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Exploring DNA Interpretation Software Using the PROVEDIt Dataset

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Overview of PROVEDIt database

Allows examination of probabilistic genotyping systems

Large publicly available database

Examine effect of analytical thresholds and peak detection parameters on downstream analysis

https://lftdi.camden.rutgers.edu/provedit/

Project Research Openness for Validation with Empirical Data

PROVEDIt

Contains over 25,000 STR profiles

Assess approaches to evaluate STR signal (genotyping software packages and validation software)

1 to 5 person mixtures varying

- contributor ratios
- DNA quality

Amplified with different STR kits
DNA quantity (0.007- 1 ng)

Analyzed with different CE instrument types and injection times

[1] L.E. Alfonse, A.D. Garrett, D.S. Lun, K.R. Duffy, C.M. Grgicak, A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVEDIt, Forensic science international. Genetics 32 (2018) 62-70.

Objective of this study

Project Research Openness for Validation with Empirical Data

PROVEDIt

STRmix v2.6

EuroForMix v2.1.0

An open-source software for statistical DNA interpretation

Interpretation parameters used for each software

Software	
STRmix v2.6	▪ ***N-1, N-2 and N+1*** stutter peaks were modeled ▪ ***Drop-in frequency*** = 0.0015 and maximum cap = 180 RFU ▪ ***Saturation threshold*** = 30,000 RFU ▪ ***MCMC settings:*** 8 chains of 100,000 burn-in accepts, 50,000 post burn-in accepts per chain ▪ Allelic, stutter, and locus-specific amplification efficiency variance were determined using ***Model Maker*** where over ***300 single source profiles*** of varying quality and quantity were assessed ▪ The **sub-source LR** is reported
EuroForMix v2.1.0	▪ ***MLE*** (Maximum likelihood estimation) approach ▪ ***Degradation and stutter models*** jointly turned on ▪ Default parameters, except for a 35 RFU ***detection threshold***, ***Pr(C)*** = 0.0015 and λ = 0.018. ▪ The **MLE based method LR** is reported
Both software	▪ Profiles were analyzed using the ***per dye ATs*** ▪ ***NIST 1036-Caucasian*** allele frequencies ▪ θ correction was applied using an $F_{st}(\theta) = 0.01$ ▪ True ***NOC*** and ***same propositions*** were used in both software

J.A. Bright et al., Internal validation of STRmix - A multi laboratory response to PCAST, Forensic science international. Genetics 34 (2018) 11-24.
O. Bleka et al., EuroForMix: An open source software based on a continuous model to evaluate STR DNA profiles from a mixture of contributors with artefacts, Forensic science international. Genetics 21 (2016) 35-44.

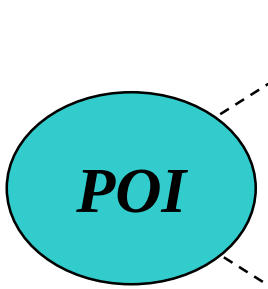
Dataset used in our study

Kit (PCR cycle no.)		CE instrument (injection time)					
GlobalFiler (29 cycles)						3500 (15 s)	
Number of contributors	**Mixture ratios**	**Pristine DNA**	**Degraded DNase I**	**Degraded Sonication**	**Damaged UV**	**Inhibited Humic Acid**	**Minor Contributor DNA amount (pg)**
2P (16 unique individuals)	1:1	x	x	x	x	x	15; 30; 62; 125
	1:2	x	x				15; 30; 62; 125
	1:4	x	x	x	x	x	15; 30; 62; 125
	1:9	x	x	x	x	x	15; 30; 54; 62; 75
Sum		88	228	44	104	108	572
3P (21 unique individuals)	1:1:1	x	x	x	x	x	15; 30; 62; 125
	1:2:1	x	x				15; 30; 62; 125
	1:2:2	x	x				15; 30; 62; 125
	1:4:1	x	x	x	x	x	15; 30; 62; 125
	1:4:4	x	x	x	x	x	15; 30; 62; 83
	1:9:1	x	x				15; 30; 45; 62
	1:9:9	x	x				15; 26; 30; 40
Sum		114	324	72	138	162	810

Dataset used in our study

Kit (PCR cycle no.)	CE instrument (injection time)
GlobalFiler (29 cycles)	3500 (15 s)

Number of contributors	
2P	Hp = POI + U1 Hd = U1 + U2
Sum	
3P	Hp = POI + U1 + U2 Hd = U1 + U2 + U3
Sum	

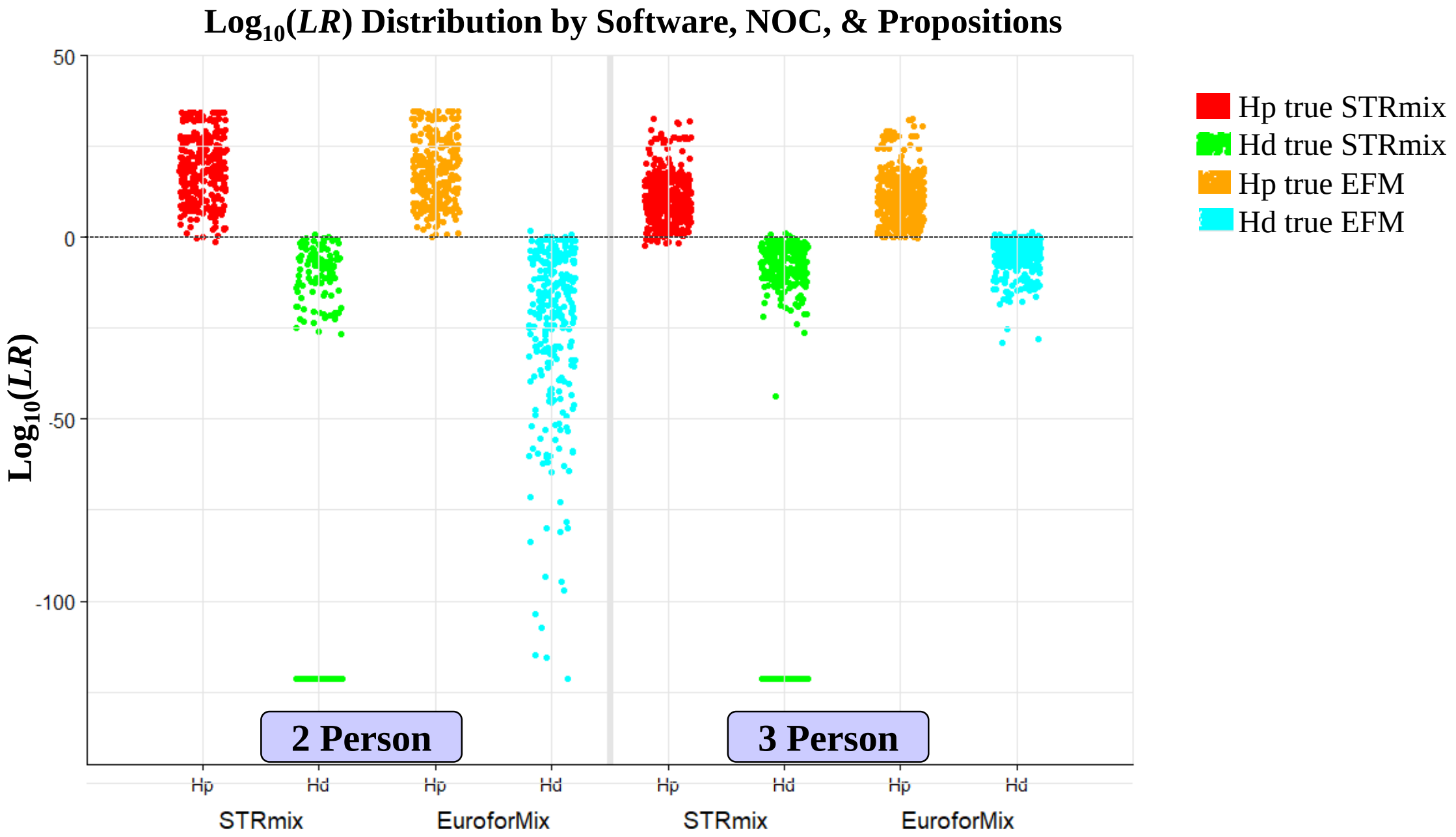


True contributors

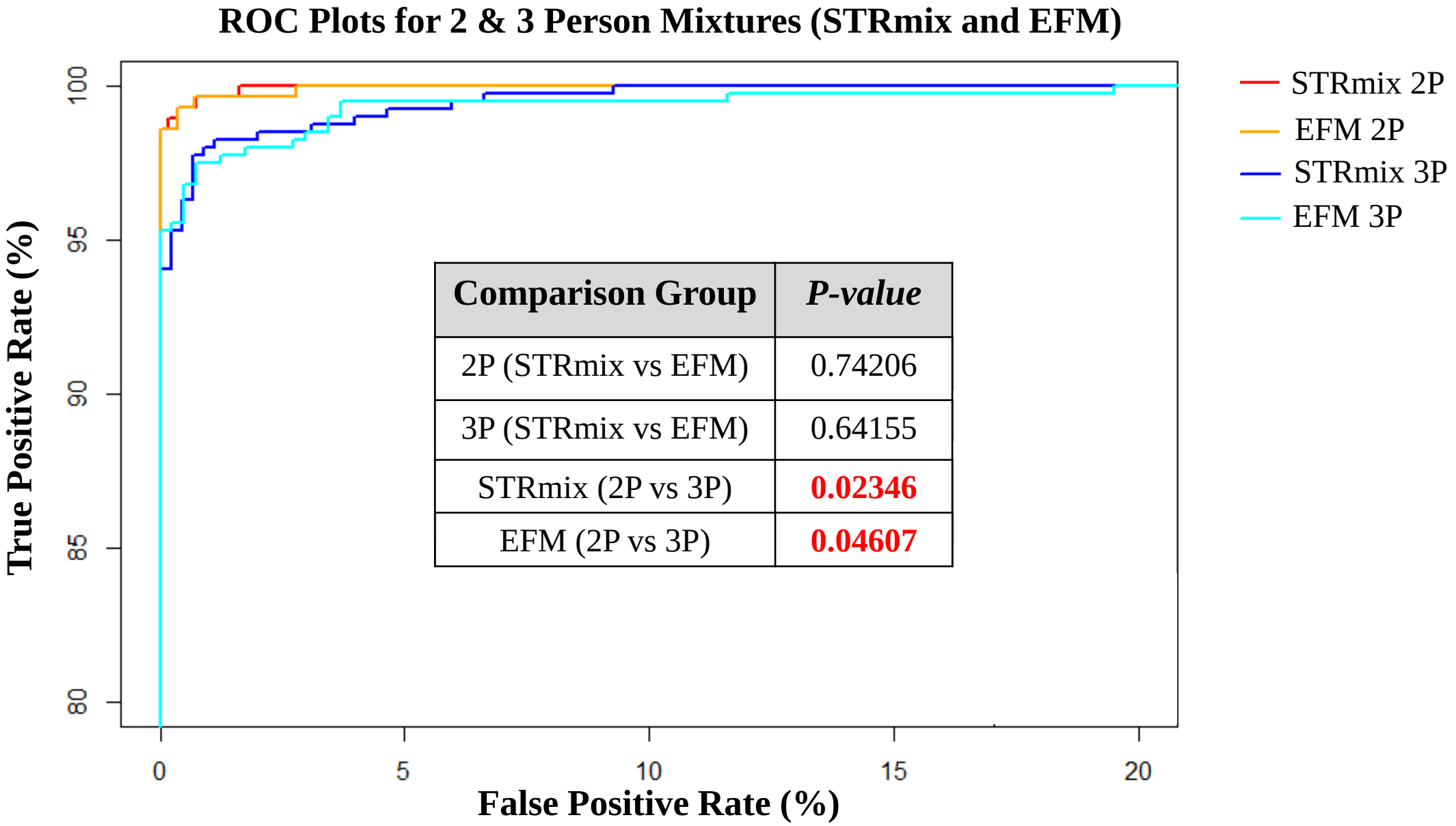
True non-contributors <----- (random person from NIST 1036)

Analysis of 2P and 3P mixtures

$Log_{10}(LR)$ for 2P and 3P mixtures



Receiver operating characteristic (ROC) plots

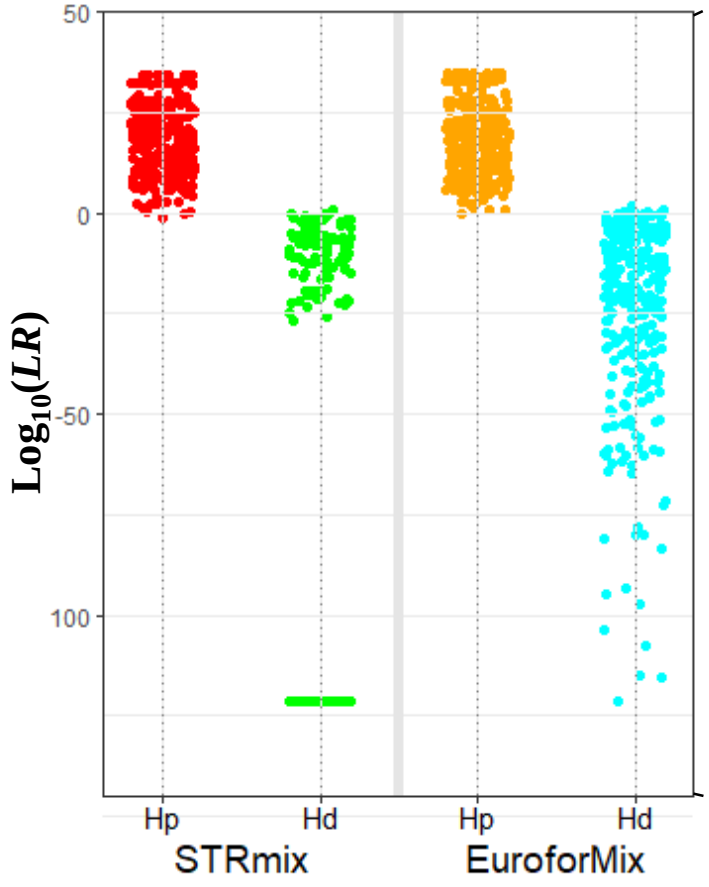


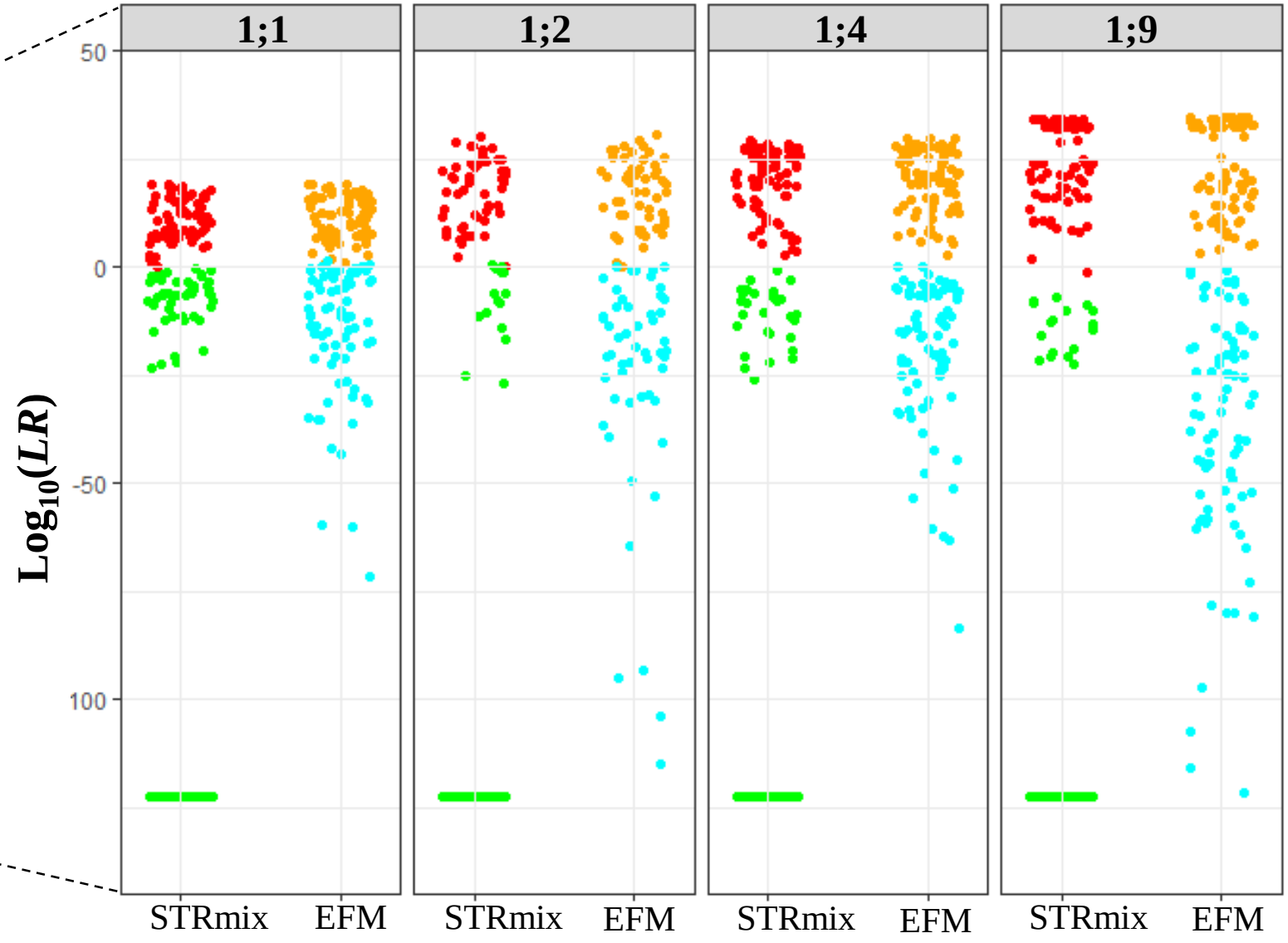
Comparison Group	*P-value*
2P (STRmix vs EFM)	0.74206
3P (STRmix vs EFM)	0.64155
STRmix (2P vs 3P)	**0.02346**
EFM (2P vs 3P)	**0.04607**

$\text{Log}_{10}(LR)$ Distribution from 2P by **software**, **contributor ratios** and **DNA treatments**

$Log_{10}(LR)$ Distribution by Software & Mixture Ratios

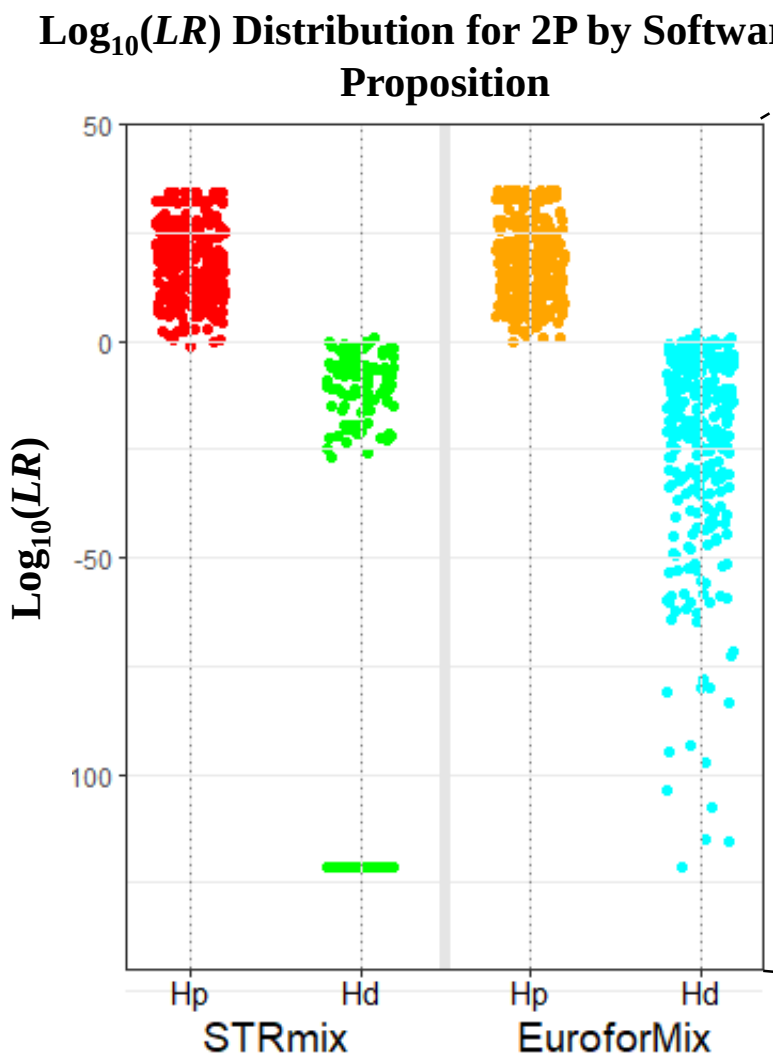
2P

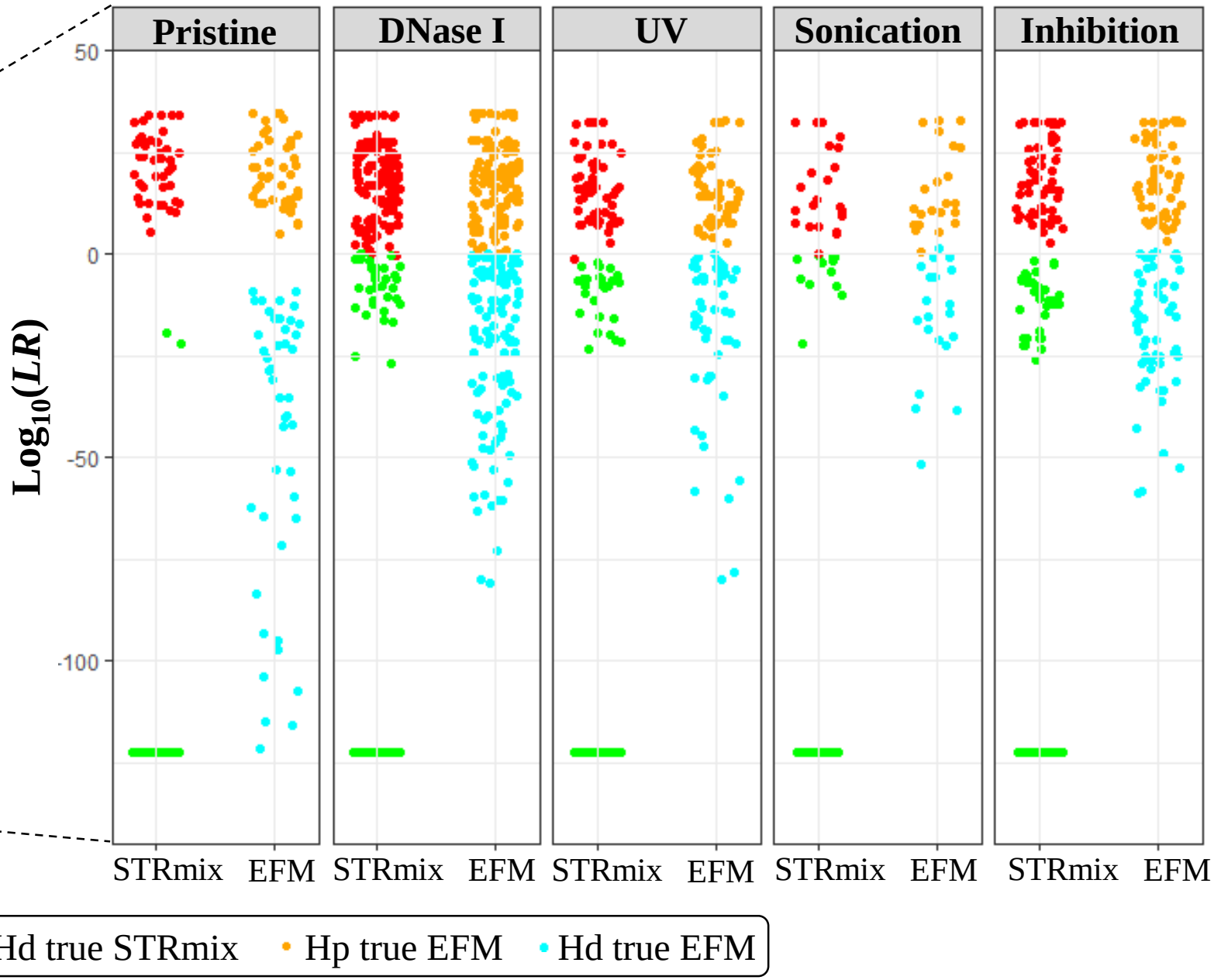




$Log_{10}(LR)$ Distribution by Software & Treatment

2P

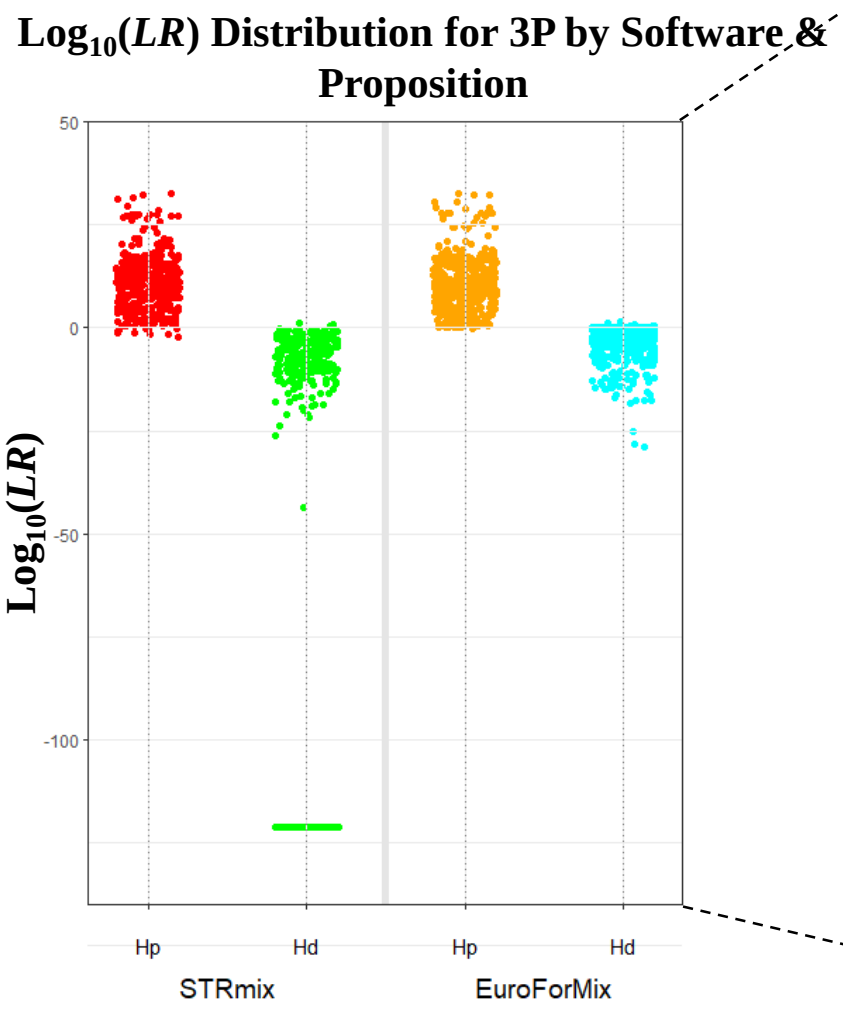


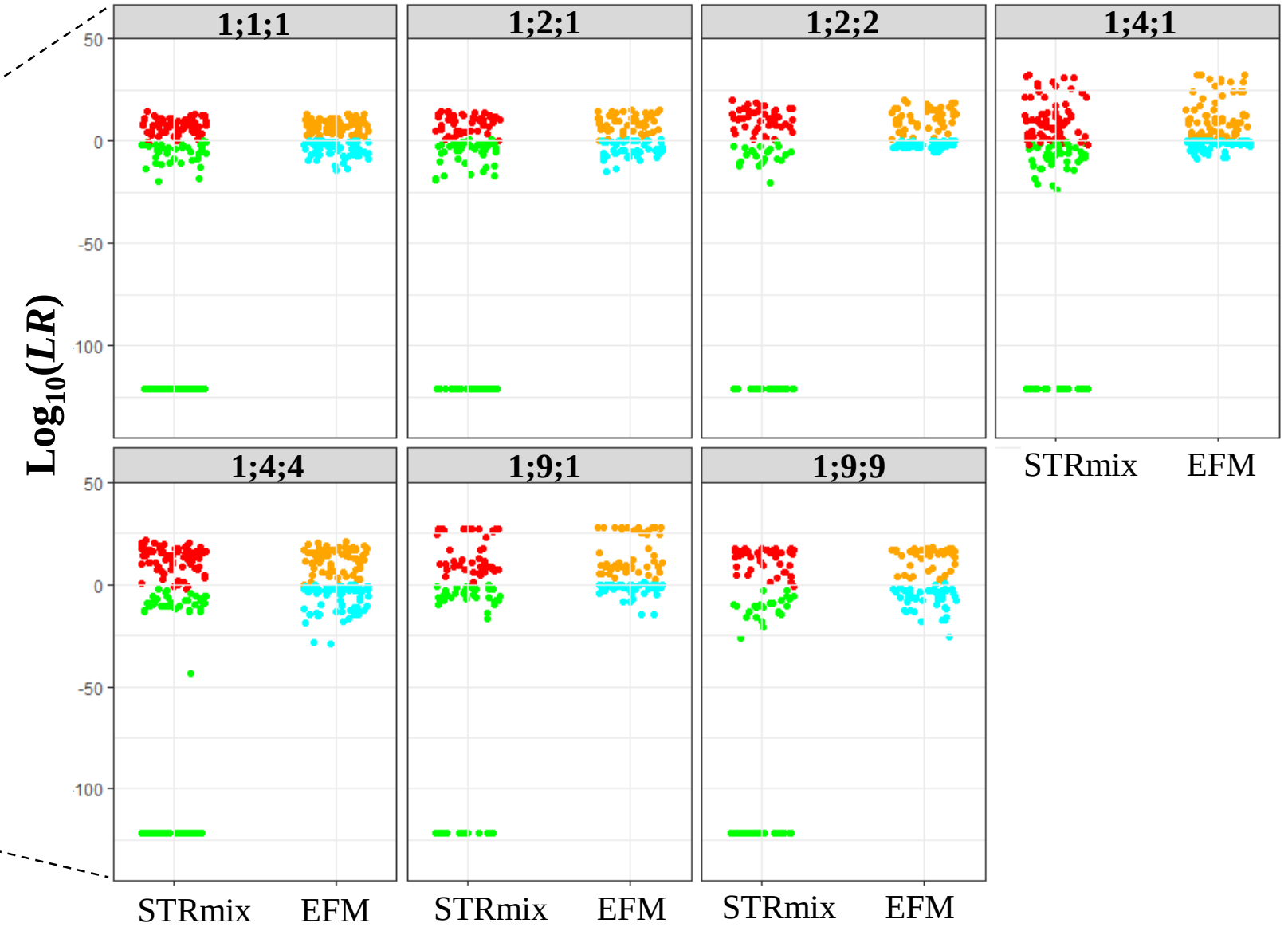


$\text{Log}_{10}(LR)$ Distribution from 3P by **software**, **contributor ratios** and **DNA treatments**

$Log_{10}(LR)$ Distribution by Software & Mixture Ratios

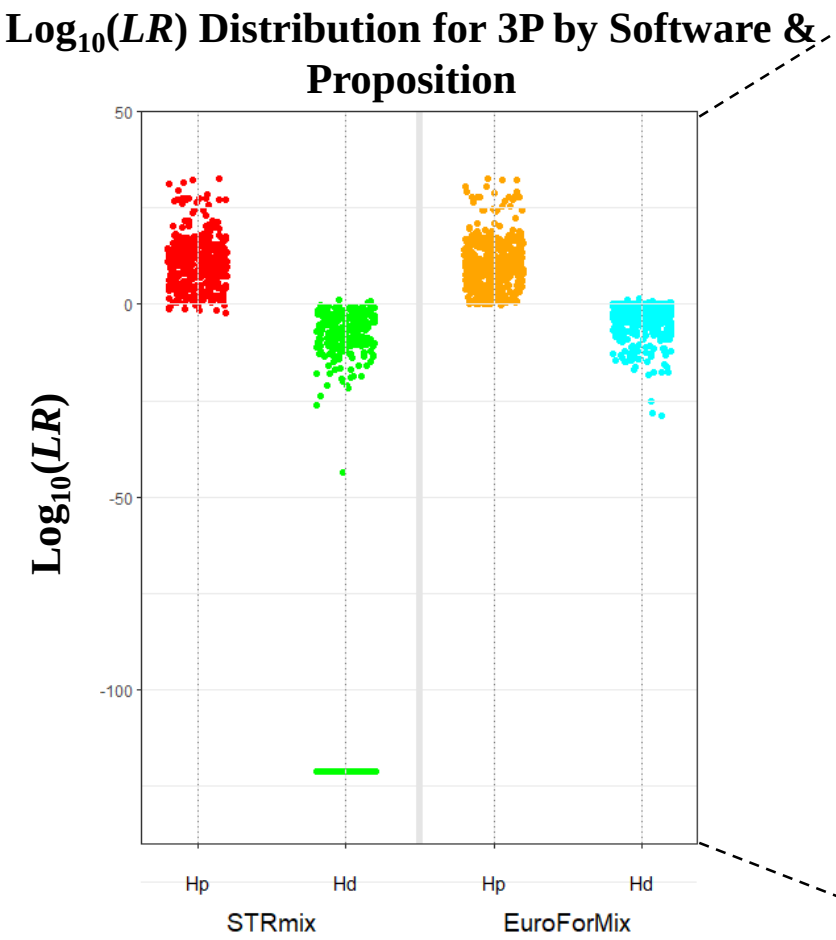
3P

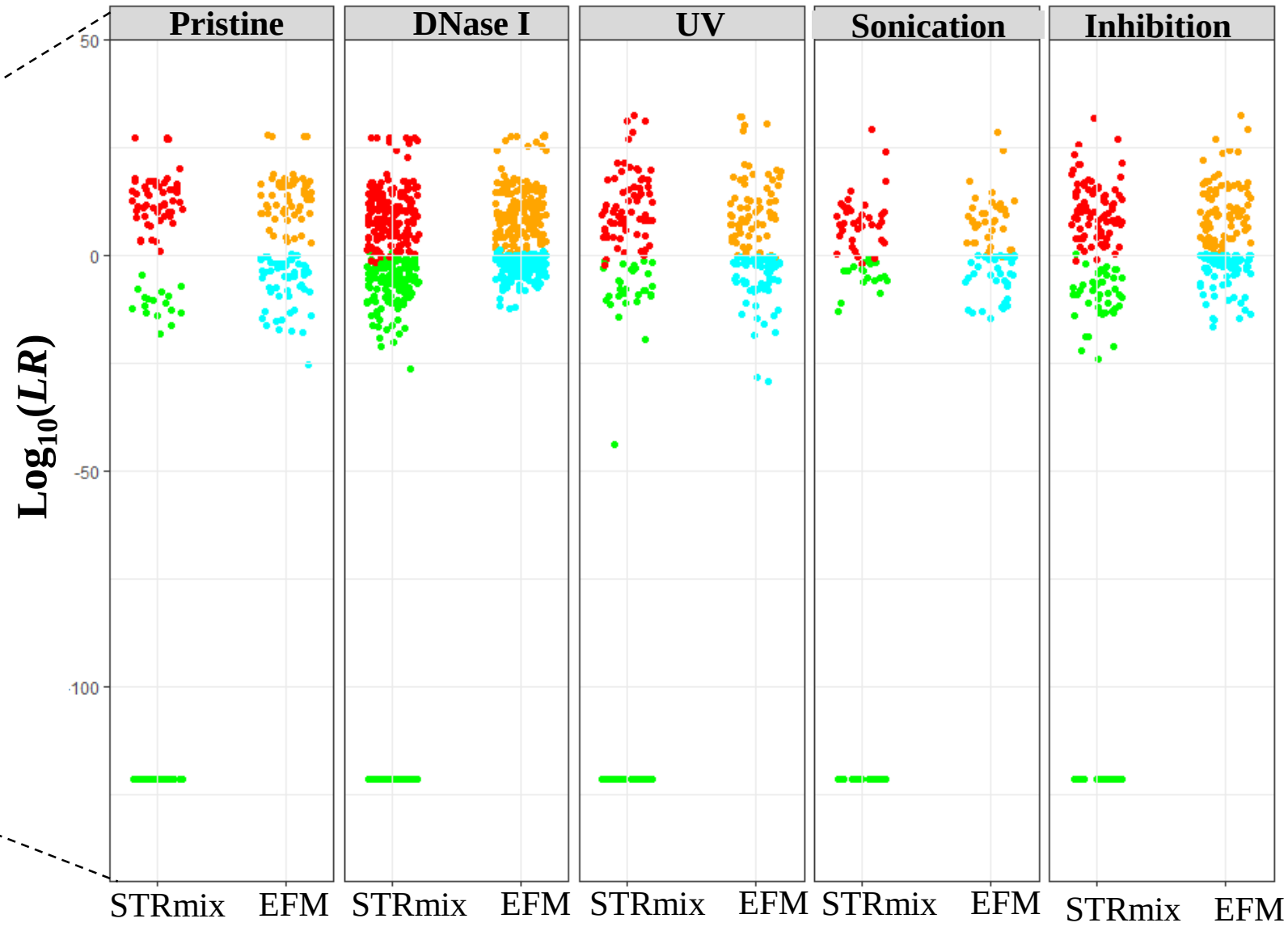




$Log_{10}(LR)$ Distribution by Software & Treatment

3P

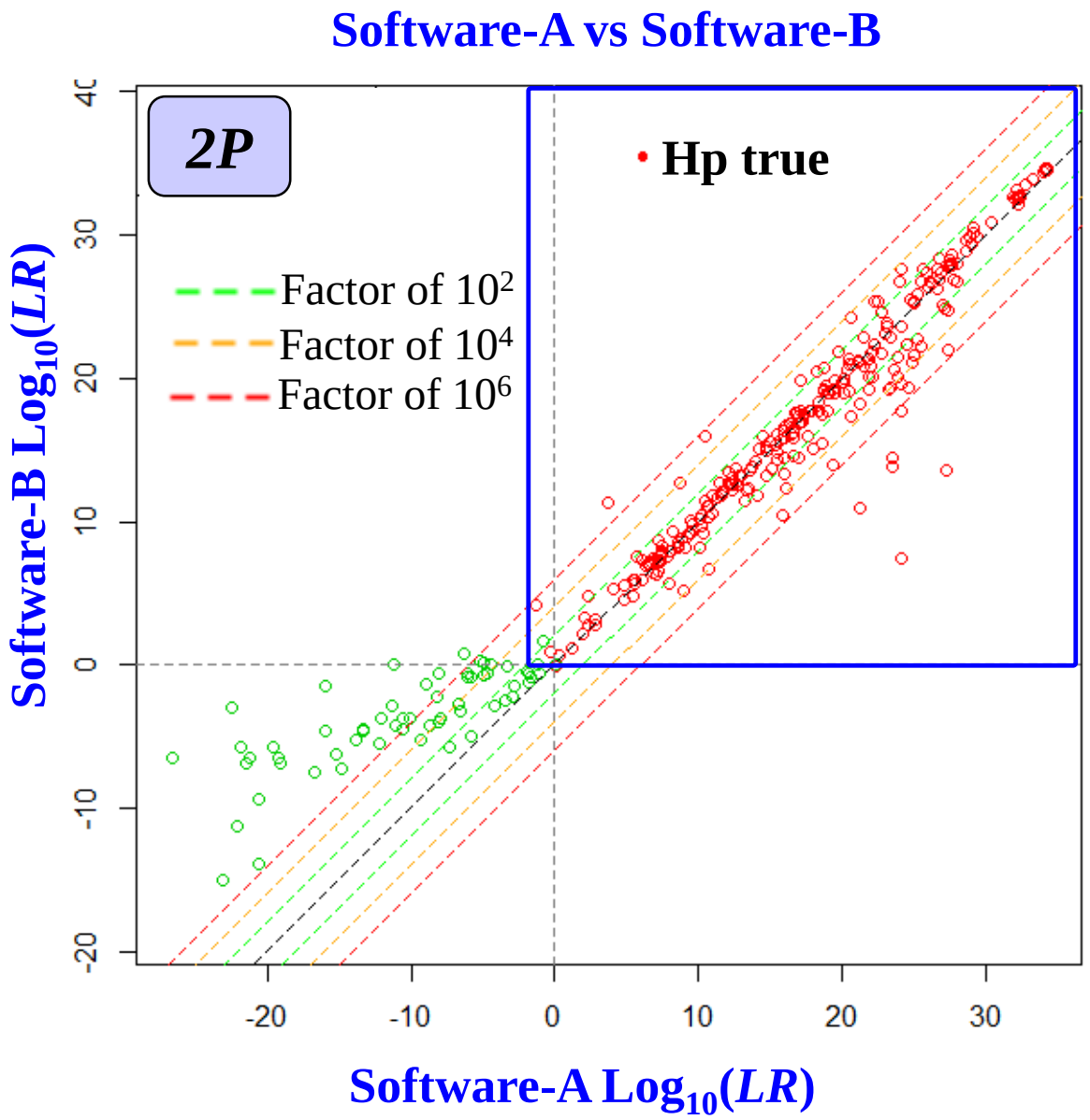


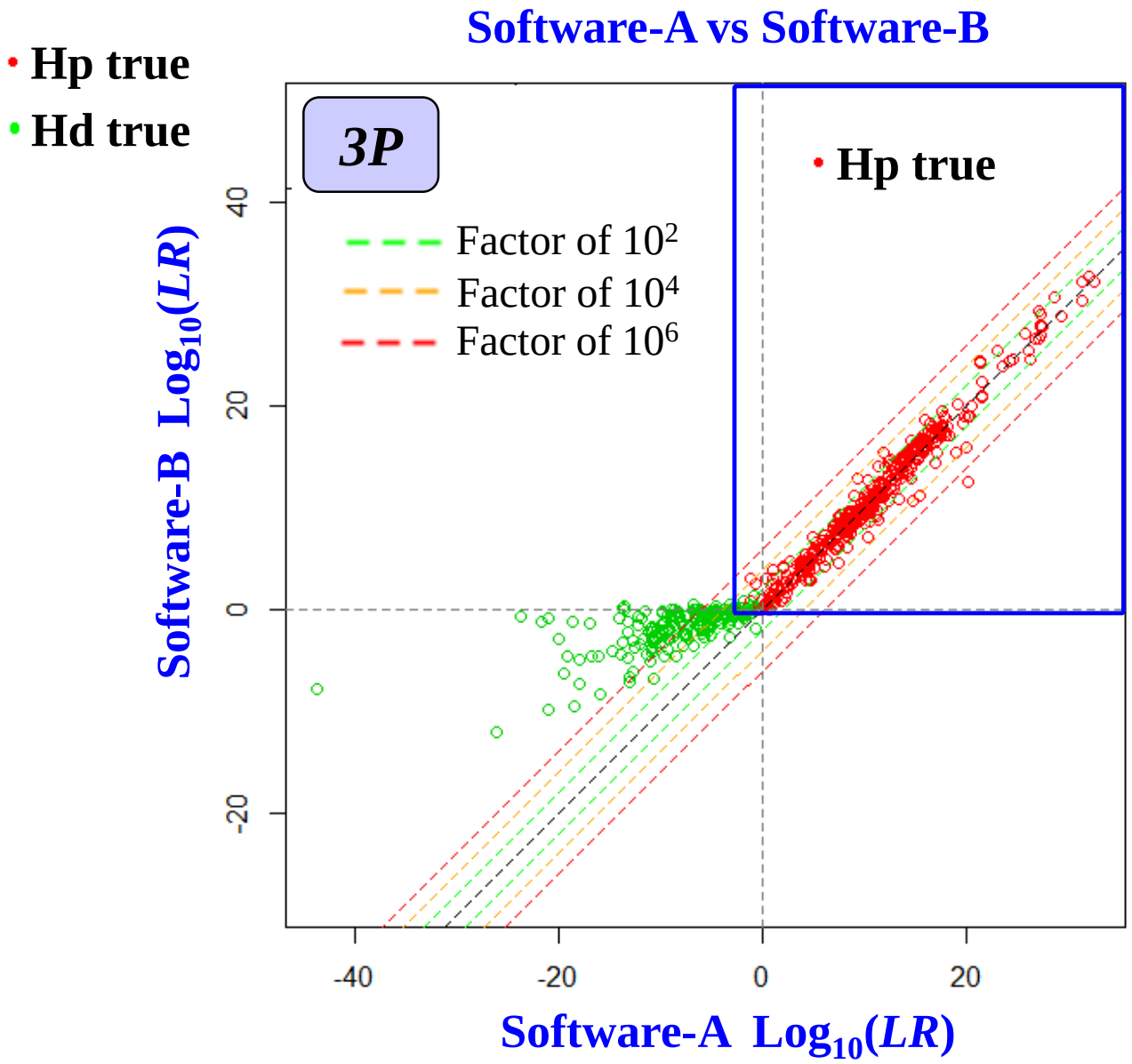


Global overall profile $\mathbf{Log_{10}(\boldsymbol{LR})}$ from each software

Note for the following plots: $\mathbf{Log_{10}(\boldsymbol{LR})}$ from each software is shown ‘as is’ without further designation of the sample type, ratio, treatment, or software run diagnostics (this will be addressed in future work)

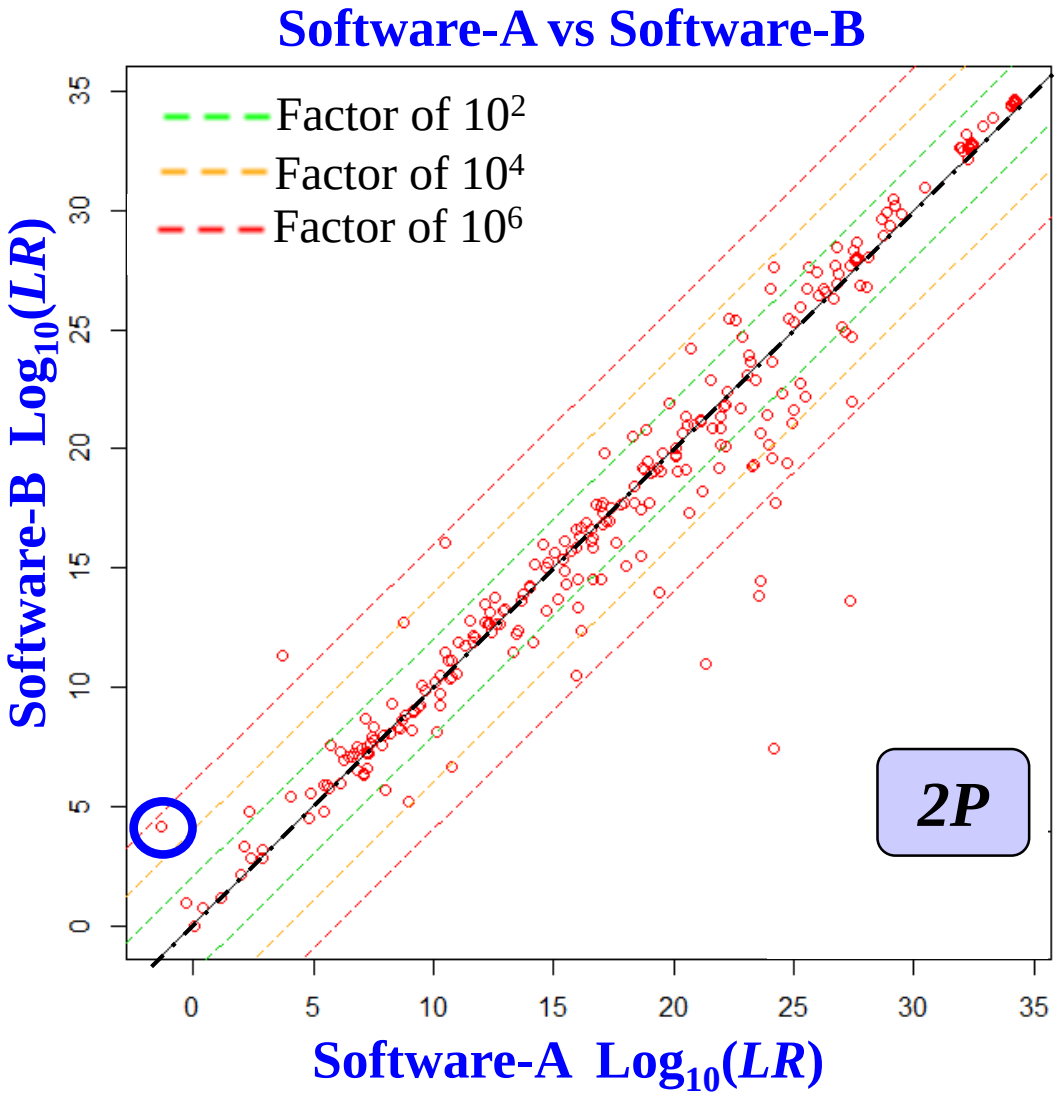
Global profile $\text{Log}_{10}(LR)$ from 2P and 3P

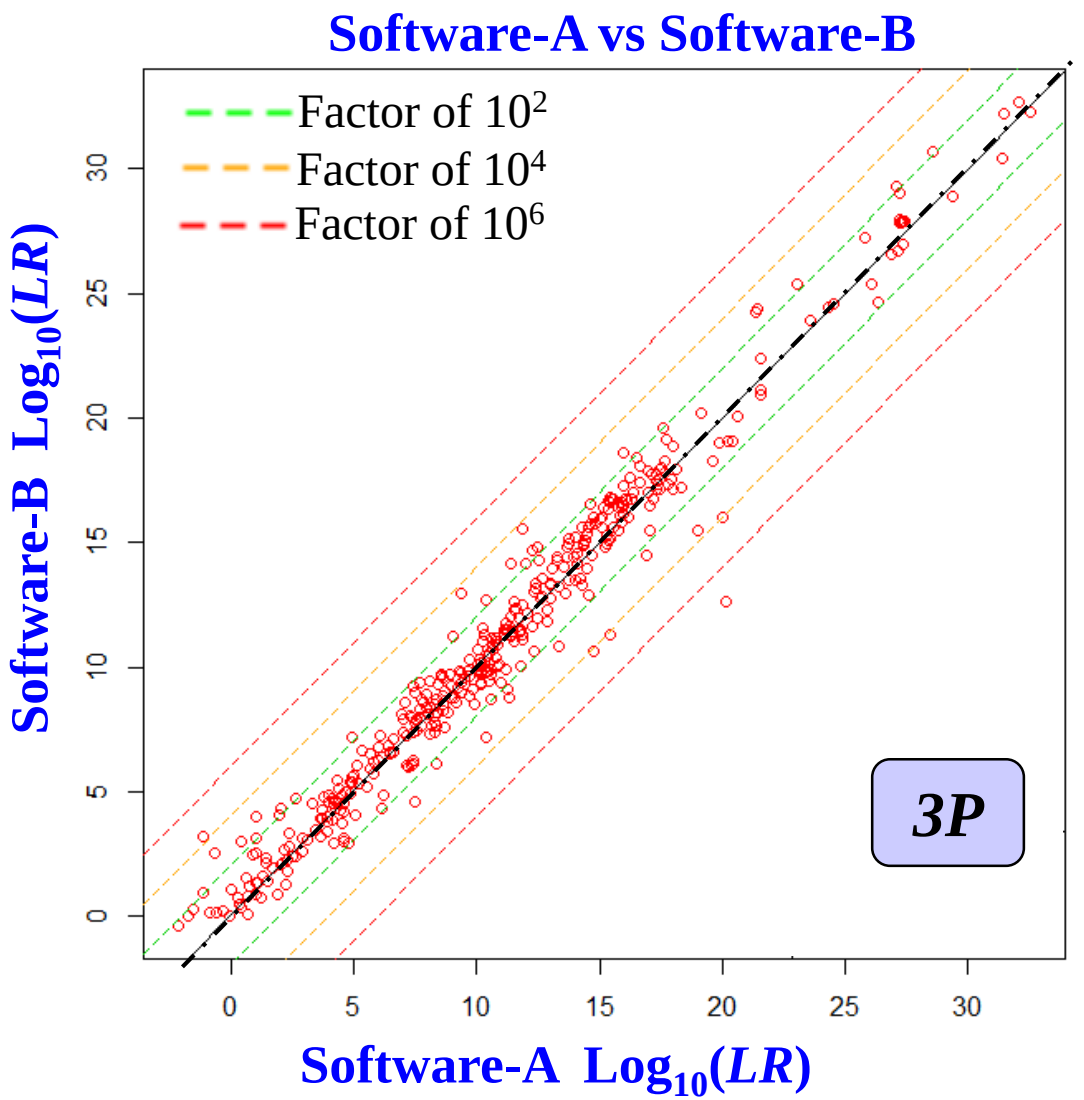




Note: $\text{Log}_{10}(LR)$ for Hd true tests with values of - ∞ from either software are not shown in these graphs

Global profile $Log_{10}(LR)$ from 2P and 3P for Hp true





Conclusions

- The publicly available PROVEDIt database is a useful resource to understand probabilistic genotyping software
- The effects of software (STRmix and EuroForMix), NOC, mixture ratios, and DNA treatments on LR assessment were examined
- As expected, both software showed high degree of discrimination between Hp TRUE and Hd TRUE distributions across different ratios and treatments for 2 and 3 contributor samples
- When it came to **sample to sample profile comparisons** the degree of agreement between the two software varied

Future work

- Further investigation is needed to **understand the source(s) behind the LR differences** (e.g. MCMC settings, diagnostics, number of iterations, stutter models on/off, seed number)
- Analyze **additional samples** at different mixture ratios, treatments, and DNA amounts
- Explore the ***4P mixtures***
- Study **deconvolution analysis** of major and minor contributors in both software
- Examine the reported LR values at a **per-locus level**

Disclaimer

Points of view in this presentation are mine and do not necessarily represent the official position of the National Institute of Standards and Technology or the U.S. Department of Commerce.

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https://strbase.nist.gov/pub_pres/2019_Riman_ISFG.pdf