CORRESPONDING REPEATS IN STRs AND THE INTERNAL STANDARD IN FRAGMENT ANALYSIS
REPRODUCIBILITY WITH THREE HYPERPOLYMORPHIC STRs

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Introduction: Automated fragment analysis with ABI Sequencer using the internal standard GS2500 or GS500 gives sufficient reproducibility to allow typing of regular tetrameric STR's. It is, however, a general experience that it does not allow fool-proof typing of alleles differing in size by one basepair only.
The main purpose of this study was to evaluate whether the use of internal standards composed of the same repeats as those of the STR to be analysed could overcome this obstacle. Here we show that this is indeed the case. The principle is then applied on the AAAG repeat STRs ACTBP2, APOAI1 and D11S554, and with fragment measurements using an internal standard composed of AAAG repeats. It is demonstrated that each of the three STRs are highly efficient, and that together (for instance in triplex runs) they constitute a powerful tool in forensic diagnostics.

Population and sample size: The material consists of blood samples from 300 Norwegians (150 of each sex), involved in consecutive paternity cases from all over Norway. Selected samples from the same population were also used for construction of the internal standard and in tests for intergel fragment sizing reproducibility.

Methods: Primers (Polymeropoulos 1992, Phromchotikul 1992): ACTBP2, APOAI1 and D11S554 were labelled with TAMRA, FAM, and JOE respectively.

PCR amplification condition: 500 mM Kcl, 100 mM Tris-HCl,10% TritonX-100, 2mM MgCl, 6 pmol of each primer in 25 ul reaction volume. Denaturation 2 min. 94°C, 28 cycles: 94° 20 sec., 58°C 45 sec., 72°C 1 min, followed by 10 min. elongation. ACTBP2 was run singleplex while APOAI1 and D11S554 were run duplex.

Electroforetic methods: 6% PAGE, 26W, 9 hours, on the ABI373A Sequencer. Software: 672 Genescan. Typing was performed by internal allelic ladder in each lane.

Internal standard: An internal standard was composed of DNA fragments containing mainly AAAG repeats. The standard includes 25 ROX labelled fragments with a relatively even size distribution from 174 to 327 bp. It is composed of four alleles from D11S554 (the smallest ones), while the rest are ACTBP2 alleles. The fragments were once and for all sized against a GS2500 standard. The samples applied to tests for intergel sizing reproducibility were also run with the GS500 internal standard.

Table 2:ALLELE MEASUREMENTS IN 300 UNRELATED NORWEGIANS

ACTBP2:

Average	SD	Tot.obs	Average	SD	Tot.obs	Average	SD	Tot.obs
231.15 *1	0.14	4	259.57	0.02	2	290.48 *9	0.09	55
234.66 *	0.04	4	260.46	0.00	1	294.31*10	0.12	51
236.47	0.00	1	261.45 *5	0.05	24	298.28*11	0.06	43
238.24	0.05	17	263.52	0.05	6	302.09*12	0.21	29
240.03	0.07	3	265.47 *6	0.07	15	304.15	0.00	1
241.88 *2	0.05	24	267.45	0.07	9	306.10 *	0.06	17
243.62	0.00	1	269.34 *	0.10	7	309.98	0.12	6
245.70	0.07	28	270.58	0.00	1	311.68 *	0.25	3
246.73	0.00	1	271.37	0.08	14	313.81	0.07	3
247.59	0.00	1	273.34 *	0.04	4	315.62 *	0.00	1
248.79	0.04	2	275.13	0.10	23	319.77 *	0.00	1
249.67 *3	0.05	38	278.93 *	0.12	18	327.52*13	0.00	1
253.52	0.09	33	282.75 *7	0.04	27			
257.47 *4	0.06	53	286.60 *8	0.14	28			

APOAI1:

Average	SD	Tot.obs	Average	SD	Tot.obs	Average	SD	Tot.obs
251.22	0.04	15	271.86	0.06	13	283.34	0.07	20
256.08	0.00	1	272.96	0.00	1	284.32	0.07	22
258.08	0.00	1	273.79	0.07	57	285.19	0.07	66
260.02	0.00	1	275.70	0.06	21	287.09	0.04	16
262.03	0.06	34	276.59	0.11	4	288.05	0.07	8
264.02	0.01	2	277.51	0.07	63	289.03	0.06	17
265.10	0.00	1	278.50	0.05	6	291.02	0.03	4
266.02	0.07	77	279.45	0.05	9	292.05	0.06	4
267.98	0.04	7	280.46	0.06	18	292.08	0.06	2
269.81	0.08	14	281.36	0.08	85	296.78	0.04	3
270.85	0.02	3	282.36	0.04	4	302.44	0.00	1

D11S554:

Average	SD	Tot.obs	Average	SD	Tot.obs	Average	SD	Tot.obs
174.42*	0.12	8	217.10	0.07	37	235.98	0.00	1
193,49*	0.10	3	218.26	0.12	19	236.62	0.00	1
197.32	0.04	24	221.38	0.11	23	237.78	0.13	6
199.26	0.05	4	222.52	0.10	42	239.59	0.04	6
200.10	0.00	1	224.49	0.00	1	241.47	0.06	4
201.12	0.07	33	225.55	0.09	7	243.31	0.11	25
203.09	0.01	2	226.65	0.10	51	245.31	0.00	1
204.95*	0.08	55	229.63	0.10	5	247.25	0.15	17
208.80	0.06	62	230.73	0.10	66	251.29	0.12	13
209.85	0.11	4	232.57	0.00	1	255.05	0.12	2
212.71*	0.10	49	233.16	0.00	1	261.13	0.00	1
213.99	0.07	4	234.30	0.10	19	282.59	0.05	2

*;used as internal standard fragments

Alleles marked [1-13] correspond to sequenced alleles (Anke Möller, pers.comm.) with the following lengths 1:223bp, 2:235bp, 3:243bp, 4:251bp, 5:255bp, 6:259bp, 7:277bp, 8:281bp, 9:285bp, 10:289bp, 11:293bp, 12:297bp, 13:321bp.

Results:

Table 1: Standard deviation in repeated intergel fragment sizing using different internal standards.

Internal Standard	ACTBP2 (AAAG) SD	APOAI1 (AAAG) SD	APOAI1 (TTTC) SD
AAAG repeat fragments	0.04	0.04-0.11	1.49-1.67
GS500	0.28-0.83	0.31-0.72	0.24-0.42

Fragment sizing reproducibility was tested in each system by rerunning 1-10 individuals on 8-10 successive gels. Table 1 demonstrates the dramatic effect the choice of internal standards has on the fragment sizing reproducibility. Repeated measurements of the same AAAG STR fragment whether in ACTBP2 or APOAI1 reveal remarkably good results, with 3 SDs being well below 0.5bp. This is a reasonable limit for allowing automated allele typing in STRs with one-bp-differences between alleles. The results with the synthetic GS500 fragment standard are significantly poorer and insufficient for our purpose, while choosing «opposite» base composition (AAAG versus TTTC) in the standard as compared to the fragments measured, clearly makes any allele typing based on fragment sizing impossible.

Having shown that the present internal standards meets our demands in terms of sizing reproducibility of identical alleles, we typed the 300 individuals of our population material in ACTBP2 (singleplex) as well as APOAI1 and D11S554 (duplex). The samples were run on 30 different gels. The results are given in Table 2 where allele sizes as well as SDs of each allele are indicated. There are discrete alleles, and with practically no exception the SDs are within acceptable limits for each allele in all three systems.

Table 3 gives some basic data concerning the efficiency of the three STRs. The probability that two unrelated individuals have the same type in all three STRs is $\mathbf{5x10^{-7}}$, and the combined paternity exclusion capacity is **99.77%.** We are currently applying these STRs (singleplex and duplex PCR, triplex run) in paternity casework and will probably introduce them for forensic casework and intelligence databases in the near future.

Table 3: Number of alleles, discrimination power (DP), and paternity exclusion power (EP) of ACTBP2, APOAI1, and D11S554.

LOCUS	NO. ALLELES	DP %	EP %
ACTBP2	40	99.45	89.19
APOAI1	33	98.81	83.85
D11S554	36	99.18	86.72

References: Phromchotikul, Hum. Mol. Gen. Vol. 1, No. 3 :214, 1992
Polymeropoulos, Nuc. Acid Res. Vol. 20, N0. 6 : 1432, 1992