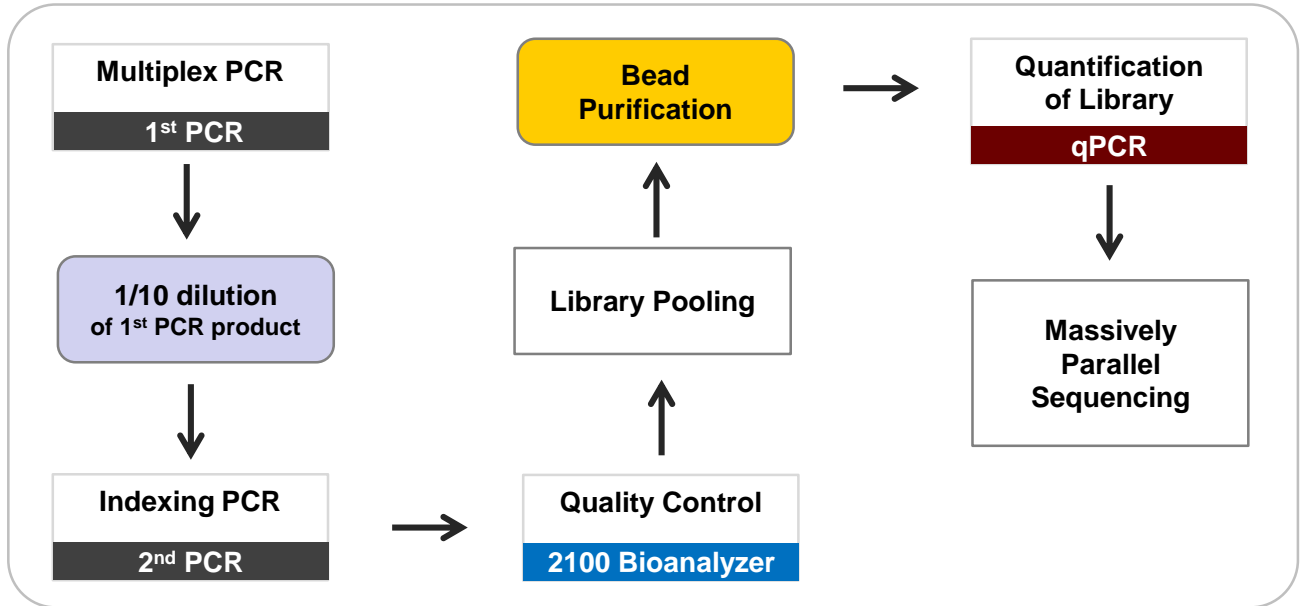


Autosomal STR Analysis of Reference Sample using In-house Massively Parallel Sequencing Panel

Workflow



Multiplex PCR

Reagents Needed

5X **KplexSeq-A25** 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

Thermal Cycling

95°C for 11 minutes, then:

94°C for 20 seconds

59°C for 60 seconds

72°C for 45 seconds

for x 26 cycles, then:

72°C for 5 minutes

4°C soak

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

Autosomal 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 1 st PCR product	1.0
Total	20.0

Thermal Cycling

95°C for 15 minutes, then:

94°C for 20 seconds

61°C for 30 seconds

72°C for 45 seconds

for x 15 cycles, then:

72°C for 5 minutes

4°C 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

Autosomal 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 (ThermoFisher Scientific, Waltham, MA)

Protocol

According to the KAPA Library Quantification Kits User's Manual