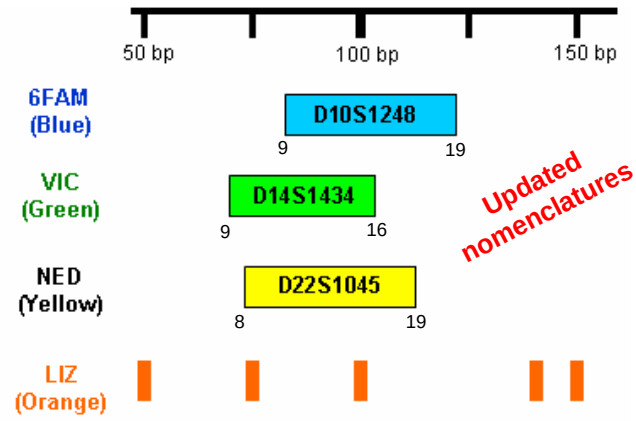
Protocol for using the miniSTR system "miniNC01" on the ABI 3100 Instrument

MiniNC01



Reagents/Materials Needed

1X Primer Mix (see below for sequence information)
$MgCl_2$ (25mM)
10X PCR Buffer
10mM dNTPs
BSA (3.2 mg/mL)
TaqGold DNA Polymerase (5 U/μL)
dH_2O
ABI 3100 Capillary Array (36cm - ABI P/N 4315931)
Matrix Standard DS-33 (ABI P/N 4318159)
Hi-Di formamide (ABI P/N 4311320)
GS500 LIZ size standard (ABI P/N 4322682)
1X Genetic Analyzer Buffer w/EDTA (ABI P/N 402824)
POP-6 polymer (ABI P/N 4316357)
GeneScan and Genotyper Software

Locus	Primer Sequences (5'-3')	Conc.
D10S1248	F **[6FAM]**-TTAATGAATTGAACAAATGAGTGAG	1.3 μM
	R **G**CAACTCTGGTTGTATTGTCTTCAT	1.3 μM
D14S1434	F **[VIC]**-TGTAATAACTCTACGACTGTCTGTCTG	1.3 μM
	R **G**AATAGGAGGTGGATGGATGG	1.3 μM
D22S1045	F **[NED]**-ATTTTCCCCGATGATAGTAGTCT	0.8 μM
	R **G**CGAATGTATGATTGGCAATATTTTT	0.8 μM

The 5' Guanine residue in each reverse primer (indicated in bold, red font) was added to promote full adenylation.

PCR Conditions

PCR Preparation

	10μL PCR 1X Reaction (μL)	25μL PCR 1X Reaction (μL)
PCR Buffer	(n+1)[a] * 1.0	(n+1)[a] * 2.5
$MgCl_2$	(n+1) * 0.8	(n+1) * 2.0
Primer Mix	(n+1) * 2.0	(n+1) * 5.0
dNTPs	(n+1) * 0.25	(n+1) * 0.625
BSA	(n+1) * 0.5	(n+1) * 1.25
TaqGold	(n+1) * 0.2	(n+1) * 0.5
dH_2O	(n+1) * 4.25	(n+1) * 8.125
M.Mix vol. + template (μL)	(n+1) * 9.0 1.0 per sample	(n+1) * 20.0 5.0 per sample

[a]The (n+1) refers to the total number of reactions (plus an additional reaction for overfill).

We use 10μL reactions for databasing high quality genomic DNA ,and 25 μL reactions for degraded templates (the amount of DNA input can be increased accordingly by adjusting the dH_2O content).

Thermal Cycling Conditions (GeneAmp 9700 – ABI)

95°C for 10 minutes

94°C for 1 minute
55°C for 1 minute
72°C for 1 minute
} 28 – 34 cycles

60°C for 45 minutes

25°C soak

Detection of PCR Products

We have used the ABI 3100 with POP6 polymer, capillaries, and buffer used for STR typing with commercial kits. The MiniNC01 assay uses 6FAM (blue), VIC (green), NED (yellow) dyes. POP4 polymer can also be used for fragment separation. For more details, refer to the Materials and Methods section of Butler et al. (2003) *JFS* 48(5): 1054-1064. Article available at: http://www.cstl.nist.gov/biotech/strbase/pub_pres/Butler2003d.pdf

ABI 3100

Prior to running any samples with the MiniNC01 STR system on the ABI 3100, a 5 dye matrix needs to be established under the "**G5 filter**" with the dyes 6FAM (blue), VIC (green), NED (yellow), PET (red), and LIZ (orange) using **matrix standard set DS-33** (P/N 4318159). Samples are typically prepared with **15 µL Hi-Di™ formamide** (Applied Biosystems, P/N 4311320), **0.35 µL GS500 LIZ** (P/N 4322682), and with **1 µL PCR product**.

The samples may be run using the default module **GeneScan36_POP4DefaultModule**, which performs an electrokinetic injection onto the 16-capillary array for 10 s at 3,000 volts. The STR alleles are then separated at 15,000 volts for approximately 30 minutes with a run temperature of 60 ºC using POP-6 sieving polymer (Applied Biosystems, P/N 4316357), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and a 36 cm array (P/N 4315931).

Updated nomenclatures

Expected Control Results

STR Locus	Control DNA 007 Genotype	Control DNA 9947A Genotype
D10S1248	12, 15	13, 15
D14S1434	11, 14	11, 13
D22S1045	11, 16	11, 14

Macro Information:

A downloadable research macro using fixed bins has been posted on the STRBase website:

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

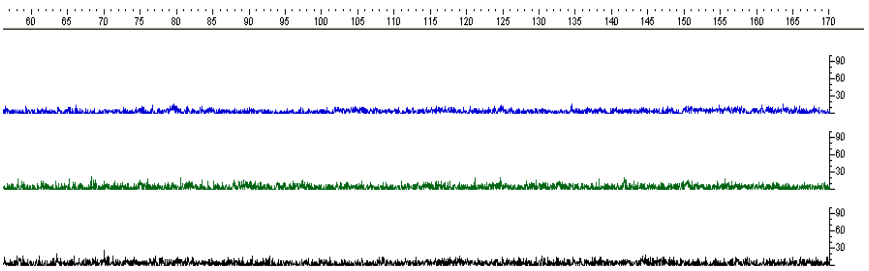
Also posted is a document with allele size ranges for each bin (for individuals to build their own macro).

Published Allele Frequencies – Coble and Butler (2005)

D10S1248					D14S1434					D22S1045				
Allele	Afr. Am.	Cauc.	Hisp.	Total	Allele	Afr. Am.	Cauc.	Hisp.	Total	Allele	Afr Am.	Cauc.	Hisp.	Total
9	0.0061			0.0021	**9**	0.0061		0.0036	0.0032	**8**	0.0061			0.0021
10	0.0031			0.0011	**10**	0.2561	0.1471	0.1857	0.1966	**9**				
11	0.0335			0.0116	**11**	0.0213	0.0294	0.0357	0.0285	**10**	0.0457		0.0179	0.0211
12	0.1189	0.0382	0.0607	0.0729	**12**	0.0945	0.0177	0.0429	0.0518	**11**	0.1128	0.1441	0.0607	0.1089
13	0.2592	**0.3441**	0.2643	0.2918	**13**	0.2317	**0.3971**	0.3321	0.3203	**12**	0.0579	0.0177	0.0179	0.0317
14	**0.2652**	0.2941	**0.3179**	0.2918	**14**	**0.3628**	0.3912	**0.3857**	0.3795	**13**	0.0061	0.0088	0.0107	0.0085
15	0.2256	0.1824	0.2357	0.2125	**15**	0.0244	0.0088	0.0143	0.0159	**14**	0.0610	0.0500	0.0250	0.0465
16	0.0640	0.1147	0.1036	0.0930	**16**	0.0031	0.0088		0.0042	**15**	**0.2683**	0.3382	**0.4536**	0.3478
17	0.0213	0.0235	0.0143	0.0201						**16**	0.2012	**0.3588**	0.3107	0.2907
18	0.0031			0.0011						**17**	0.2195	0.0794	0.0964	0.1321
19		0.0029	0.0036	0.0021						**18**	0.0183	0.0029	0.0071	0.0095
										19	0.0031			0.0011

Updated nomenclatures

Negative control from sensitivity series using 32 cycles, 2U Taq



Scale: 100 RFUs

References

Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA.*J. Forensic Sci.* 50: 43-53. PDF version of this article can be downloaded at: http://www.cstl.nist.gov/biotech/strbase/pub_pres/Coble2005miniSTR.pdf

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