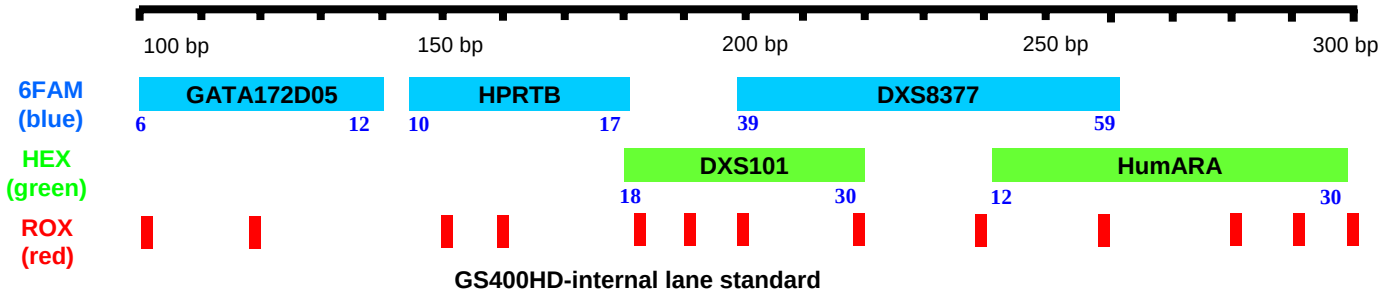
X-STR multiplex system I

Multiplex I- GATA172D05, HPRTB, DXS8377, DXS101, HumARA



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)
DMSO

10 X Primer Mix for Quadruplex:

STR Loci		Primer Sequence (5'-3')	Conc.
GATA172D05	F	6FAM-TAG TGG TGA TGG TTG CAC AG	0.7 µM
	R	ATA ATT GAA AGC CCG GAT TC	0.7 µM
HPRTB	F	6FAM-TCT CTA TTT CCA TCT CTG TCT CC	0.8 µM
	R	TCA CCC CTG TCT ATG GTC TCG	0.8 µM
DXS8377	F	6FAM- CAC TTC ATG GCT TAC CAC AG	1.5 µM
	R	GAC CTT TGG AAA GCT AGT GT	1.5 µM
DXS101	F	HEX-ACT CTA AAT CAG TCC AAA TAT CT	2.5 µM
	R	AAA TCA CTC CAT GGC ACA TGT AT	2.5 µM

10 X Primer Mix for HumARA:

STR Loci		Primer Sequence (5'-3')	Conc.
HumARA	F	HEX-TCC AGA ATC TGT TCC AGA GCG TGC	5.0 µM
	R	GCT GTG AAG GTT GCT GTT CCT CAT	5.0 µM

PCR Mixture:

PCR Component	Quadruplex	HumARA
dH_2O	6.7 µl	5.2 µl
Gold ST*R 10 X Buffer	1.0 µl	1.6 µl
50 % DMSO	-	1.0 µl
10 X Primer Mix	1.0 µl	1.0 µl
AmpliTaq Gold	0.3 µl	0.2 µl
DNA Template	1.0 µl	1.0 µl
Total	10 µl	10 µl

Thermal Cycling:

95ºC for 11 minutes, then:

94ºC for 1 minutes
59ºC 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 I- GATA172D05, HPRTB, DXS8377, DXS101, HumARA

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 I macro