APPLICATION OF THE 3700 DNA ANALYZER FOR FORENSIC FRAGMENT ANALYSIS

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Introduction

The 3700 DNA Analyzer is an automated high through-put capillary electrophoresis system that can analyze up to 96 capillaries of fluorescently labelled DNA fragments within one run. It is primarily constructed for large scale sequencing efforts. Additionally, its high through-put capacity makes it a valuable instrument for STR analysis, especially for DNA databasing projects. In contrast to the single-capillary electrophoresis instrument, with the CE 310 Genetic Analyzer, the fluorescently labelled STR amplification products are detected outside the capillary. This is made possible by a sheath-flow detection system, where the amplicons migrate from the end of the capillary through a continuous fluid through the detection area of a laser-beam. The benefit of this technique is that signal-loss and background-noise caused by light-scattering from the capillary walls are reduced. In contrast to the CE 310, which uses a permanently coated capillary, the 3700 DNA Analyzer is equipped with an array of bare silica capillaries which are coated dynamically when the capillaries are filled with polymer (POP 6). Therefore they can be regenerated and reused.

The aim of the current study was to evaluate the 3700 DNA Analyzer for forensic purposes by investigating precision data of STR fragment size determination.

3700 run conditions

Electrophoresis conditions were set as recommended by the manufacturer (1), e.g. 10kV injection voltage, 10s injection time, 7.5kV run voltage, and 40°C cuvette temperature. The washing volume was increased from 20µl to 120µl in order to prevent sample carry-over during pipetting. 3700 Collection Software Version 1.1 was used to perform and monitor the run and analyze the raw data. STR profiles were analyzed using GeneScan® NT Version 3.5 and Genotyper® Software Version 3.6 NT.

Precision data within one run/ array

Control DNA 007 was amplified using AmpF*l*STR SGM Plus following the manufacturer´s protocol (2) in a 96-well microtiterplate. 2µl of the amplification products were combined with 20µl HiDi-formamide including 0.6µl HD 400 Rox internal lane standard into another 96-well microtiterplate and loaded on the 3700. Fragment sizes of each allele were collected, the mean values and the standard deviations were calculated, as well as the differences between the smallest and the largest fragment sizes observed for each allele (d-value; Table 1).

Table 1: Mean fragment sizes, standard deviations and smallest/ largest fragment sizes are listed for each SGM Plus 007 allele on the basis of 96 samples within one electrophoresis run.

3700 Precision within one run/ array (N=96)						
STR	**Allele**	**Size (m)**	**std**	**min**	**max**	**d-value**
D3	15	125.03	**0.07**	124.89	125.21	**0.32**
	16	129.21	**0.07**	129.08	129.36	**0.28**
VWA	14	167.15	**0.08**	167.01	167.39	**0.38**
	16	175.28	**0.08**	175.12	175.49	**0.37**
D16	9	247.90	**0.06**	247.74	248.04	**0.30**
	10	251.92	**0.05**	251.82	252.01	**0.19**
D2	20	312.12	**0.07**	311.92	312.28	**0.36**
	23	324.37	**0.09**	324.19	324.57	**0.38**
D8	12	141.92	**0.09**	141.76	142.19	**0.43**
	13	146.04	**0.10**	145.84	146.31	**0.47**
D21	28	202.30	**0.06**	202.14	202.45	**0.31**
	31	214.21	**0.06**	214.08	214.39	**0.31**
D18	12	284.60	**0.12**	284.38	284.90	**0.52**
	15	296.76	**0.12**	296.55	297.12	**0.57**
D19	14	123.87	**0.04**	123.76	123.96	**0.20**
	15	127.87	**0.04**	127.75	127.99	**0.24**
TH01	7	175.84	**0.07**	175.71	175.99	**0.28**
	9	184.11	**0.06**	183.96	184.26	**0.30**
FGA	24	243.08	**0.15**	242.85	243.50	**0.65**
	26	251.22	**0.16**	250.90	251.64	**0.74**

Standard deviations of the mean fragment sizes of the SGM Plus 007 alleles were below 0.1 bp (D3, VWA, D16, D2, D8, D21, D19, TH01) and below 0.2 bp, respectively (D18 and FGA). The largest differences between the maximum and minimum fragment size for an allele was observed for FGA alleles 24/26 and D18 alleles 12/15, exceeding half a basepair (bp). The d-values for all other alleles were below 0.5 bp.

Precision data between different runs

Fragment sizes of 007 SGM Plus profiles were obtained from 159 runs/ 96-well plates, in which this sample served as PCR positive control. Amplification and electrophoresis conditions were as mentioned above. Similar to the precision data observed within one run, fragment lengths of all alleles were collected, mean values, standard deviations and d-values were calculated (Table 2).
Standard deviations from the mean fragment sizes of the alleles obtained from different runs as well as the d-values, were comparable to the data obtained for the precision study within one run. Again, STR loci D18 and FGA displayed the highest standard deviations (between 0.1 and 0.2 bp) and the largest d-values (between 0.5 and 0.7 bp). All other alleles showed standard deviations below 0.1 bp and d-values below 0.5 bp.

Table 2: Mean fragment sizes, standard deviations and smallest/ largest fragment sizes are listed for each SGM Plus 007 allele on the basis of 159 runs.

3700 Precision between runs (N=159)						
STR	**Allele**	**Size (m)**	**std**	**min**	**max**	**d-value**
D3	15	125.14	**0.08**	124.96	125.31	**0.35**
	16	129.21	**0.08**	129.03	129.38	**0.35**
VWA	14	167.13	**0.07**	166.98	167.31	**0.33**
	16	175.22	**0.08**	175.02	175.41	**0.39**
D16	9	247.80	**0.06**	247.64	247.98	**0.34**
	10	251.85	**0.05**	251.71	252.01	**0.30**
D2	20	312.08	**0.07**	311.87	312.27	**0.40**
	23	324.33	**0.08**	324.14	324.52	**0.38**
D8	12	141.90	**0.09**	141.71	142.10	**0.39**
	13	145.93	**0.10**	145.71	146.17	**0.46**
D21	28	202.25	**0.05**	202.10	202.39	**0.29**
	31	214.21	**0.06**	214.05	214.33	**0.28**
D18	12	284.58	**0.13**	284.36	284.87	**0.51**
	15	296.74	**0.14**	296.46	297.10	**0.64**
D19	14	123.87	**0.05**	123.74	123.98	**0.24**
	15	127.87	**0.05**	127.72	127.98	**0.26**
TH01	7	175.80	**0.07**	175.65	175.97	**0.32**
	9	187.24	**0.07**	187.09	187.40	**0.31**
FGA	24	243.05	**0.16**	242.75	243.43	**0.68**
	26	251.19	**0.15**	250.91	251.53	**0.62**

Single basepair resolution

Microvariants, i.e. two alleles differing by 1 bp in length, were analyzed on the 3700 DNA Analyzer in order to examine whether the two alleles could clearly be separated from each other. Apart from the well described microvariant TH01 9.3/10, the STR locus SE33 includes a number of microvariants, whose fragment lengths exceed 250 bp. SE33 alleles 17.3/18 were amplified and electrophoresed on the 3700 DNA Analyzer applying standard conditions (Figure 1, Table 3).

Figure 1: Electropherogram of a SE33 17.3/18 genotype on the 3700 applying standard conditions (POP 6 3700, 7.5 kV run voltage).

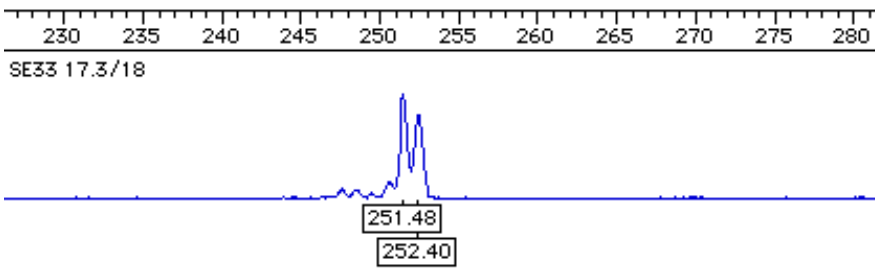

Table 3: Mean fragment sizes, standard deviations and smallest/ largest fragment sizes are listed for the two SE33 alleles 17.3 and 18 on the basis of 96 samples within one electrophoresis run.

3700 Single basepair resolution (N=96)						
STR	**Allele**	**Size (m)**	**std**	**min**	**max**	**d-value**
SE33	17.3	251.59	**0.08**	251.48	251.67	**0.19**
	18	252.52	**0.08**	252.40	252.59	**0.19**

Concordance study

In order to compare STR profiles and their allelic designation between two capillary electrophoresis instruments, the CE 310 Genetic Analyzer and the 3700 DNA Analyzer, 1216 samples were Chelex®-extracted (3), amplified using AmpFlSTR SGM Plus and electrophoresed as described above. In total, 22619 alleles were analyzed on each instrument and called by the respective software package. The STR profiles were then imported into a self-programmed Access 2.0 Laboratory Information Management System and automatically compared by the software. No deviations were found between the allele designations of both instruments.

Conclusions

The precision data, i.e. standard deviations of the mean fragment lengths and differences between the smallest and largest fragment sizes of an allele, gave comparable results within one array compared to different runs. The results suggest, that the electrophoretic separation on the 3700 DNA Analyzer is very accurate, and independent of the capillary used for analysis. The precision data are well in the range of other electrophoresis systems already in use (e.g. CE 310, 377).

The results obtained for the one-basepair-microvariant study supports the use of the 3700 for the analysis of STR markers for forensic purposes. The latter is also demonstrated by the results of the concordance study.

References

(1) ABI Prism® 3700 DNA Analyzer, User´s manual, PE Corporation, 1999
(2) AmpF*l*STR™ SGM Plus, PCR Amplification Kit, User´s manual, PE Corporation, 1999
(3) Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, Biotechniques 1991; 10:506-513.