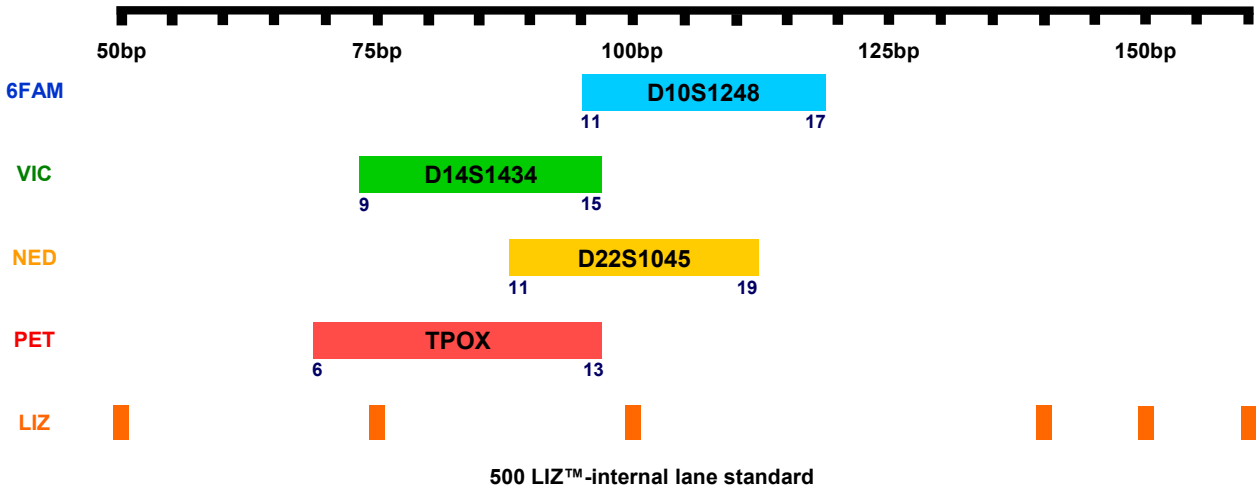
Miniplex NC01 plus system

D10S1248, D14S1434, D22S1045, TPOX



500 LIZ™-internal lane standard

Reagents Needed:

10X Primer Mix

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)

Gold ST*R 10X Buffer (Promega, Madison, WI)

10X Primer mix:

STR Loci		Primer Sequence (5'→3')	Conc.
D10S1248	F	6FAM-TTA ATG AAT TGA ACA AAT GAG TGA G	13.0 µM
	R	GCA ACT CTG GTT GTA TTG TCT TCA T	13.0 µM
D14S1434	F	VIC-TGT AAT AAC TCT ACG ACT GTC TGT CTG	2.0 µM
	R	GAA TAG GAG GTG GAT GGA TGG	2.0 µM
D22S1045	F	NED-ATT TTC CCC GAT GAT AGT AGT CT	1.3 µM
	R	GCG AAT GTA TGA TTG GCA ATA TTT TT	1.3 µM
TPOX	F	PET-CTT AGG GAA CCC TCA CTG AAT G	5.5 µM
	R	GTC CTT GTC AGC GTT TAT TTG C	5.5 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	5.7 µL
Gold ST*R 10X Buffer	1.0 µL
10X Primer Mix	1.0 µL
AmpliTaq Gold (5U/µL)	0.3 µL
DNA Template (100pg/µL)	2.0 µL
Total	10.0 µL

Thermal Cycling:

95°C for 11 minutes, then:

94°C for 20 seconds
59°C for 2 minutes
72°C for 1 minute
for 30 cycles, then:

60°C for 45 minutes
4°C soak

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Miniplex NC01 plus system

Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler
310 capillaries, 47 cm x 50 ㎛ (Applied Biosystems, Foster City, CA)
Performance Optimized Polymer (POP-4™ Polymer, Applied Biosystems, Foster City, CA)
DS-33 Matrix Standard Set [6FAM™, VIC®, NED™, PET®, and LIZ® dyes]
(Applied Biosystems, Foster City, CA)
Run module GS STR POP4 (1mL) G5
GeneScan™ 500 LIZ™ Size Standard (Applied Biosystems, Foster City, CA)
Hi-Di™ Formamide (Applied Biosystems, Foster City, CA)

Creating Matrix:

According to the ABI PRISM® 310 Genetic Analyzer User's Manual

Preparing the Sample:

1. Prepare a loading cocktail by combining and mixing the 0.2 μL GeneScan™ 500 LIZ™ Size Standard and 20.0 μL Hi-Di™ Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.2 μL of the prepared loading cocktail and 1.0 μL of the PCR product.
4. Preparing the allelic ladder, combine 20.0 μL of the prepared loading cocktail and 1.0 μL of the allelic ladder mix. Vortex the allelic ladder mix prior to pipetting.
5. Denature the samples and ladder by heating at 95°C for 5 minutes and immediately chill on crushed ice. Denature the samples just prior to loading.
6. Assemble the tubes in the appropriate autosampler, and place the autosampler tray in the instrument.

310 Data Collection Software:

Prepared the samples are run using the Run module **GS STR POP4 (1mL) G5** and a described above **matrix**.
Samples are injected for 5 seconds at 15,000 V and separate at 15,000 V for 17 minutes with run temperature of 60°C.

Genotyper Macro:

NC01 plus.gta

Electropherogram of NC01 plus Allelic Ladder and Genotyping of Standard 9948 DNA

NC01 plus