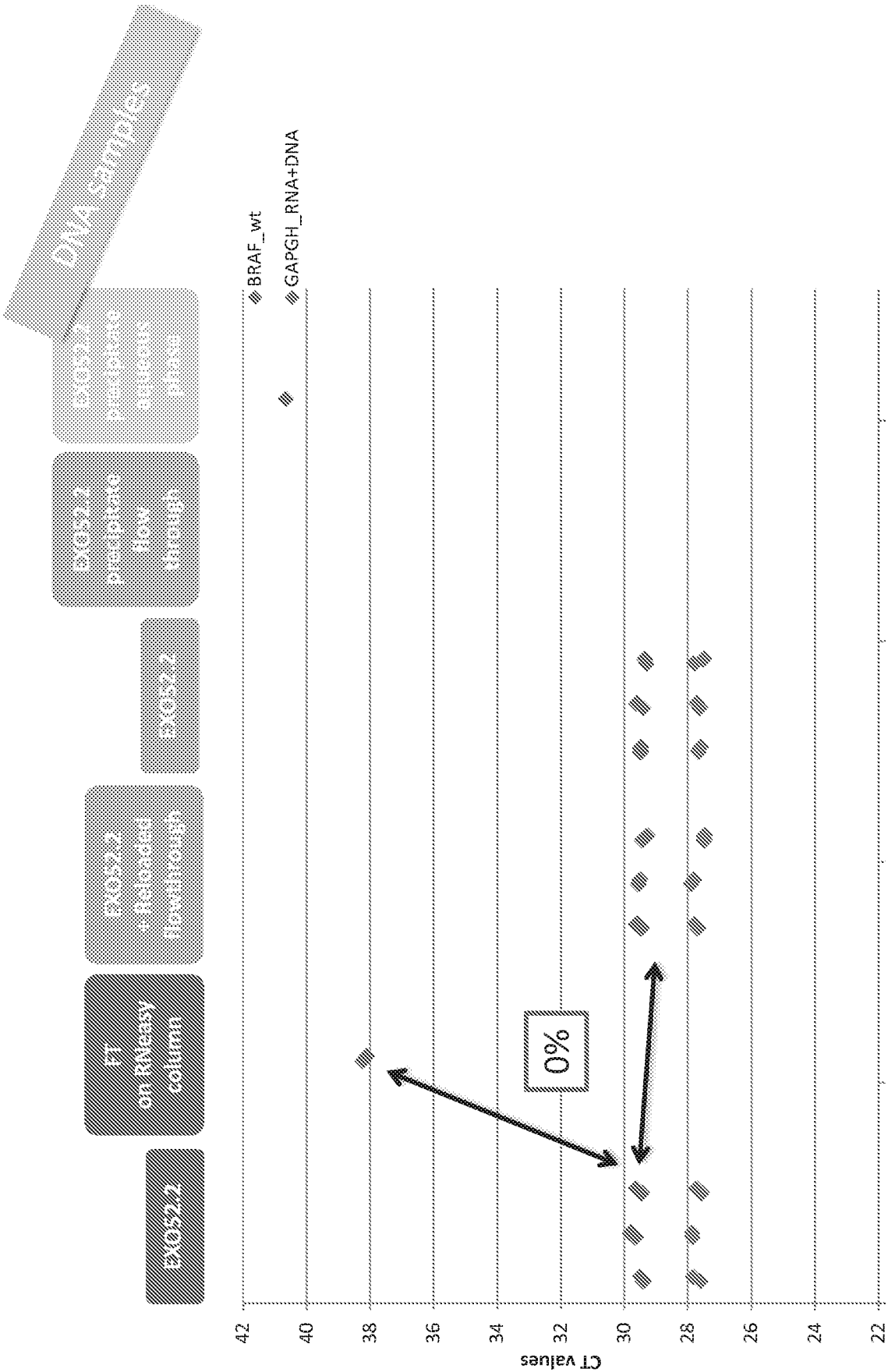
Figure

EXO502.2 DNA-Isolation: multiple Qiazol extraction steps



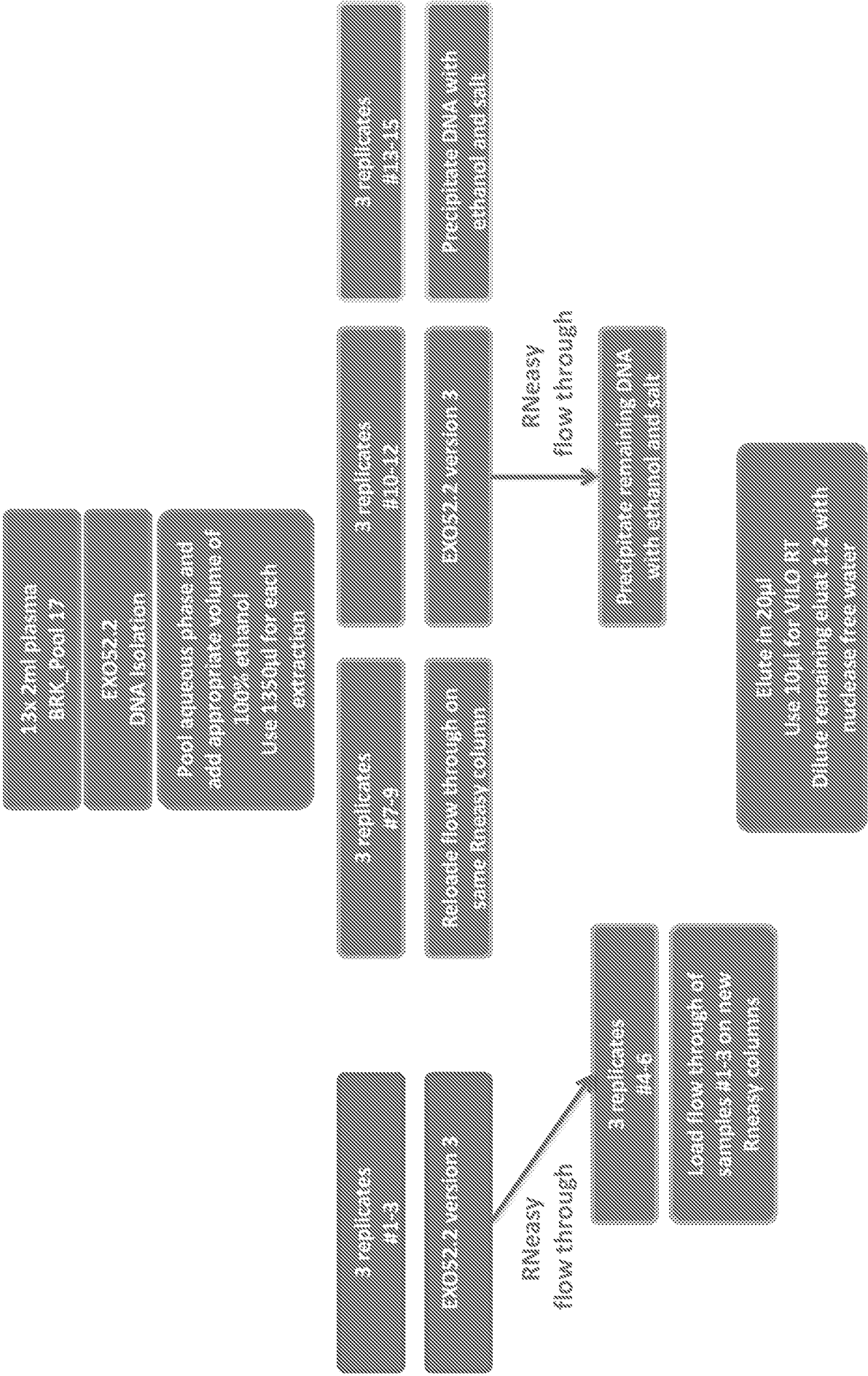
Figure

EXO52.2 DNA-Isolation: double RNeasy loading steps – ethanol precipitation



Figure

EXO52.2 DNA-Isolation: double RNeasy loading steps – ethanol precipitation



Protocol ethanol precipitation

Nucleic acid ethanol precipitation from aqueous phase (EXO52.2 procedure):

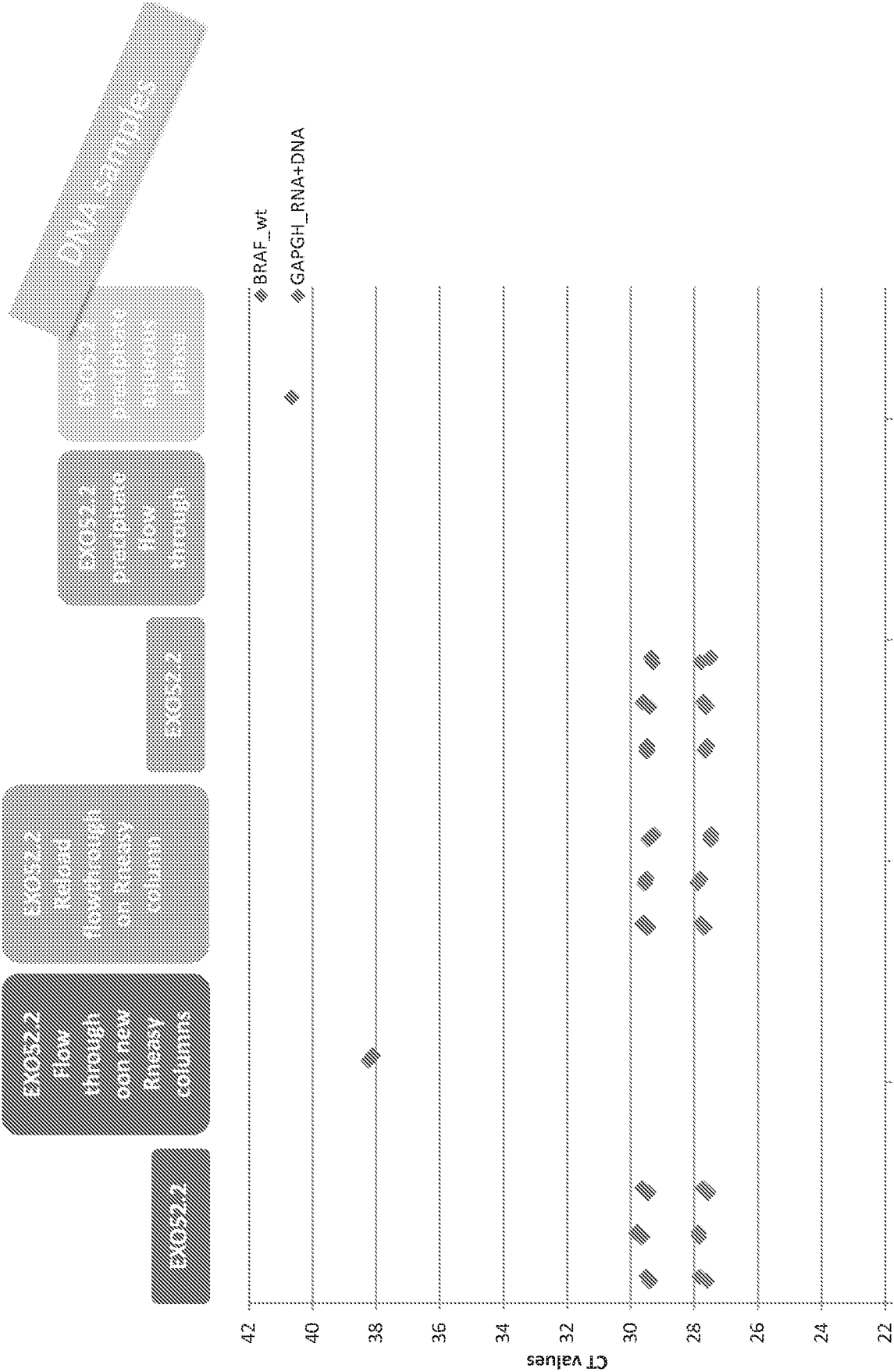
Reagents:

- * 3M sodium acetate pH 5.5
- * 100% ethanol
- * 20µg/µl Glycogen

Protocol:

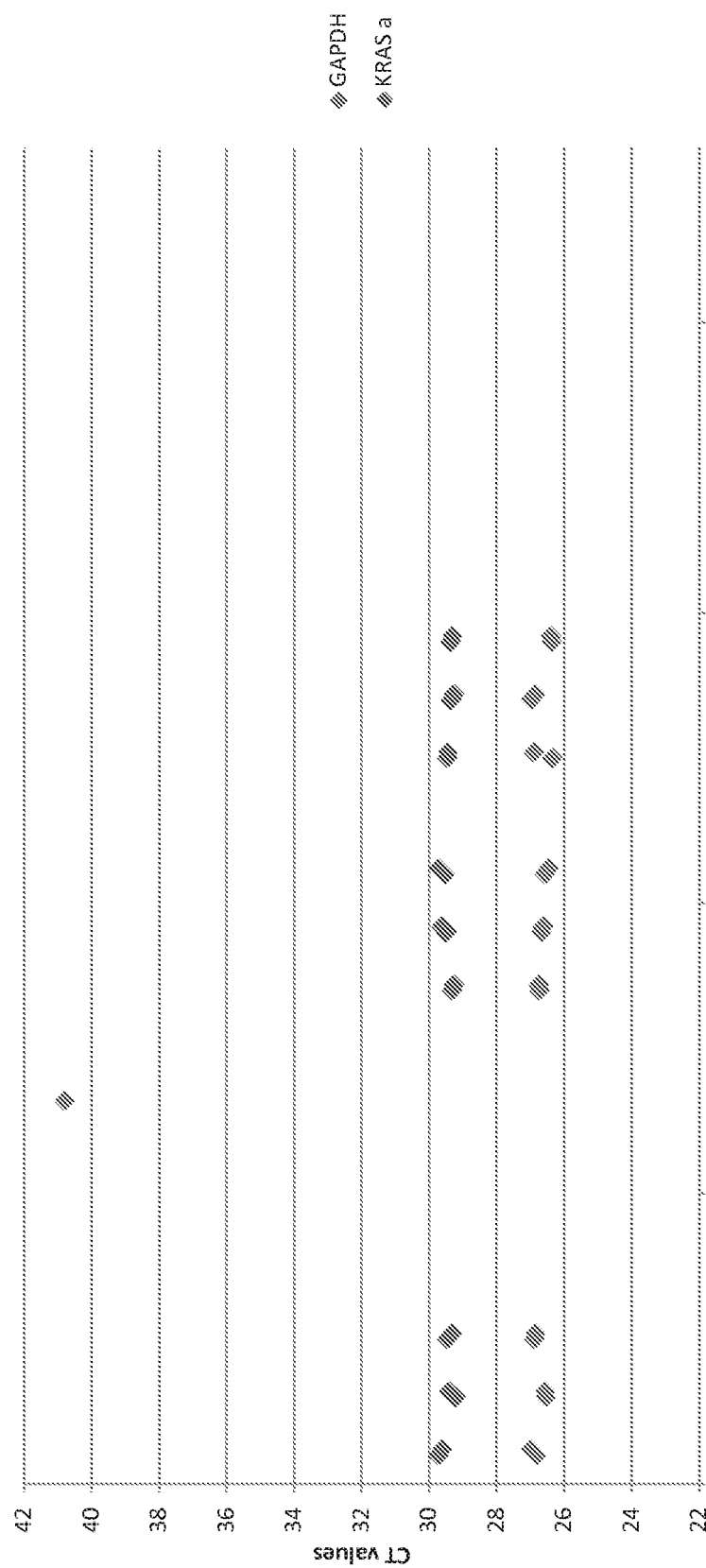
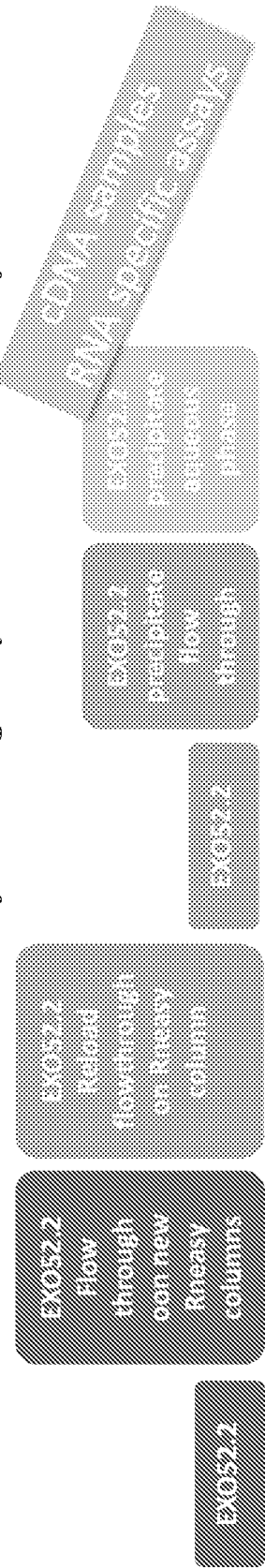
1. Transfer the upper aqueous phase to a new collection tube. Avoid transfer of any interphase material. Add 2 volumes of 100% ethanol and mix thoroughly by pipetting up and down several times. Do not centrifuge
2. Measure the volume of the aqueous phase (containing RNA and DNA) and added ethanol 1350µl (450µl aqueous phase + 900µl 100% ethanol)
3. Add 1/10 volume (relative to aqueous phase volume) of sodium acetate pH 5.2. → 45µl
4. Add up to 2.5 volumes of 100% ethanol (450µl aqueous phase + 45µl 3M sodium acetate) → 1237.5µl 100% ethanol (900µl already added to aqueous phase see step 1.) → add remaining 100% ethanol (337.5µl)
5. Mix thoroughly by pipetting up and down several times. Do not centrifuge
6. Add 25µg Glycogen (1.25µl Glycogen 20µg/µl)
7. Mix thoroughly by pipetting up and down several times. Do not centrifuge
8. Place tubes in the freezer @ -20°C over night
9. Thaw samples on the next day
10. Mix thoroughly by inverting the tube several time
11. Spin @ maximum speed (16000xg) for 15 min @ 4°C
12. Carefully remove supernatant without destroying nucleic acid pellet
13. If pellet come off the tube wall, re-spin for 2 min @ full speed
14. Add 1ml 70% ethanol. Mix and spin briefly. Carefully remove supernatant
15. If pellet come off the tube wall, re-spin for 2 min @ full speed
16. Remove remaining 70% ethanol using a small pipette
17. Air dry pellet
18. Resuspend pellet in 20µl EB

EXO52.2 DNA-Isolation: double RNeasy loading steps – ethanol precipitation



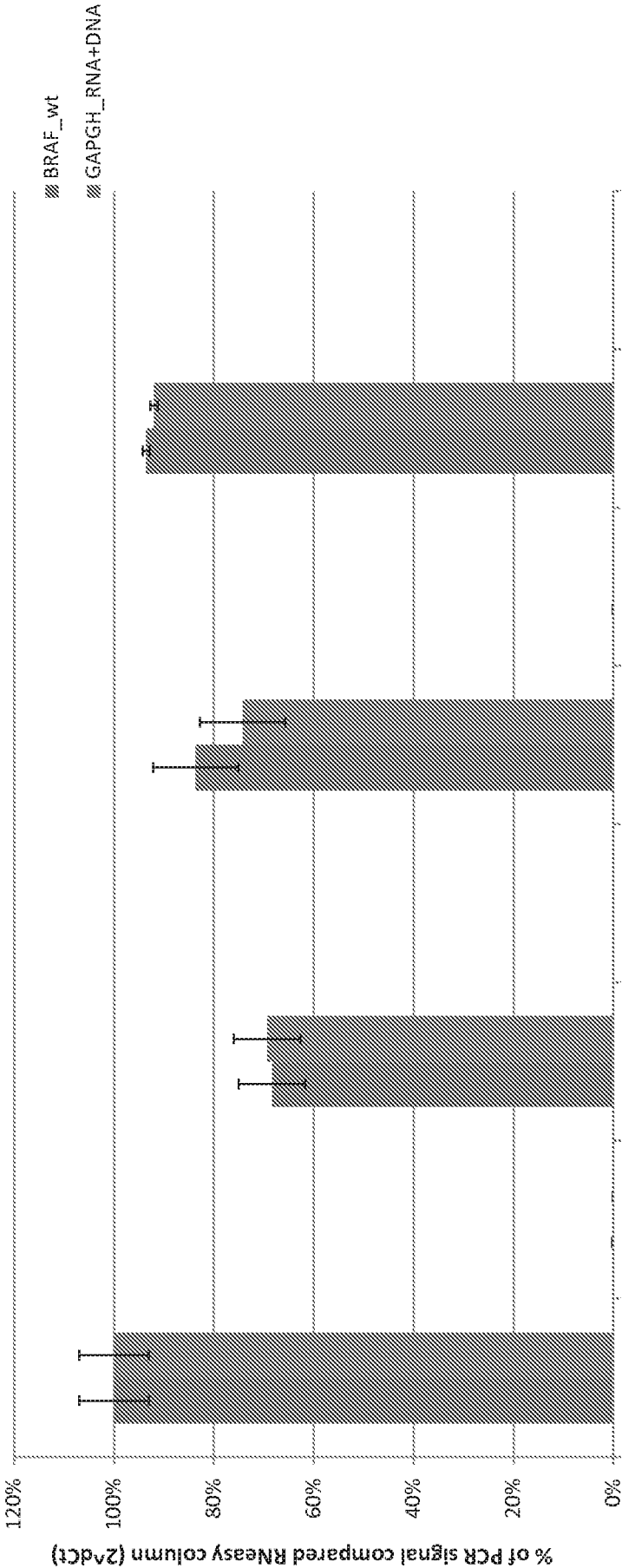
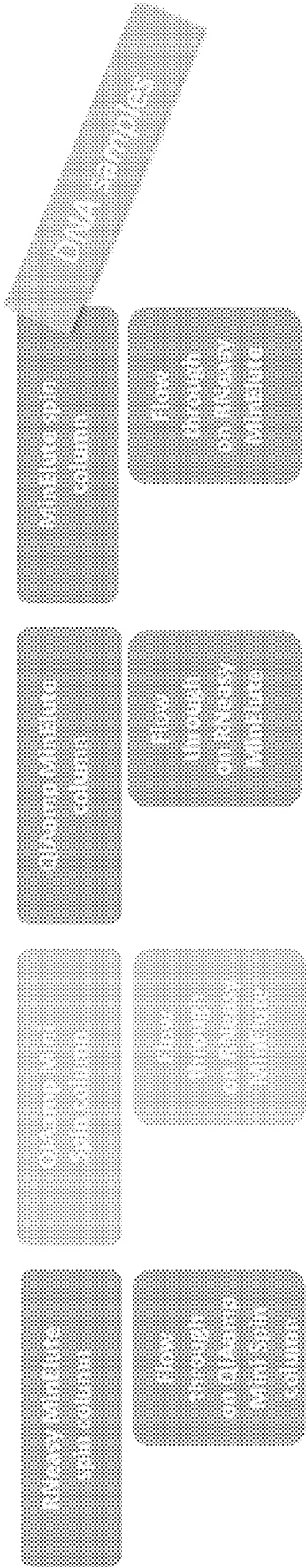
Figure

EXO52.2 DNA-Isolation: double RNeasy loading steps – ethanol precipitation



Figure

EXO52.2 DNA-Isolation: use different downstream columns



Figure

EXO52.2 DNA-Isolation: use different downstream columns

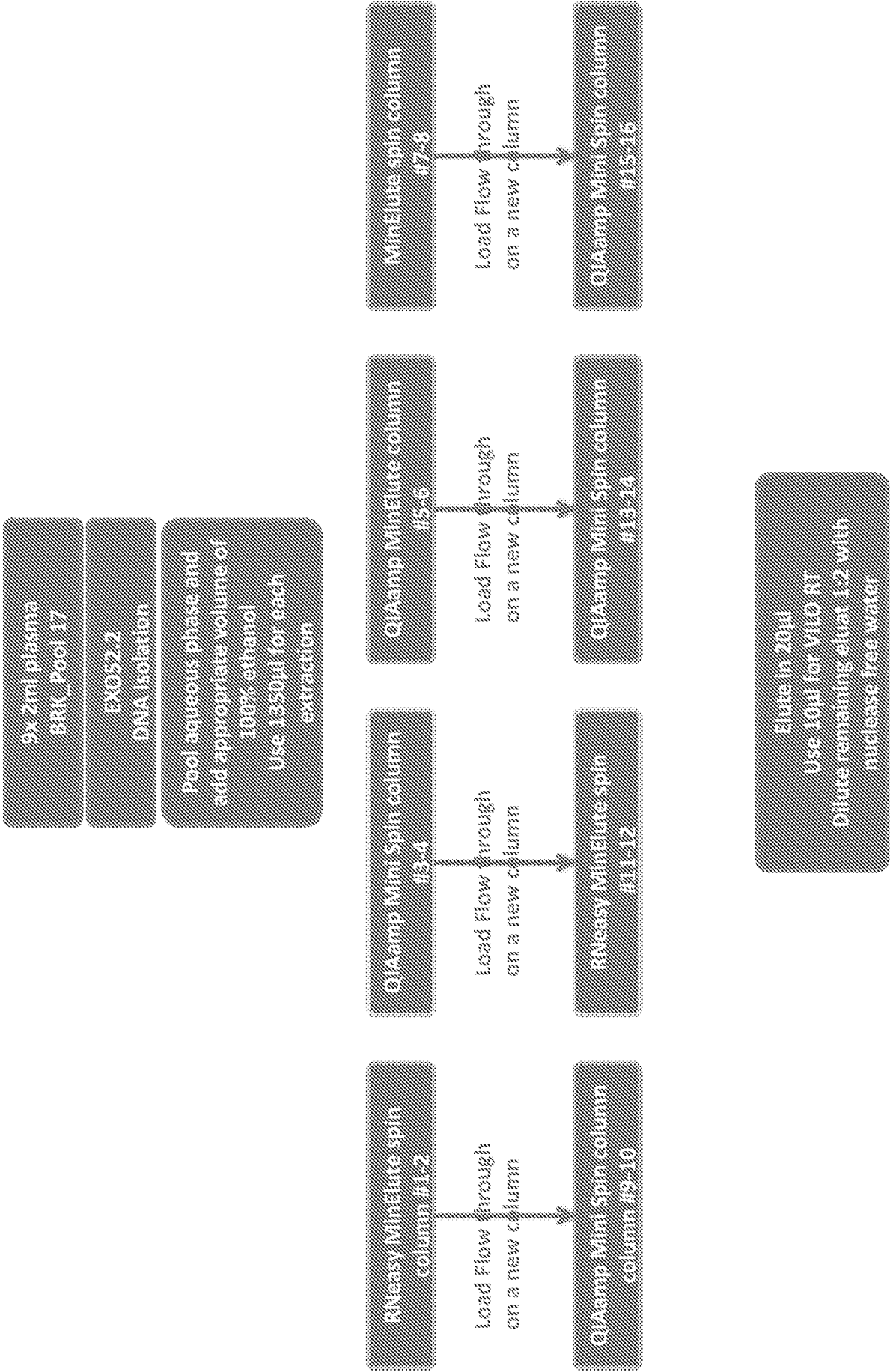
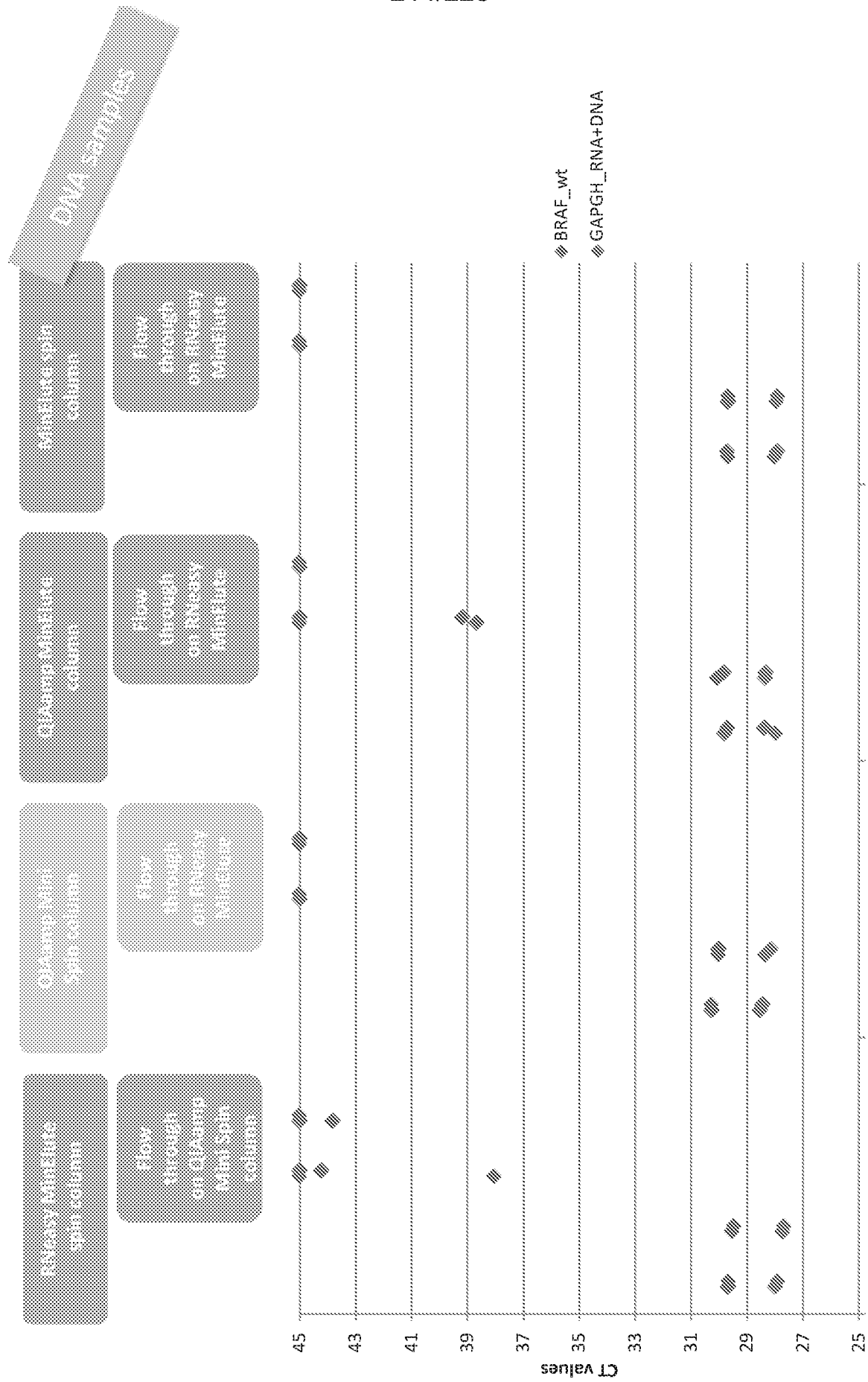


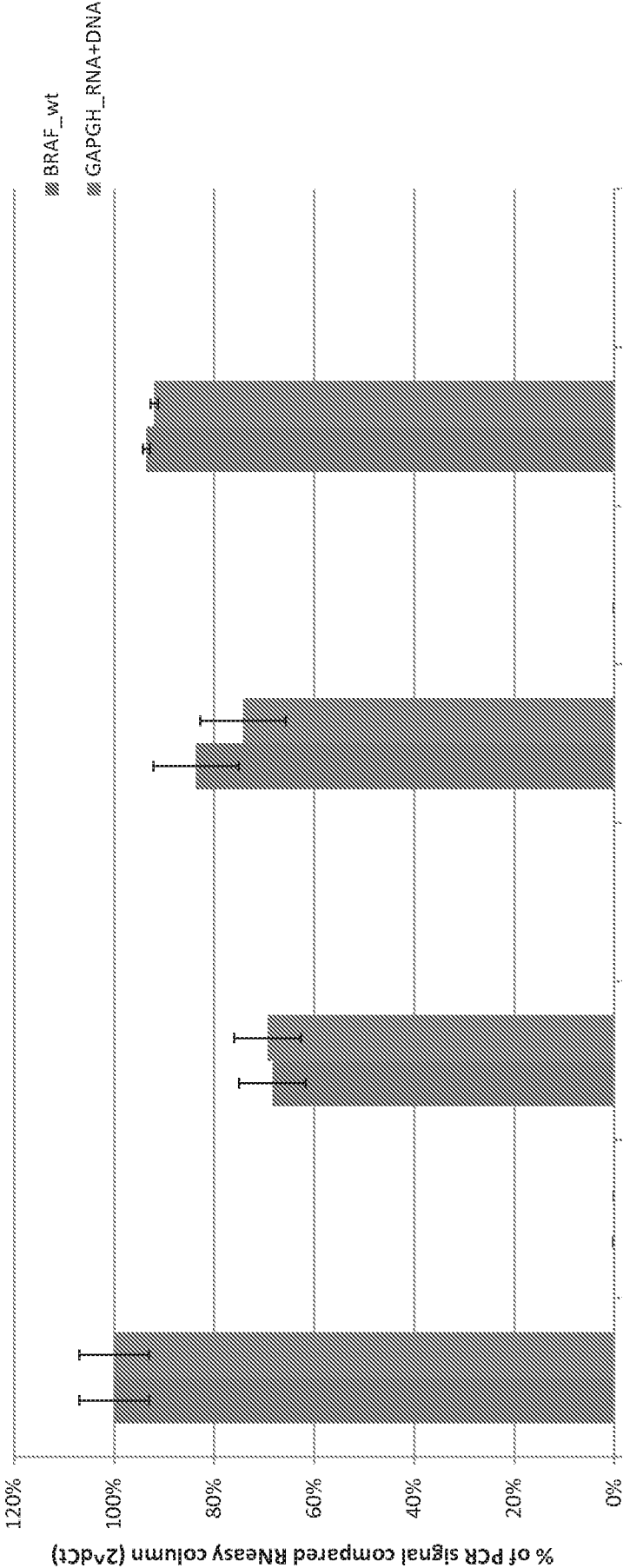
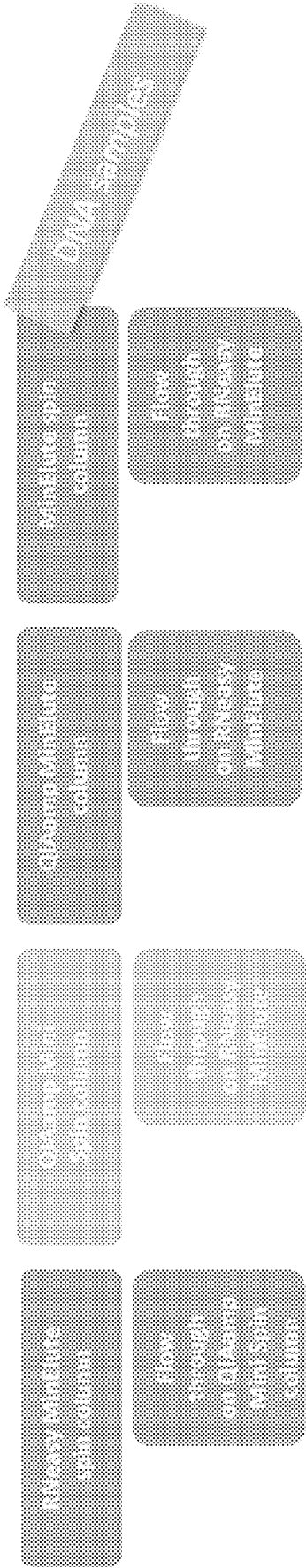
Figure Note : 350µl Chloroform /2.0x EtOH 213

EXO52.2 DNA-Isolation: use different downstream columns



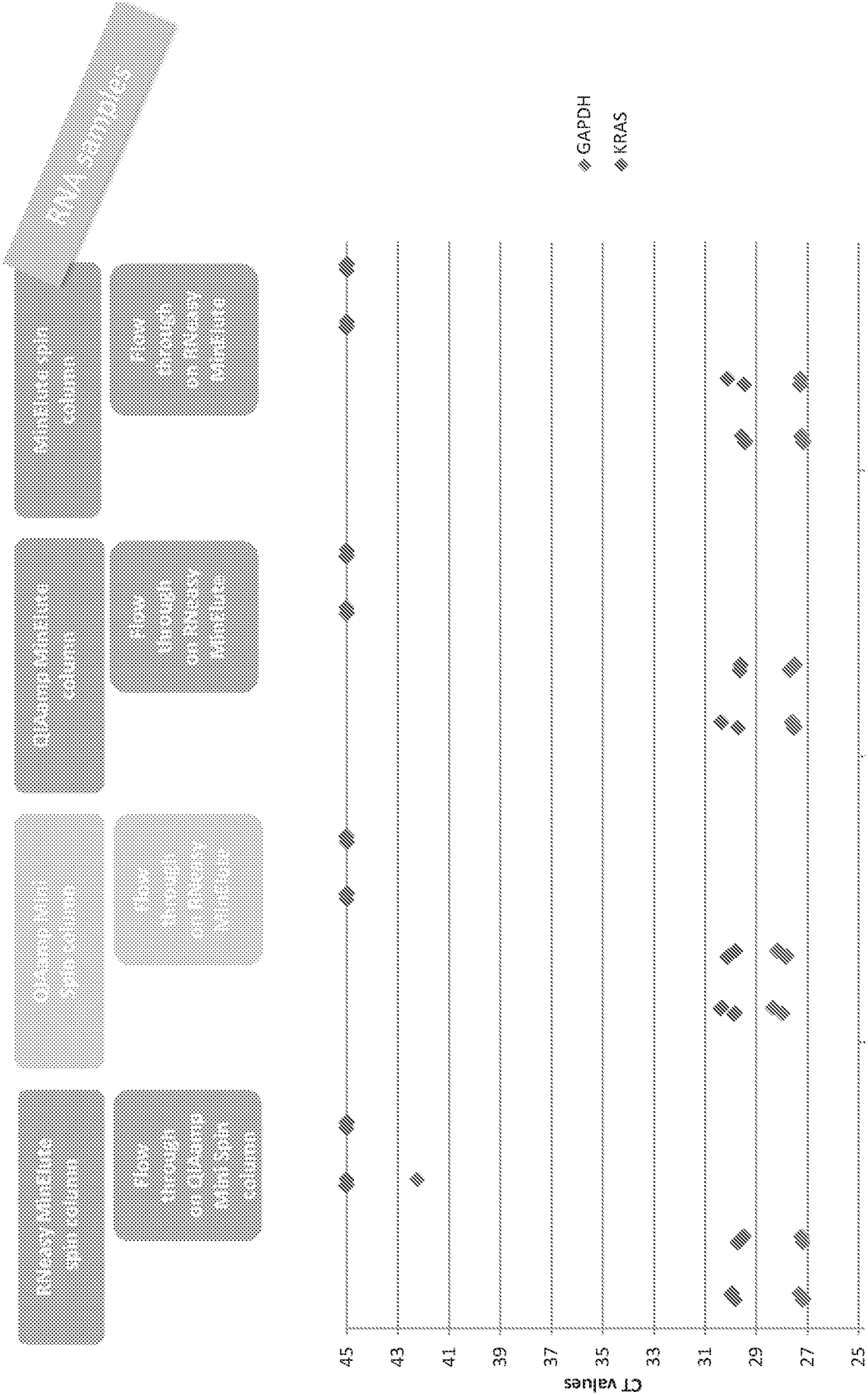
324

EXO52.2 DNA-Isolation: use different downstream columns



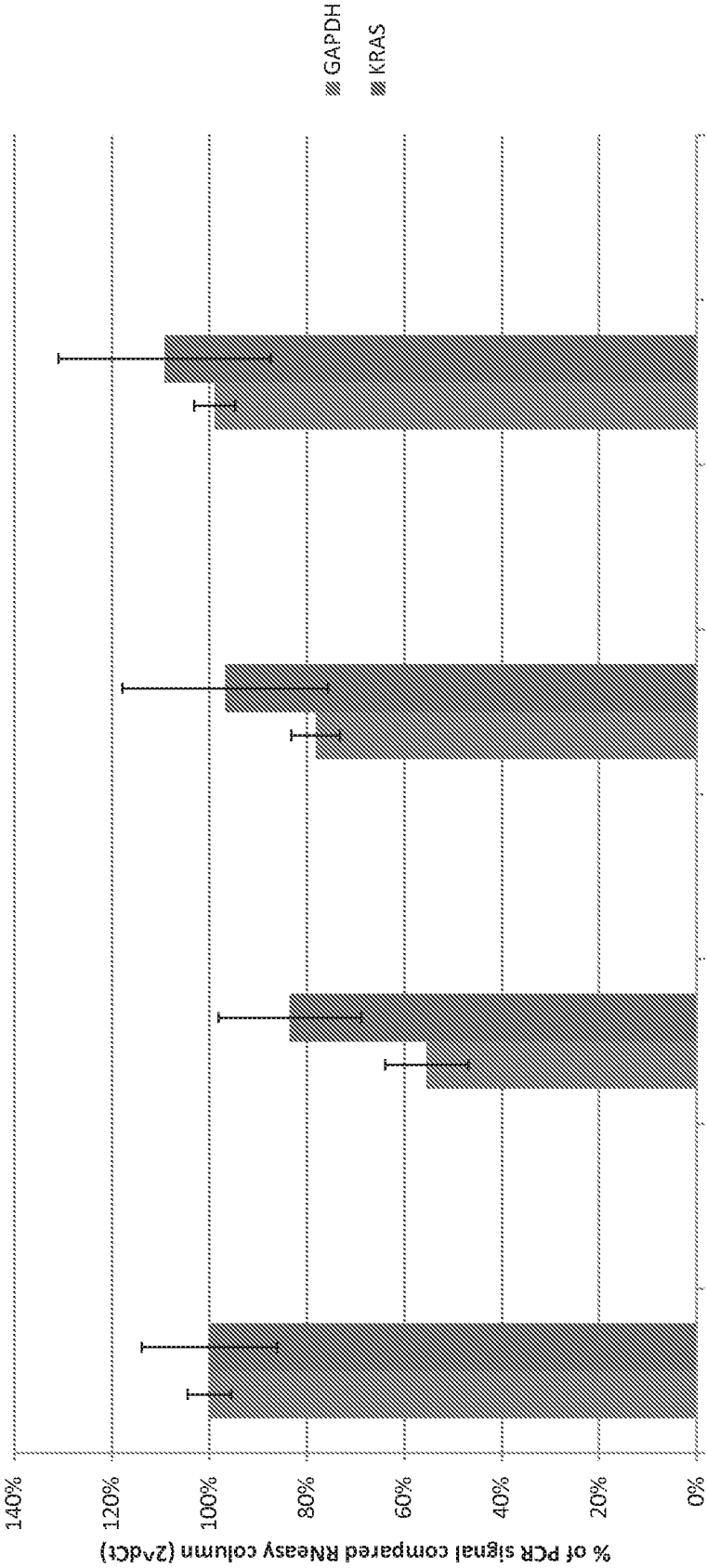
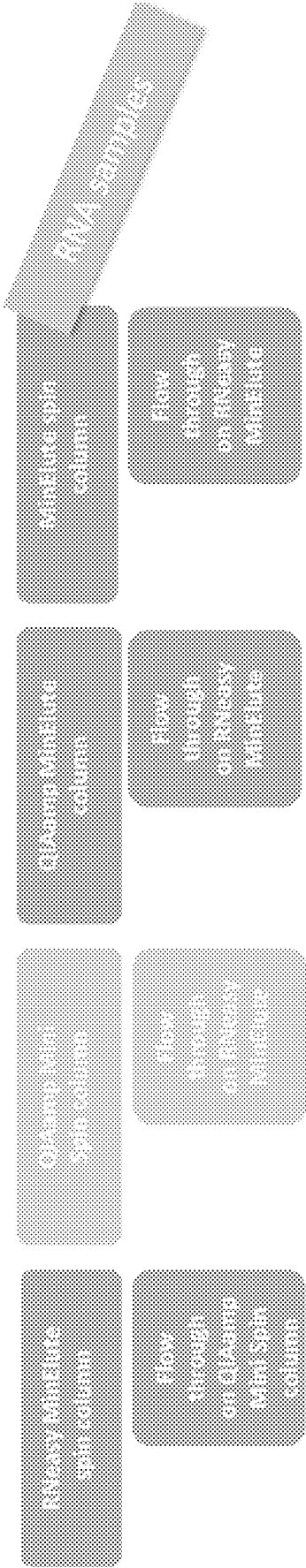
Figure

EXO52.2 DNA-Isolation: use different downstream columns



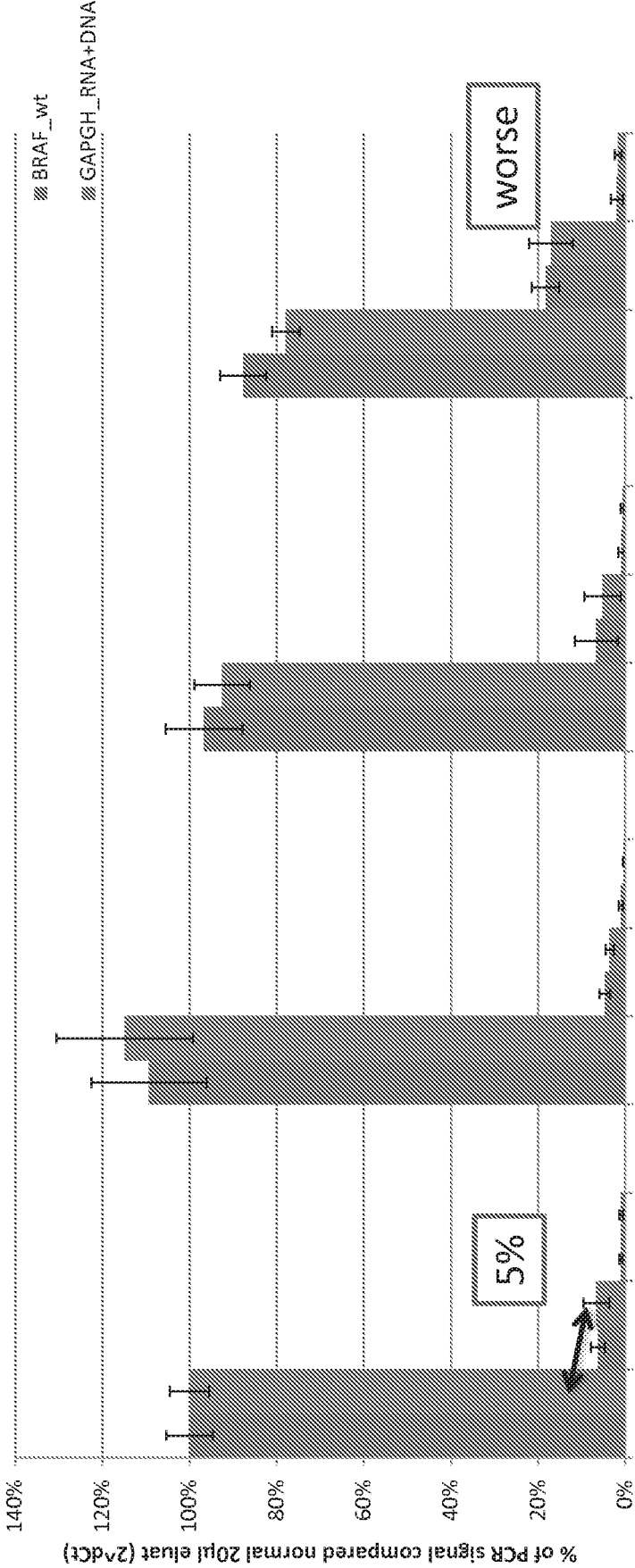
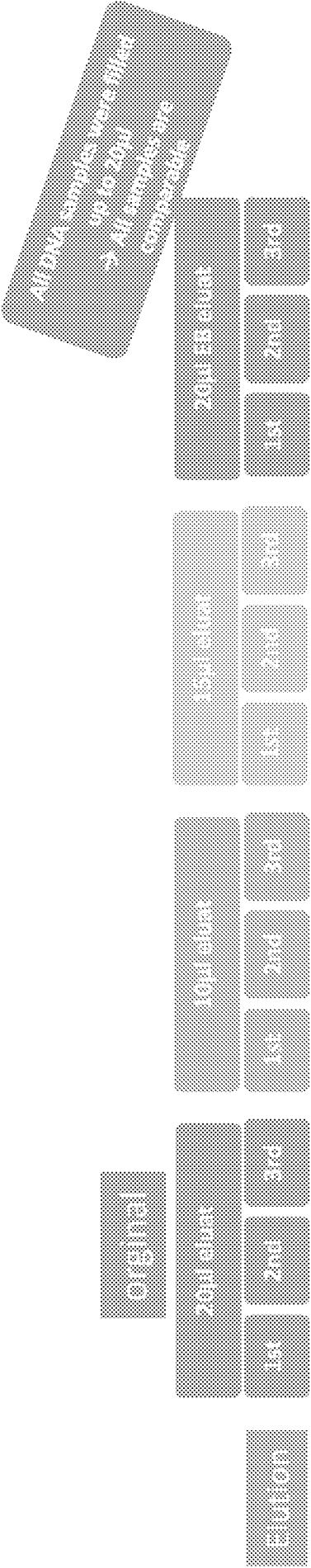
Figure

EXO52.2 DNA-Isolation: use different downstream columns



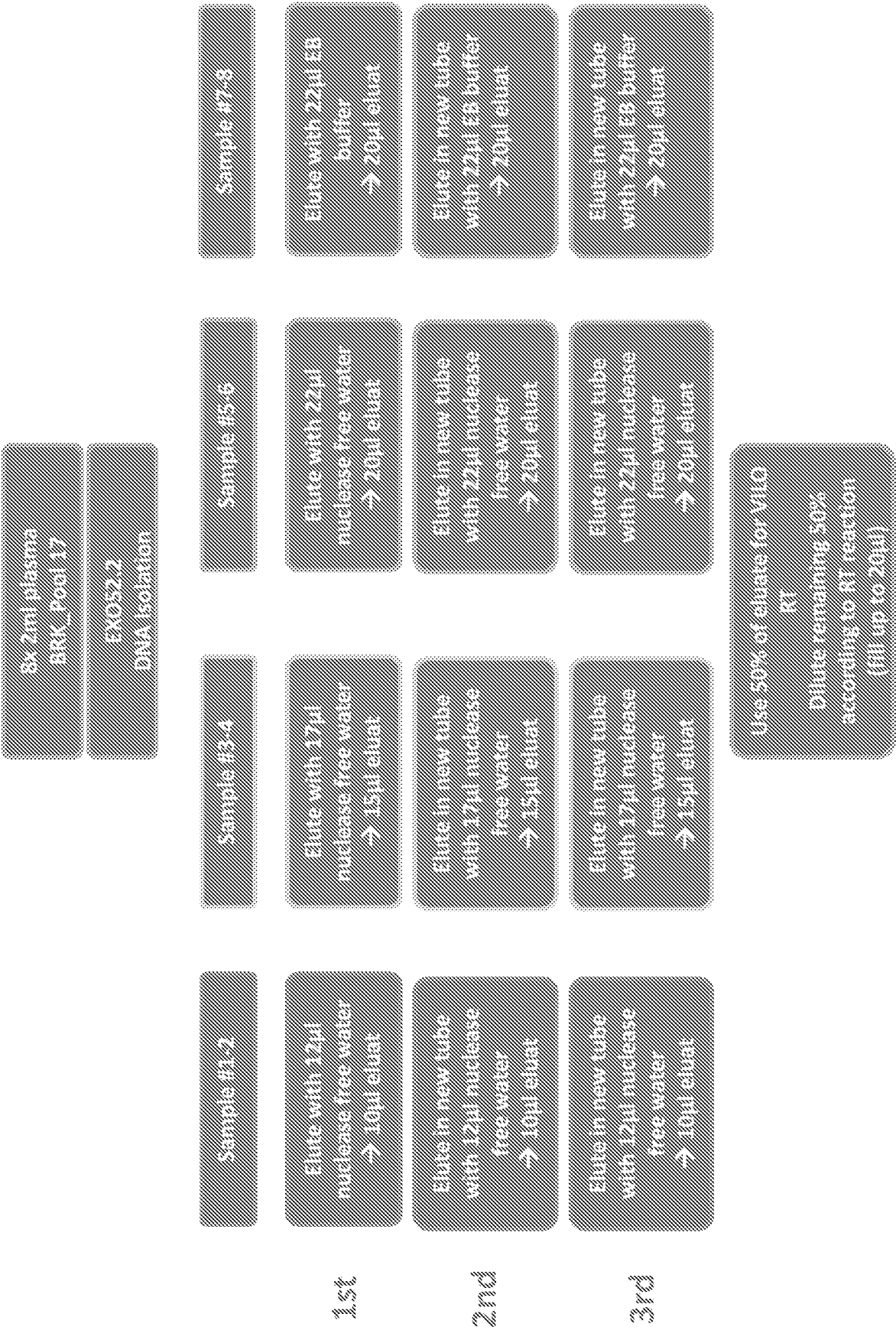
Figure

EXO52.2 DNA-Isolation: multiple RNeasy elution steps



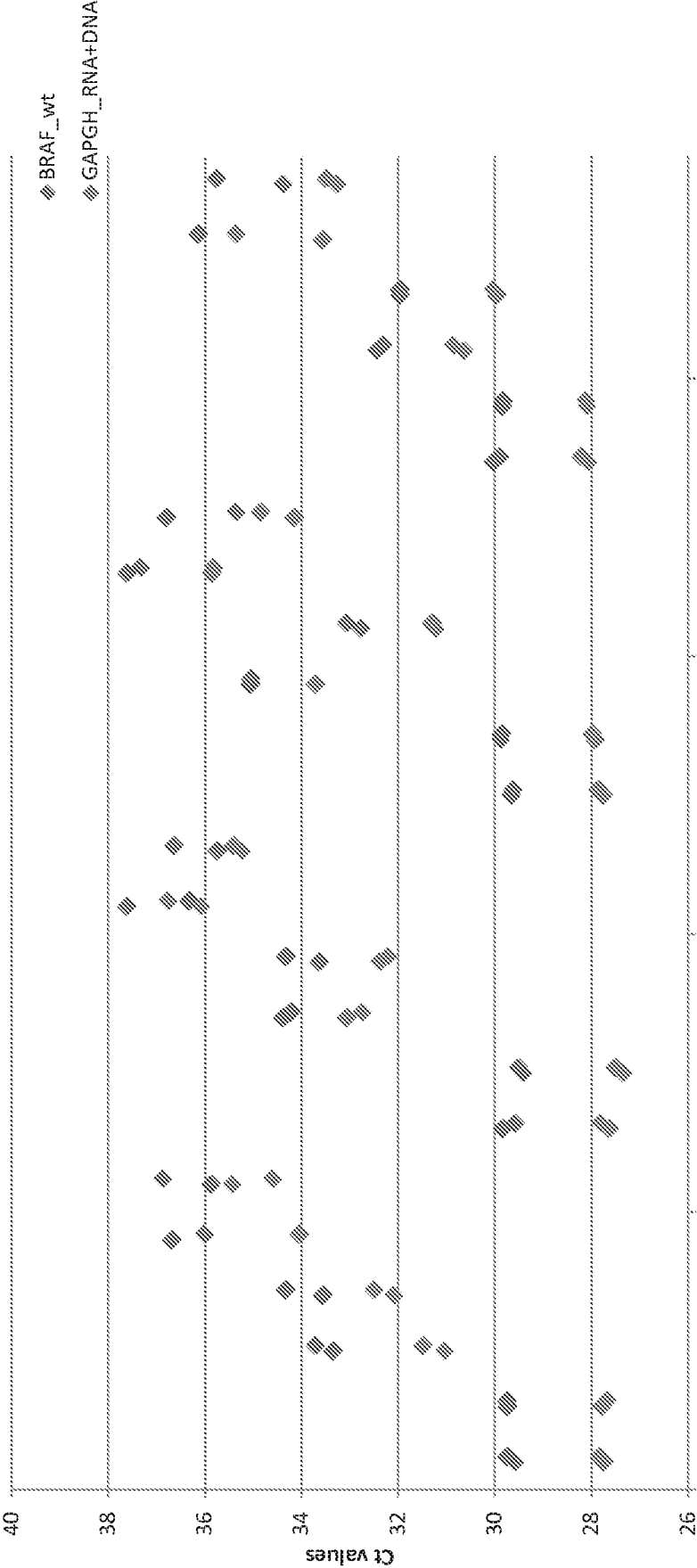
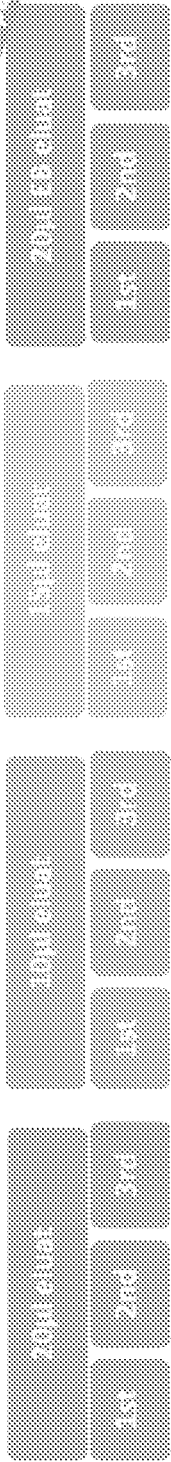
Figure

EXO52.2 DNA-Isolation: multiple RNeasy elution steps



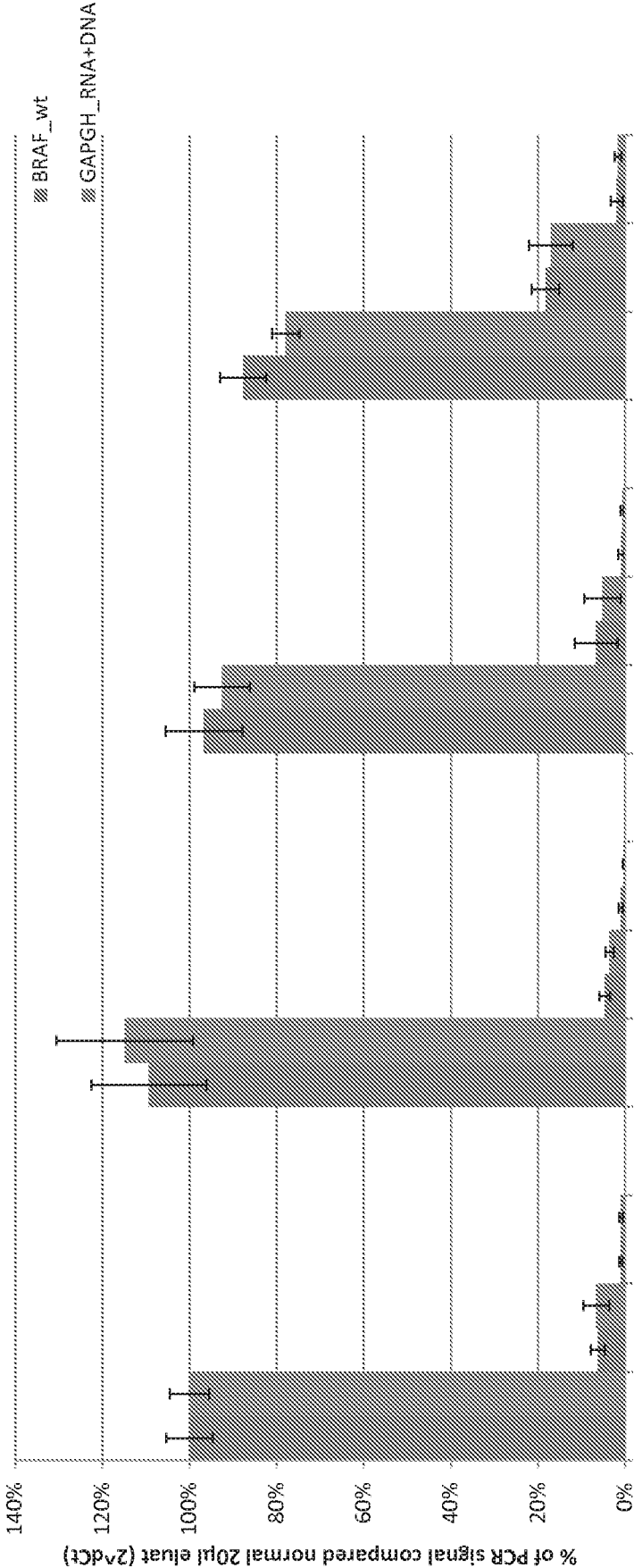
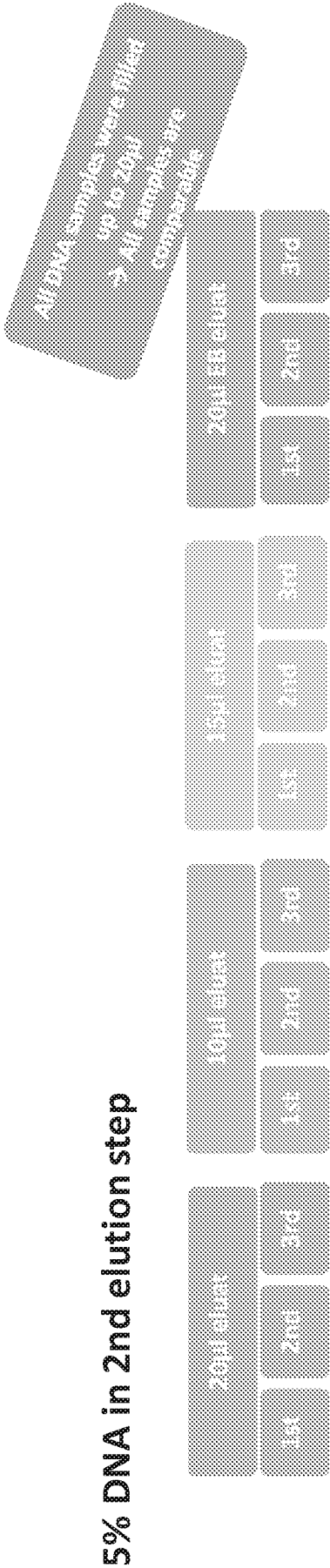
EXO52.2 DNA-Isolation: multiple RNeasy elution steps

All DNA samples were fitted
up to 20µl
→ All samples are
comparable



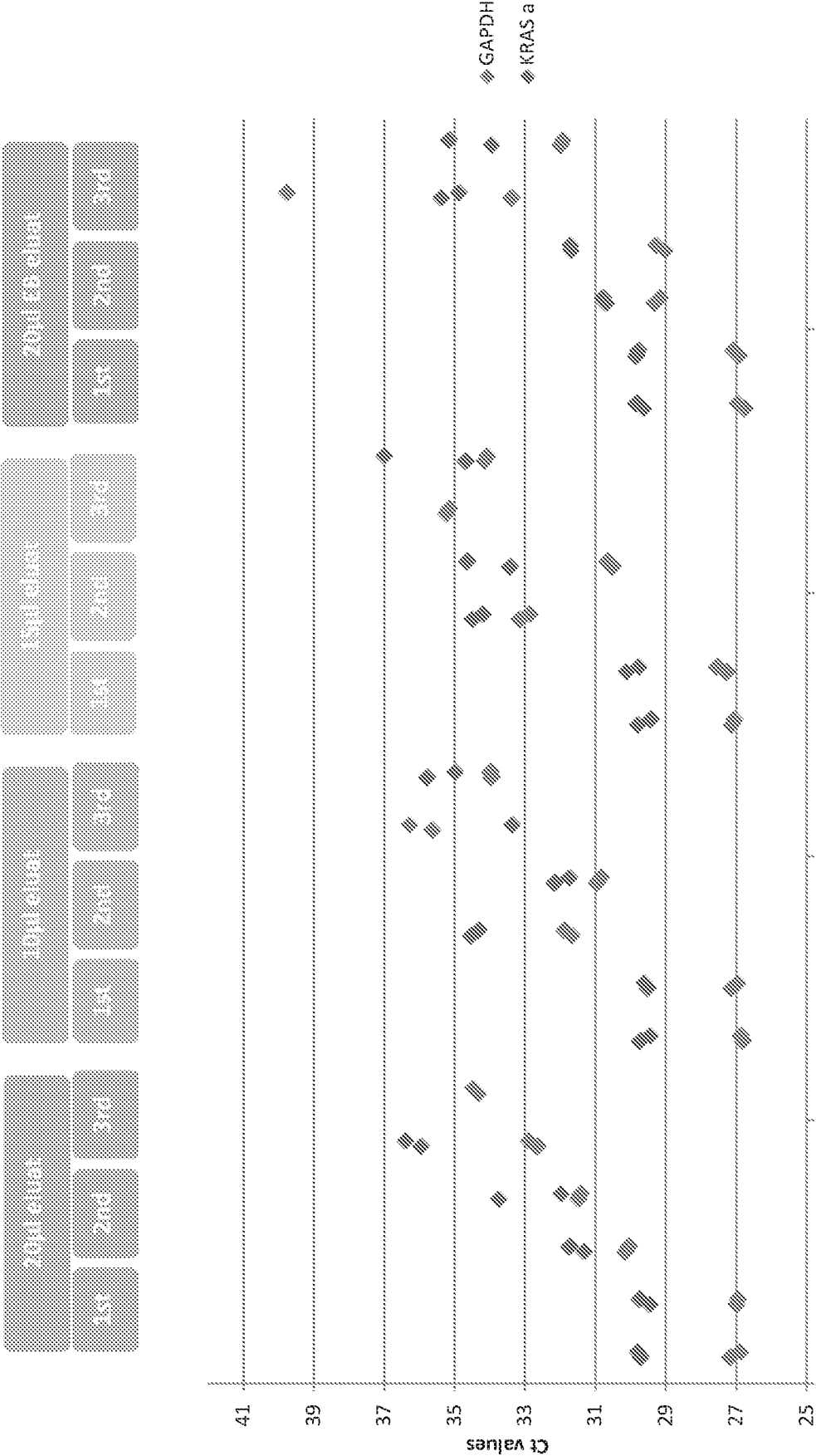
Figure

EXO52.2 DNA-Isolation: multiple RNeasy elution steps



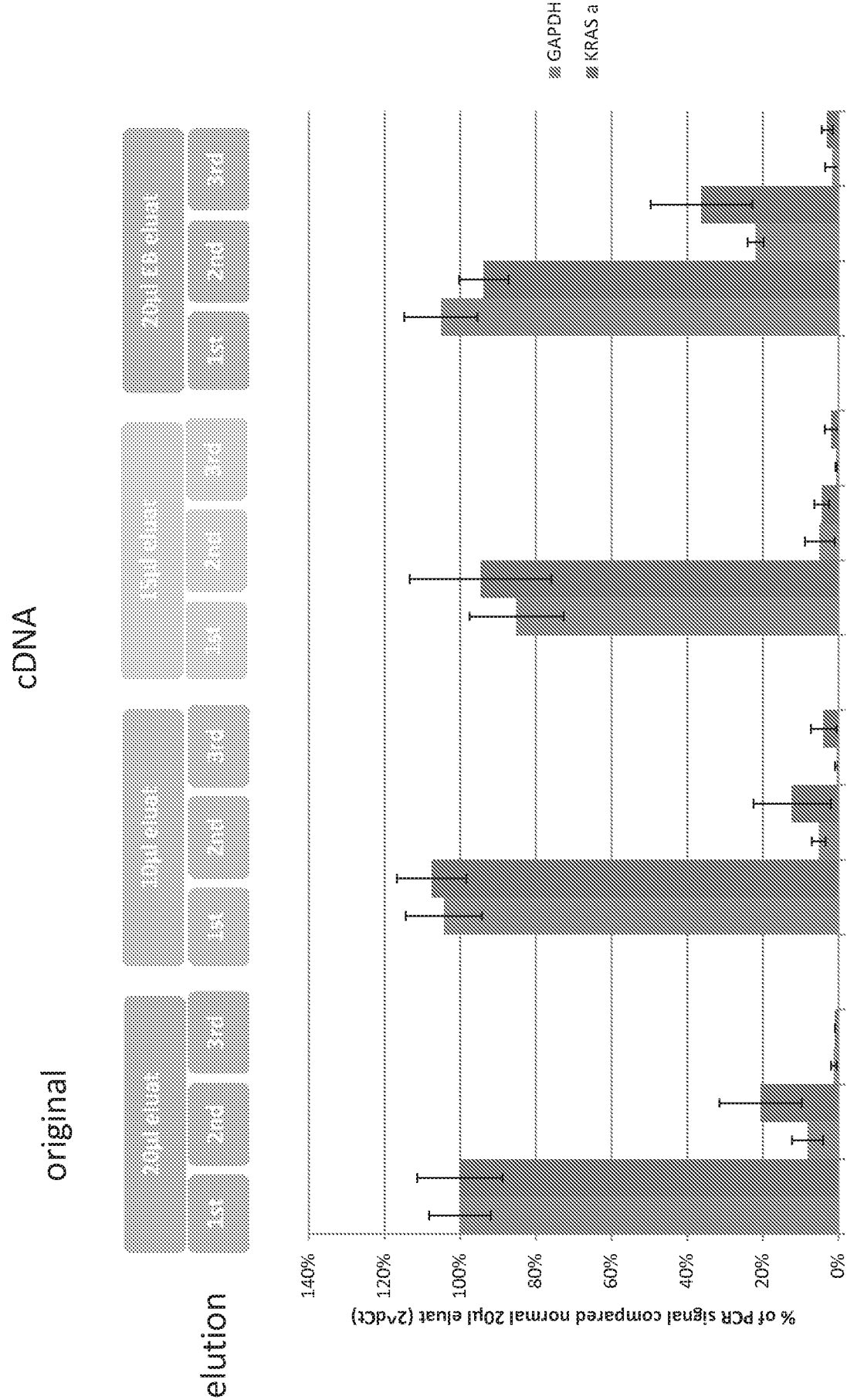
Used average of all isolation replicates and use Error Bars with standard deviation

EXO52.2 DNA-Isolation: multiple RNeasy elution steps



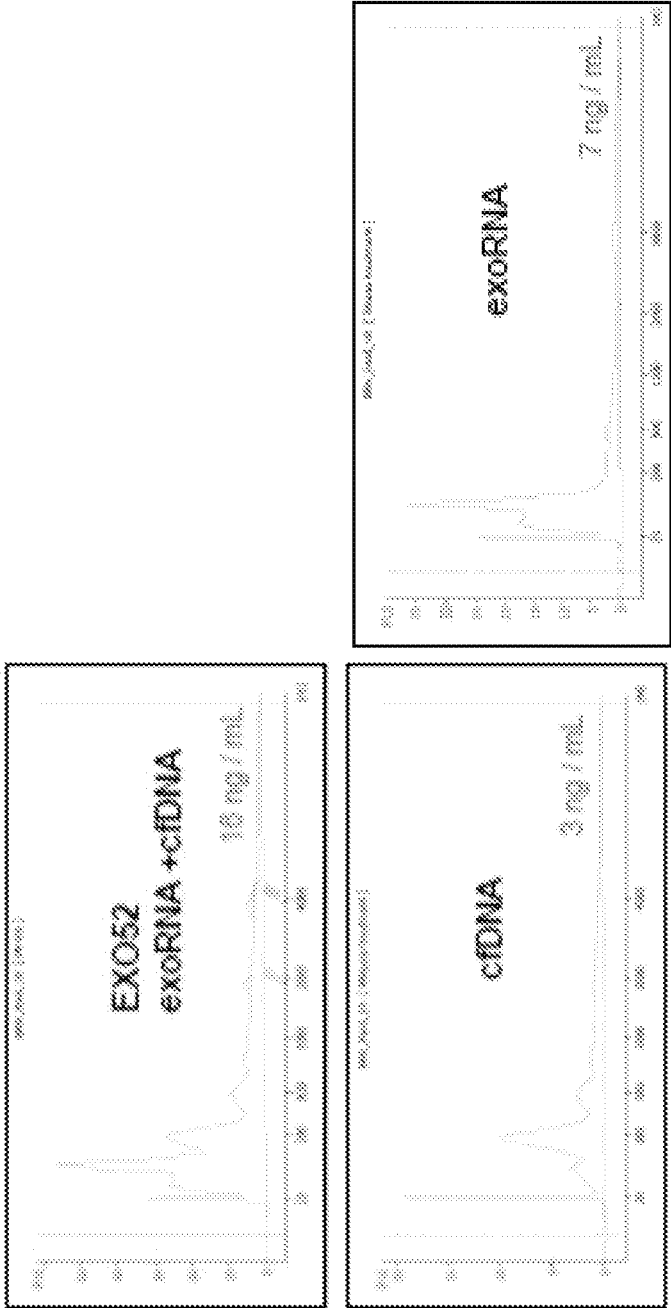
Figure

EXO52.2 DNA-Isolation: multiple RNeasy elution steps



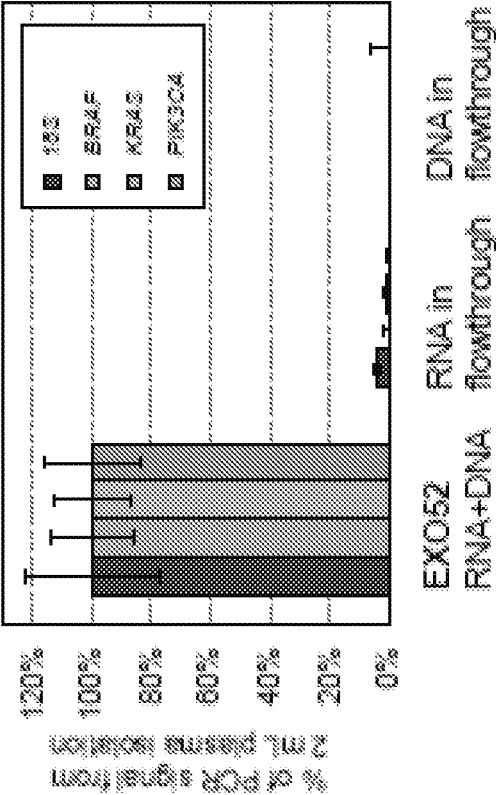
Figure

Size Distribution of nucleic acids in plasma

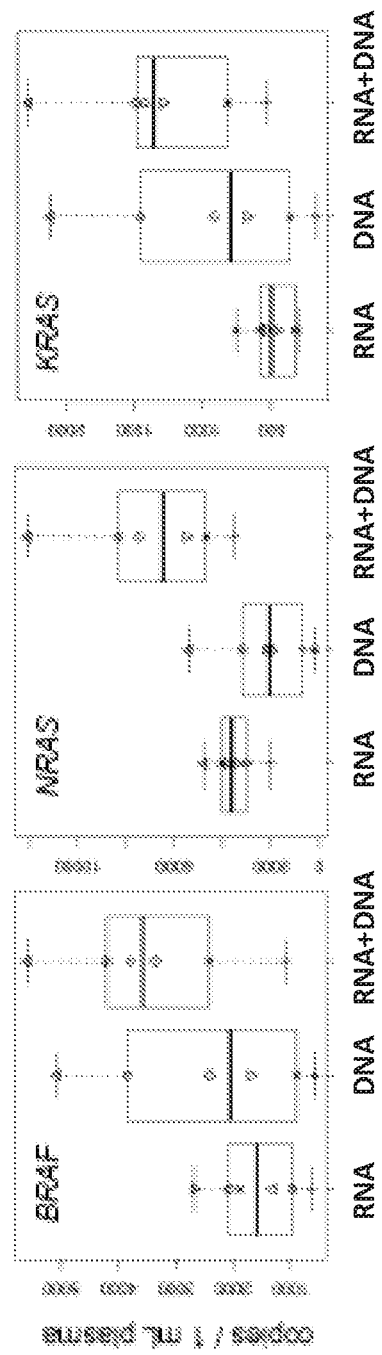


Figure

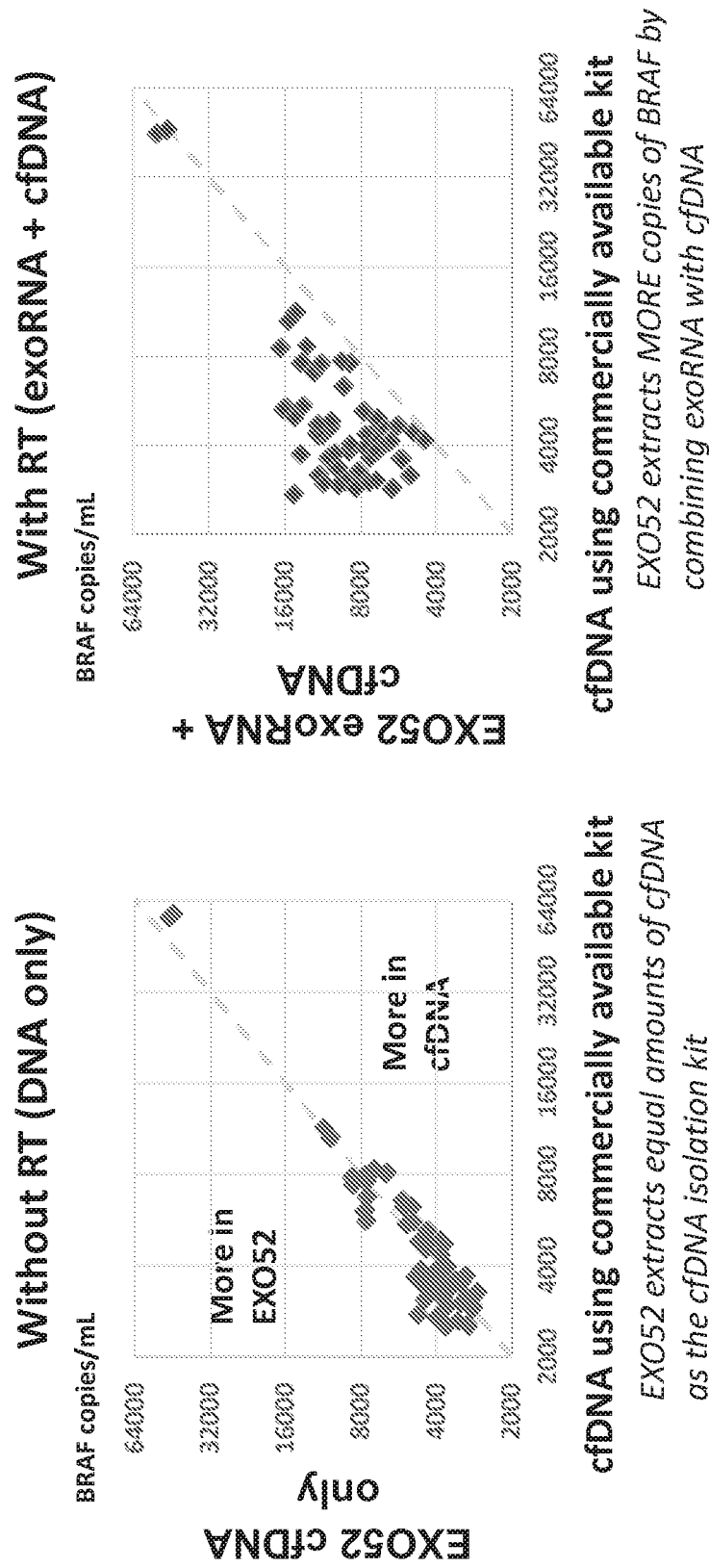
EXO52 columns bind all mRNA and DNA in plasma

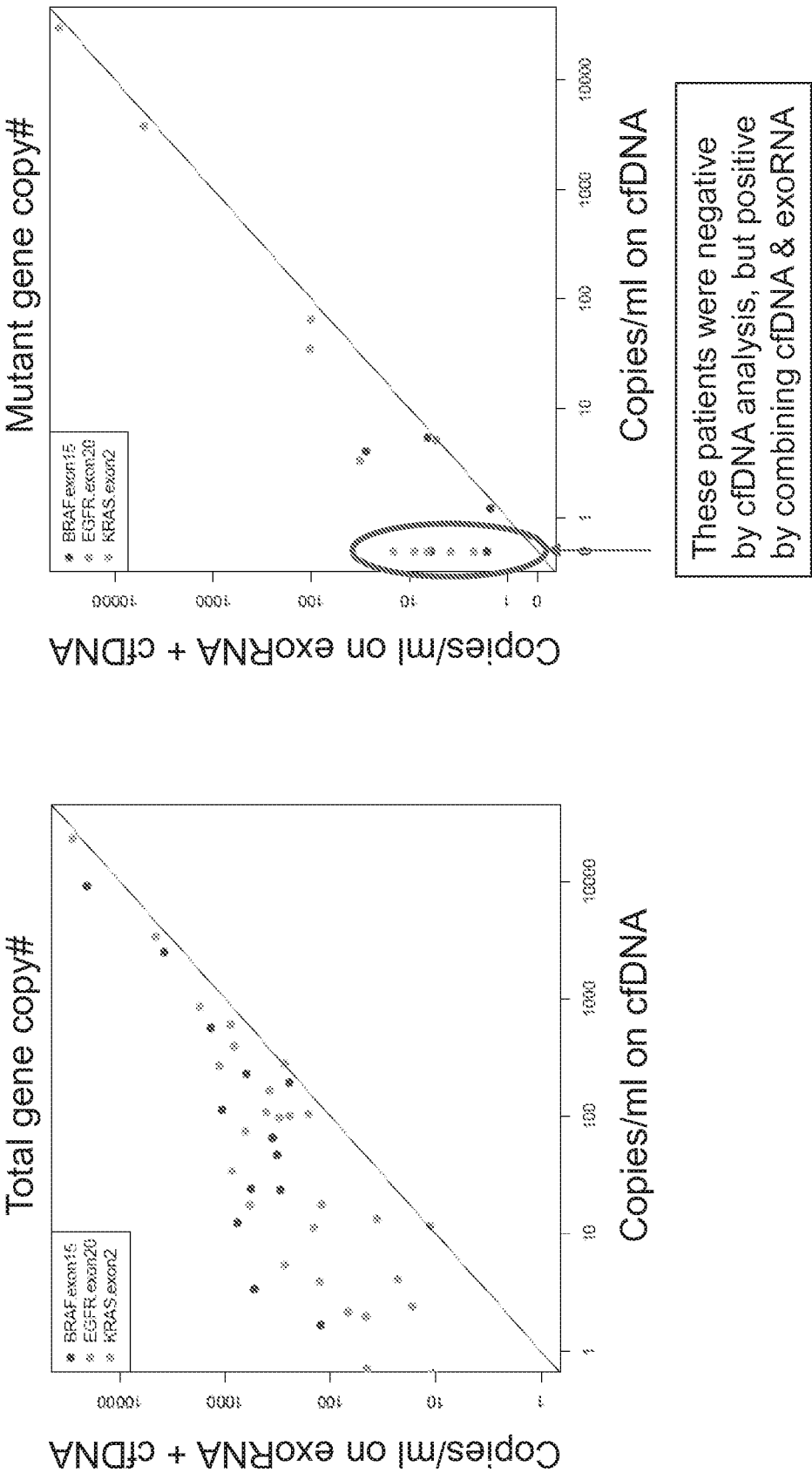


Figure



Figure





Figure