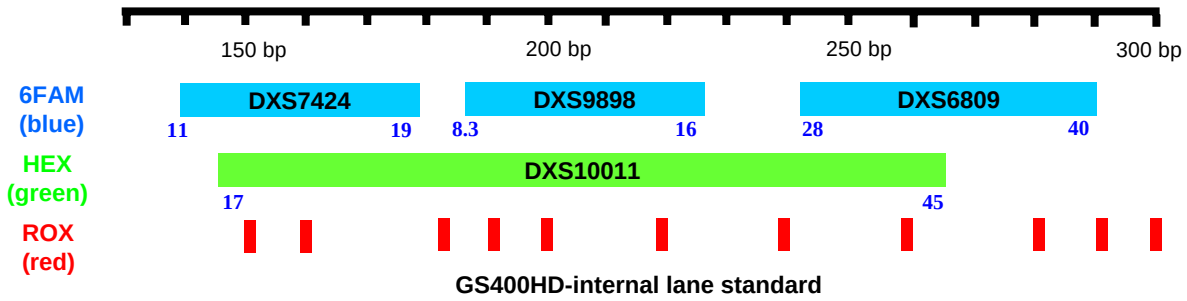
X-STR multiplex system II

Multiplex II- DXS7424, DXS9898, DXS6809, DXS10011



GS400HD-internal lane standard

Pre-amplification

Reagents Needed:

Primer Mix

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

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

10 X Primer Mix:

STR Loci		Primer Sequence (5'-3')	Conc.
DXS7424	F	6FAM-CTG CTT GAG TCC AGG AAT TCA A	3.3 µM
	R	GAA CAC GCA CAT TTG AGA ACA TA	3.3 µM
DXS9898	F	CGA GCA CAC CTA CAA AAG CT	1.2 µM
	R	6FAM-TCG ATT AGG TTC AGT TCC CA	1.2 µM
DXS6809	F	TGA ACC TTC CTA GCT CAG GA	3.8 µM
	R	6FAM-TCT GGA GAA TCC AAT TTT GC	3.8 µM
DXS10011	F	GGA GTG AAC TCT GAA AAA AAA	6.5 µM
	R	HEX-TGA AAT CAT CTA TCT TTC TTT C	6.5 µM

PCR Mixture:

PCR Component	Volume per Sample
dH_2O	6.1 µl
Gold ST*R 10 X Buffer	1.6 µl
10 X Primer Mix	1.0 µl
AmpliTaq Gold	0.3 µl
DNA Template	1.0 µl
Total	10 µl

Thermal Cycling:

95ºC for 11 minutes, then:

94ºC for 1 minutes
55ºC for 1 minutes
72ºC for 1 minutes
For 30 cycles, then:

60ºC for 45 minutes
4ºC soak

Electrophoresis on the ABI PRISM 310 Genetic Analyzer

Multiplex II- DXS7424, DXS9898, DXS6809, DXS10011

Post-amplification

Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler

310 capillaries, 47cm x 50 µm (Applied Biosystems, Foster City, CA)

Performance Optimized Polymer (POP4, Applied Biosystems, Foster City, CA)

Flourescent Amidite Matrix Standards [6FAM™, TET™, HEX™, TAMRA™, ROX™]
(Applied Biosystems, Foster City, CA)

Run module GS STR POP4 (1 mL) D

GS ROX-400HD 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 GS ROX-400HD and 20 µl Hi-Di formamide per sample.
2. Vortex for 10 s.
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 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 95ºC 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 (1 mL) D** and a described above **matrix**.

Samples are injected for 5s at 15,000 V and separate at 15,000V for 24 min with run temperature of 60 ºC.

Genotyper Software:

X-STR II macro