

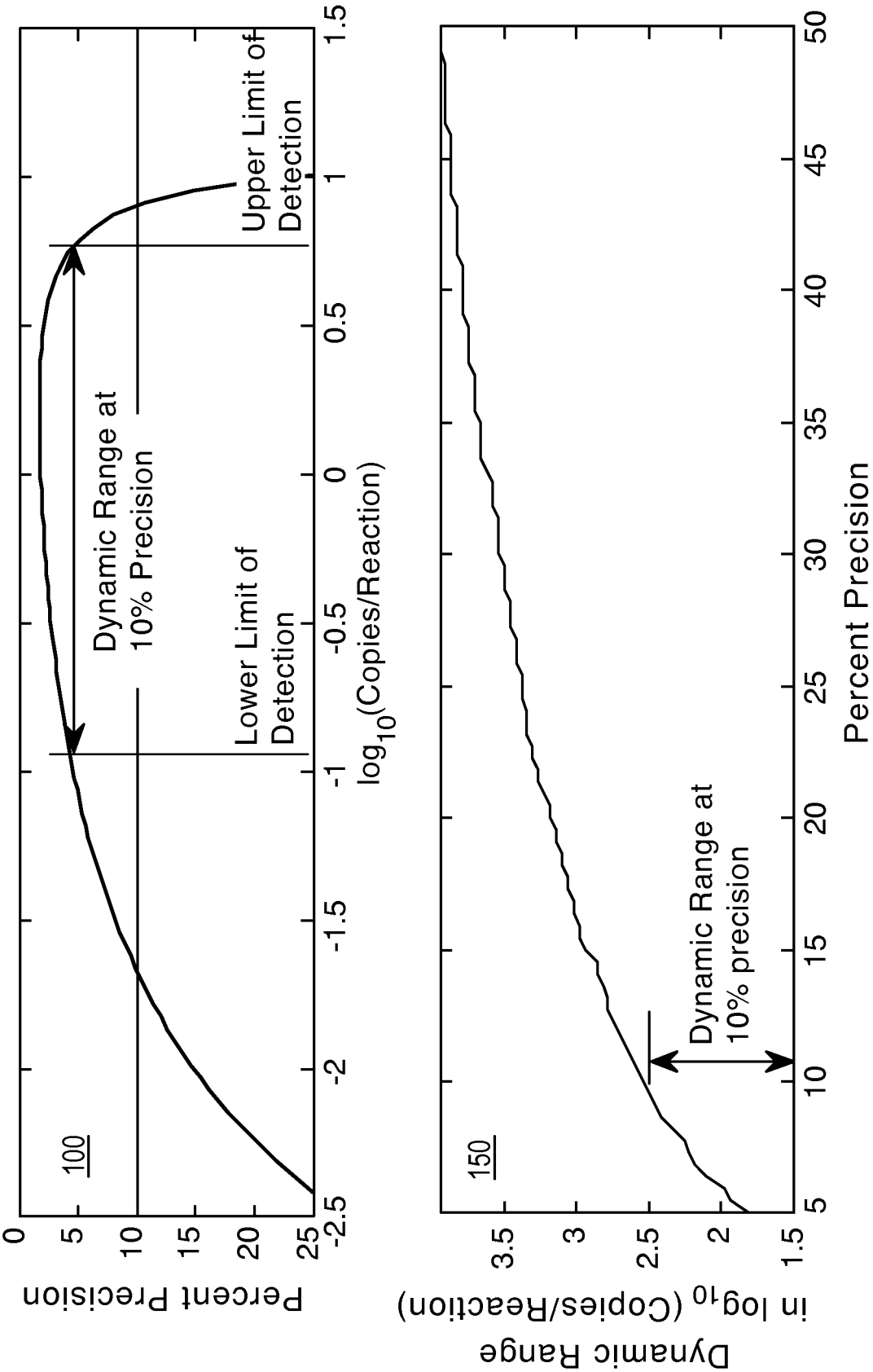


FIG. 1A

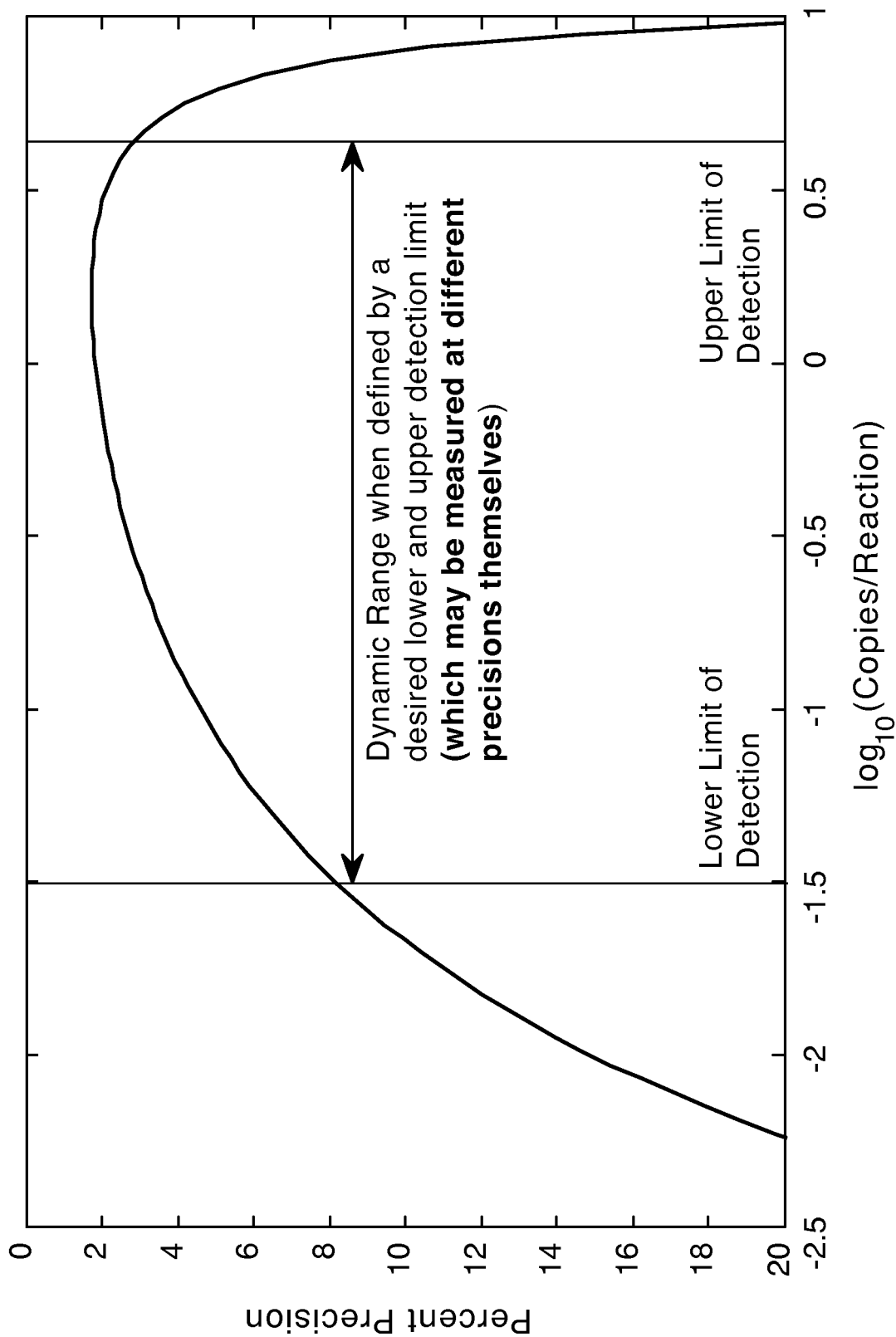


FIG. 1B

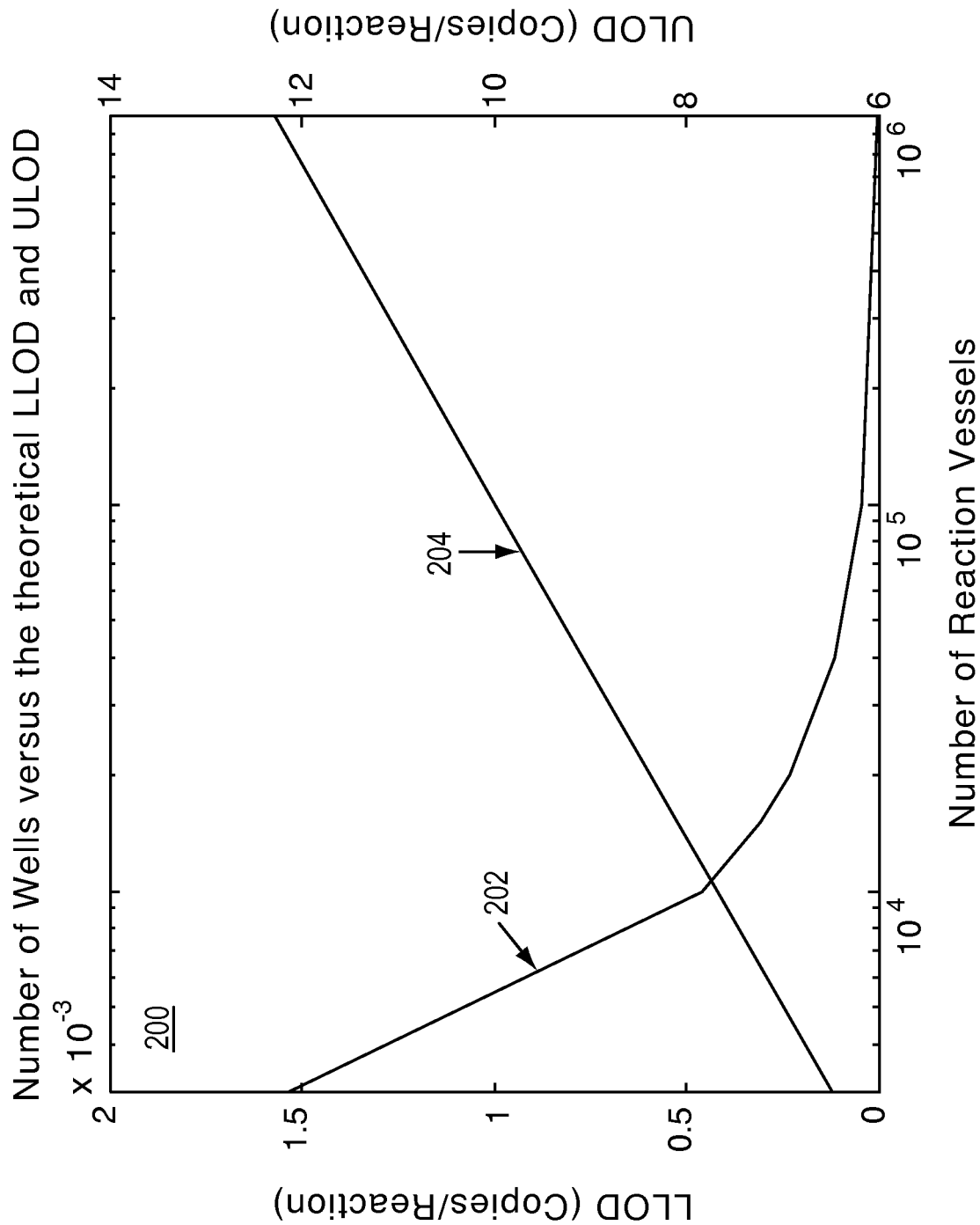


FIG. 2

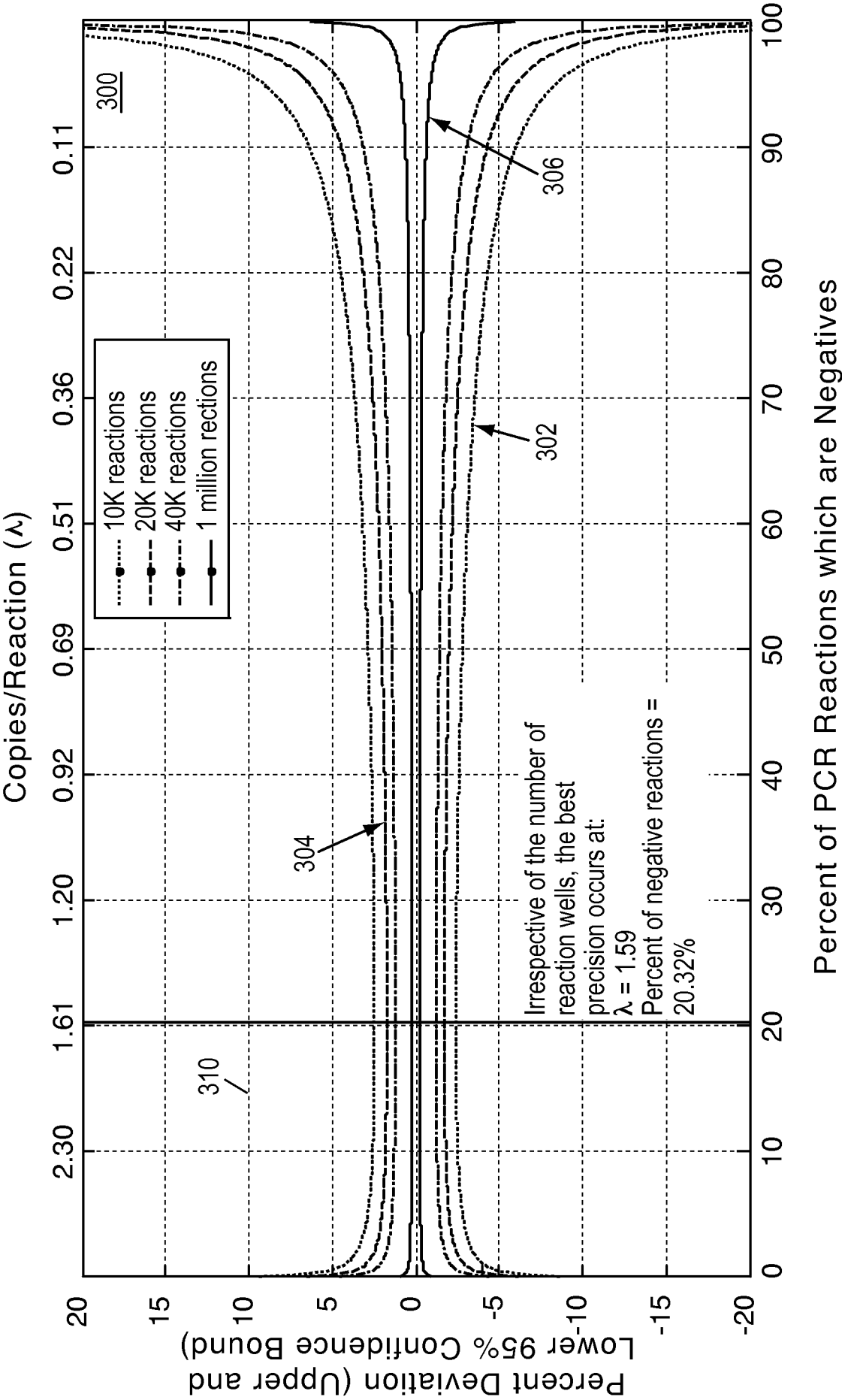


FIG. 3

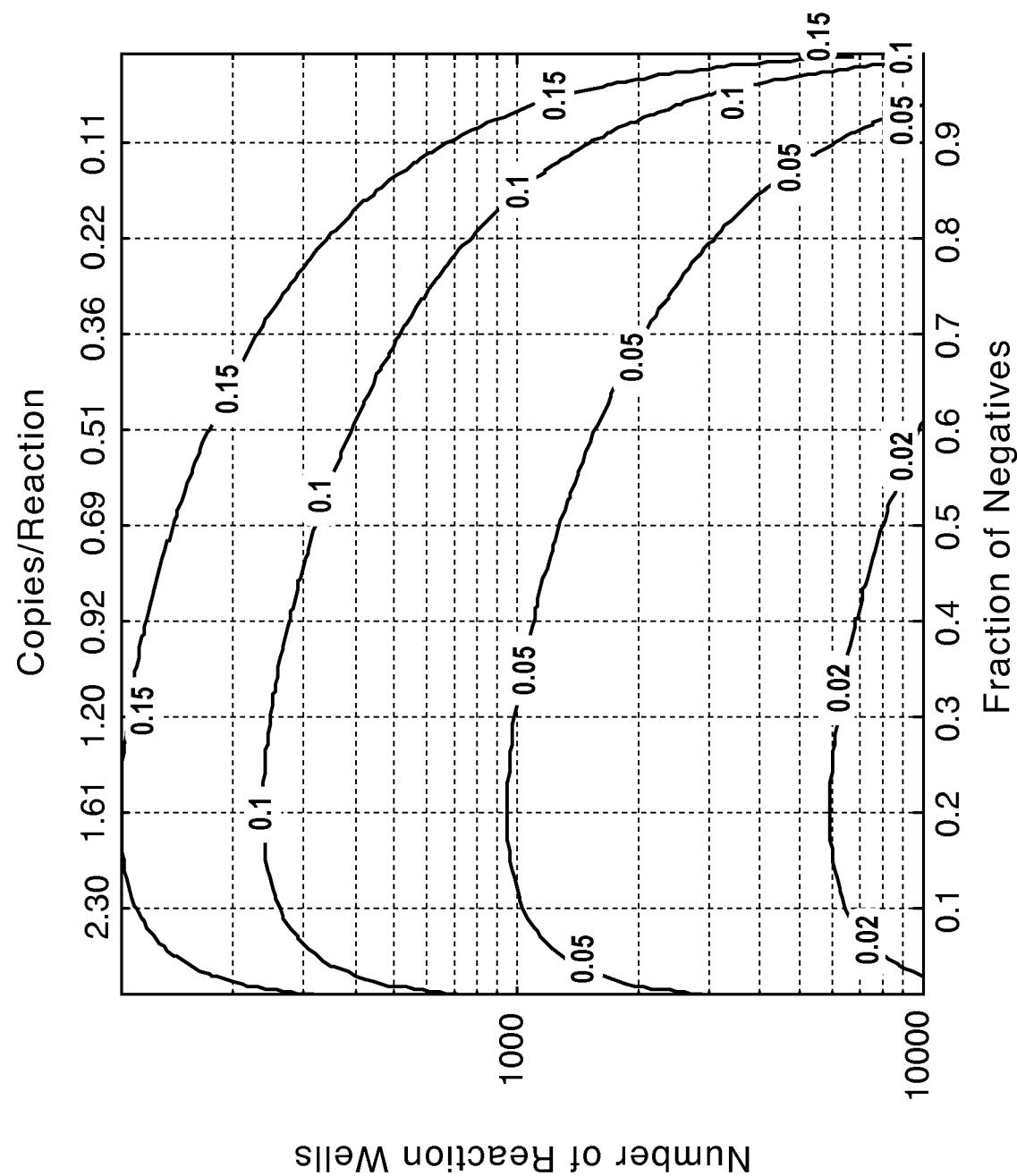


FIG. 4

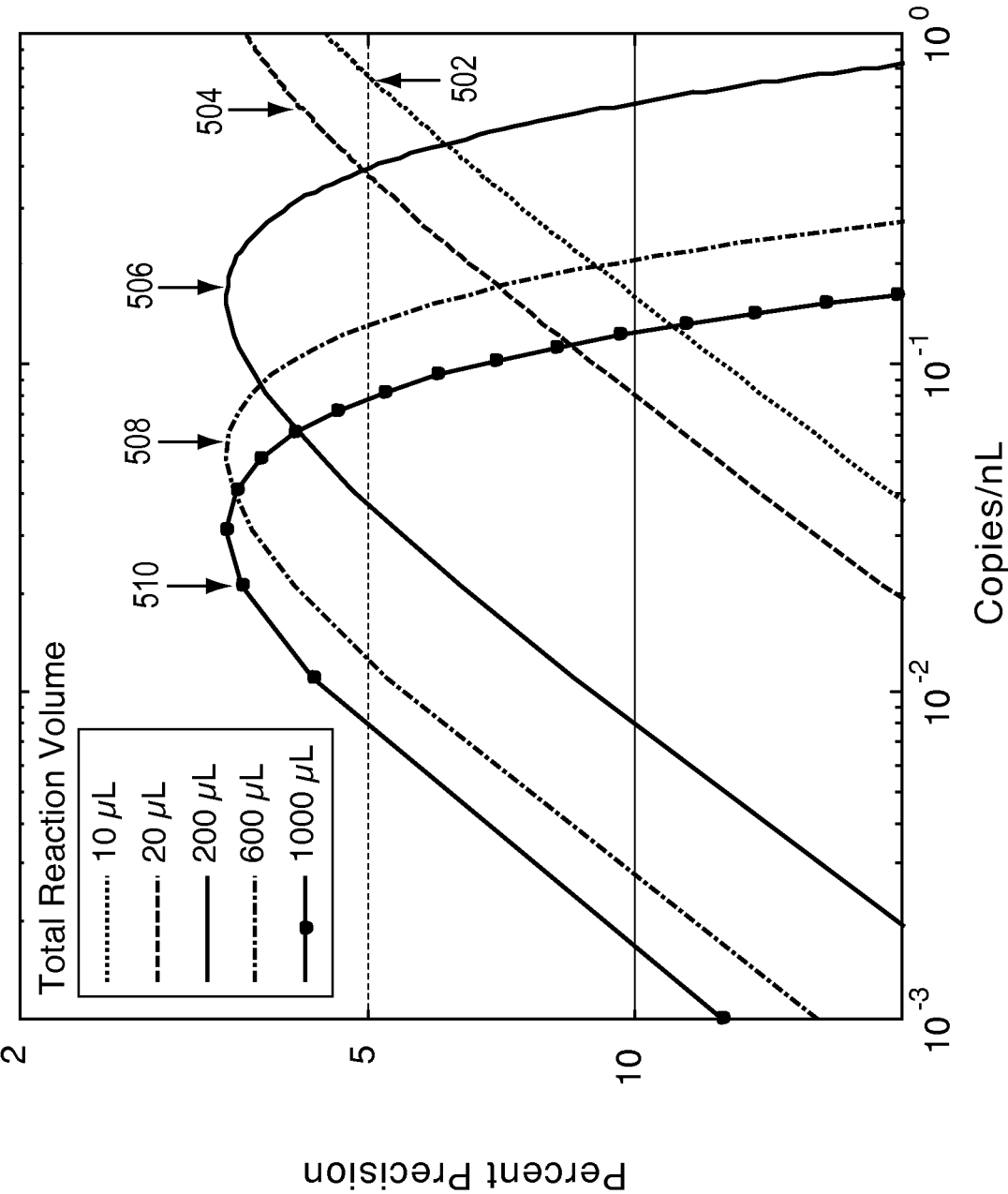
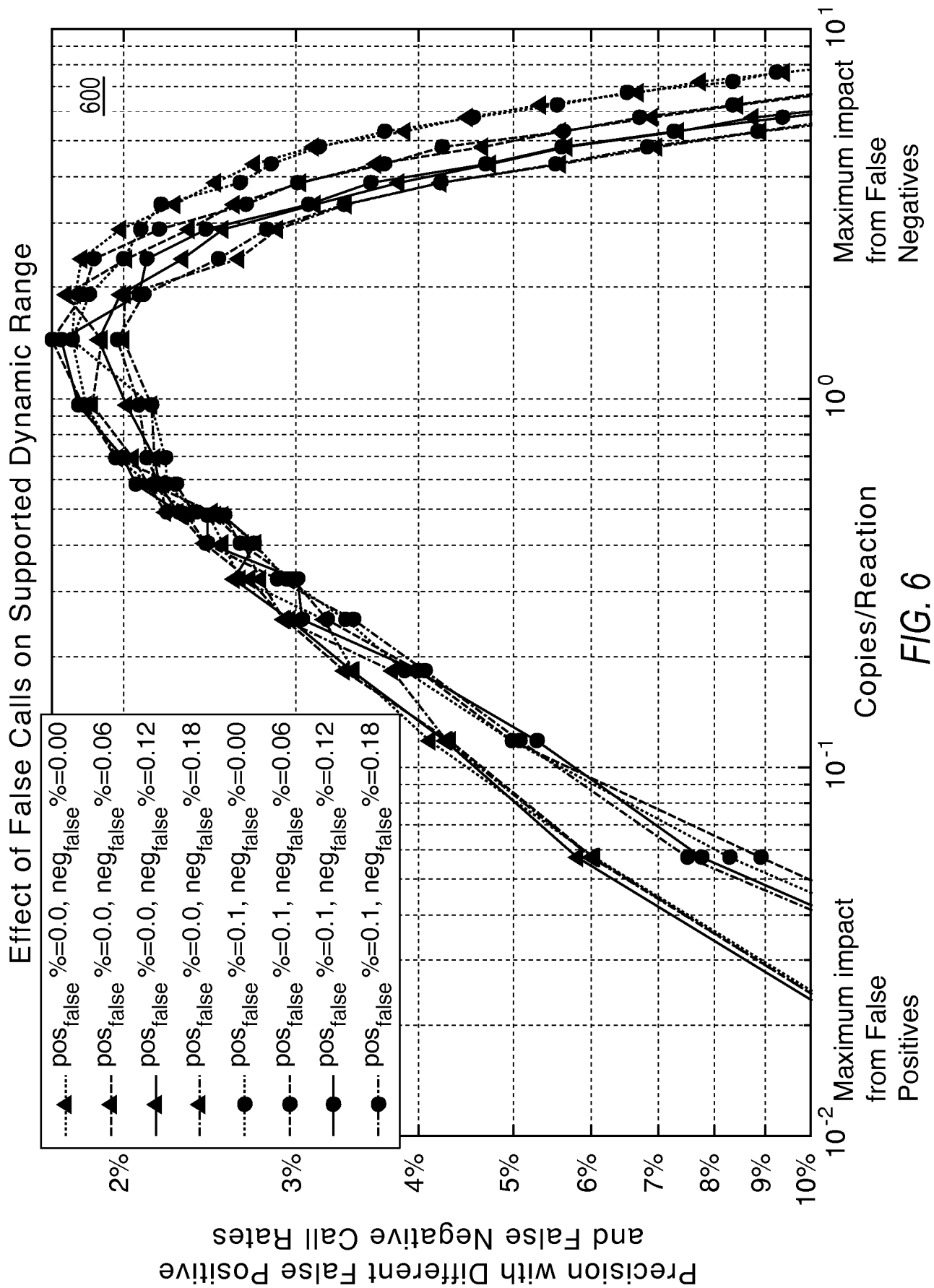


FIG. 5

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Target Load In Percent Negative Reactions For Best Precision
For a System with Non-zero False Call Rates

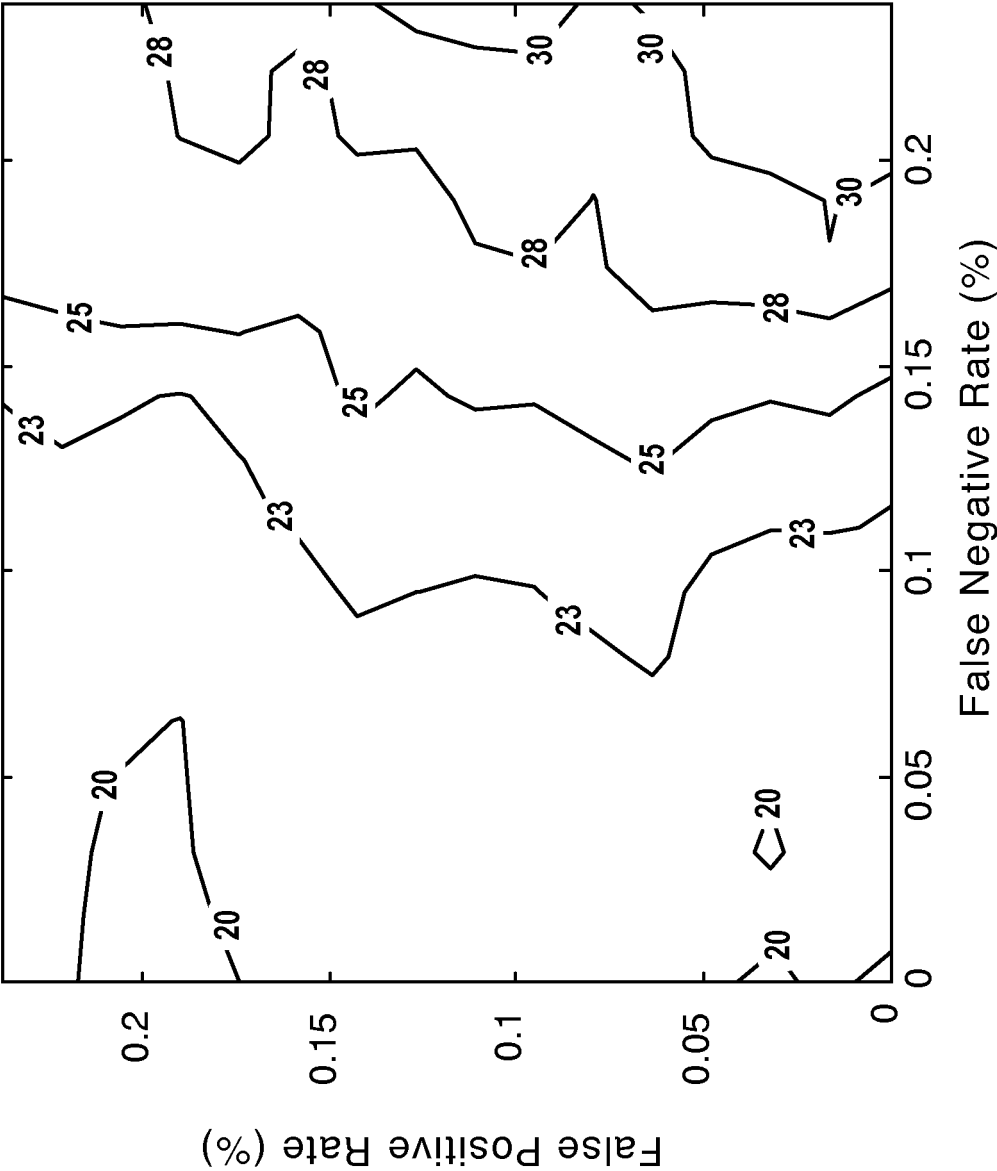


FIG. 7

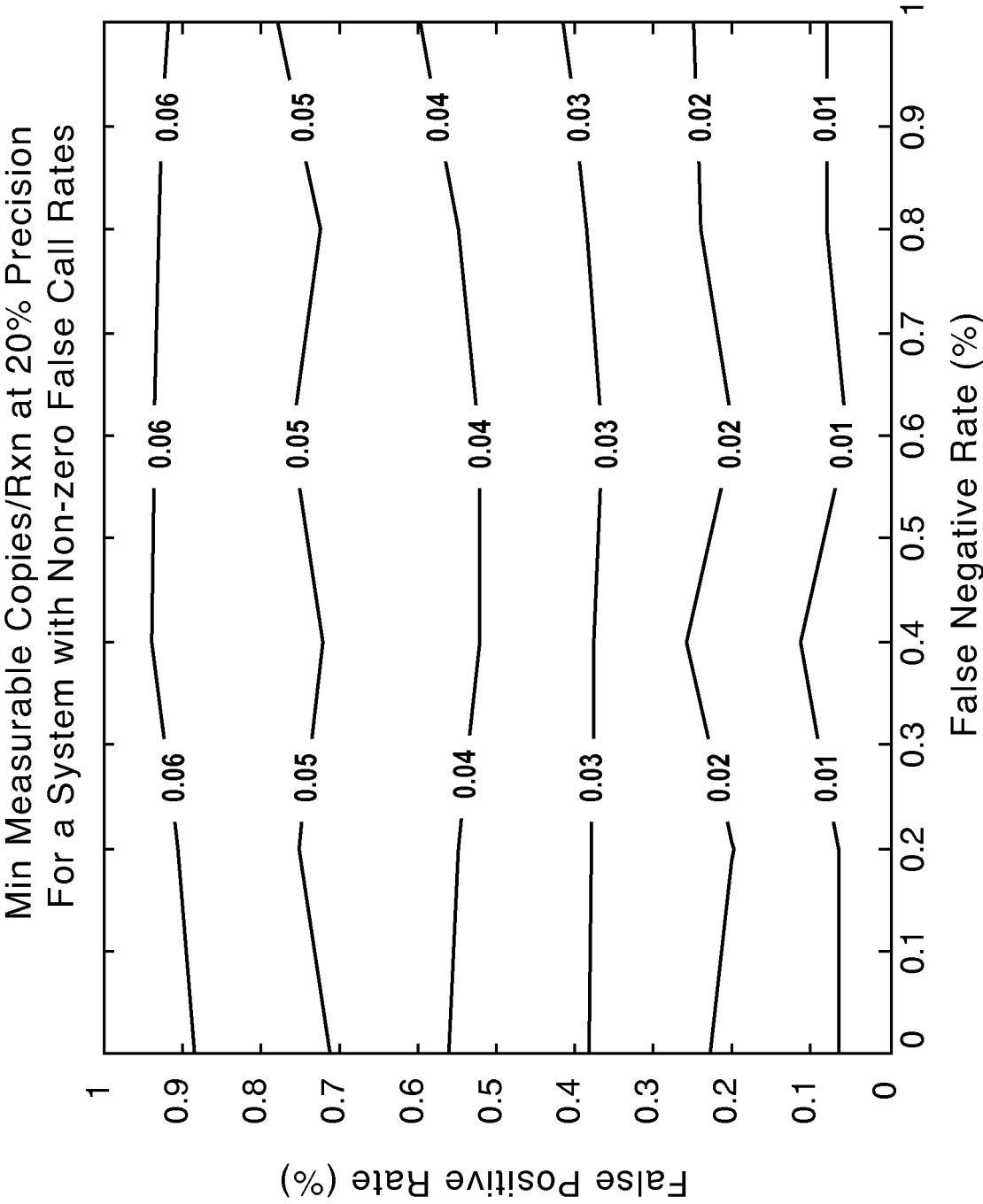


FIG. 8

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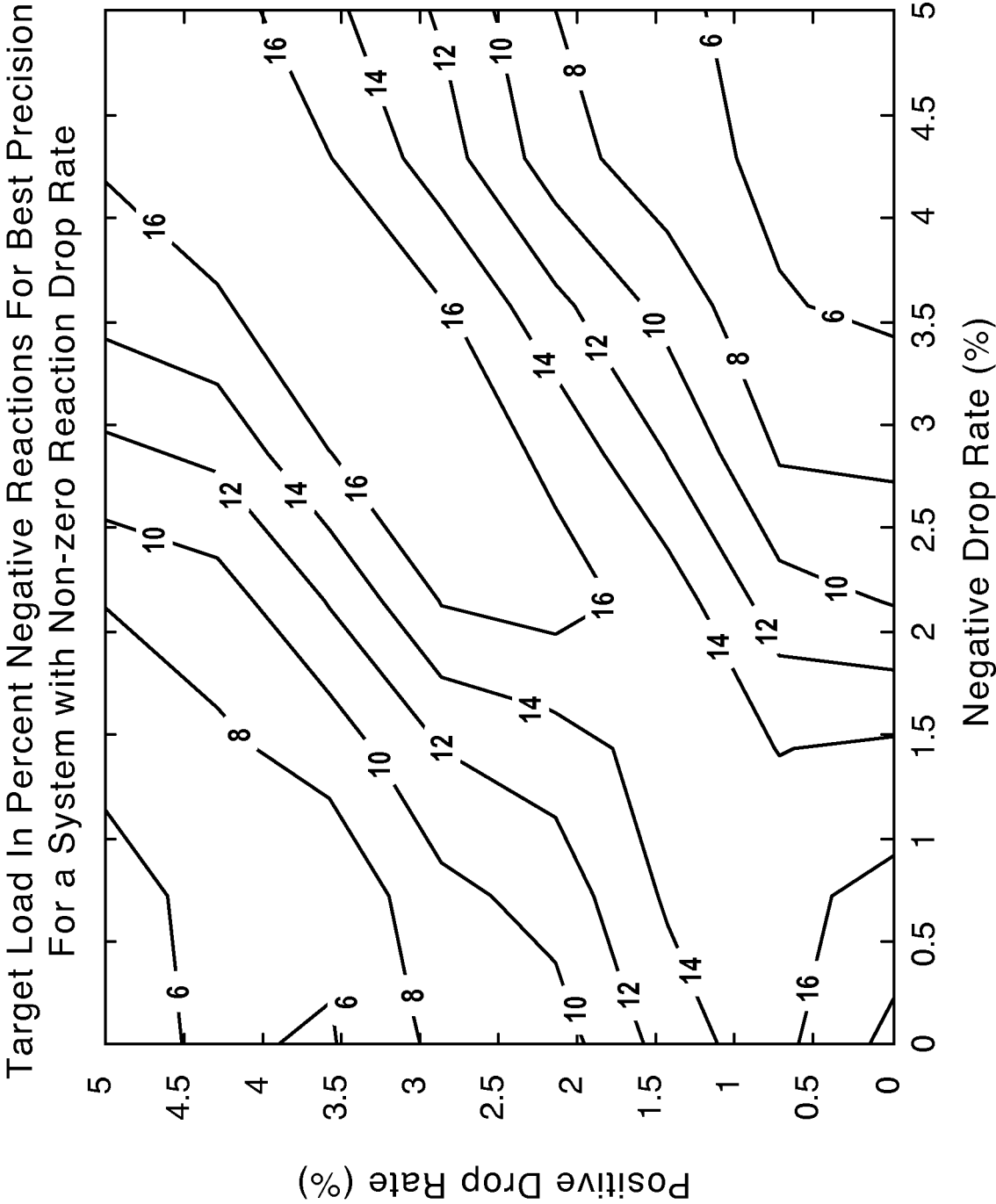
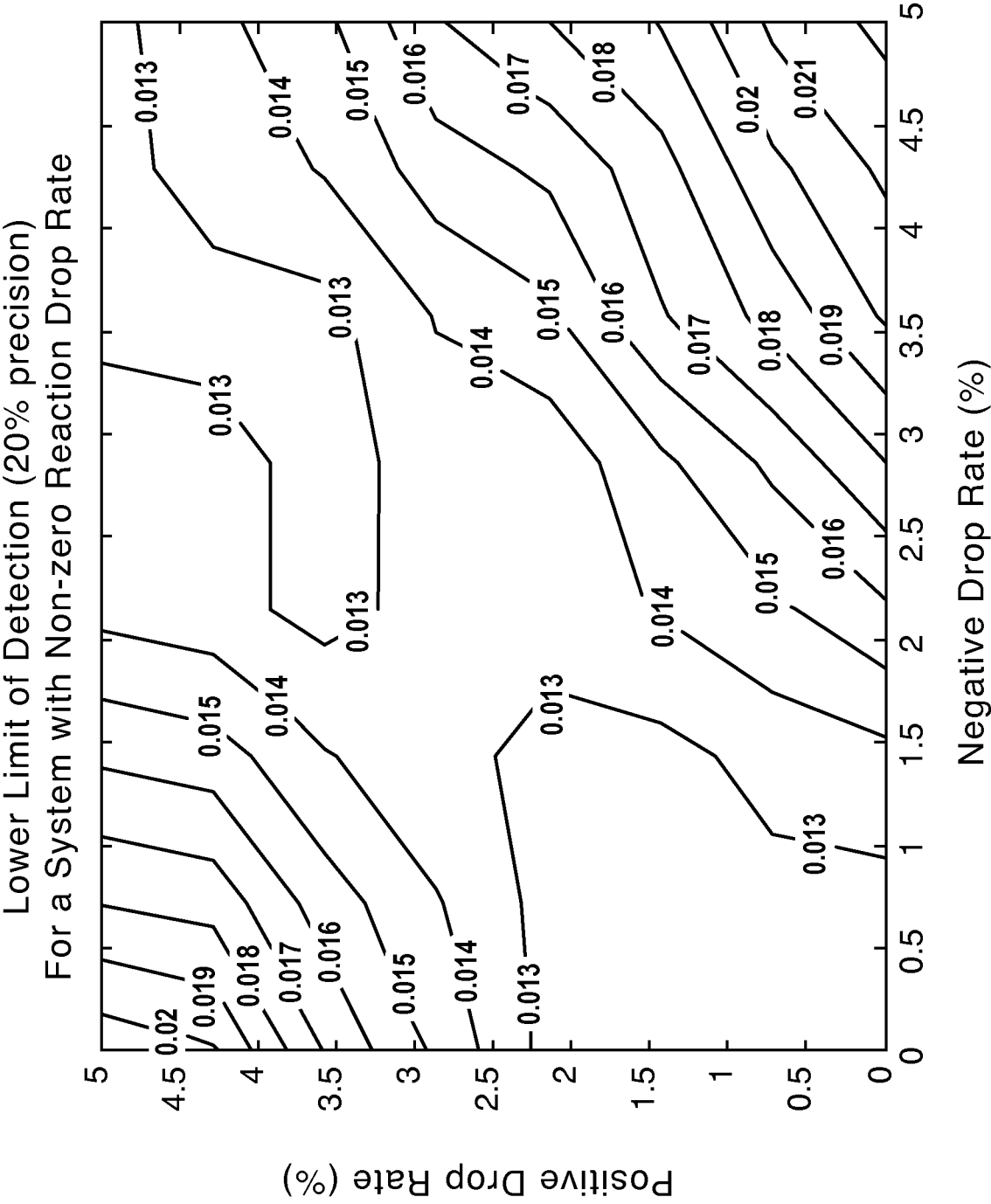


FIG. 9



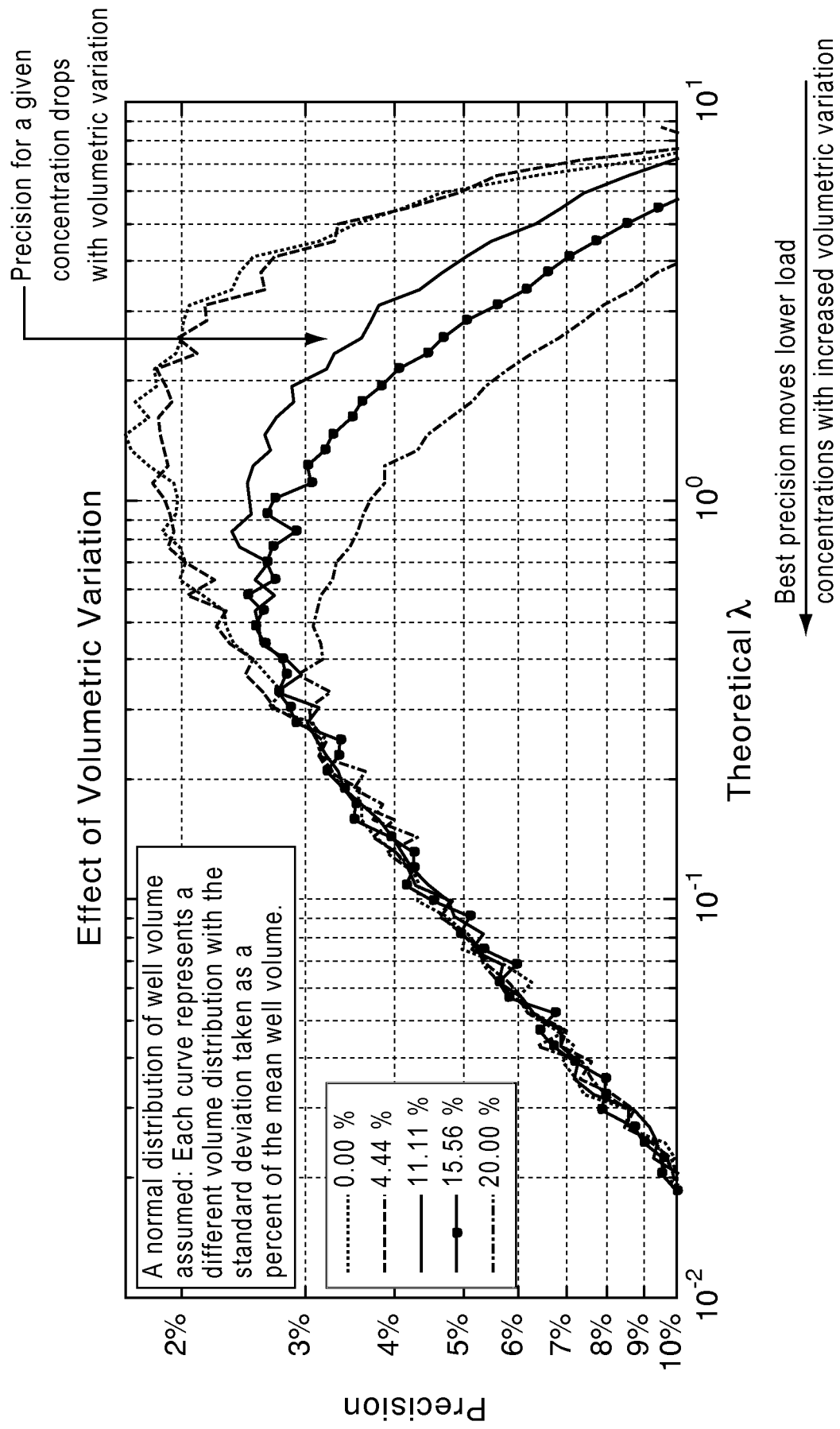


FIG. 11

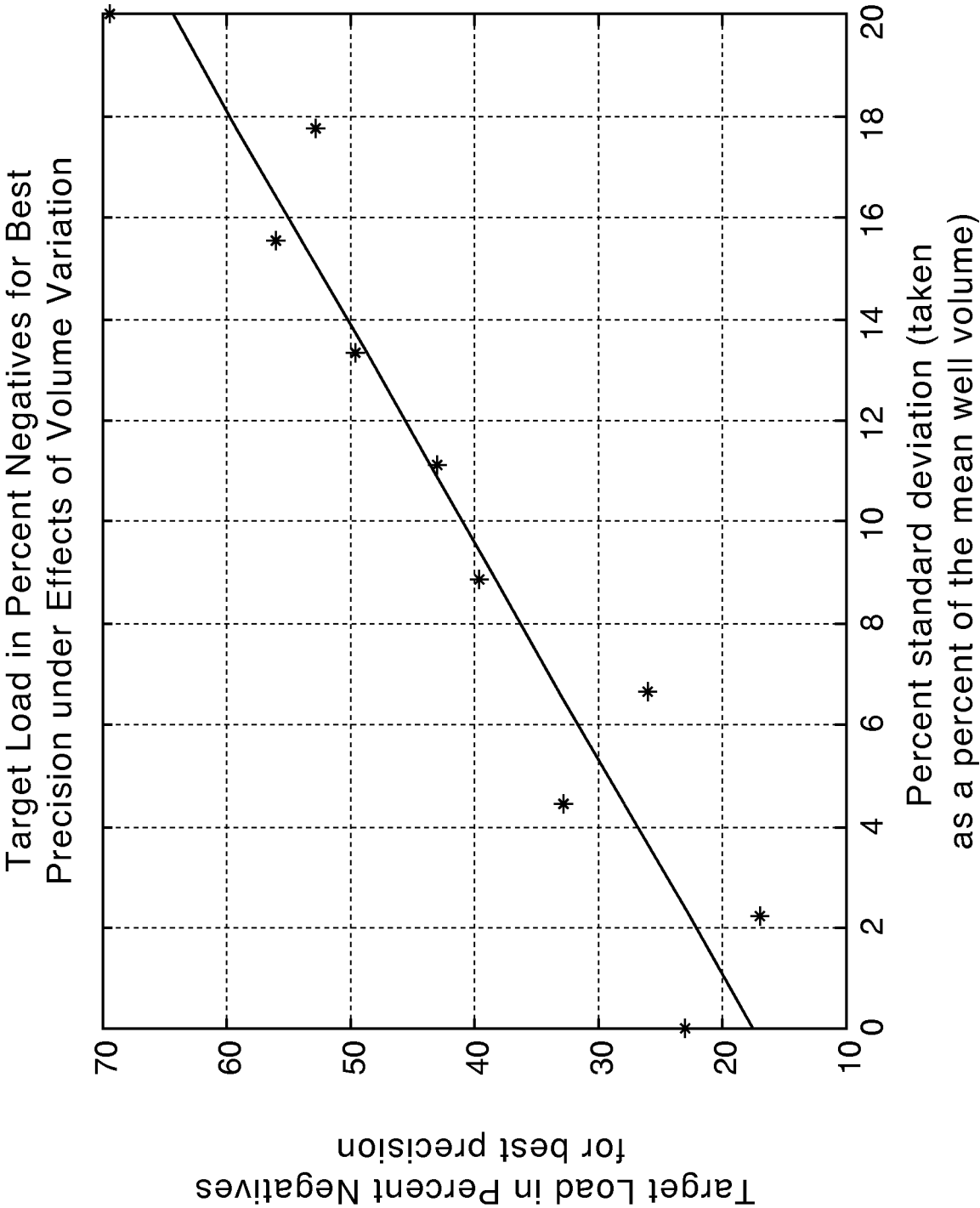


FIG. 12

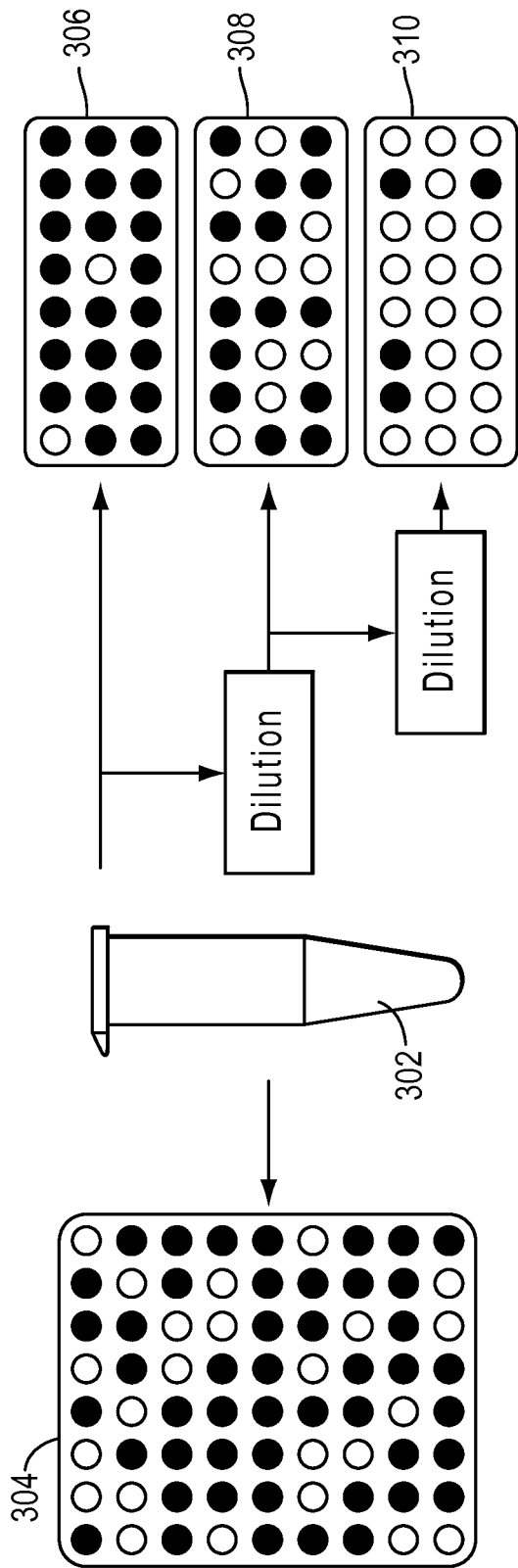


FIG. 13

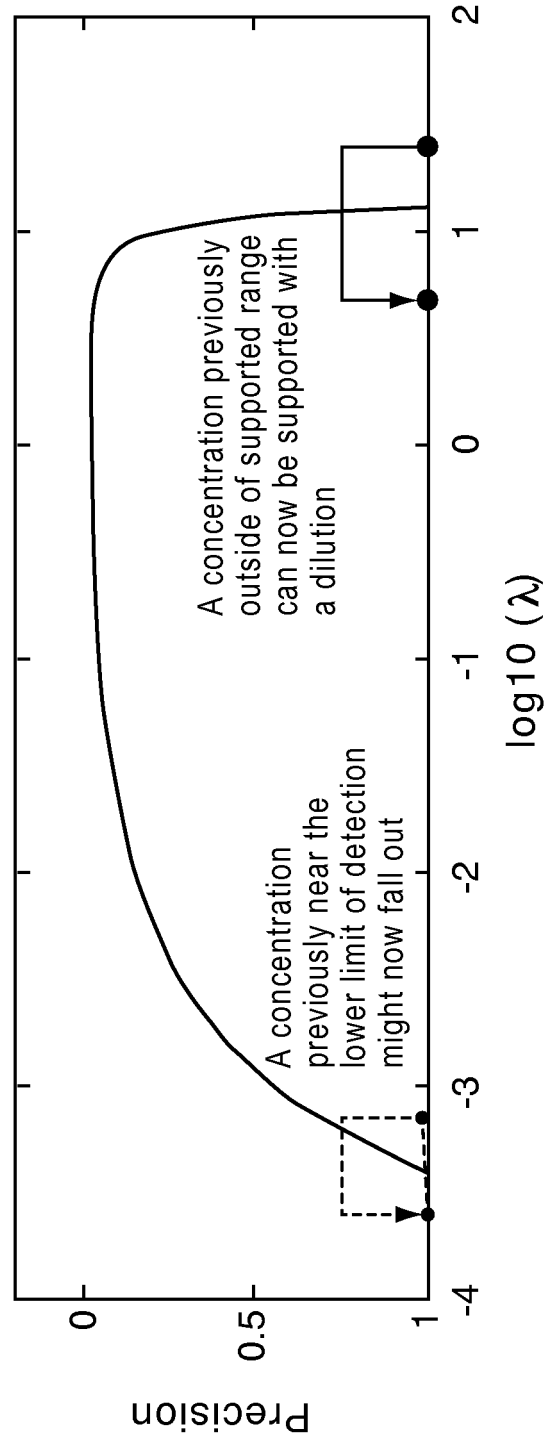


FIG. 14

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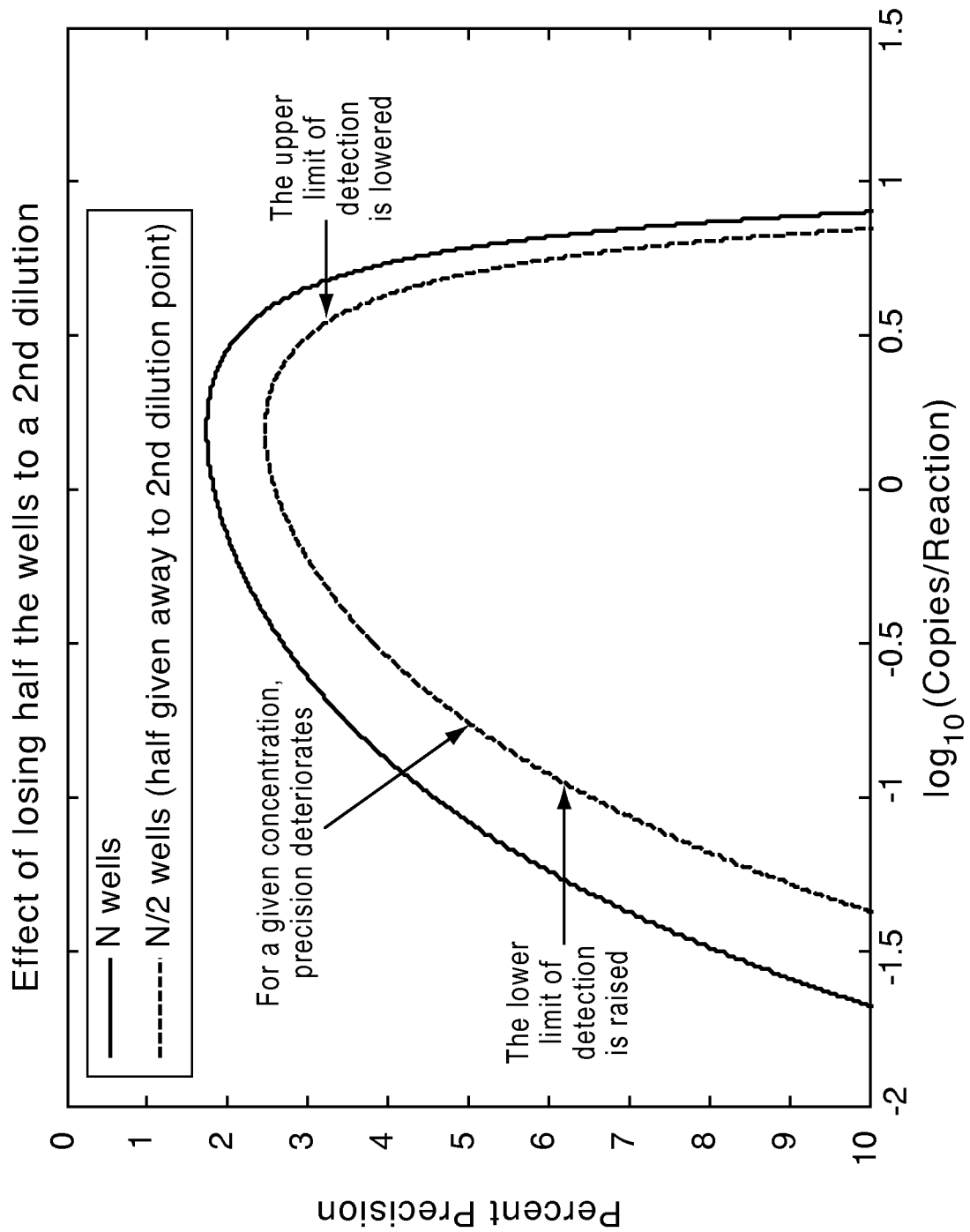


FIG. 15

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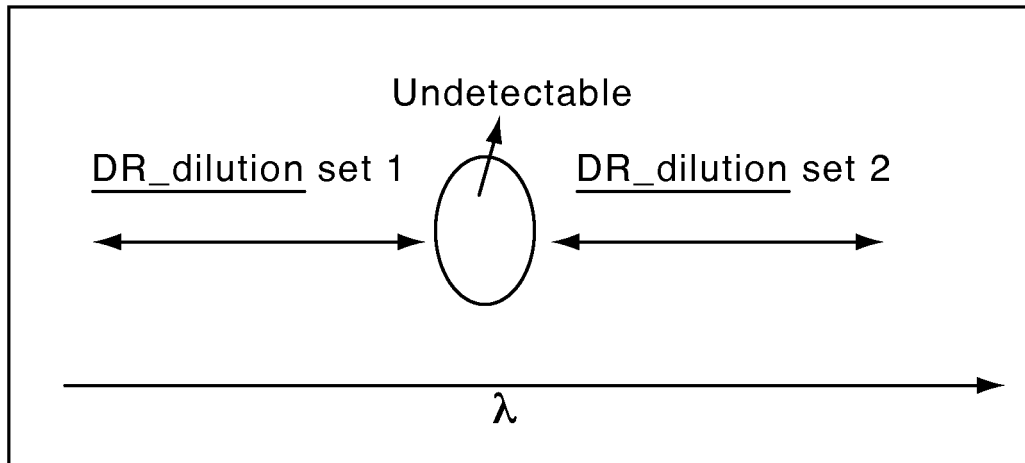


FIG. 16A

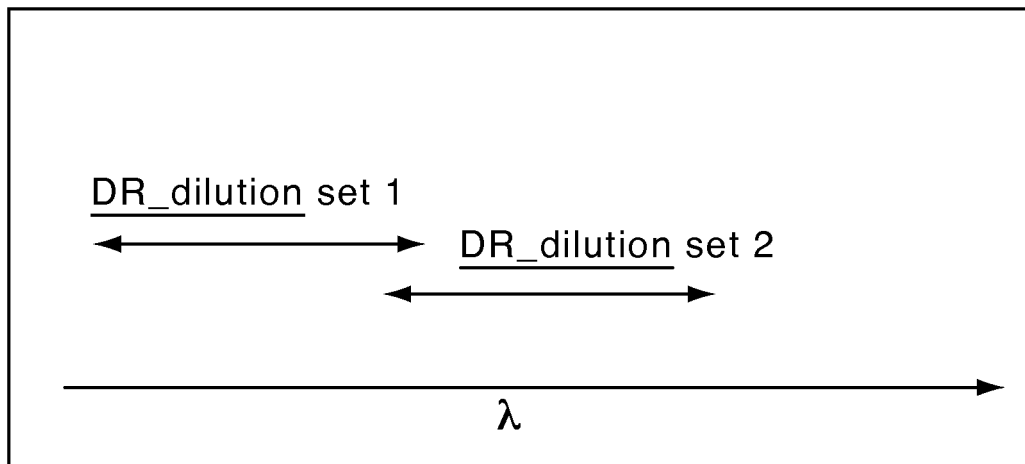


FIG. 16B

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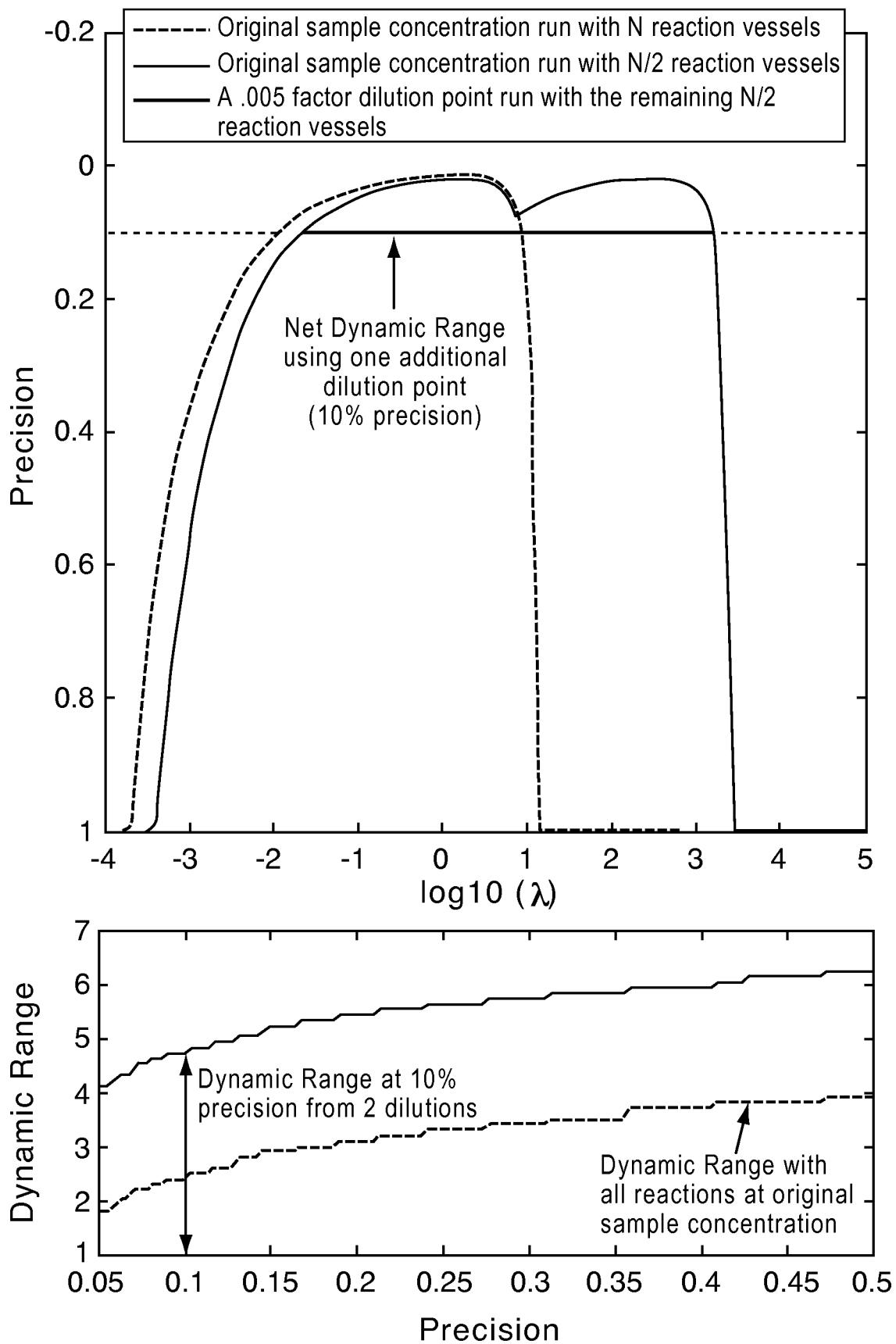


FIG. 17

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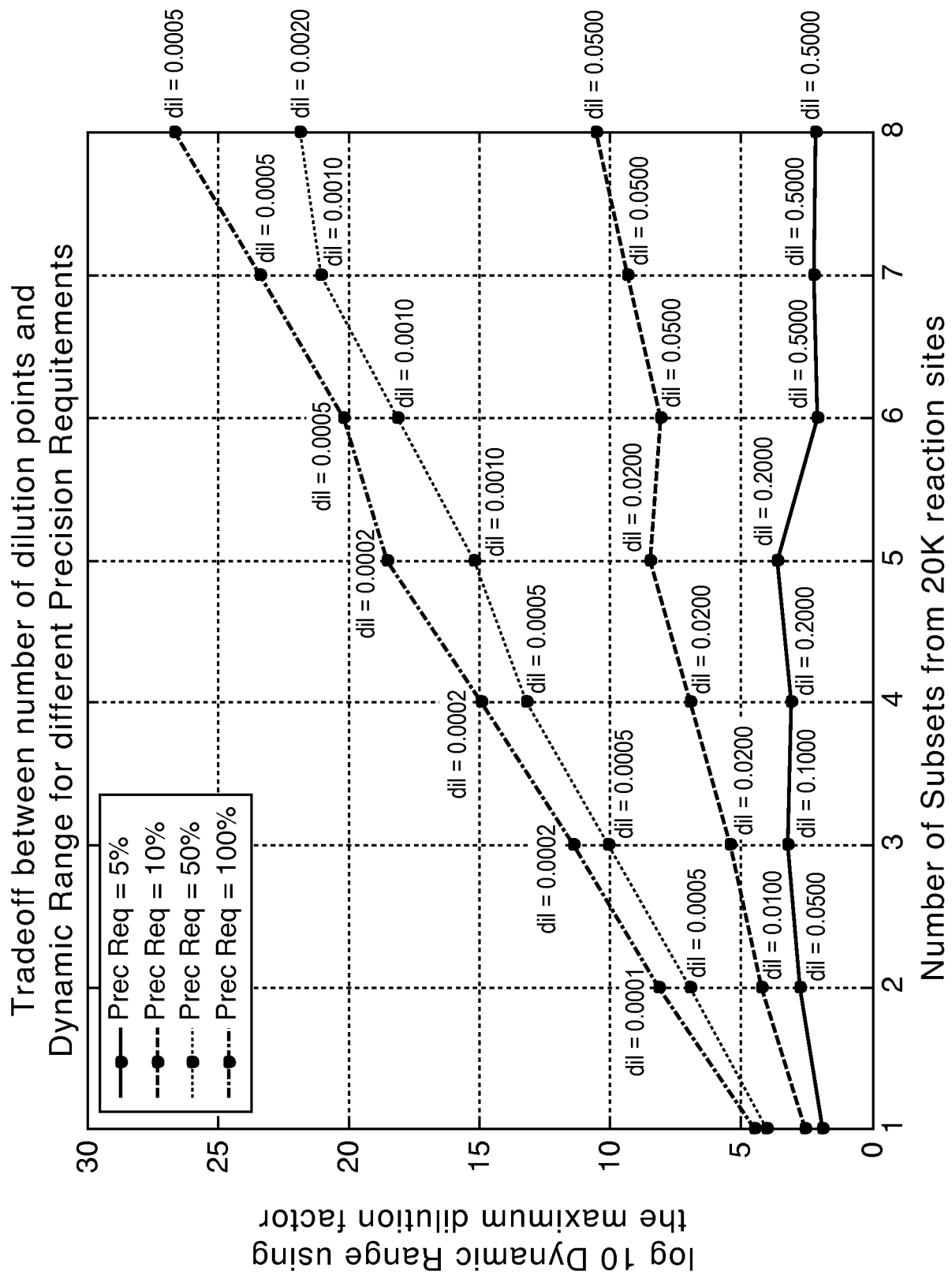


FIG. 18A

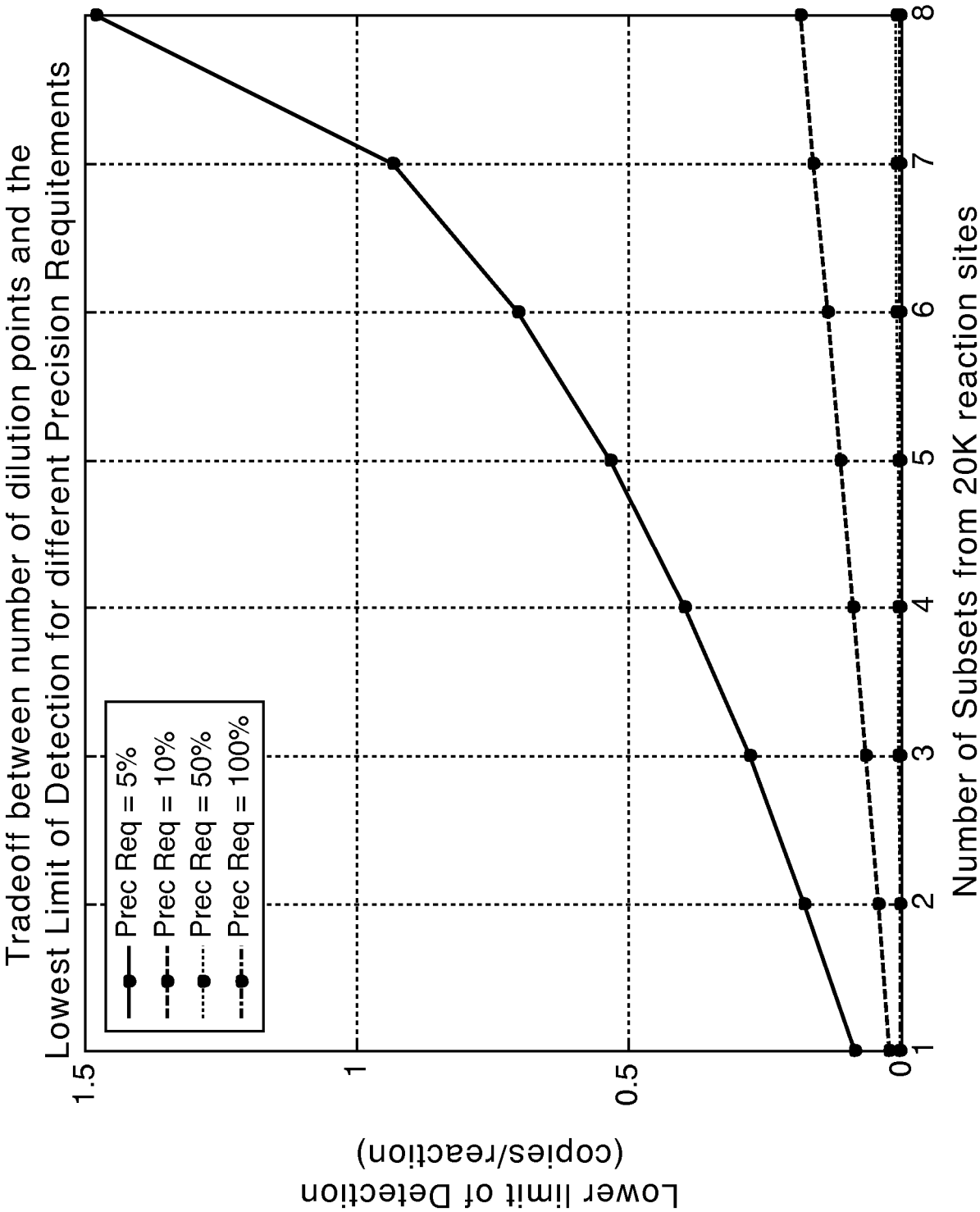


FIG. 18B

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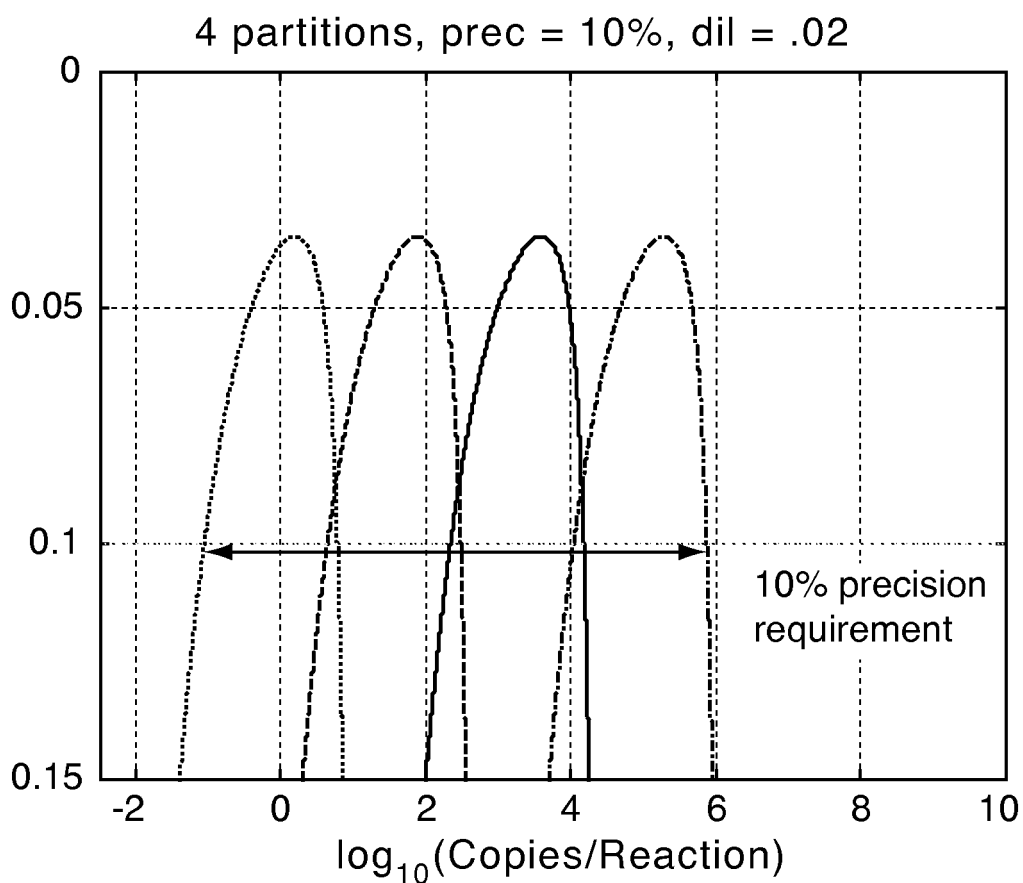
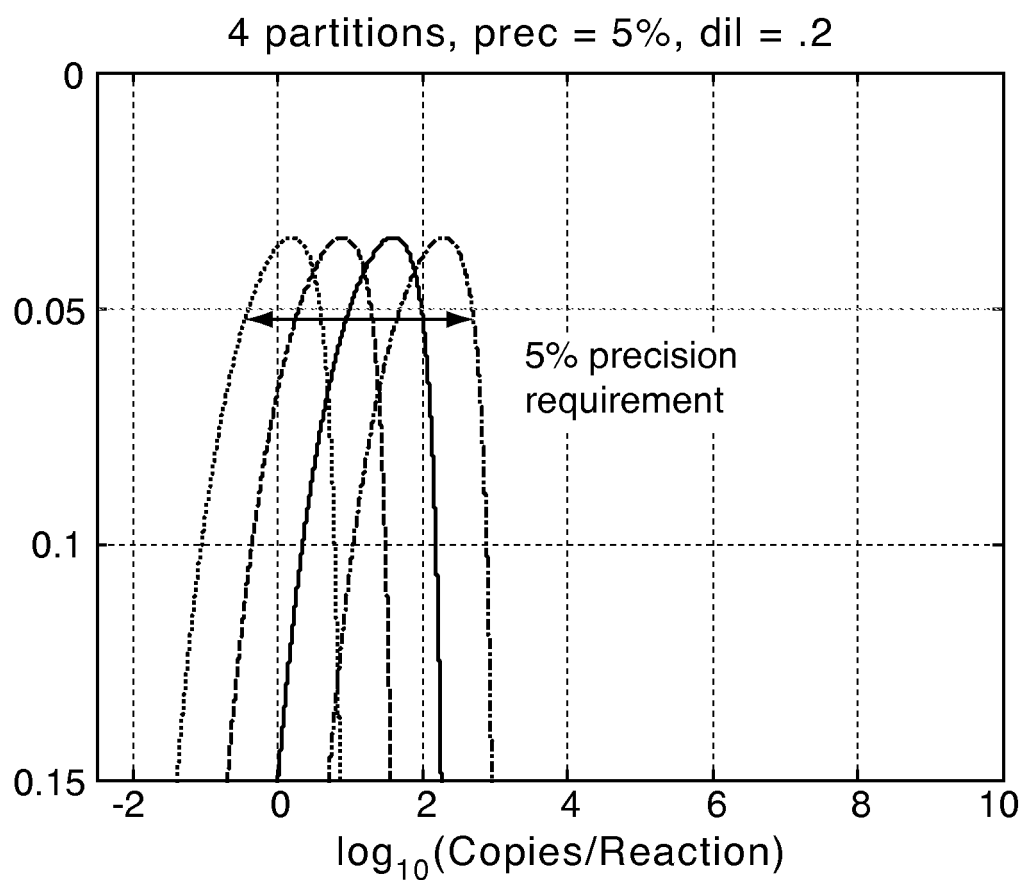


FIG. 18C

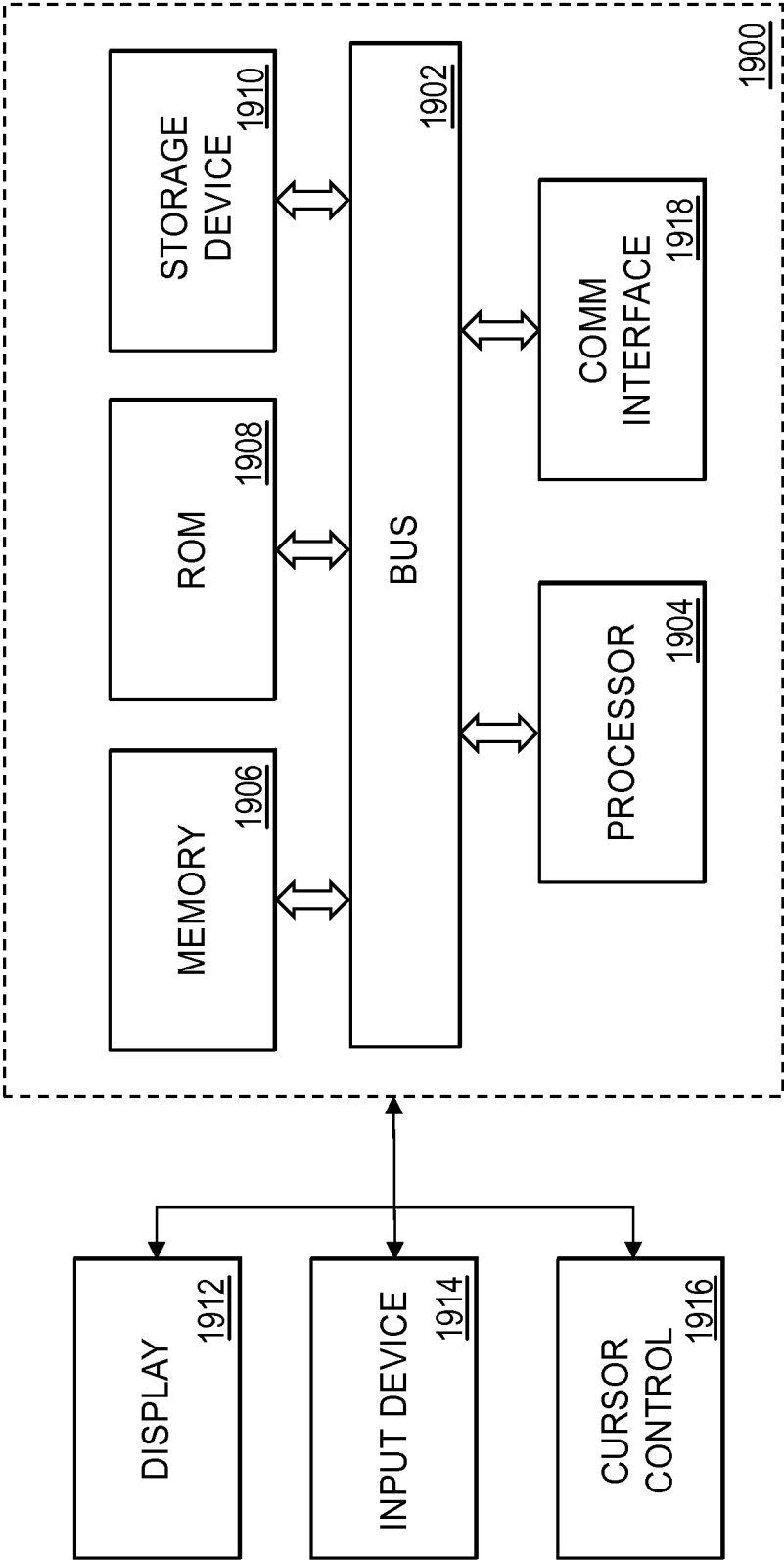


FIG. 19

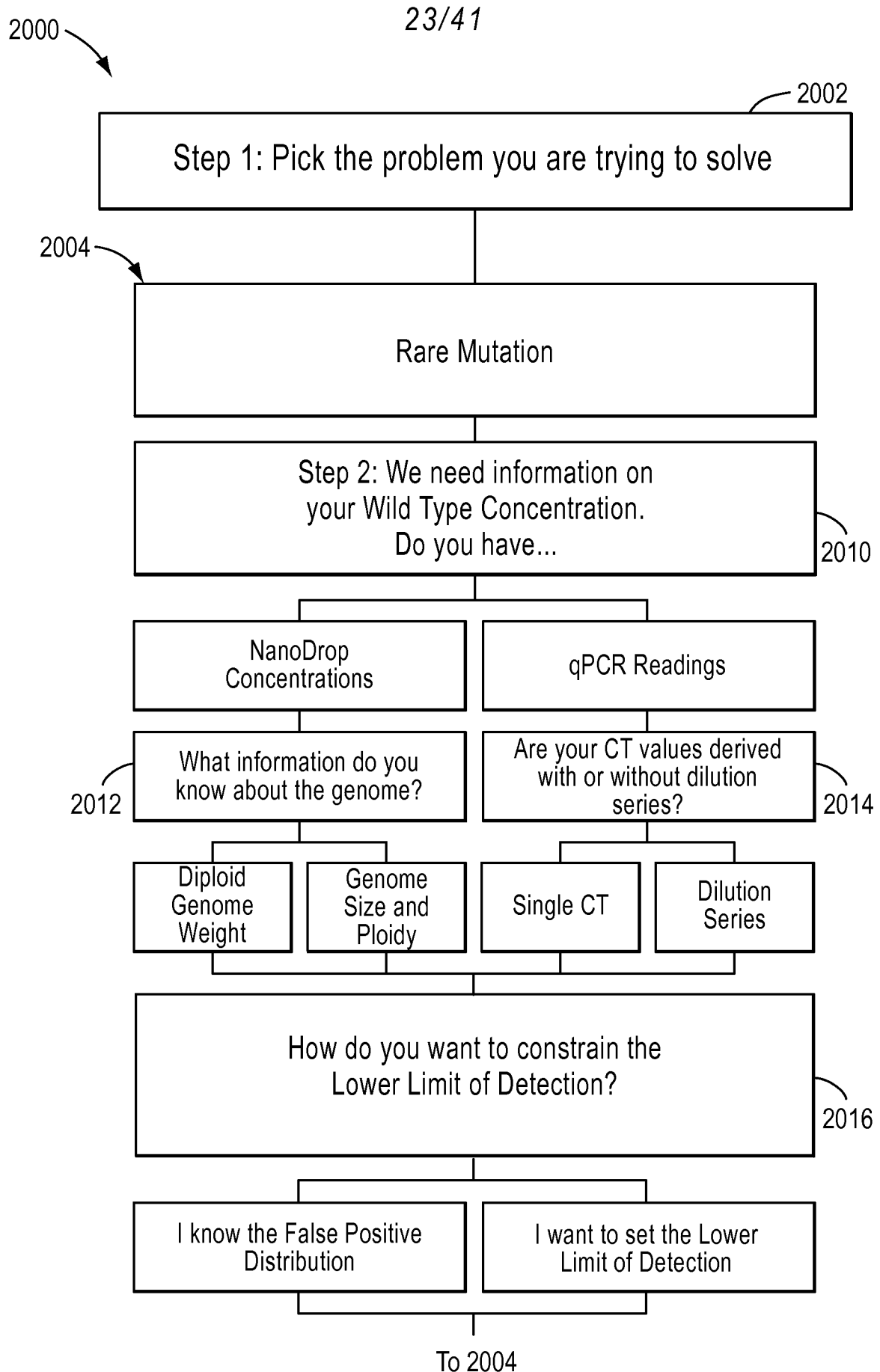


FIG. 20A-1

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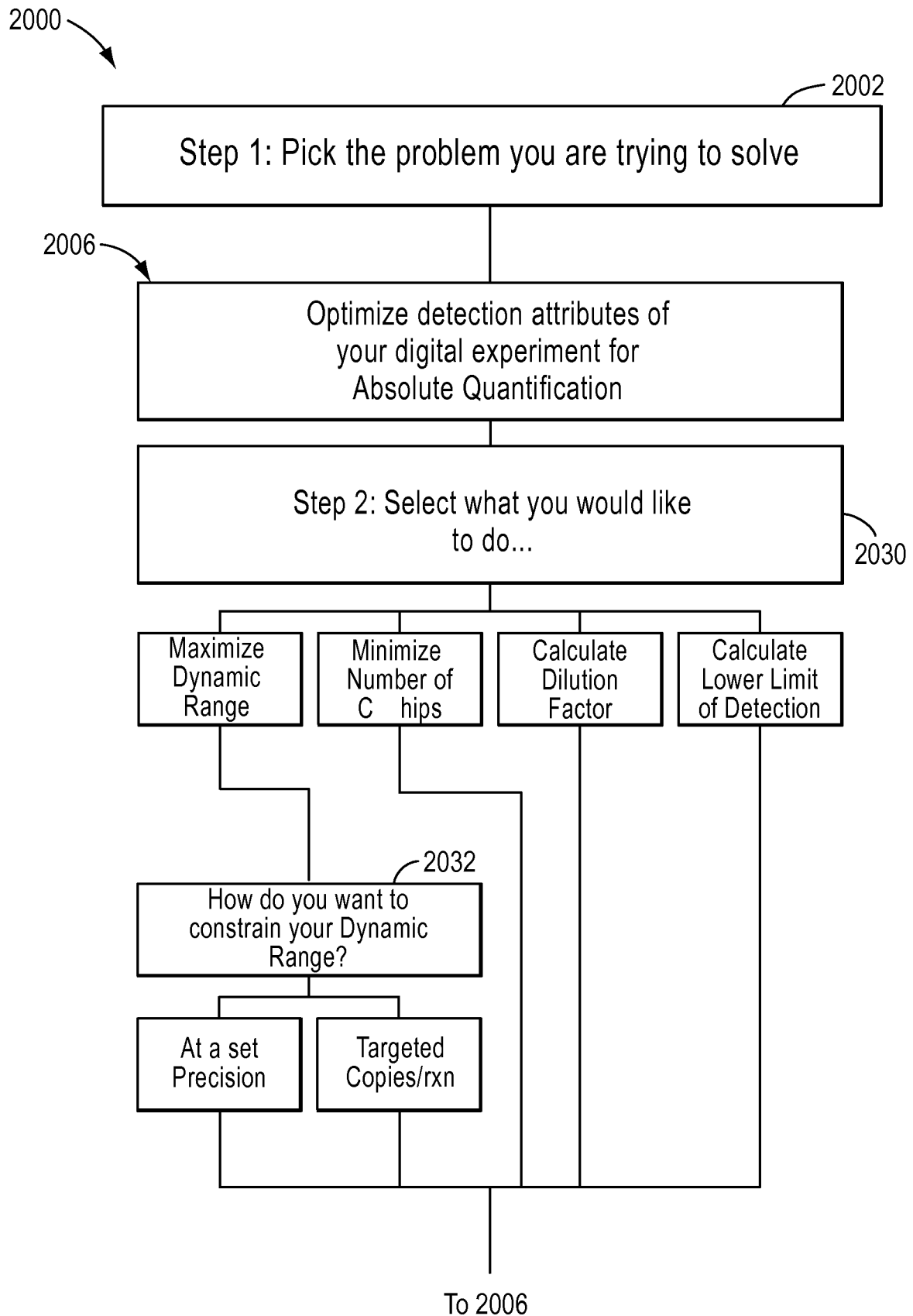


FIG. 20A-2

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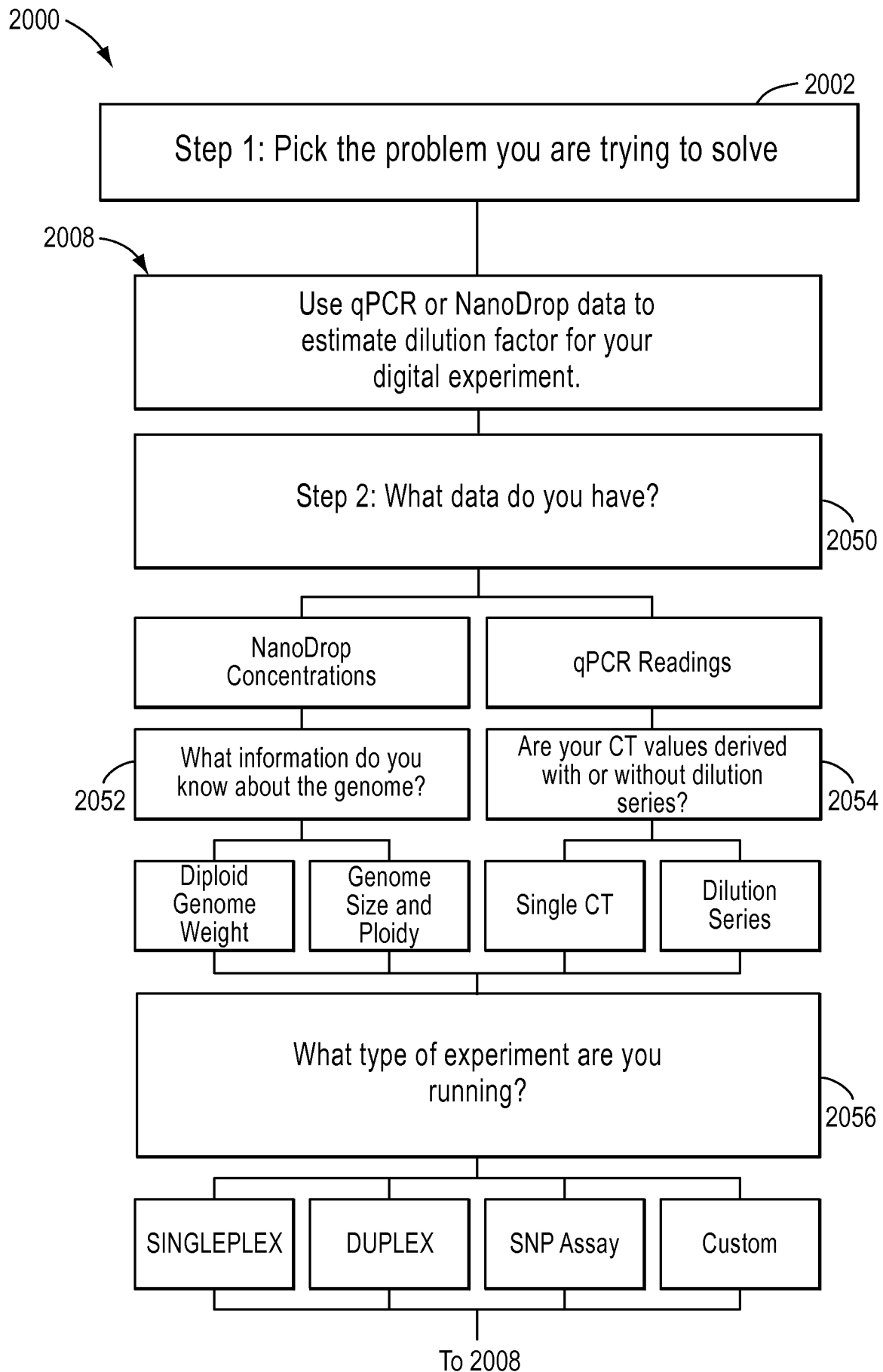


FIG. 20A-3

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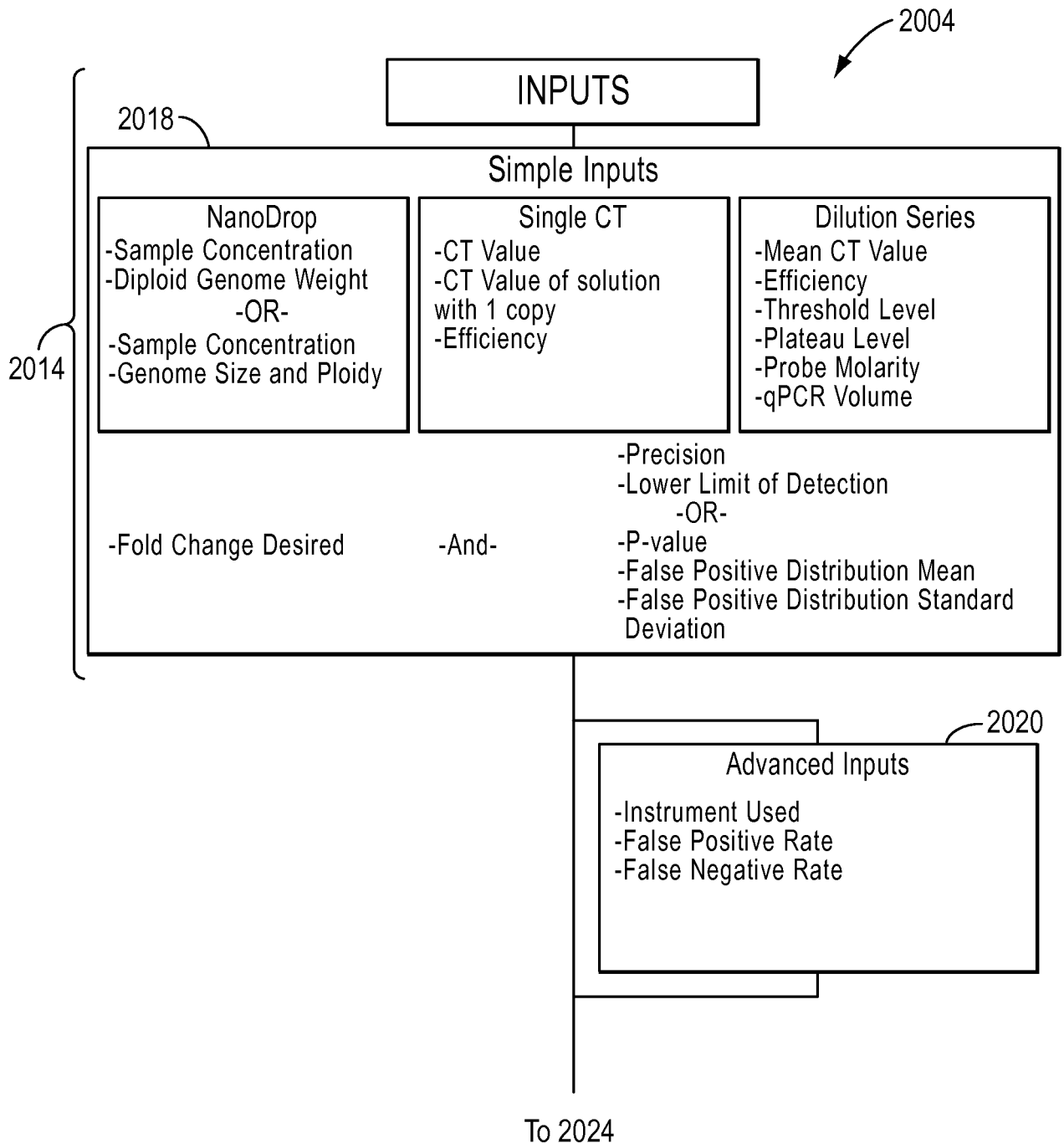


FIG. 20B-1a

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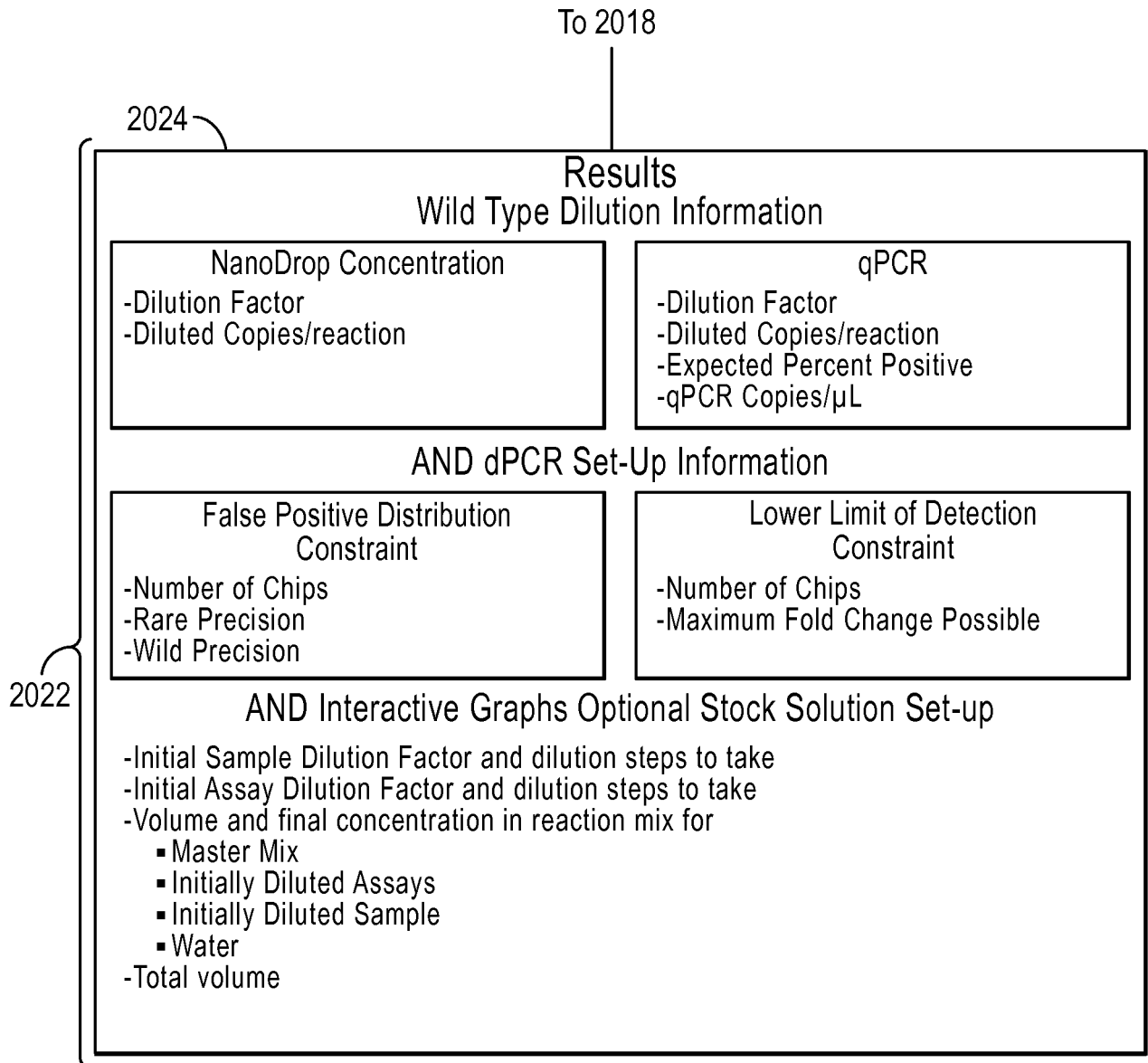


FIG. 20B-1b

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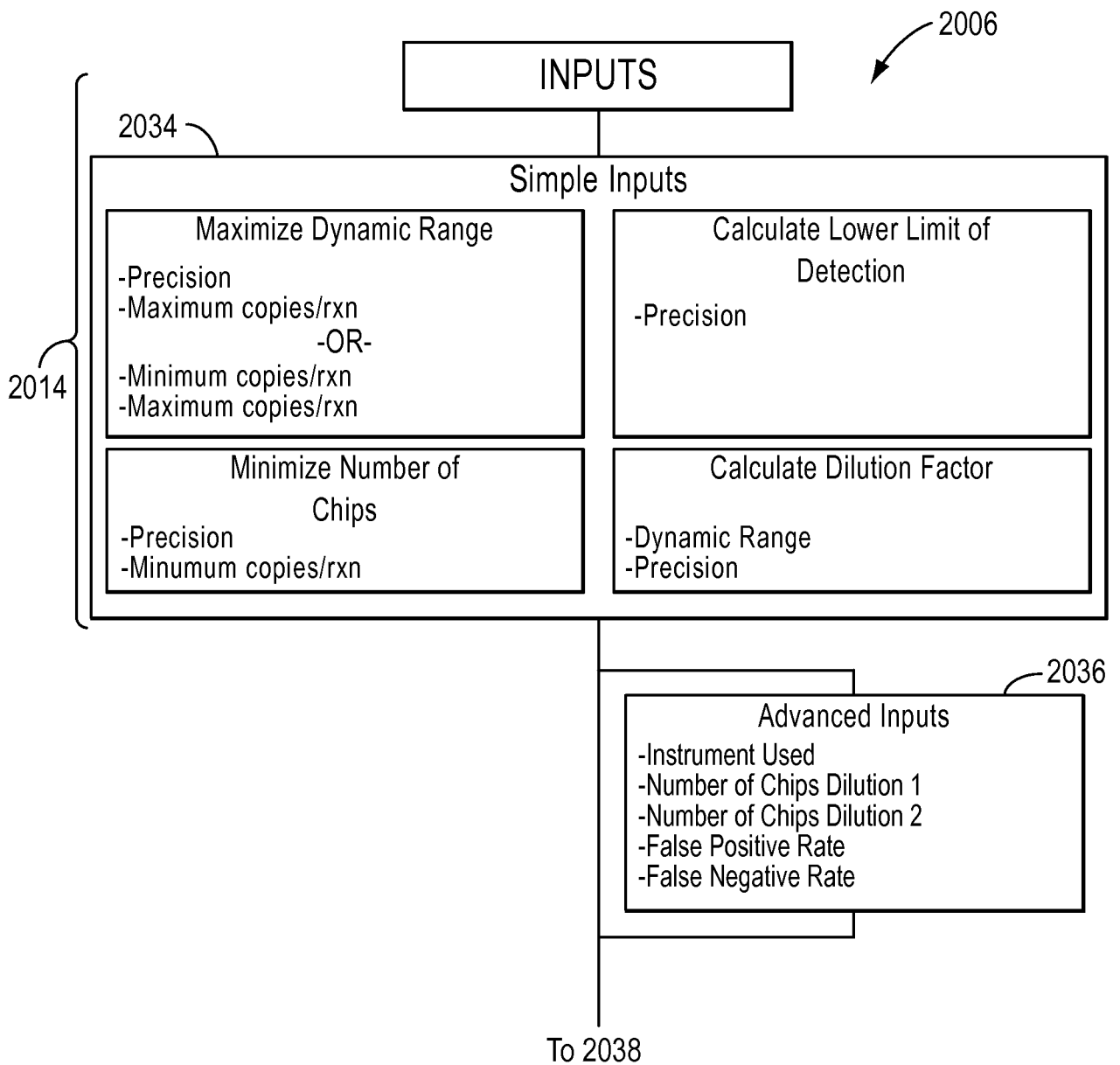


FIG. 20B-2a

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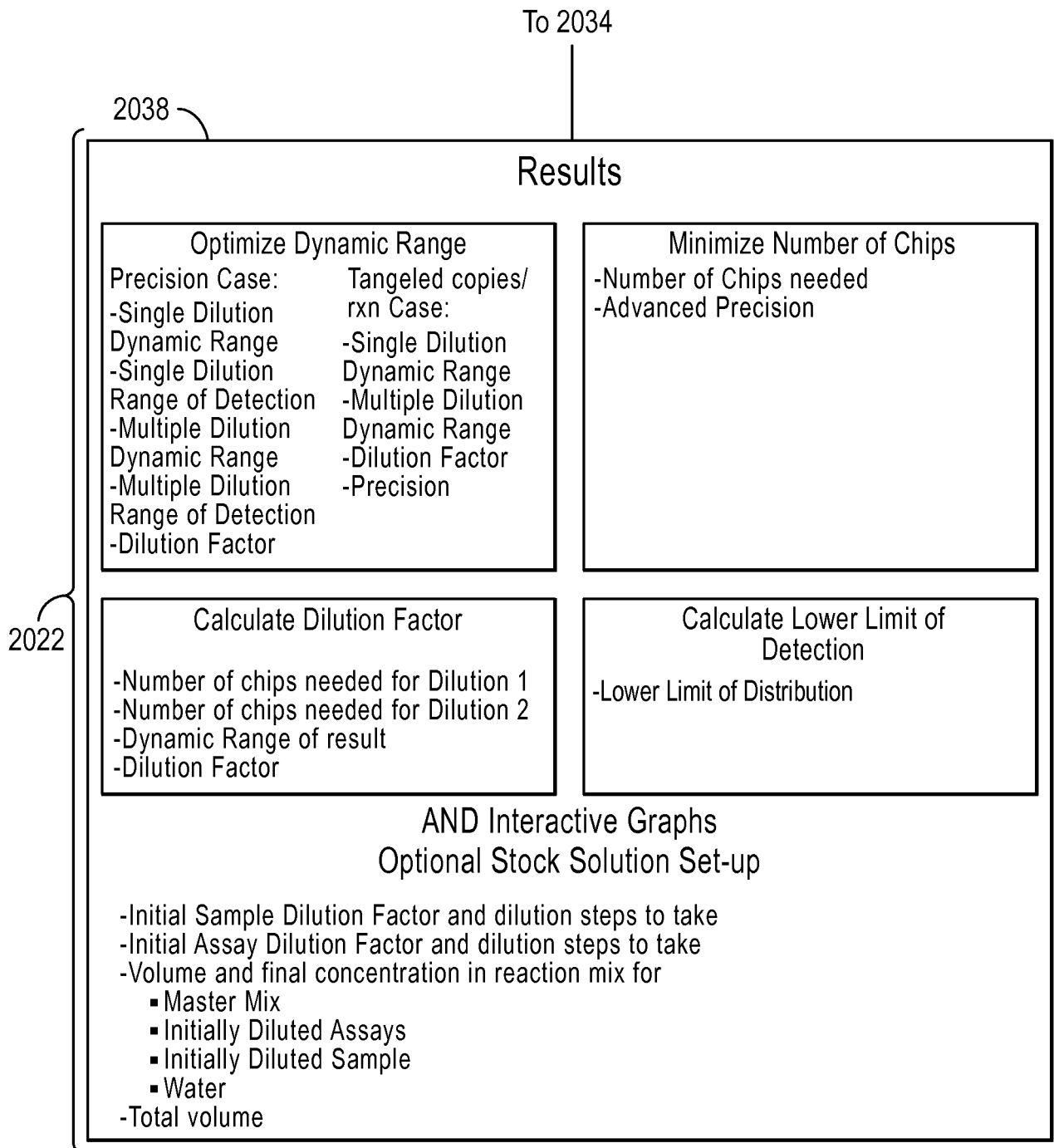


FIG. 20B-2b

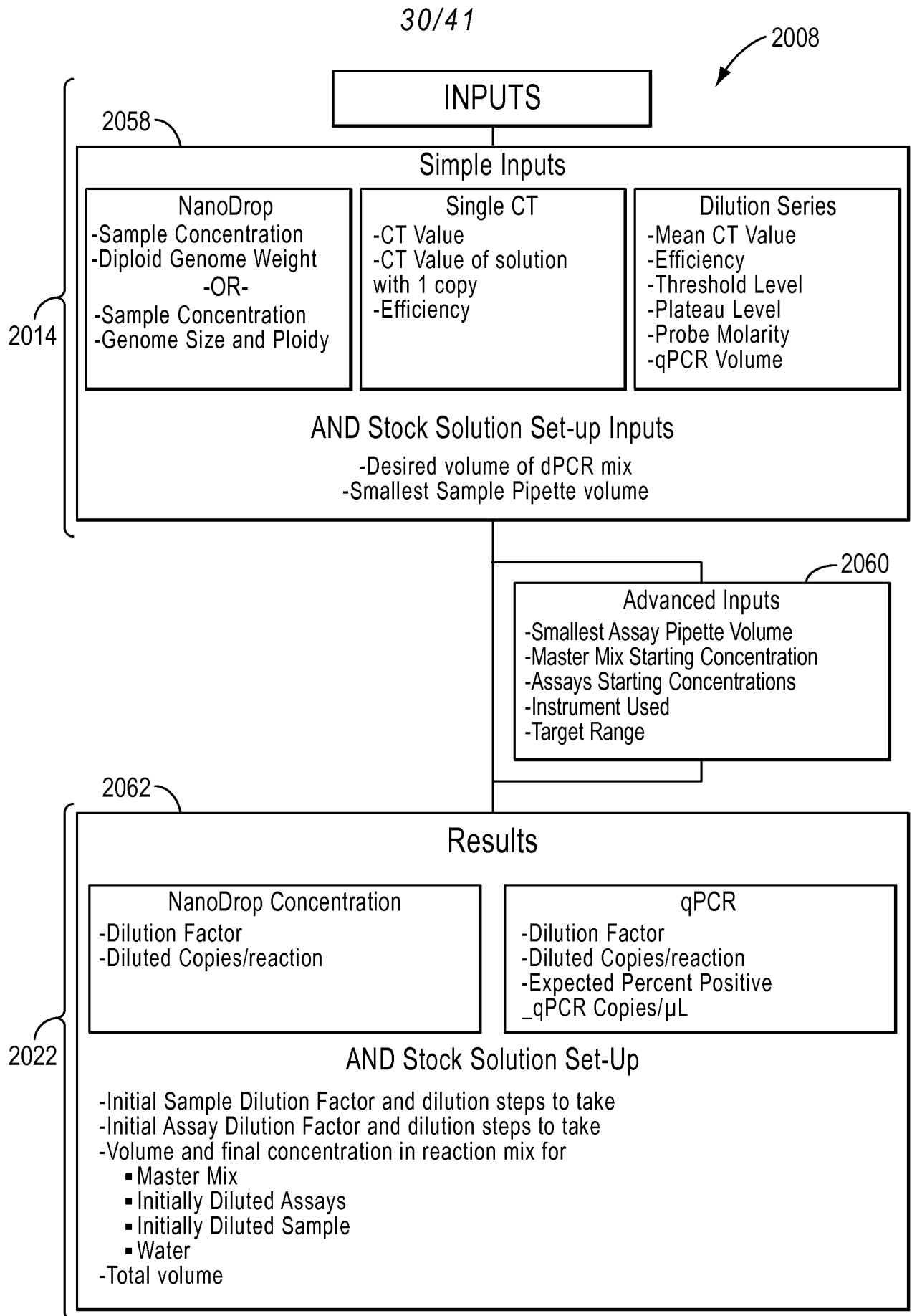


FIG. 20B-3

dPCR Experiment Setup Calculator

A tool for doing the calculations necessary to design a dPCR experiment using what information is available about the consumable, the system in use, the sample, and targets of interest.

1 Pick the problem you are trying to solve

☒ Rare Mutation

Consider putting some sort of verbage here to explain their choice?

☒ Optimize detection attributes of your digital experiment for Absolute Quantification

☐ Use qPCR or NanoDrop data to estimate dilution factor for your digital experiment.

2 Select what you'd like to do

☒ Maximize dynamic range

☐ Minimize number of chips

☐ Calculate Dilution Factor

☐ Calculate lower limit of detection

How do you want to constrain this?

Precision

Standard Inputs

Precision:

10

%

Minimum copies/rxn:

0.01

copies/rxn

Describes how close two quantities can be and still be distinguished by the system, at a certain confidence level. It is calculated as the ratio of the spread of the confidence interval around the copies/rxn compared to the true value of the copies/rxn.

The minimum readable concentration, anything lower will be ignored.

Show Advanced Inputs

Optimize

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FIG. 21A

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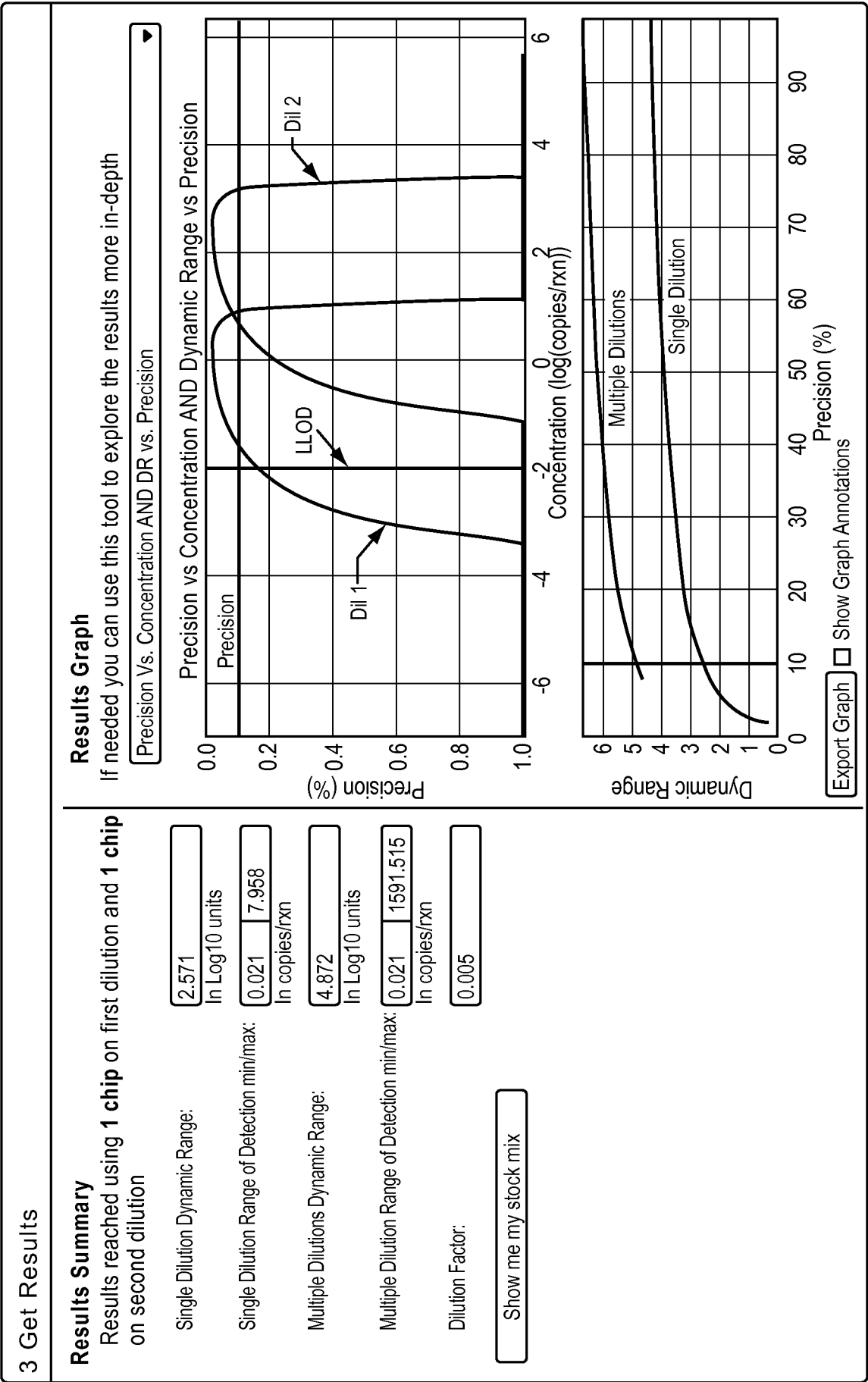


FIG. 21B

3 Get Results	
Results Summary Results reached using 1 chip on first dilution and 1 chip on second dilution Single Dilution Dynamic Range: 2.571 In Log10 units Single Dilution Range of Detection min/max: 0.021 7.958 In copies/rxn Multiple Dilutions Dynamic Range: 4.872 In Log10 units Multiple Dilution Range of Detection min/max: 0.021 1591.515 In copies/rxn Dilution Factor: 0.005 Show me my stock mix	Results Graph (HIDDEN) Stock Concentration Inputs What type of experiment are you running? SINGLEPLEX Desired volume of dPCR mix: 20 uL Smallest Sample Pipette Volume: 1 uL Calculate

FIG. 21C

Results reached using 1 chip on first dilution and 1 chip on second dilution

Single Dilution Dynamic Range: In Log10 units

Single Dilution Range of Detection min/max: In copies/rxn

Multiple Dilutions Dynamic Range: In Log10 units

Multiple Dilution Range of Detection min/max: In copies/rxn

Dilution Factor:

Stock Concentration Inputs

What type of experiment are you running?

Desired volume of dPCR mix:

Smallest Sample Pipette Volume:

Stock Solution Set-up Dilution 1

Item	Final Concentration in Reaction Mix	Volume
2x Master Mix	1x	10
20x FAM Assay	1x	1
Sample	0.45x	9
Water	1x	0
Total Volume		20

Stock Solution Set-up Dilution 2

Initial Sample Dilution (apart from reaction mix): Dilution Factor: Series:

*Dilute Sample by this amount before creating final mix

Item	Final Concentration in Reaction Mix	Volume
2x Master Mix	1x	10
20x FAM Assay	1x	1
Sample	0.00225x	9
Water	1x	0
Total Volume		20

FIG. 21D

dPCR Experiment Setup Calculator

A tool for doing the calculations necessary to design a dPCR experiment using what information is available about the consumable, the system in use, the sample, and targets of interest.

1 Pick the problem you are trying to solve

☐ Rare Mutation

Consider putting some sort of verbage here to explain their choice?

☐ Optimize detection attributes of your digital experiment for Absolute Quantification

☐ Use qPCR or NanoDrop data to estimate dilution factor for your digital experiment.

2 We need information on your stock solution. Please select what data you have.

☐ NanoDrop Concentration

What information do you know about the genome?

How do you want to constrain the Lower Limit of Detection?

Select

Select

☐ Single CT

☐ Multiple CT (Swillens)

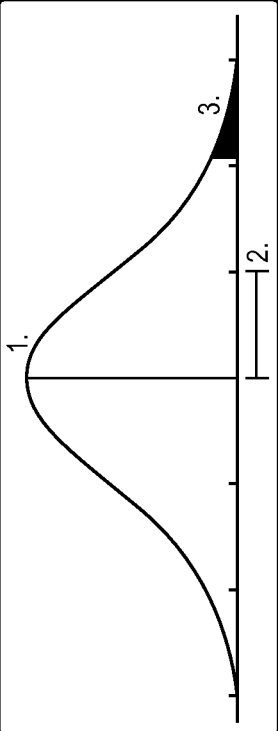
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FIG. 22A

SUBSTITUTE SHEET (RULE 26)

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2 We need information on your stock solution. Please select what data you have.	
☒ NanoDrop Concentration	☐ Single CT
☐ Multiple CT (Swillens)	
What information do you know about the genome?	
I know my Diploid Genome Weight	
How do you want to constrain the Lower Limit of Detection?	
I know the False Positive Distribution	
Show Advanced Inputs	
Dilution Factor Inputs Diploid Genome Weight: 3.3 pg The weight of the Diploid Genome. Sample Concentration: 50 ng/ul The concentration from the NanoDrop reading.	Standard Inputs Fold Change Desired: 0.01 rare/wild P-Value: 0.025 Decimal False Positive distribution Mean: 10 Positive/chip False Positive distribution Standard Deviation: 2 Positives/chip
The desired fold change from rare to wild that you are expecting to detect.	
The False Positive distribution can be found by running Non Template Control (NTC) 1. Mean 2. Standard Deviation 3. P-value: Probability that result belongs to False Positive distribution	
	
Solve	
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FIG. 22B

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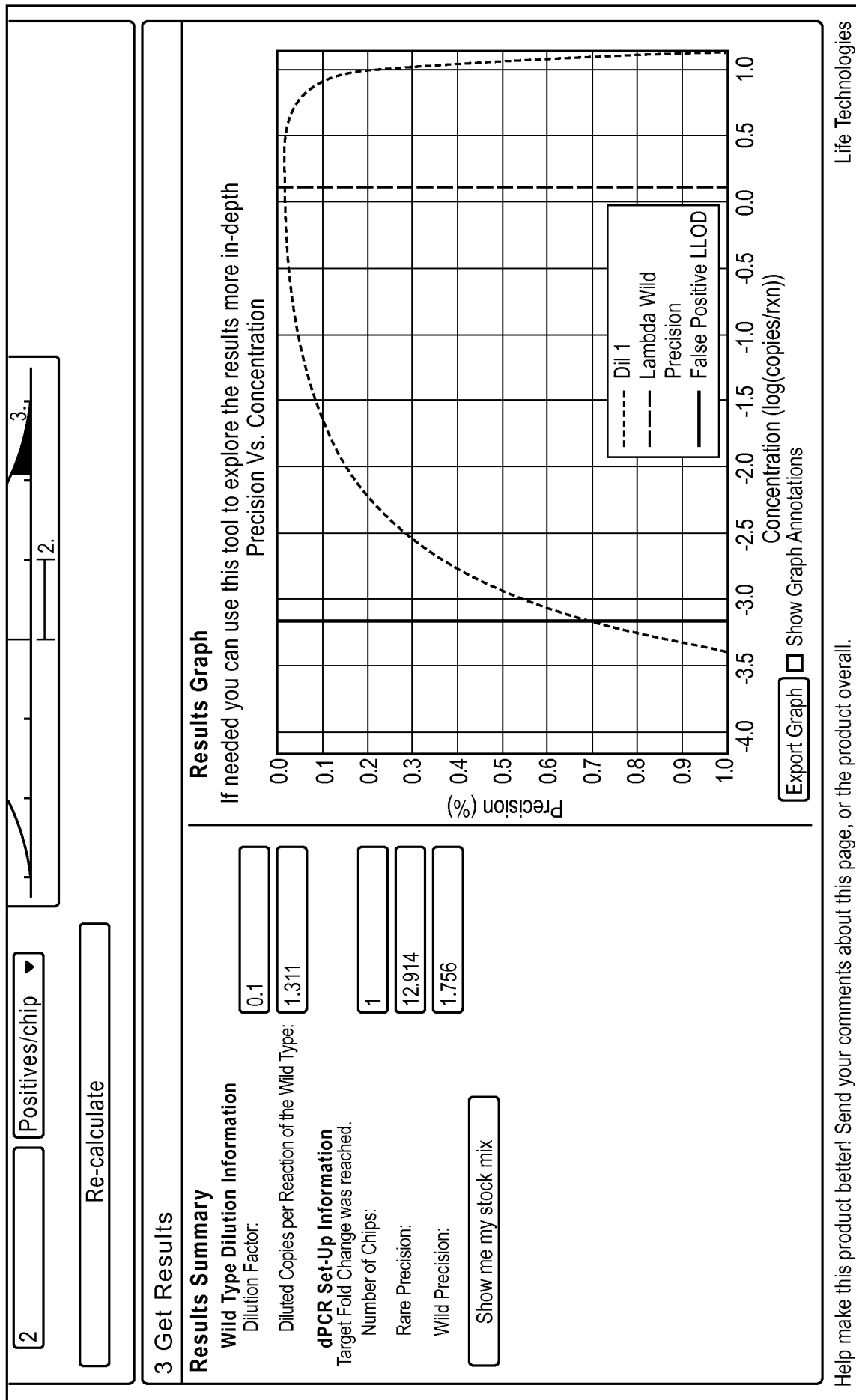


FIG. 22C

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False Positive distribution Mean: Positive/chip ▾
False Positive distribution Standard Deviation: Positives/chip ▾

2. Standard Deviation
3. P-value: Probability that result belongs to False Positive distribution

3 Get Results

Results Summary

Wild Type Dilution Information
Dilution Factor:
Diluted Copies per Reaction of the Wild Type:

dPCR Set-Up Information
Target Fold Change was reached.
Number of Chips:
Rare Precision:
Wild Precision:

Results Graph (HIDDEN)

Stock Concentration Inputs

What type of experiment are you running?

Desired volume of dPCR mix: ▾

Smallest Sample Pipette Volume: ▾

Stock Solution Set-up

Initial Assay Dilution Factor (apart from reaction mix): Equivalent Dilution Series: 1/2

*Dilute Assays by this amount before creating final mix

Item	Final Concentration in Reaction Mix	Mix	Volume
2x Master Mix	1x		8.5
20x FAM Assay	1x		1.7
Sample	0.1x		1.7
Water	1x		5.1
Total Volume			17

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FIG. 22D

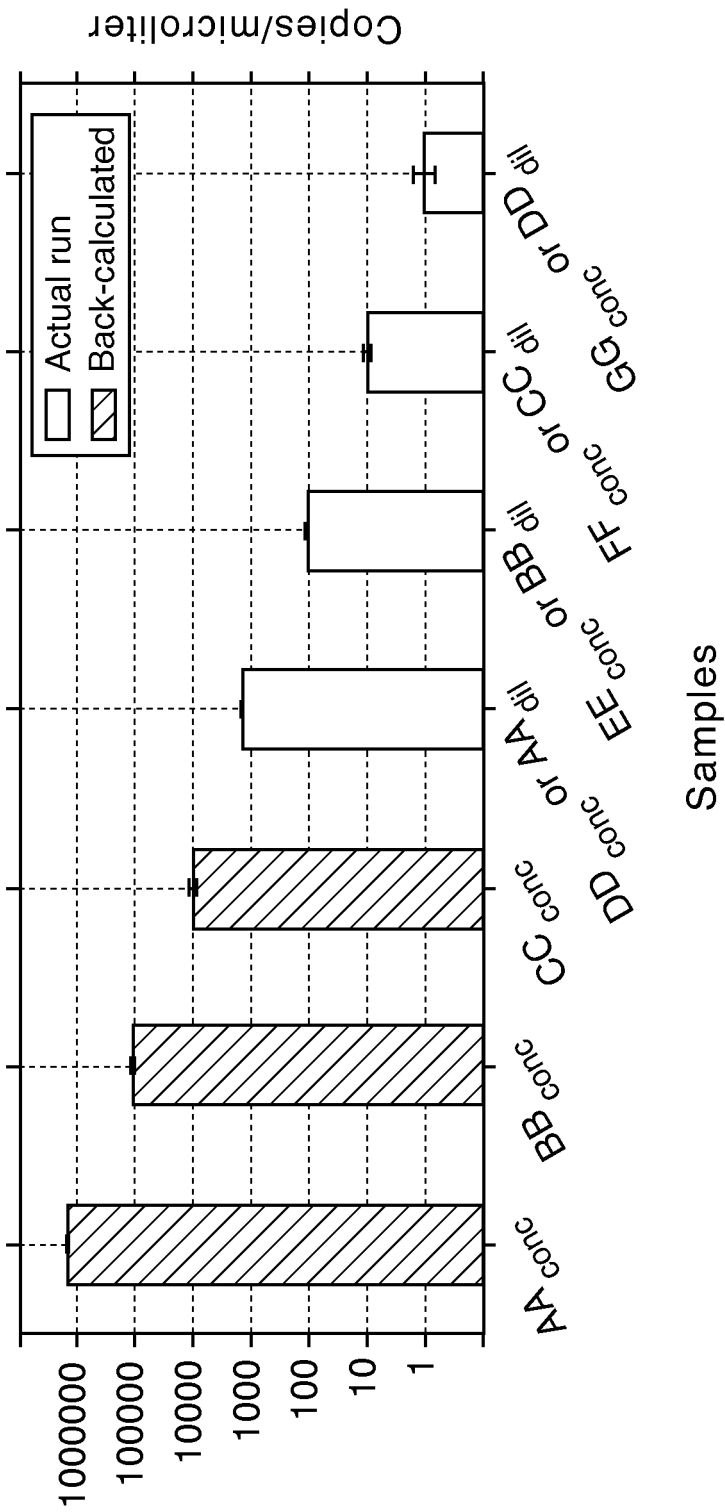


FIG. 23A

Model using 20K Reactions, False Positive = 10/20K, False Negative = 5/20K
Detect between 0.0005 to 5.0000 copies/reaction (denoted by--line for each set of reactions)

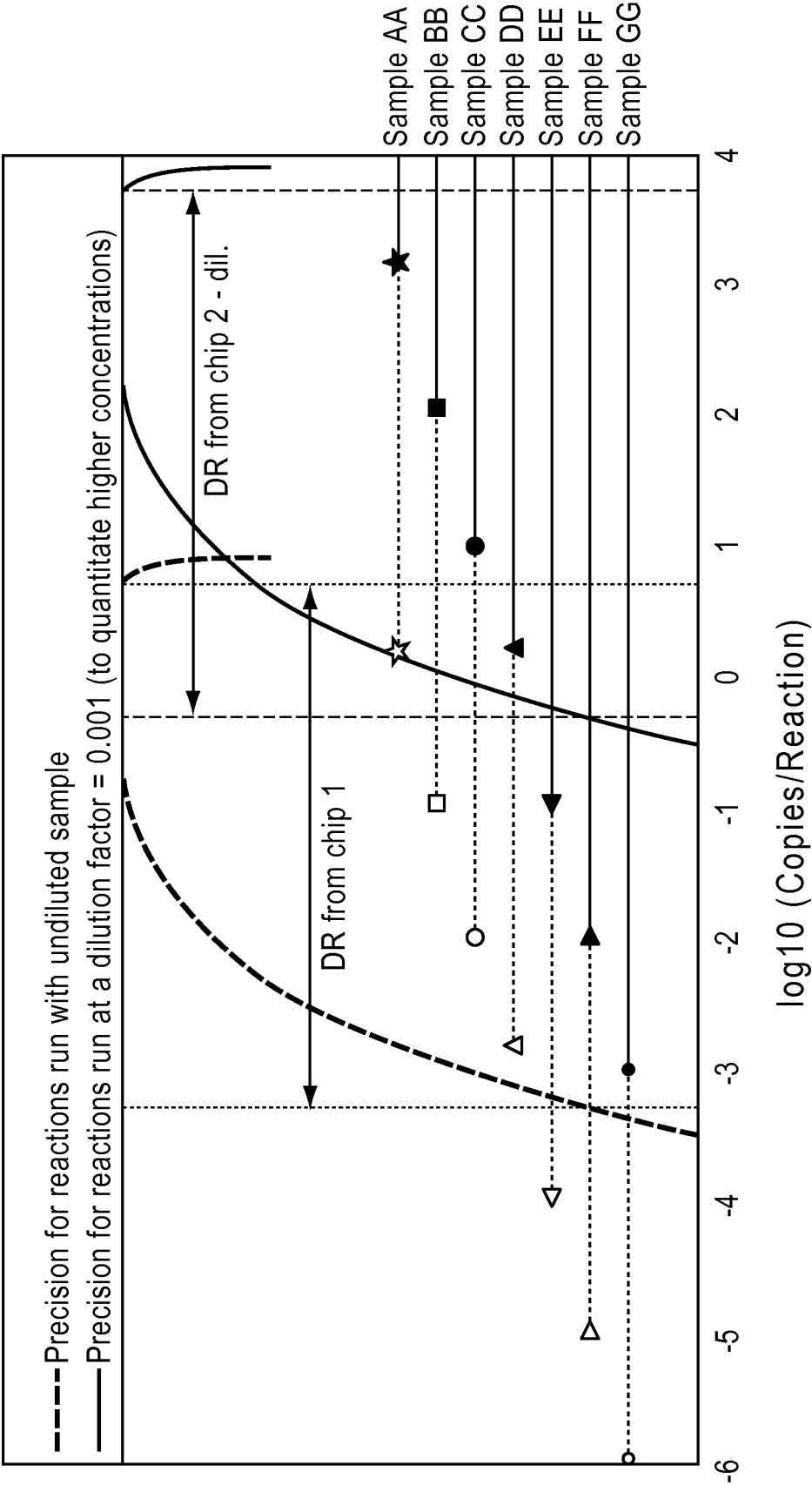


FIG. 23B

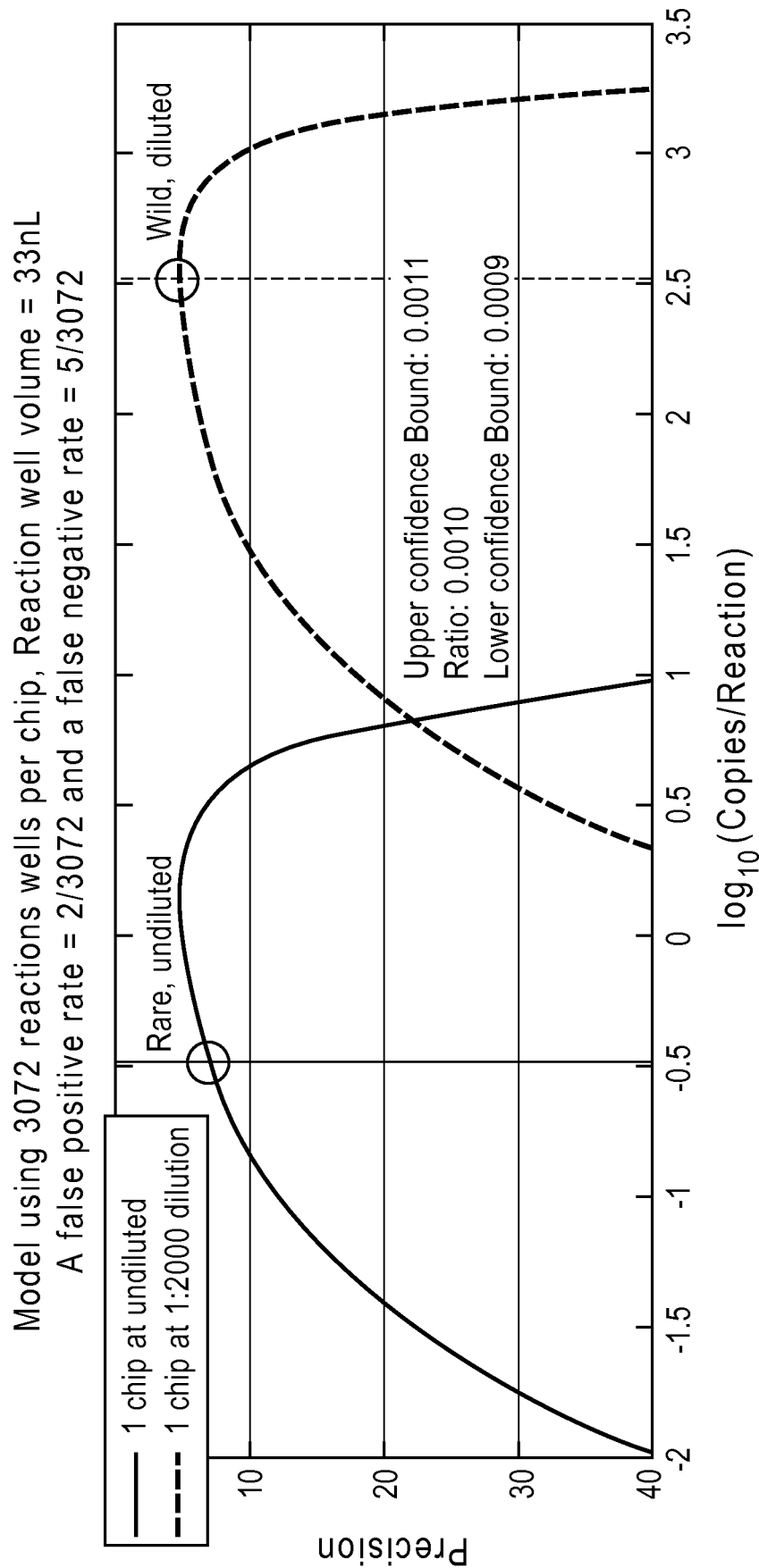


FIG. 24