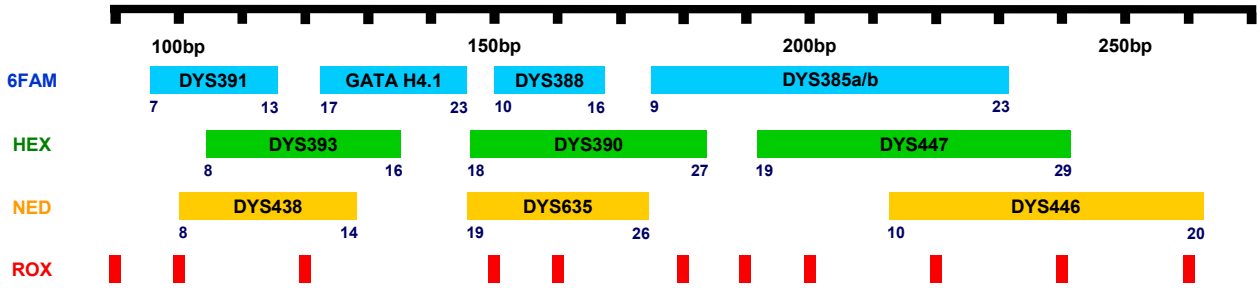
Y-miniplex II System

DYS385, DYS388, DYS390, DYS391, DYS393, DYS438, DYS446, DYS447, DYS635, GATA H4.1



400HD ROX™-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.
DYS385	F	GAA GGA AGG AAG GAA GGG AAA	1.2 µM
	R	6FAM-TAA GGG CTG CTG ACC AGA TT	1.2 µM
DYS388	F	6FAM-GAA TTC ATG TGA GTT AGC CGT TTA GC	4.2 µM
	R	GAG GCG GAG CTT TTA GTG AG	4.2 µM
DYS390	F	HEX-CTG CAT TTT GGT ACC CCA TA	0.8 µM
	R	GCA ATG TGT ATA CTC AGA AAC AAG G	0.8 µM
DYS391	F	6FAM-TTC AAT CAT ACA CCC ATA TCT GTC	2.4 µM
	R	GAT AGA GGG ATA GGT AGG CAG GC	2.4 µM
DYS393	F	HEX-GTG GTC TTC TAC TTG TGT CAA TAC	1.2 µM
	R	GAA CTC AAG TCC AAA AAA TGA GG	1.2 µM
DYS438	F	NED-TGG GGA ATA GTT GAA CGG TAA	2.0 µM
	R	GGC AAC AAG AGT GAA ACT CCA	2.0 µM
DYS446	F	GCC TTC ACT TCC ACA CAC GTT	6.5 µM
	R	NED- GAG CTT GTA CCA CTG CAC TCA	6.5 µM
DYS447	F	HEX-GGT CAC AGC ATG GCT TGG TT	1.8 µM
	R	GGG CTT GCT TTG CGT TAT CTC T	1.8 µM
DYS635	F	GGC TTC TCA CTT TGC ATA GAA TC	1.4 µM
	R	NED- ACC AGC CCA AAT ATC CAT CA	1.4 µM
GATA H4.1	F	6FAM-ATG CTG AGG AGA ATT TCC AA	2.4 µM
	R	GCT ATT CAT CCA TCT AAT CTA TCC ATT	2.4 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	4.9 µL
Gold ST*R 10X Buffer	1.6 µL
10X Primer Mix	1.0 µL
AmpliTaq Gold (5U/µL)	0.5 µL
DNA Template (100pg/µL)	2.0 µL
Total	10.0 µL

Thermal Cycling:

95°C for 11 minutes
96°C for 1 minute, then:

94°C for 30 seconds
59°C for 30 seconds
70°C for 45 seconds
for 10 cycles, then:

→

90°C for 30 seconds
59°C for 30 seconds
70°C for 45 seconds
for 21 cycles, then:

60°C for 45 minutes
4°C soak

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Y-miniplex II 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)
Fluorescent Amidite Matrix Standards [6FAM™, TET, HEX, TAMRA™, ROX™]
(Applied Biosystems, Foster City, CA)
NED™ Matrix Standard (Applied Biosystems, Foster City, CA)
Run module GS STR POP4 (1mL) D
GeneScan™ 400HD ROX™ 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 0.3 μL GeneScan™ 400HD ROX™ Size Standard and 20.0 μL Hi-Di™ Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.3 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) D** and a described above **matrix**.
Samples are injected for 5 seconds at 15,000 V and separate at 15,000 V for 22 minutes with run temperature of 60°C.

Genotyper Macro:

Y-miniplex II.gta

Electropherogram of Y-miniplex II Allelic Ladder and Genotyping of Standard 9948 DNA