

TECHNICAL NOTE

GeneMapper™ PG Software: Developmental Validation

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1. Introduction

GeneMapper™ PG Software is a deterministic probabilistic genotyping system for forensic DNA mixture analysis. GeneMapper™ PG Software employs a fully continuous, deterministic framework based on adaptive grid search rather than Markov Chain Monte Carlo (MCMC) sampling. This enables identical results across repeated analyses of the same inputs, and traceability of computational steps. GeneMapper™ PG Software is also fully integrated with the Applied Biosystems™ GeneMapper™ ID-X Software ecosystem.

GeneMapper™ PG Software includes a suite of complementary algorithms for estimating the number of contributors (NOC), encompassing supervised machine learning models and simulation-based methods, as well as a deterministic maximum likelihood deconvolution engine. Likelihood ratios (LRs) are calculated using a Balding-Nichols framework with full transparency, and robustness analyses are conducted following the methodology of Dørum, *et al.* [1], providing an objective measure of the risk of misleading evidence.

This technical note summarizes developmental validation testing for GeneMapper™ PG Software. The validation adheres to Scientific Working Group on DNA Analysis Methods (SWGDM) Guidelines for the Validation of Probabilistic Genotyping Systems [2].

2. System Control

SWGDM guidelines for the validation of probabilistic genotyping software detail several requirements for system control of the software. These include:

- A requirement that laboratories verify that the software is installed on suitable computers and properly installed. Release notes which detail the installation procedure and the required hardware specifications for GeneMapper™ PG Software have been published and are publicly available [3].
- A requirement that every software release have a unique version number and that this version be referenced in any validation documentation. GeneMapper™ PG Software has been released at version 1.0. The version number is visible in the header bar of the software and under 'Help' > 'About'. This document covers the validation of GeneMapper™ PG Software v1.0.

- A requirement that only authorized users can access the software, that audit trails track changes to data each time a calculation is run, and that users can only perform authorized actions. In GeneMapper™ PG Software, analyses performed on profiles and system settings are tracked as calculations are run. Details of this tracking are available in the software. The software includes roles allowing users varying levels of access to actions, parameter changes, and data. Roles were verified as part of internal testing of the software.

3. Developmental Validation

SWGDM guidelines for the validation of probabilistic genotyping software outline the requirements for the developmental validation of probabilistic genotyping software by the manufacturer. This includes a requirement to publish the underlying scientific principles of the probabilistic genotyping methods and characteristics of the software in a peer-reviewed scientific journal. Published studies that address the sensitivity, specificity, precision and accuracy of the software, and which cover case-type and control samples, are also required.

The following sections describe how GeneMapper™ PG Software has met these criteria:

3.1. *Peer-reviewed Publication*

The underlying scientific principles of GeneMapper™ PG Software have been published in a technical note by Thermo Fisher Scientific entitled '*GeneMapper™ PG Software: Scientific Principles*' [4]. The content of this technical note is planned to be submitted for publication to a peer-reviewed scientific journal on full commercial release of GeneMapper™ PG Software (September 2025).

3.2. *Sensitivity*

3.2.1. Introduction

Studies are required which assess the ability of GeneMapper™ PG Software to reliably determine the presence of a contributor's DNA over a broad variety of evidence, including mixtures and low-level DNA quantities. This should be evaluated using various sample types (e.g. different numbers of contributors, mixture proportions, and template quantities.). The sensitivity studies should demonstrate the potential for Type I errors (incorrect rejection of a true hypothesis) and should demonstrate the range of LR values that can be expected for contributors.

3.2.2. Methods

Single-source and mixed DNA samples were prepared in multiple different configurations with various mixture proportions and template quantities. See Section 3.5.2 for more details on sample setup.

PCR amplification was performed with the GlobalFiler™ PCR Amplification Kit (PN 4476135) with 29 PCR cycles. Capillary electrophoresis (CE) was performed with GeneScan™ 600 LIZ Size Standard v2 (PN 4408399) on both the 3500xL Genetic Analyzer and the SeqStudio™ Flex Genetic Analyzer (24-capillary). Default amplification, run and injection parameters were used.

Data was analyzed in GeneMapper™ ID-X v1.7 with an analytical threshold of 20 RFU. No stutter filter was applied to the data in GeneMapper™ ID-X, with all above-threshold potential stutter peaks being labelled and taken into GeneMapper PG™ Software for further analysis.

LR values were calculated for multiple scenarios with different NOC. LRs were calculated with NOC equal to the correct value (true-NOC), as well as with NOC equal to true-NOC minus 1 and to true-NOC plus 1 to understand the range of possible LRs given correct and incorrect NOC settings.

See Section 3.5.2 for more detail on the scenarios tested. Calculations in GeneMapper™ PG Software were performed with factory-default settings and the default model file for GlobalFiler.

Pre-normalized NIST population frequencies relevant to the ethnicity of the major sample donor were used. $5/2N$ where N is the number of individuals sampled from the population was used to calculate unobserved frequencies.

False exclusions of a true contributor from a mixture are counted as $LR < 1$ or as $\log_{10}(LR) < 0$.

3.2.3. Sensitivity Results

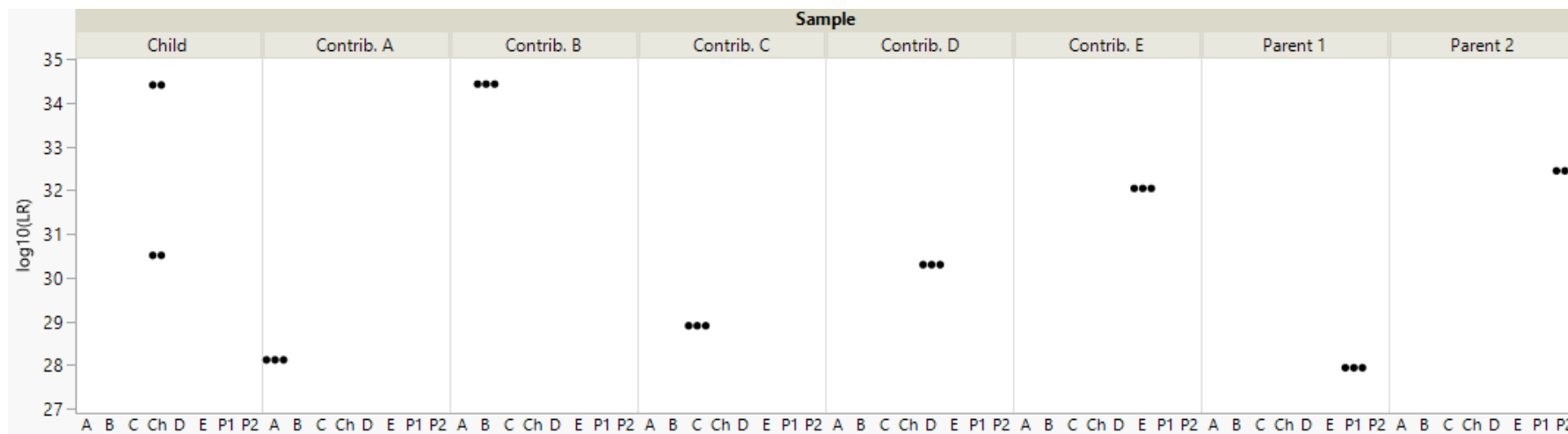


Figure 1: Sensitivity result for 1-person, 1 ng DNA input on 3500xL. NOC = true-NOC. Sample names are: Child (Ch), Contributors A, B, C, D and E, and Parents 1 and 2 (P2 and P2). All samples tested in triplicate, except (Ch) which was tested in duplicate. 'Ch' is of mixed heritage and LR's were calculated with two sets of allele frequencies, one corresponding to the ethnicity of each parent. This results in four results for 'Ch' on the chart. LR tested as H_p / H_d where H_p = the probability of seeing the observed profile given that the named person contributed and H_d = the probability of seeing the observed profile given that an unknown person contributed. All LR's are supportive of H_p over H_d .

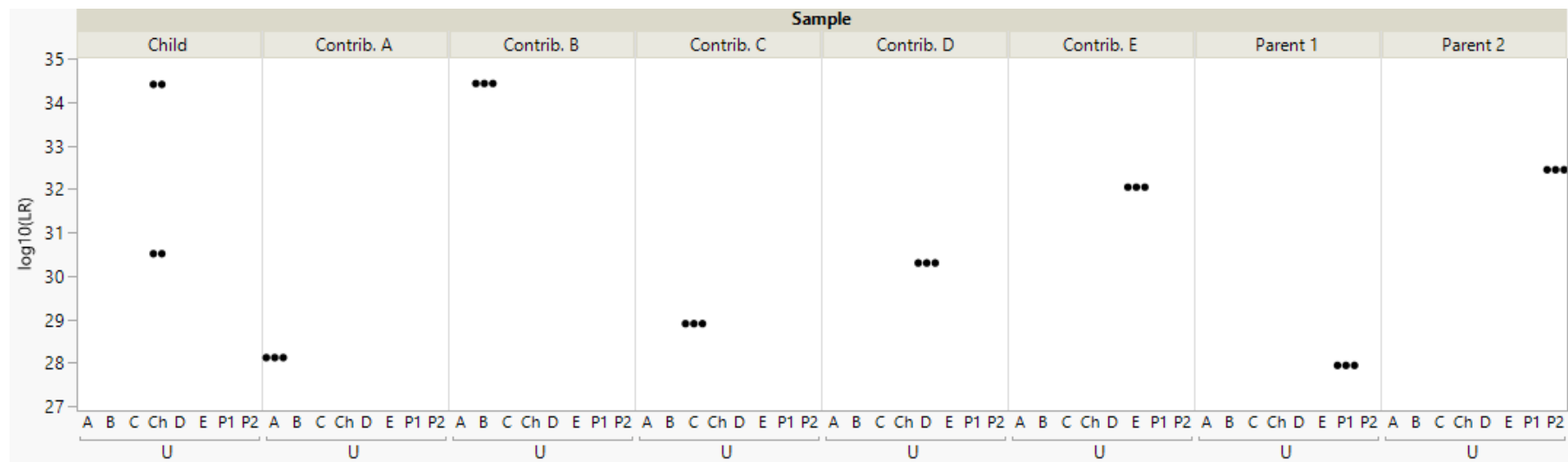


Figure 2: Sensitivity result for 1-person, 1 ng DNA input on SeqStudio Flex. NOC = true-NOC. 8 samples tested with same sample names and LR hypotheses as in Figure 1. All LRs are supportive of H_p over H_d . No significant difference between instruments observed.

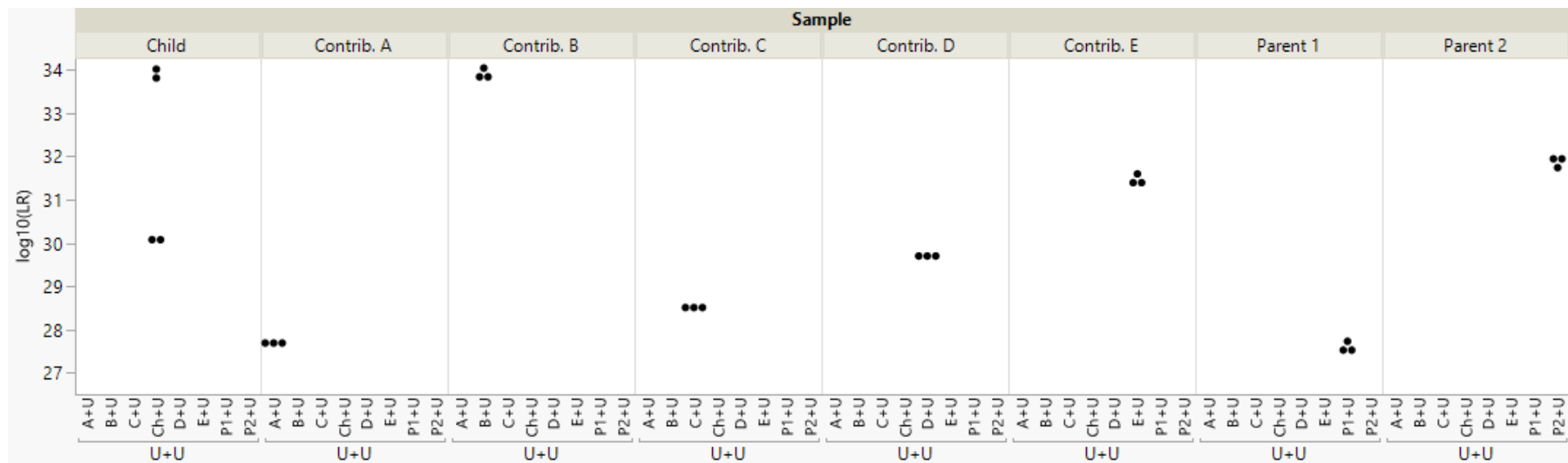


Figure 3: Sensitivity result for 1-person, 1 ng DNA input on 3500xL. NOC = true-NOC + 1 (i.e. NOC incorrectly set to 2). Individual samples have same sample names as *Figure 1*. LR tested as H_p / H_d where H_p = the probability of seeing the observed profile given that the named person + one unknown person ('U') contributed and H_d = the probability of seeing the observed profile given that an unknown person contributed. All LRs are supportive of H_p over H_d . Magnitude of LRs near equal to *Figure 1*.

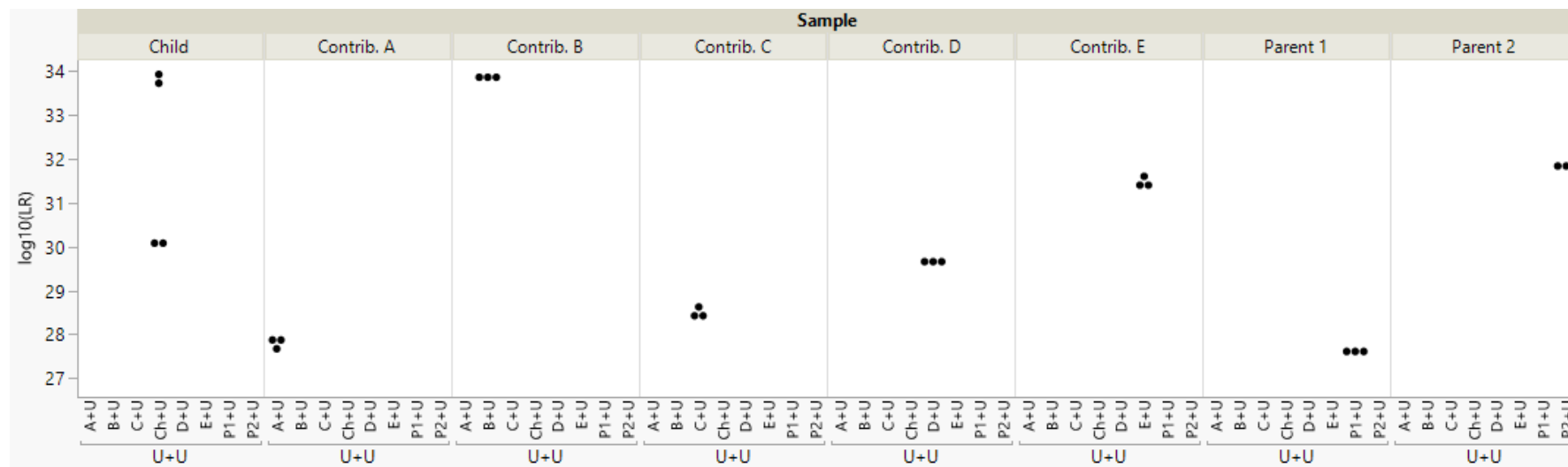


Figure 4: Sensitivity result for 1-person, 1 ng DNA input on SeqStudio Flex. NOC = true-NOC + 1 (i.e. NOC incorrectly set to 2). 8 samples tested with same sample names as in Figure 1. LR tested with hypotheses as in Figure 3. Magnitude of LRs near equal to Figure 2. No significant difference between CE instruments observed.

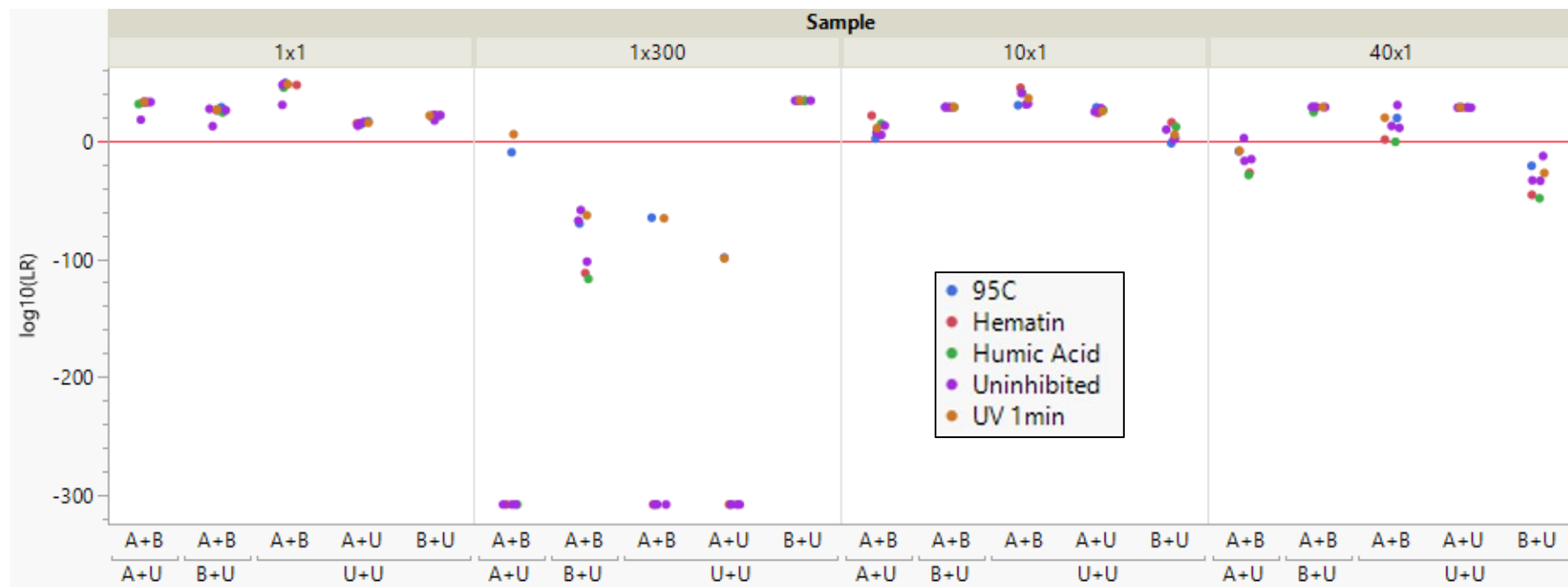


Figure 5 Sensitivity result for 2-person samples on 3500xL. NOC = true-NOC. Two contributors (A and B) mixed in varying ratios (1 to 1; 1 to 300; 10 to 1 and 40 to 1) and exposed to varying artificial degradation/inhibition conditions (heated to 95°C for 15 minutes; UV treated for 1 minute; inhibited with humic acid, inhibited with hematin, and uninhibited/undegraded). (See color key on chart). Total of 1 ng of DNA amplified. LR_s calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where U = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. All log₁₀(LR)_s calculated using the 1 to 1 mixture profiles and most log₁₀(LR)_s calculated using the 10 to 1 and 40 to 1 mixture profiles are positive. Result shows low sensitivity of detection of the very minor mixture contributor in the 1 to 300 sample. The estimated mean peak height for the minor contributor in the 1 to 300 mixture profiles were < 15 RFU in all cases, below the 20 RFU analytical threshold used in this analysis.

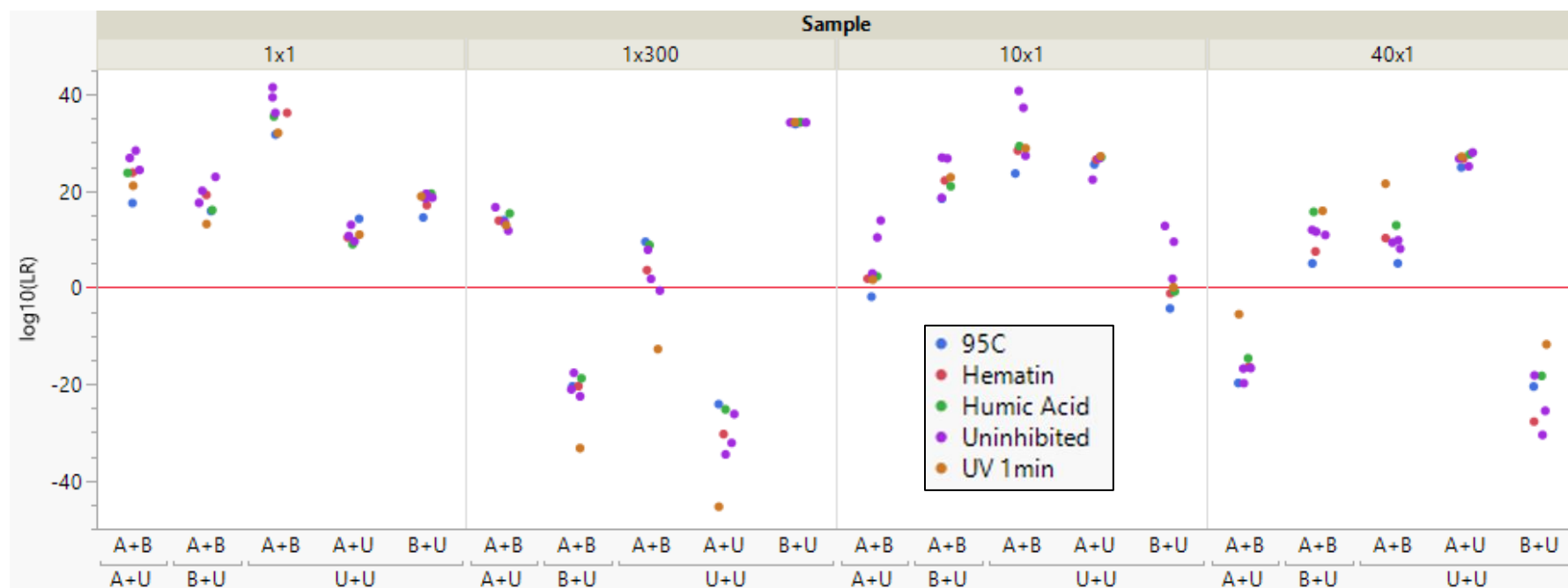


Figure 6: Sensitivity result for 2-person samples on 3500xL. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 5. Total of 0.125 ng of DNA input per sample. More LR's calculated given the 1 to 300 profiles are supportive of H_p over H_d (positive $\log_{10}(\text{LR})$) than in Figure 5. Positive $\log_{10}(\text{LR})$'s calculated for all 1 to 1 and most 10 to 1 mixture samples.

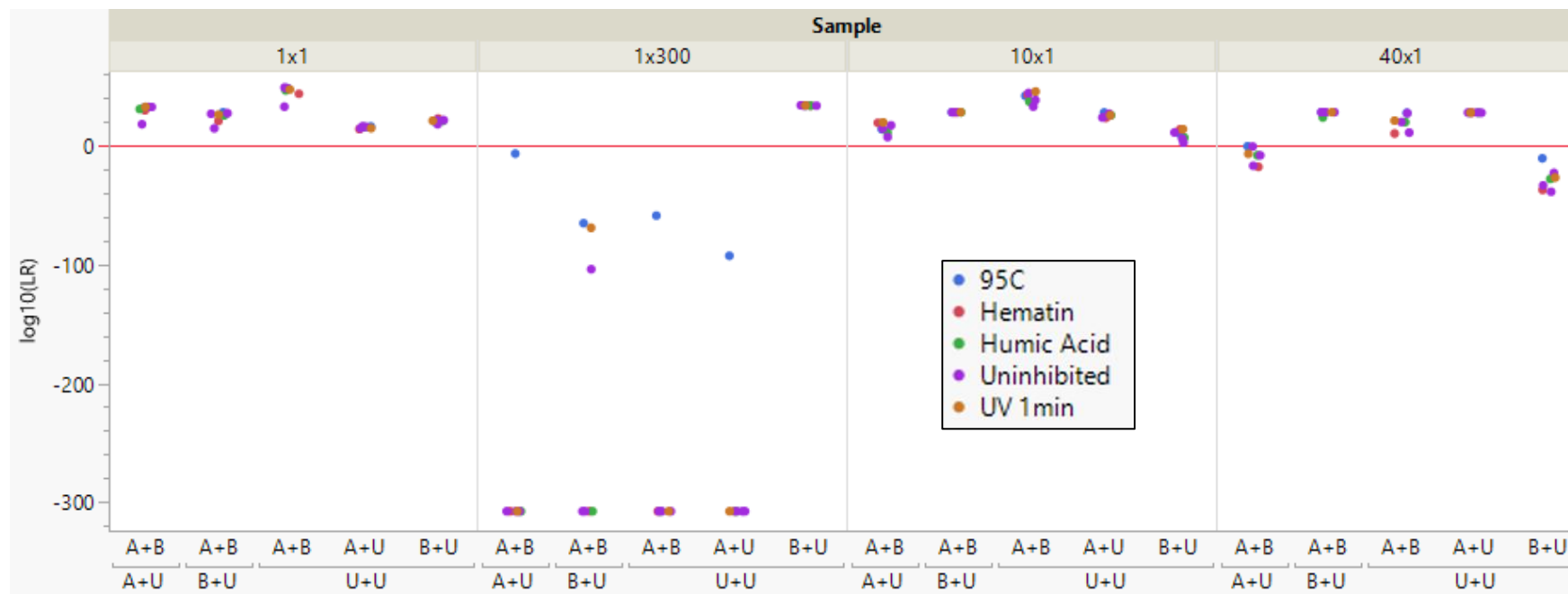


Figure 7: Sensitivity result for 2-person samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition, DNA input and LR hypotheses as in Figure 5. Total of 1 ng of DNA input per sample. Results show no significant difference between CE instruments.

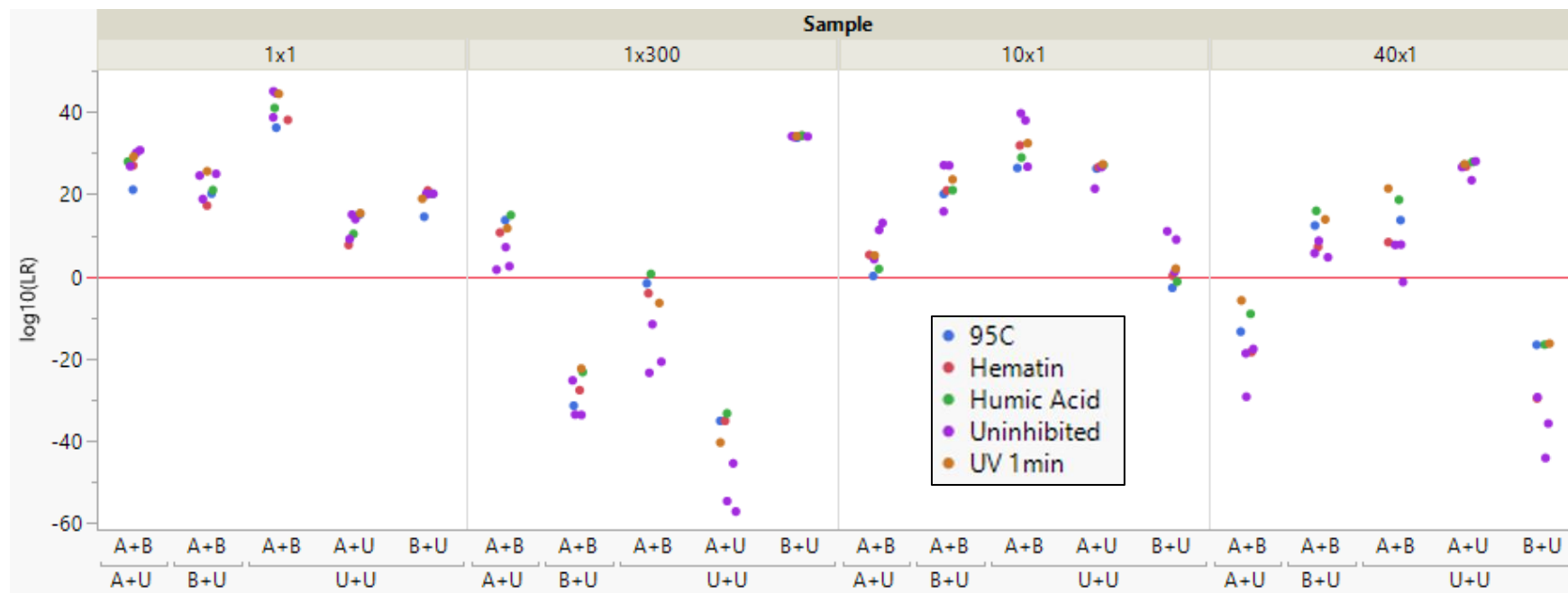


Figure 8: Sensitivity result for 2-person samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition, DNA input and LR hypotheses as in Figure 6. Total of 0.125 ng of DNA input per sample. Results show no significant difference between CE instruments.

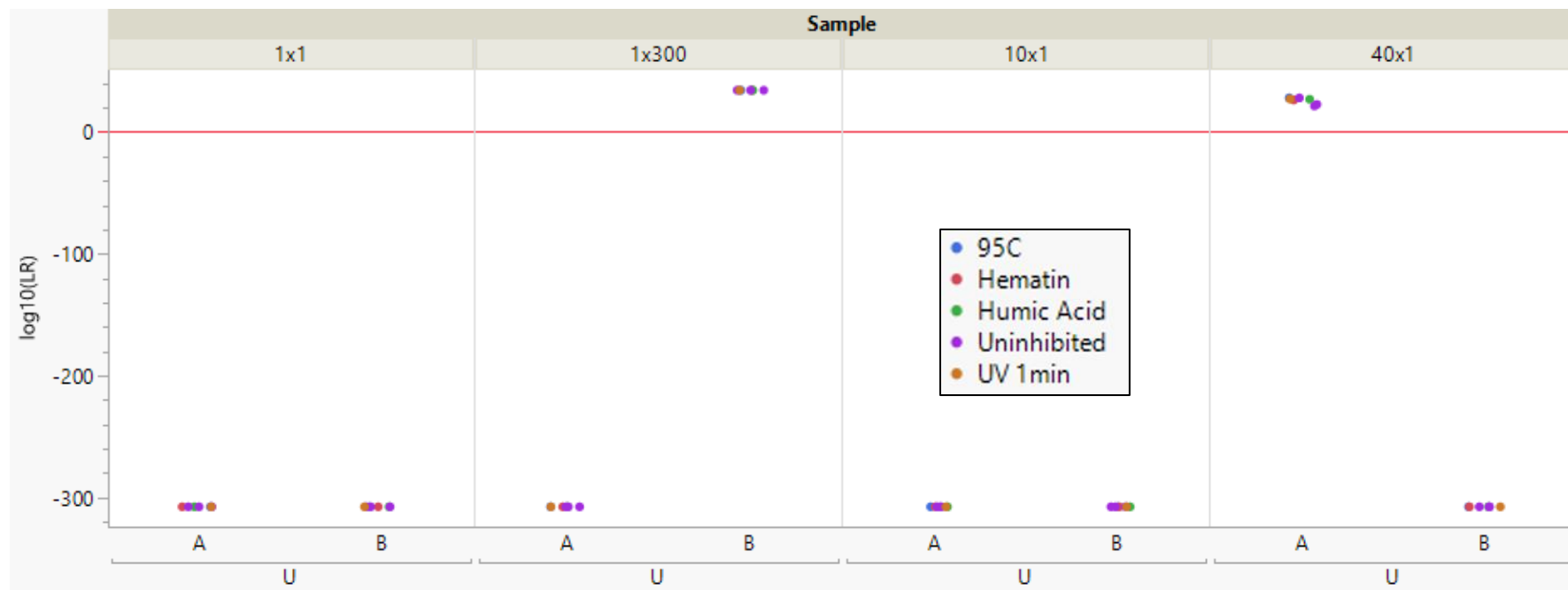


Figure 9: Sensitivity result for 2-person samples on 3500xL. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 1). Sample set up, naming, degradation/inhibition as in Figure 5. Total of 1 ng of DNA input per sample. LR tested as H_p / H_d where H_p = the probability of seeing the observed profile given that the named person contributed and H_d = the probability of seeing the observed profile given that an unknown person contributed. Positive $\log_{10}(\text{LR})$ seen where H_p was conditioned on observing the major contributor in 1 to 300 and 40 to 1 mixtures, likely caused by the partial or complete dropout of the minor contributor. Otherwise, $\log_{10}(\text{LR})$ s were negative, indicating 1-person was not a likely solution.

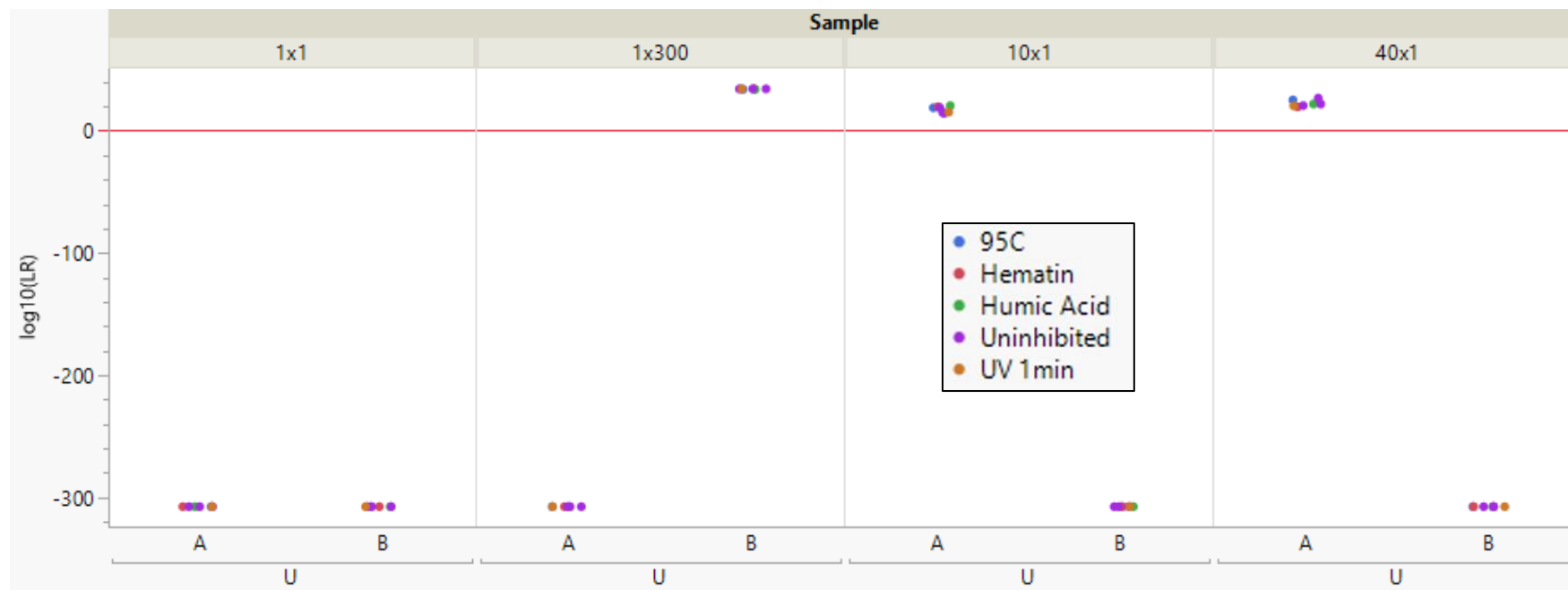


Figure 10: Sensitivity result for 2-person samples on 3500xL. $NOC = \text{true-NOC} - 1$. (i.e. NOC incorrectly set to 1). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 9. Total of 0.125 ng of DNA input per sample. Results similar to 1 ng result (Figure 9) except where H_p was the probability of observing the profile given that 'A' contributed in the 10 to 1 mixture. This gave a positive $\log_{10}(LR)$, likely due to dropout of the minor contributor. As such, all $\log_{10}(LR)$ s calculated assessing the likelihood of observing the profiles derived from all mixture ratios, other than 1 to 1, gave a positive result suggesting it is more likely to observe these profiles given the major contributor is contributing rather than an unrelated unknown individual. Otherwise, results were negative, indicating 1-person was not a likely solution.

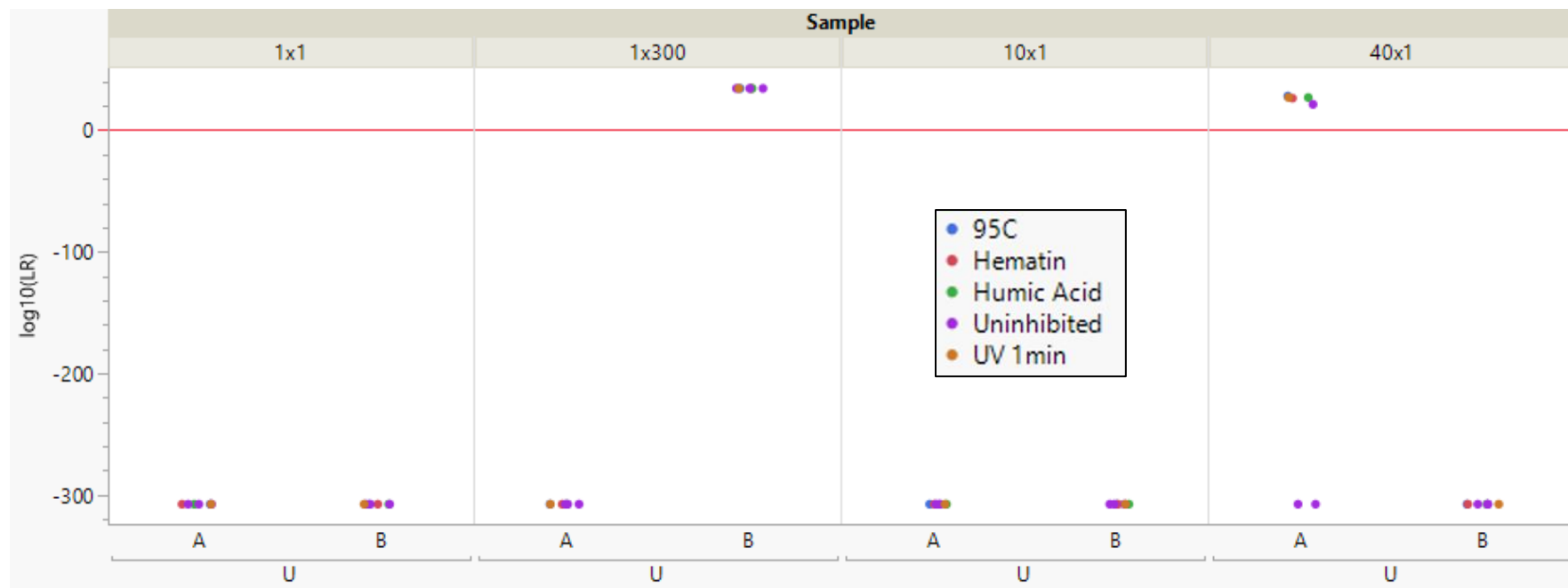


Figure 11: Sensitivity result for 2-person samples on SeqStudio Flex. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 1). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 9. Total of 1 ng of DNA input per sample. Similar results seen as Figure 9, indicating no significant difference between CE instruments.

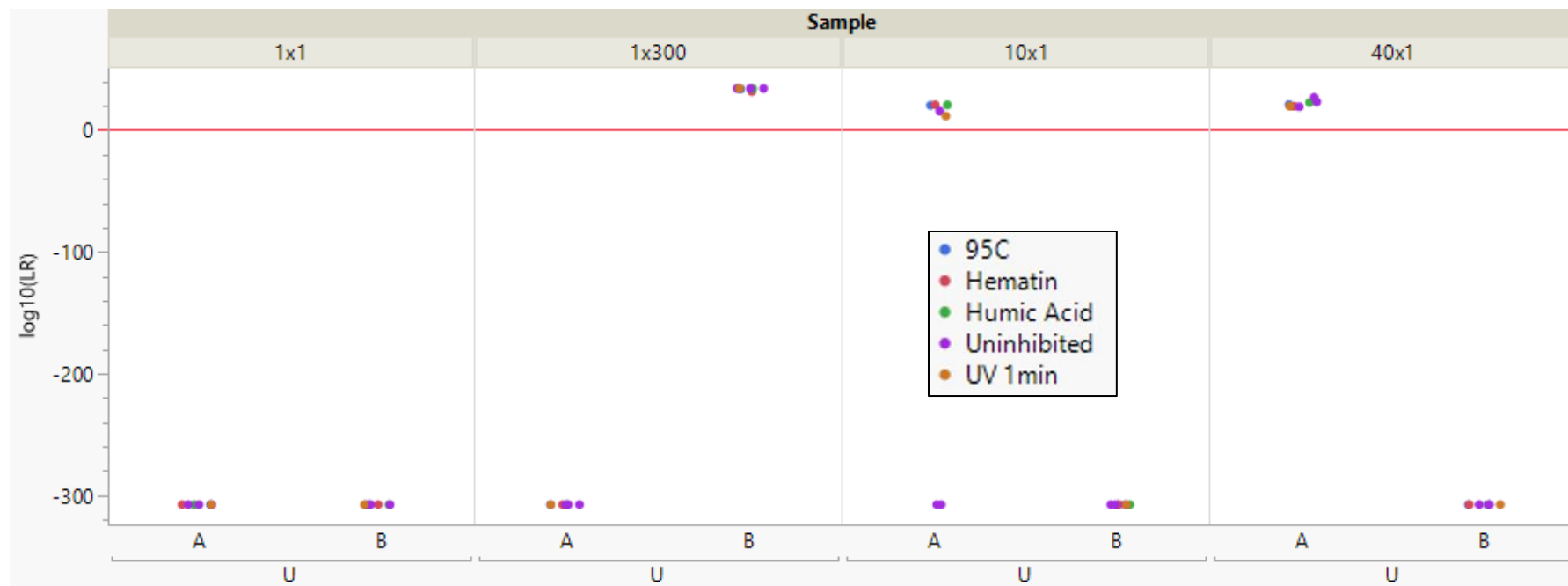


Figure 12: Sensitivity result for 2-person samples on SeqStudio Flex. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 1). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 10. Total of 0.125 ng of DNA input per sample. Similar results seen as Figure 10, indicating no significant difference between CE instruments.

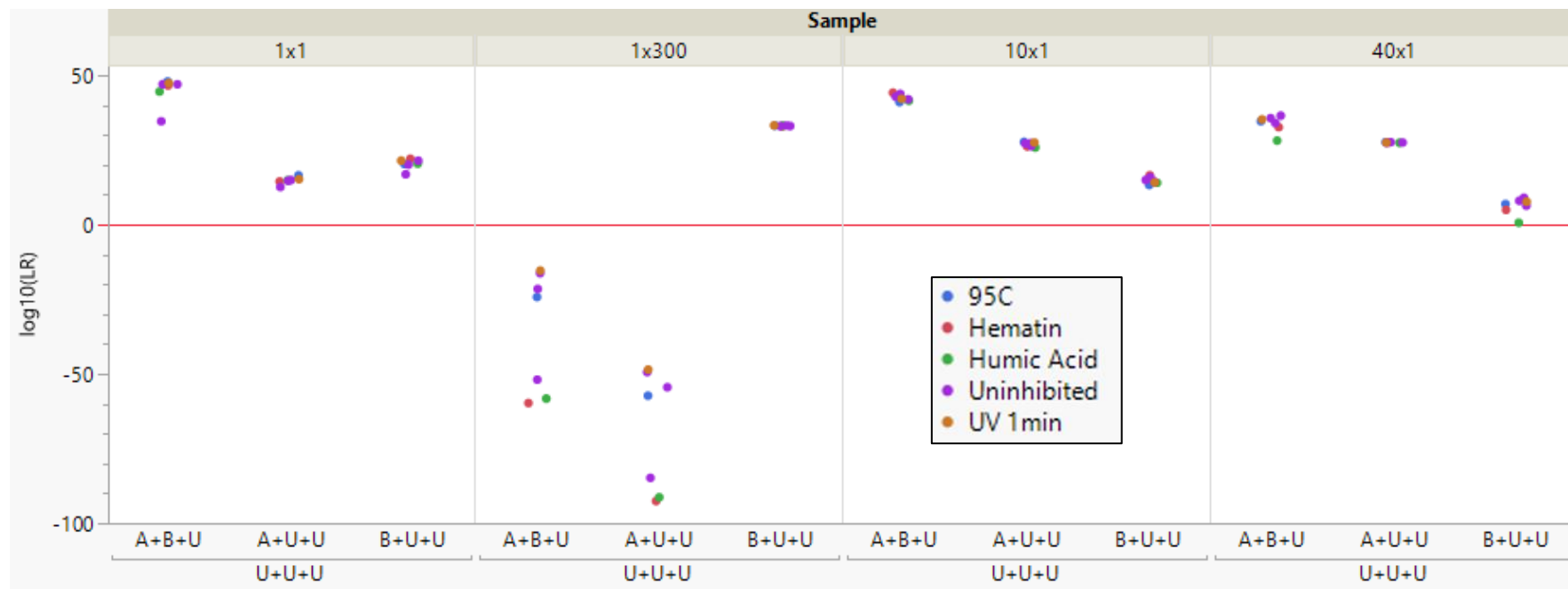


Figure 13: Sensitivity result for 2-person samples on 3500xL. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition as in Figure 5. Total of 1 ng of DNA input per sample. LRs calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where U = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. When H_p involved the major contributor, LR was of near equal magnitude to the NOC = 2 (true) scenario (Figure 5).

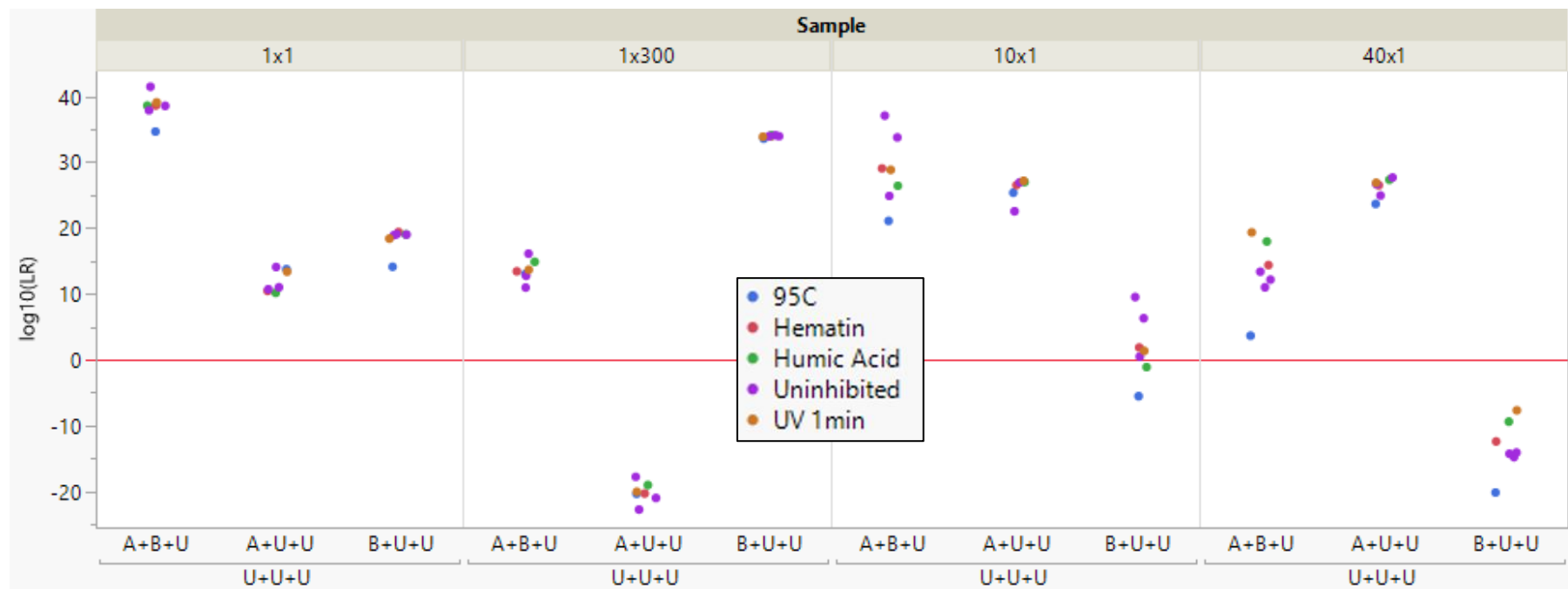


Figure 14: Sensitivity result for 2-person samples on 3500xL. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 13. Total of 0.125 ng of DNA input per sample. When H_p involved the major contributor, LR was of near equal magnitude to the NOC = 2 (true) scenario (Figure 6).

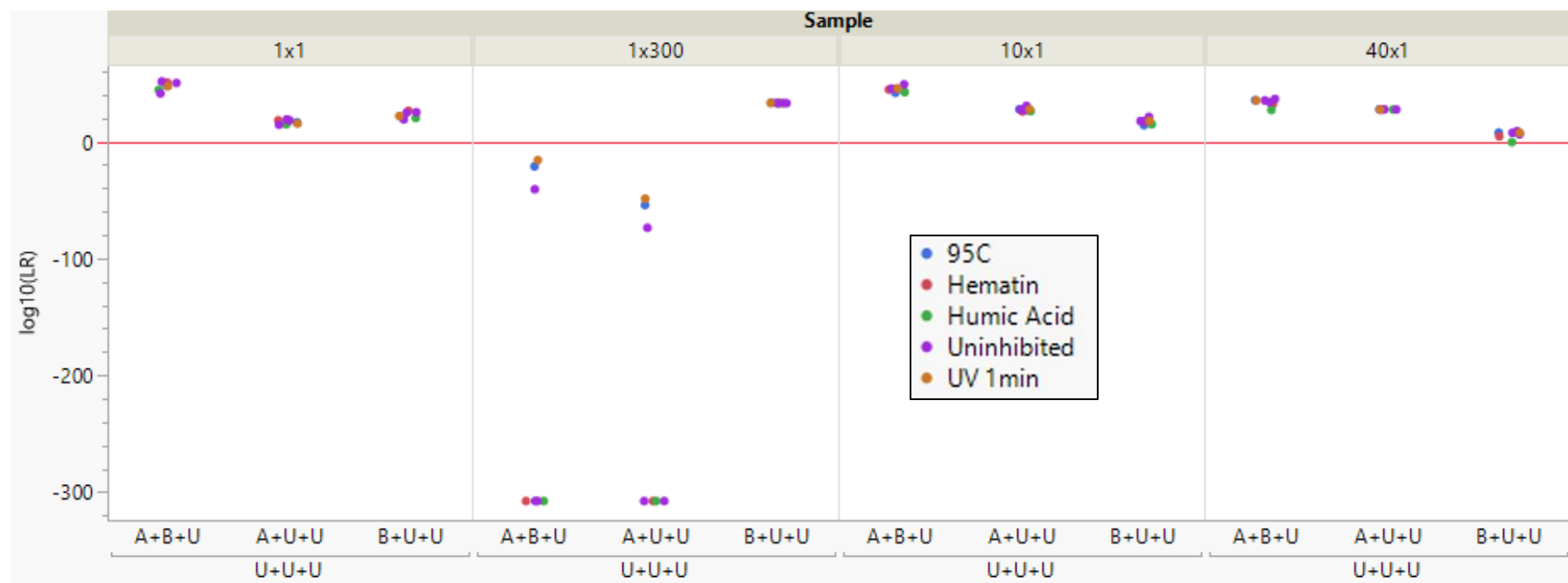


Figure 15: Sensitivity result for 2-person samples on SeqStudio Flex. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 13. Total of 1 ng of DNA input in sample. Similar results seen as Figure 13, indicating no significant difference between CE instruments.

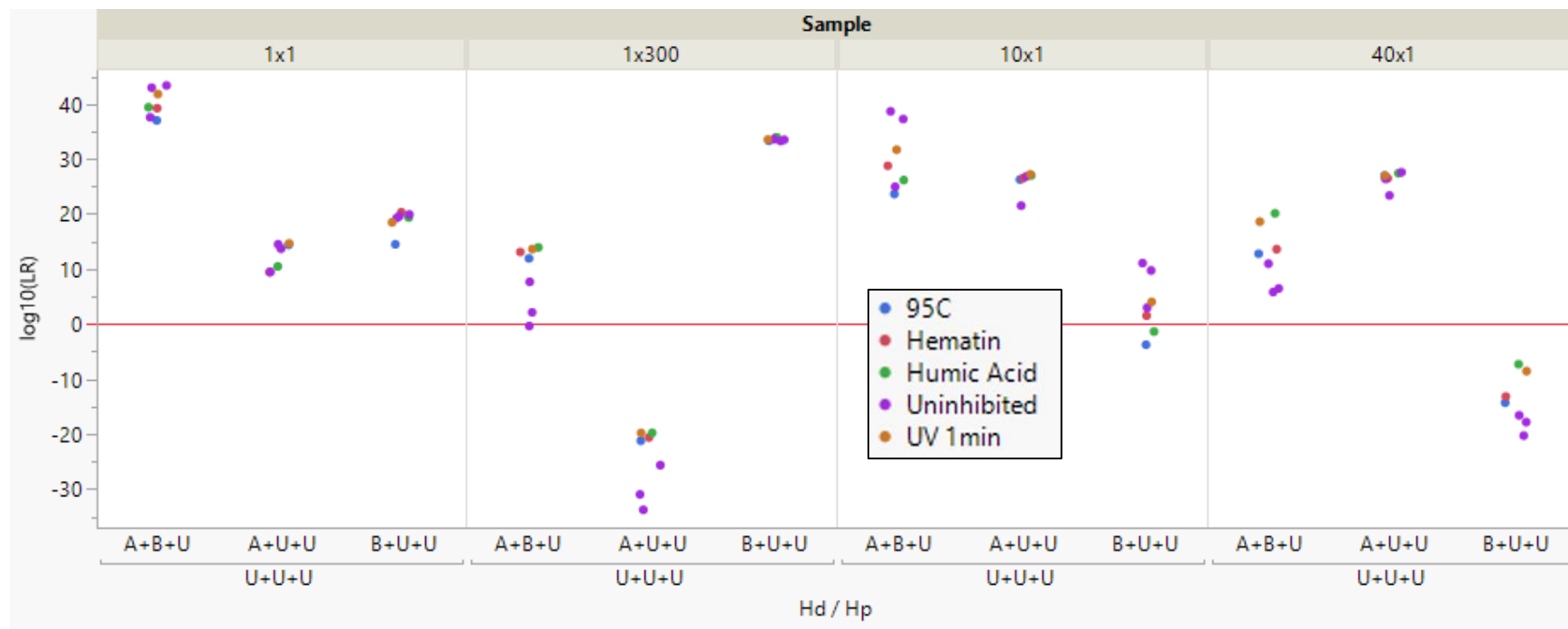


Figure 16: Sensitivity result for 2-person samples on SeqStudio Flex. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 14. Total of 0.125 ng of DNA input per sample. Similar results seen as Figure 14, indicating no significant difference between CE instruments.

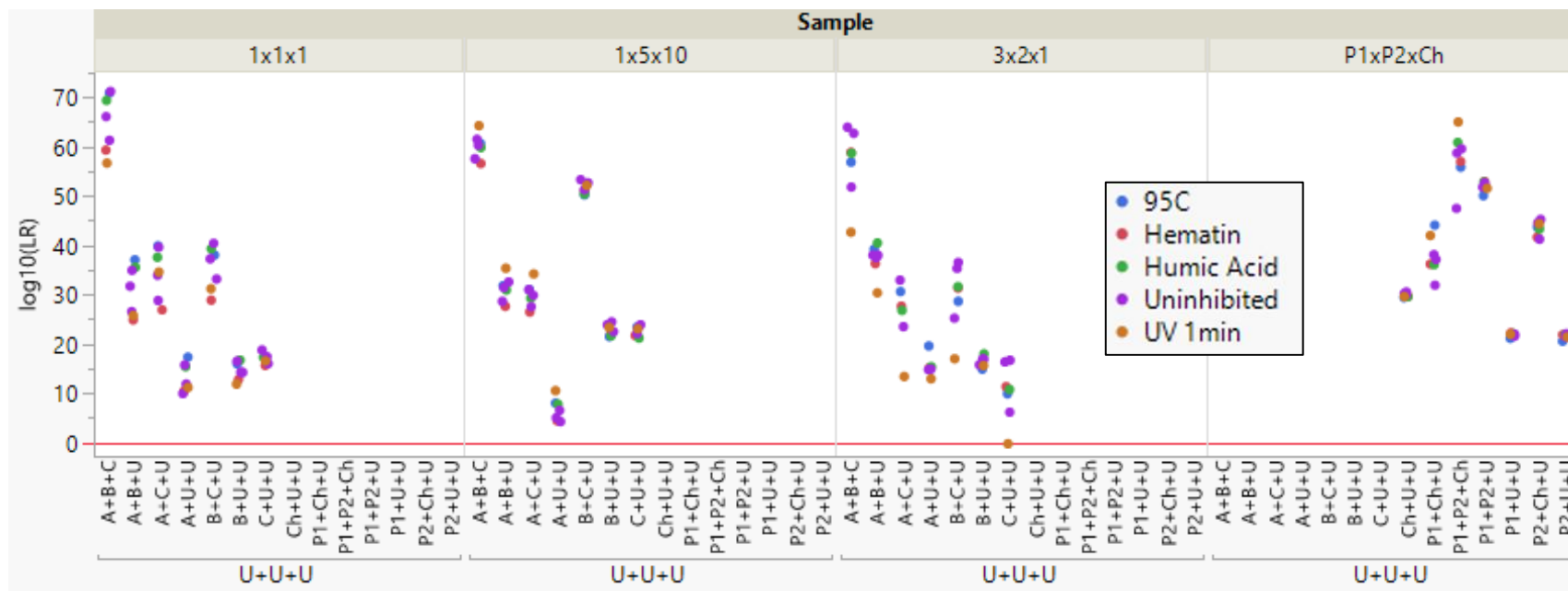


Figure 17: Sensitivity result for 3-person 1 ng DNA input samples on 3500xL. NOC = true-NOC. Individual contributors have the same names as Figure 1. Contributors mixed in varying ratios (1 to 1 to 1; 1 to 5 to 10; 3 to 2 to 1). 'P1 x P2 x Ch' tests a 1 to 1 to 1 combination of related samples where 'P1' and 'P2' are parents of 'Ch'. LR tested as H_p / H_d where: H_p = the probability of seeing the mixture profile given that the named persons in the top row of the x-axis label, where 'U' = unknown contributed. H_d = the probability of seeing the mixture profile given that three unknown persons contributing the profile. The mixtures were treated with varying degradation/inhibition conditions as in Figure 5. Positive $\log_{10}(LR)$ supportive of H_p over H_d observed in all cases, except for LR where H_p involved the minor component of the UV degraded 3 to 2 to 1 mixture sample, which was inconclusive.

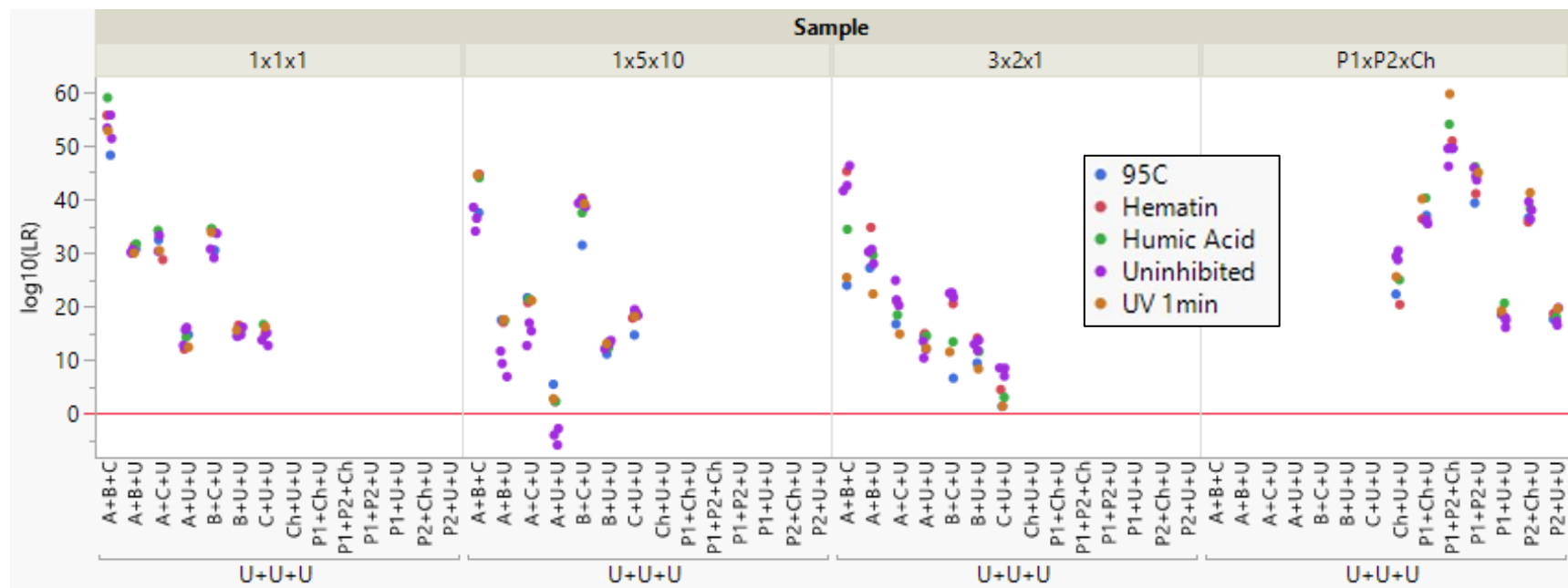


Figure 18: Sensitivity result for 3-person samples on 3500xL. NOC = true-NOC. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 17. Total of 0.125 ng of DNA input per sample. Positive $\log_{10}(\text{LR})$ s suggesting support of H_p over H_d in all cases, except for LR with H_p conditioned on the minor component of uninhibited 1 to 5 to 10 mixture sample, which supported H_d over H_p .

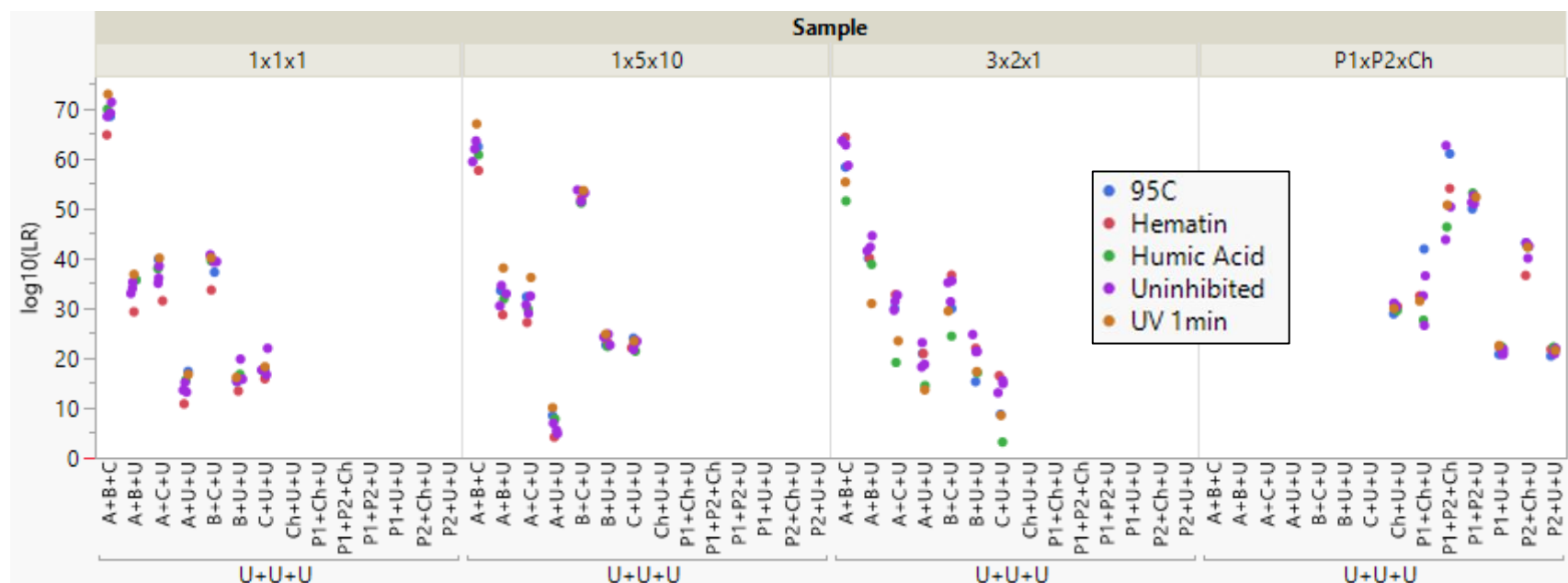


Figure 19: Sensitivity result for 3-person samples on SeqStudio Flex. NOC = true-NOC. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 17. Total of 1 ng of DNA input per sample. Similar results seen as Figure 17, indicating no significant difference between CE instruments.

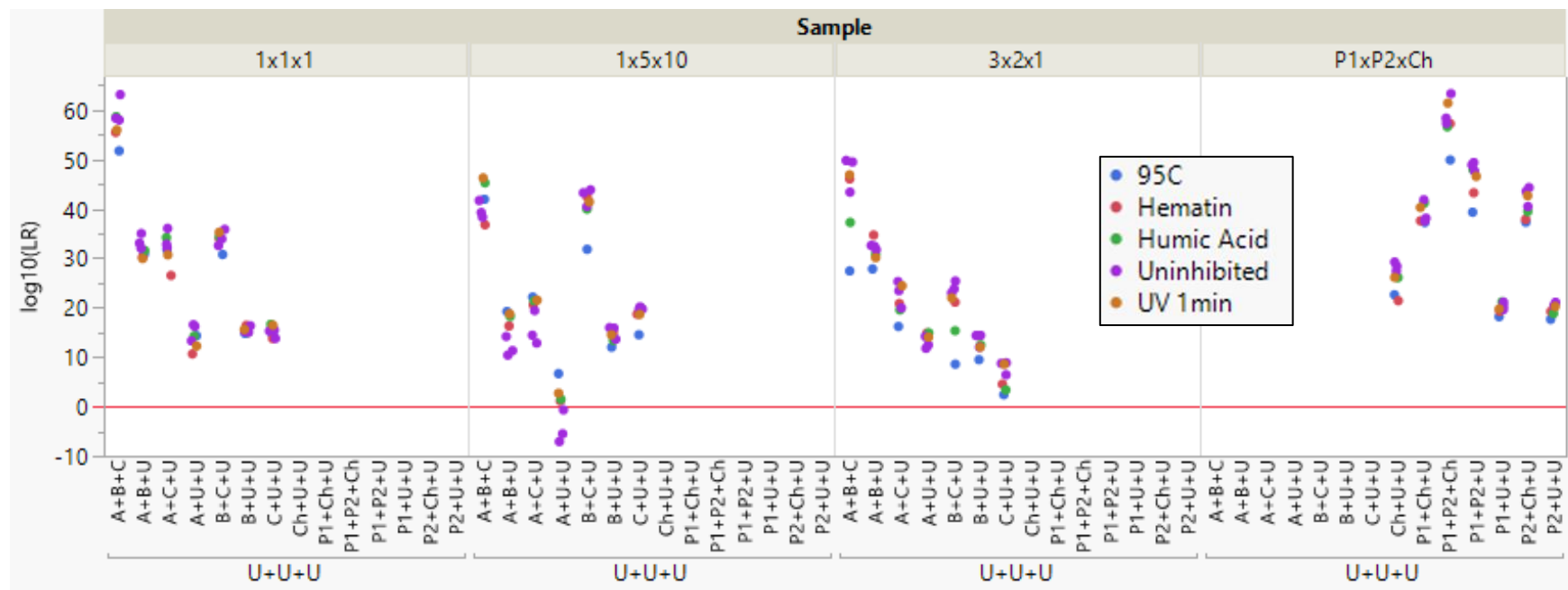


Figure 20: Sensitivity result for 3-person samples on SeqStudio Flex. NOC = true-NOC. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 17. Total of 0.125 ng of DNA input per sample. Similar results seen as Figure 18, indicating no significant difference between CE instruments.

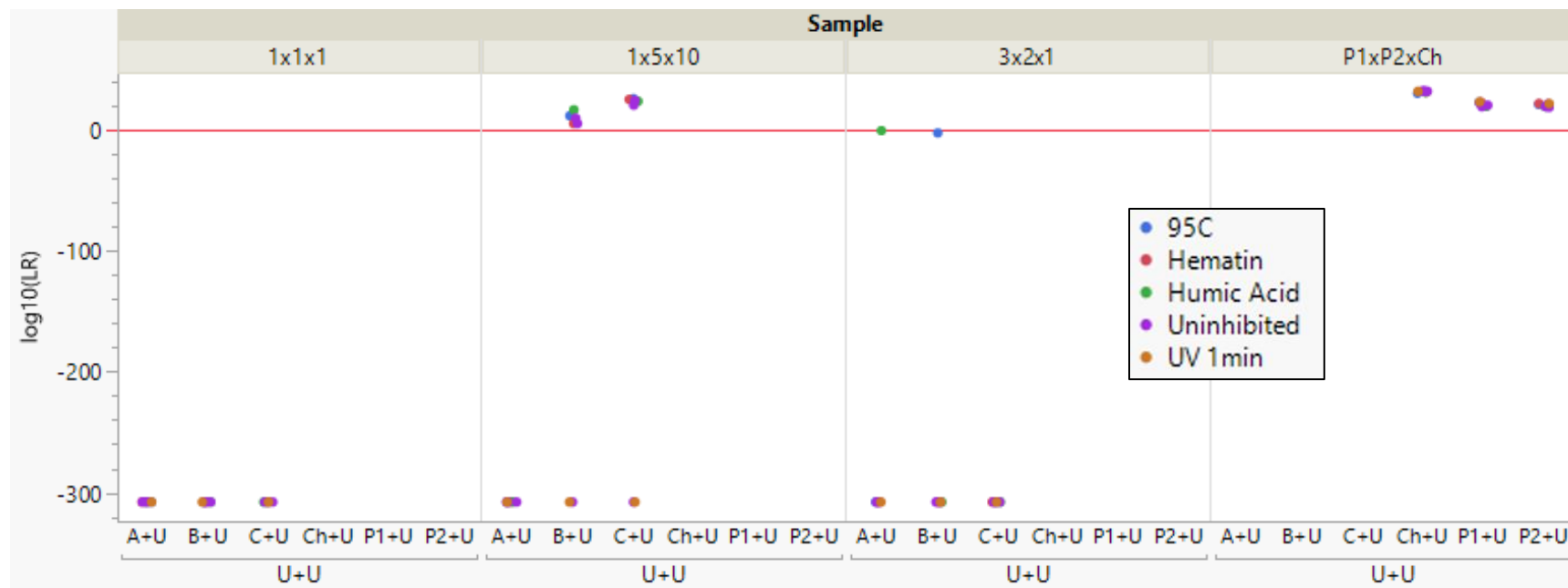


Figure 21: Sensitivity result for 3-person samples on 3500xL. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 2). Sample set up, naming, degradation/inhibition as in Figure 17. LR tested as H_p / H_d where H_p = the probability of seeing the observed profile given that the named person + one unknown person ('U') contributed and H_d = the probability of seeing the observed profile given that an two unknown persons contributed. Total of 1 ng of DNA input per sample. Most LRs supportive of H_d over H_p . Several LRs calculated using the 1 to 5 to 10 mixture profiles support H_p over H_d due to high peak imbalance. LRs supportive of H_p over H_d observed for parent/child scenarios (P1 x P2 x Ch) due to sharing of alleles between parent and child samples.

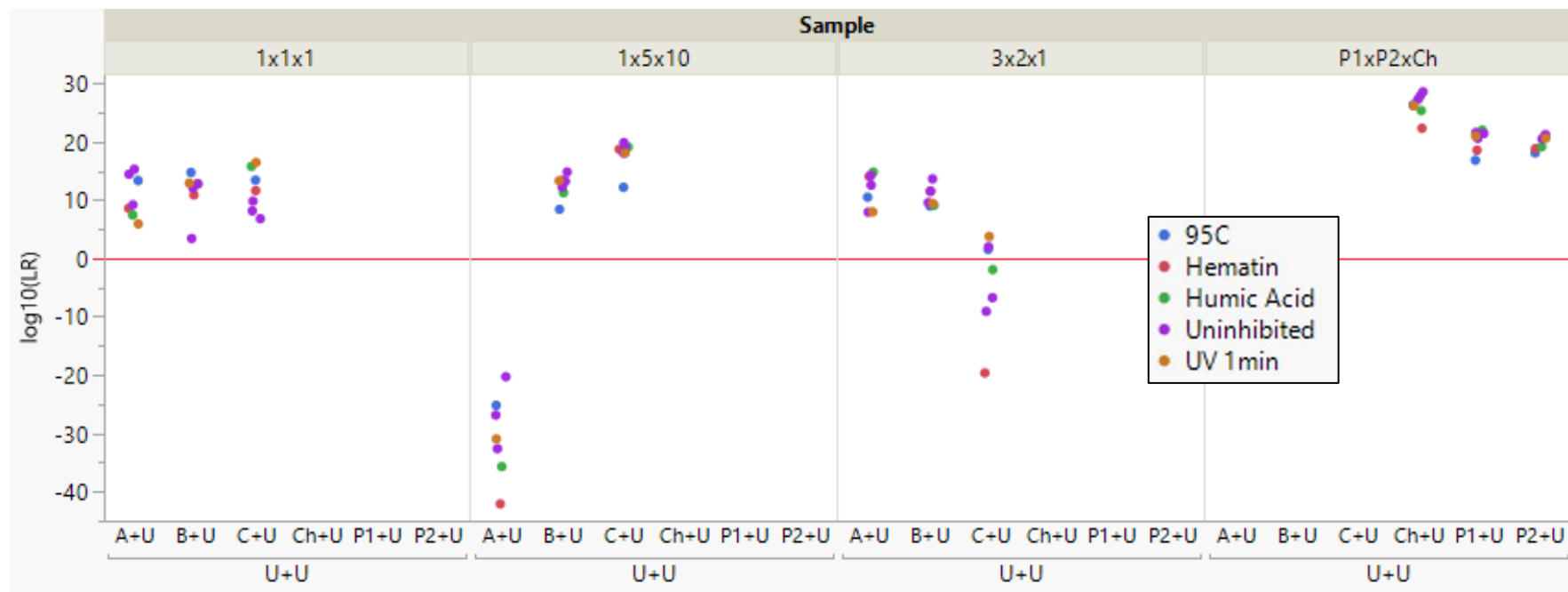


Figure 22: Sensitivity result for 3-person samples on 3500xL. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 2). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 21. Total of 0.125 ng of DNA input per sample. Most LR supportive of H_p over H_d . Several LRs which query the minor contributor of 1 to 5 to 10 and 3 to 2 to 1 mixtures are supportive of H_d over H_p due to high peak imbalance.

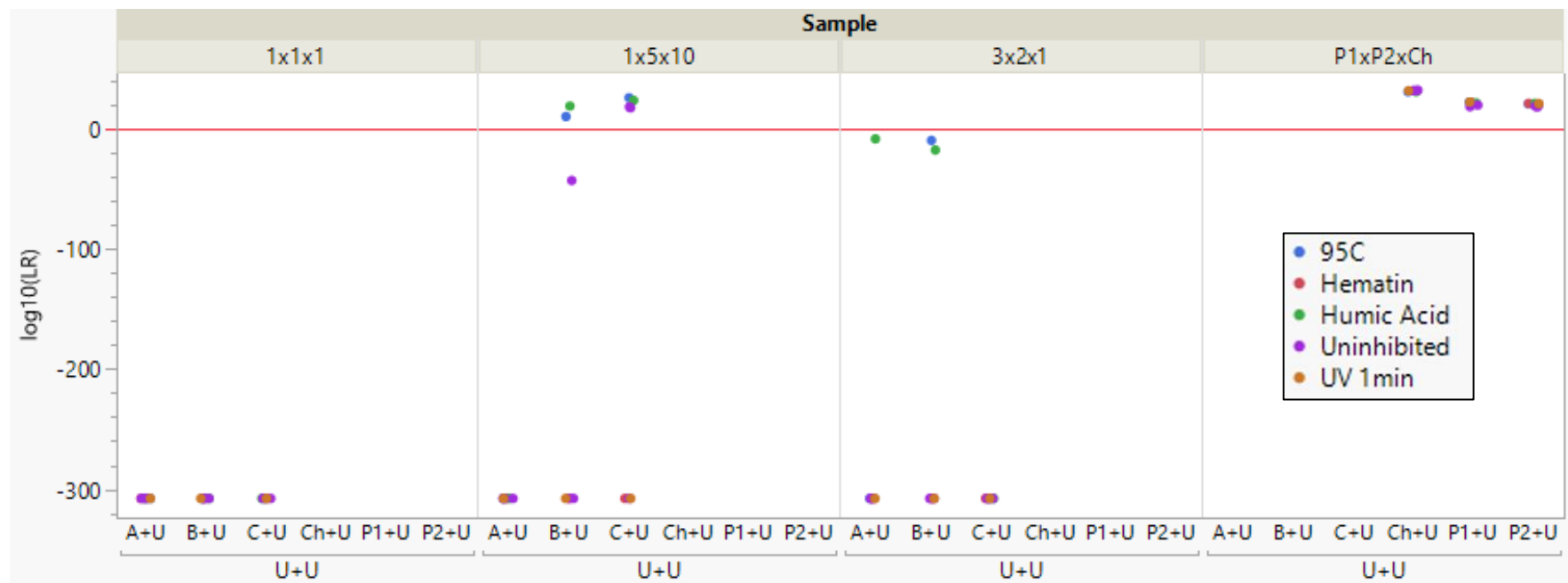


Figure 23: Sensitivity result for 3-person samples on SeqStudio Flex. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 2). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 21. Total of 1 ng of DNA input per sample. Results similar to Figure 21 indicating no significant difference between CE instruments.

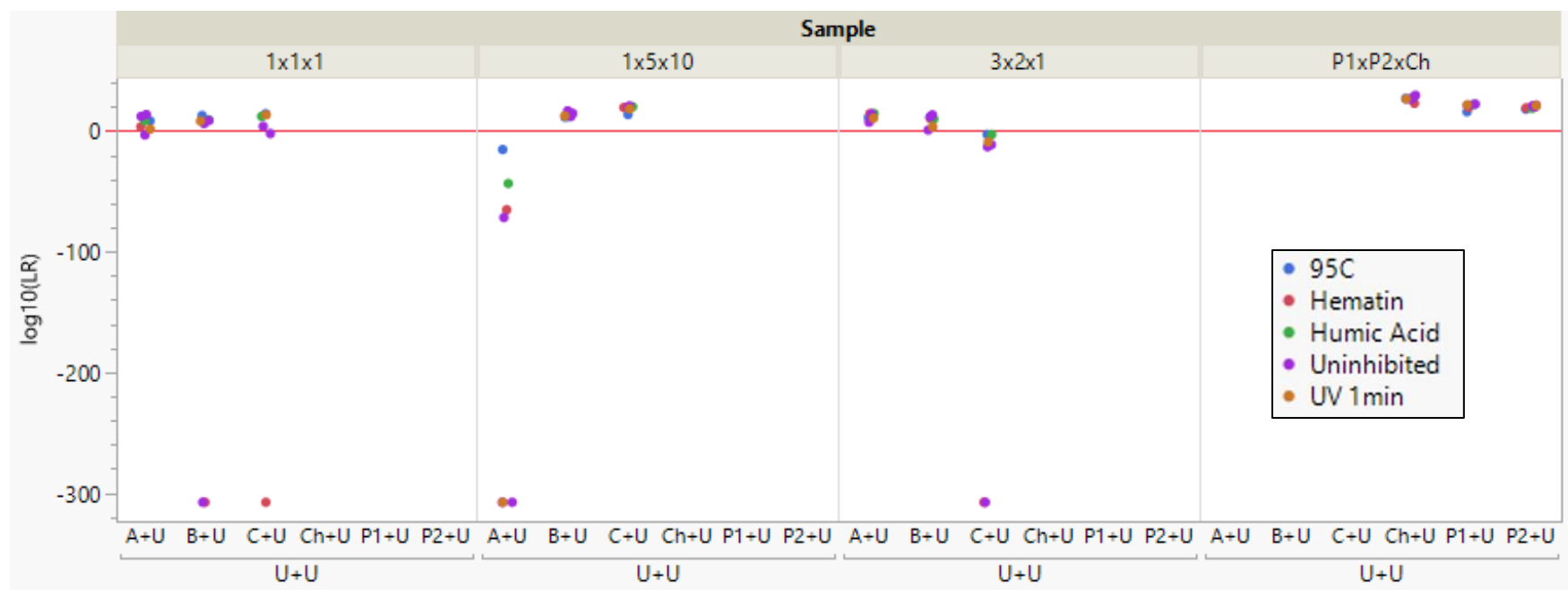


Figure 24: Sensitivity result for 3-person samples on SeqStudio Flex. $\text{NOC} = \text{true-NOC} - 1$. (i.e. NOC incorrectly set to 2). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 22. Total of 0.125 ng of DNA input per sample. LRs that query the minor contributors of 1 to 5 to 10 and 3 to 2 to 1 mixtures support H_d over H_p due to peak height imbalance. Some 1 to 1 to 1 scenarios support H_d over H_p due to the presence of too many peaks to support a 2-person solution.

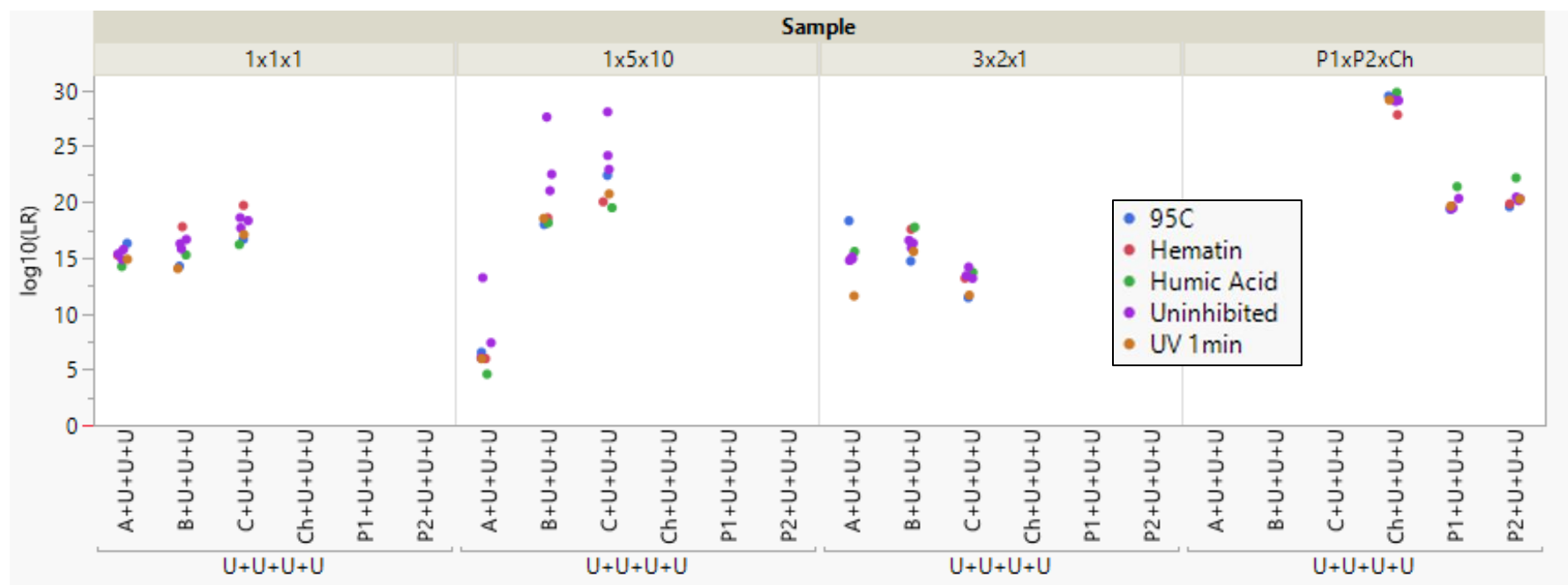


Figure 25: Sensitivity result for 3-person samples on 3500xL. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 4). Sample set up, naming, degradation/inhibition as in Figure 17. Total of 1 ng of DNA input per sample. LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that four unknown persons contributed. 'U' = an unknown contributor. Results showed LR supportive of H_p over H_d with similar magnitude to when NOC = 3 (true) (see Figure 17).

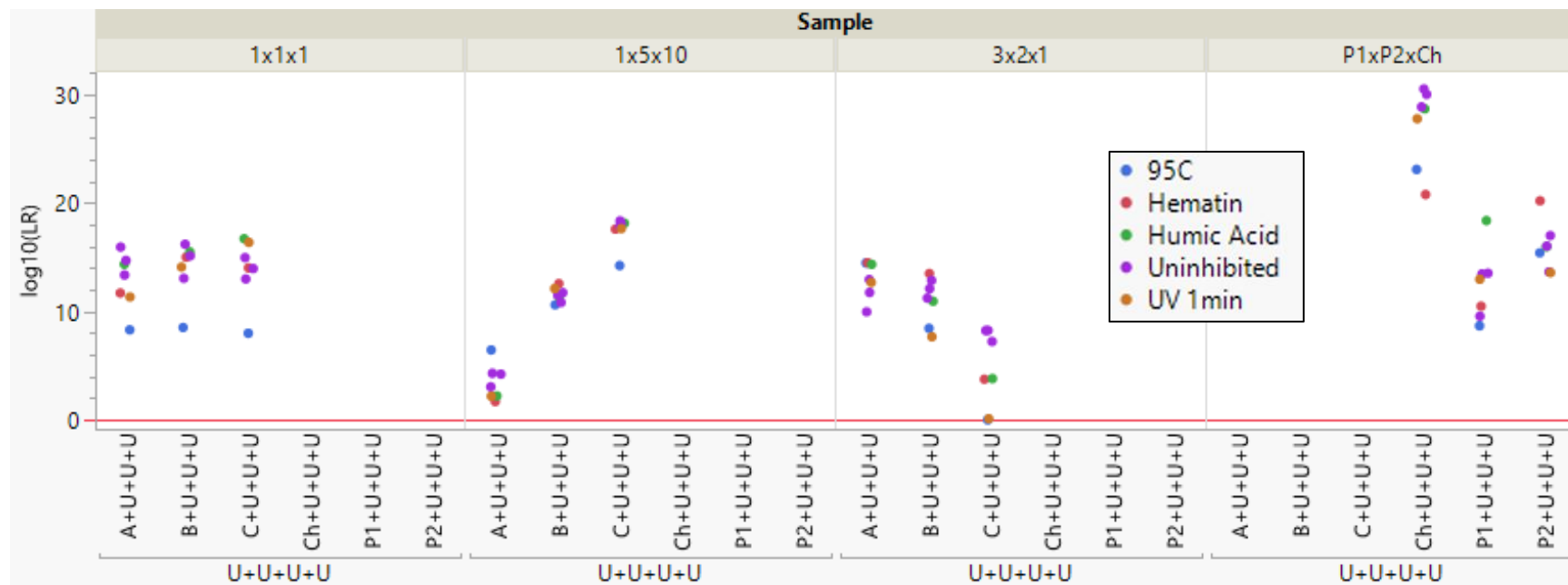


Figure 26: Sensitivity result for 3-person samples on 3500xL. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 4). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 25. Total of 0.125 ng of DNA input per sample. Results showed LR supportive of H_p over H_d for the majority of scenarios compared to when NOC = 3 (true) (see Figure 18).

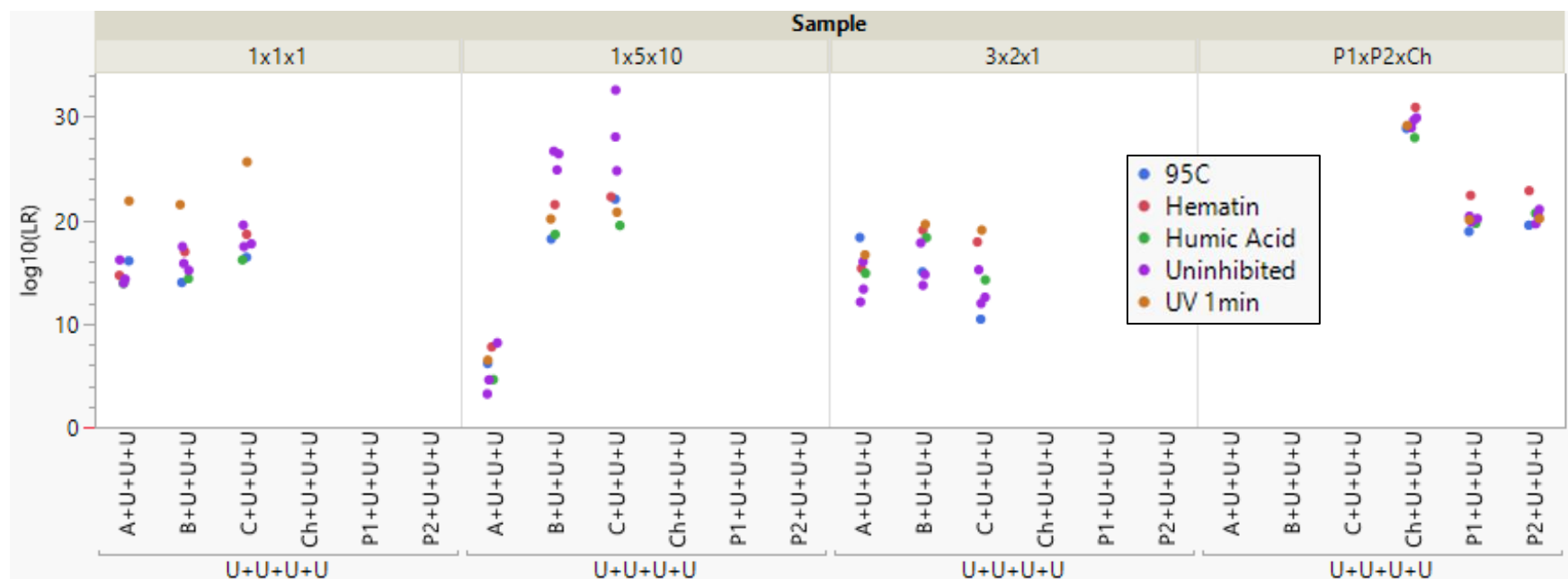


Figure 27: Sensitivity result for 3-person samples on SeqStudio Flex. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 4). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 25. Total of 1 ng of DNA input per sample. Results similar to Figure 25 indicating no significant difference between CE instruments.

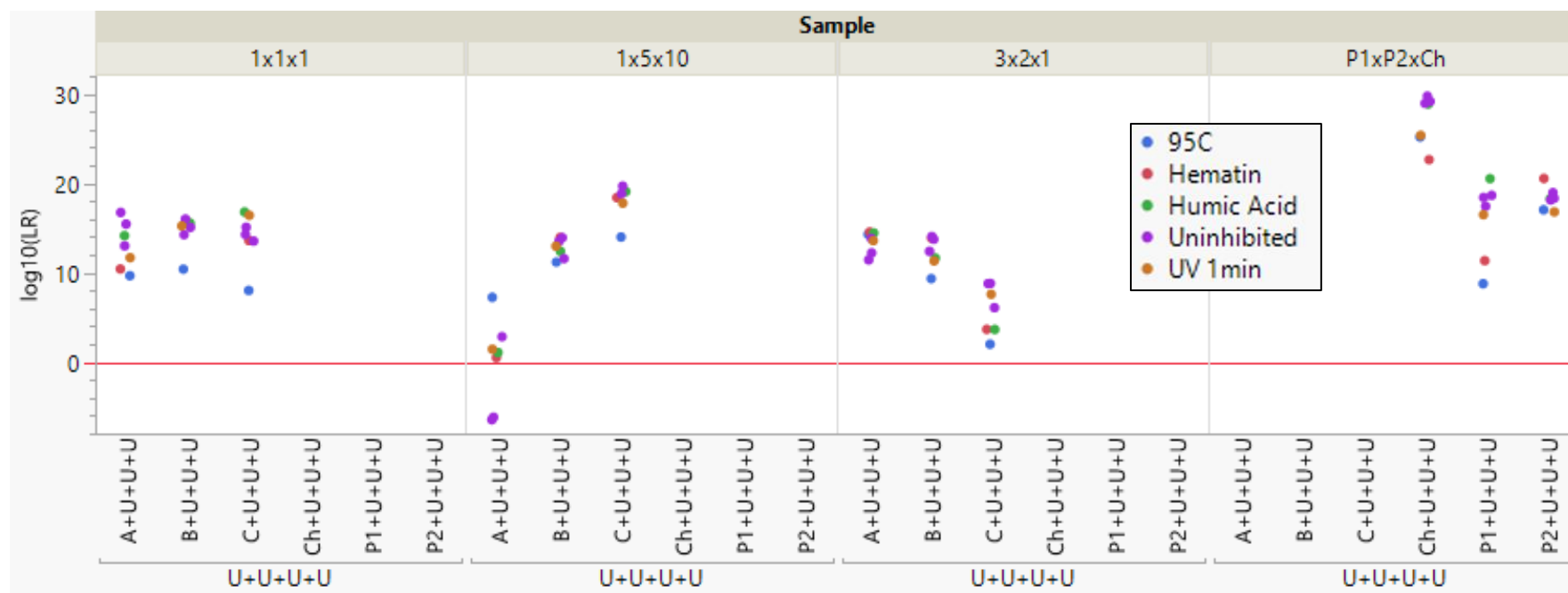


Figure 28: Sensitivity result for 3-person samples on SeqStudio Flex. NOC = true-NOC plus 1. (i.e. NOC incorrectly set to 4). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 26. Total of 0.125 ng of DNA input per sample. LRs evaluating the likelihood of observing the profiles of two out of three amplifications of the uninhibited 1 to 5 to 10 sample given the minor contributor and three unknown individuals make up the mixture versus the likelihood of observing the profiles given four unknown individuals make up the mixture are supportive of H_d over H_p . Results are similar to Figure 26 indicating no significant difference between CE instruments.

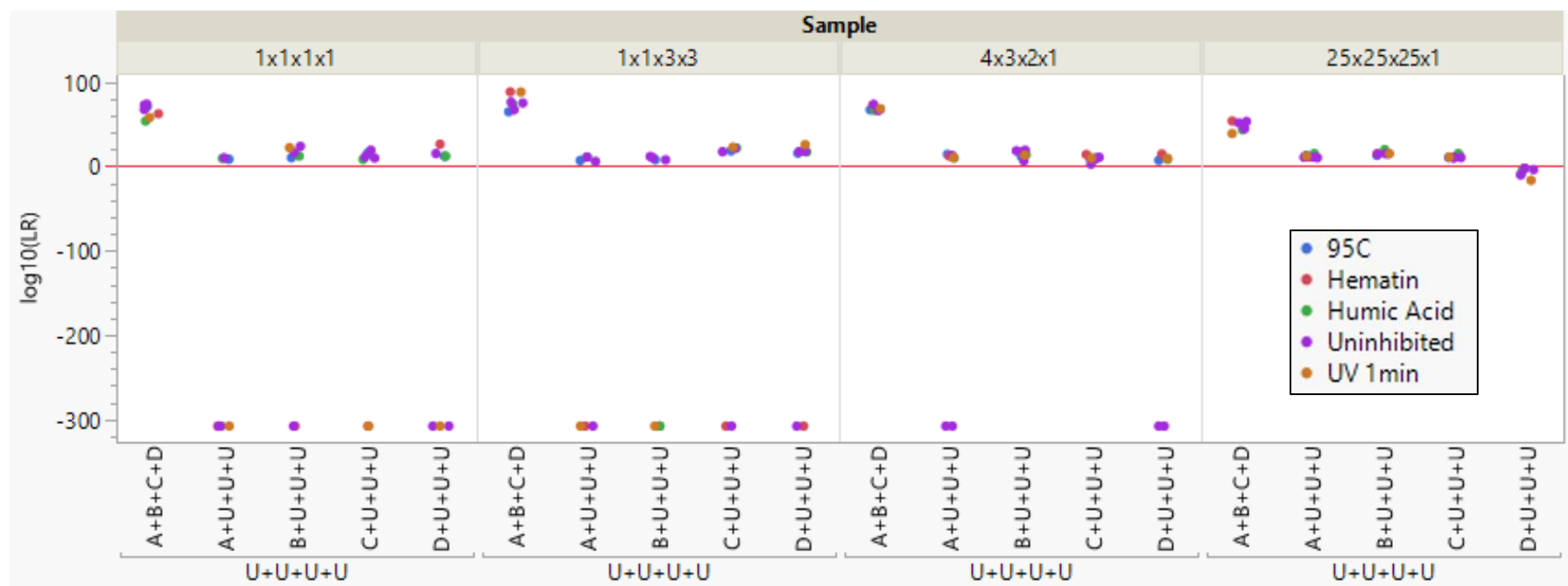


Figure 29: Sensitivity result for 4-person 1 ng DNA input samples on 3500xL. NOC = true-NOC. Known individuals named 'A', 'B', 'C', and 'D'. Samples mixed in varying ratios (1 to 1 to 1 to 1; 1 to 1 to 3 to 3; 4 to 3 to 2 to 1; and 25 to 25 to 25 to 1). LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that four unknown persons contributed. 'U' = an unknown contributor. Mixtures treated with varying degradation/inhibition conditions as in Figure 5. Positive $\log_{10}(\text{LR})$ s supportive of H_p over H_d observed in many cases. Multiple cases also show inconclusive or negative $\log_{10}(\text{LR})$, possibly caused by allele sharing between samples..

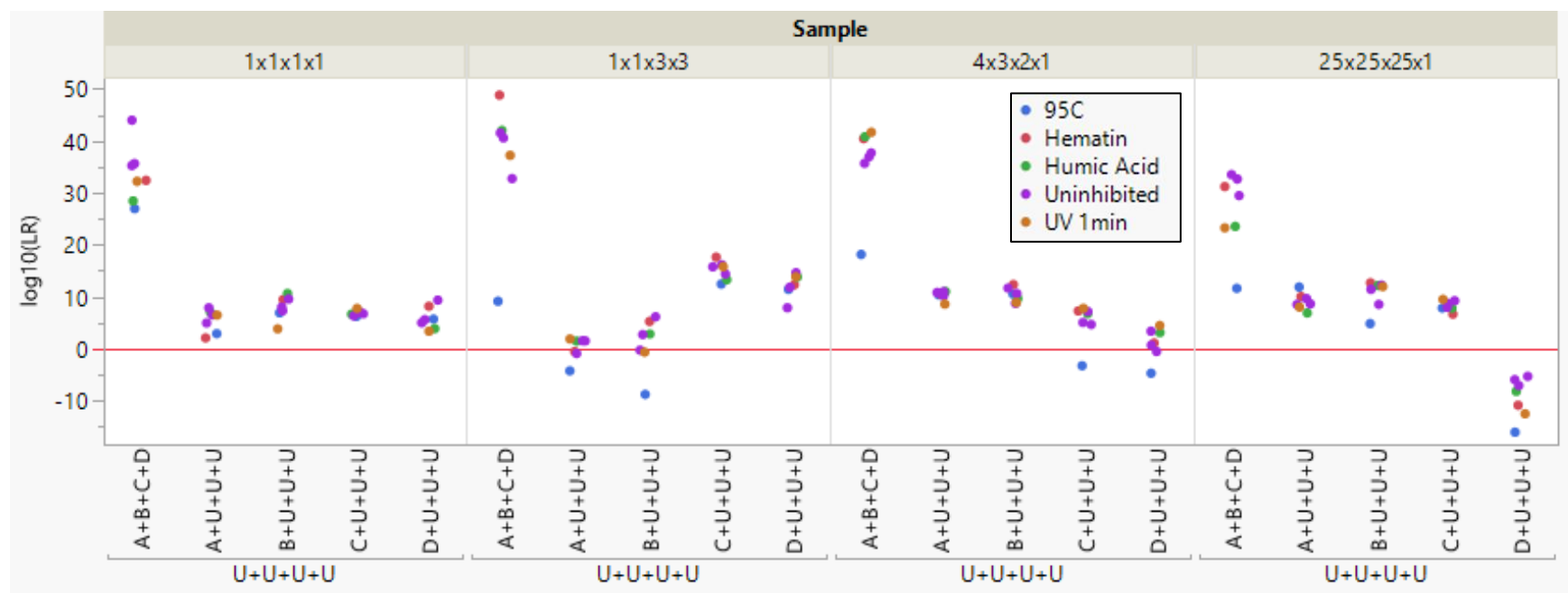


Figure 30: Sensitivity result for 4-person samples on 3500xL. NOC = true-NOC. Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 29. Total of 0.125 ng of DNA input per sample. LRs supportive of H_p over H_d observed in most cases. 95°C degraded samples featured lower total peak count, indicating dropout, which results in negative $\log_{10}(\text{LR})$ in some cases.

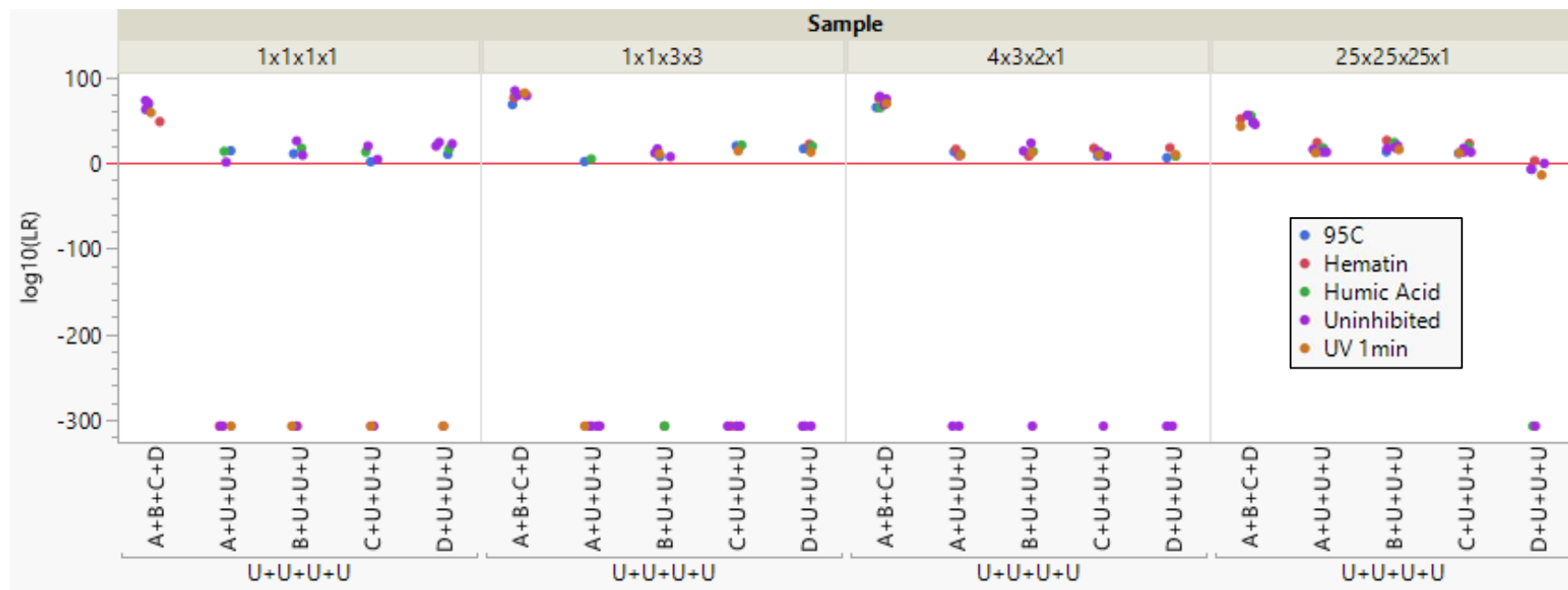


Figure 31: Sensitivity result for 4-person samples on SeqStudio Flex. NOC = true-NOC. Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 29. Total of 1 ng of DNA input per sample. Results are similar to Figure 29, indicating no significant difference between CE instruments.

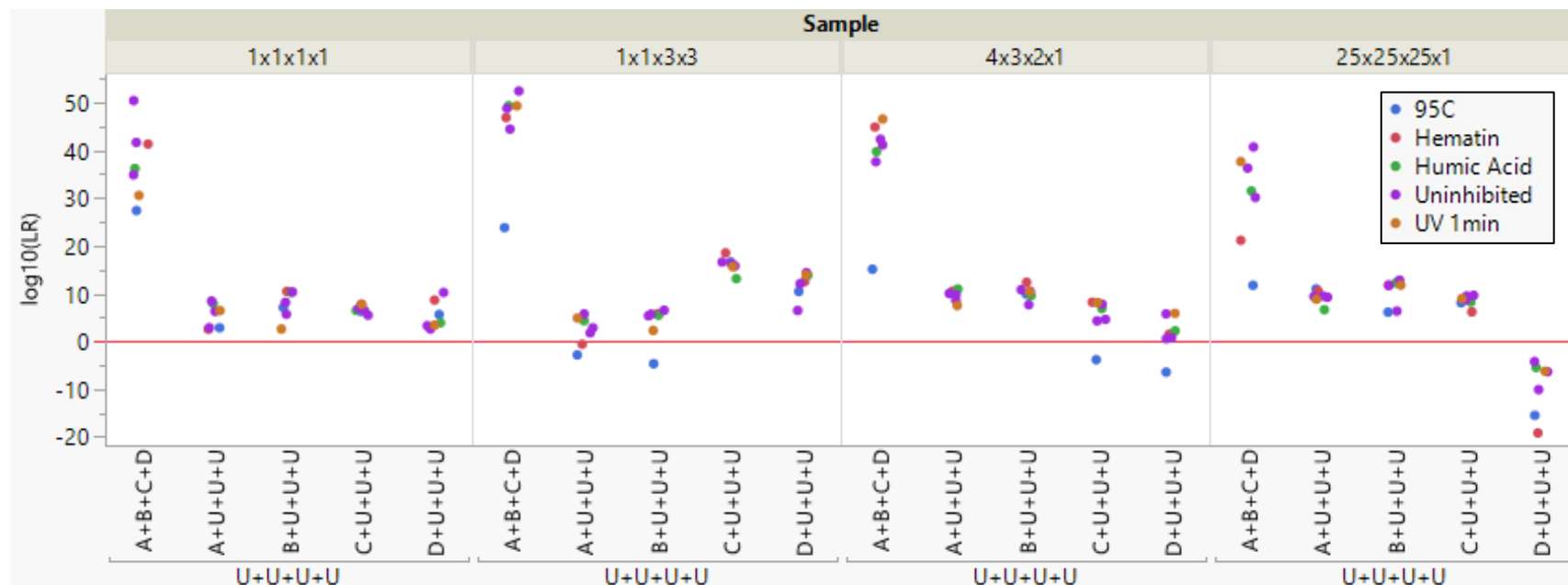


Figure 32: Sensitivity result for 4-person samples on SeqStudio Flex. NOC = true-NOC. Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 30. Total of 0.125 ng of DNA input per sample. Results are similar to Figure 30, indicating no significant difference between CE instruments.

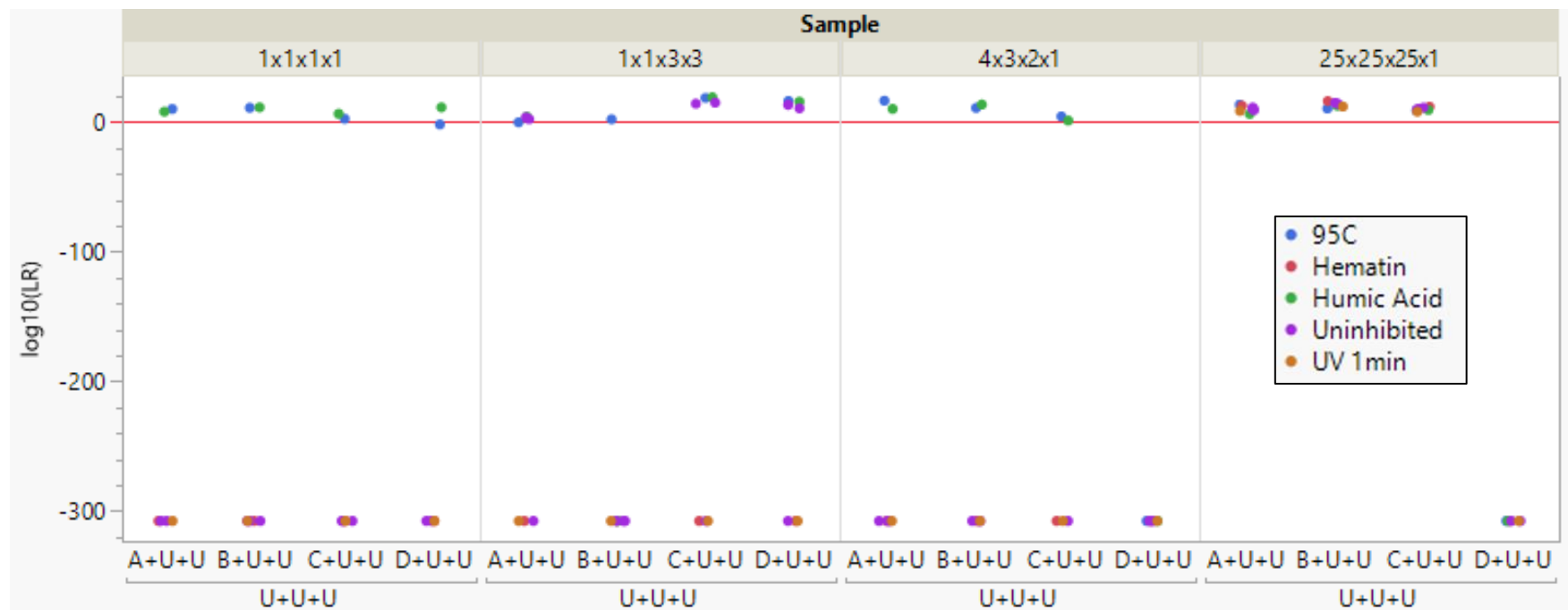


Figure 33: Sensitivity result for 4-person samples on 3500xL. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition as in Figure 29. LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that three unknown persons contributed. 'U' = an unknown contributor. Total of 1 ng of DNA input per sample. LRs are supportive of H_p over H_d for 95°C degraded and Humic acid inhibited samples and for the major contributors of the most unbalanced mixtures. These have fewer peaks than other profiles so NOC = 3 is a more reasonable solution.

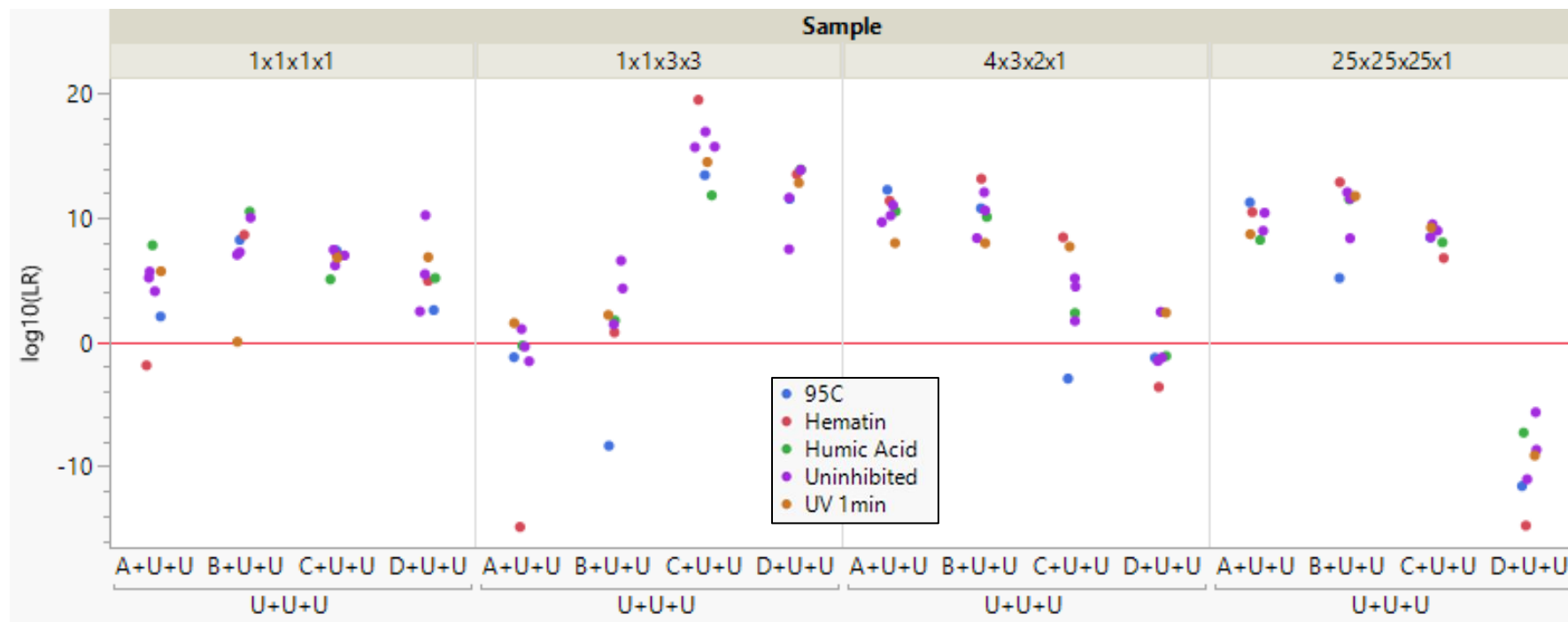


Figure 34: Sensitivity result for 4-person samples on 3500xL. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 33. Total of 0.125 ng of DNA input per sample. Results of similar magnitude to when NOC = 4 (true) (see Figure 30). More stochastic effects are expected to be observed at 0.125 ng of input, so a 4-person mixture can appear similar to a 3-person mixture.

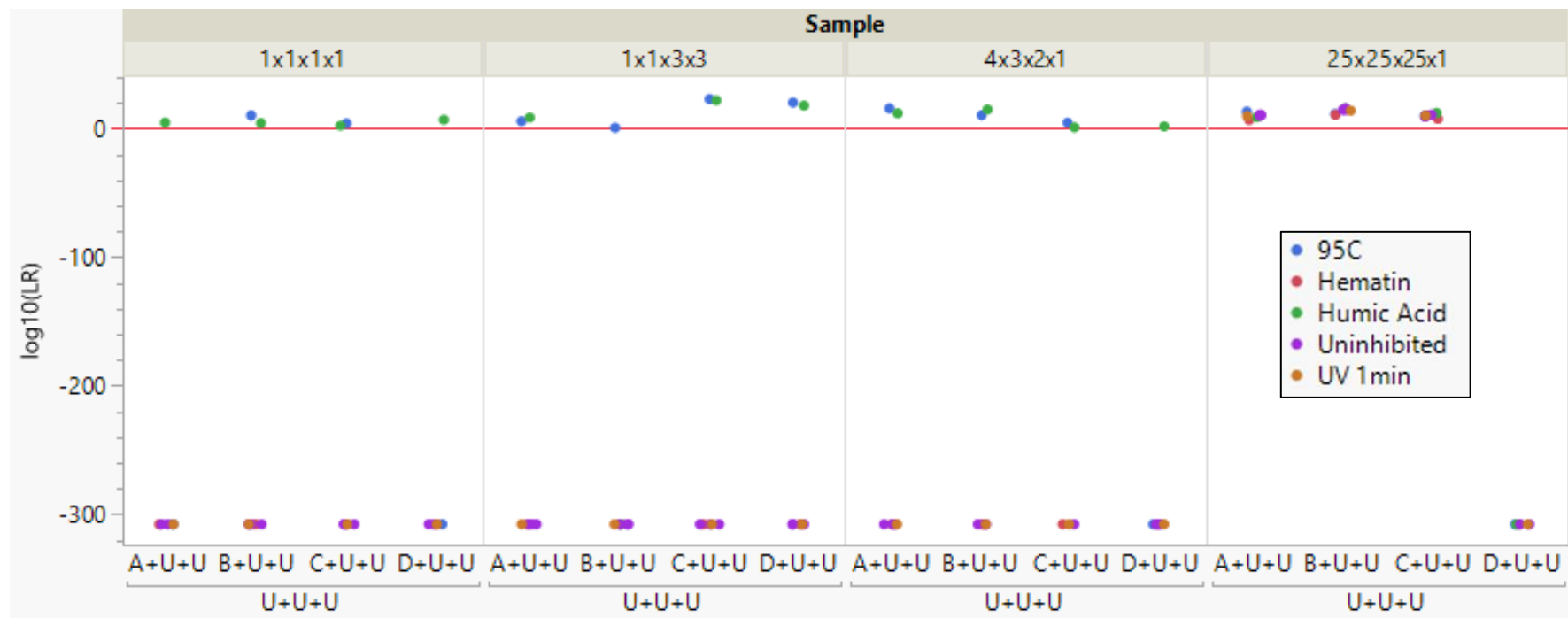


Figure 35: Sensitivity result for 4-person samples on SeqStudio Flex. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 33. Total of 1 ng of DNA input per sample. Results broadly similar to 3500xL (Figure 33) with exception of more LR calculations of uninhibited 1 to 1 to 3 to 3 samples that are supportive of H_d over H_p .

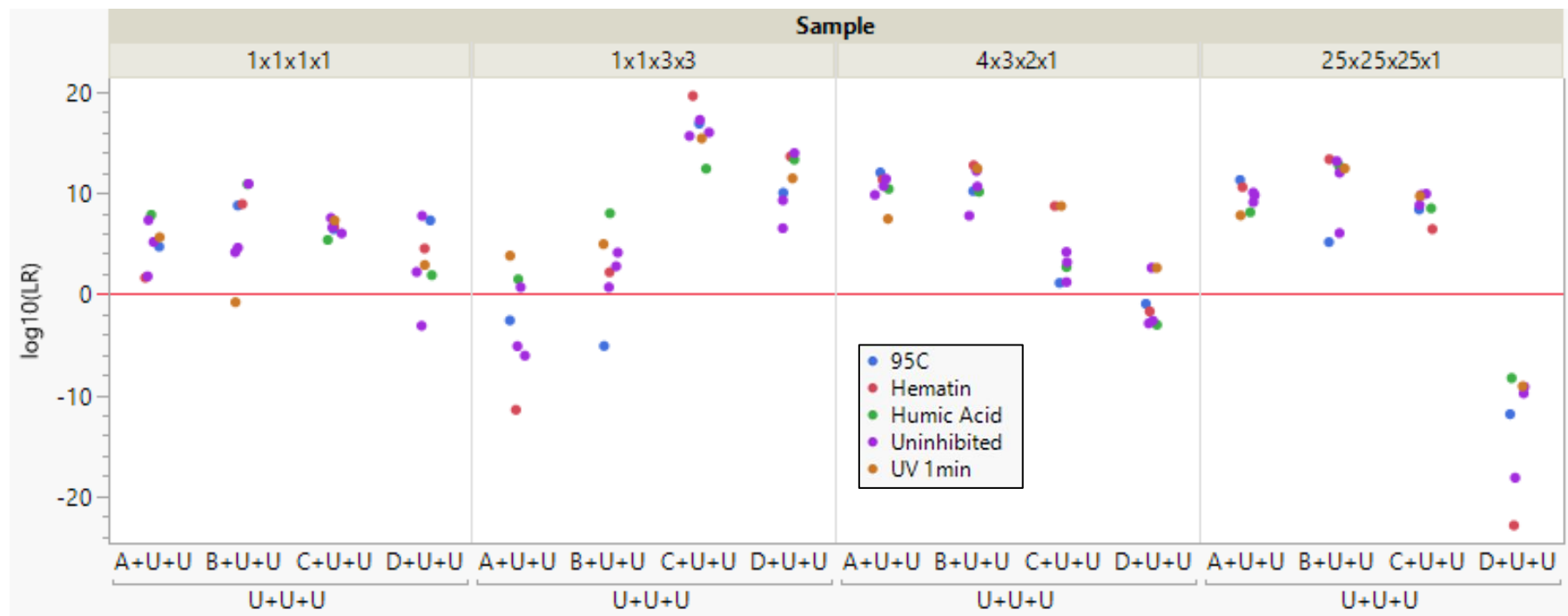


Figure 36: Sensitivity result for 4-person samples on SeqStudio Flex. NOC = true-NOC minus 1. (i.e. NOC incorrectly set to 3). Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 34. Total of 0.125 ng of DNA input per sample. Results similar to Figure 34, indicating no significant difference between CE instruments.

3.3. *Specificity*

3.3.1. Introduction

Studies are required which evaluate the ability of GeneMapper PG Software to provide reliable results for non-contributors over a broad variety of evidence types, including mixtures and low-level DNA quantities. This should incorporate various numbers of contributors, mixture proportions and template quantities. Specificity studies should demonstrate the potential for Type II errors (failure to reject a false hypothesis) and should demonstrate the range of LR values that can be expected for non-contributors.

3.3.2. Methods

Studies were performed which calculated the p-value for multiple LR scenarios. NOC was set to the true NOC for all scenarios. The p-value is the probability that a contributor, substituted for the person of interest (POI) in a given LR scenario would result in a LR at least as large as the one observed with the POI [1]. To calculate this p-value, alternative contributors were simulated as unrelated or related to the POI based on the possible genotypes that can explain the evidence profile. These simulated profiles are the non-contributors by which the range of LR values expected for non-contributors was assessed.

If the original $\log_{10}(\text{LR})$ was negative, no p-value was calculated and the result appears blank. This is because negative $\log_{10}(\text{LR})$ suggests, in this study, the evidence is more likely to be seen if an unrelated, unknown individual were a contributor to the profile than if the POI were a contributor, thus suggesting the POI is not a likely contributor to the mixture. In other words, the POI behaves like the other non-contributors that were simulated as part of p-value determination.

No p-value was calculated for scenarios with multiple POI in H_p . GeneMapper PG Software only calculates the p-value for the first POI of any scenario. Therefore, it is equivalent to look at the p-values generated for LRs with only one POI in H_p .

3.3.3. Specificity Results

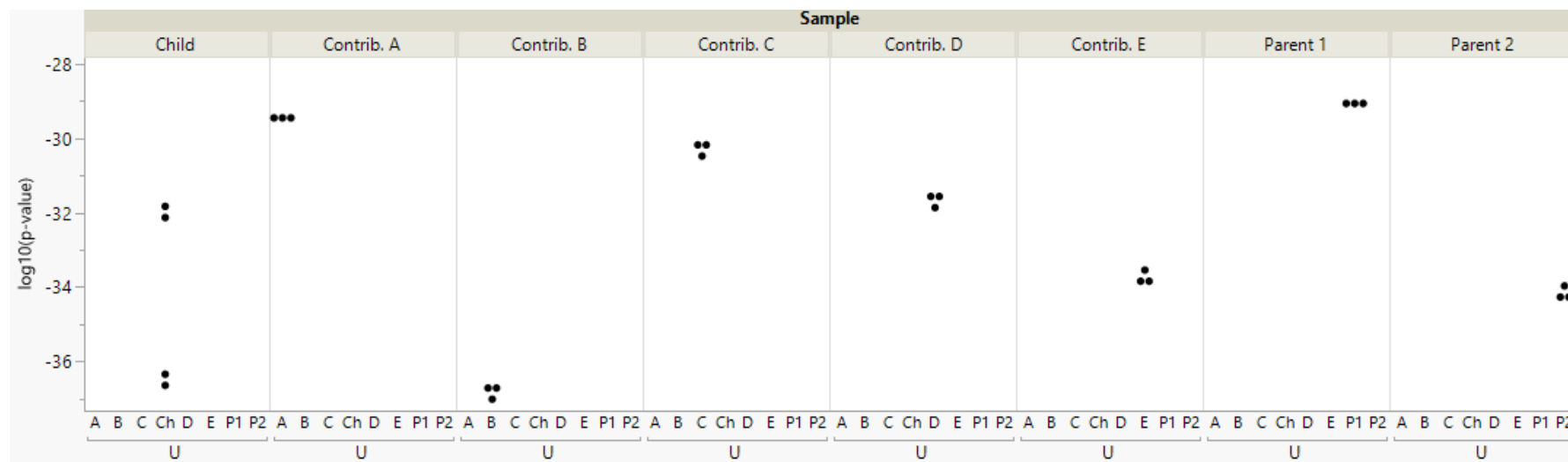


Figure 37: Specificity result for 1-person, 1 ng DNA input samples on 3500xL. Sample names are: Child (Ch), Contributors A, B, C and D, and Parents 1 and 2 (P1 and P2). LR tested as H_p / H_d where H_p = the probability of seeing the sample's profile given that the named person contributed and H_d = the probability of seeing the observed profile given that an unknown person contributed. All p-values are low (i.e., $\log_{10}(\text{p-value})$ in order of approximately -30 to -36, indicating p-value = approximately 10^{-30} to 10^{-36}). This suggests a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

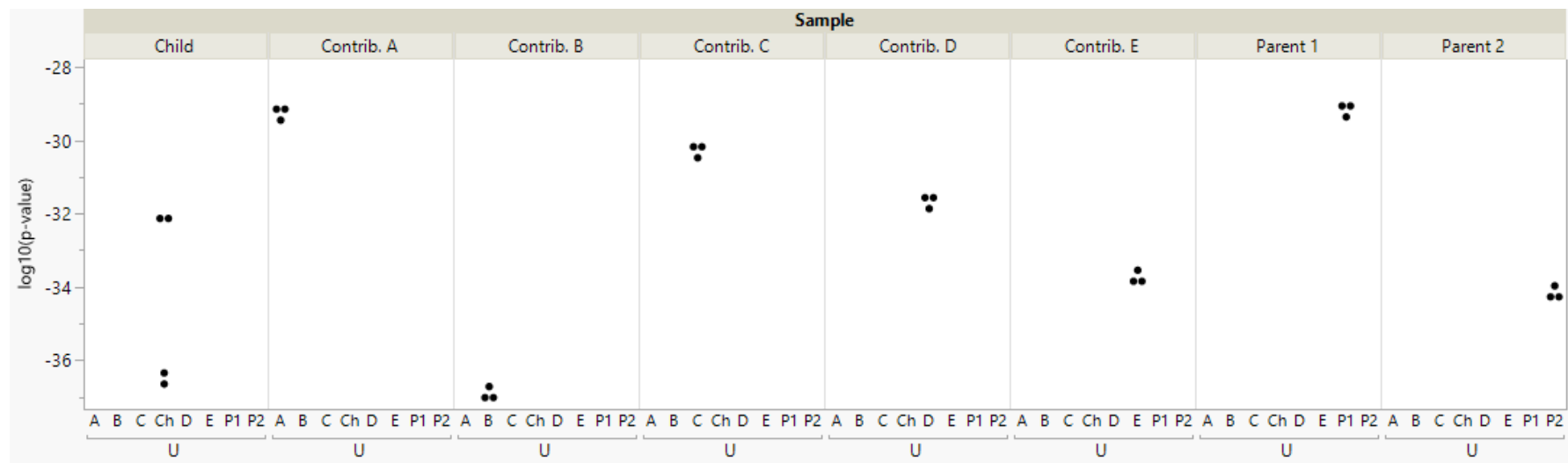


Figure 38: Specificity result for 1-person, 1 ng DNA input samples on SeqStudio Flex. Sample set up, naming, and LR hypotheses as in Figure 37. Results similar to Figure 37, indicating no significant difference between CE instruments.

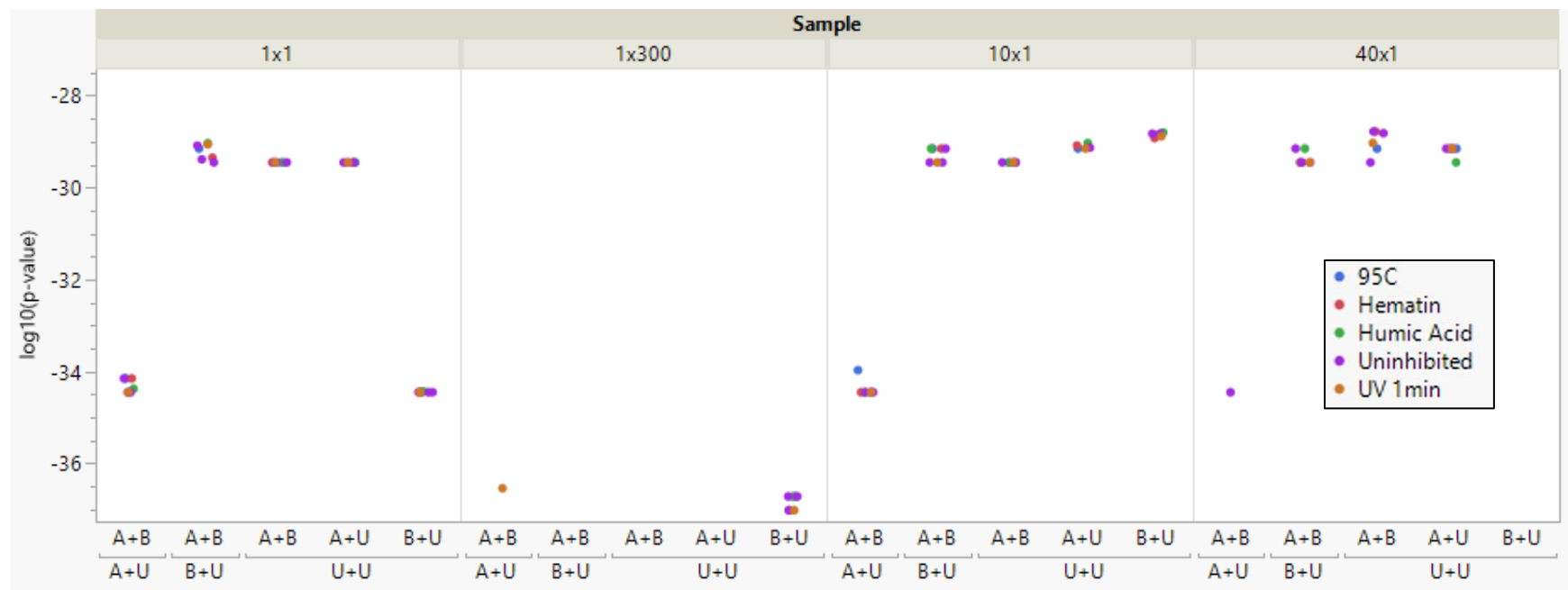


Figure 39: Specificity result for 2-person, 1 ng DNA input samples on 3500xL. Two individual's DNA samples (A and B) tested in varying ratios (1 to 1; 1 to 300; 10 to 1 and 40 to 1) and with varying artificial degradation/inhibition conditions (incubated for 15 minutes at 95°C; UV treated for 1 minute; inhibited with humic acid and hematin, and uninhibited/undegraded). (See color key on chart). LRs calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where 'U' = an unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. All calculated p-values are low, indicating a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR. (Note that some tests are blank on the chart as the log₁₀(LR) was negative, so no p-value was calculated. See 'Methods' section).

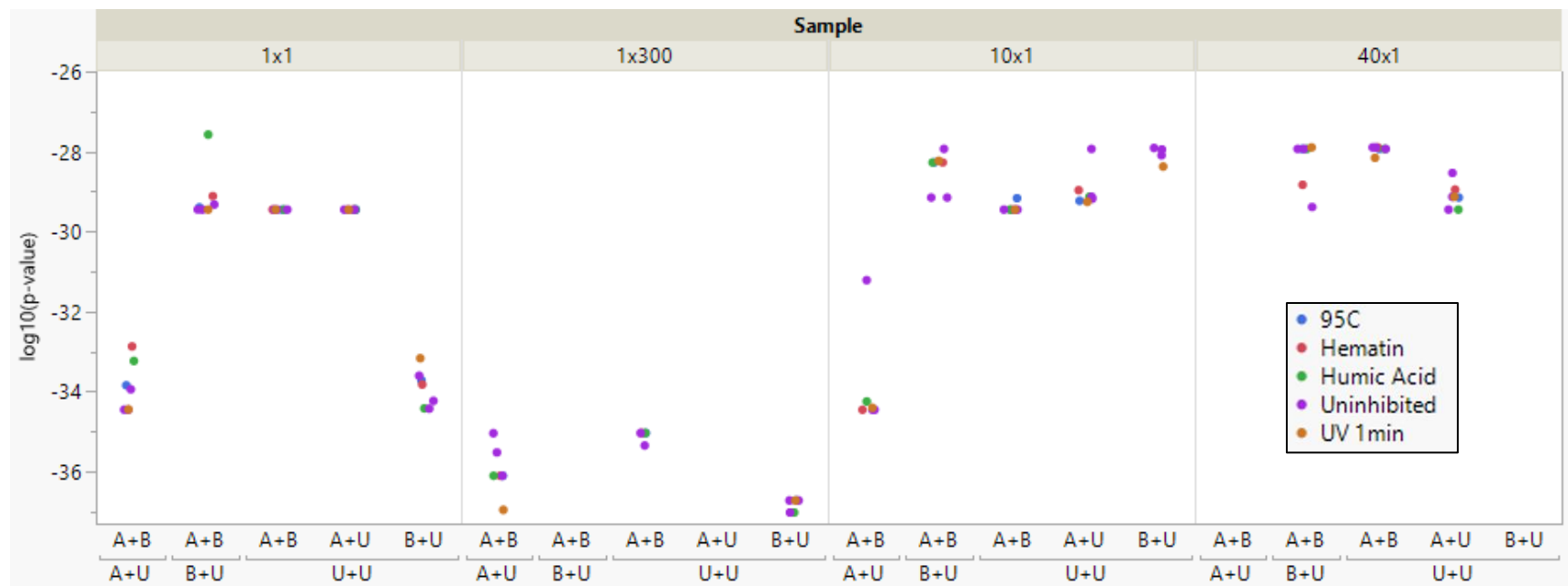


Figure 40: Specificity result for 2-person samples on 3500xL. Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 39. Total of 0.125 ng of DNA input per sample. All calculated p-values are low, indicating a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

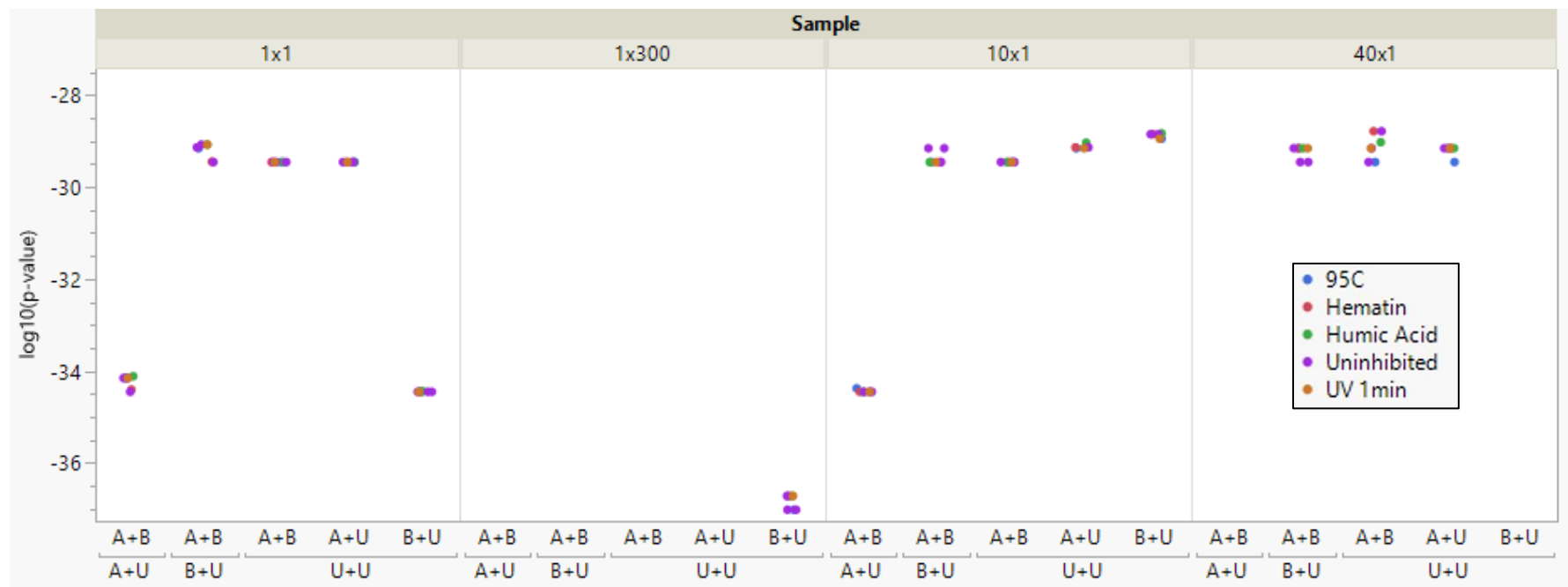


Figure 41: Specificity result for 2-person, 1 ng DNA input samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 39. Results are similar to Figure 39, indicating no significant difference between CE instruments.

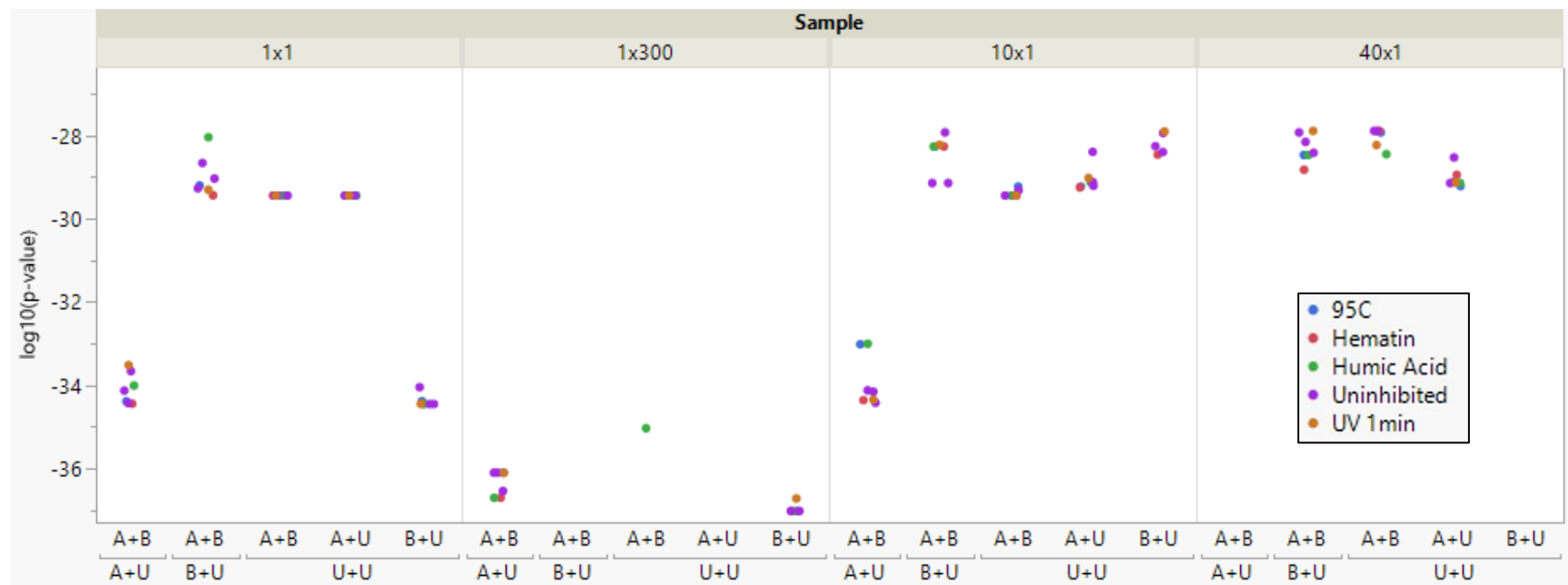


Figure 42: Specificity result for 2-person samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 40. Total of 0.125 ng of DNA input per sample. Results are similar to Figure 40, indicating no significant difference between CE instruments.

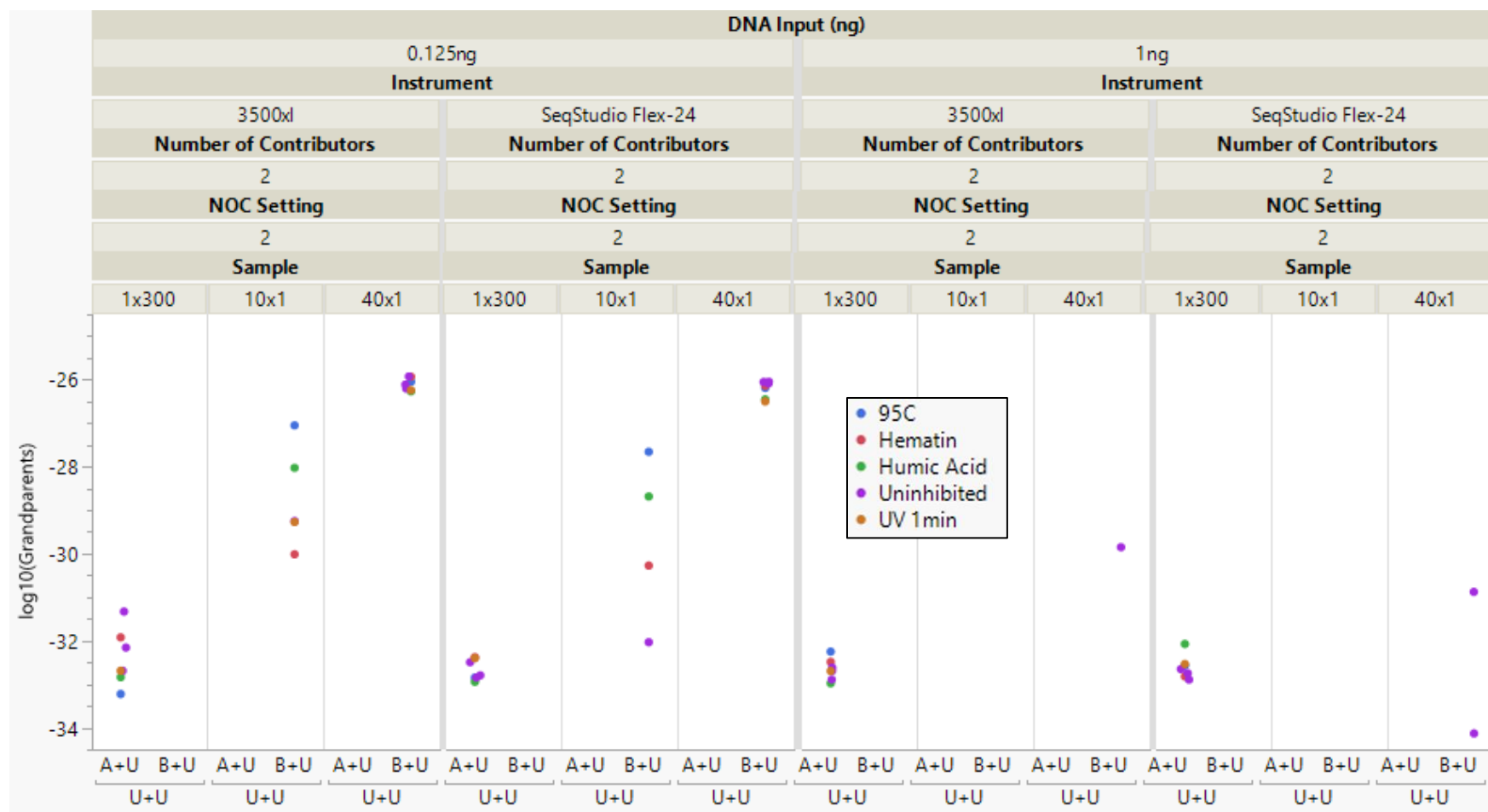


Figure 43 Specificity result for 2-person samples with sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 39. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA input are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being the grandparent of the POI. All calculated p-values are low, indicating a low probability that a grandparent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

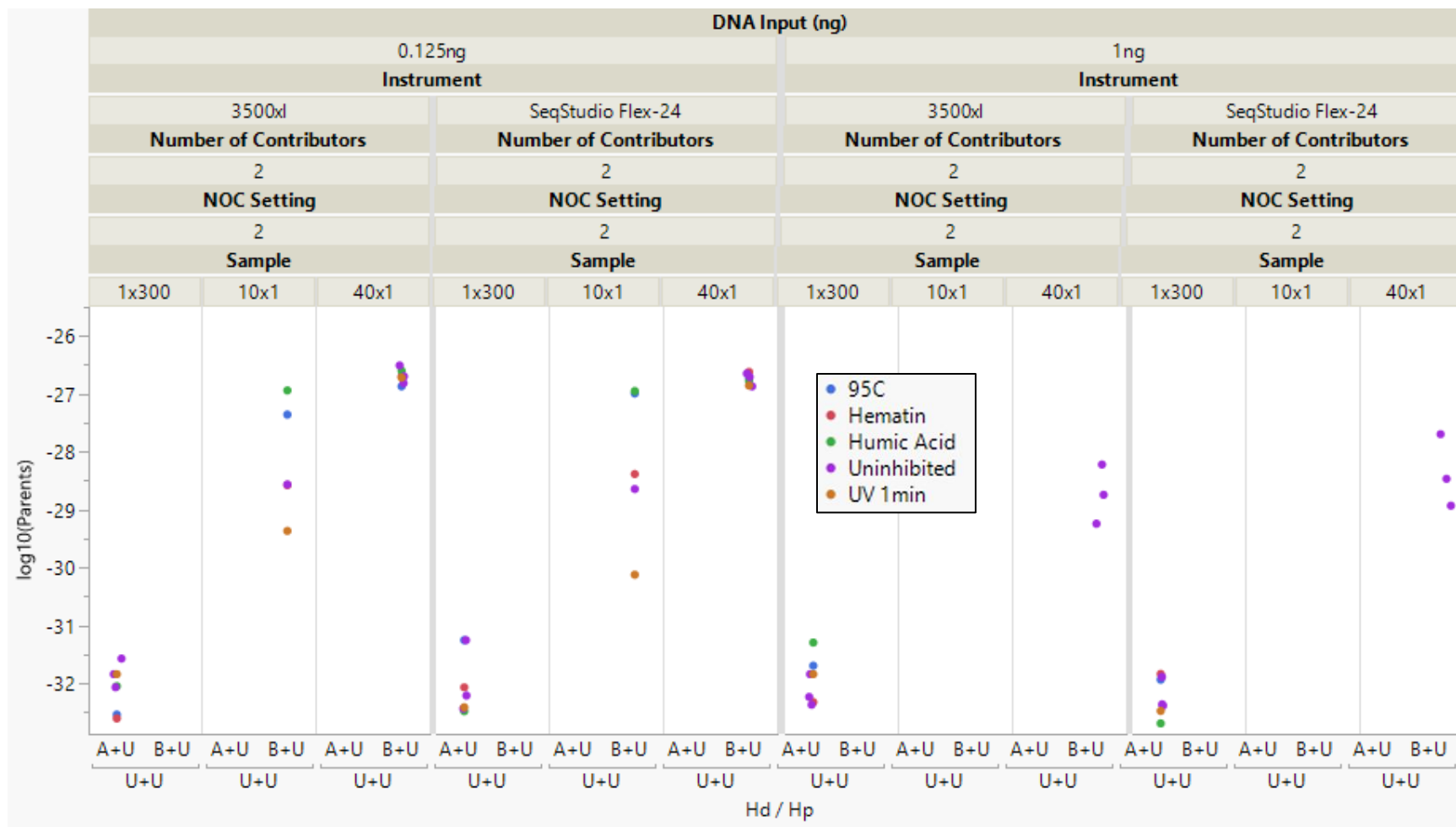


Figure 44: Specificity result for 2-person samples with sample set up, naming, degradation/inhibition, DNA input, instrument type and LR hypotheses as in Figure 43. The y-axis shows $\log_{10}(\text{p-value})$ using simulated profiles consistent with being the parent of the POI. All calculated p-values are low, indicating a low probability that a parent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

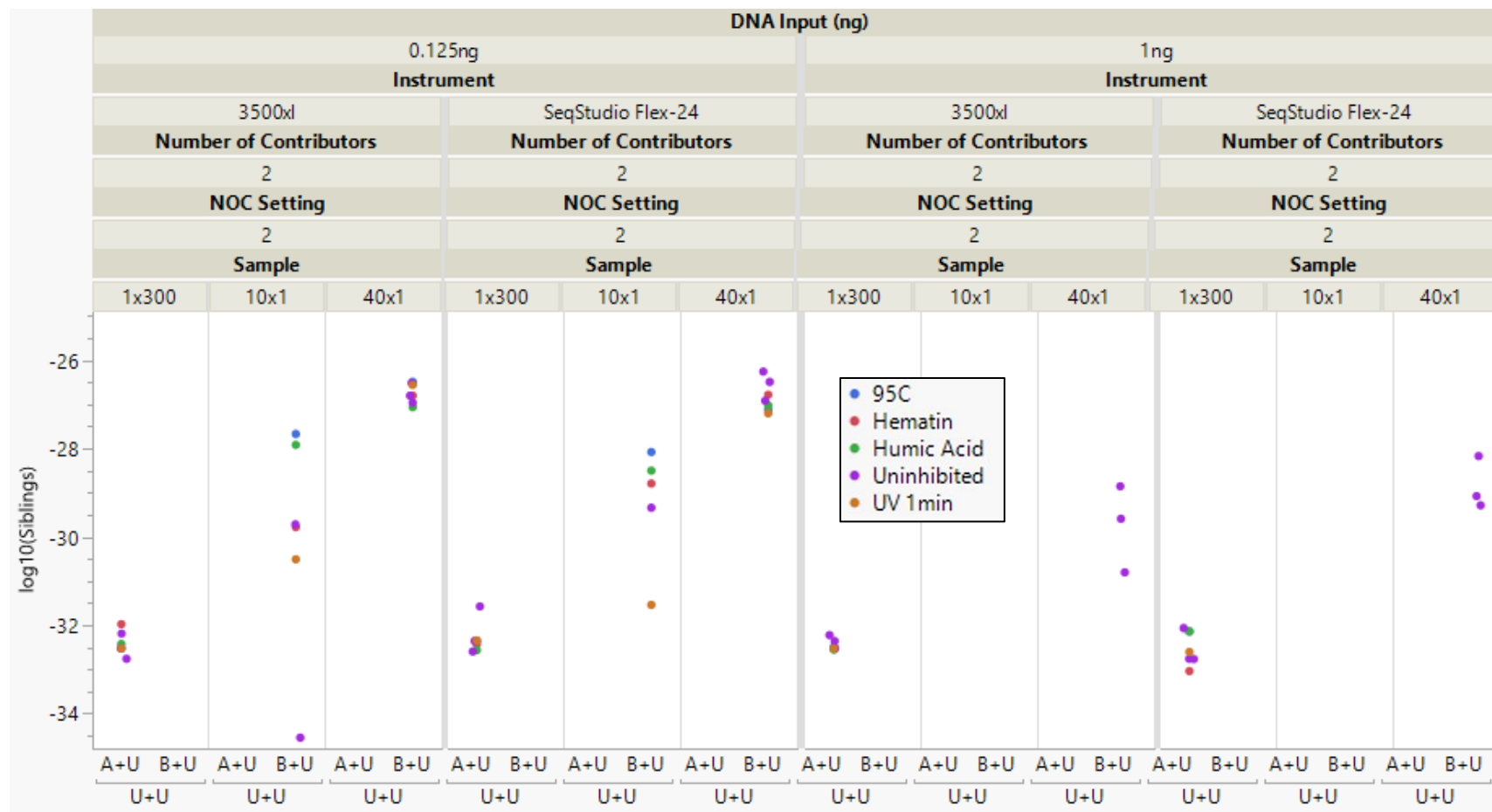


Figure 45: Specificity result for 2-person samples with sample set up, naming, degradation/inhibition, DNA input, instrument type and LR hypotheses as in Figure 43. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being a sibling of the POI. All calculated p-values are low, indicating a low probability that a sibling of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

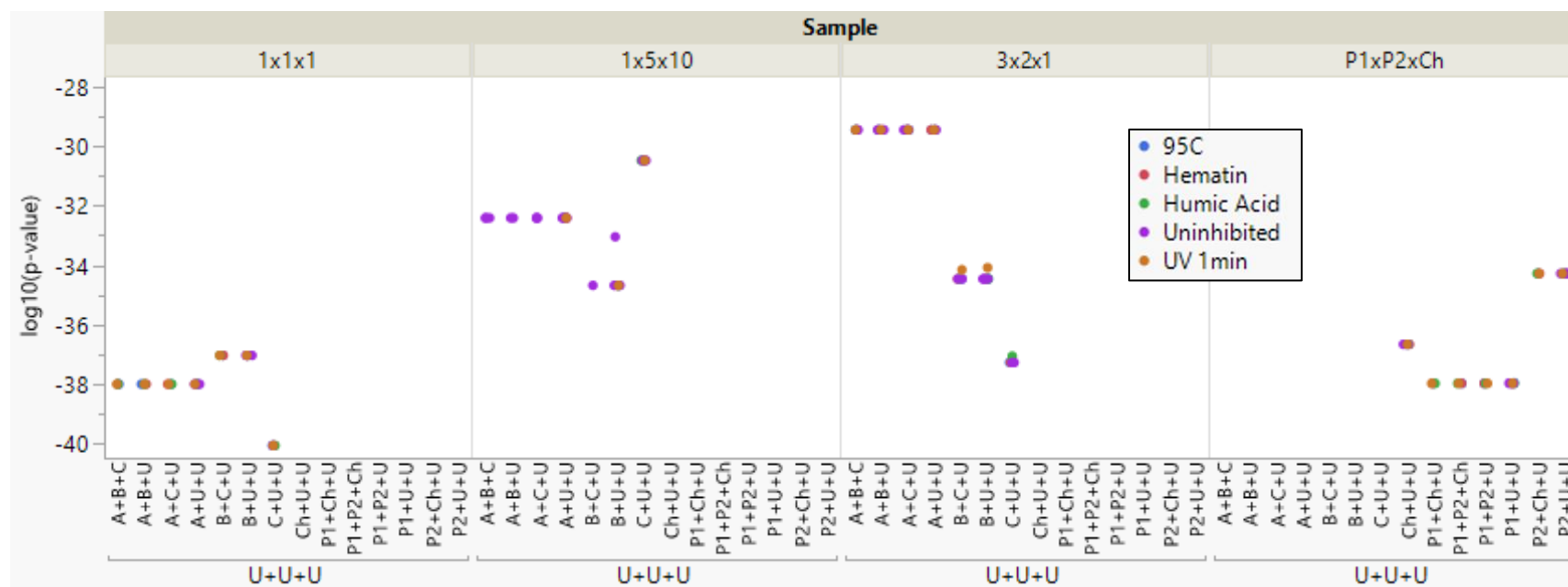


Figure 46: Specificity result for 3-person, 1 ng DNA input samples on 3500xL. Individual contributors' names are: Child (Ch), Contributors A, B, and C, and Parents 1 and 2 (P1 and P2). Contributors mixed in varying ratios (1 to 1 to 1; 1 to 5 to 10; and 3 to 2 to 1). 'P1 x P2 x Ch' tests a 1 to 1 to 1 combination of related individuals where 'P1' and 'P2' are parents of 'Ch'. Mixtures were exposed to varying artificial degradation/inhibition conditions. (See color key on chart). LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that three unknown persons contributed. 'U' = an unknown contributor. All calculated p-values are low, which suggest a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

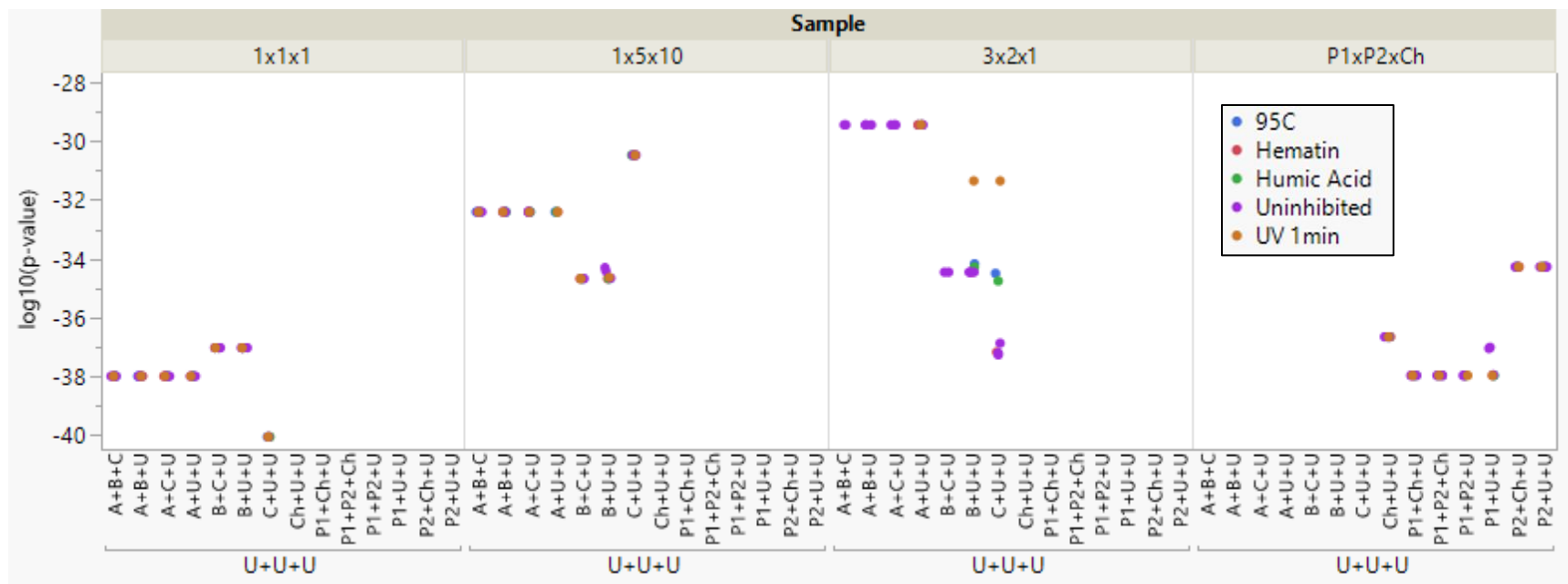


Figure 47: Specificity result for 3-person, 0.125 ng DNA input samples on 3500xL. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 46. All calculated p-values are low, indicating a low probability that that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

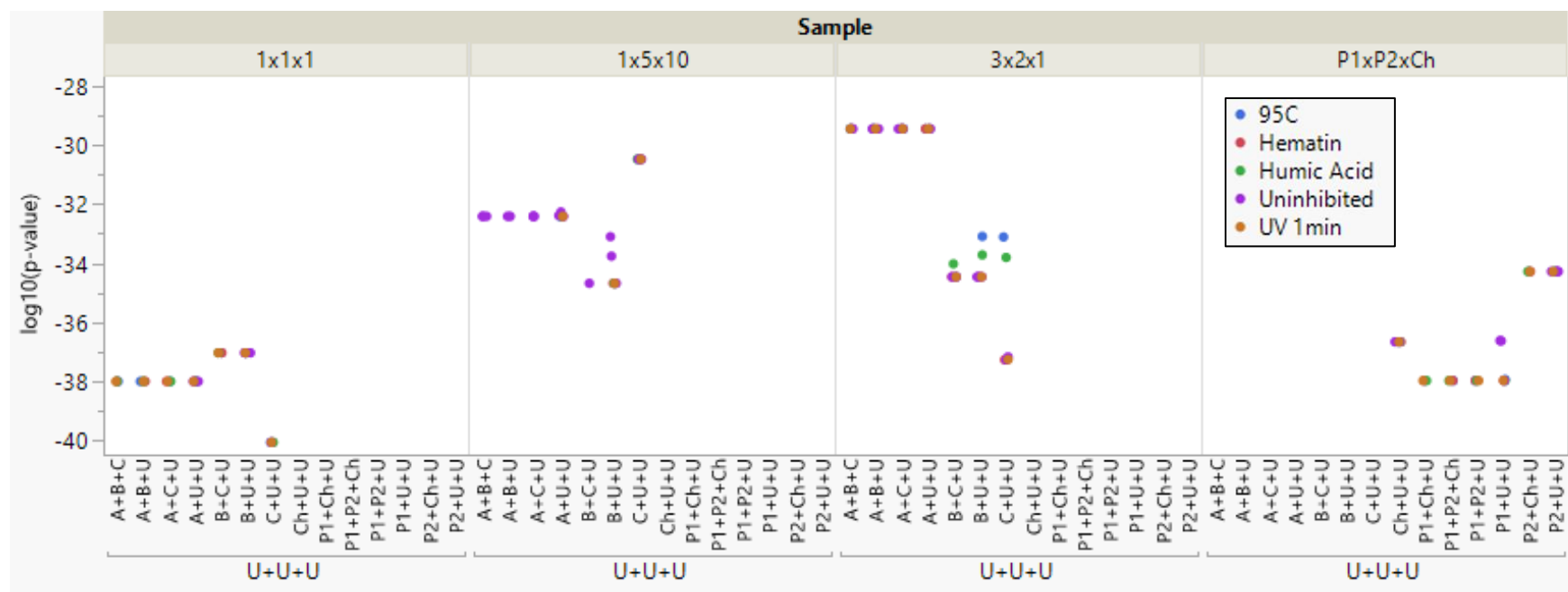


Figure 48: Specificity result for 3-person, 1 ng DNA input samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 46. Results are similar to Figure 46Figure 40, indicating no significant difference between CE instruments.

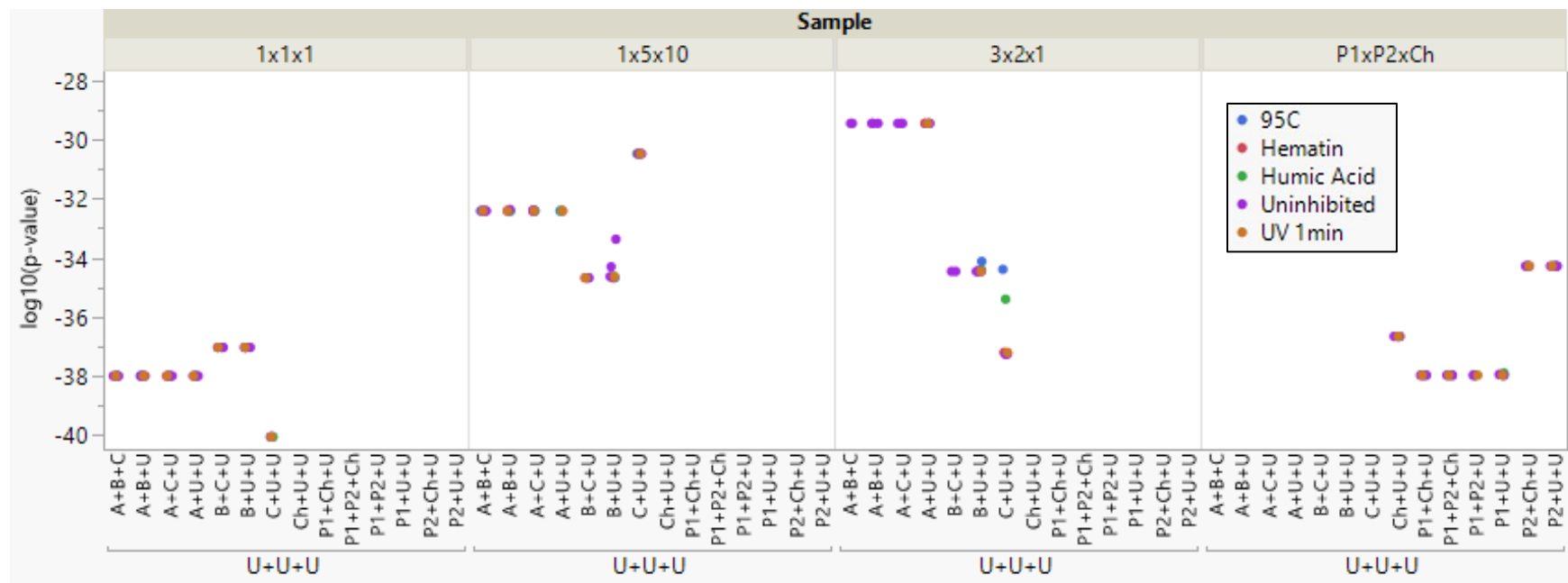


Figure 49: Specificity result for 3-person, 0.125 ng DNA input samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 47. Results are similar to Figure 47, indicating no significant difference between CE instruments.

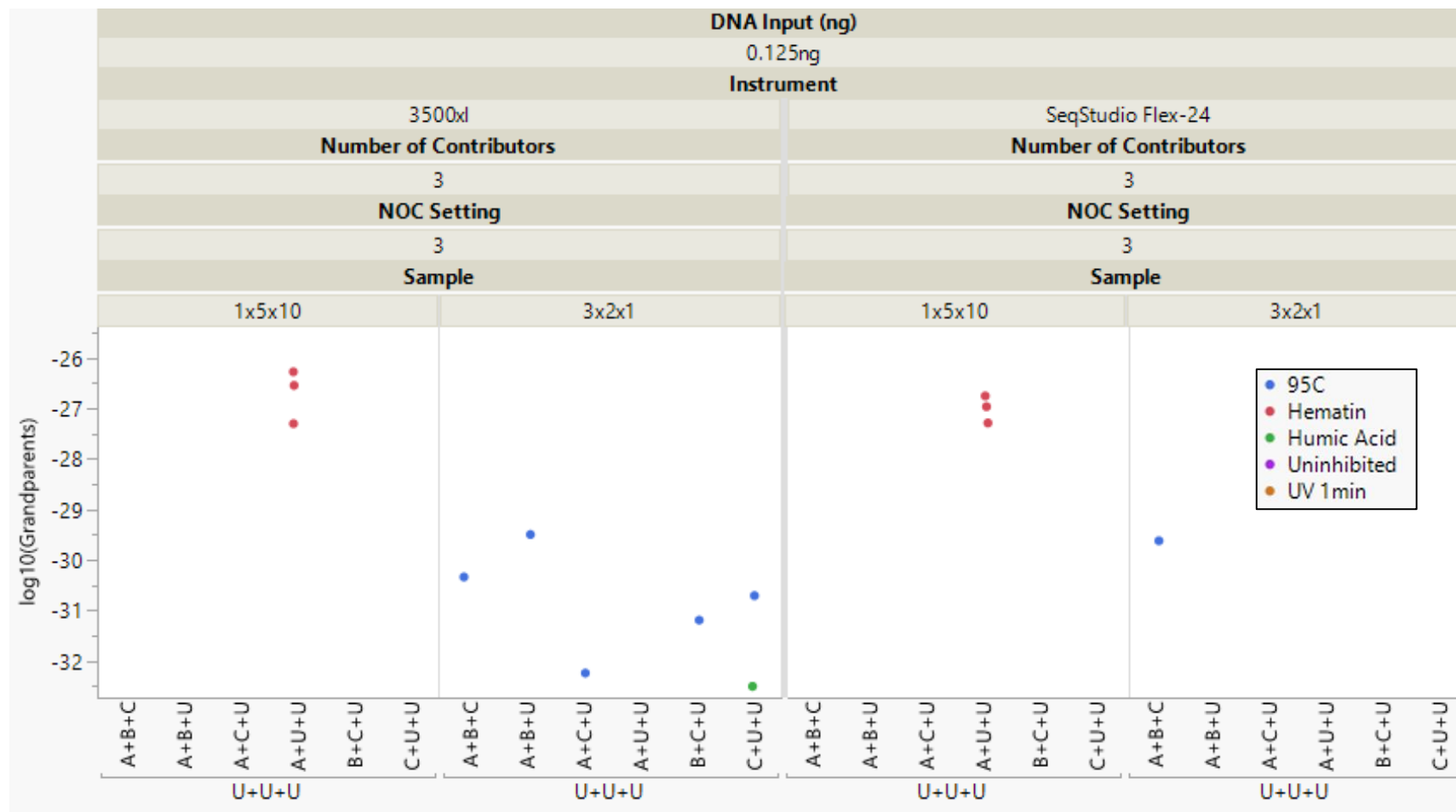


Figure 50: Specificity result for 3-person samples with sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 46. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng DNA inputs. The y-axis shows log₁₀(p-value) result using simulated profiles consistent with being the grandparent of the POI. All calculated p-values are low, indicating a low probability that a grandparent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR. Note that all 1 ng DNA input results and multiple 0.125 ng DNA input results are not shown because these scenarios resulted in a null result: i.e. the p-value was too low for the software to calculate.

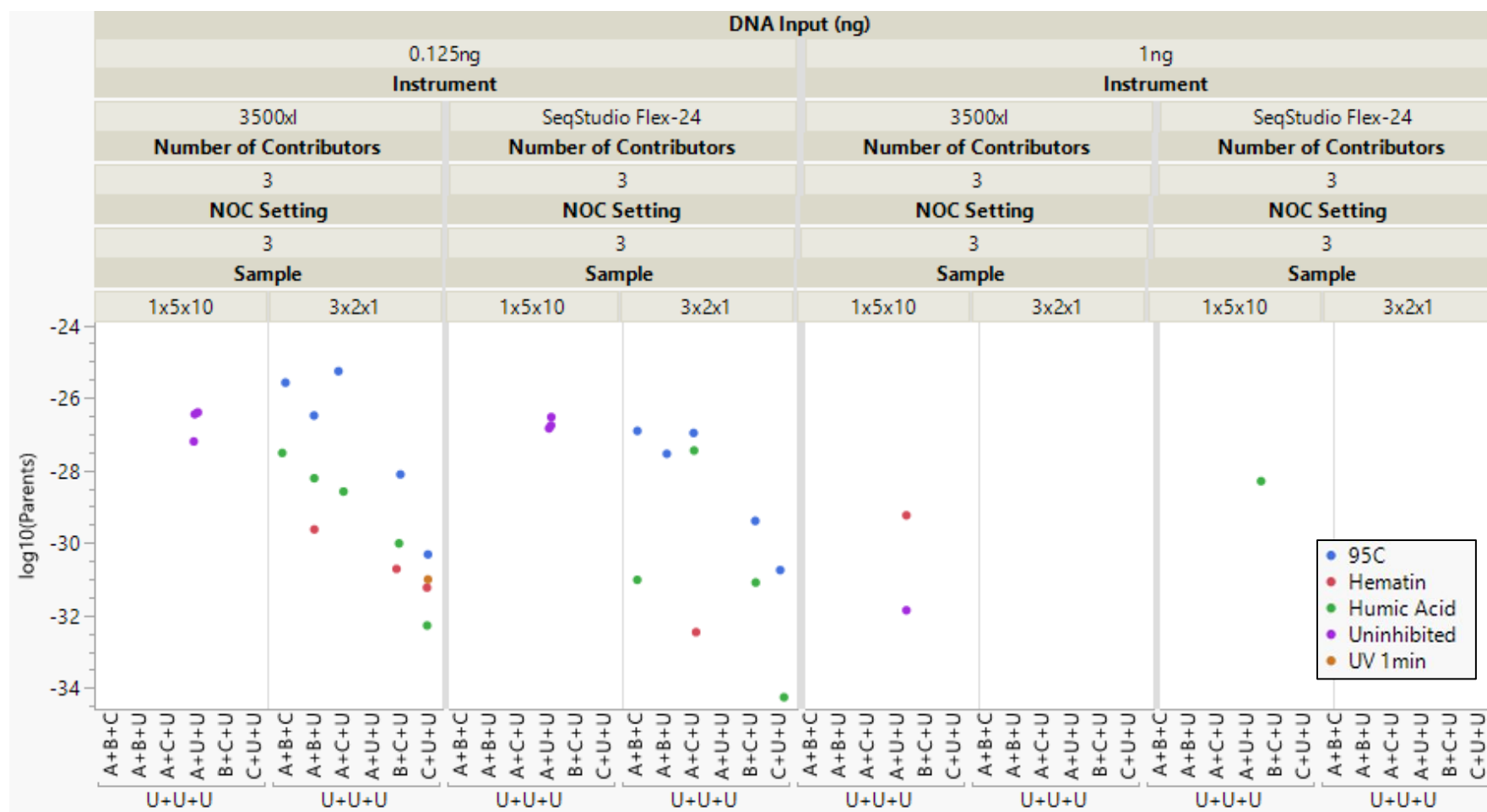


Figure 51: Specificity result for 3-person samples with sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 46. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA inputs are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being the parent of the POI. All calculated p-values are low, indicating a low probability that a parent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR. Note that multiple results are not shown on the chart because these scenarios resulted in a null result: i.e. the p-value was too low for the software to calculate.

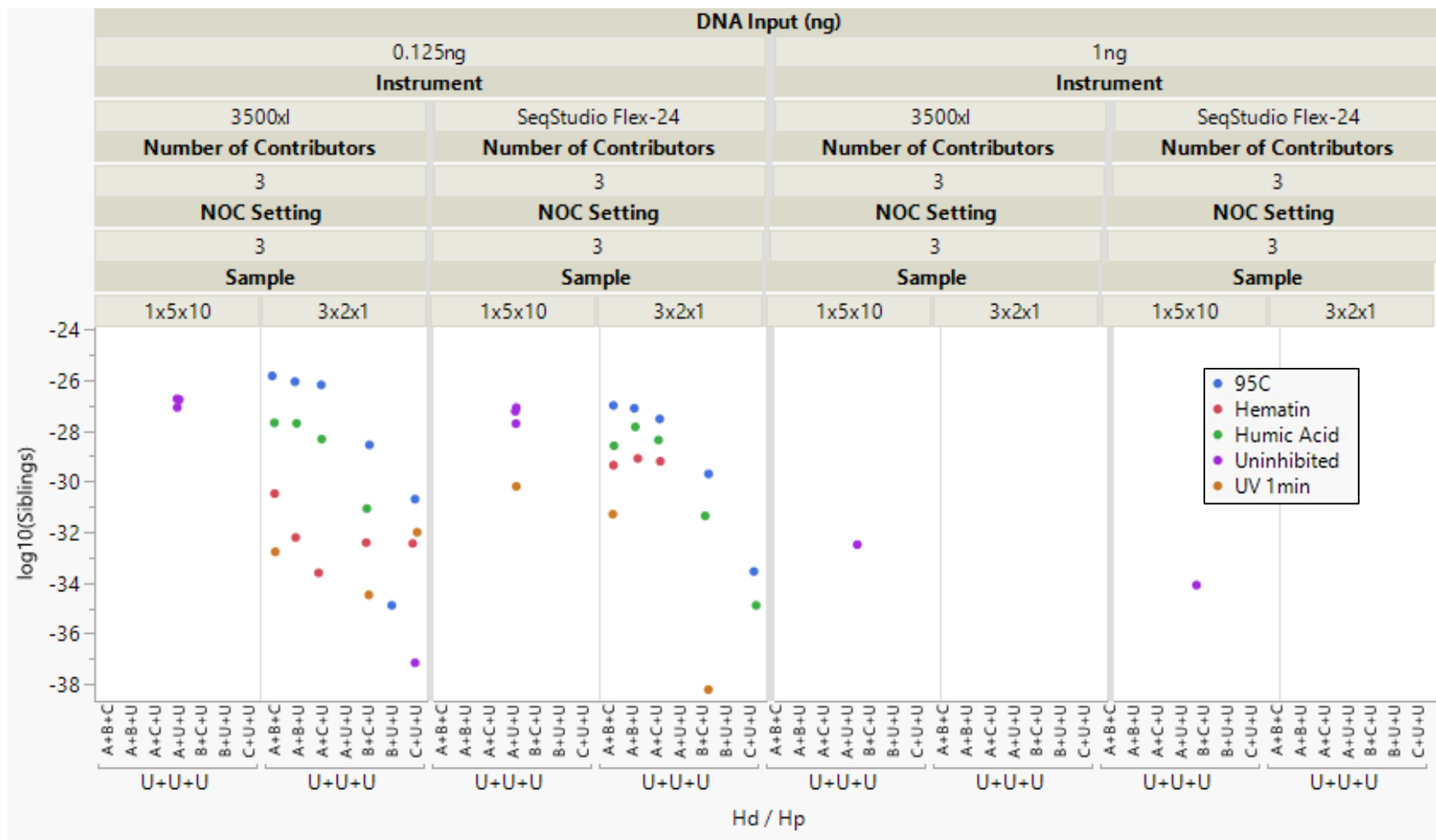


Figure 52: Specificity result for 3-person samples with sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 46. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA input are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being a sibling of the POI. All calculated p-values are low, indicating a low probability that a sibling of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR. Note that multiple results are not shown on the chart because these scenarios resulted in a null result: i.e. the p-value was too low for the software to calculate.

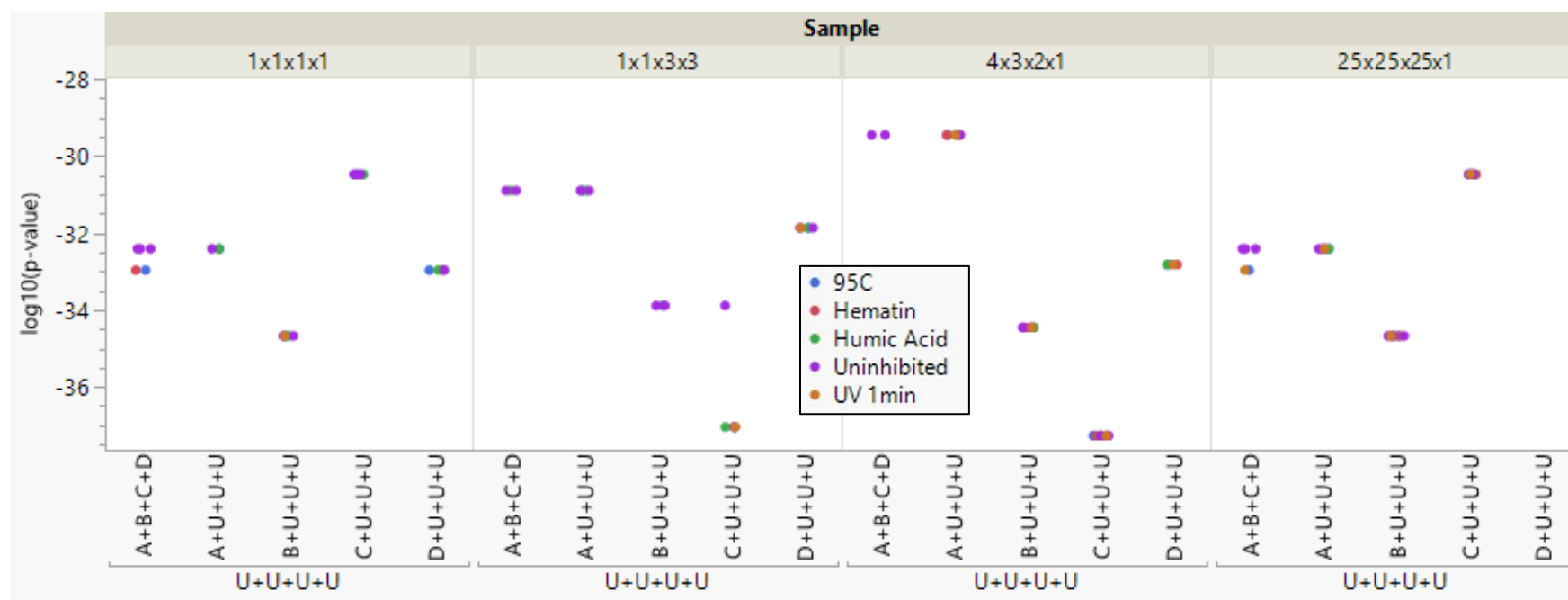


Figure 53: Specificity result for 4-person, 1 ng DNA input samples on 3500xL. Four samples (A, B, C and D) mixed in varying ratios (1 to 1 to 1 to 1; 1 to 1 to 3 to 3; 4 to 3 to 2 to 1; and 25 to 25 to 25 to 1) and exposed to varying artificial degradation/inhibition conditions (incubated for 15 minutes at 95°C; UV treated for 1 minute; inhibited with humic acid and hematin, and uninhibited/undegraded). (See color key on chart). LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that four unknown persons contributed. 'U' = an unknown contributor. All calculated p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

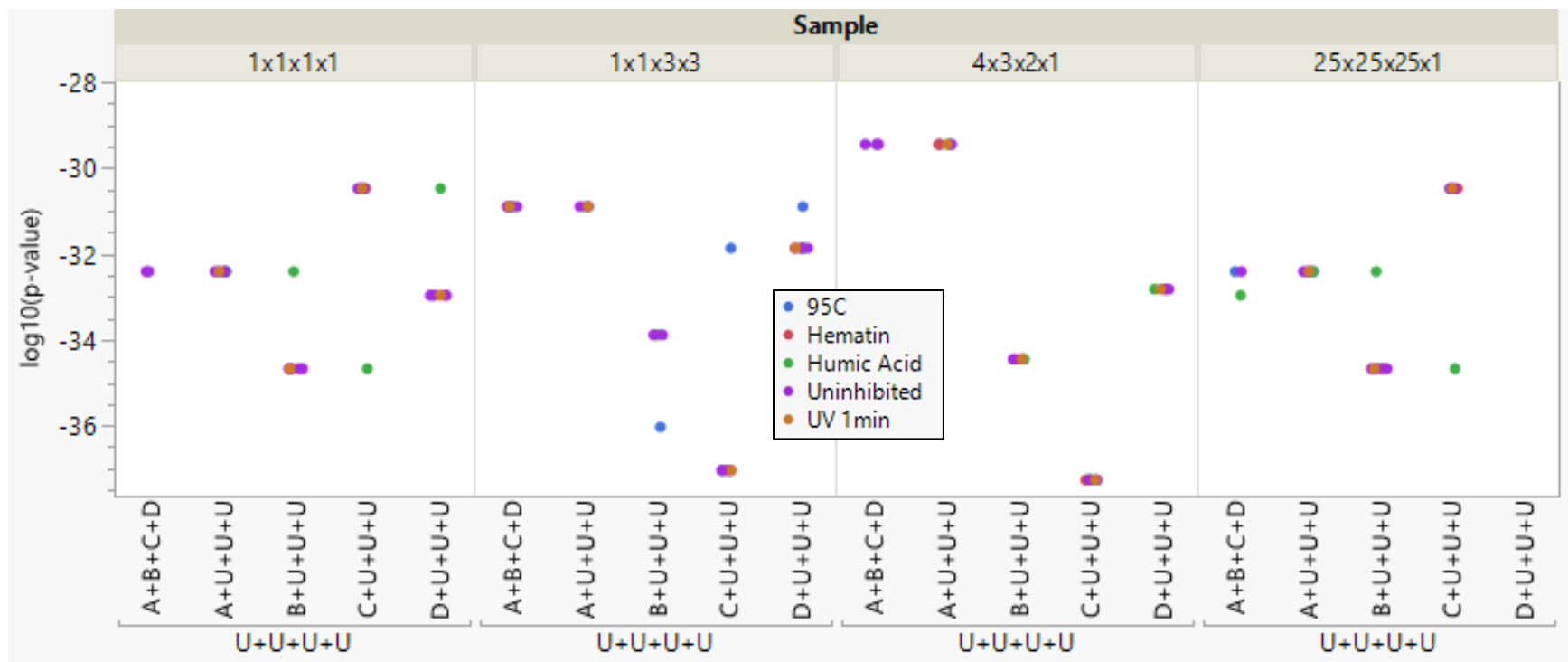


Figure 54: Specificity result for 4-person, 0.125 ng DNA input samples on 3500xL. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 53. All calculated p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

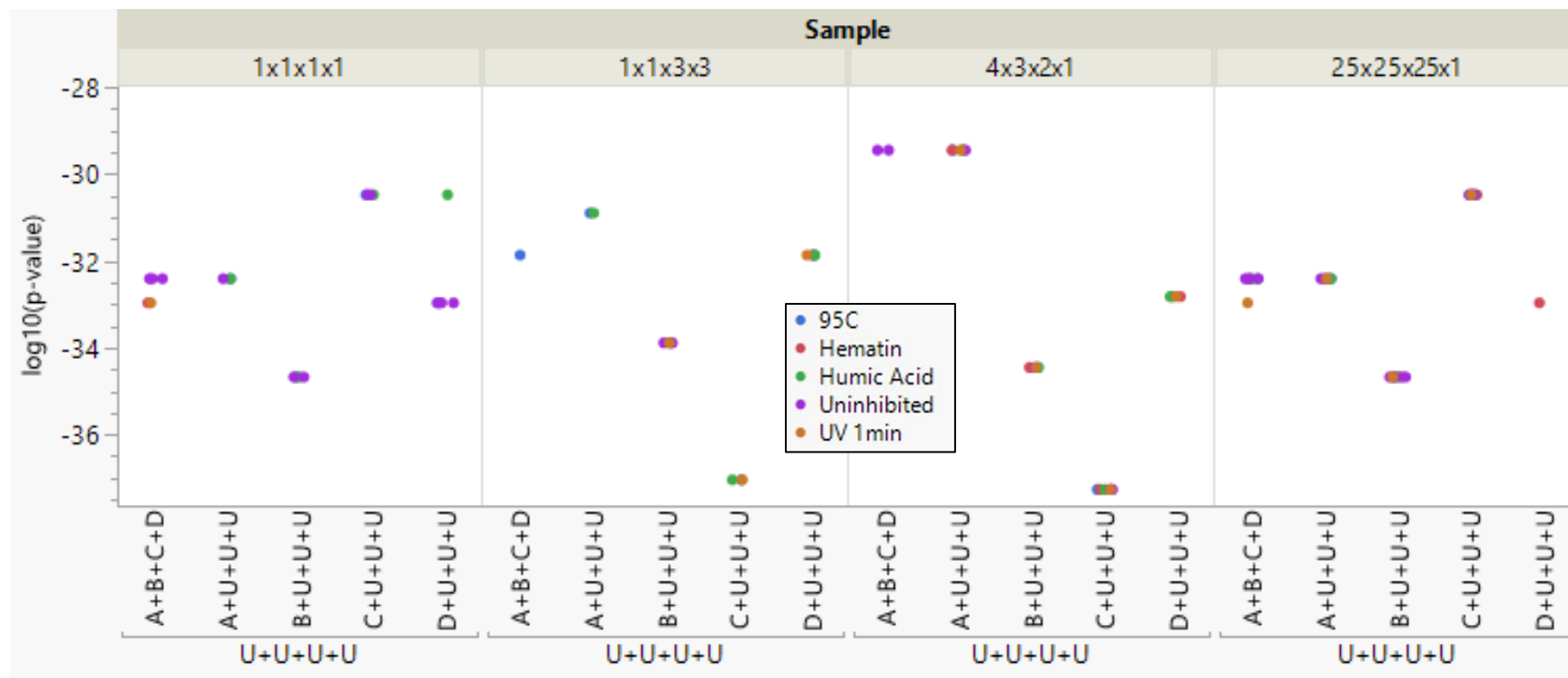


Figure 55: Specificity result for 4-person, 1 ng DNA input samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 53. Results are similar to Figure 53 indicating no significant difference between CE instruments.

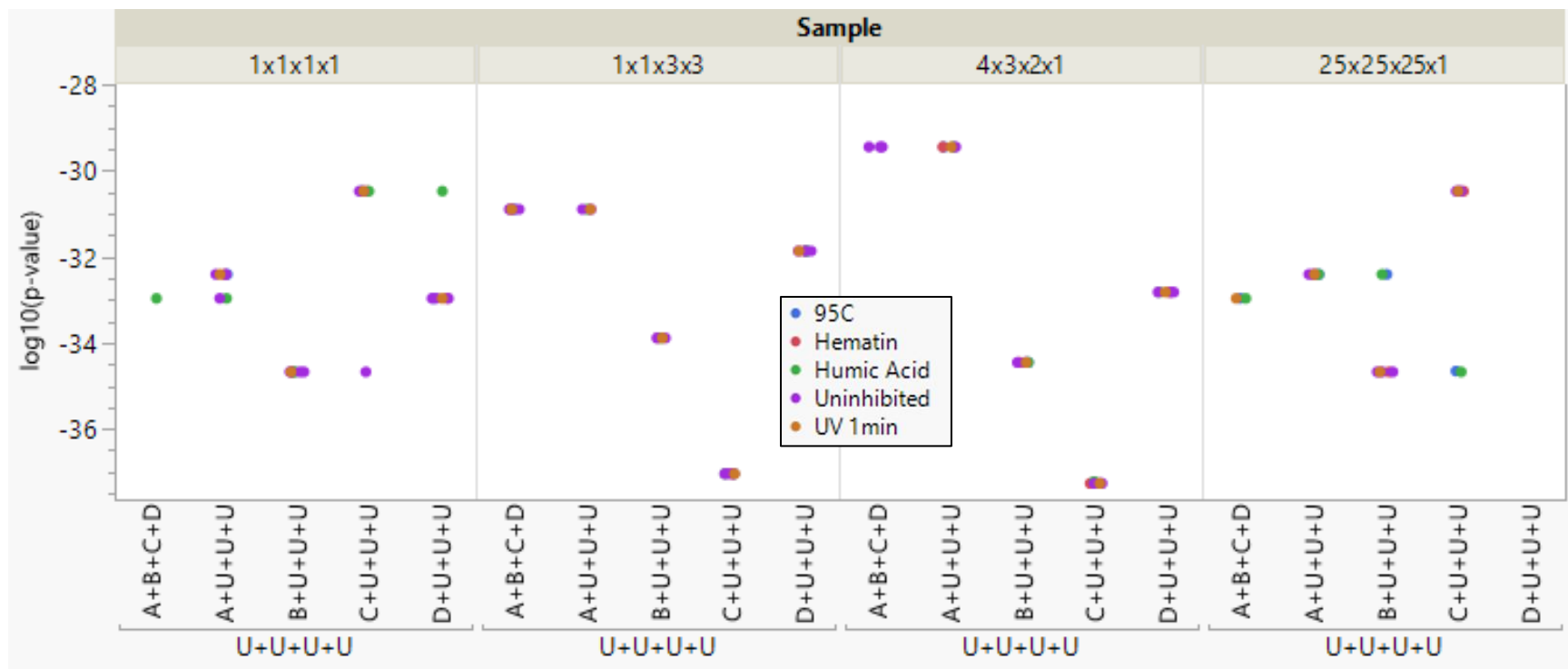


Figure 56: Specificity result for 4-person, 0.125 ng DNA input samples on SeqStudio Flex. Sample set up, naming, degradation/inhibition and LR hypotheses as in Figure 54. Results are similar to Figure 53 indicating no significant difference between CE instruments.

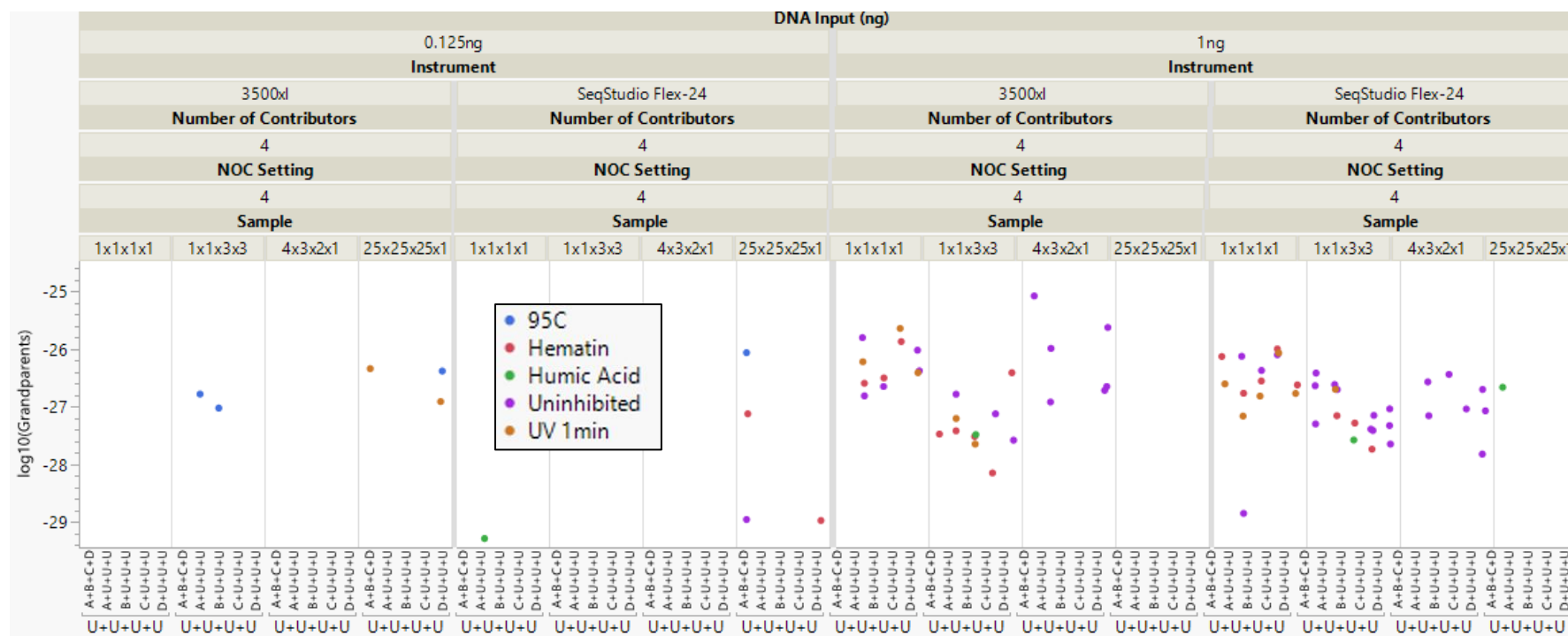


Figure 57: Specificity result for 4-person samples with sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 53. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA inputs are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being the grandparent of the POI. All calculated p-values are low, indicating a low probability that a grandparent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

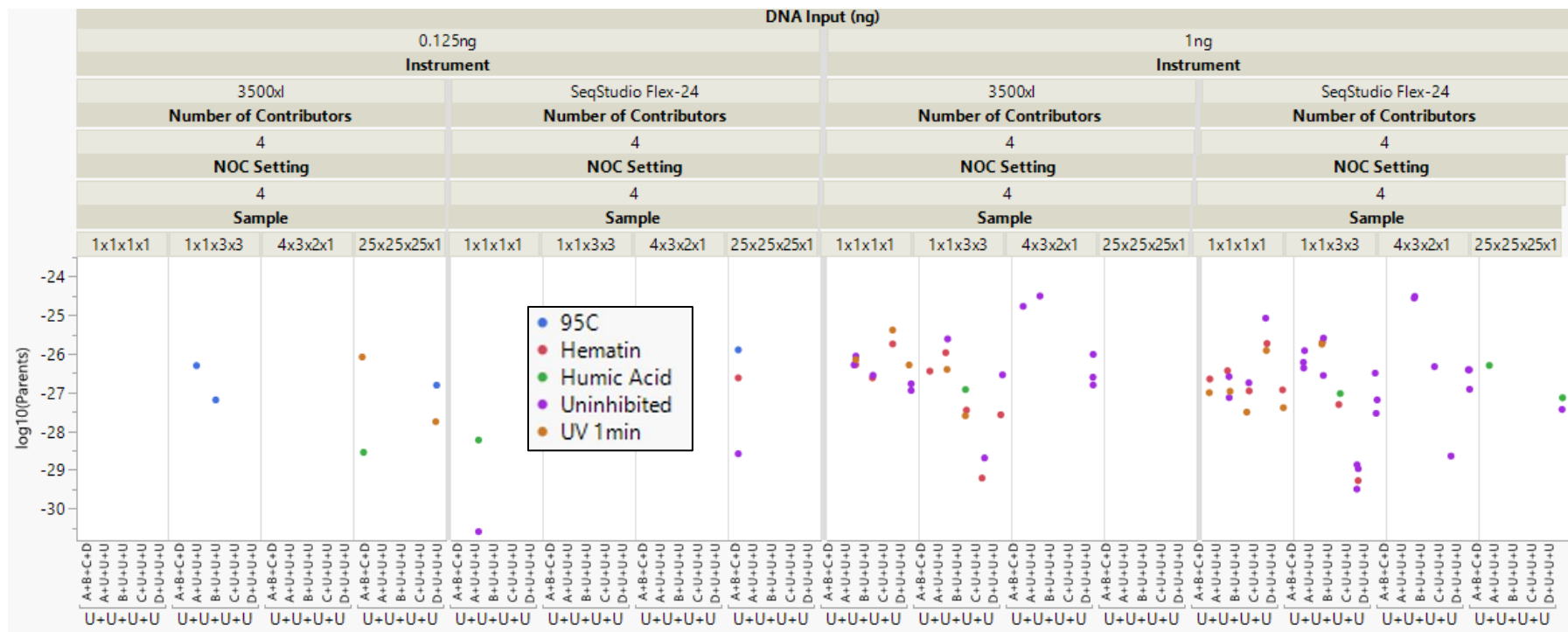


Figure 58: Specificity result for 4-person samples with sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 53. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA inputs are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being the parent of the POI. All calculated p-values are low, indicating a low probability that a parent of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

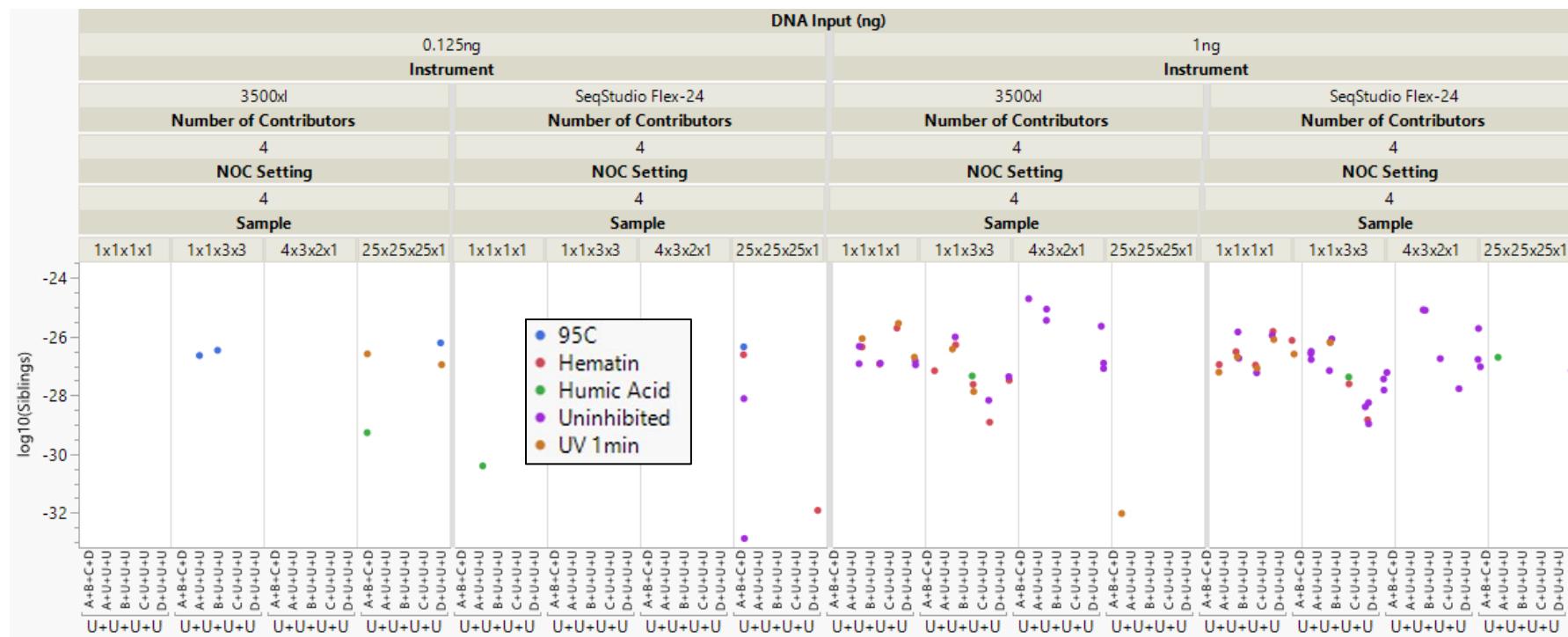


Figure 59: Specificity result for 4-person samples with sample set up, naming, degradation/inhibition, and LR hypotheses as in Figure 53. Results for the same scenarios run on 3500xL and SeqStudio Flex and with 0.125 ng and 1 ng DNA input are shown. The y-axis shows $\log_{10}(\text{p-value})$ result using simulated profiles consistent with being a sibling of the POI. All calculated p-values are low, indicating a low probability that a sibling of the POI would attain a LR at least as large as the one observed for the POI with the given model for LR.

3.4. Precision

3.4.1. Introduction

Studies should evaluate the variation in Likelihood Ratios calculated from repeated software analyses of the same input data. Some probabilistic genotyping software packages contain a random element to the algorithm, which means that identical results are not obtained from repeated analyses of the same data. GeneMapper PG Software employs a fully deterministic algorithm with no random element, which means that identical results are expected to be obtained for repeated analyses of the same data.

3.4.2. Methods and Results

To test whether an identical result was obtained in repeated analyses of the same input data, 24 LR scenarios from the Sensitivity and Specificity sections were run twice on different computers. An identical $\log_{10}(\text{LR})$ result was obtained in each case.

Two further LR scenarios were each run twice – once in the GeneMapper PG Software user interface (UI) and once via the command line interface (CLI). Identical results were obtained for same scenario run in the UI and CLI.

3.5. Case Type Samples

3.5.1. Introduction

Studies are required that assess a range of data types exhibiting features representative of those typically encountered by testing laboratories. These include those derived from mixtures and single-source samples, such as stutter, masked/shared alleles, differential and preferential amplification, degradation and inhibition.

These studies should demonstrate sample and/or data types that can be reliably evaluated using the probabilistic genotyping system.

3.5.2. Methods

4,244 LR_s were calculated in this developmental validation, covering profiles obtained from mixed and single-source samples, different degradation/inhibition conditions, and include many examples of stutter, masked/shared alleles and preferential amplification.

Thirteen mixtures were prepared as per Table 1.

Table 1: Preparation of the 13 mixed samples used in this work. A, B, C, and D refer to single-source samples from known donors, with sex and ethnicity as described in Table 2. P1 and P2 are single-source samples from individuals who are the parents of the Ch (child) sample. 'NIST Control' is a mixture of two control DNA samples, as described in Section 3.6.2. Numerical values in the table refer to the relative proportions of each of the given individual samples in the mixed sample. e.g. Mixture 2' is a 40 to 1 mixture of individuals A and B. (Table continues on next page).

Mixture No.	Contributors to Mixture							
	A	B	C	D	NIST Control	P1	P2	Ch
1	1	1						
2	40	1						
3	10	1						
4	1	300						
5	1	1	1					
6	3	2	1					
7	1	5	10					

Mixture No.	Contributors to Mixture							
	A	B	C	D	NIST Control	P1	P2	Ch
8	1	1	1	1				
9	1	1	3	3				
10	4	3	2	1				
11	25	25	25	1				
12					1			
13						1	1	1

Table 2: Sex and Ethnicity of the individual samples in Table 1. (Note that Sample 'E' was analyzed as a single-source sample, but not included in mixed samples)

Individual Sample	Sex	Ethnicity
A	Male	Caucasian
B	Female	Asian
C	Male	African
D	Female	South American
E	Female	Caucasian

Preparations of the mixed samples from Table 1 were each subjected to degradation and inhibition as per Table 3.

Table 3: Degradation and inhibition treatments performed on the mixed samples from Table 1. (Abbreviations in table are used in the Figures in the Results section).

Degradation / Inhibition	Abbreviation
Uninhibited / no degradation	X
UV degradation for 1 minute	UV
Hematin inhibition (400µM)	HE
95°C heat degradation for 15 minutes	95C
Humic acid degradation (70ng/µL)	HA

The mixtures described in Table 1 had many masked/shared alleles. An illustration of those alleles in Mixtures 1 to 11 is shown in Table 4.

Table 4. Genotypes of individual samples used to make mixed samples. Samples A, B, C, and D refer to sample names from Table 1. Blue shaded cells are alleles shared between one or more individuals. Gray shaded cells are loci with no genotypic data

Locus	Genotypes of Individual Samples							
	A		B		C		D	
D3S1358	16	17	14	17	15	16	15	17
vWA	16	17	14	19	18	18	14	15
D16S539	12	12	9	9	11	11	10	13
CSF1PO	9	10	10	12	7	11	12	13
TPOX	10	11	11	12	8	9	8	11
Yindel	2	2			2	2		
AMEL	X	Y	X	X	X	Y	X	X
D8S1179	13	15	13	13	12	13	14	14
D21S11	28	30	30	30	29	30	29	30
D18S51	12	14	15	17	16	17	16	20
DYS391	11	11			10	10		
D2S441	14	14	10	12	11	14	10	14
D19S433	13	14	12	14	12.2	12.2	12	13
TH01	9.3	9.3	7	7	8	9	9	9.3
FGA	21	24	21	23	22	24	24	26
D22S1045	11	15	15	17	10	11	15	16
D5S818	11	11	11	12	10	12	10	12
D13S317	11	14	9	9	12	13	8	8
D7S820	11	12	7	11	8	12	8	11
SE33	17	25.2	22.2	28.2	19	22	14	16
D10S1248	14	16	12	15	12	14	14	15
D1S1656	13	16	14	16	14	14	14	17.3
D12S391	21	22	15	20	15	18	16	22
D2S1338	19	20	19	22	20	21	19	24

These mixtures were then tested by calculating multiple LR_s. The LR scenarios for Mixtures 1 to 11 were as described in Table 5.

Table 5: LR hypotheses tested for Mixtures 1 to 11. U = an unrelated, unknown contributor. Scenarios highlighted in yellow were also tested with calculation set to NOC = true-NOC plus one and NOC = true-NOC minus one where applicable.

Number of Contributors	H _p	H _d
1	A	U
	B	U
	C	U
	D	U
	E	U
2	A + U	U + U
	B + U	U + U
	A + B	A + U
	A + B	B + U
	A + B	U + U
3	A + U + U	U + U + U
	B + U + U	U + U + U
	C + U + U	U + U + U
	A + B + U	U + U + U
	A + C + U	U + U + U
	B + C + U	U + U + U
	A + B + C	U + U + U
4	A + U + U + U	U + U + U + U
	B + U + U + U	U + U + U + U
	C + U + U + U	U + U + U + U
	D + U + U + U	U + U + U + U
	A + B + C + D	U + U + U + U

3.5.3. Case Type Samples Results

The results for the LR scenarios for the case-type samples are those described in the previous Sensitivity and Specificity sections. The same data applies to all of these sections. The following results give further detail on the peak heights and number of peaks observed in the mixed and single-source samples used, the effect of the degradation/inhibition and the effect of varying input DNA and instrument platform.

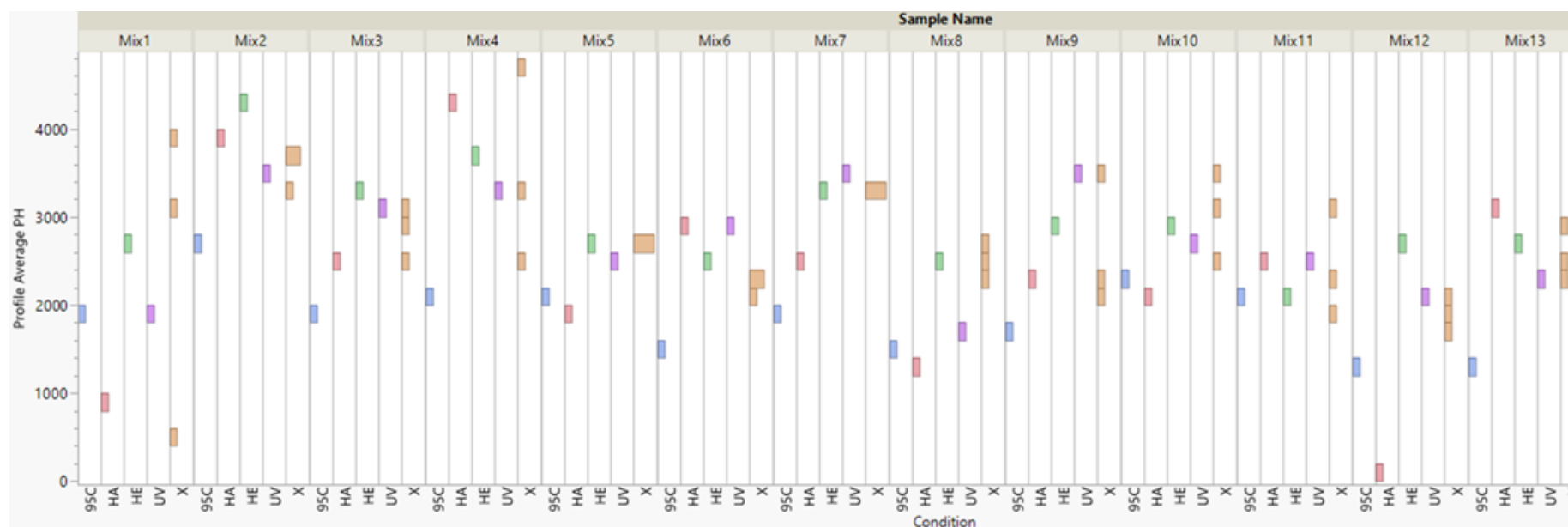


Figure 60: Average profile peak height (PH) in RFU observed for Mixtures 1-13, as described in Table 1. 'Condition' in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on 3500xL with 1 ng of total input DNA. Undegraded / uninhibited samples ('X') were run in triplicate. Other samples run once.

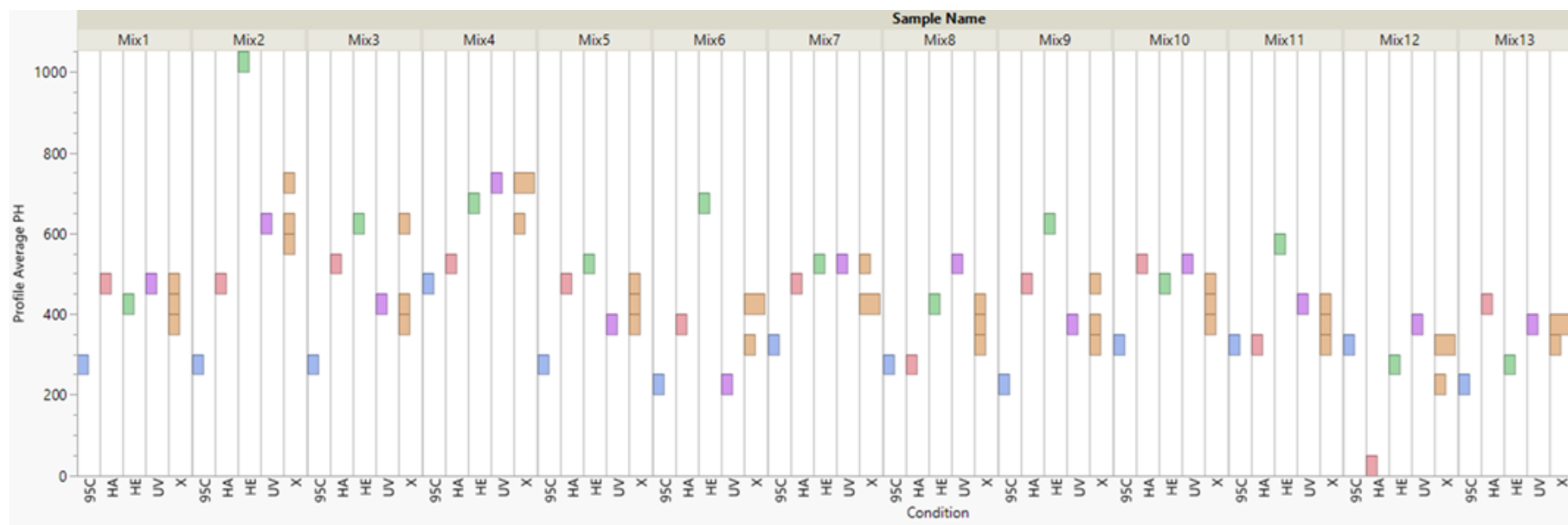


Figure 61: Average profile peak height (PH) in RFU observed for Mixtures 1-13, as described in Table 1. ‘Condition’ in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on 3500xL with 0.125 ng of total input DNA. Undegraded / uninhibited samples (‘X’) were run in triplicate. Other samples run once.

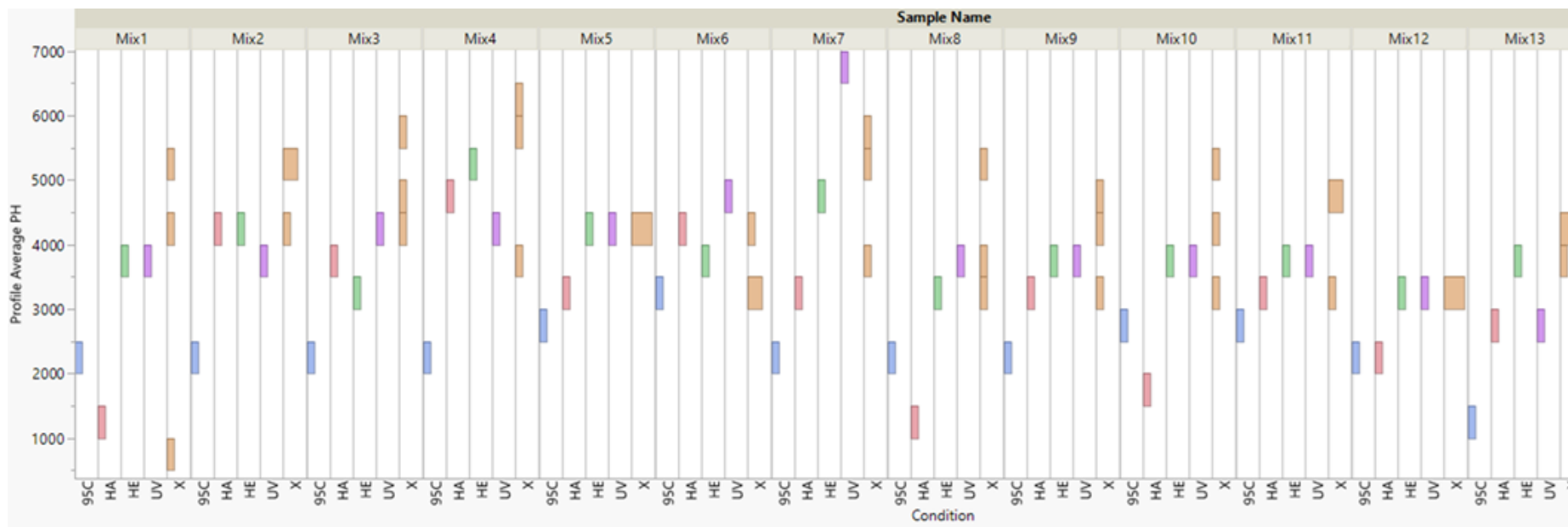


Figure 62: Average profile peak height (PH) in RFU observed for Mixtures 1-13, as described in Table 1. ‘Condition’ in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on SeqStudio Flex with 1 ng of total input DNA. Undegraded / uninhibited samples (‘X’) were run in triplicate. Other samples run once.

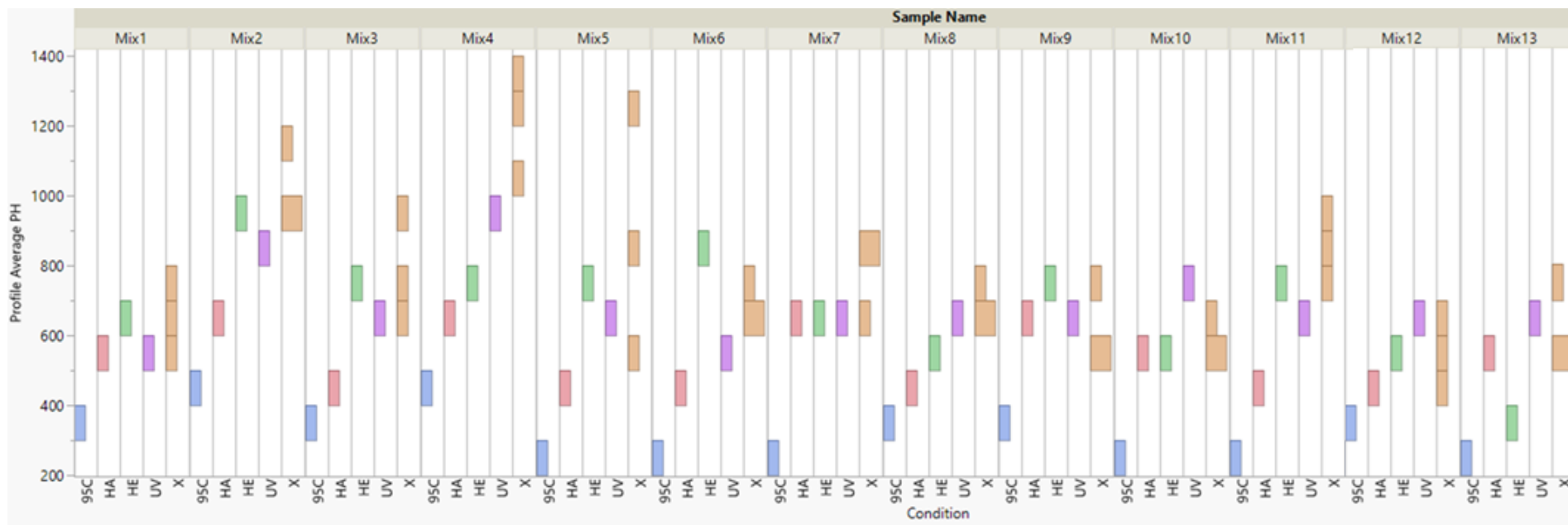


Figure 63: Average profile peak height (PH) in RFU observed for Mixtures 1-13, as described in Table 1. 'Condition' in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on SeqStudio Flex with 0.125 ng of total input DNA. Undegraded / uninhibited samples ('X') were run in triplicate. Other samples run once.

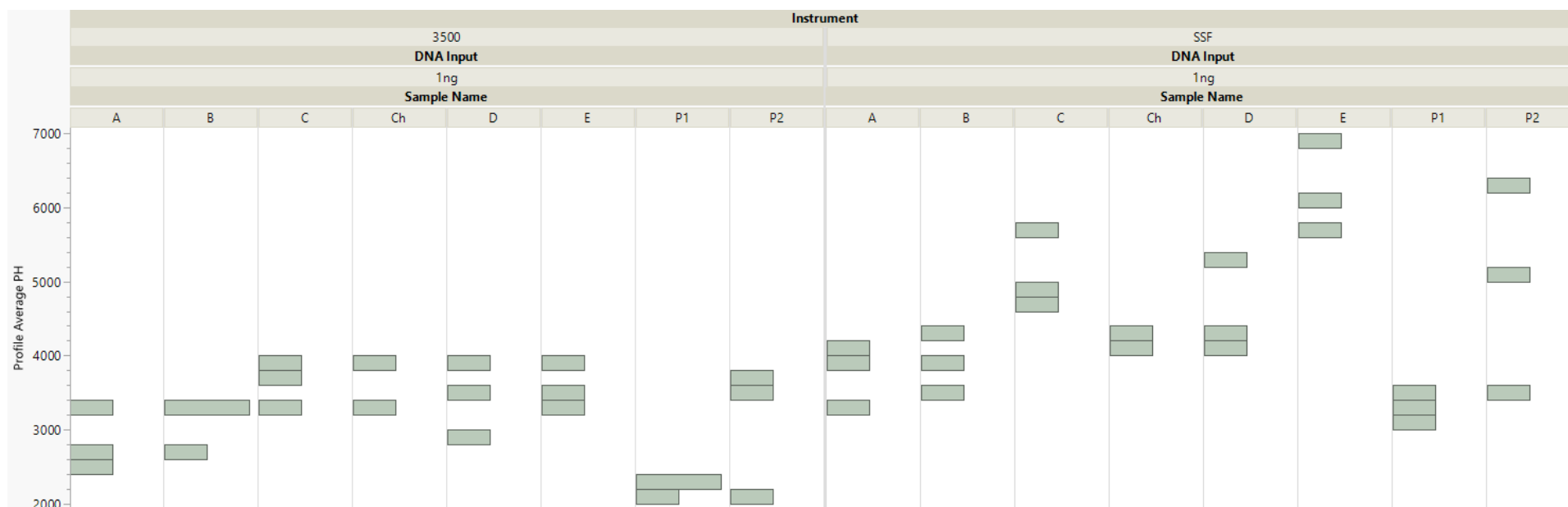


Figure 64: Average profile peak height (PH) in RFU observed for single source samples, as described in Table 1. Samples were not exposed to any degradation or inhibition and were run with 1 ng of input DNA. Data for 3500xL (3500) and SeqStudio Flex (SSF) shown. All samples run in triplicate.

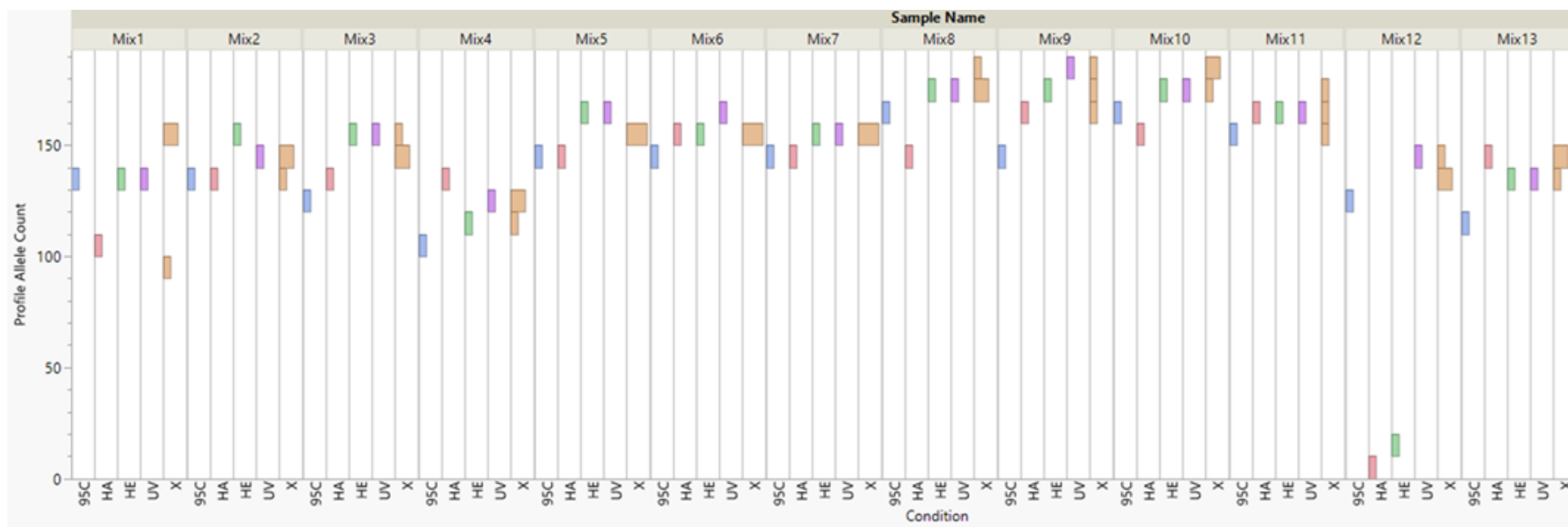


Figure 65: Profile peak count (y-axis) observed for Mixtures 1-13, as described in Table 1. 'Condition' in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on 3500xL with 1 ng of total input DNA. Undegraded / uninhibited samples ('X') were run in triplicate. Other samples run once. (Note that the number shown represents 'peaks', not necessarily 'alleles': data was analyzed with no stutter filter in GeneMapper ID-X. As a result, many peaks in the input data are likely to be stutter.)

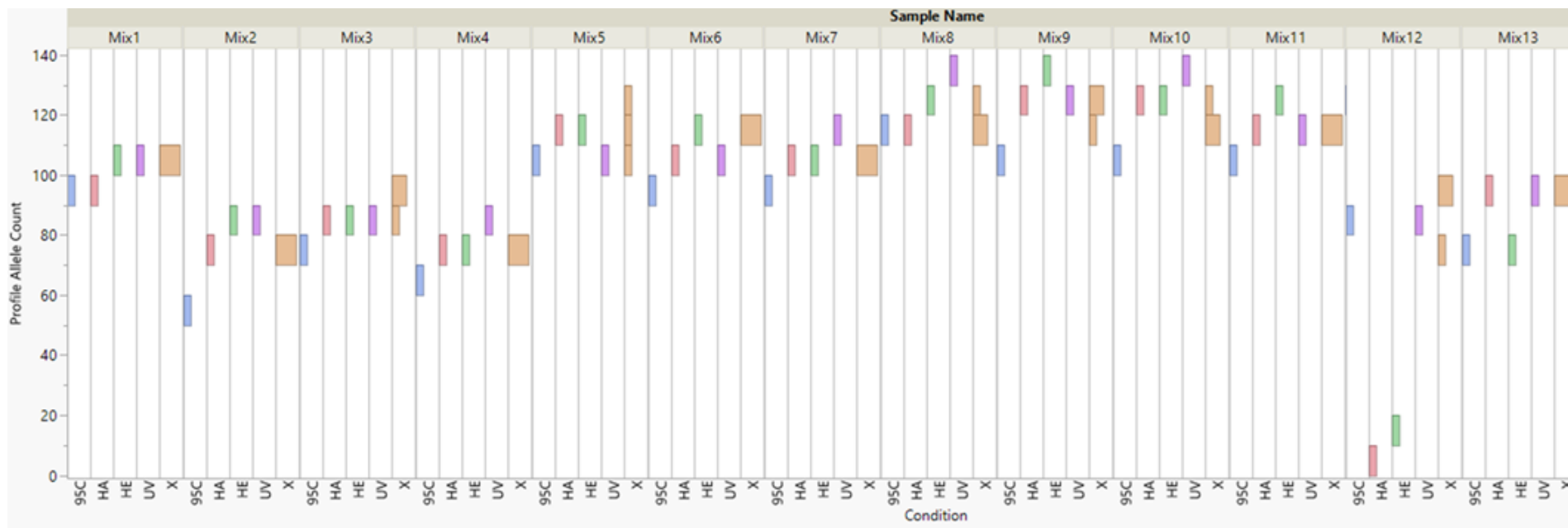


Figure 66: Profile peak count (y-axis) observed for Mixtures 1-13, as described in Table 1. 'Condition' in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on 3500xL with 0.125 ng of total input DNA. Undegraded / uninhibited samples ('X') were run in triplicate. Other samples run once. (Note that the number shown represents 'peaks', not necessarily 'alleles': data was analyzed with no stutter filter in GeneMapper ID-X. As a result, many peaks in the input data are likely to be stutter.)

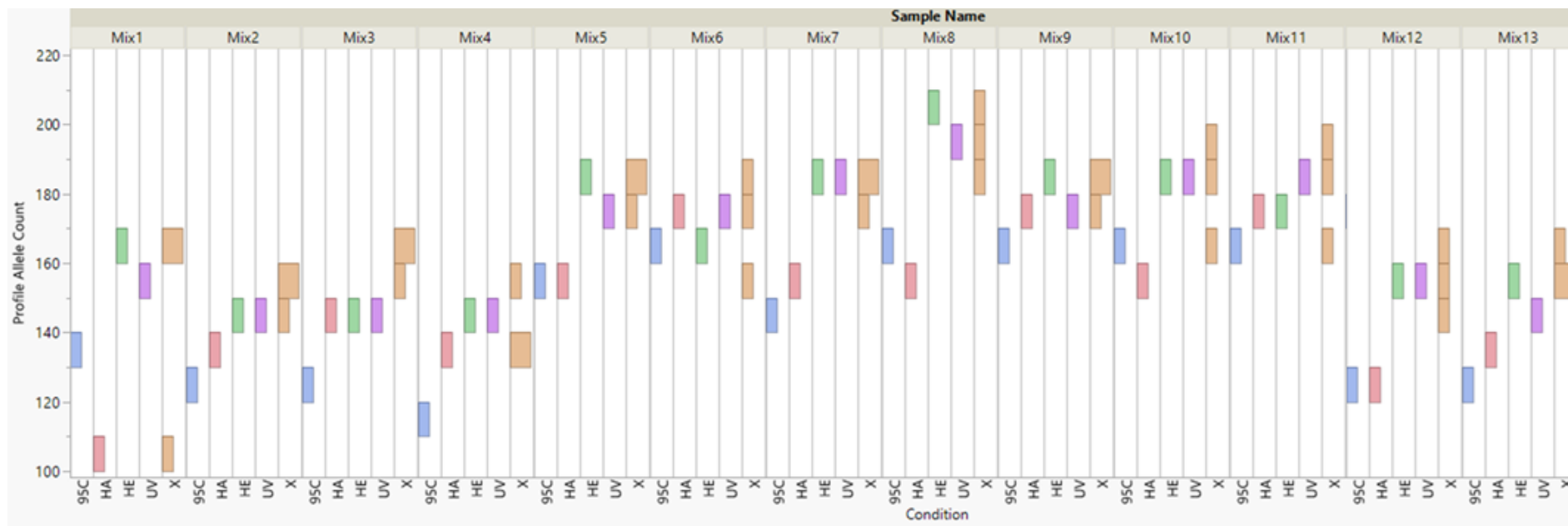


Figure 67: Profile peak count (y-axis) observed for Mixtures 1-13, as described in Table 1. ‘Condition’ in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on SeqStudio Flex with 1 ng of total input DNA. Undegraded / uninhibited samples (‘X’) were run in triplicate. Other samples run once. (Note that the number shown represents ‘peaks’, not necessarily ‘alleles’: data was analyzed with no stutter filter in GeneMapper ID-X. As a result, many peaks in the input data are likely to be stutter.)

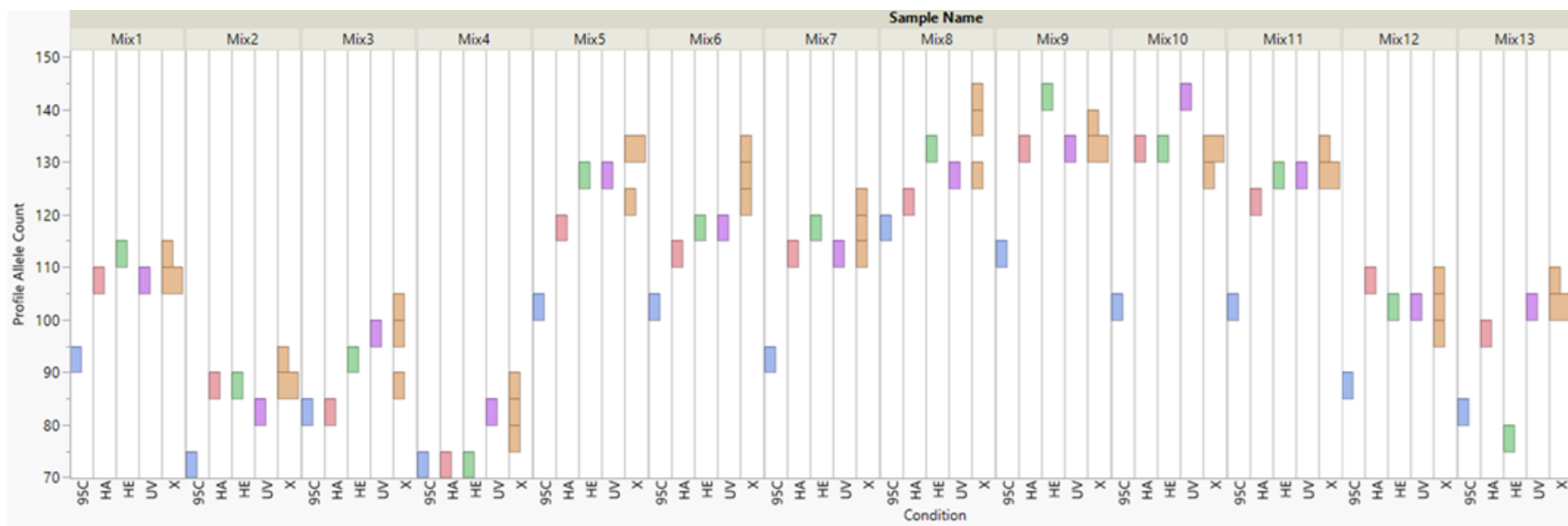


Figure 68: Profile peak count (y-axis) observed for Mixtures 1-13, as described in Table 1. 'Condition' in the x-axis describes the inhibition/degradation applied to each sample, as in Table 3. Samples run on SeqStudio Flex with 0.125 ng of total input DNA. Undegraded / uninhibited samples ('X') were run in triplicate. Other samples run once. (Note that the number shown represents 'peaks', not necessarily 'alleles': data was analyzed with no stutter filter in GeneMapper ID-X. As a result, many peaks in the input data are likely to be stutter.)

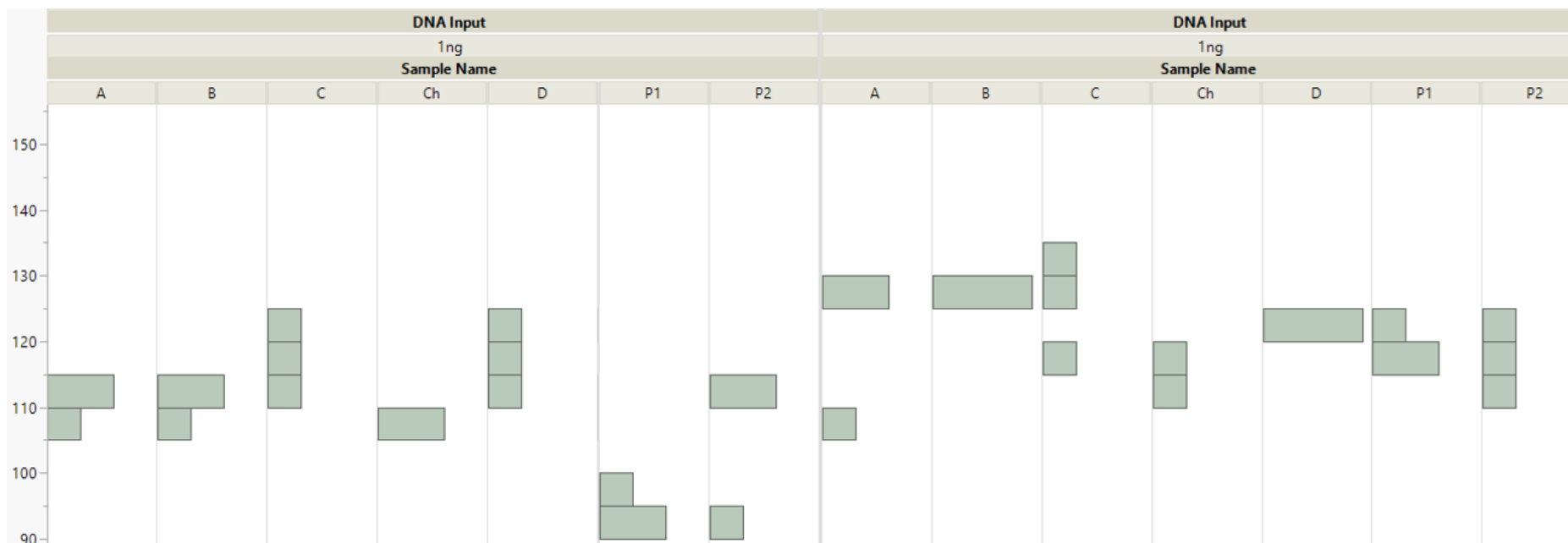


Figure 69: Profile peak count observed for single source samples, as described in Table 1. Samples were not exposed to any degradation or inhibition and were run with 1 ng of input DNA. Data for 3500xL (3500) and SeqStudio Flex (SSF) shown. All samples run in triplicate. (Note that the number shown represents 'peaks', not necessarily 'alleles': data was analyzed with no stutter filter in GeneMapper ID-X. As a result, many peaks in the input data for single-source samples are likely to be stutter.)

3.6. Control Samples

3.6.1. Introduction

Studies are required to evaluate whether correct results are obtained with control samples.

3.6.2. Methods

Two mixed National Institute of Standards and Technology (NIST) control DNA samples were analyzed in this developmental validation. These were NIST Standard Reference Material (SRM) 2391c and NIST SRM 2391d. The single-source samples that comprise these mixed samples are detailed in Table 6.

Table 6: Sample configuration for Control Sample testing

NIST SRM	Mixed component label (NIST)	Single-source label (NIST)	Single-source name used in this testing	Sex of single-source Donor
2391c	D	A	F2	Female
		C	M2	Male
2391d	D	A	F	Female
		C	M	Male

These samples were prepared and analyzed with the same methods described in Section 3.2.2 and were exposed to the degraded and inhibitory conditions as described in Table 3.

3.6.3. Control Sample Results

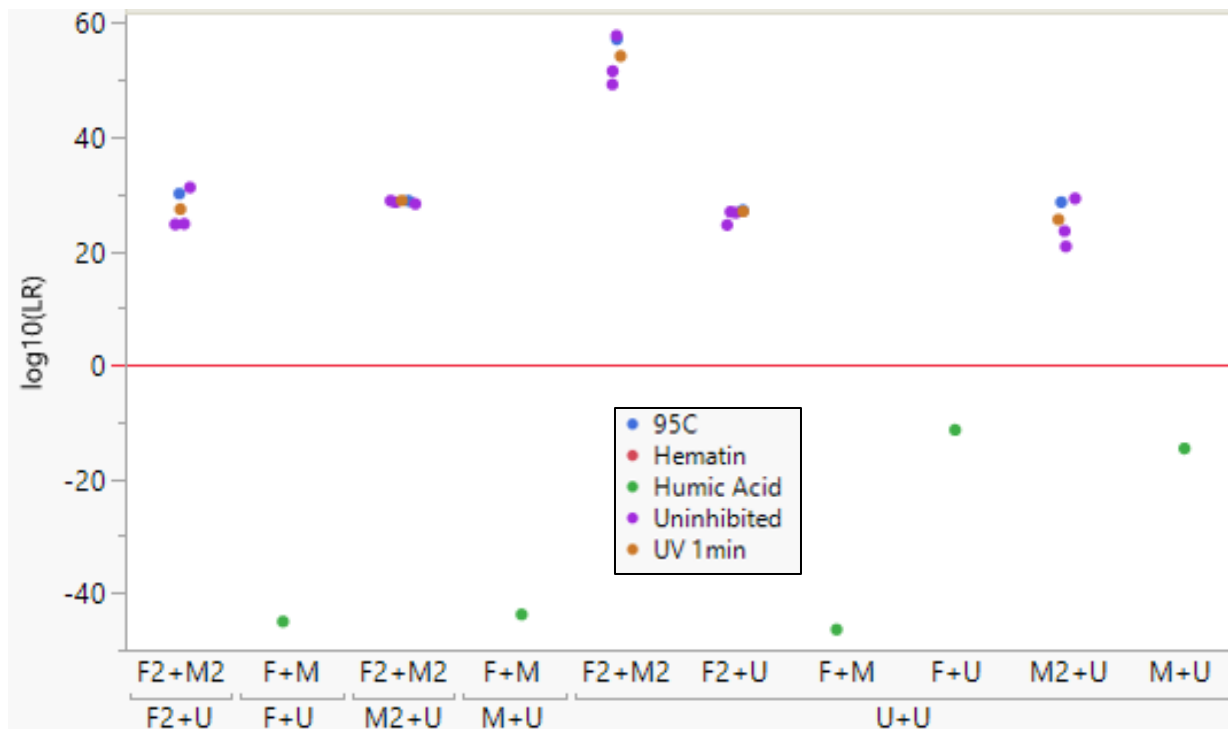


Figure 70: Sensitivity results for Control Samples. 1 ng of total input DNA run on 3500xL. NOC = true-NOC. Degradation/inhibition performed on samples as per color key on chart. (See Table 3 for full details). LRs calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where 'U' = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. Generally, LR was supportive of H_p over H_d . Samples treated with hematin and humic acid either produced too few peaks for an LR result to be calculated, or produced an LR supportive of H_d over H_p .

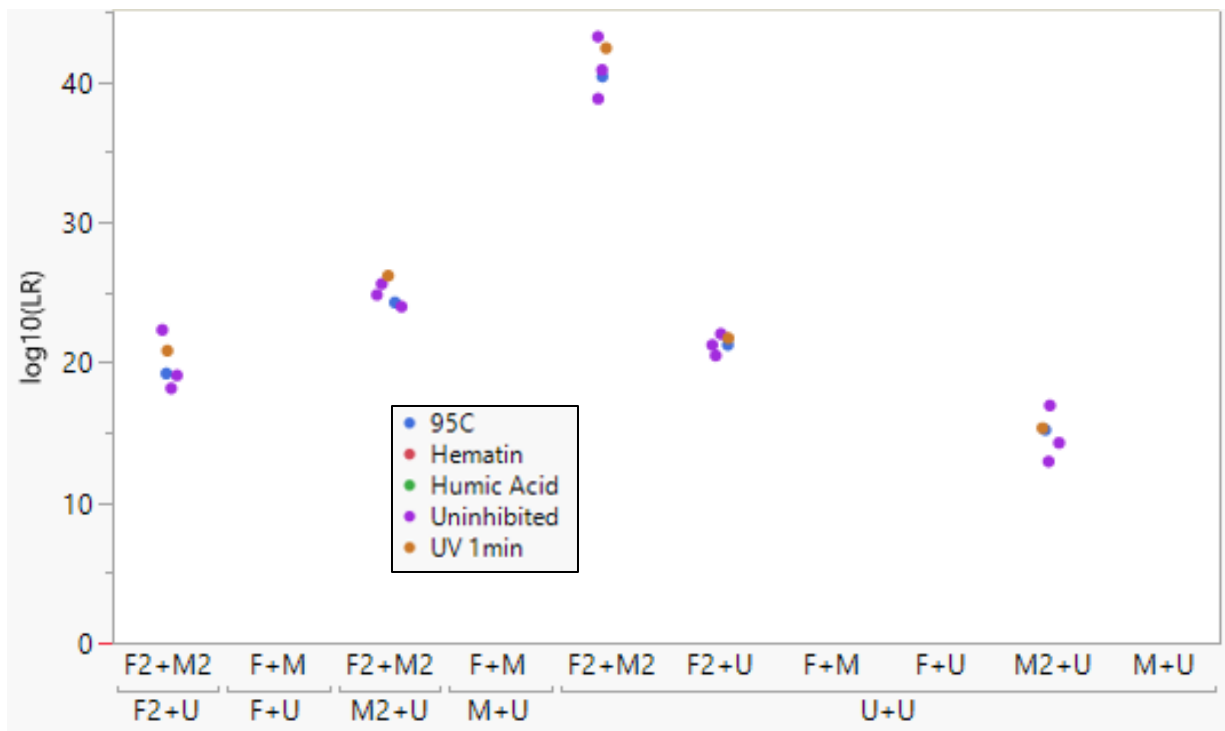


Figure 71: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on 3500xL. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 70. Generally LR is supportive of H_p over H_d . Samples treated with hematin and humic acid produced too few peaks to calculate an LR result.

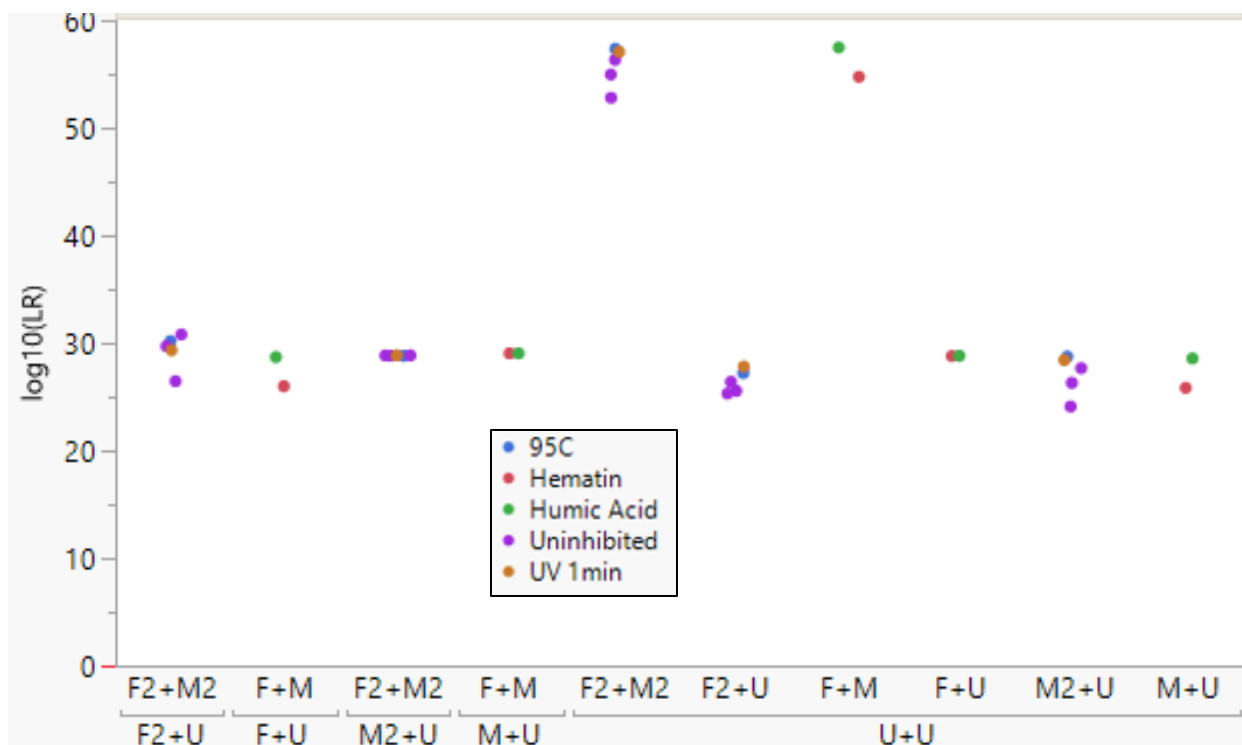


Figure 72: Sensitivity results for Control Samples. 1 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 70. Generally LR is supportive of H_p over H_d .

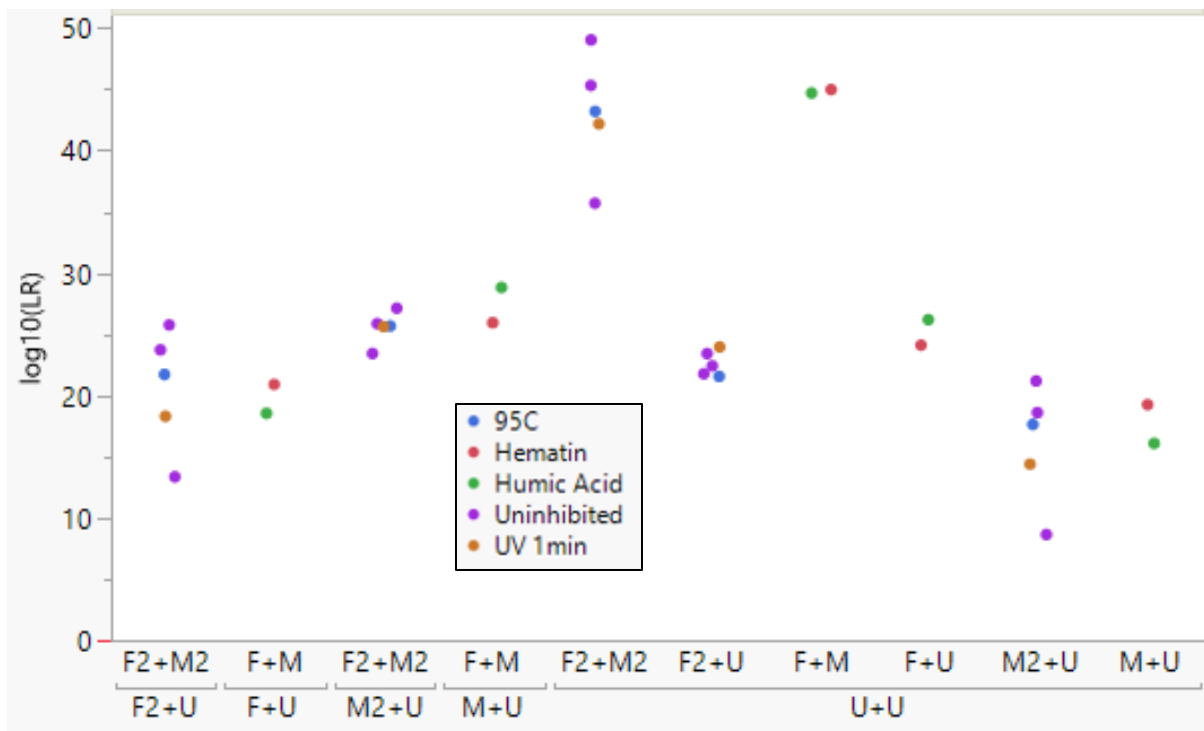


Figure 73: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 70. Generally LR is supportive of H_p over H_d .

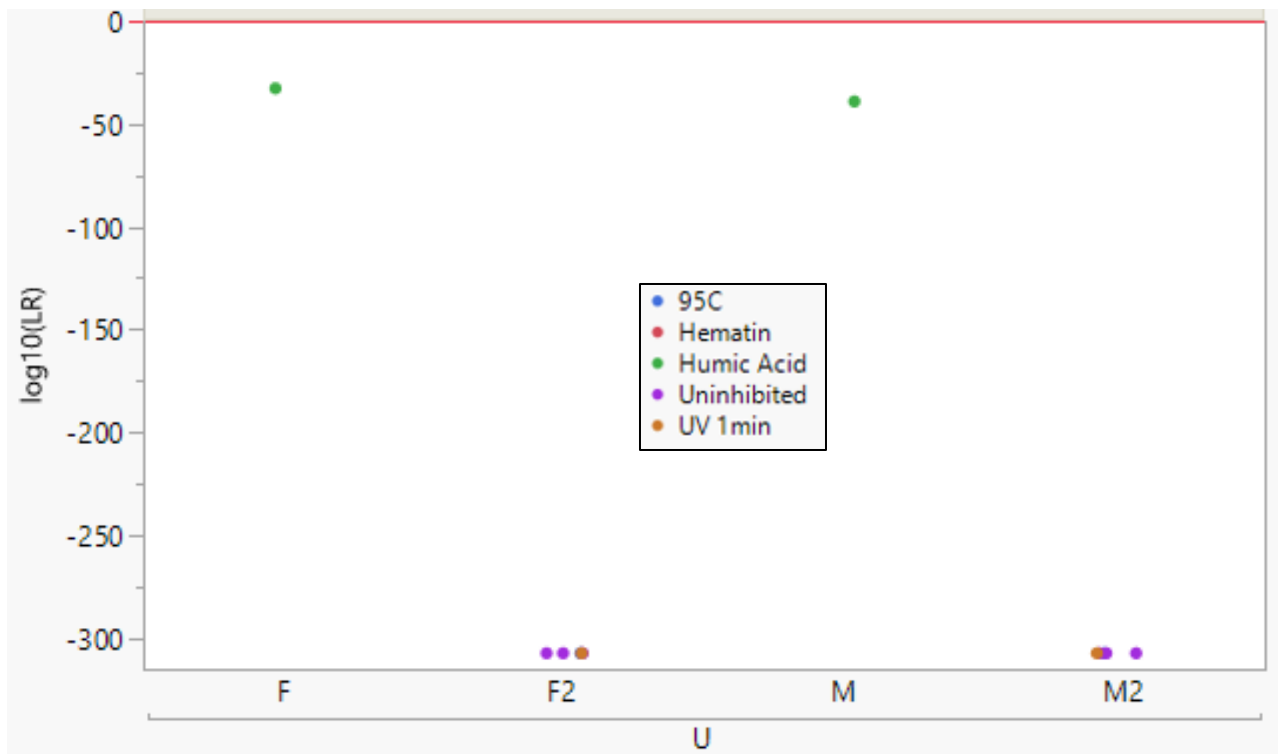


Figure 74: Sensitivity results for Control Samples. 1 ng of total input DNA run on 3500xL. NOC = true-NOC minus – 1 (i.e. NOC incorrectly set to 1). Degradation/inhibition as in Figure 70. LRs calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where ‘U’ = unknown contributor. Calculated as the probability of observing the profile given that the named individual or ‘U’ contributed. LR is supportive of H_d over H_p as there are too many peaks for 1-person to be a reasonable solution.

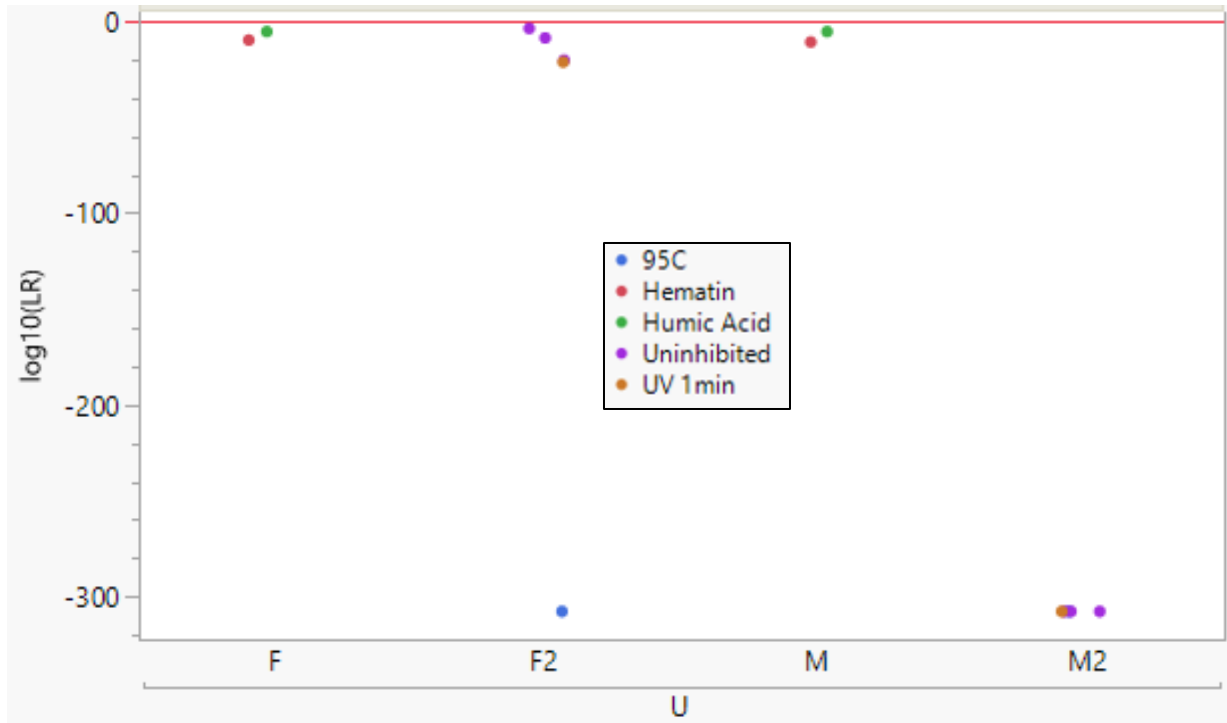


Figure 75: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on 3500xL. NOC = true-NOC minus – 1 (i.e. NOC incorrectly set to 1). Degradation/inhibition and LR hypotheses as in Figure 74. LR is supportive of H_d over H_p as there are too many peaks for 1-person to be a reasonable solution.

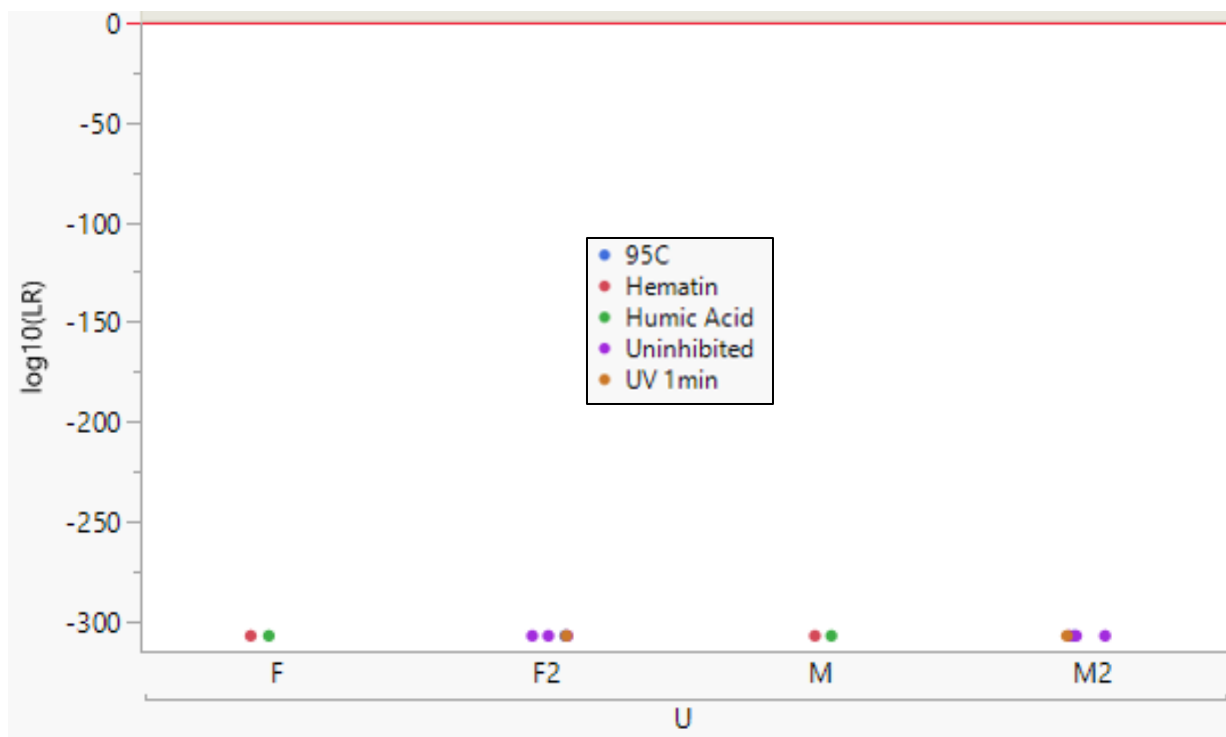


Figure 76: Sensitivity results for Control Samples. 1 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC minus – 1 (i.e. NOC incorrectly set to 1). Degradation/inhibition and LR hypotheses as in Figure 74. LR is supportive of H_d over H_p as there are too many peaks for 1-person to be a reasonable solution.

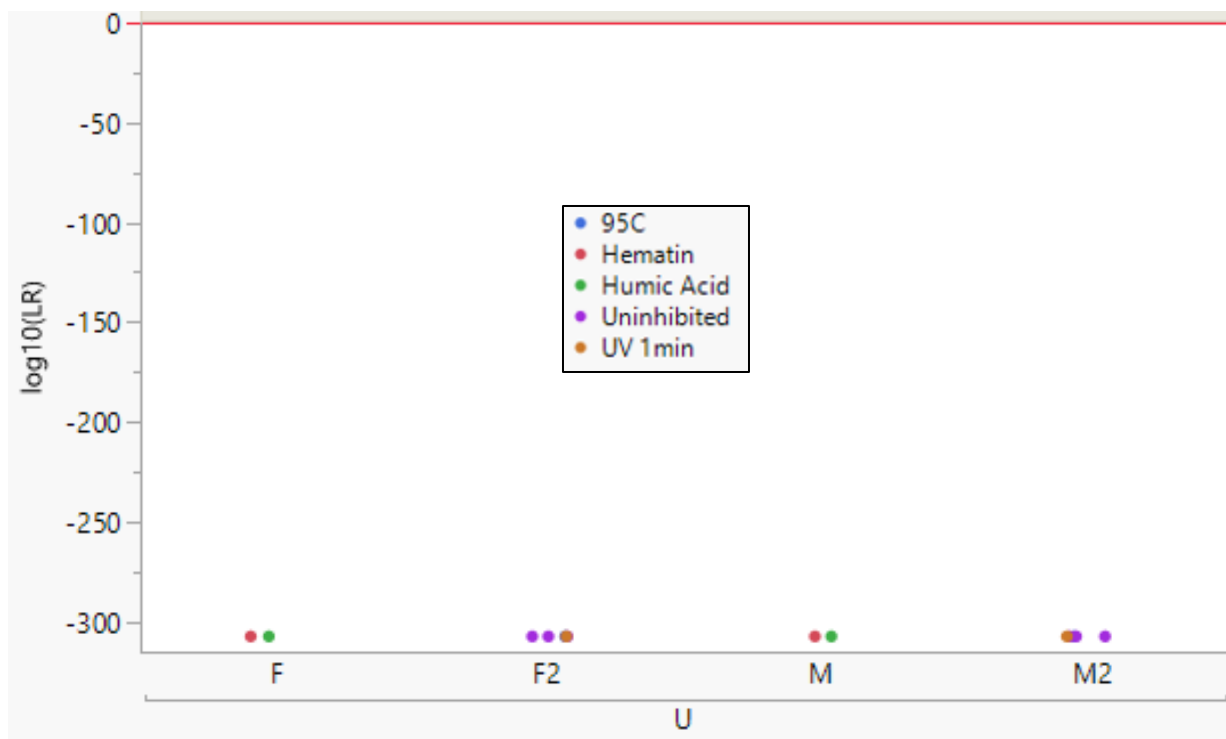


Figure 77: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC minus – 1 (i.e. NOC incorrectly set to 1). Degradation/inhibition and LR hypotheses as in Figure 74. LR is supportive of H_d over H_p as there are too many peaks for 1-person to be a reasonable solution.

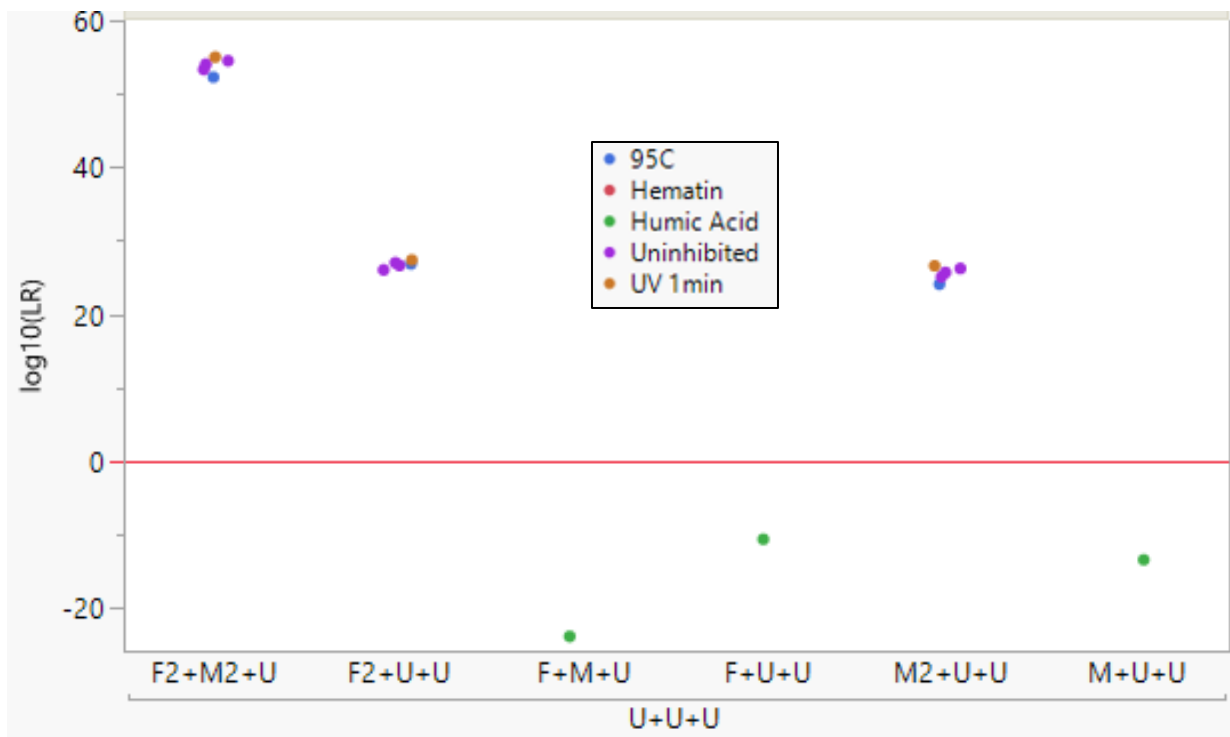


Figure 78: Sensitivity results for Control Samples. 1 ng of total input DNA run on 3500xL. NOC = true-NOC plus 1 (i.e. NOC incorrectly set to 3). Degradation/inhibition as in Figure 70. LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that three unknown persons contributed. 'U' = an unknown contributor. LR is mostly supportive of H_p over H_d – most samples have enough peaks present for 3-person to be a supported solution. Humic acid treated samples show support for H_d over H_p , likely due to the presence of fewer peaks.

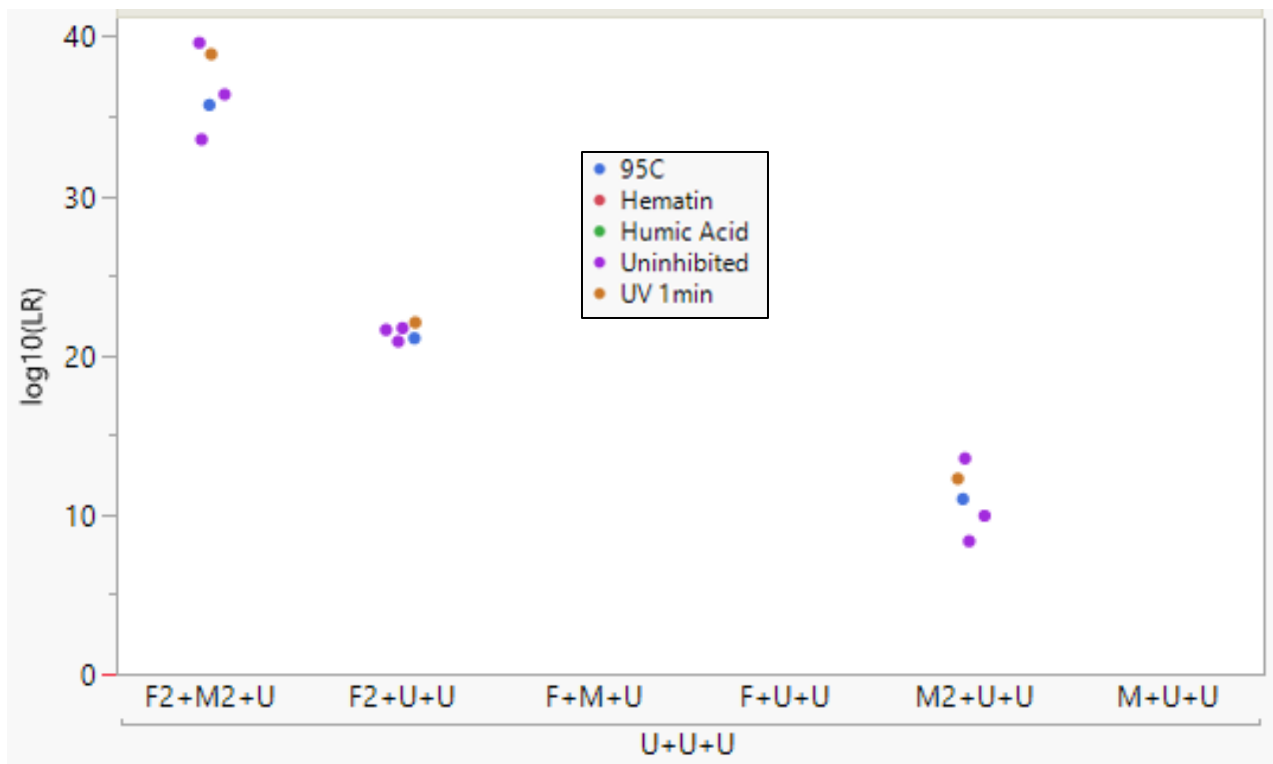


Figure 79: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on 3500xL. NOC = true-NOC plus 1 (i.e. NOC incorrectly set to 3). Degradation/inhibition and LR hypotheses as in Figure 78. LR is generally supportive of H_p over H_d – most samples have enough peaks for 3-person to be a supported solution. Humic acid treated are missing due to having too few peaks present to analyze.

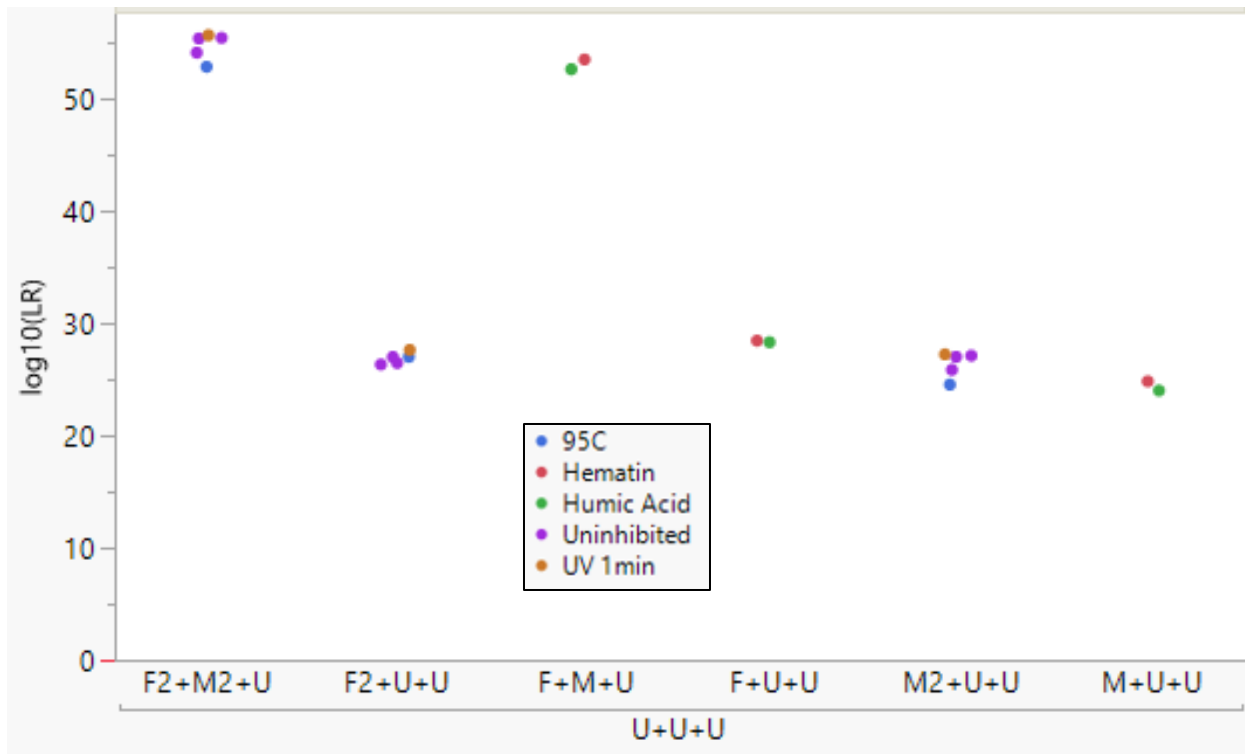


Figure 80: Sensitivity results for Control Samples. 1 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC plus 1 (i.e. NOC incorrectly set to 3). Degradation/inhibition and LR hypotheses as in Figure 78. LR is supportive of H_p over H_d – samples have enough peaks for 3-person to be a supported solution.

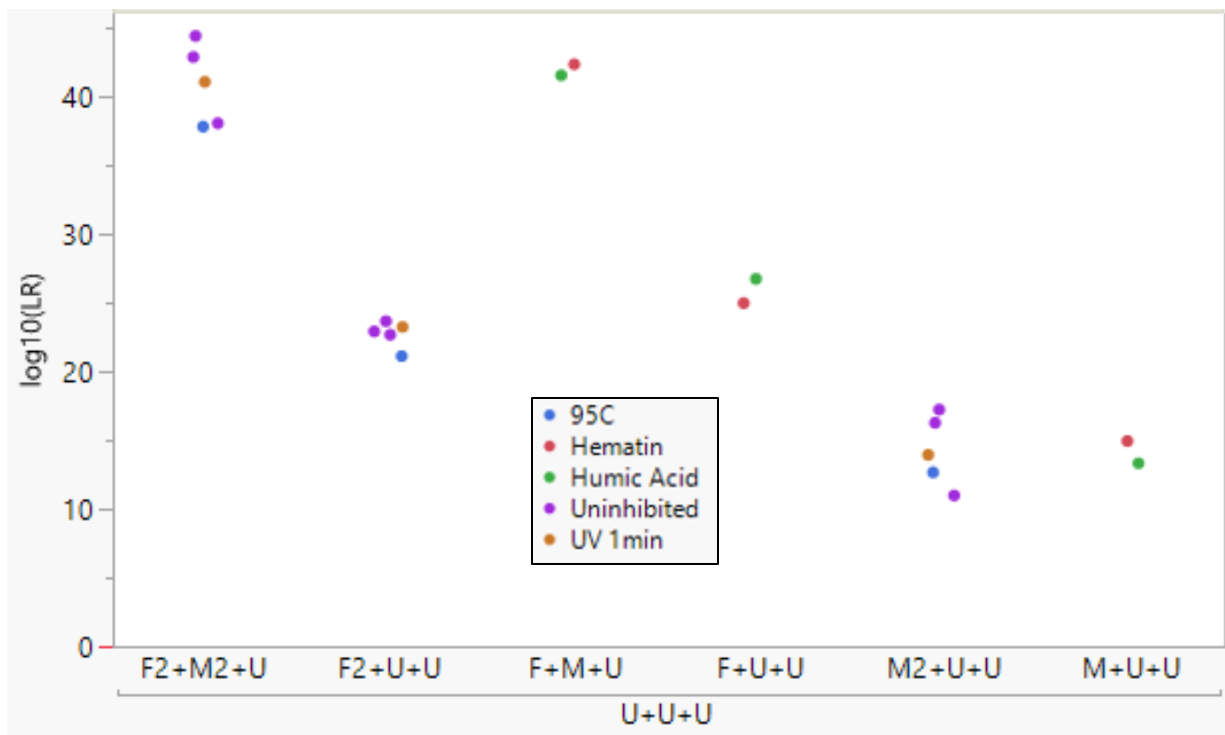


Figure 81: Sensitivity results for Control Samples. 0.125 ng of total input DNA run on SeqStudio Flex. NOC = true-NOC plus 1 (i.e. NOC incorrectly set to 3). Degradation/inhibition and LR hypotheses as in Figure 78. LR is supportive of H_p over H_d – samples have enough peaks for 3-person to be a supported solution.

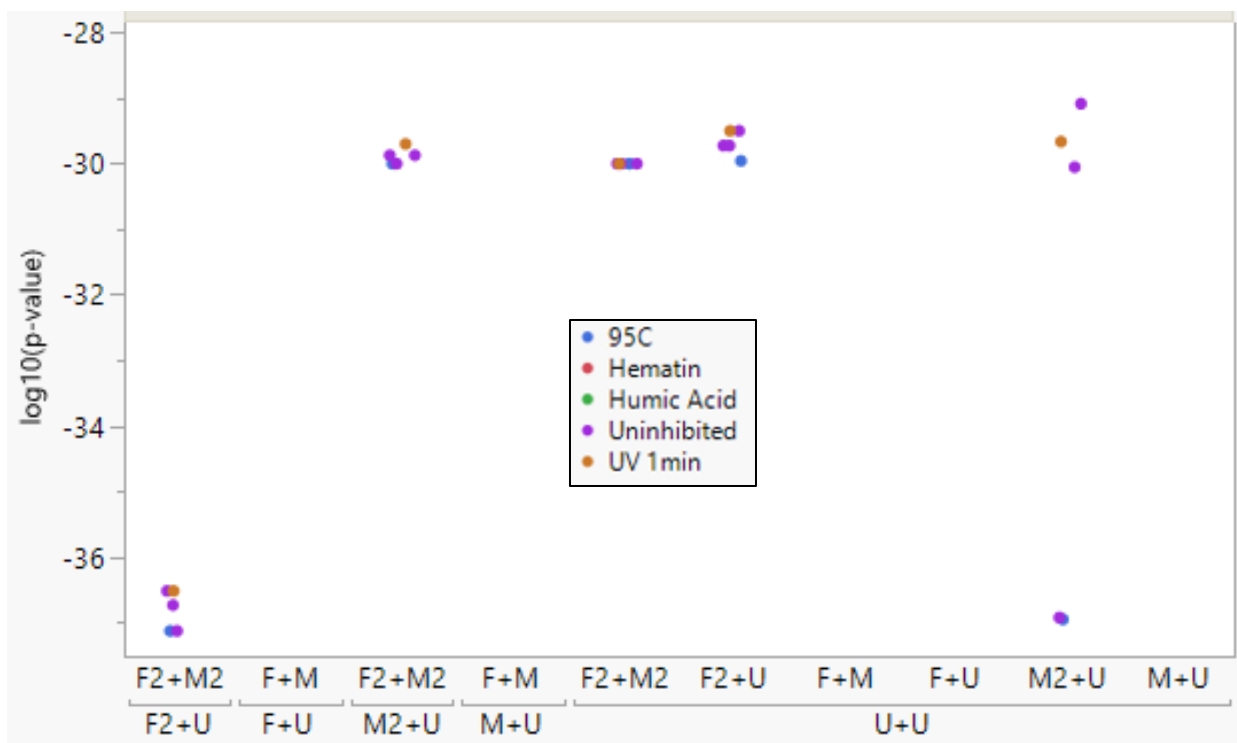


Figure 82: Specificity results for Control Samples. 1 ng of total input DNA run on 3500xL. Degradation/inhibition as in Figure 70. LR_s calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where 'U' = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. All p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

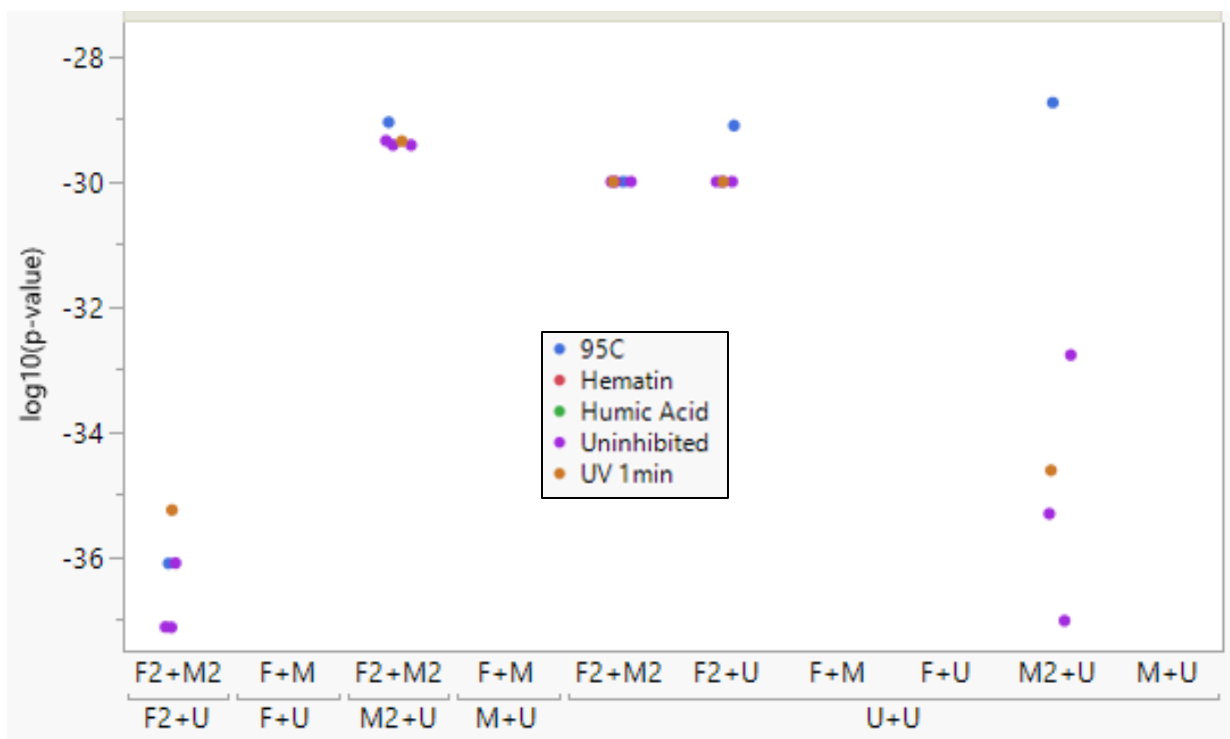


Figure 83: Specificity results for Control Samples. 0.125 ng of total input DNA run on 3500xL. Degradation/inhibition and LR hypothesis as in Figure 82. All p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

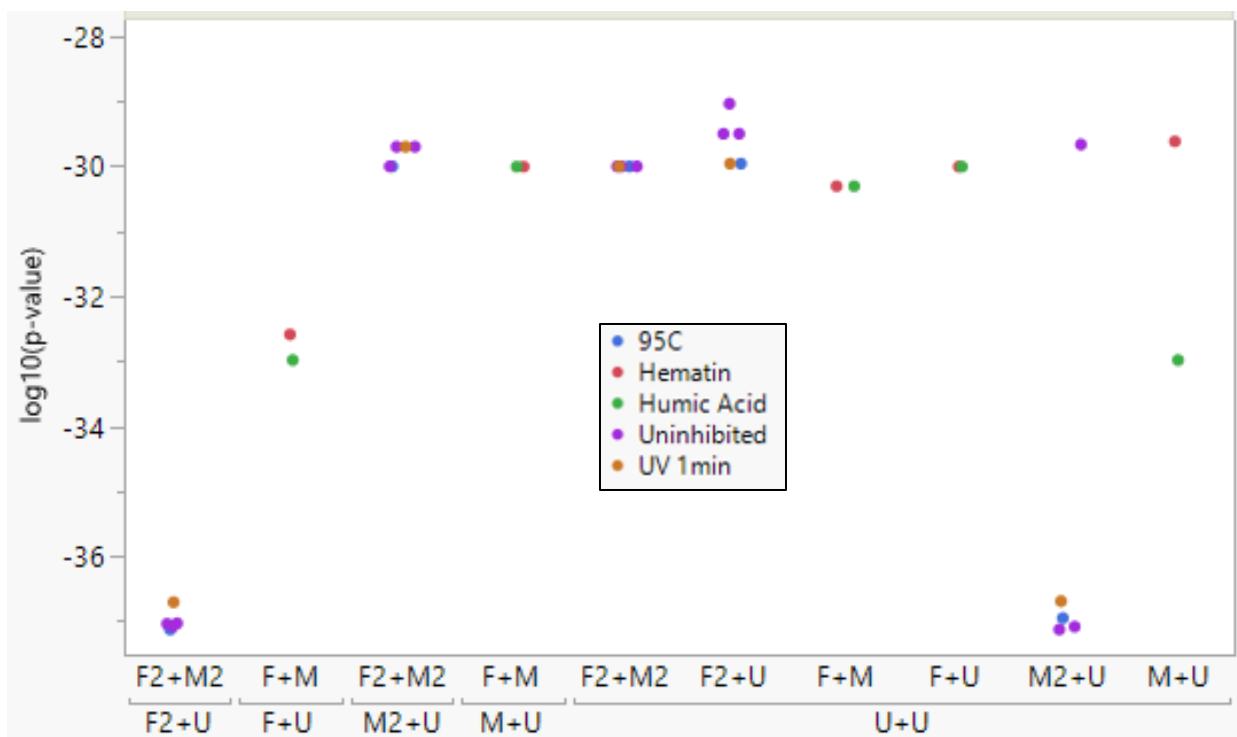


Figure 84: Specificity results for Control Samples. 1 ng of total input DNA run on SeqStudio Flex. Degradation/inhibition and LR hypothesis as in Figure 82. All p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

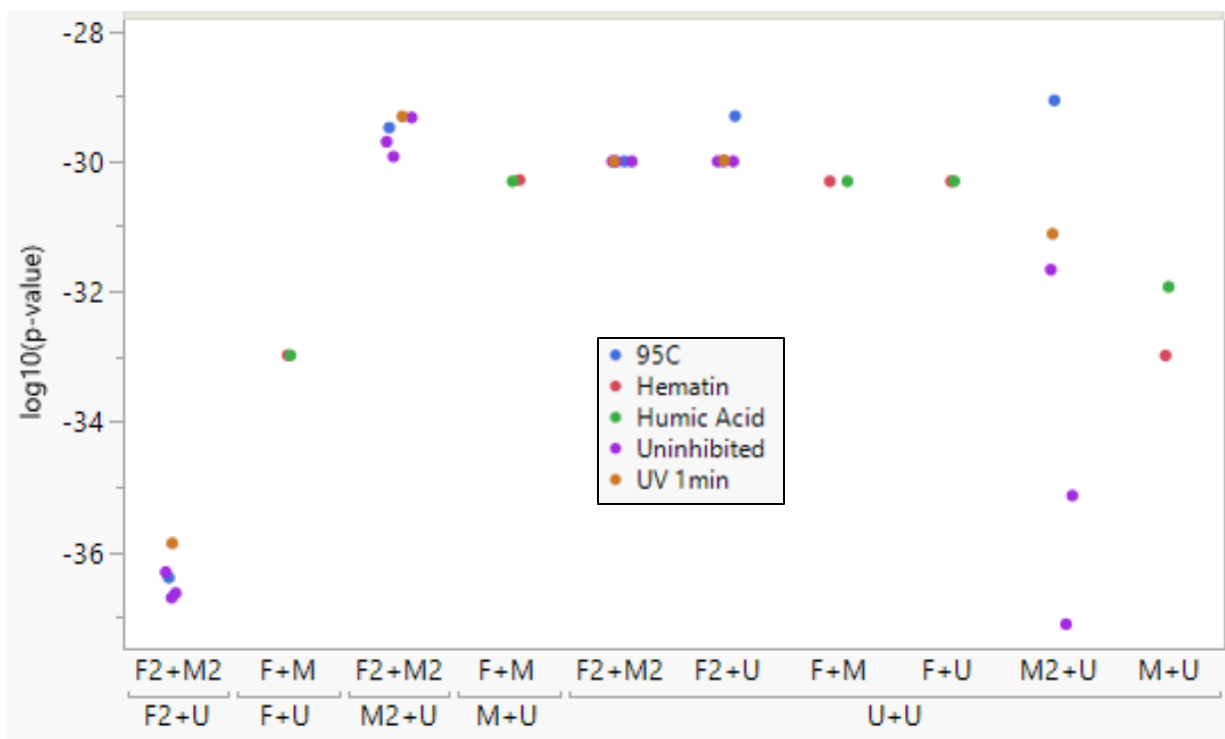


Figure 85: Specificity results for Control Samples. 0.125 ng of total input DNA run on SeqStudio Flex. Degradation/inhibition and LR hypothesis as in Figure 82. All p-values are low, suggesting a low probability that a random person in the population would attain a LR at least as large as the one observed for the POI with the given model for LR.

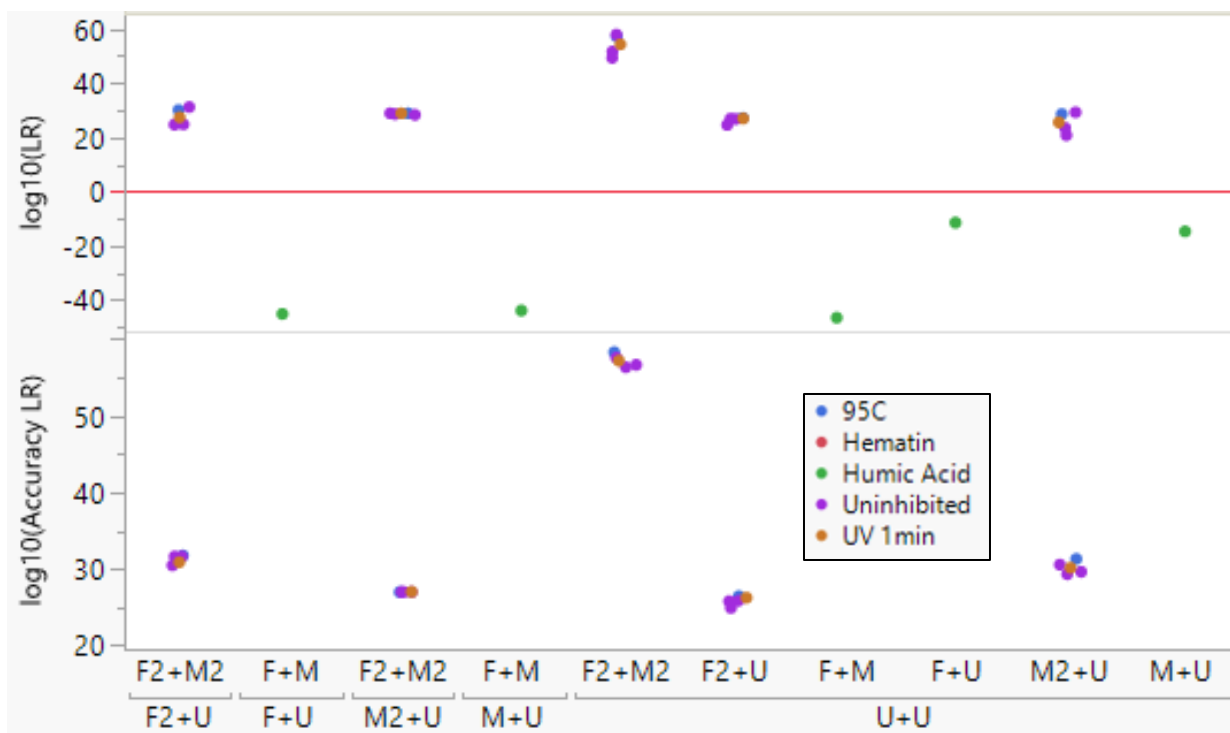


Figure 86: Accuracy result for Control Samples. 1 ng of total DNA input run on 3500xL. NOC = true-NOC. Degradation/inhibition as in Figure 70. LR_s calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where 'U' = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. GeneMapper™ PG Software result shown at top of chart (y-axis = log₁₀(LR)). EuroForMix result shown at bottom of chart (y-axis = log₁₀(Accuracy LR)). LR_s calculated are of similar magnitudes between the two softwares. (Humic acid inhibited samples produced too few peaks for analysis in EuroForMix. Hematin inhibited samples produced too few peaks for analysis in both softwares).

For more details on Accuracy analysis, refer to Section 3.7.2, where Accuracy methods for this developmental validation in their entirety are described.

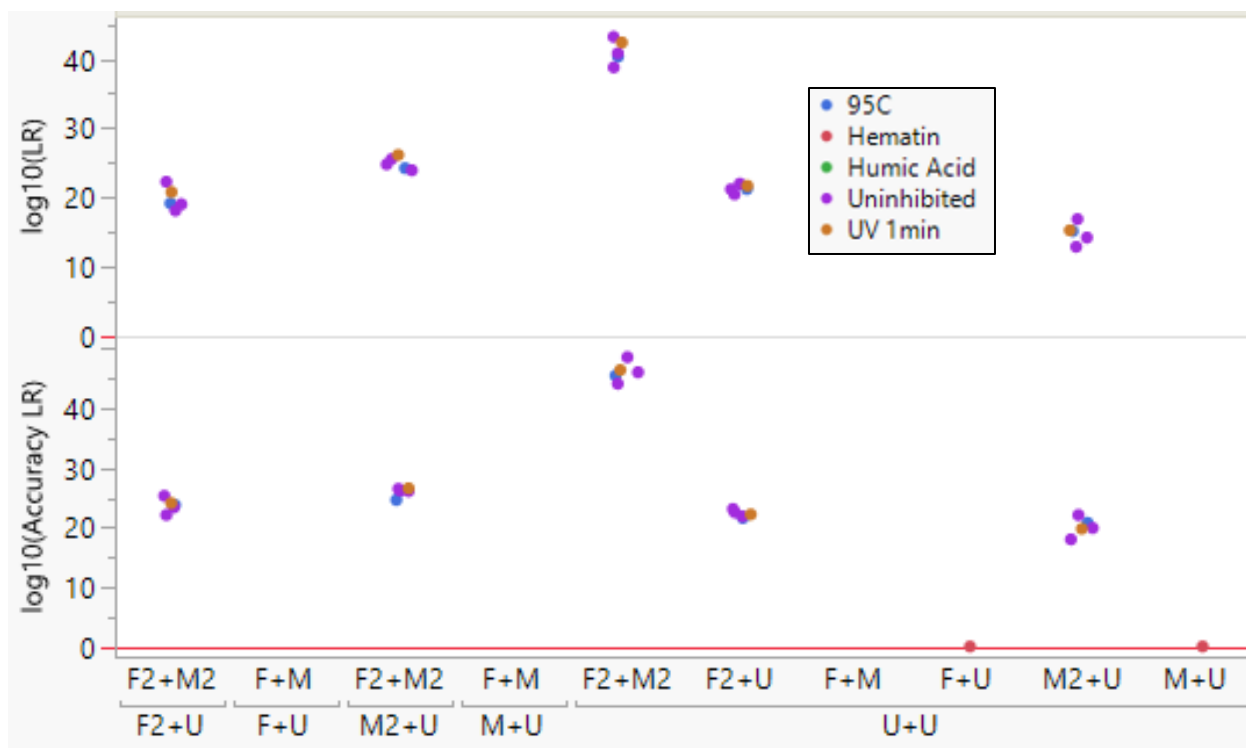


Figure 87: Accuracy result for Control Samples. 0.125 ng of total DNA input run on 3500xL. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 86. GeneMapper™ PG Software result shown at top of chart (y-axis = log10(LR)). EuroForMix result shown at bottom of chart (y-axis = log10(Accuracy LR)). LRs calculated are of similar magnitudes between the two softwares.

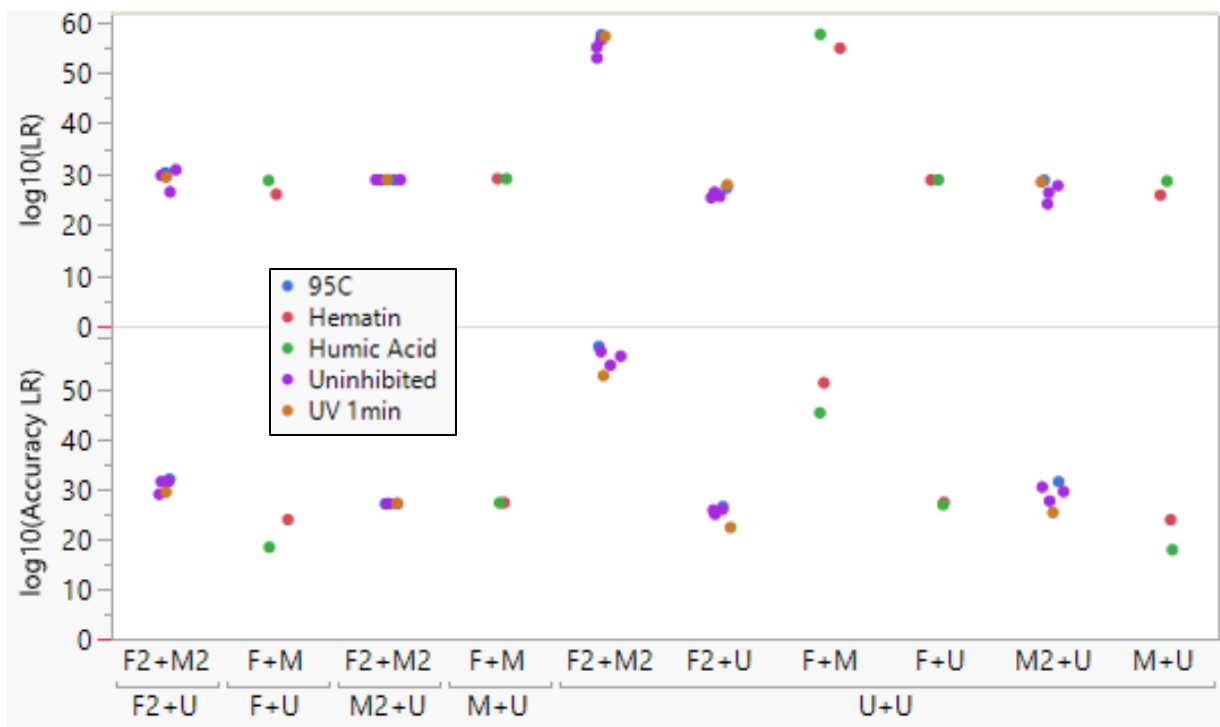


Figure 88: Accuracy result for Control Samples. 1 ng of total DNA input run on SeqStudio Flex. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 86. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). LRs calculated are of similar magnitudes between the two softwares.

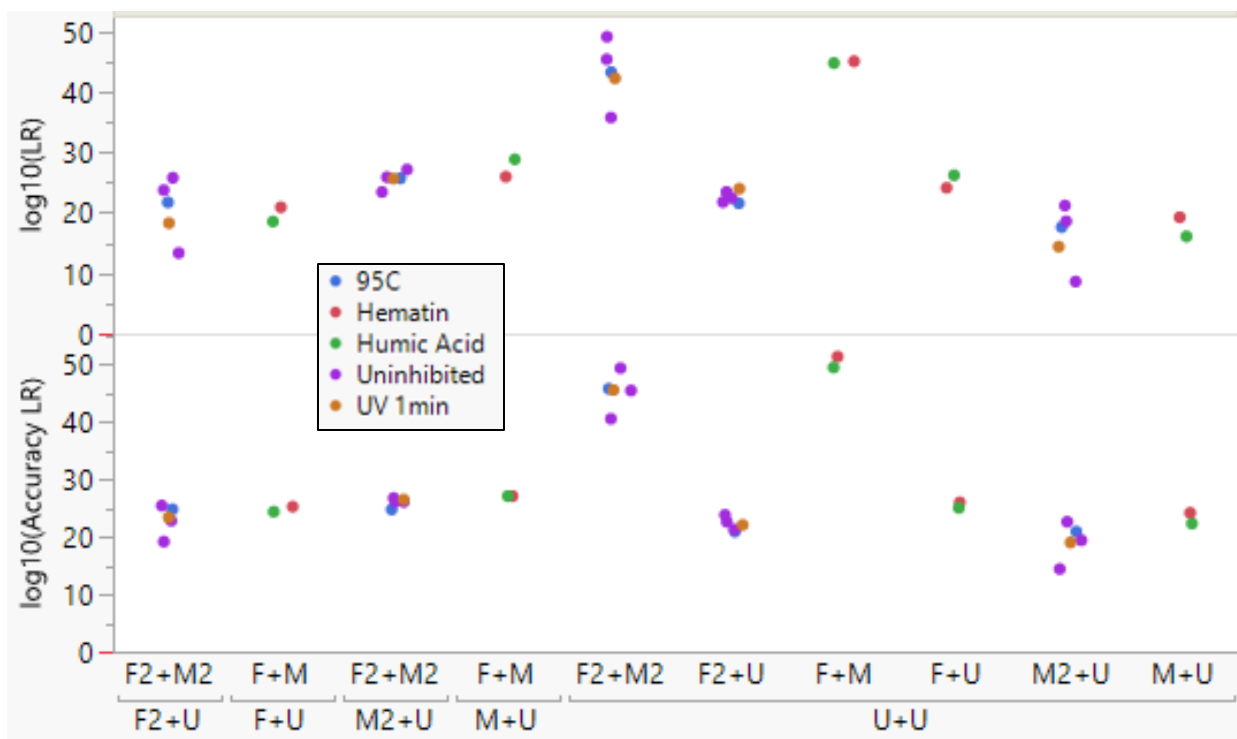


Figure 89: Accuracy result for Control Samples. 0.125 ng of total DNA input run on SeqStudio Flex. NOC = true-NOC. Degradation/inhibition and LR hypotheses as in Figure 86. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). LRs calculated are of similar magnitudes between the two softwares.

3.7. Accuracy

3.7.1. Introduction

Studies should assess the accuracy of the calculations performed by the system. These studies should include the comparison of the results produced by the probabilistic genotyping software to manual calculations, or results produced with an alternate software program or application, to aid in assessing accuracy of results generated by the probabilistic genotyping system.

3.7.2. Methods

Two LR results generated by GeneMapper™ PG Software were compared to a manual calculation of the LR result using Microsoft Excel workbooks. This manual calculation used the same deterministic algorithm as that in GeneMapper™ PG Software and were calculated for both $\theta = 0$ and $\theta = 0.01$ (default), where θ is the co-ancestry co-efficient used to account for population structure.

1-person and 2-person mixtures where LR was calculated assuming true-NOC in GeneMapper™ PG Software were compared to calculations for the same scenarios in EuroForMix, an open-source fully continuous model for the evaluation of STR DNA profiles [5].

3-person and 4-person mixtures where LR was calculated assuming true-NOC in GeneMapper™ PG Software were compared to calculations for the same scenarios in LRmixStudio, an open-source expert system for the interpretation of forensic DNA profiles that employs a semi-continuous model [6].

3.7.3. Accuracy Results

Manual calculation of the LR scenarios in Microsoft Excel produced identical results to the same scenarios calculated in GeneMapper™ PG Software. Detail of LR calculations can be seen in the GeneMapper™ PG Software UI through use of the 'LRrecorder' parameter.

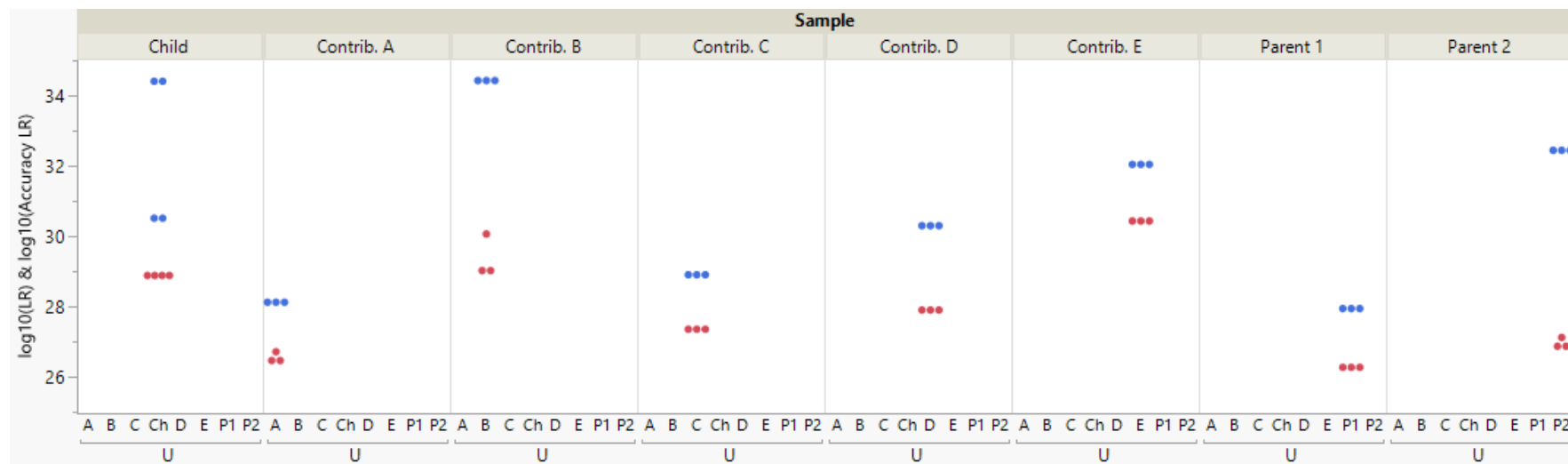


Figure 90: Accuracy result for 1-person 1 ng samples on 3500xL. NOC = true-NOC. Samples are named as described in Table 1. LR tested as H_p / H_d where H_p = the probability of seeing the observed profile given that the named person contributed and H_d = the probability of seeing the observed profile given that an unknown person contributed. Blue data points are GeneMapper™ PG Software result. Red data points are EuroForMix result. LR values for GeneMapper™ PG Software were all of greater magnitude than EuroForMix but support the same interpretation of the result.

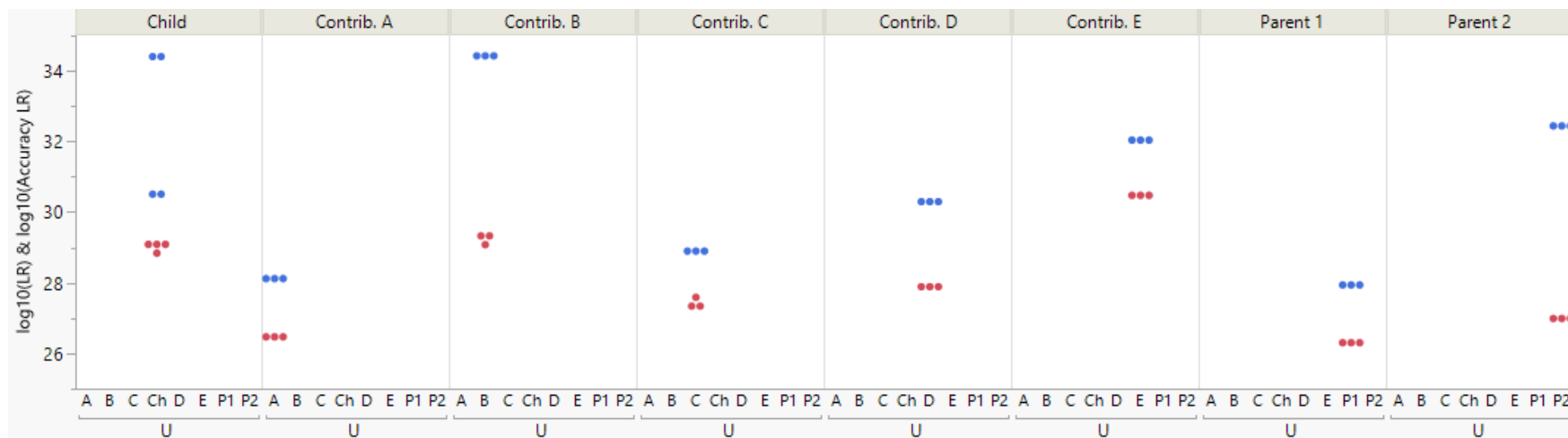


Figure 91: Accuracy result for 1-person 1 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names and LR hypotheses as in Figure 90. Blue data points are GeneMapper™ PG Software result. Red data points are EuroForMix result. Similar results seen as in Figure 90, indicating no significant difference between CE instruments.

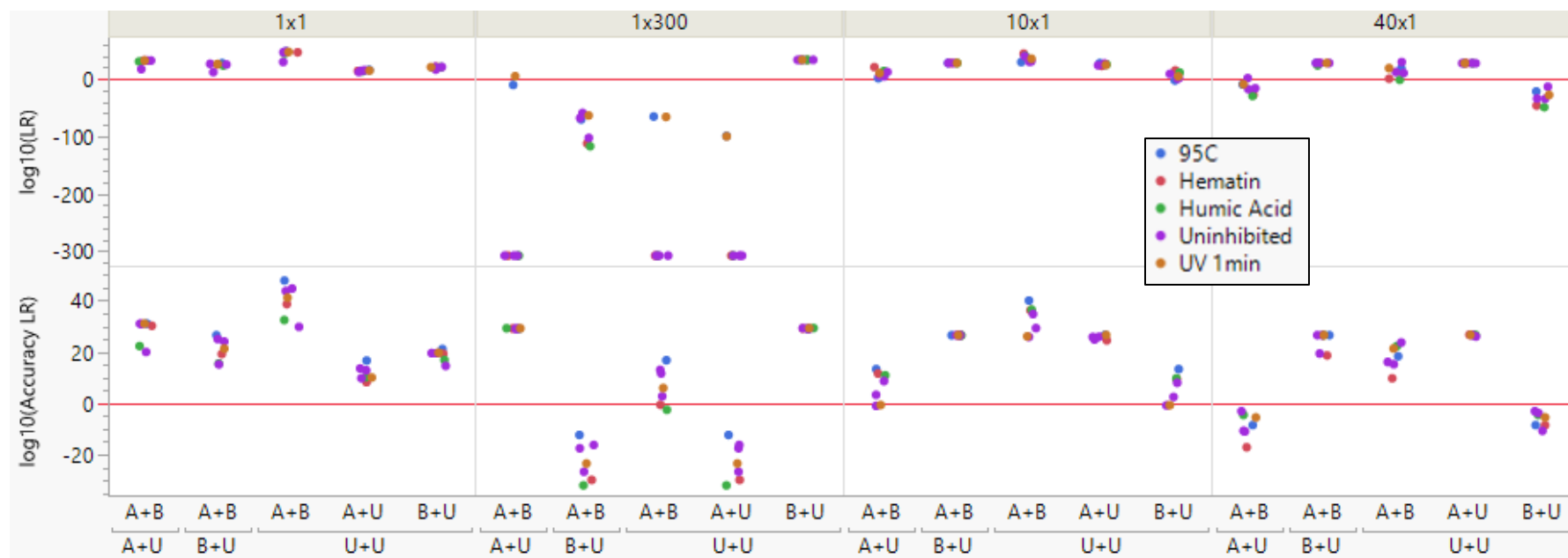


Figure 92: Accuracy result for 2-person 1 ng samples on 3500xL. NOC = true-NOC. Samples are named as described in Table 1. Degradation/inhibition performed on samples as per color key on chart. (See Table 3 for full details). LRs calculated with hypotheses as listed in x-axis, with H_p in top row, H_d in bottom row and where 'U' = unknown contributor. Calculated as the probability of observing the profile given that the named individual(s) and/or 'U' contributed. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). GeneMapper™ PG Software produced more conservative results than EuroForMix, especially for the 1 to 300 mixture.

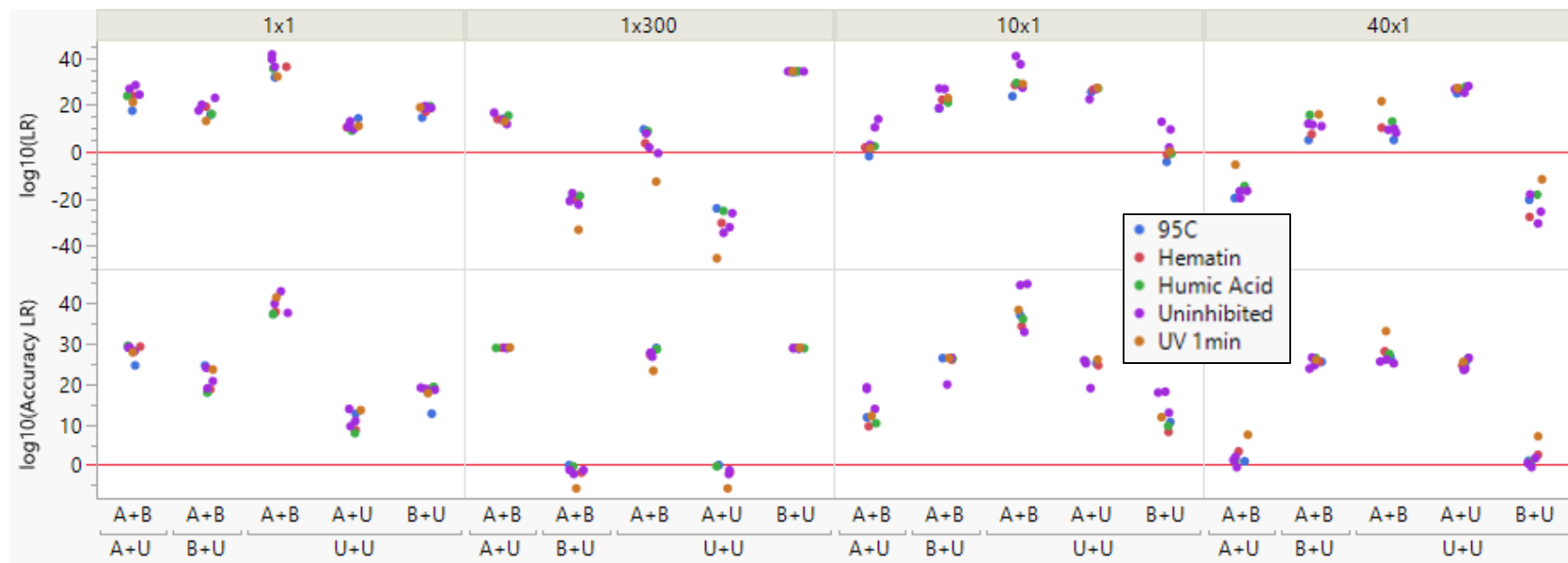


Figure 93: Accuracy result for 2-person 0.125 ng samples on 3500xL. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 92. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). GeneMapper™ PG Software produced more conservative results than EuroForMix, especially for the 1 to 300 mixture.

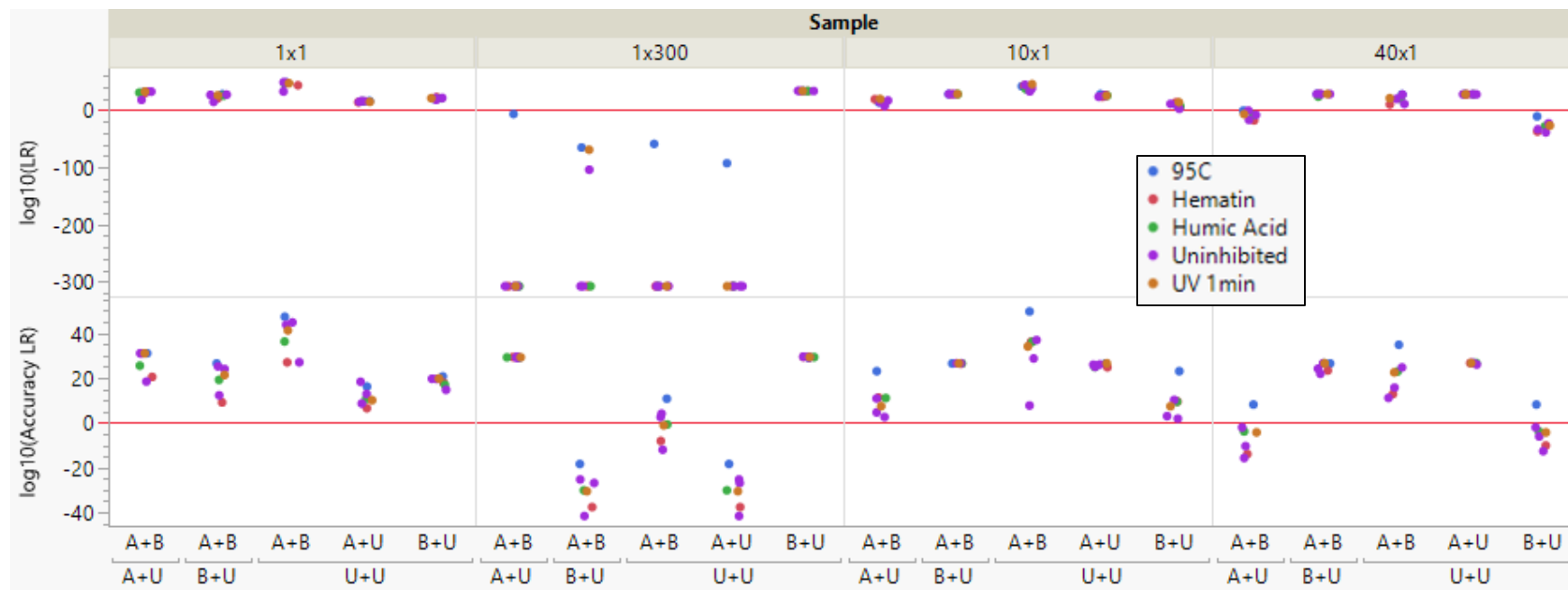


Figure 94: Accuracy result for 2-person 1 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 92. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Results similar to Figure 92, indicating no significant difference between CE instruments.

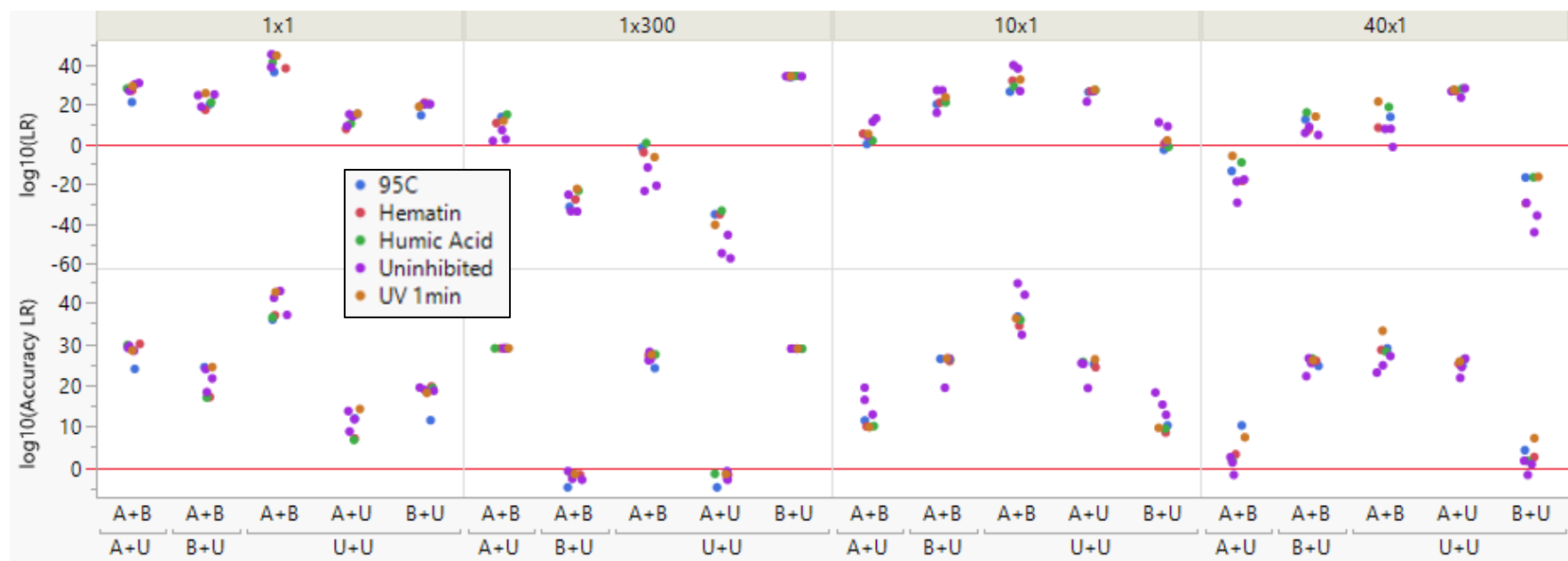


Figure 95: Accuracy result for 2-person 0.125 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 93. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). EuroForMix result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Results similar to Figure 93, indicating no significant difference between CE instruments.

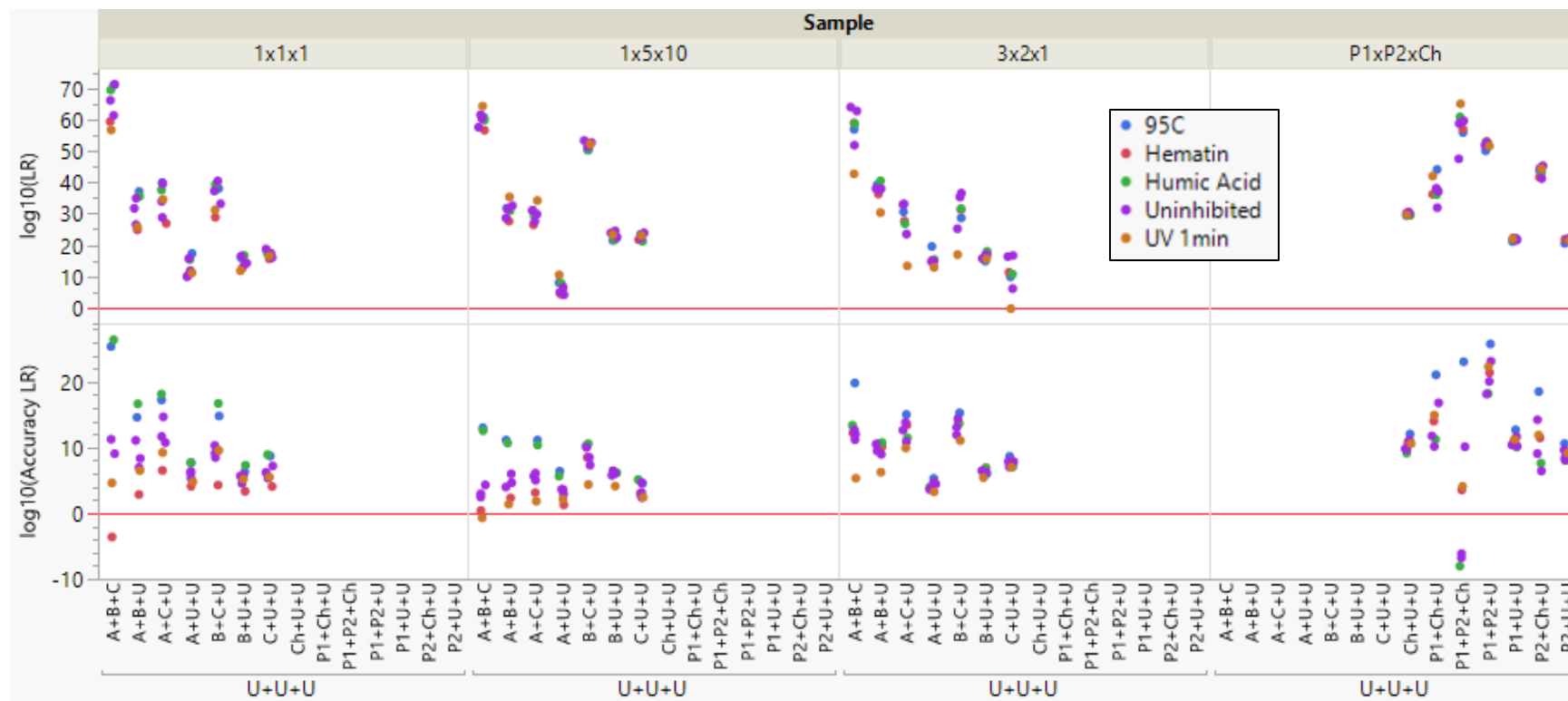


Figure 96: Accuracy result for 3-person 1 ng samples on 3500xL. NOC = true-NOC. Samples are named as described in Table 1. Degradation/inhibition performed as in Figure 92. LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that three unknown persons contributed. 'U' = an unknown contributor. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRMixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). LRMixStudio LRs are comparable, but generally lower in magnitude than GeneMapper™ PG Software result, due to the fact LRMixStudio uses a semi-continuous method of LR calculation.

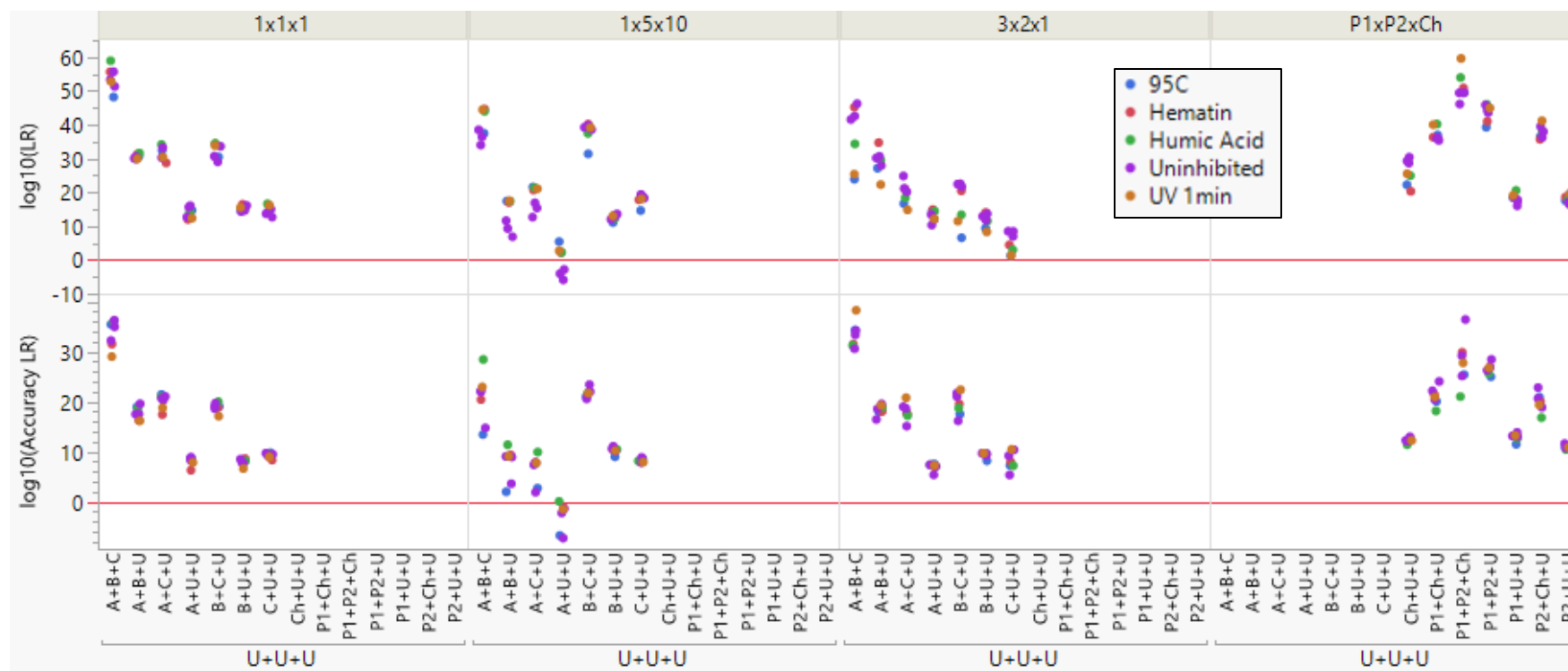


Figure 97: Accuracy result for 3-person 0.125 ng samples on 3500xL. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 96. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Comparable results between the two systems observed.

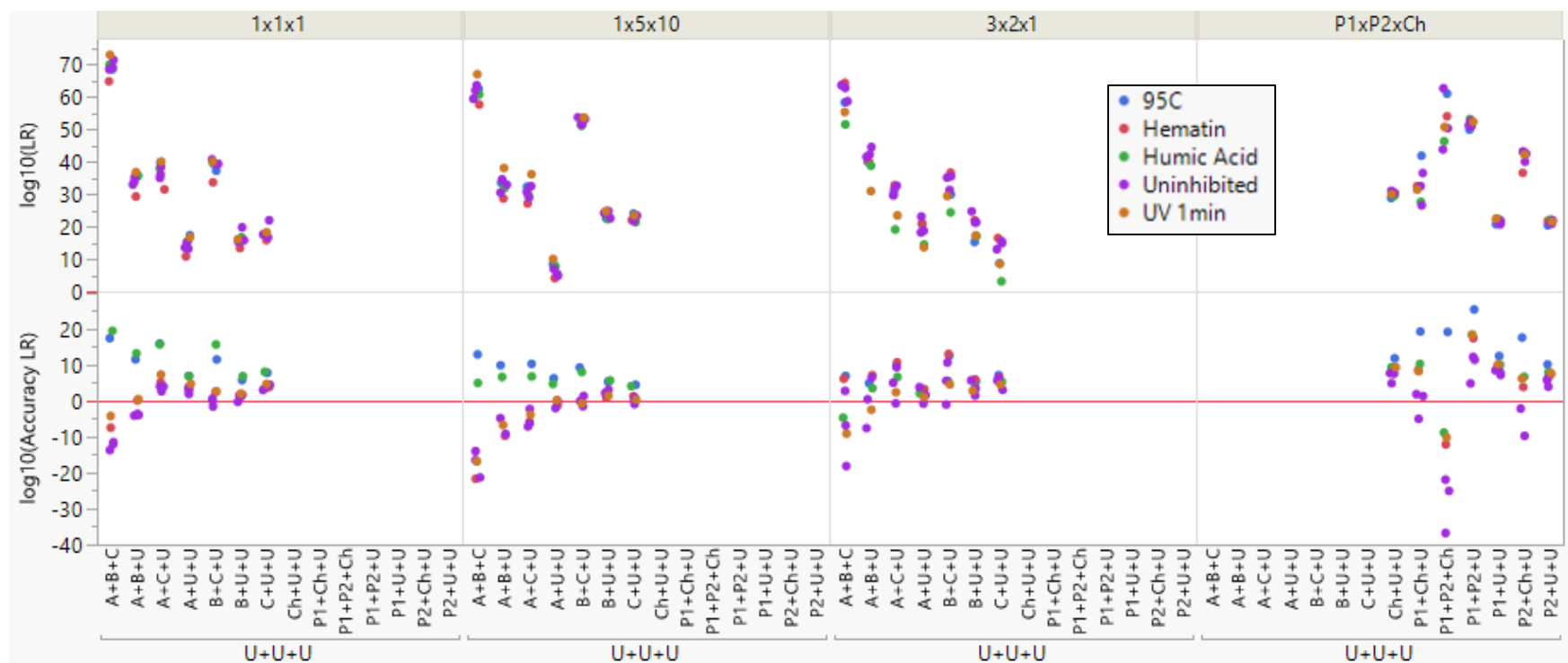


Figure 98: Accuracy result for 3-person 1 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 96. GeneMapper™ PG Software result shown at top of chart (y-axis = log10(LR)). LRMixStudio result shown at bottom of chart (y-axis = log10(Accuracy LR)). LRMixStudio model is likely a poor fit to SeqStudio Flex data for stronger samples.

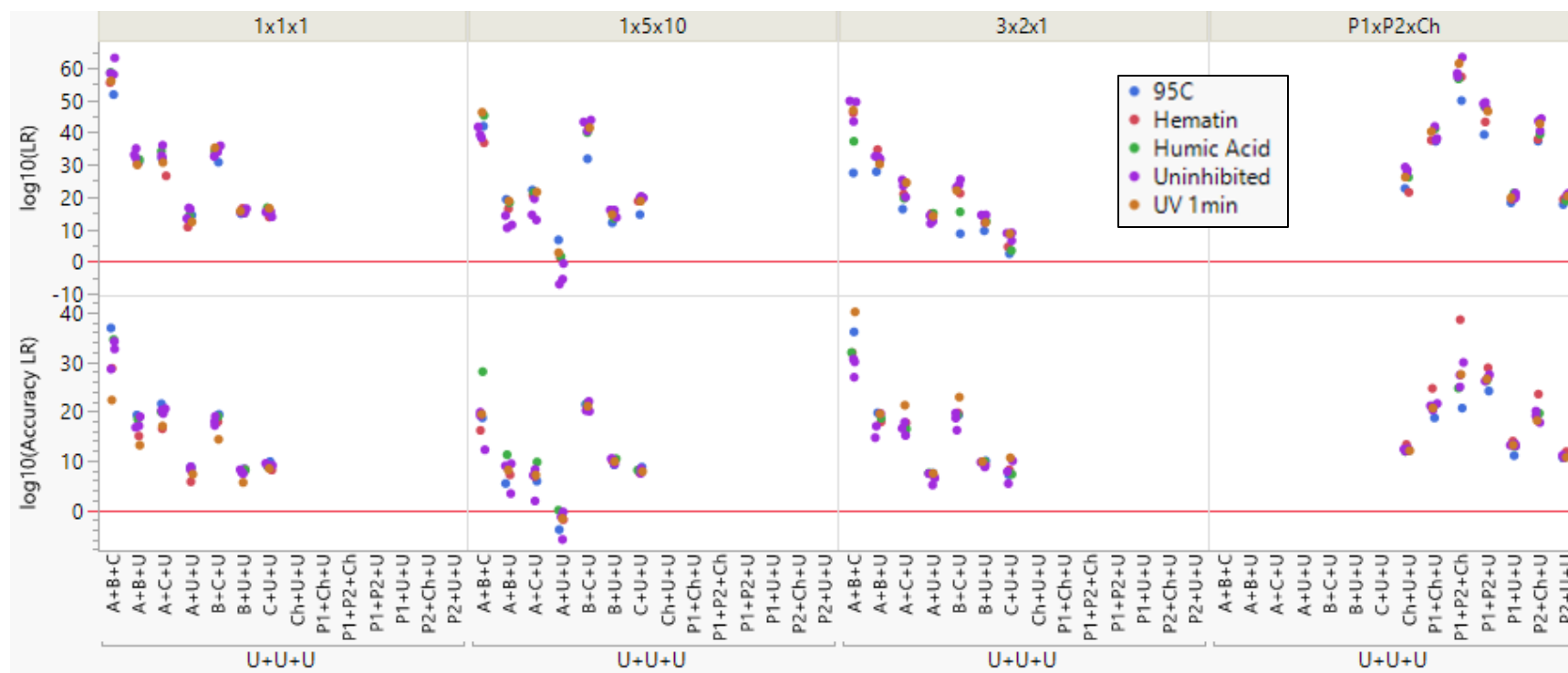


Figure 99: Accuracy result for 3-person 0.125 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 97. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Results similar to Figure 97, indicating no significant difference between CE instruments. Compared to Figure 98, LRmixStudio model appears a better fit for lower DNA input SeqStudio Flex samples.

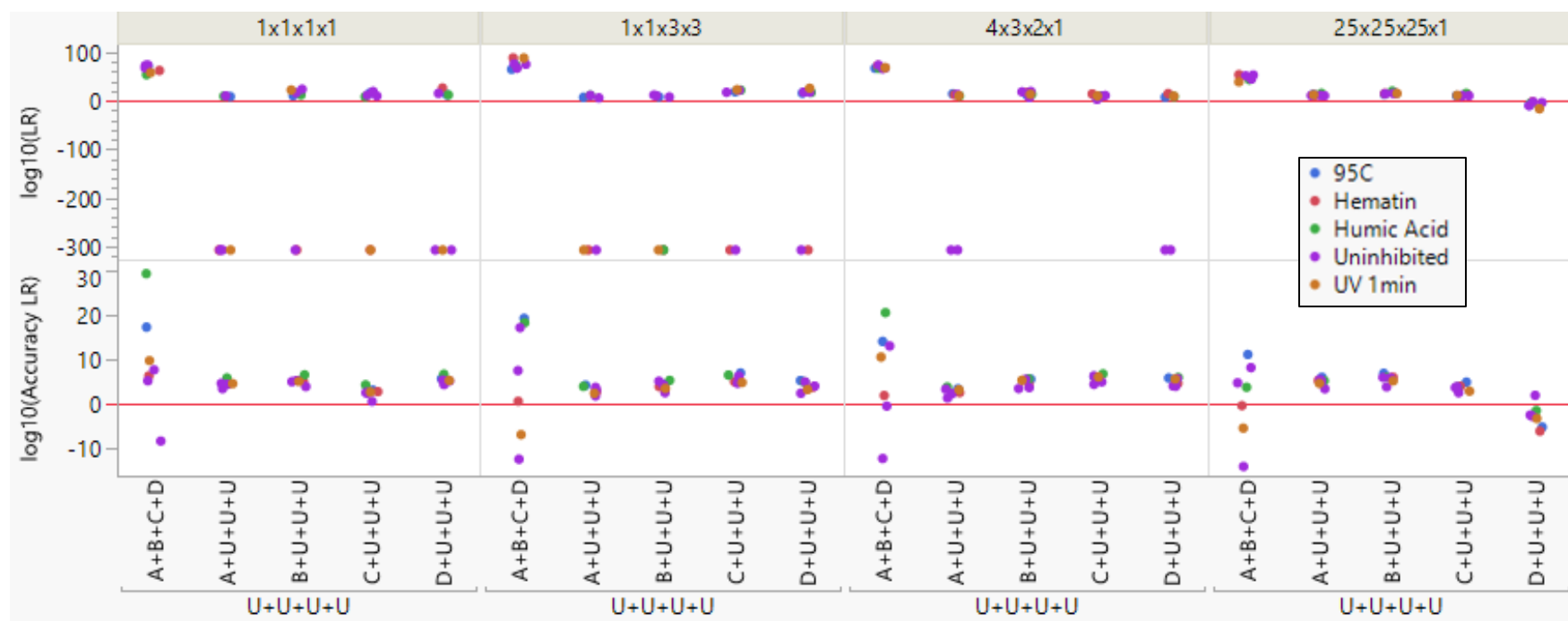


Figure 100: Accuracy result for 4-person 1 ng samples on 3500xL. NOC = true-NOC. Samples are named as described in Table 1. Degradation/inhibition performed as in Figure 92. LR tested as H_p / H_d where: H_p = the probability of seeing the profiles in the top row of the x-axis label, given that the named persons contributed. H_d = the probability of observing the profile given that four unknown persons contributed. 'U' = an unknown contributor. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). More LRs considering all true contributors support H_p over H_d in GeneMapper™ PG Software. More LRs queried on individual contributors support H_d over H_p in GeneMapper™ PG Software.

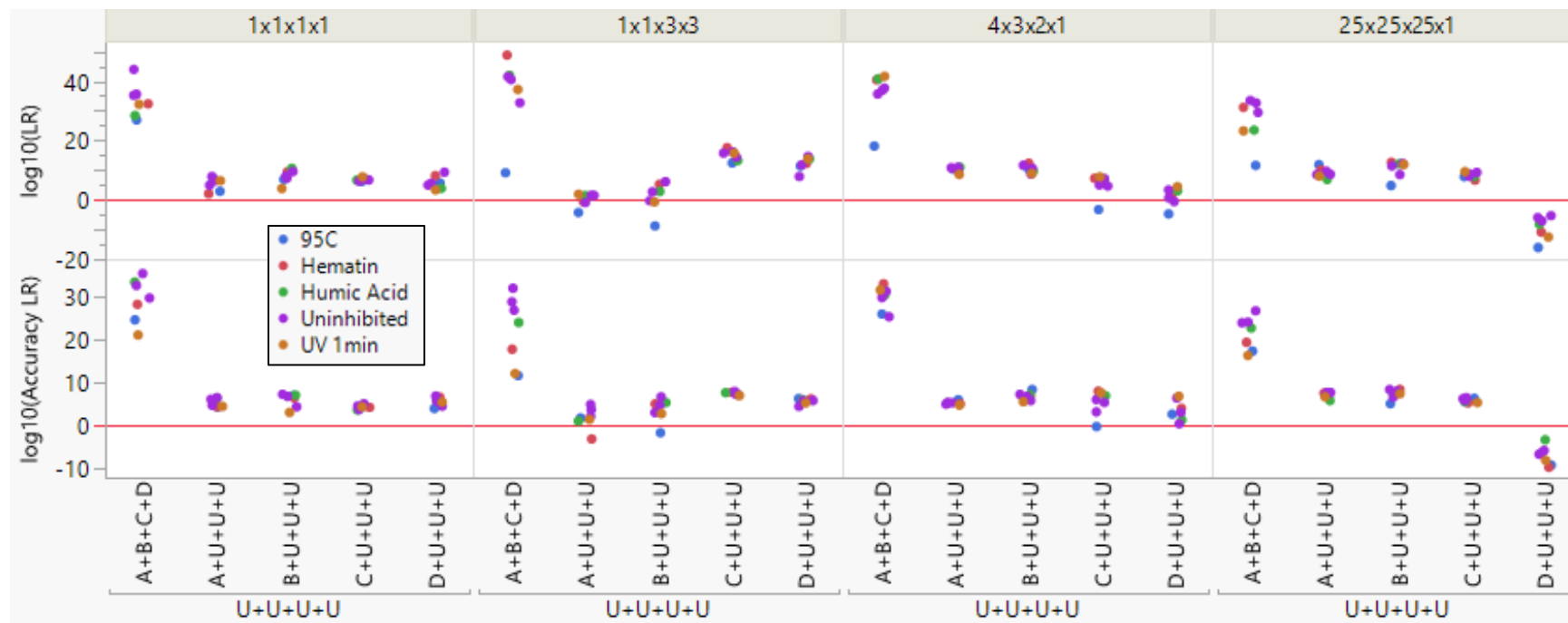


Figure 101: Accuracy result for 4-person 0.125 ng samples on 3500xL. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 100. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). LRs that query the minor contributors in the 95°C degraded 4 to 3 to 2 to 1 samples support H_d over H_p in GeneMapper™ PG Software and H_p over H_d in LRmixStudio. Otherwise, relative magnitude of LRs is similar between programs.

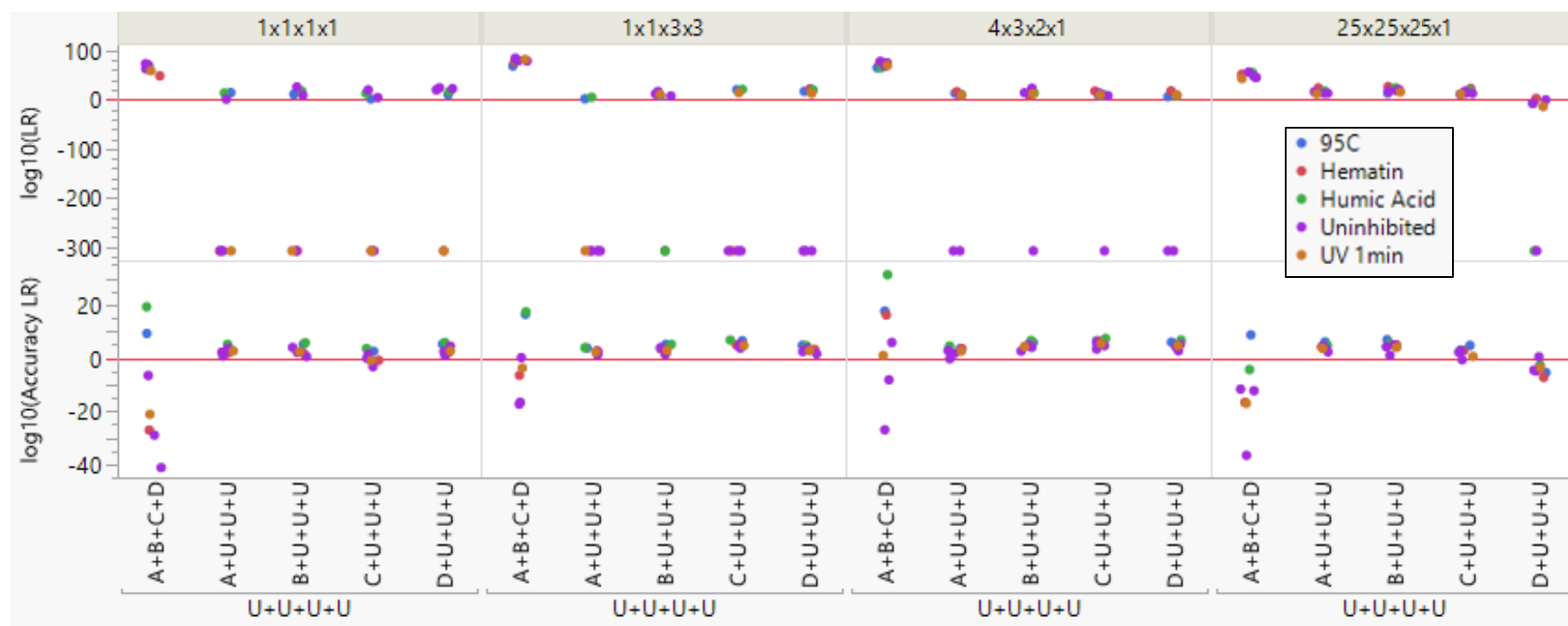


Figure 102: Accuracy result for 4-person 1 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 100. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Results similar to Figure 100, indicating no significant difference between CE instruments.

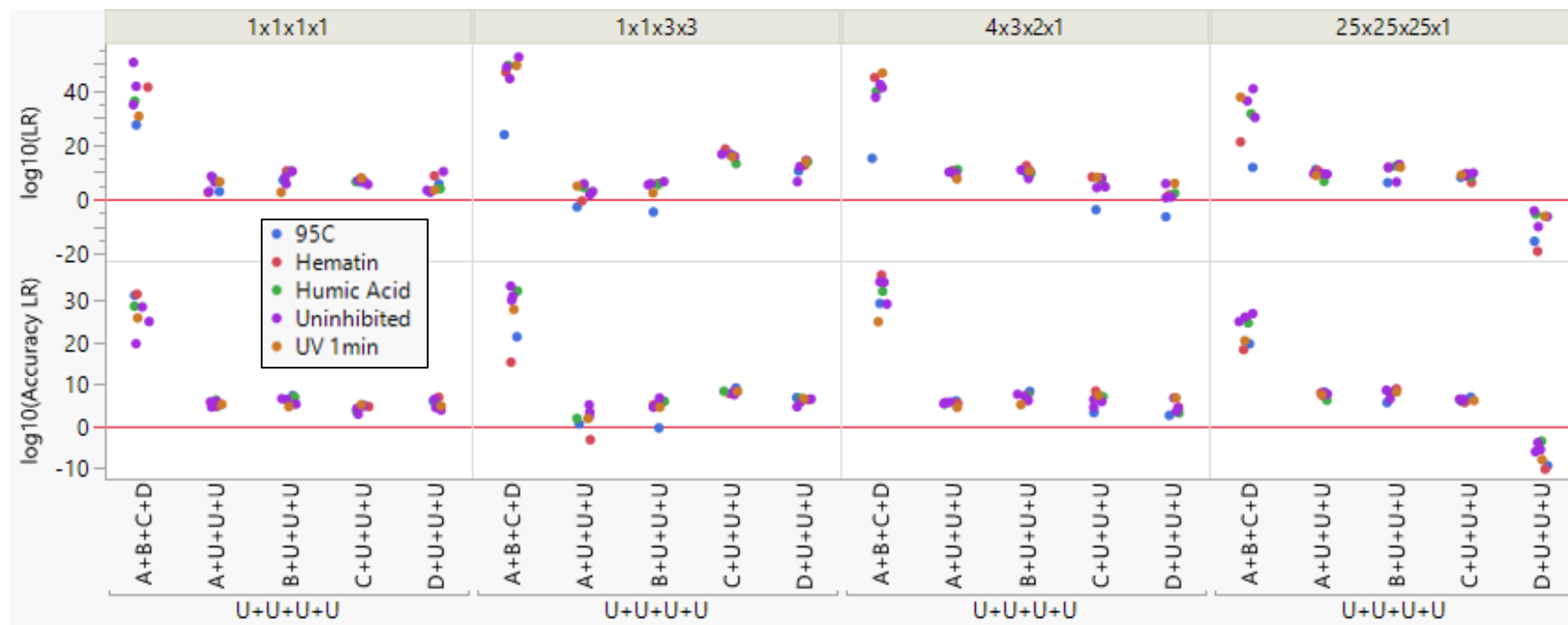


Figure 103: Accuracy result for 4-person 1 ng samples on SeqStudio Flex. NOC = true-NOC. Sample names, degradation/inhibition and LR hypotheses as in Figure 101. GeneMapper™ PG Software result shown at top of chart (y-axis = $\log_{10}(\text{LR})$). LRmixStudio result shown at bottom of chart (y-axis = $\log_{10}(\text{Accuracy LR})$). Results similar to Figure 101, indicating no significant difference between CE instruments.

4. Discussion

GeneMapper™ PG Software has been developmentally validated in accordance with the SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems. The validation encompassed testing of sensitivity, specificity, precision and accuracy, using a diverse range of sample types and conditions.

Sensitivity testing involved analysis on both the 3500xL and SeqStudio 24 Flex instruments of single-source and mixture samples with varying DNA input amounts (0.125 ng and 1 ng), different degradation/inhibition conditions and with one to four contributors. Results for one-contributor samples showed robust LR results, even if NOC is incorrectly set to two. Two-contributor samples showed sensitive detection of minor contributors to mixtures, including for degraded and inhibited samples. Only for the 1 to 300 2-person mixtures, when the scenario tested the presence of the minor contributor, was H_d favored over H_p , due to the minor contributor being below the analytical threshold. For 40 to 1 mixtures, H_p was favored over H_d for a number of these same scenarios, despite the effect of degradation and inhibition. This was the case at both 1 ng and 0.125 ng of DNA input. Consistent results were observed on both 3500xL and SeqStudio Flex. The 2-person mixtures also showed comparable results when NOC was incorrectly set to true-NOC plus 1 (i.e. NOC = 3). With NOC incorrectly set to true-NOC minus 1 (i.e. NOC = 1), the LR tended to support H_d over H_p due to there being an unrealistically high number of peaks to support a 1-person solution.

Three-contributor sensitivity samples showed LRs supportive of H_p over H_d across a wide range of samples with 1 ng and 0.125 ng of input DNA, with an exception for the most minor component in the 3 to 2 to 1 UV treated 1ng DNA input sample, and for minor components in the 1 to 5 to 10 sample with 0.125 ng of input DNA. Minor contributors, particularly when they are present at a much lower amount compared to other contributors, may not be recognized as a real contributor and instead be categorized along with stutter or noise. When NOC was incorrectly set to true-NOC minus 1 for the 3-person mixtures (i.e. to NOC = 2), LR results for 1 ng DNA input samples were mostly supportive of H_d over H_p , except for cases with high peak imbalance, or with increased allele sharing due to the parent/child relationship of the samples. In cases of high peak imbalance and high allele sharing, the total peak count may be as well explained as coming from two vs three contributors – particularly the two contributors present at the highest amounts in a mixture. The 0.125 ng DNA input samples were more broadly supportive of H_p over H_d , likely due to the decreased peak height and increased permissible imbalance in these samples making more combinations of contributors, even those present in low amounts, appear more plausible. When NOC was incorrectly set to true-NOC plus 1 (i.e. to NOC = 4) for the 3-

person samples, most LR values were supportive of H_p over H_d for both 1 ng and 0.125 ng samples. This is because the distinction between three and four contributors is typically not as clear-cut as that between lower numbers of contributors.

The four-contributor sensitivity samples often showed LR values supportive of H_p over H_d across the 0.125 and 1 ng DNA input samples. The minor contributor in the 25 to 25 to 25 to 1 mixture was not detected in both the 1 ng and 0.125 ng DNA input samples. This meant that, while some peaks consistent with the minor contributor were above AT and were observed in the profile, the software did not consider such a minor contributor to be a reasonable solution to the mixture. Some additional 4-person LR scenarios also showed LR values supportive of H_d over H_p at 1 ng DNA input, likely due to the sharing of alleles between contributors. Similar results were seen with NOC incorrectly set to true-NOC minus 1 (i.e. to $NOC = 3$) for the 4-person mixtures, especially for some of the degraded or lower input samples. This is likely because the decreased peak height can make 3-contributors look like a reasonable solution in these cases, especially as the distinction between three and four contributors is typically not as clear-cut as that between lower numbers of contributors. Similar results were gained for both the 3 and 4-contributor sensitivity samples on both 3500xL and SeqStudio Flex.

Specificity studies evaluated GeneMapper™ PG Software's ability to provide reliable results for non-contributors across various evidence types. These studies assessed the potential for failure to reject a false hypothesis and to demonstrate the range of LR values that can be expected for non-contributors. The specificity testing focused on calculating the p-value for different LR scenarios, evaluating the probability that a random person would attain a LR at least as large as the one observed with the POI in the given LR scenario. The results generally showed low calculated p-values across the different scenarios, indicating low probability that a random person would attain as large a LR result.

Specificity testing included the analysis of single-source, 2-person, 3-person, and 4-person samples with varying mixture ratios, degradation/inhibition conditions and DNA inputs. The results demonstrated that the software can accurately assess non-contributors as having a low probability of an LR comparable to the POI, even in challenging scenarios such as when the non-contributor is a close relative (sibling, parent, or grandparent) of the true POI. Note that charts showing the probability of single-source samples having a close relative that give a LR comparable to the POI are not shown because this testing resulted in null results for all scenarios: the p-value was too low for the software to calculate. Samples were tested on both 3500xL and SeqStudio Flex and showed minimal difference between the two platforms.

Precision studies assessed the reproducibility and repeatability of the results obtained from GeneMapper™ PG Software. These studies involved multiple analyses of the same

samples under the same parameters to evaluate the consistency of the results. Because GeneMapper™ PG Software uses a deterministic algorithm for deconvolution and LR calculation, no differences were expected to be found between repeated analyses of the same data. This conclusion was supported, with all samples tested showing identical results on repeat analysis of the same scenarios, regardless of whether run in the GeneMapper™ PG Software user interface (UI) or in the command line interface (CLI).

SWGDAM guidelines further require that case-type and control samples be evaluated to assess the same range of samples encountered by testing laboratories. The sensitivity and specificity testing described above included analysis of 4,244 LR scenarios including samples with 1 ng and 0.125 ng of DNA input, samples degraded by UV and heat treatment and inhibited with humic acid and hematin. Many samples in this developmental validation included shared and masked alleles, and there were many profiles with low-level and imbalanced peaks. NIST control samples were also evaluated in a separate section, producing results aligned with the case-type samples, and demonstrating that GeneMapper PG Software is capable of correctly interpreting control samples with a published genotype.

The accuracy studies compared the results produced by GeneMapper™ PG Software to those obtained from alternative software programs, specifically EuroForMix and LRmixStudio. LRs calculated in GeneMapper™ PG Software using 1-person and 2-person samples were compared to results from EuroForMix and LRs calculated using 3-person and 4-person mixture samples were compared to results from LRmixStudio. The results showed that the LRs calculated by GeneMapper™ PG Software were generally of similar magnitudes to those produced by the alternative software programs, confirming the accuracy of the GeneMapper™ PG Software calculations.

Specifically in the accuracy analysis, 1-person samples showed generally higher LRs in GeneMapper™ PG Software than in EuroForMix but were supportive of the same general result. This was true for samples processed on both 3500xL and SeqStudio Flex. 2-person samples produced broadly similar LR results between GeneMapper™ PG Software and EuroForMix, but GeneMapper™ PG Software produced more conservative results than EuroForMix regarding support for observing minor contributors, especially for the 1 to 300 mixture. Again, results for this were the same on both 3500xL and SeqStudio Flex. 3-person mixtures showed comparable accuracy results between GeneMapper™ PG Software and LRmixStudio for the 0.125 ng DNA input samples, but generally lower results for LRmixStudio in the 1 ng DNA input samples and on the SeqStudio Flex. This did not affect the overall conclusion of these LRs however, with both software programs generally favoring H_p over H_d for these scenarios. This could be due to the semi-continuous LRmixStudio algorithm which naturally results in lower magnitude LR calculations and a worse fit of the LRmixStudio model to SeqStudio Flex data as the software was developed

before the instrument's release. The 4-person accuracy samples again showed a slightly lower magnitude of result for the LRmixStudio 1 ng DNA input results, but the results were generally comparable between both software and instrument platforms.

Finally, the system control features of GeneMapper™ PG Software meet the validation requirements specified in the SWGDAM guidelines. The software includes published release notes with installation procedures and hardware specifications, unique version numbering (current release is v1.0), access controls, audit trails, and user role management. The underlying scientific principles of GeneMapper PG Software have been published in a technical note by Thermo Fisher Scientific entitled '*GeneMapper™ PG Software: Scientific Principles*'. The content of this technical note is planned to be submitted for publication to a peer-reviewed scientific journal upon the full commercial release of GeneMapper™ PG Software in September 2025.

5. Conclusion

GeneMapper™ PG Software has been demonstrated to offer reliable results that meet SWGDAM guidelines for developmental validation of probabilistic genotyping systems. The software has been shown to produce sensitive, specific and accurate results on a wide range of evidence-type and control samples commonly encountered by forensic laboratories. The deterministic nature of the GeneMapper™ PG Software algorithm enables precision with identical results generated with repeated analysis of the same input data. GeneMapper™ PG Software offers forensic laboratories a powerful tool for addressing the challenges associated with the interpretation of complex DNA mixtures.

6. References

- [1] *Exact Computation of the Distribution of Likelihood Ratios with Forensic Applications.*
G. Dørum, Ø. Bleka, P. Gill, and G. Storvik.
Forensic Science International: Genetics 9:93-101, 2014
- [2] *SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems.*
<https://www.swgdam.org>
- [3] *GeneMapper™ PG Software v1.0 Release Notes.*
<https://www.thermofisher.com>
- [4] *GeneMapper™ PG Software: Scientific Principles.*
<https://www.thermofisher.com>
- [5] *EuroForMix: An open source software based on a continuous model to evaluate STR DNA profiles from a mixture of contributors with artefacts*
Ø. Bleka, G. Storvik, P. Gill.
Forensic Sci. Int. Genet., 21:35-44, 2016
- [6] *LRmixStudio.*
<https://www.isfg.org/Software#dna021>

7. Revision History

Revision	Date	Description
A00	05 SEP 2025	Initial publication

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