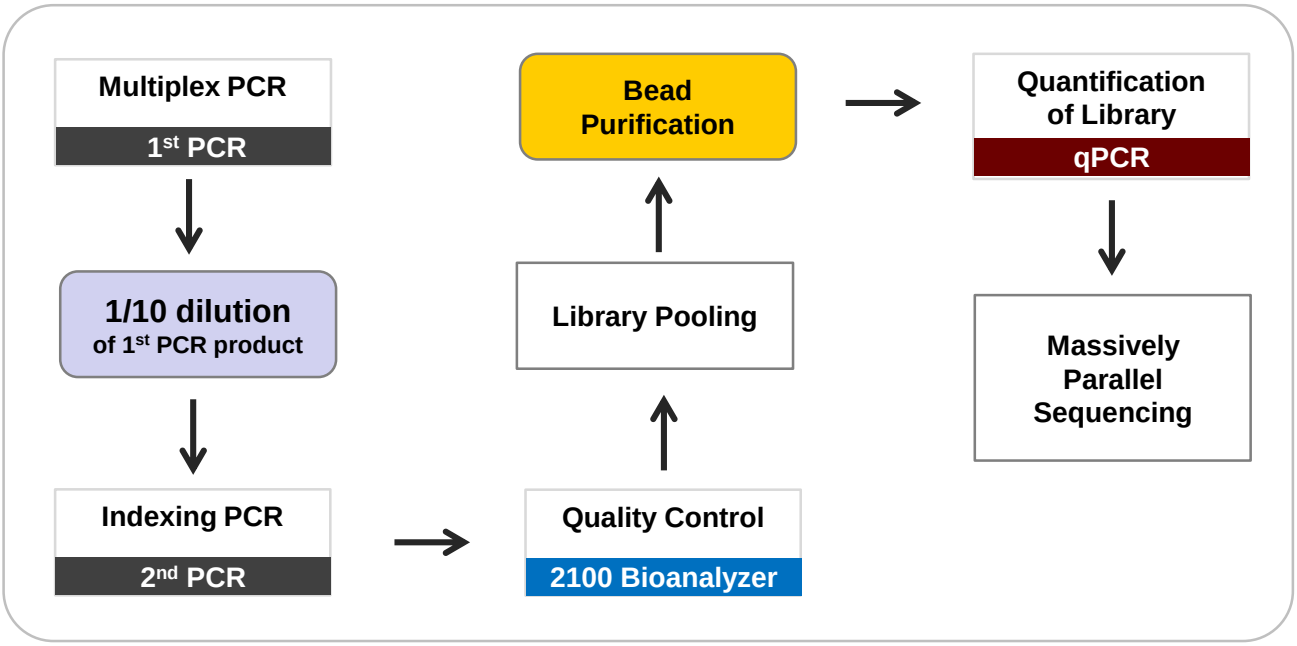
Y-STR Analysis of Reference Sample
using In-house Massively Parallel Sequencing Panel

Workflow



Multiplex PCR

Reagents Needed

5X KplexSeq-Y24 Primer Mix

AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)

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

PCR Mixture

PCR Component	Vol. (µl)
Nuclease-Free Water	12.0
10X Gold ST*R Buffer	2.0
5X Primer Mix¥	4.0
AmpliTaq Gold (5U/µl)	1.0
Template DNA (1ng/ul)	1.0
Total	20.0

¥5X primer mix can be divided into 2 ~ 3 sets

Thermal Cycling

95℃ for 11 minutes, then:

94℃ for 20 seconds
60℃ for 60 seconds
72℃ for 45 seconds
for x 27 cycles, then:

72℃ for 5 minutes
4℃ soak

Y-STR Analysis of Reference Sample
using In-house MPS Panel (continued)

Indexing PCR

Reagents Needed

Nextera® XT v2 index kit (Illumina®, Inc., San Diego, CA)

AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)

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

PCR Mixture

PCR Component	Vol. (µl)
Nuclease-Free Water	12.5
10X Gold ST*R Buffer	2.0
Index 1 (i7)	2.0
Index 2 (i5)	2.0
AmpliTaq Gold (5U/µl)	0.5
1/10 diluted 1st PCR product	1.0
Total	20.0

Thermal Cycling

95℃ for 15 minutes, then:

94℃ for 20 seconds
61℃ for 30 seconds
72℃ for 45 seconds
for x 16 cycles, then:

72℃ for 5 minutes
4℃ soak

Quality Control

Materials and Reagents Needed

Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA)

Agilent DNA 1000 kit (Agilent Technologies, Inc., Santa Clara, CA)

Protocol

According to the Agilent 2100 Bioanalyzer and DNA 1000 kit User's Manuals

Finally, normalize each library to 10ng/ul and pool them in equal volume

Y-STR Analysis of Reference Sample
using In-house MPS Panel (continued)

Bead-based Purification of the Pooled Library

Materials and Reagents Needed

Agencourt® AMPure® XP beads (Beckman Coulter, Indianapolis, IN)

Freshly prepared 80% ethanol

DynaMag™-2 Magnet (ThermoFisher Scientific, Waltham, MA)

Elution buffer or Nuclease-Free Water

Dry heating block (for optional incubation in 37°C)

Protocol

1. Remove the AMPure® XP beads from storage and let it stand for at least 30 minutes for equilibration to room temperature
2. Vortex the AMPure® XP beads to homogenize the suspension before use
3. Add x1.2 well-mixed AMPure® XP beads to 50µl pooled library, then carry out procedures according to manufacturer's instructions
4. Elute library in 50µl of the elution buffer or Nuclease-Free Water

Quantify Libraries

Materials and Reagents Needed

KAPA Library Quantification Kits (KAPA Biosystems, Wilmington, MA)

AB 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA)

Protocol

According to the KAPA Library Quantification Kits User's Manual