http://www.cstl.nist.gov/biotech/strbase/str26plex.htm

Protocol for using the NIST Autosomal STR 26plex:

Reagents/materials required

25mM $MgCl_2$
10x PCR Buffer
10 mM dNTPs
3.2 mg/mL BSA
5 U/µL TaqGold DNA Polymerase
TE-4 buffer (10 mM Tris, 0.1 mM EDTA, pH 8)
Primer mix (~2 µM)
dH_2O
36 cm ABI 3100/3130xl capillary array
Matrix Standard SD-33 (G5)
Hi-Di Formamide
GS500 Liz size standard
1x Genetic Analyzer Buffer w/EDTA
POP-6 polymer
GeneMapper ID software

Primer Mix prep

Locus	Marker Name	Final Stock Rev Conc (uM)	Dye	singleplex	2.00	uM	Dye labeled	100	uM	Water	100	uL total vol
1	D1GATA113	143.6	PET	0.70			1			98.30		
2	D1S1627	195.9	NED	1.02			2			96.98		
3	D1S1677	98.1	FAM	3.06			3			93.94		
4	D2S441	141.8	NED	0.71			1			98.29		
5	D2S1776	137.9	VIC	1.09			1.5			97.41		
6	D3S3053	103.1	VIC	1.94			2			96.06		
7	D3S4529	147.4	NED	0.51			0.75			98.74		
8	D4S2364	149.0	VIC	0.50			0.75			98.75		
9	D4S2408	152.4	NED	1.31			2			96.69		
10	D5S2500	184.3	VIC	1.08			2			96.92		
11	D6S474	190.9	FAM	0.79			1.5			97.71		
12	D6S1017	153.6	NED	1.30			2			96.70		
13	D9S1122	184.4	VIC	0.41			0.75			98.84		
14	D9S2157	79.5	NED	6.29			5			88.71		
15	D10S1248	154.2	FAM	0.65			1			98.35		
16	D10S1435	154.8	VIC	0.65			1			98.35		
17	D11S4463	127.0	FAM	2.36			3			94.64		
18	D12ATA63	192.8	FAM	0.52			1			98.48		
19	D14S1434	181.4	PET	1.10			2			96.90		
20	D17S974	78.4	NED	2.55			2			95.45		
21	D17S1301	212.5	PET	0.94			2			97.06		
22	D18S853	214.2	PET	0.93			2			97.07		
23	D20S482	180.0	PET	0.56			1			98.44		
24	D20S1082	200.0	FAM	0.50			1			98.50		
25	D22S1045	146.1	FAM	0.68			1			98.32		
Amel	Amelogenin	187.8	PET	1.06			2			96.94		
				33.21			44.25			22.54		

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*The unlabeled reverse primers are quantified using a spec at Abs 260. The dye labeled forward primers are not quantified and are all reconstituted with TE^{-4} to 100 µM. The primer concentrations used in primer mix calculations are the reverse primer quants. The forward primers use 100 µM for all calculations. This example primer mix set-up is for a 100 µL final volume. The target primer mix concentration is 2 µM, but should be empirically adjusted for locus-to-locus balance.

PCR Conditions

	Stock	09-22-2008 miniSTRs	Desired PCR conc	Volumes		# of Reactions
	conc.	Total volume of Reaction	20	to add		10
mM	25	Mg concentration (micromolar)	2	1.6	uL	16
uM	2	Primer concentration (micromolar)	0.2	2	uL	20
U/uL	5	units of Taq (units)	1	0.2	uL	2
mM	10	dNTP concentration (micromolar)	250	0.5	uL	5
x	10	PCR Buffer	1	2	uL	20
	3.2	BSA	0.16	1	uL	10
		Water to add		11.7	uL	117
		Master Mix volume		19		190
		Volume of added template (uL)	1			

add 19µL MM +
1µL sample = 20 µL rxn

Thermal Cycling Conditions (GeneAmp 9700 – ABI)

95ºC for 11 minutes

94ºC for 45 seconds
59ºC for 2 minutes } 30 cycles
72ºC for 1 minute

60ºC for 60 minutes

25ºC soak

*Use 0.5 ng – 1.0 ng of DNA

Post PCR/Running on the 3100/3130xl

Combine the following amounts:
8.7 µL Hi-Di
0.3 µL GS 500
1 µL PCR product

**Use the G5 filter when running these samples
***All of our bins and panels are based off of results obtained by using a 36 cm capillary array and POP-6 polymer. There may be OL alleles that need adjusting if POP-4 is used instead.

Inject for 10 s at 3 kV and separated at 15 kV at a run temp of 60 ºC
****If there is blown-out signal, reduce injection to 2 kV for 5 s

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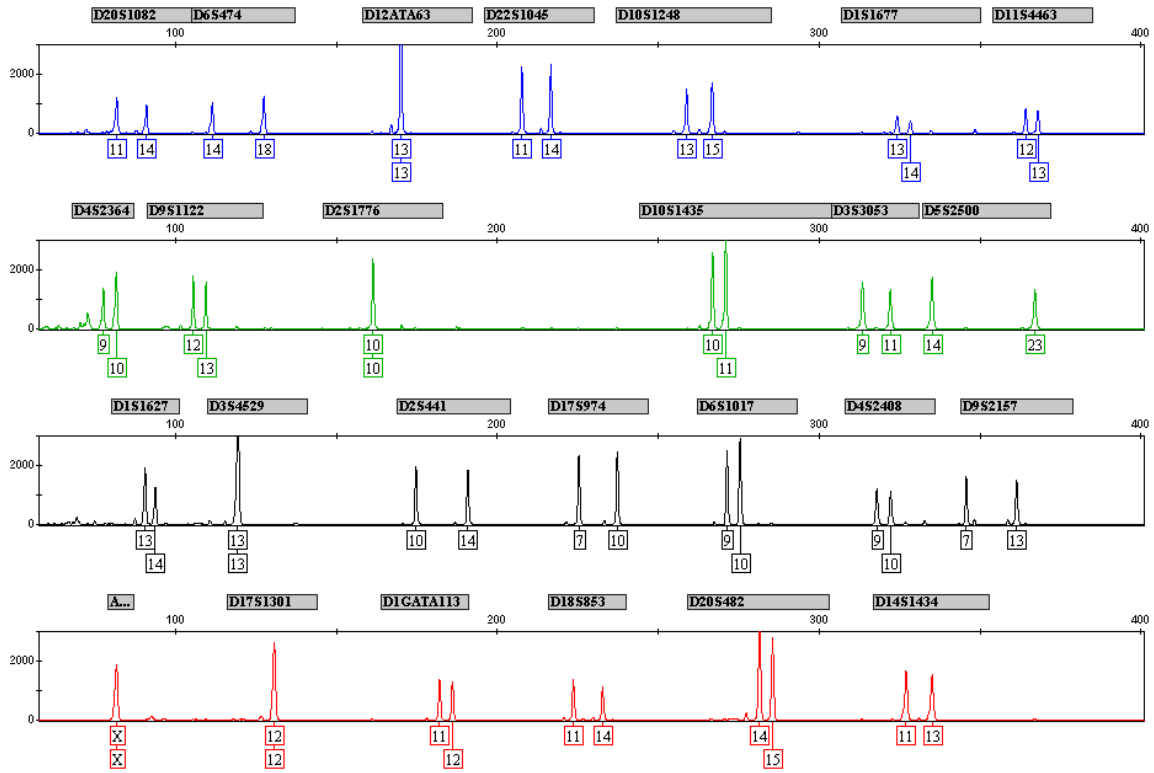
Bins and Panels for GeneMapperID v3.2:
http://www.cstl.nist.gov/biotech/strbase/str26plex.htm#Bins-and-Panels

26plex Image (9947A), 1 ng DNA, 30 cycles



For more information, contact:
Carolyn R. (Becky) Hill, MS
Human Identity Project Team
Biochemical Science Division
National Institute of Standards and Technology
100 Bureau Drive, MS 8311
Gaithersburg, MD 20899
Email: becky.hill@nist.gov
Phone: 301-975-4275
Fax: 301-975-8505