

Supplementary data

Supplementary Table S1. STR profile of DNA A5 and DNA B5 of the Human Random Control DNA Panel, and of HeLa DNA, as determined by capillary electrophoresis.....	3
Suppl. Figures S1-S6: Sensitivity study	4
Suppl. Figure S1. Sensitivity study: Sample 1 ng, human DNA A5	4
Suppl. Figure S2. Sensitivity study: Sample 500 pg, human DNA A5	4
Suppl. Figure S3. Sensitivity study: Sample 250 pg, human DNA A5	5
Suppl. Figure S4. Sensitivity study: Sample 125 pg, human DNA A5	5
Suppl. Figure S5. Sensitivity study: Sample 62.5 pg, human DNA A5	6
Suppl. Figure S6. Sensitivity study: Sample 31.25 pg, human DNA A5	6
Suppl. Figures S7-S11: Degradation study	7
Suppl. Figure S7. Degradation study: Sample 0 mU/μL DNaseI, HeLa DNA	7
Suppl. Figure S8. Degradation study: Sample 0.3 mU/μL DNaseI, HeLa DNA	7
Suppl. Figure S9. Degradation study: Sample 0.6 mU/μL DNaseI, HeLa DNA	8
Suppl. Figure S10. Degradation study: Sample 1.19 mU/μL DNaseI, HeLa DNA	8
Suppl. Figure S11. Degradation study: Sample 2.38 mU/μL DNaseI, HeLa DNA	9
Suppl. Figures S12-S17: Mixture study	9
Suppl. Figure S12. Mixtures study: Sample 500 pg DNA, human DNA B5	9
Suppl. Figure S13. Mixtures study: Sample 50:50 mixture, human DNA A5 and B5	10
Suppl. Figure S14. Mixtures study: Sample 75:25 mixture, human DNA A5 and B5	10
Suppl. Figure S15. Mixtures study: Sample 90:10 mixture, human DNA A5 and B5	11
Suppl. Figure S16. Mixtures study: Sample 95:5 mixture, human DNA A5 and B5	11
Suppl. Figure S17. Mixtures study: Sample 98:2 mixture, human DNA A5 and B5	12
Suppl. Figures S18-S35: Inhibitor study	12
Suppl. Figure S18. Inhibitor study, 30 μM hematin, human DNA A5	12
Suppl. Figure S19. Inhibitor study, 60 μM hematin, human DNA A5	13
Suppl. Figure S20. Inhibitor study, 120 μM hematin, human DNA A5	13
Suppl. Figure S21. Inhibitor study, 120 μM hematin with PowerPlex MM, human DNA A5	14
Suppl. Figure S22. Inhibitor study, 120 μM hematin with BSA, human DNA A5	14
Suppl. Figure S23. Inhibitor study, 50 μM humic acid, human DNA A5	15
Suppl. Figure S24. Inhibitor study, 100 μM humic acid, human DNA A5	15
Suppl. Figure S25. Inhibitor study, 200 μM humic acid, human DNA A5	16
Suppl. Figure S26. Inhibitor study, 200 μM humic acid with PowerPlex MM, human DNA A5	16
Suppl. Figure S27. Inhibitor study, 200 μM humic acid with BSA, human DNA A5	17

Suppl. Figure S28. Inhibitor study, 25µM melanin, human DNA A5	17
Suppl. Figure S29. Inhibitor study, 50 µM melanin, human DNA A5	18
Suppl. Figure S30. Inhibitor study, Sample 100 µM melanin, human DNA A5	18
Suppl. Figure S31. Inhibitor study, 100 µM melanin with PowerPlex MM, human DNA A5	19
Suppl. Figure S32. Inhibitor study, Sample 100 µM melanin with BSA, human DNA A5	19
Suppl. Figure S33. Inhibitor study, 200 µM indigo, human DNA A5	20
Suppl. Figure S34. Inhibitor study, 400 µM indigo, human DNA A5	20
Suppl. Figure S35. Inhibitor study, 800 µM indigo, human DNA A5	21
Suppl. Figures S36-S38: Electropherograms of DNA A5, B5, and HeLa DNA	22
Suppl. Figure S36. Electropherogram of DNA A5 (500 pg, analysed with PowerPlex ESX17)	22
Suppl. Figure S37. Electropherogram of DNA B5 (500 pg, analysed with PowerPlex ESX17)	23
Suppl. Figure S38. Electropherogram of HeLa DNA (500 pg, analysed with PowerPlex ESX17)	24
Suppl. Figure S39: Intra- and inter-locus balances of profiles obtained with maSTR assay or CE analysis of PCR inhibitor-supplemented DNA samples	25
Suppl. Figure S40: Intra-locus balance of HeLa DNA obtained with maSTR assay or CE analysis	26
Supplementary Table S2. Calculation of sequencing costs	27

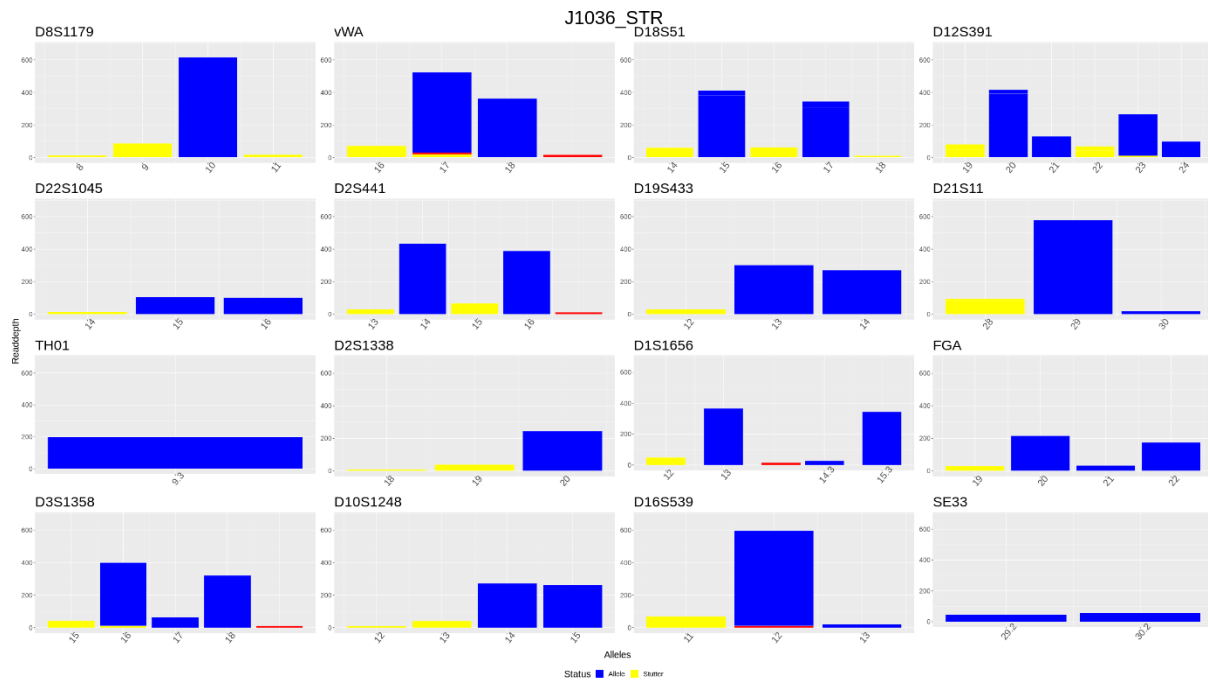
Supplementary Table S1. STR profile of DNA A5 and DNA B5 of the Human Random Control DNA Panel, and of HeLa DNA, as determined by capillary electrophoresis (see Figures S36-S38).

Locus	A5	B5*	HeLa
<i>D10S1248</i>	14, 15	<u>14</u> , 16	13, 15
<i>D12S391</i>	20, 23	19, 22	20, 25
<i>D16S539</i>	12	11	9, 10
<i>D18S51</i>	15, 17	14, 16	16
<i>D19S433</i>	13, 14	<u>13</u> , 14.2	13, 14
<i>D1S1656</i>	13, 15.3	<u>15.3</u> , 17.3	12, 15
<i>D21S11</i>	29	30, 32.2	27, 28
<i>D22S1045</i>	15, 16	14, <u>15</u>	16, 17
<i>D2S1338</i>	20	17, <u>20</u>	17
<i>D2S441</i>	14, 16	11, <u>14</u>	10, 11
<i>D3S1358</i>	16, 18	15, 17	15, 18
<i>D8S1179</i>	10	9, 14	12, 13
<i>FGA</i>	20, 22	23, 25	18, 21
<i>SE33</i>	29.2, 30.2	28.2, <u>30.2</u>	20
<i>TH01</i>	9.3	6	7
<i>vWA</i>	17, 18	<u>18</u>	16, 18

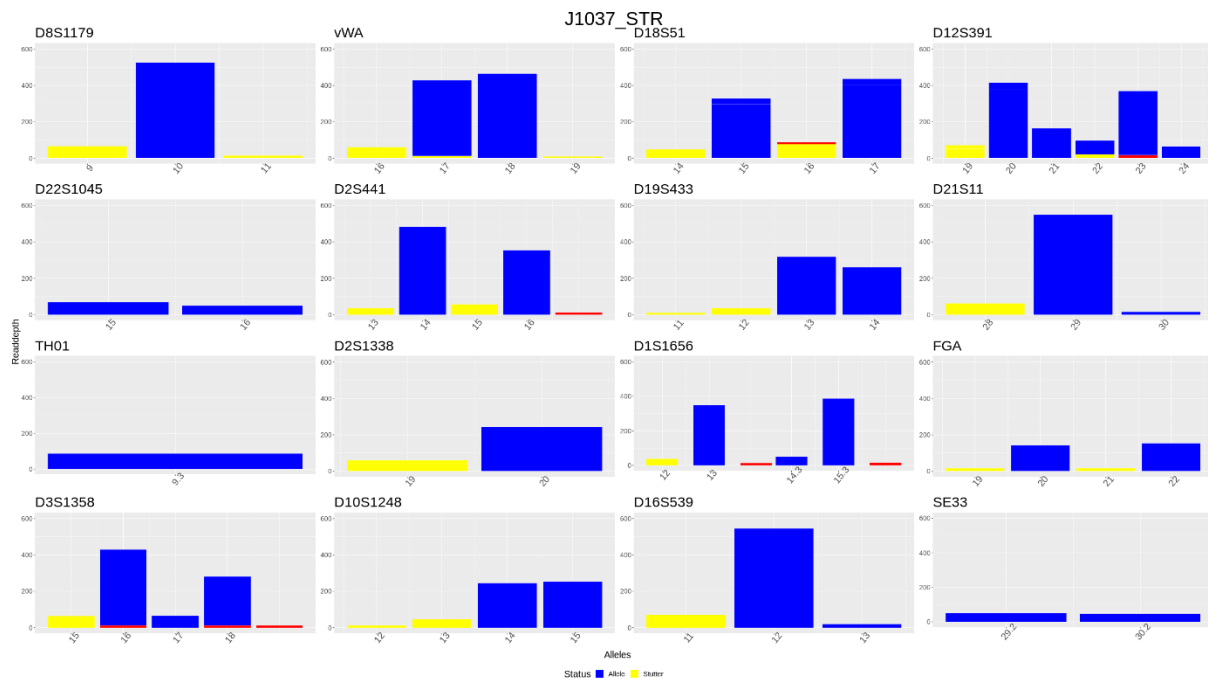
*underlined: alleles shared with DNA A5

Suppl. Figures S1-S6: Sensitivity study

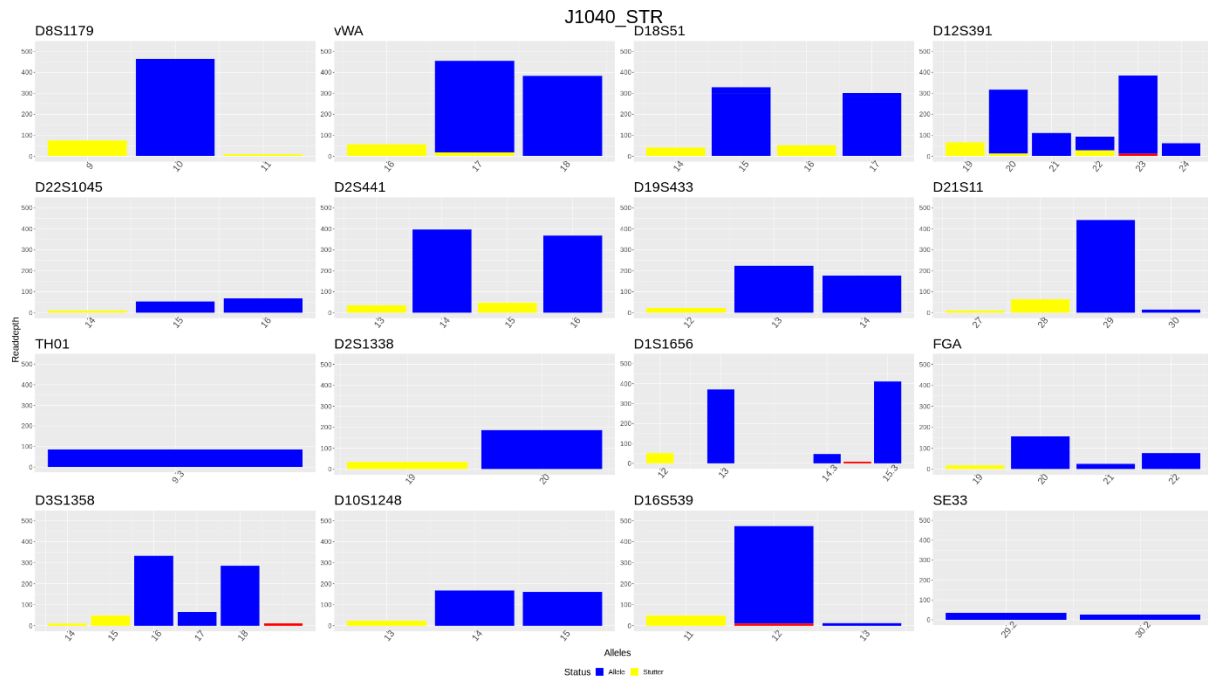
Suppl. Figure S1. Sensitivity study: Sample 1 ng, human DNA A5



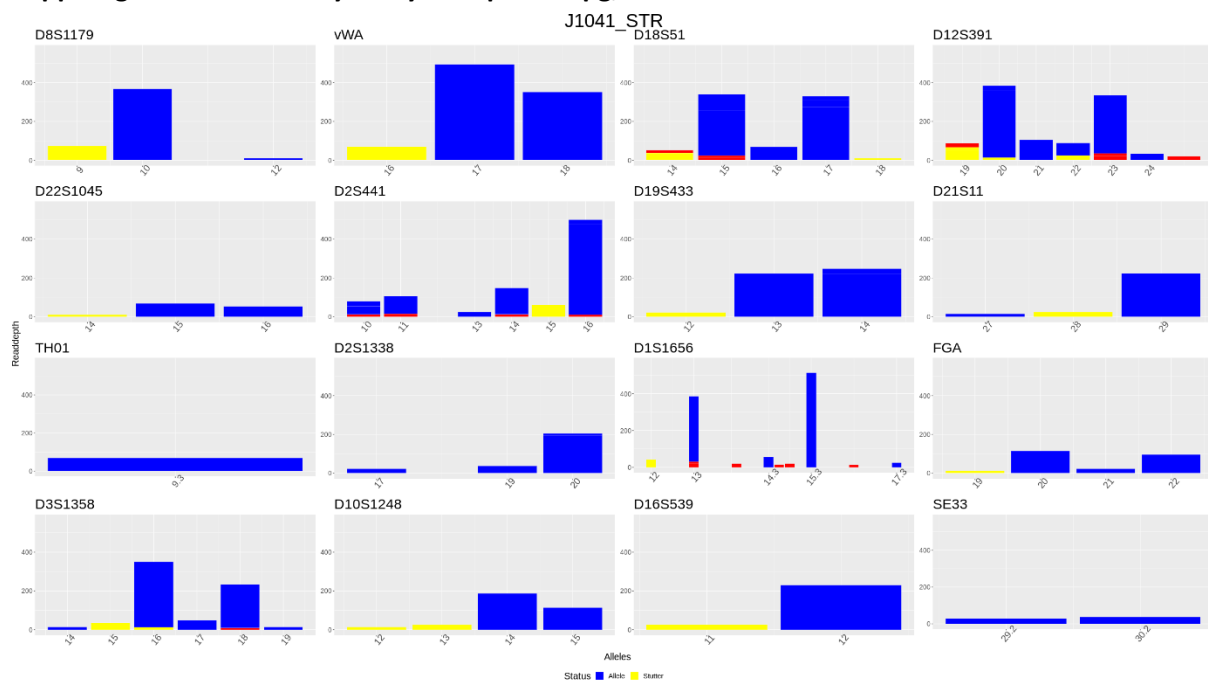
Suppl. Figure S2. Sensitivity study: Sample 500 pg, human DNA A5



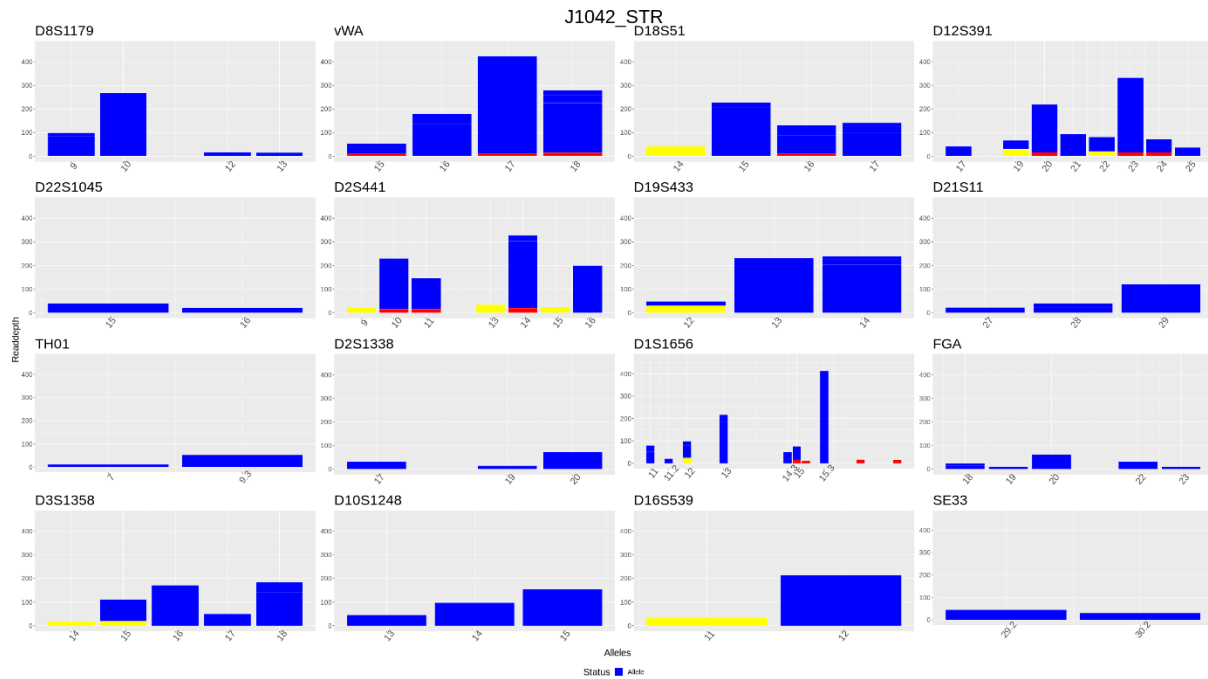
Suppl. Figure S3. Sensitivity study: Sample 250 pg, human DNA A5



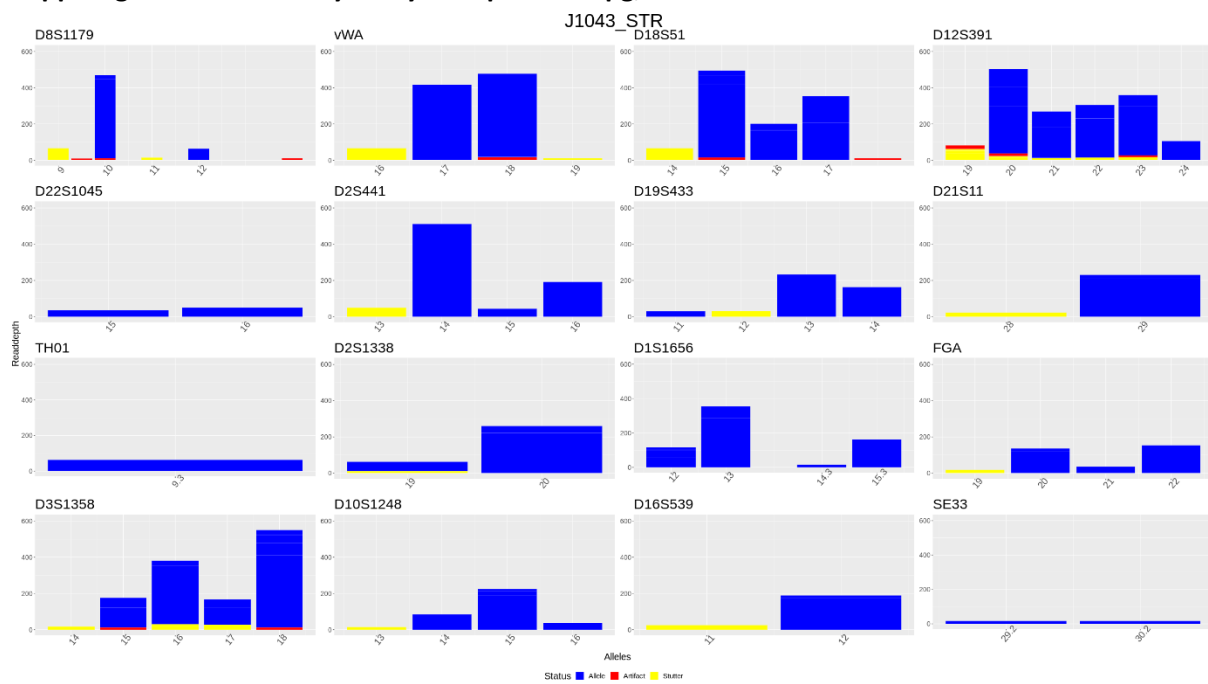
Suppl. Figure S4. Sensitivity study: Sample 125 pg, human DNA A5



Suppl. Figure S5. Sensitivity study: Sample 62.5 pg, human DNA A5

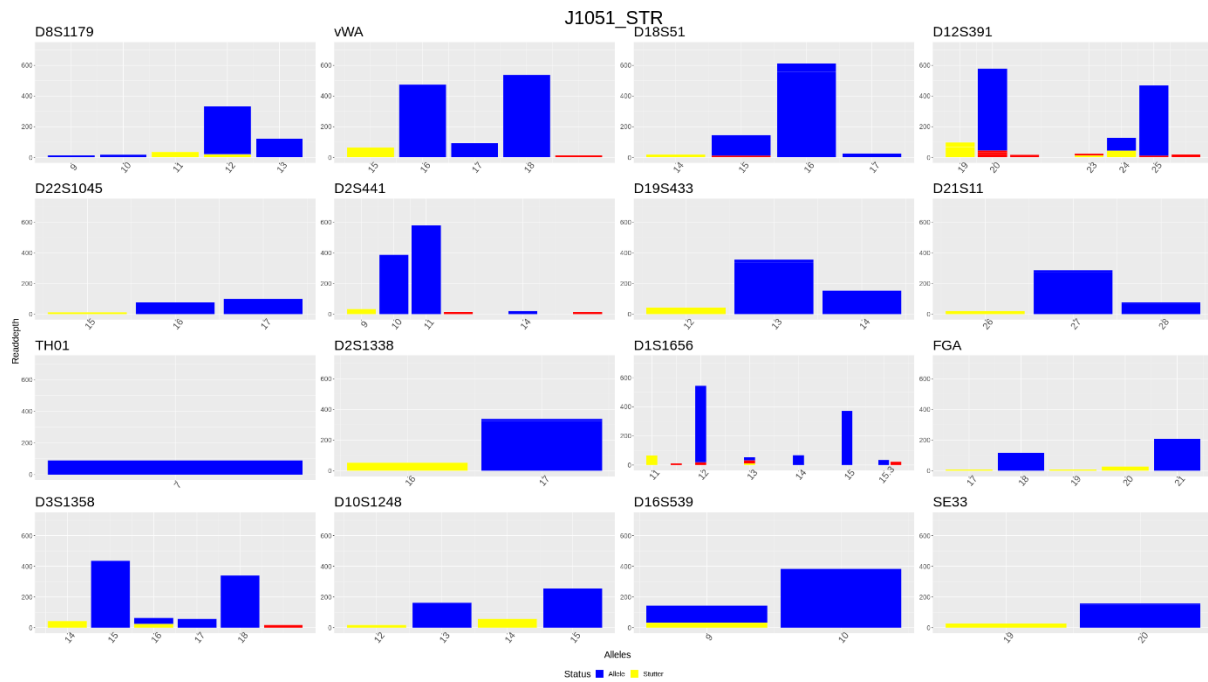


Suppl. Figure S6. Sensitivity study: Sample 31.25 pg, human DNA A5

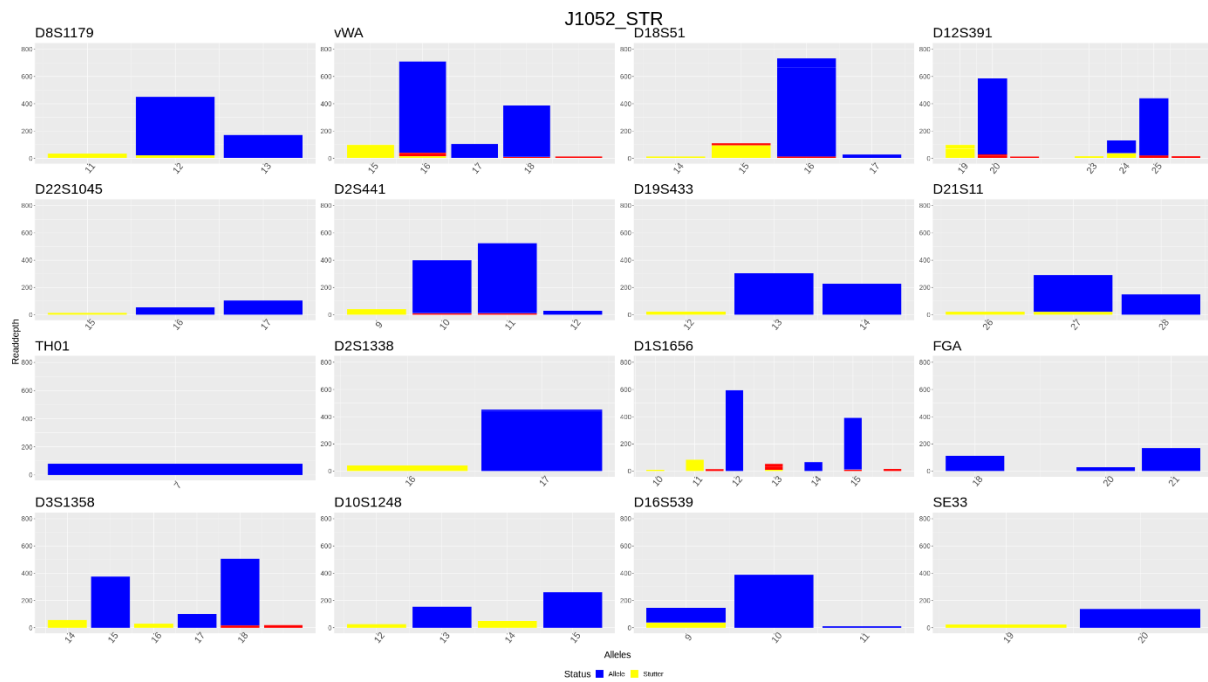


Suppl. Figures S7-S11: Degradation study

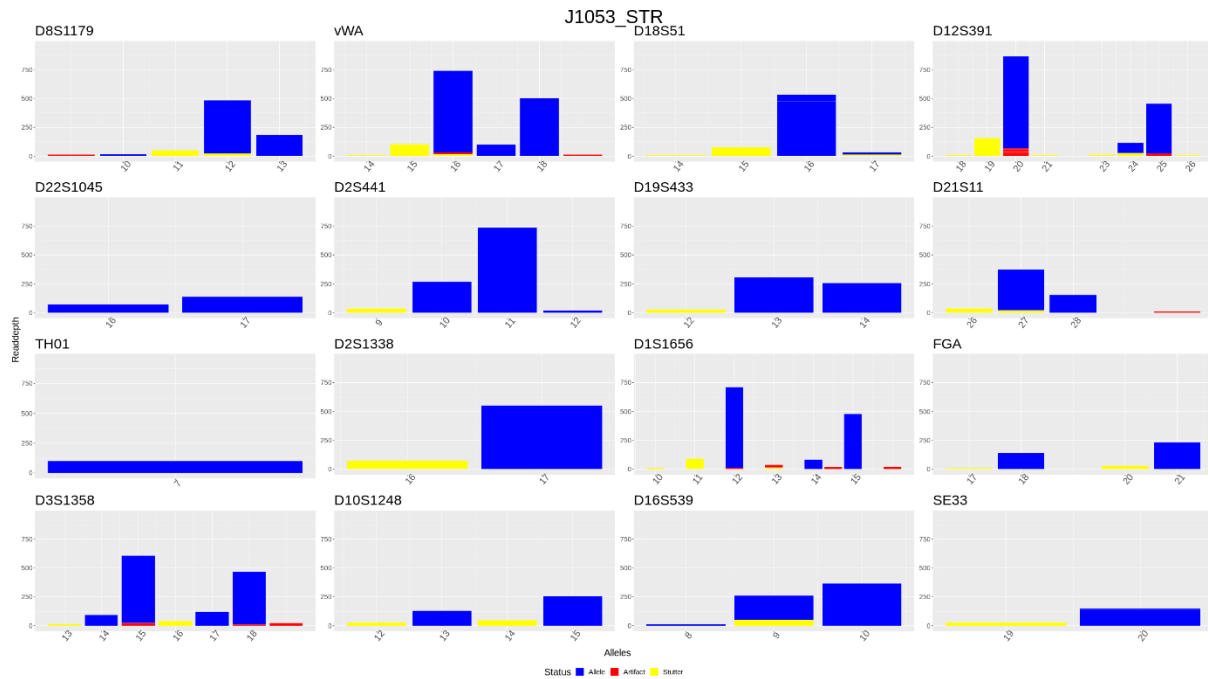
Suppl. Figure S7. Degradation study: Sample 0 mU/μL DNaseI, Hela DNA



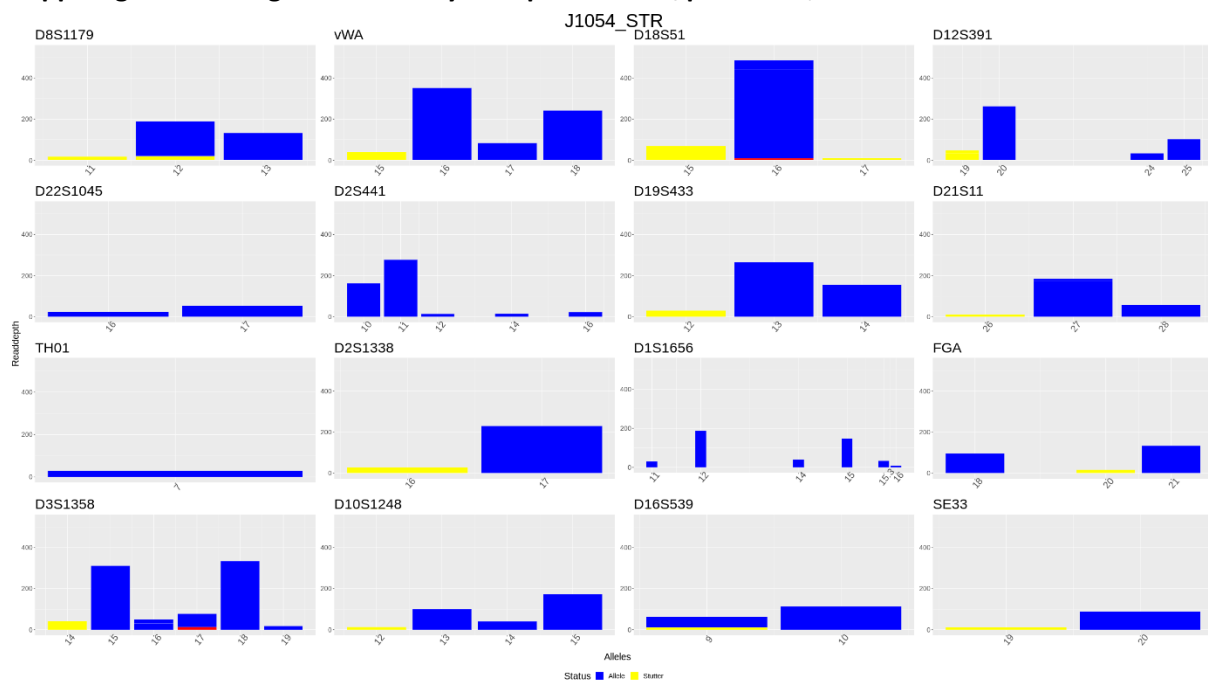
Suppl. Figure S8. Degradation study: Sample 0.3 mU/μL DNaseI, Hela DNA



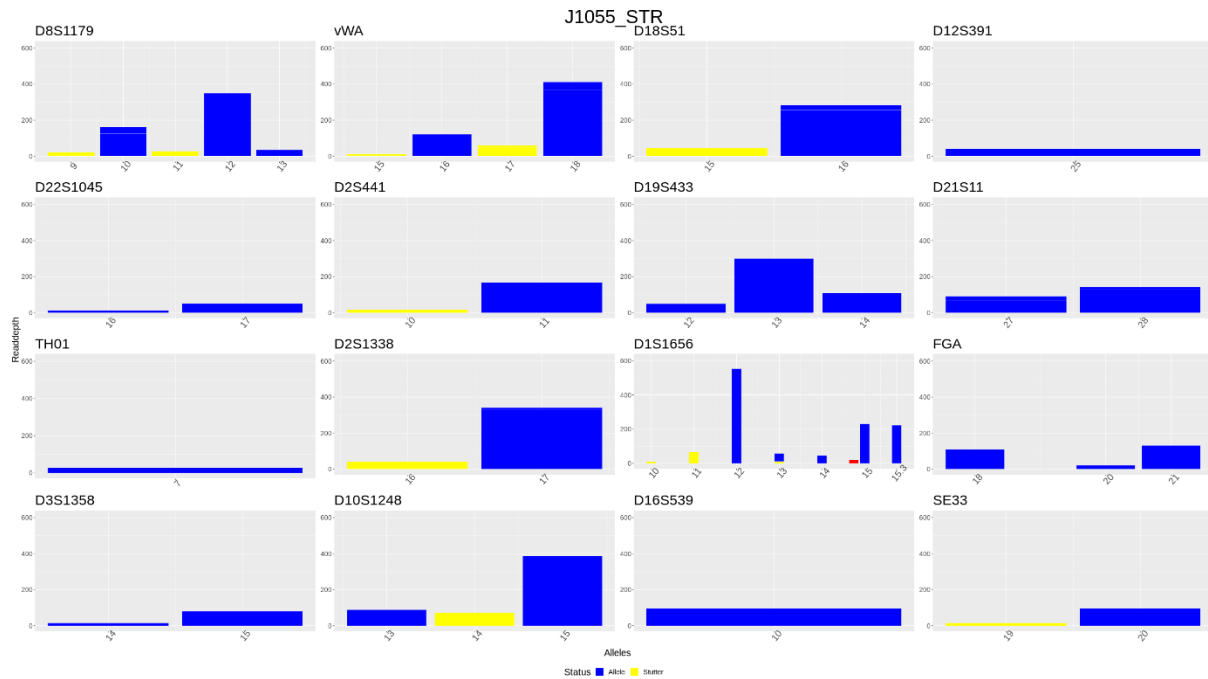
Suppl. Figure S9. Degradation study: Sample 0.6 mU/μL DNaseI, Hela DNA



Suppl. Figure S10. Degradation study: Sample 1.19 mU/μL DNaseI, Hela DNA

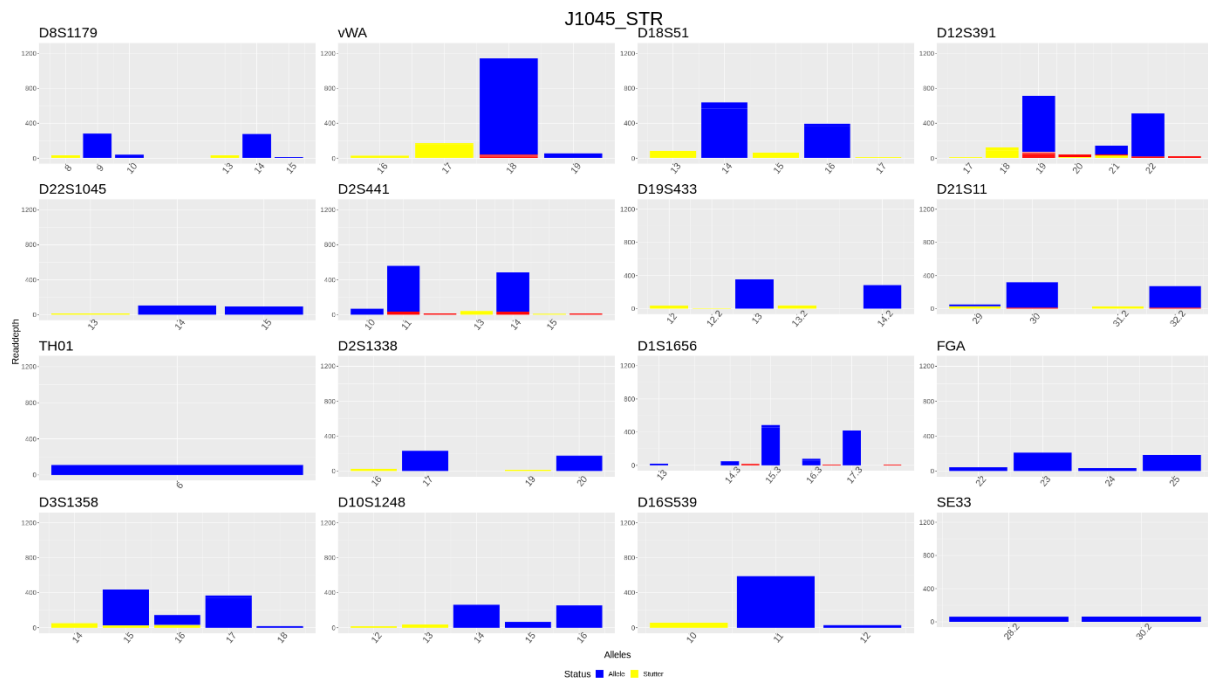


Suppl. Figure S11. Degradation study: Sample 2.38 mU/μL DNaseI, Hela DNA

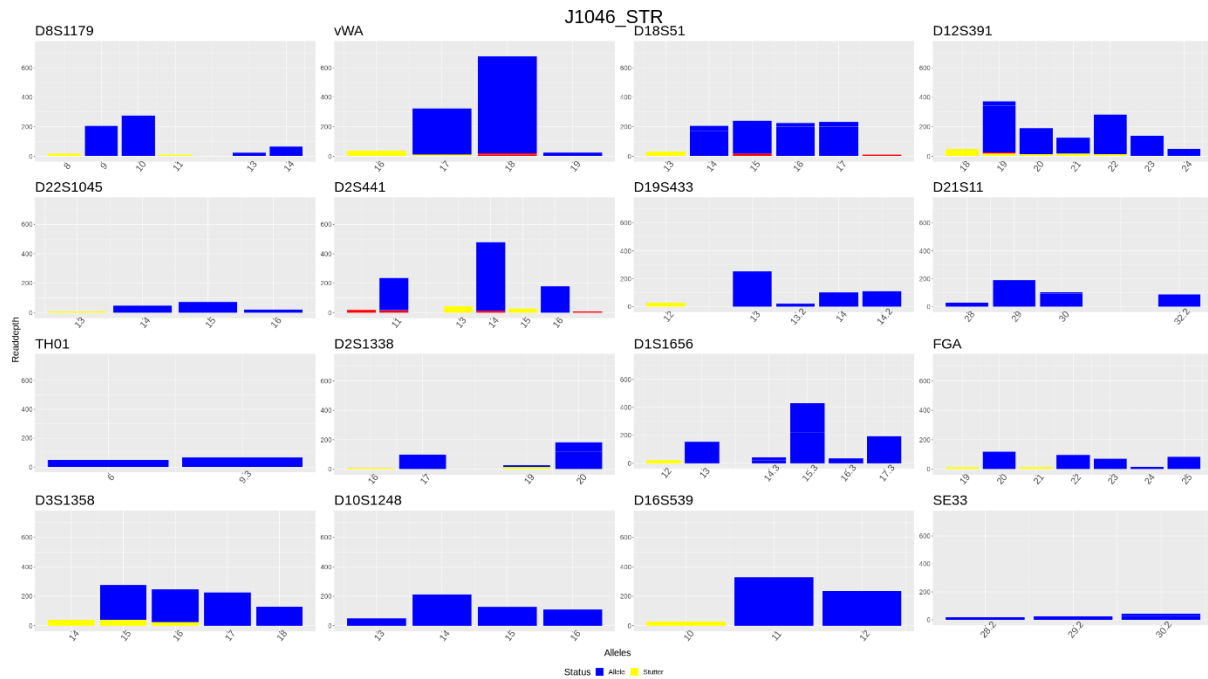


Suppl. Figures S12-S17: Mixture study

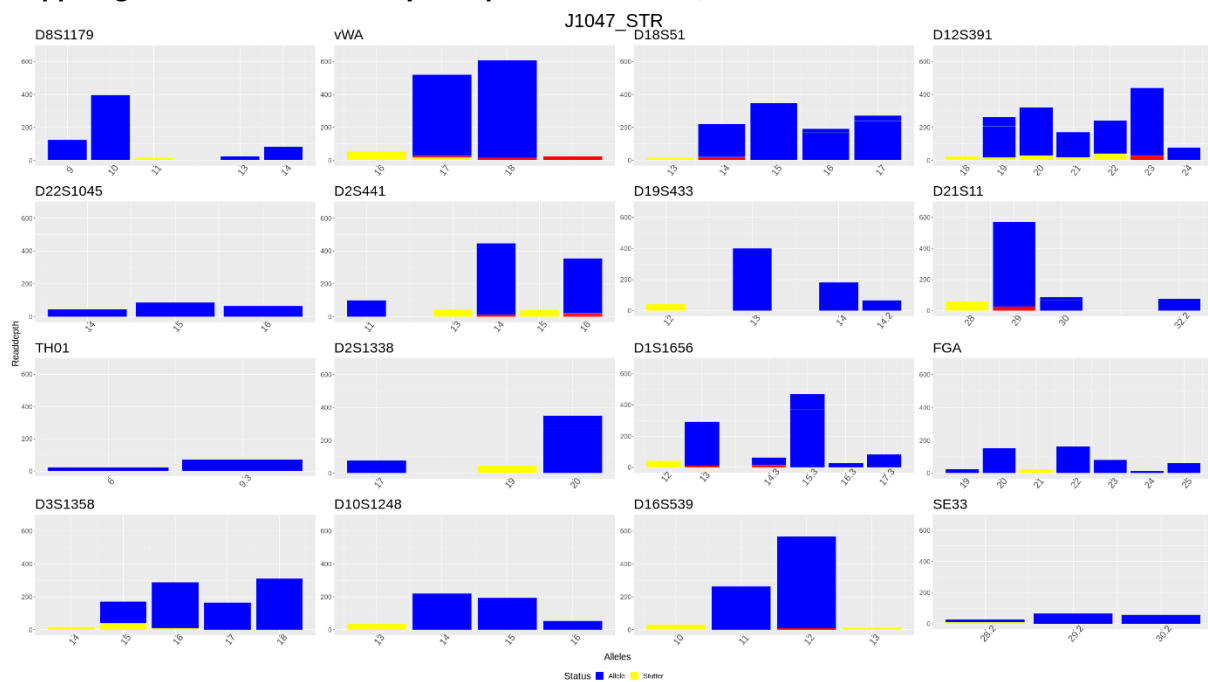
Suppl. Figure S12. Mixtures study: Sample 500 pg DNA, human DNA B5



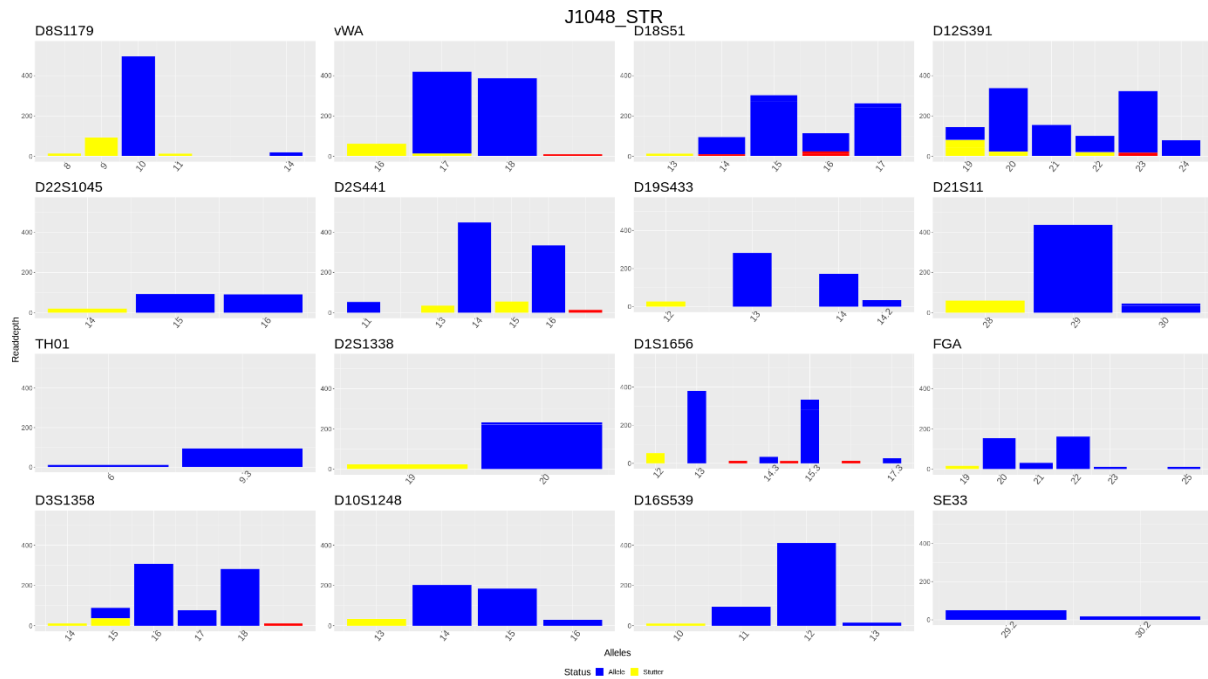
Suppl. Figure S13. Mixtures study: Sample 50:50 mixture, human DNA A5 and B5



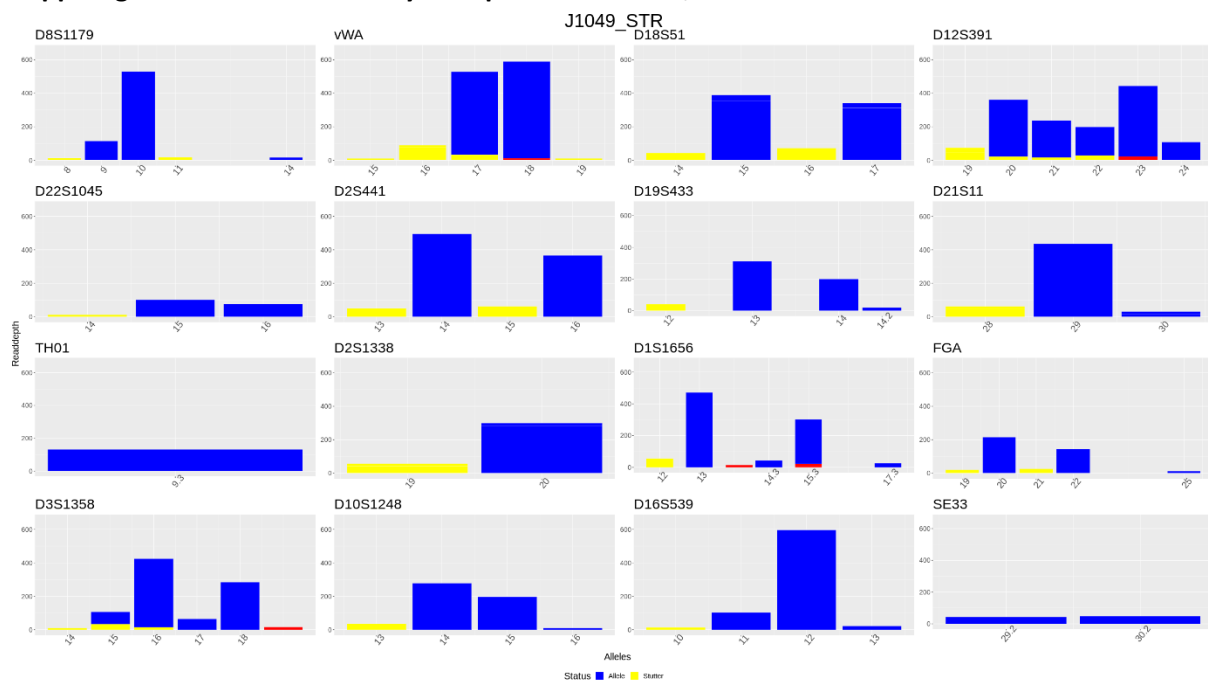
Suppl. Figure S14. Mixtures study: Sample 75:25 mixture, human DNA A5 and B5



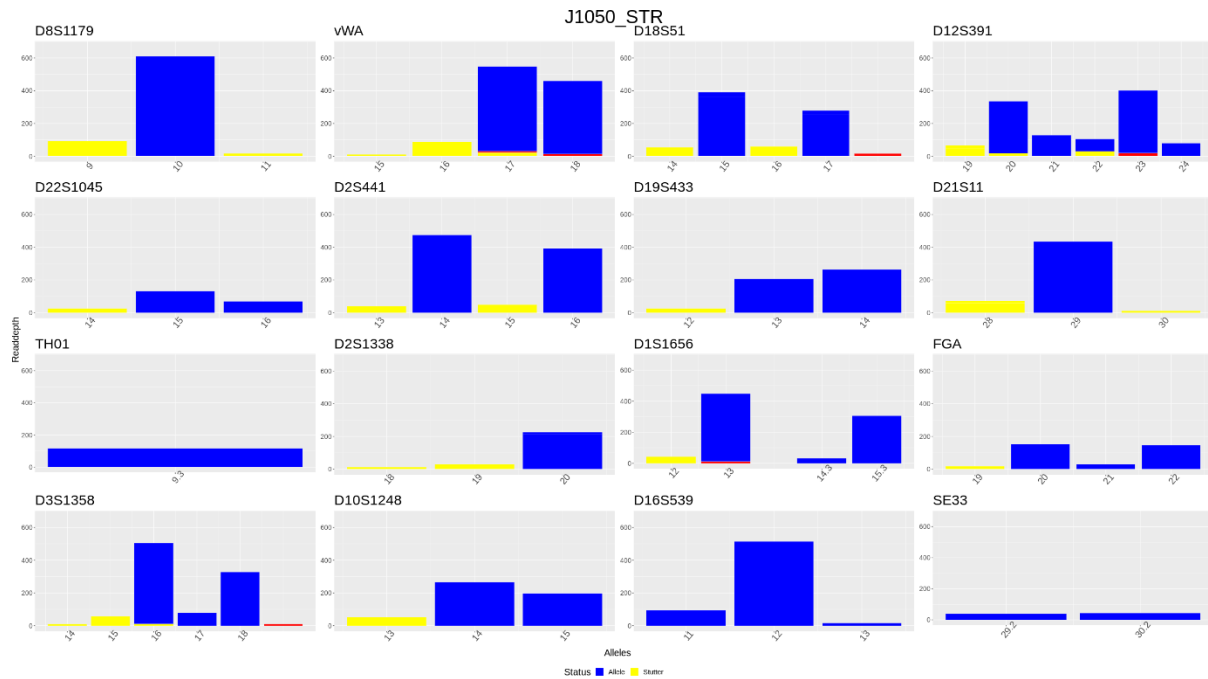
Suppl. Figure S15. Mixtures study: Sample 90:10 mixture, human DNA A5 and B5



Suppl. Figure S16. Mixtures study: Sample 95:5 mixture, human DNA A5 and B5

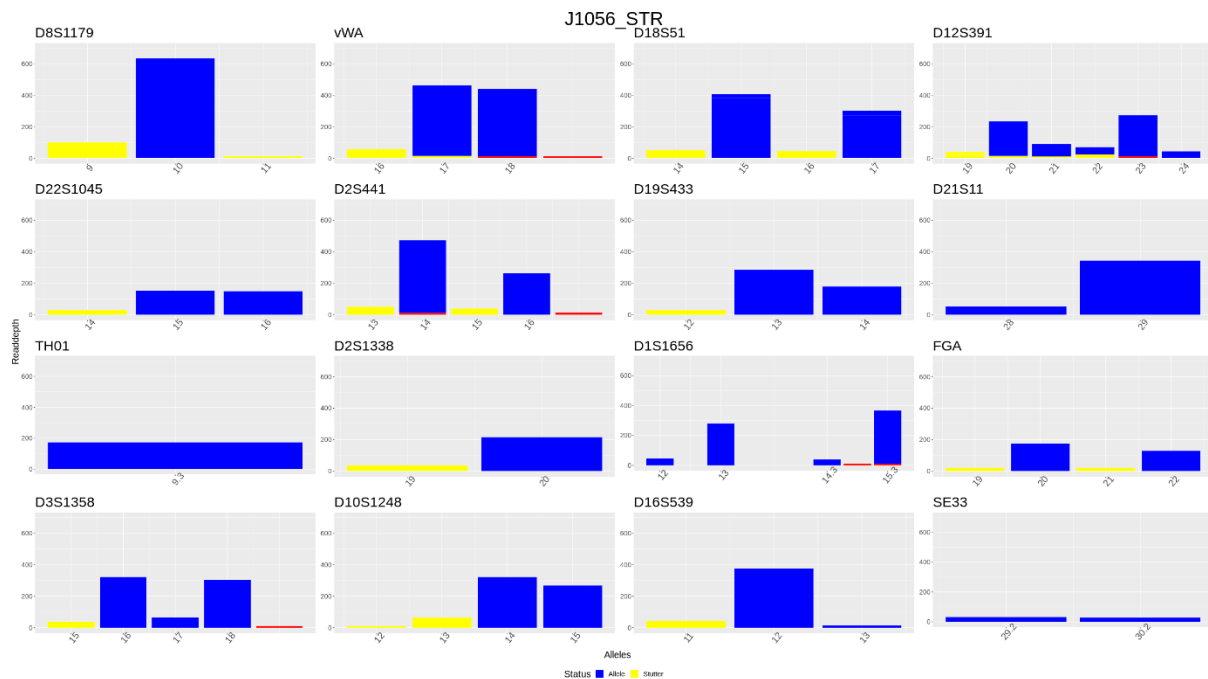


Suppl. Figure S17. Mixtures study: Sample 98:2 mixture, human DNA A5 and B5

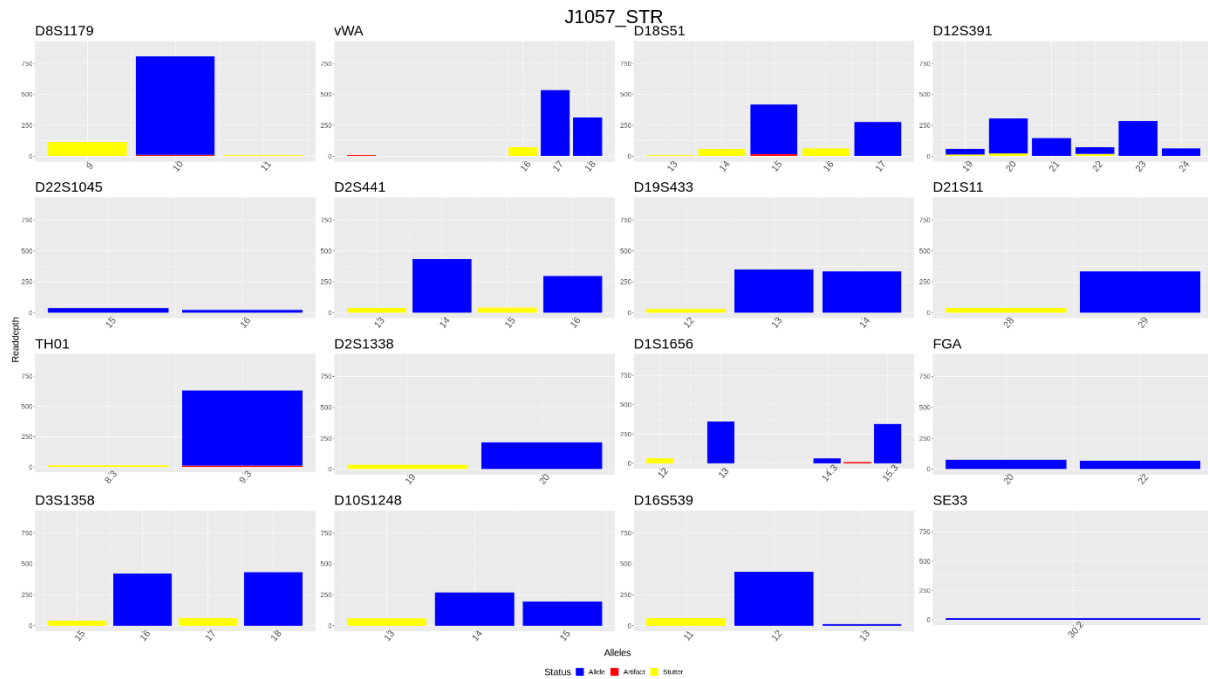


Suppl. Figures S18-S35: Inhibitor study

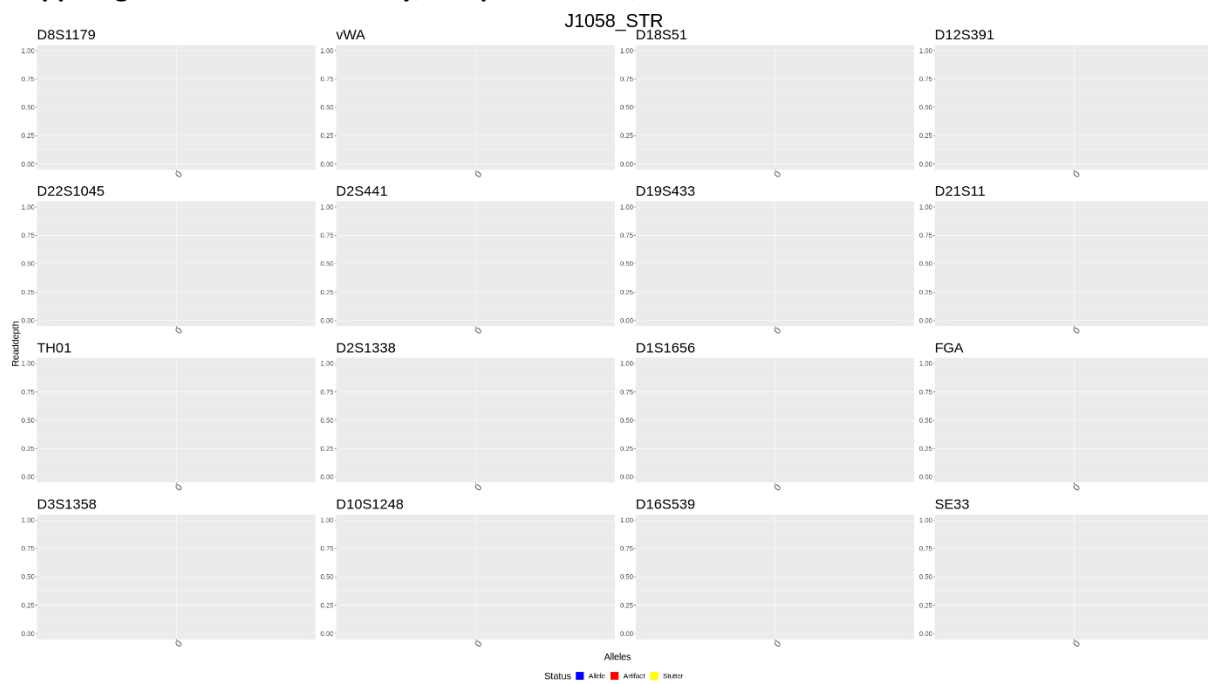
Suppl. Figure S18. Inhibitor study, 30 μ M hematin, human DNA A5



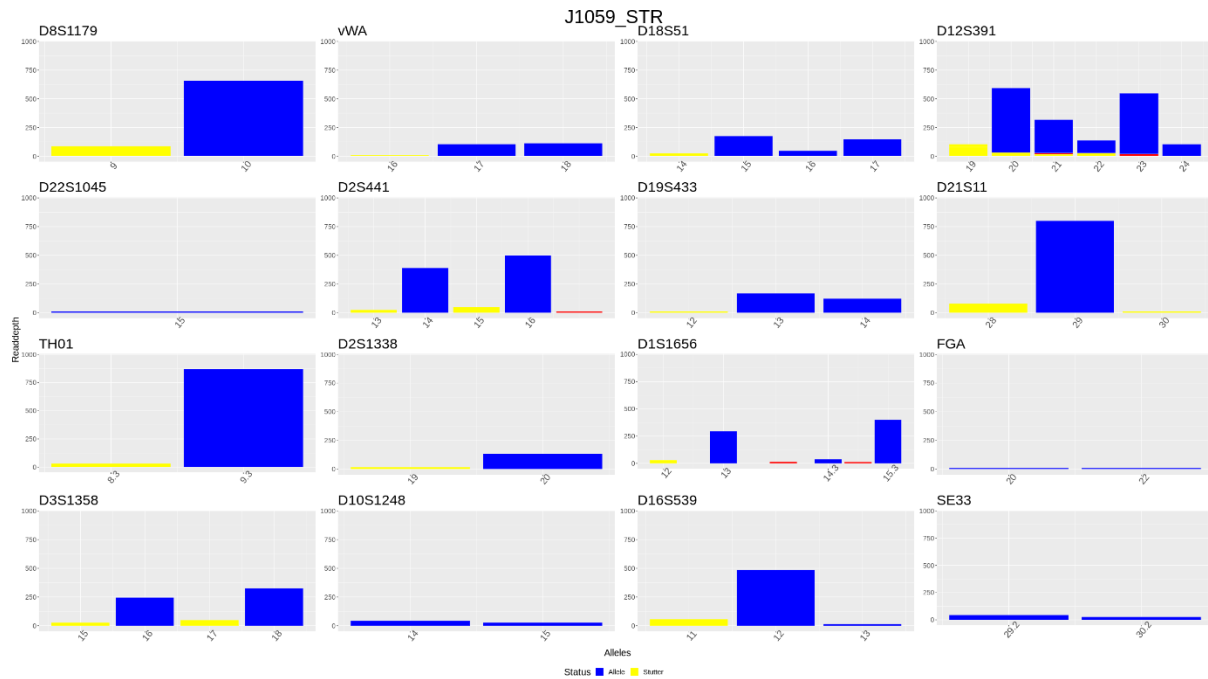
Suppl. Figure S19. Inhibitor study, 60 μ M hematin, human DNA A5



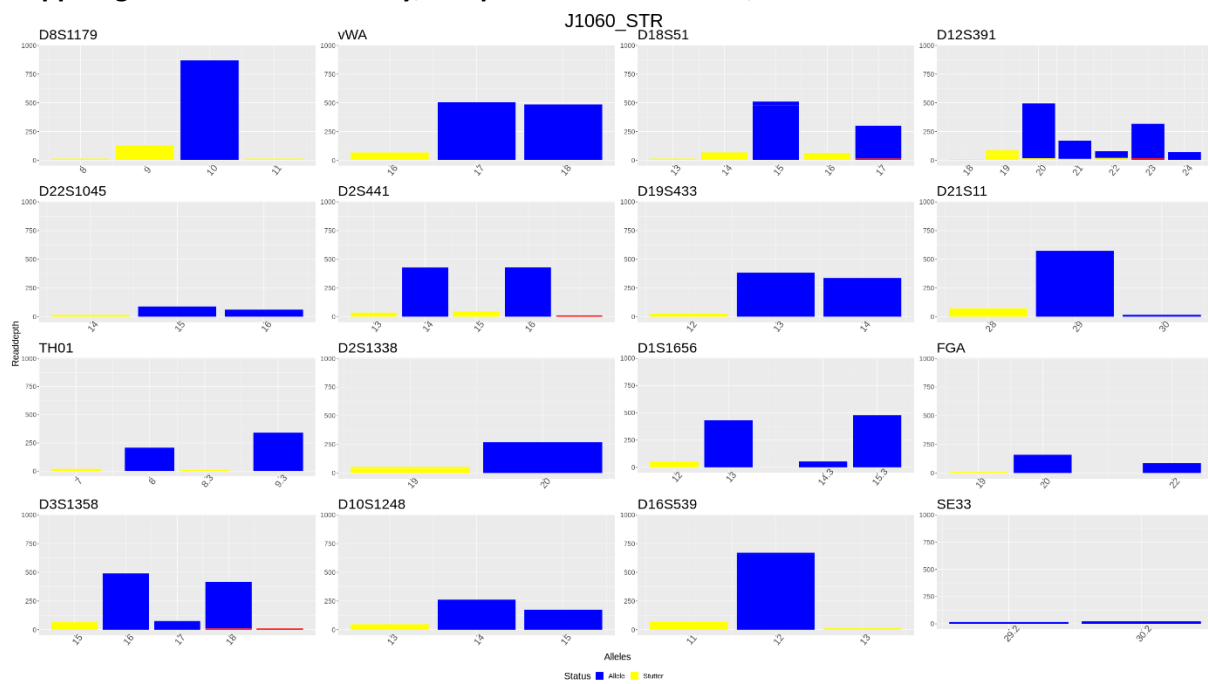
Suppl. Figure S20. Inhibitor study, 120 μ M hematin, human DNA A5



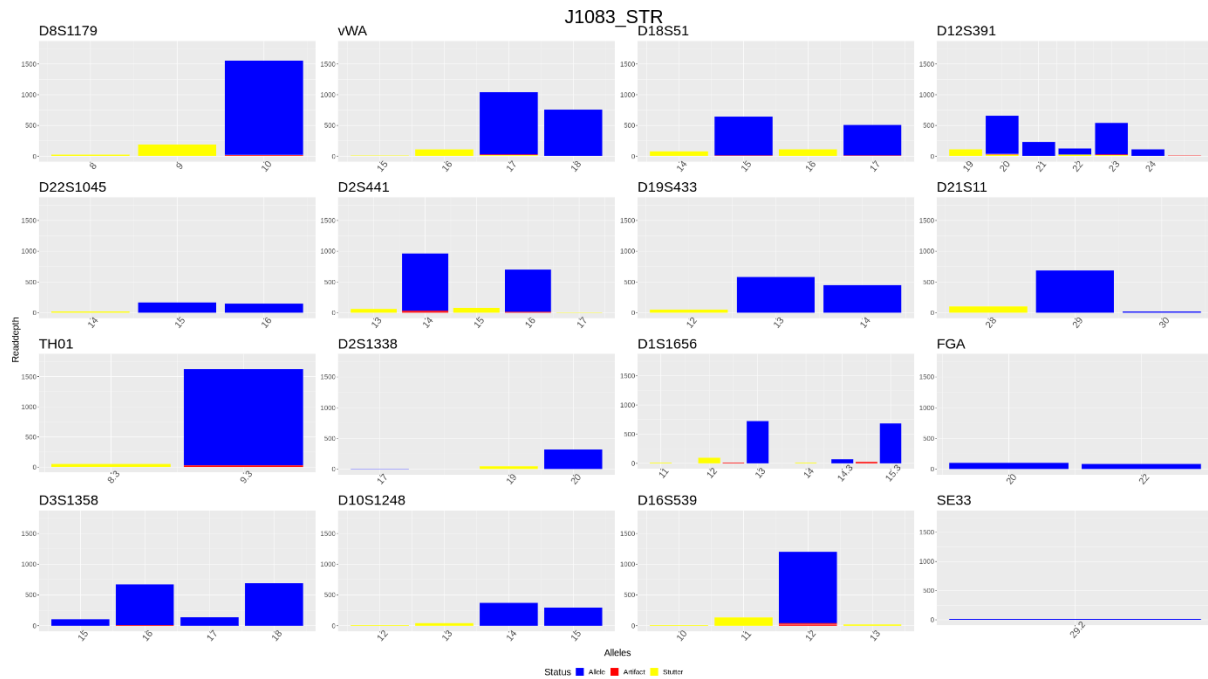
Suppl. Figure S21. Inhibitor study, 120 μ M hematin with PowerPlex MM, human DNA A5



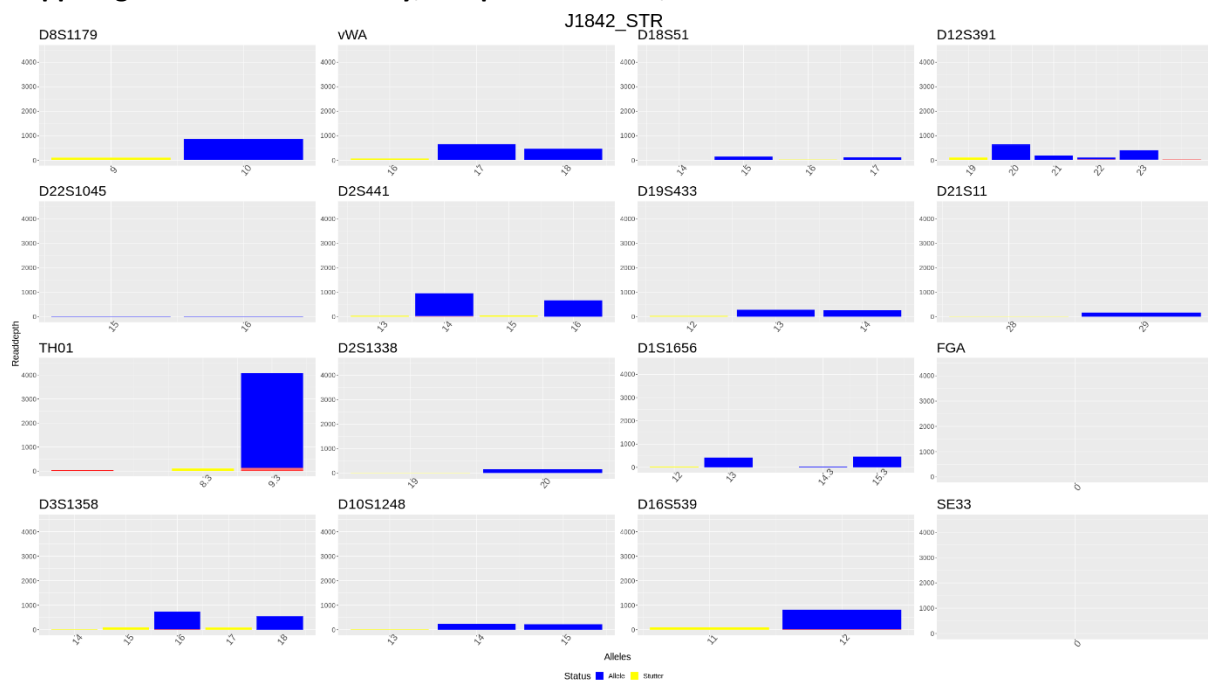
Suppl. Figure S22. Inhibitor study, 120 μ M hematin with BSA, human DNA A5



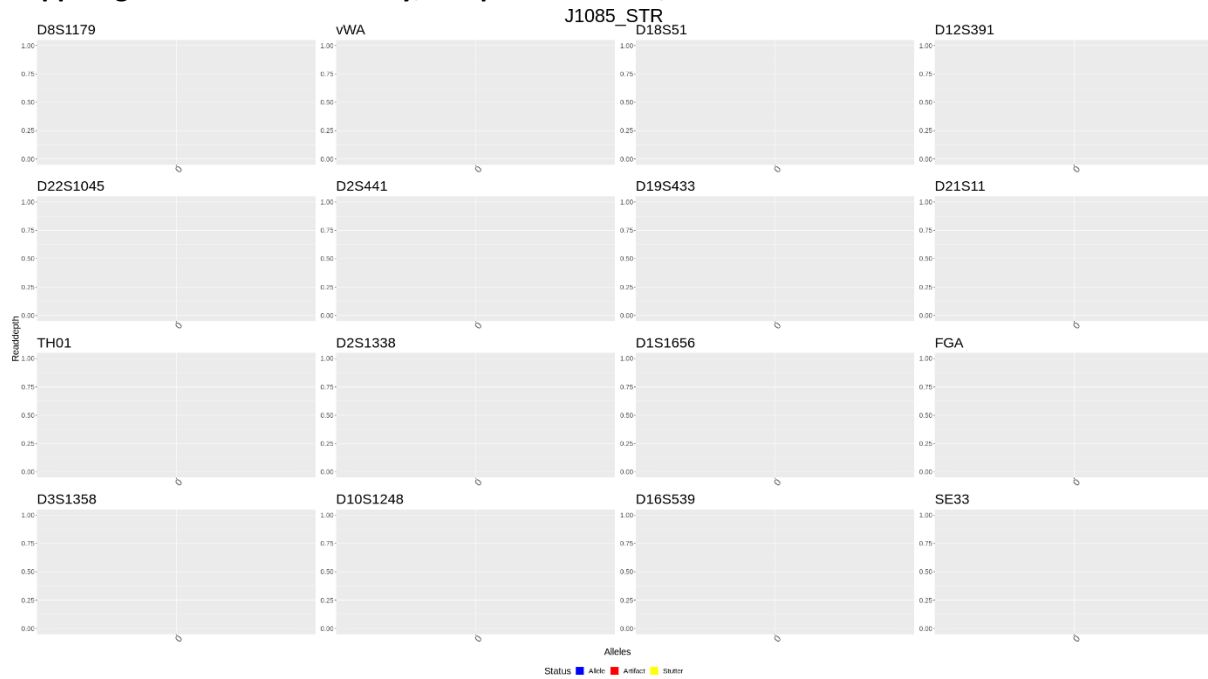
Suppl. Figure S23. Inhibitor study, 50 μ M humic acid, human DNA A5



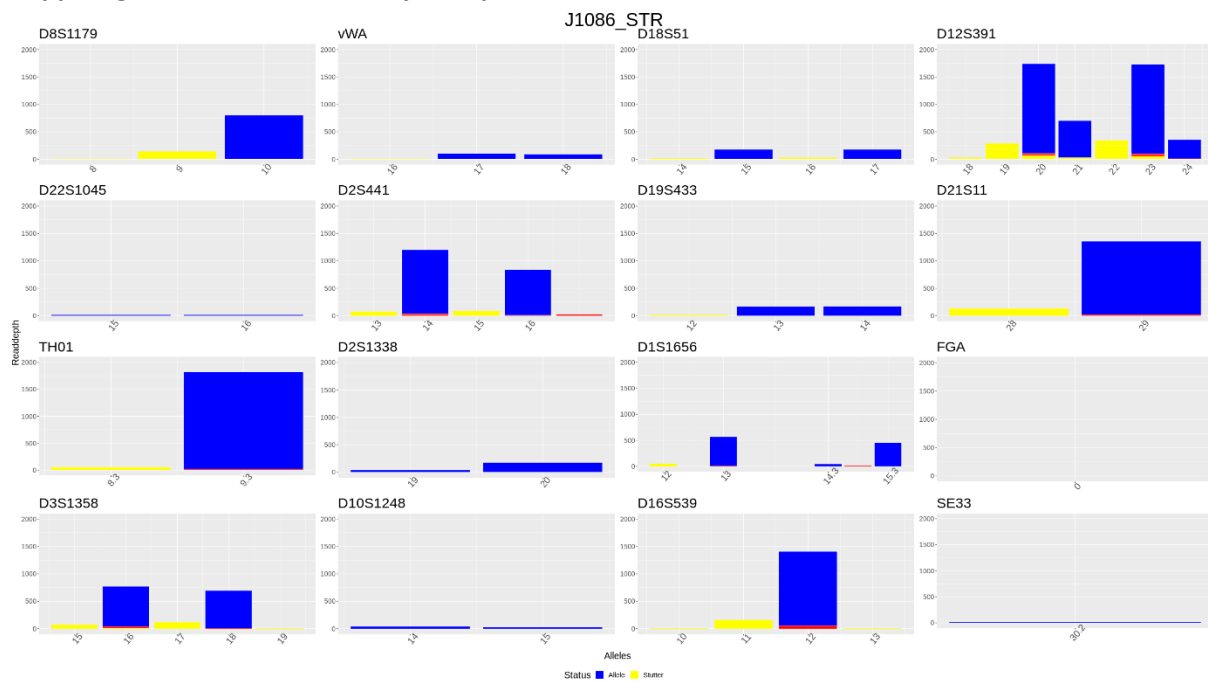
Suppl. Figure S24. Inhibitor study, 100 μ M humic acid, human DNA A5



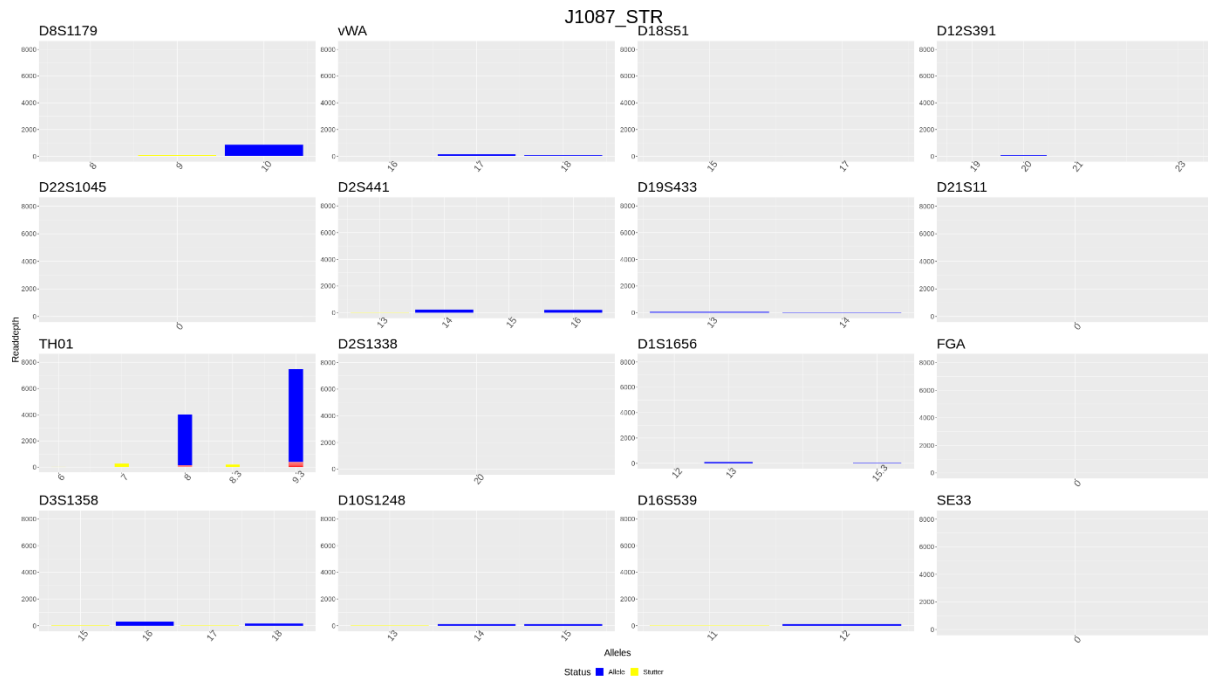
Suppl. Figure S25. Inhibitor study, 200 μ M humic acid, human DNA A5



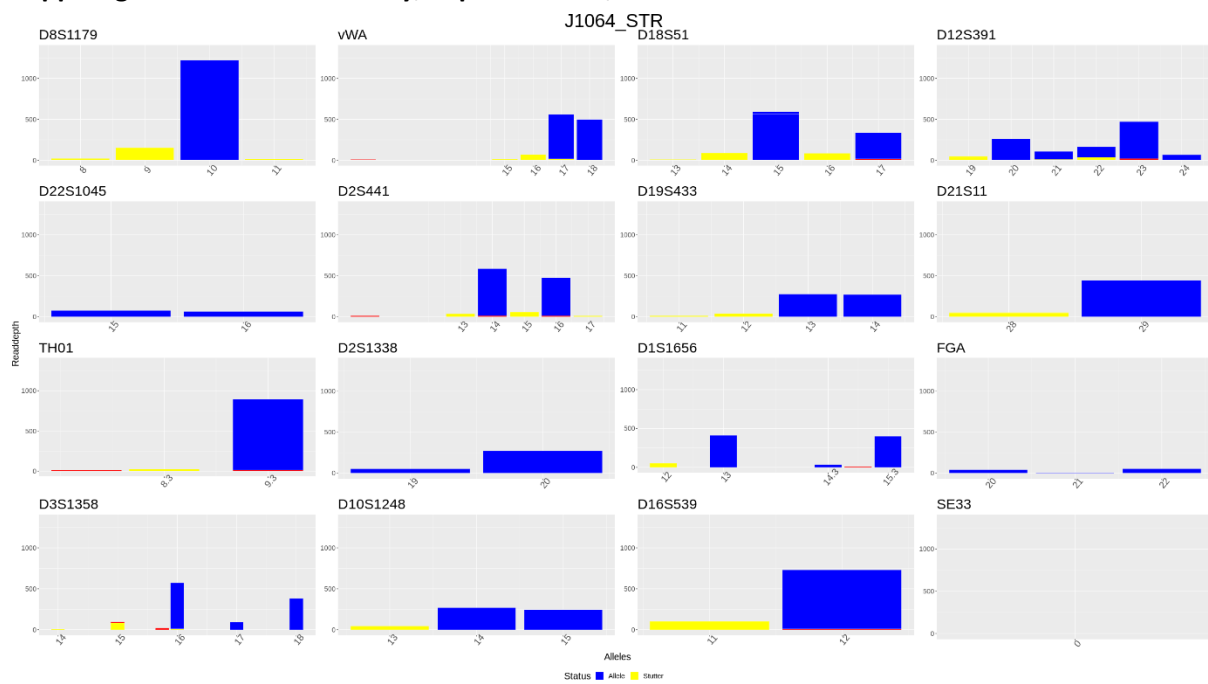
Suppl. Figure S26. Inhibitor study, 200 μ M humic acid with PowerPlex MM, human DNA A5



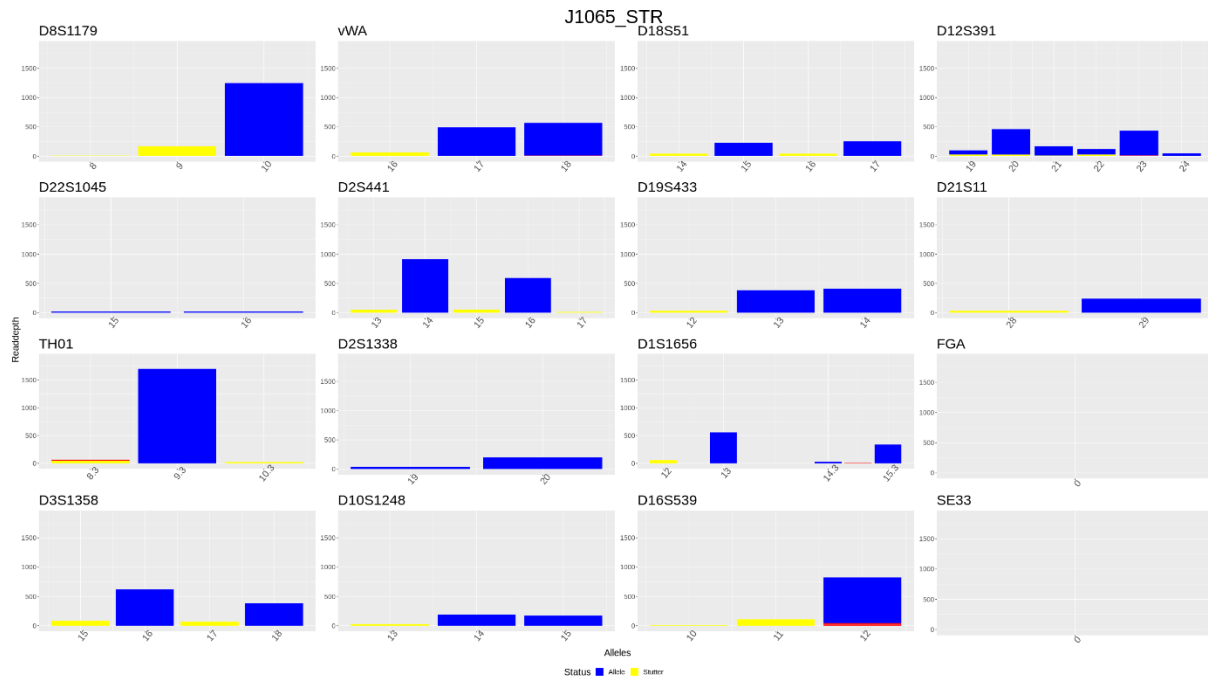
Suppl. Figure S27. Inhibitor study, 200 μ M humic acid with BSA, human DNA A5



Suppl. Figure S28. Inhibitor study, 25 μ M melanin, human DNA A5



Suppl. Figure S29. Inhibitor study, 50 μ M melanin, human DNA A5



Suppl. Figure S30. Inhibitor study, Sample 100 μ M melanin, human DNA A5

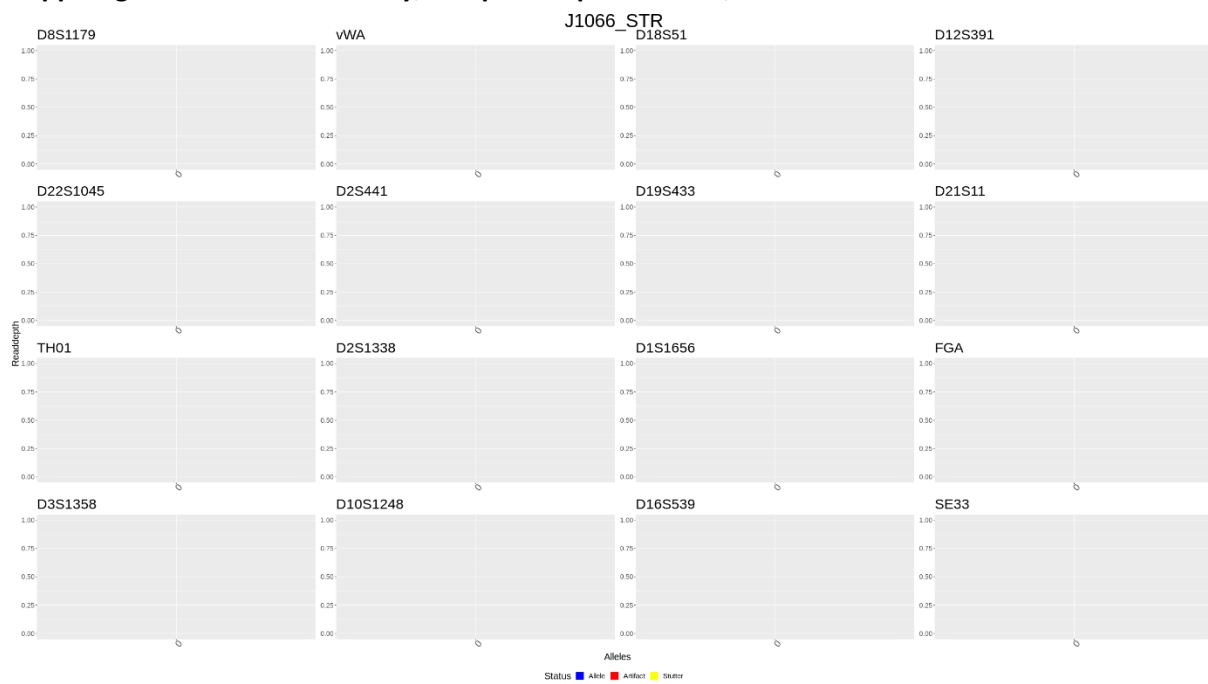
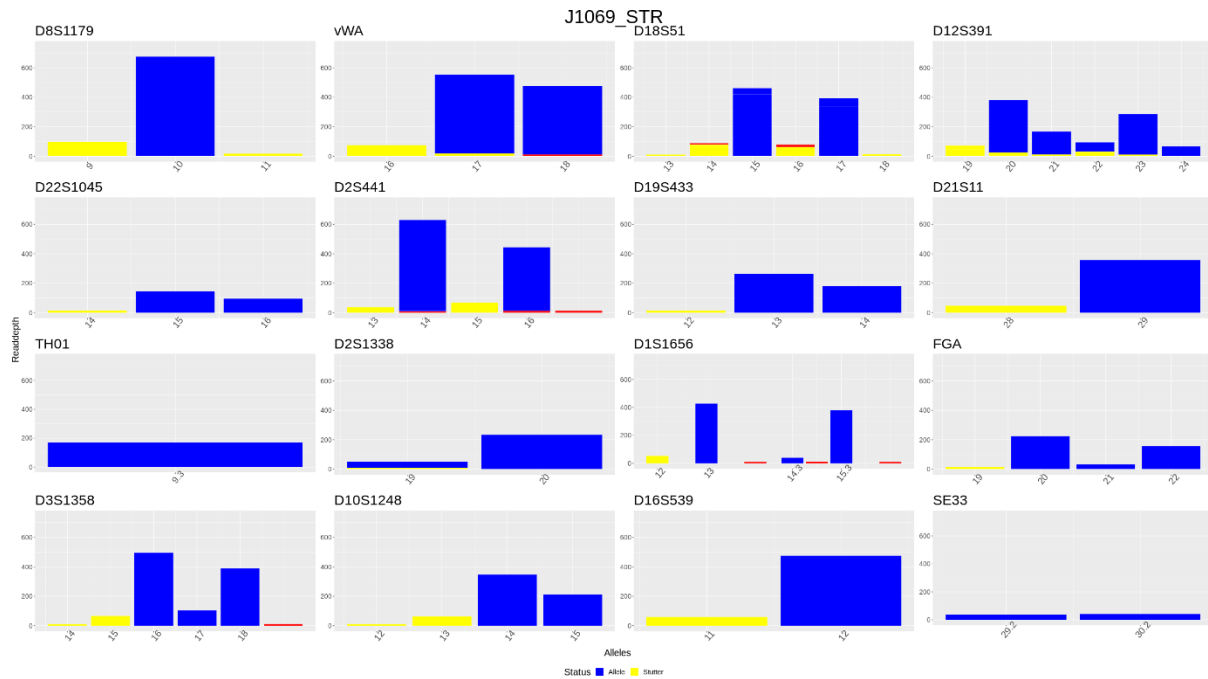
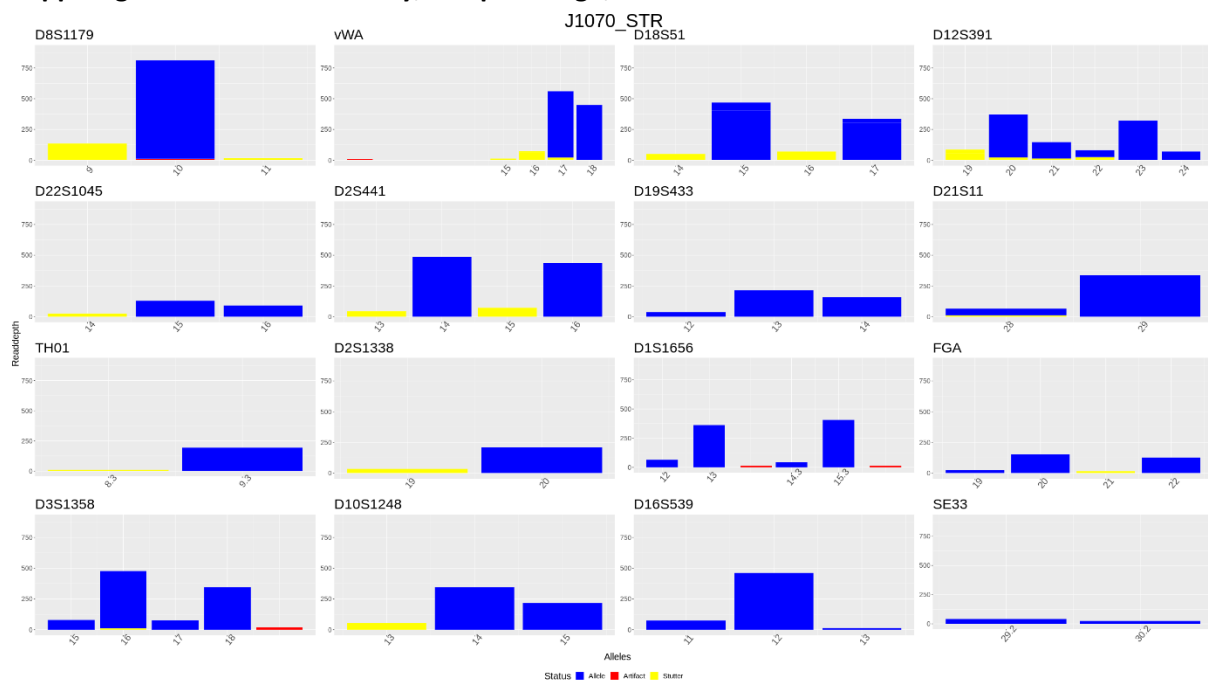


Figure 1 displays 15 allele frequency plots for various STR loci. Each plot shows ReadDepth (Y-axis, 0 to 1500) versus Alleles (X-axis, 0 to 26). The plots are arranged in a 4x4 grid, with the last cell empty. The loci are: D8S1179, D22S1045, TH01, D3S1358, vWA, D2S441, D2S1338, D10S1248, J1068_STR, D18S51, D19S433, D1S1656, D16S539, D12S391, D21S11, FGA, and SE33. The plots show the distribution of alleles for two status groups: 'Allic' (blue bars) and 'Stutter' (yellow bars). The 'Allic' status shows a single peak for each locus, while the 'Stutter' status shows multiple peaks at lower depths.

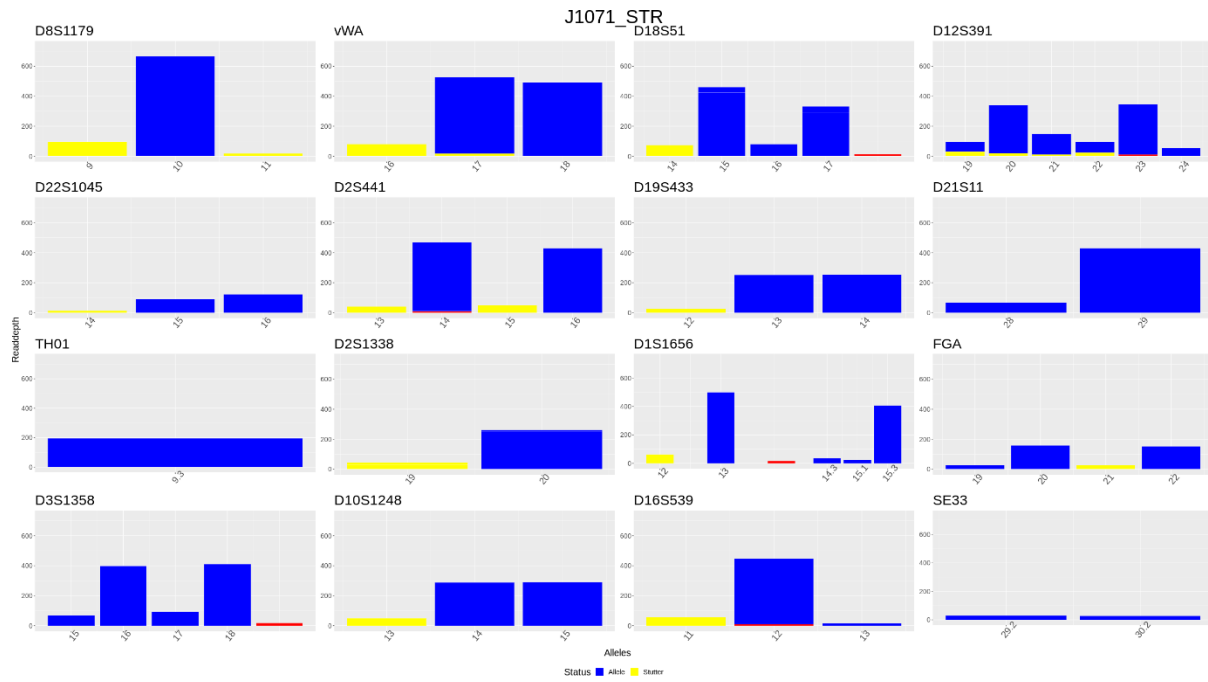
Suppl. Figure S33. Inhibitor study, 200 μ M indigo, human DNA A5



Suppl. Figure S34. Inhibitor study, 400 μ M indigo, human DNA A5

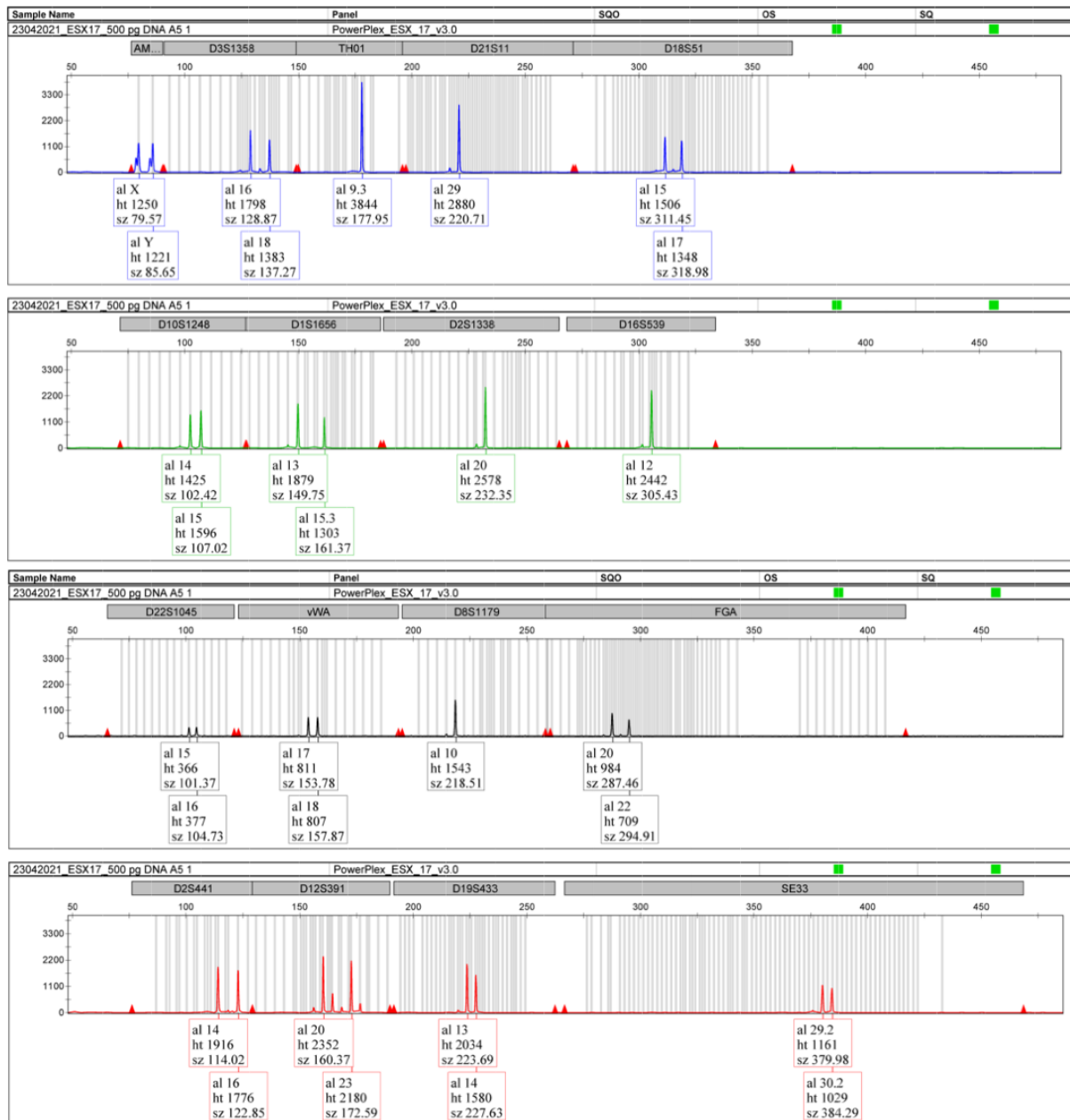


Suppl. Figure S35. Inhibitor study, 800 μ M indigo, human DNA A5

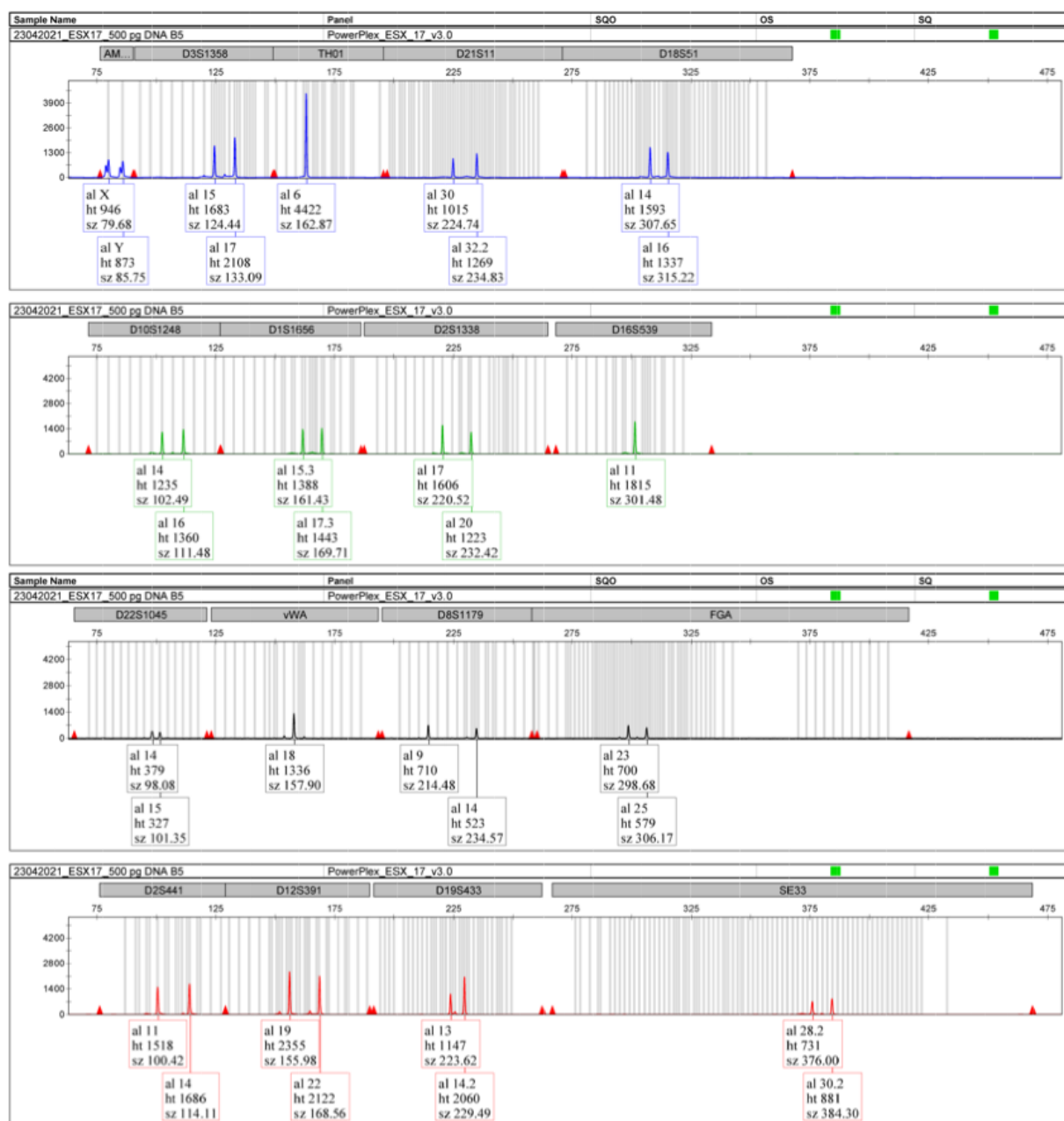


Suppl. Figures S36-S38: Electropherograms of DNA A5, B5, and HeLa DNA

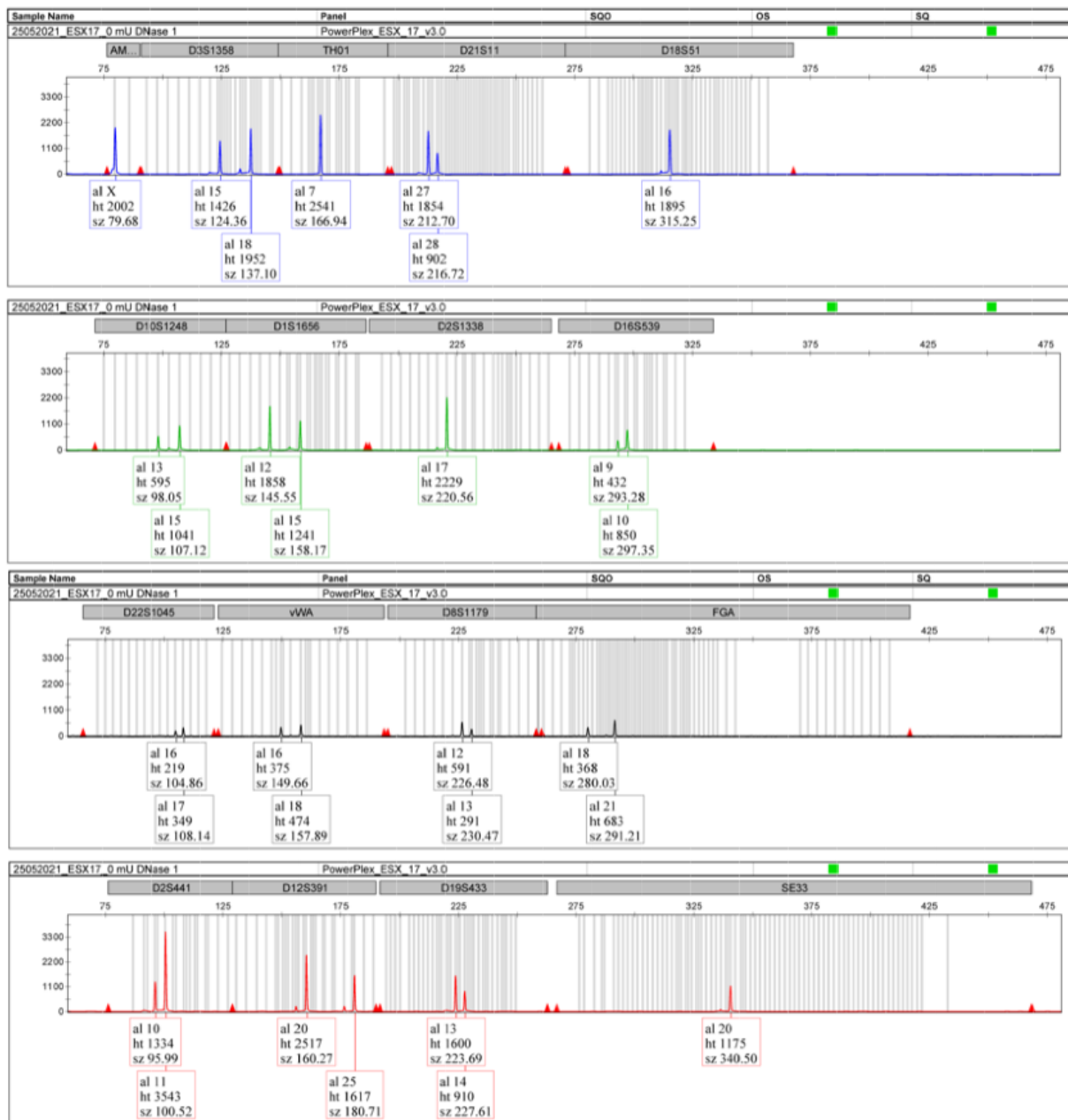
Suppl. Figure S36. Electropherogram of DNA A5 (500 pg, analysed with PowerPlex ESX17)



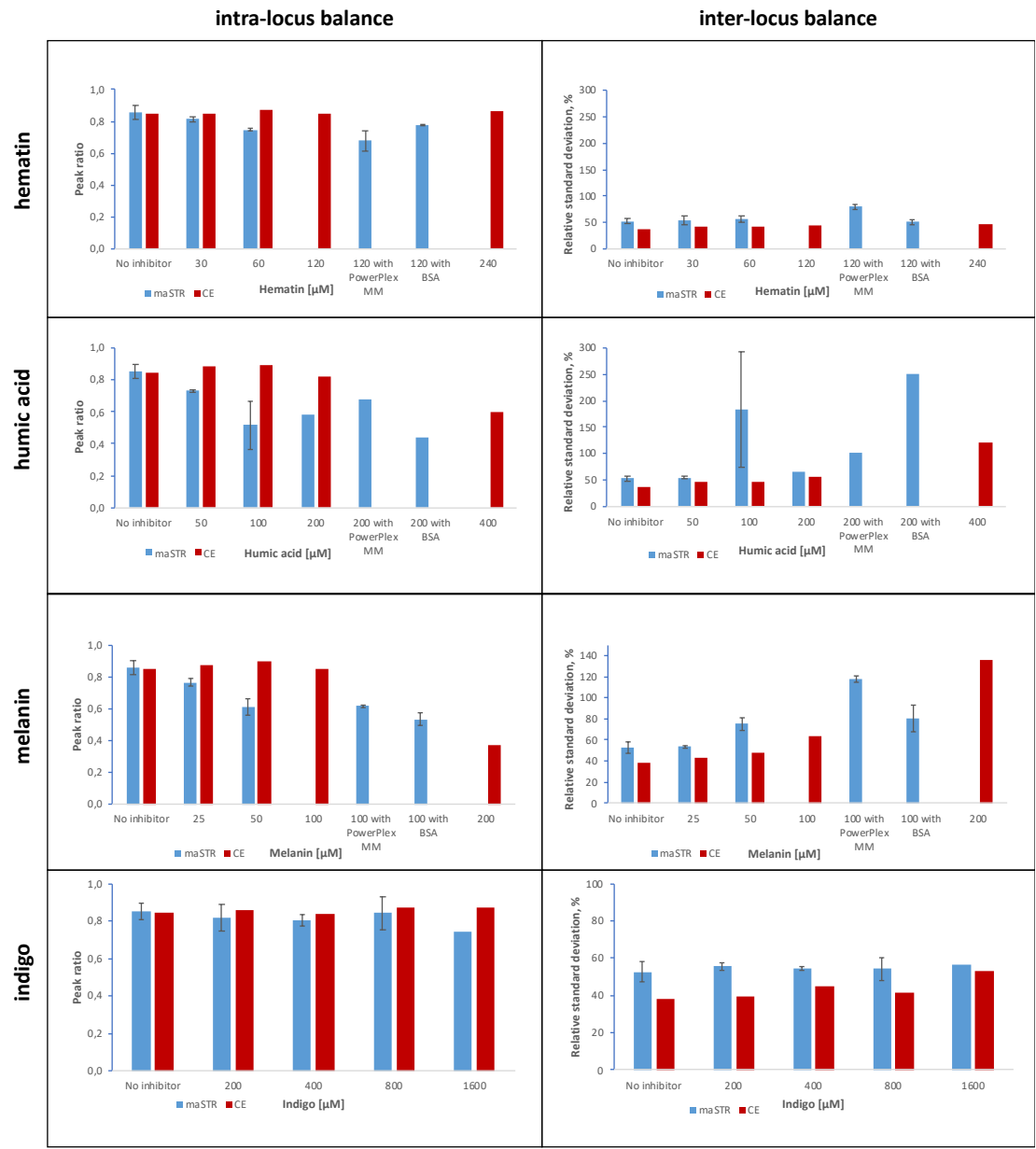
Suppl. Figure S37. Electropherogram of DNA B5 (500 pg, analysed with PowerPlex ESX17)



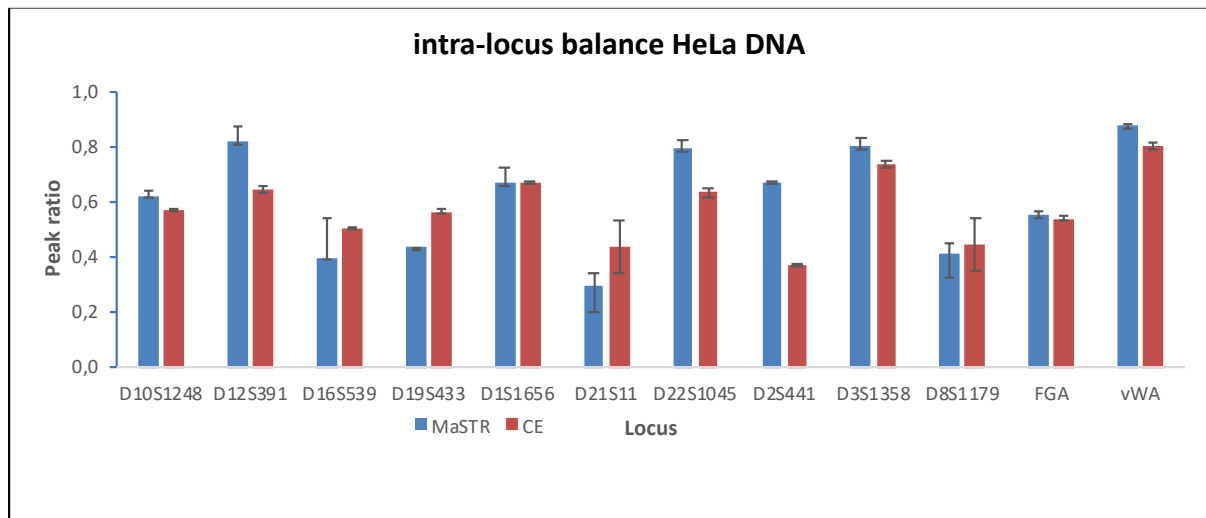
Suppl. Figure S38. Electropherogram of HeLa DNA (500 pg, analysed with PowerPlex ESX17)



Suppl. Figure S39: Intra- and inter-locus balances of profiles obtained with maSTR assay or CE analysis of PCR inhibitor-supplemented DNA samples



Suppl. Figure S40: Intra-locus balance of HeLa DNA obtained with maSTR assay or CE analysis



Supplementary Table S2. Calculation of sequencing costs

Library preparation costs					
Kits	Cost of the kit [€]	Number of samples that can be processed with this amount of reagent	Cost per sample [€]		
maSTR					
QIAGEN Multiplex PCR <i>Plus</i> Kit	258	200	1,29		
AMPure XP Reagent, 60 mL	960,10	789	1,22		
KapaHiFi HotStart ReadyMix*	450	250	1,8		
Index kit	266	96	2,77		
Promega ONEdsDNA quantification kit	308	500	0,62		
Primers (25 nmole)	580	10.000	0,06		
Total			7,75		
Verogen					
ForenSeq DNA Signature Prep Kit	6586	96	68,60		
Promega					
Promega PowerSeq® 46GY	5247	100	52,47		
Sequencing costs					
Workflow	Sequencing Kits involved	Cost of the kit [€]	Costs per sample, if to sequence 12 samples [€]	Costs per sample, if to sequence 32 samples [€]	Costs per sample, if to sequence 96 samples [€]
maSTR	Miseq Reagent Nano Kit (500 cycles)	394	32,83	12,31	N/A
Standard Verogen	Verogen MiSeq FGx Reagent Kit	1836	153,00	57,38	19,13
Micro Verogen	Verogen MiSeq FGx Reagent Micro Kit	791	65,92	24,72	N/A
Promega PowerSeq	MiSeq Reagent Kit v3 (600 cycles)	1725	143,75	53,90	17,97
Total costs (library preparation + sequencing)					
Workflow	Sequencing Kits involved	Total costs per sample, if to sequence 12 samples [€]	Total costs per sample, if to sequence 32 samples [€]	Total costs per sample, if to sequence 96 samples [€]	
maSTR	Miseq Reagent Nano Kit (500 cycles)	40,59	20,06	N/A	
Standard Verogen	Verogen MiSeq FGx Reagent Kit	221,60	125,98	87,73	
Micro Verogen	Verogen MiSeq FGx Reagent Micro Kit	134,52	93,32	N/A	
Promega PowerSeq	MiSeq Reagent Kit v3 (600 cycles)	196,22	106,37	70,44	