



TECHNICAL MANUAL

PowerSeq[®] 46GY System

Instructions for Use of Product
PS4600

PowerSeq[®] 46GY System

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Manual.
 E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

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1. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–9). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using massively parallel sequencing and bioinformatic analysis.

The PowerSeq® 46GY System is a 46-locus multiplex for human identification applications including forensic analysis, relationship testing and research use. This system allows co-amplification of 22 autosomal loci (CSF1PO, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D22S1045, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, FGA, Penta D, Penta E, TH01, TPOX, vWA), 23 Y-STR loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481, DYS533, DYS549, DYS570, DYS576, DYS635, DYS643, Y-GATA-H4) and Amelogenin. This extended panel of STR markers is intended to satisfy both CODIS and ESS recommendations (10,11).

STR markers on the Y chromosome (Y-STR) have qualities that are distinct from autosomal markers and are useful for human identification (12–18). Y-STR markers are found on the nonrecombining region of the Y chromosome (NRY) and produce a haploid profile when amplified from male DNA. This quality simplifies male/female mixture interpretation by removing the female contribution from an amplification profile (19,20). Strict paternal inheritance of these markers makes them useful for paternity and kinship studies.

Massively parallel sequencing data of these STR markers can be used to identify any potential sequence variation with an STR allele in addition to the length variation (21–23). Isoalleles (alleles that are the same length but have different sequences) in flanking regions identified by sequencing can provide additional power of discrimination, but may cause results that appear discordant with CE-based data (24). Investigation of flanking region insertions or deletions may often explain any encountered discrepancies. The PowerSeq® 46GY System is compatible with the Illumina MiSeq® System and the MiSeq FGx™ Forensic Genomics System. Amplification instrumentation may vary. You may need to optimize protocols, including amount of template DNA and cycle number for your laboratory instrumentation. In-house validation should be performed (25). PowerSeq® 46GY System provides all materials necessary to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the PowerSeq® 5X Master Mix. This manual contains protocols for use of the PowerSeq® 46GY System with the GeneAmp® PCR System 9700 thermal cycler, Veriti® 96-Well Thermal Cycler and the Applied Biosystems® ProFlex® PCR System in addition to protocols to prepare sample libraries using TruSeq® DNA PCR-Free High Throughput Library Prep Kit. A protocol to operate the massively parallel sequencing instrument should be obtained from the instrument manufacturer.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerSeq® 46GY System	100 reactions	PS4600

Not for Medical Diagnostic Use. This system includes sufficient reagents for 100 reactions at 25µl per reaction. Components are shipped on dry ice.

- 1 × 500µl PowerSeq® 5X Master Mix
- 1 × 500µl PowerSeq® 46GY 5X Primer Pair Mix
- 1 × 25µl 2800M Control DNA (10ng/µl)
- 2 × 1,250µl Water, Amplification Grade
- 1 × 10mg Proteinase K

Storage Conditions: Store all components at -30°C to -10°C in a nonfrost-free freezer. Store the 2800M Control DNA at $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$ for at least 24 hours before use. Dispense reconstituted Proteinase K into single-use aliquots and stored at -20°C , where it is stable for 2–3 months. Store these aliquots in the post-amplification area. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes because these can reduce product stability. All other components can be stored at $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$ for up to 3 months.

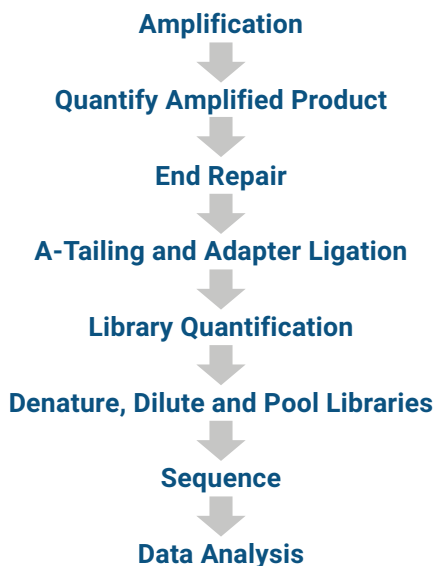


Figure 1. Overview of the PowerSeq® 46GY System workflow.

3. Before You Begin

Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual. Please refer to the appropriate guidelines (25,26).

The quality of purified DNA or direct-amplification samples, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and library preparation. Additional research and validation are required if any modifications are made to the recommended protocols.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and preparing sequencing libraries. Always include a negative-control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

Some reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Read the warning label, take appropriate precautions when handling these substances, and wear personal protective equipment, including eye protection, gloves and a lab coat. The reagents in the MiSeq® Reagent Cartridge contain formamide. Ensure that disposal of waste generated from the MiSeq® sequencing run complies with laboratory and governmental safety standards. Please refer to the MiSeq® System Guide #15027617 or the MiSeq FGx™ Instrument Reference Guide #15050524 for additional information on MiSeq® instrument waste.

Determine the concentration of genomic DNA for your samples with a DNA quantification method (e.g., PowerQuant® System). Note that different quantification methods yield different quantification values. We strongly recommend that you perform experiments to determine the optimal DNA template amount for the PowerSeq® 46GY amplification reaction based on your DNA quantification method.

The type and quantity of DNA samples to be sequenced should be considered when determining the number of samples to be multiplexed (i.e., pooled for simultaneous sequencing). Up to 96 reference samples can be sequenced simultaneously. Multiplexing should be limited to 32 samples or less for more challenging samples (e.g., mixtures, degraded DNA samples). These are suggested guidelines; the number of samples that can be successfully multiplexed should be established through internal validation.

Deviations from the instructions provided in this technical manual are likely to impact system performance.

Please refer to the MiSeq® System Guide #15027617 or the MiSeq FGx™ Instrument Reference Guide #15050524 for troubleshooting instrument performance.

4. Protocols for DNA Amplification Using the PowerSeq® 46GY System

The PowerSeq® 46GY System is developed for amplification of extracted DNA and direct-amplification samples. (For direct-amplification system requirements, refer to Appendix 9.A.) Slight protocol variations are recommended for optimal performance with each template source. The PowerSeq® 46GY System is compatible with the GeneAmp® PCR System 9700 Thermal Cycler with a gold-plated silver or silver sample block, the Veriti® 96-Well Thermal Cycler and the Applied Biosystems® ProFlex® PCR System. This system has not been tested with the Veriti® 96-Well Fast Thermal Cycler or GeneAmp® PCR System 9700 with an aluminum block. We recommend using gloves and aerosol-resistant pipette tips to prevent cross-contamination. Keep all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup. Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 8.

Materials to Be Supplied by the User

Instrumentation and Equipment

- GeneAmp® PCR System 9700, 96-well with a gold-plated silver or silver sample block, Applied Biosystems Veriti® 96-well Thermal Cycler or ProFlex® PCR System (ThermoFisher Scientific)
- fluorometer [For tubes, we recommend Quantus™ Fluorometer (Cat.# E6150). For 96-well plates, we recommend the GloMax® Discover (Cat.# GM3000) or the GloMax® Explorer System (Cat.# GM3500 or GM3510).]
- magnetic separation stand [For tubes, we recommend MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342). For 96-well plates, we recommend ThermoFisher DynaMag™-96 Side (Part# 12331D).]
- centrifuge compatible with a 96-well plate
- Deep Well MagnaBot® Magnetic Separation Device (Cat.# V3031)
- 96-Well Format Spacer, 1/4 inch (Cat.# Z3301)
- 96-Well Format Spacer, 1/8 inch (Cat.# V8581)
- Illumina MiSeq® or Illumina MiSeq FGx™ Instrument

4. Protocols for DNA Amplification Using the PowerSeq® 46GY System (continued)

Reagents

- fluorometric-based quantification system [We recommend the QuantiFluor® dsDNA System (Cat.# E2670) or the QuantiFluor® ONE dsDNA System Cat.# E4871 or E4870).]
- TruSeq® DNA PCR-Free High Throughput Library Prep Kit (Illumina Cat.# 20015963)
- IDT for Illumina - TruSeq® DNA UD Indexes (Illumina Cat.# 20022370)
- MiSeq® Reagent Kit v3 (600 cycles, Illumina Cat.# MS-102-3003)
- PowerSeq® Quant MS System (Cat.# PS5000)
- absolute ethanol (200 proof, molecular biology-grade)
- Nuclease-Free Water (Cat.# P1193, P1195, or P1197)
- 2N NaOH (molecular biology-grade)
- PhiX Control v3 (Illumina Cat.# FC-110-3001)
- **optional:** 10mM Tris-HCl (pH 8.5)

Note: Sample Purification Beads are included in the TruSeq® DNA PCR-Free High Throughput Library Prep Kit. Based on the amount of overage factored into dispensed volumes, the volume of beads provided in the kit may be insufficient for your laboratory needs. If additional purification beads are needed, we recommend purchasing Agencourt AMPure® XP System beads, 60ml (Beckman Coulter Life Sciences Cat.# A63881).

Plastics

- aerosol-resistant pipette tips
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- MicroAmp® Optical 8-Cap Strips or MicroAmp® Optical 12-Cap Strips (Applied Biosystems)
- 1.2ml, Round-Bottom Deep Well Plate (Cat.# V6771) **Note:** Using the Deep Well MagnaBot® 96 Magnetic Separation Device and 1.2ml, Round-Bottom Deep Well Plate may require the use of extended length pipette tips.
- adhesive seals for 96-well plates

4.A. Amplification Setup

We routinely amplify 1ng of template DNA in a 25µl reaction volume using the following protocol.

1. At the first use, thaw the PowerSeq® 5X Master Mix, PowerSeq® 46GY 5X Primer Pair Mix and Water, Amplification Grade, completely. After the first use, store the reagents at +2°C to +8°C.
2. Ensure that the 2800M Control DNA has been stored at +2°C to +8°C for at least 24 hours before use.

Note: Centrifuge tubes briefly to bring contents to the bottom, and then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

- Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix (see table below).

Component	Volume Per Reaction	Number of Reactions	Final Volume
Water, Amplification-Grade ¹	To a final volume of 25µl		
PowerSeq [®] 5X Master Mix ¹	5µl		
PowerSeq [®] 46GY 5X Primer Pair Mix	5µl		
Template DNA (1ng) ¹⁻³	(up to 15µl)		
total reaction volume	25µl		

¹Add Water, Amplification Grade, to the tube first, and then add PowerSeq[®] 5X Master Mix and PowerSeq[®] 46GY 5X Primer Pair Mix. The template DNA will be added at Step 8.

²Store DNA templates in TE⁻⁴ buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE⁻⁴ buffer with 20µg/ml glycogen. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of DNA added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations, depending on the source of the template DNA and the extraction procedure used.

³Apparent DNA concentrations can differ, depending on the DNA quantification method used (27). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

- Use a clean MicroAmp[®] plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean 0.2ml reaction tubes required and label appropriately.
- Add the final volume of each of the components in table above to a separate, sterile tube. Prepare the PCR amplification mix by combining volumes calculated in Step 3 of Amplification-Grade Water, PowerSeq[®] 5X Master Mix and PowerSeq[®] 46GY 5X Primer Pair Mix in the order listed. **Do not** add the template DNA until directed to do so in Step 8.
- Vortex the PCR amplification mix for 5–10 seconds, and then pipet PCR amplification mix into each reaction well.
Note: Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.
- Add the template DNA (1ng) for each sample to the respective well containing PCR amplification mix.
Note: The PowerSeq[®] 46GY System was optimized and balanced using 1ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

8. For the positive amplification control, vortex the 2800M Control DNA and then dilute an aliquot to 0.2ng/μl. Add 5μl (1ng) of diluted DNA to a reaction well containing PCR amplification mix.
Note: The dilution of 0.2ng/μl 2800M Control DNA may be stored at 4°C for 24 hours.
9. For the negative amplification control, pipet Water, Amplification Grade, or TE⁻⁴ buffer instead of template DNA into a reaction well containing PCR amplification mix.
10. Seal or cap the plate, or close the tubes.
Optional: Briefly centrifuge the plate or tubes to bring contents to the bottom of the wells, and remove any air bubbles.

4.B. Thermal Cycling

Amplification instrumentation may vary. You may need to optimize protocols including the amount of template DNA and cycle number for your laboratory instrumentation. Testing at Promega shows that 29 cycles works well for 1ng of purified DNA template.

After completion of the thermal cycling protocol, prepare amplification products for sequencing or store amplified samples at -20°C. For short-term storage, amplified samples can be stored at 4°C.

1. Place the MicroAmp® plate or reaction tubes in the thermal cycler.
2. Select and run the recommended protocol, which is provided in the table below. The total cycling time is less than 1 hour.

Thermal Cycling Protocol	
96°C for 1 minute, then:	
29 cycles of:	96°C for 5 seconds
	60°C for 35 seconds,
	72°C for 5 seconds, then:
60°C for 2 minutes	
4°C soak	

Notes:

When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%.

When using the ProFlex® PCR System, select **Advanced Options** followed by **Simulation Mode** and **GeneAmp™ PCR System 9700**.

When using the GeneAmp® PCR System 9700, the program must be run with 'Max Mode' as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

3. After completion of the thermal cycling protocol, proceed to amplification product purification or store amplified samples at -20°C. For short-term storage, amplified samples may be stored at 4°C.

5. Preparing Amplification Products for Sequencing

5.A. Purifying Amplification Products

To avoid bead clumping during resuspension steps, treat the amplification reactions with Proteinase K immediately before bead purification.

Preparing Proteinase K Solution

Proteinase K is supplied as a lyophilized powder. Reconstitute in Nuclease-Free Water to make a 20mg/ml stock solution. Alternatively Proteinase K can be purchased as a solution (Cat.# MC5005).

Note: Dispense the reconstituted protease into single-use aliquots and store at -20°C , where it is stable for 2–3 months. Store these aliquots in the post-amplification area. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes because these can reduce product stability.

Purifying DNA Using the Illumina TruSeq[®] DNA PCR-Free High Throughput Sample Preparation Kit

Purify amplification products using the Sample Purification Beads provided in the Illumina[®] TruSeq DNA PCR-Free High Throughput Sample Preparation Kit using the directions below. Refer to the TruSeq[®] DNA PCR-Free Reference Guide, Document# 1000000039279 for additional information.

Notes:

- a. This protocol and the sections following refer specifically to 96-well plates, but reactions may be performed in tubes with appropriate adjustments.
- b. Thorough and complete bead mixing is critical to the success of this procedure. Before you begin, you can view a [short video](#) illustrating these steps.
 1. Thaw the Resuspension Buffer at room temperature. Store at $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$ after thawing.
 2. Let the Sample Purification Beads stand for 30 minutes to equilibrate to room temperature.
 3. Prepare fresh 80% ethanol.
 4. Dilute the 20mg/ml Proteinase K by adding 25 μl of 20mg/ml Proteinase K to 967 μl of Nuclease-Free Water to make a 504 $\mu\text{g}/\text{ml}$ solution.
 5. Add 5 μl of the 504 $\mu\text{g}/\text{ml}$ Proteinase K to the 25 μl amplification reaction.

Note: If the volume of the amplification reaction to be purified is not 25 μl , then the final concentration of the Proteinase K must be adjusted so that, in a final reagent volume that includes the amplification reaction, Sample Purification Beads and Proteinase K, the final concentration of Proteinase K is 30 $\mu\text{g}/\text{ml}$.
 6. Vortex the Sample Purification beads for at least 1 minute until they are well dispersed.
 7. Add 54 μl of the Sample Purification beads to each well (1.8X ratio to the amplification reaction volume and Proteinase K). Vortex the beads frequently to make sure that the beads are evenly distributed to the samples.

5.A. Purifying Amplification Products (continued)

8. Mix thoroughly by gently pipetting 10 times using a 200µl single-channel or multichannel pipette set to 70µl.
Note: The amplification reaction and purification beads are both viscous solutions that do not mix easily. Optimal yield and balanced representation of all STR loci is dependent on thorough mixing of the beads and samples. The solution in the well should be a consistent light brown color after mixing. Continue mixing if the color of the solution is lighter or clear near the top of the well.
9. Incubate at room temperature for 5 minutes.
10. Place the plate on a magnetic stand at room temperature for approximately 5 minutes or until liquid is clear.
11. Remove and discard the supernatant from each well.
12. With the plate on the magnetic stand, wash 2 times with 80% ethanol as follows:
 - a. Add 200µl of the freshly prepared 80% ethanol to each well.
 - b. Incubate on the magnetic stand for 30 seconds.
 - c. Remove and discard the supernatant from each well, taking care not to disturb the bead pellet.
 - d. Repeat Steps 12.a–c for the second wash.
13. Use a 20µl pipette to remove residual ethanol from each well.
14. Air-dry the samples on the magnetic stand for 5 minutes.
15. Add 30µl of the Resuspension Buffer to each well.
16. Remove the plate from the magnetic stand.
17. Mix thoroughly by gently pipetting 10 times.
18. Incubate at room temperature for 2 minutes.
19. Place the plate on the magnetic stand, and wait until the liquid is clear (2–5 minutes).
20. Being careful not to disturb the bead pellet, transfer 28µl of the supernatant to a new 96-well reaction plate.
Note: This is a safe stopping point. Seal the plate and store at –25°C to –15°C for up to 7 days.
21. Determine the concentration of the purified amplification products using a fluorescence-based quantification method. We recommend the QuantiFluor® dsDNA System (Cat.# E2670) or the QuantiFluor® ONE dsDNA System (Cat.# E4871 or E4870) with the Quantus™ Fluorometer or the GloMax® Discover System. Follow the instructions in the Technical Manual provided for each system.

5.B. Preparing the DNA Library with the Illumina TruSeq® DNA PCR-Free High-Throughput Library Prep Kit

Refer to the TruSeq® DNA PCR-Free Reference Guide, Document# 1000000039279 for a list of components in the TruSeq® DNA PCR-Free Library Prep Kit and storage conditions.

End Repair Reactions

1. Thaw the End Repair Mix 2 at room temperature, and then place on ice. Be sure to return the End Repair Mix to -20°C after use.
2. Leave the Resuspension Buffer out for 30 minutes to equilibrate to room temperature.
3. Let the Sample Purification Beads stand for 30 minutes to equilibrate to room temperature.
4. Dilute each purified amplification product in Resuspension Buffer with a final mass of 500ng in a final volume of 60 μl .

Note: If the total amount of purified amplification product is less than 500ng, add all volume to the end repair reaction.

5. Assemble the end repair reactions for each sample in a 96-well plate as shown below:

Component	Volume Per Sample
500ng purified amplification product diluted in Resuspension Buffer (from Step 4)	60 μl
End Repair Mix 2	40 μl
total volume	100μl

6. Mix each end repair reaction thoroughly by gently pipetting 10 times.
7. Program the thermal cycler with the end repair program described below:

End Repair Program
Preheat lid to 100 $^{\circ}\text{C}$
30 $^{\circ}\text{C}$ for 30 minutes
4 $^{\circ}\text{C}$ hold

8. Seal the plate with strip caps, and place the plate in the thermal cycler. Run the program, and remove the plate from the thermal cycler.

5.B. Preparing the DNA Library with the Illumina TruSeq® DNA PCR-Free High-Throughput Library Prep Kit (continued)

Purifying End-Repaired Products

Purify the end-repaired amplification products using the Sample Purification Beads provided in the Illumina TruSeq® DNA PCR-Free High Throughput Sample Preparation Kit.

1. If the Sample Purification Beads are not already at room temperature, equilibrate to room temperature for 30 minutes.
2. Prepare fresh 80% ethanol (if needed).
3. To accommodate the volume of Sample Purification Beads needed for purifying the end repair products, transfer the end repair products from Step 8, End Repair Reaction, into a 1.2ml round-bottom deep-well plate.
4. Vortex the beads for at least 1 minute until they are well dispersed.
5. Add 180µl of the Sample Purification beads to each well (1.8X ratio to the end repair reaction volume). Vortex the beads frequently to make sure that the beads are evenly distributed to the samples.
Note: Optimal yield and balanced representation of all STR loci is dependent on thorough mixing of the beads and samples.
6. Mix thoroughly by gently pipetting 10 times using a 200µl single-channel or multichannel pipette set to 180µl.
7. Incubate at room temperature for 5 minutes.
8. Place the deep well plate on the Deep Well MagnaBot® 96 Magnetic Separation Device (with 1/4 inch and 1/8 inch foam spacers) at room temperature for approximately 10 minutes or until liquid is clear.
9. Remove and discard the supernatant from each well.
10. With the plate on the magnetic stand, wash two times with 80% ethanol as follows:
 - a. Add 200µl of the freshly prepared 80% ethanol to each well.
 - b. Incubate on the magnetic stand for 30 seconds.
 - c. Remove and discard the supernatant from each well, taking care not to disturb the bead pellet.
 - d. Repeat Steps 10.a–c for the second wash.
11. Use a 20µl pipette to remove residual ethanol from each well.
12. Air-dry the samples on the magnetic stand for 5 minutes.
13. Add 20µl of the Resuspension Buffer to each well.
14. Remove from the magnetic stand.
15. Cover the 1.2ml round-bottom, deep-well plate with an adhesive seal for a 96-well plate. Vortex the covered deep-well plate for 30 seconds.
16. Incubate at room temperature for 2 minutes.
17. Place on the magnetic stand, and wait until the liquid is clear.
18. Being careful not to disturb the bead pellet, transfer 17.5µl of the supernatant to a new PCR plate.

Note: This is a safe stopping point. Seal the plate and store at –25 to –15°C for up to 7 days (based on storage conditions recommended in the TruSeq® DNA PCR-Free Reference Guide, Document# 1000000039279).

A-Tailing Reaction

1. Thaw the A-Tailing Mix at room temperature. Be sure to return it to -20°C after use.
2. Assemble the A-tailing reactions in a 96-well plate as described below:

Component	Volume Per Sample
Purified end-repair product from Step 18 above	17.5 μl
A-Tailing Mix	12.5 μl
total volume	30μl

3. Mix each A-tailing reaction thoroughly by gently pipetting 10 times.
4. Program the thermal cycler with the A-tailing program described below:

A-Tailing Program
Preheat lid to 100°C
37°C for 30 minutes
70°C for 5 minutes
4°C for 5 minutes
4°C hold

5. Seal the plate with strip caps, and place the plate in the thermal cycler. Run the program, and remove the plate from the thermal cycler.

Adaptor Ligation Reactions

1. Leave the Resuspension Buffer and Sample Purification Beads out for 30 minutes to equilibrate to room temperature.
2. Thaw the Ligation Mix 2 at room temperature. If the Ligation Mix 2 will not be used immediately, store it on ice. (Return to -20°C immediately after use.)
3. Thaw the DNA Adapters in the IDT for Illumina TruSeq[®] DNA UD Index Plate at room temperature for 10 minutes.
4. Briefly centrifuge the IDT for Illumina TruSeq[®] DNA UD Index Plate to bring the contents to the bottom of the wells.
5. Pierce the foil seal on the IDT for Illumina TruSeq[®] DNA UD Index Plate with the pipette tips to assemble the adaptor ligation reactions described in the following step. (Return IDT for Illumina TruSeq[®] DNA UD Index Plate to -20°C after use for up to four freeze-thaw cycles.) Cover the used wells with an adhesive seal.

5.B. Preparing the DNA Library with the Illumina TruSeq® DNA PCR-Free High-Throughput Library Prep Kit (continued)

6. Assemble the adapter ligation reactions in the same plate and wells used for the A-tailing reaction as shown below:

Component	Volume Per Sample
A-Tailing Reaction (from Step 5 above)	30µl
Resuspension Buffer	2.5µl
Ligation Mix 2	2.5µl
DNA Adapter (from the IDT for Illumina TruSeq® DNA UD Index Plate)	2.5µl
total volume	37.5µl

Note: If fewer than 16 libraries will be sequenced simultaneously, please refer to the Illumina Index Adapters Pooling Guide Document# 1000000041074 for strategies to select compatible adapters.

7. Mix each adapter ligation reaction thoroughly by gently pipetting 10 times.
 8. Program the thermal cycler with the adaptor ligation program described below:

Adapter Ligation Program
Preheat lid to 100°C
30°C for 20 minutes
4°C hold

9. Seal the plate with strip caps, and place the plate in the thermal cycler. Run the program, and remove the plate from the thermal cycler.
 10. Thaw the Stop Ligation Buffer at room temperature.
 11. Add 5µl of Stop Ligation Buffer to each well. The final volume in each well is 42.5µl.

Initial Purification of Adapter-Ligated Products

Purify the adapter-ligated products (libraries) using the Sample Purification Beads provided in the Illumina TruSeq® DNA PCR-Free High Throughput Library Preparation Kit.

1. If the Sample Purification Beads are not already at room temperature, equilibrate for 30 minutes at room temperature.
2. Prepare fresh 80% ethanol (if needed).
3. Vortex the beads for at least 1 minute until well dispersed.
4. Add 48µl of the Sample Purification beads to each well of the plate containing ligation products (1.13X ratio to the end repair reaction volume). Vortex the beads frequently to make sure that the beads are evenly distributed to the samples.
5. Mix thoroughly by gently pipetting 10 times using a 200µl single-channel or multichannel pipette set to 80µl.
Note: Optimal yield and balanced representation of all STR loci is dependent on thorough mixing of the beads and samples.
6. Incubate at room temperature for 5 minutes.
7. Place the plate on a magnetic stand at room temperature for approximately 5 minutes.
8. Remove and discard the supernatant from each well.
9. With the plate on the magnetic stand, wash two times with 80% ethanol as follows:
 - a. Add 200µl of the freshly prepared 80% ethanol to each well.
 - b. Incubate on the magnetic stand for 30 seconds.
 - c. Remove and discard the supernatant from each well, taking care not to disturb the bead pellet.
 - d. Repeat Steps 9.a–c for the second wash.
10. Use a 20µl pipette to remove residual ethanol from each well.
11. Air-dry the samples on the magnetic stand for 5 minutes.
12. Add 52.5µl of the Resuspension Buffer to each well.
13. Remove from the magnetic stand.
14. Mix thoroughly by gently pipetting 10 times.
15. Incubate at room temperature for 2 minutes.
16. Place the plate on the magnetic stand, and wait until the liquid is clear.
17. Being careful not to disturb the bead pellet, transfer 50µl of the supernatant to a new 96-well plate.

5.B. Preparing the DNA Library with the Illumina TruSeq® DNA PCR-Free High-Throughput Library Prep Kit (continued)

Second Purification of Adapter-Ligated Products

Perform a second purification the adapter-ligated products (libraries) as follows:

1. Vortex the beads for at least 1 minute until they are well dispersed.
2. Add 55µl of the Sample Purification beads to each well of the plate containing ligation products (1.1X ratio to the end repair reaction volume). Vortex the beads frequently to make sure that the beads are evenly distributed to the samples.
3. Mix thoroughly by gently pipetting 10 times using a 200µl single-channel or multichannel pipette set to 80µl.
Note: Optimal yield and balanced representation of all STR loci is dependent on thorough mixing of the beads and samples.
4. Incubate at room temperature for 5 minutes.
5. Place the plate on a magnetic stand at room temperature for approximately 5 minutes.
6. Remove and discard the supernatant from each well.
7. Wash 2 times with 80% ethanol as follows:
 - a. Add 200µl of the freshly prepared 80% ethanol to each well.
 - b. Incubate the plate on the magnetic stand for 30 seconds.
 - c. Remove and discard the supernatant from each well, taking care not to disturb the bead pellet.
 - d. Repeat Steps 7.a–c for the second wash.
8. Use a 20µl pipette to remove residual ethanol from each well.
9. Air-dry the samples on the magnetic stand for 5 minutes.
10. Add 22.5µl of the Resuspension Buffer to each well.
11. Remove from the magnetic stand.
12. Mix thoroughly by gently pipetting 10 times.
13. Incubate at room temperature for 2 minutes.
14. Place the plate on the magnetic stand, and wait until the liquid is clear.
15. Being careful not to disturb the bead pellet, transfer 20µl of the supernatant to a new PCR plate.
Note: This is a safe stopping point. Seal the plate and store at –25°C to –15°C for up to 7 days (based on storage conditions recommended in the TruSeq® DNA PCR-Free Reference Guide, Document# 1000000039279).

5.C. Quantify and Normalize Libraries

1. For optimal results from your sequencing run we strongly recommend quantifying the libraries using a qPCR-based quantification kit for Illumina libraries. We recommend the PowerSeq® Quant MS System. Follow the qPCR instructions in the PowerSeq® Quant MS System protocol provided with the kit.
Note: We recommend testing each library in triplicate. The reagents provided in the kit are sufficient for testing 100 samples in triplicate.
2. If the PowerSeq® Quant MS System is used for quantification, dilute the PowerSeq® 46GY libraries 1:10,000.
 - a. Dilute the PowerSeq® System DNA libraries 1:100 by carefully adding 2µl of the undiluted library to 198µl of the PowerSeq® Quant MS 1X Dilution Buffer.
 - b. Mix the dilution thoroughly by gently pipetting 10 times using a 200µl single-channel or multichannel pipette set to 198µl.
 - c. Dilute the PowerSeq® System DNA libraries 1:10,000 by carefully adding 2µl of the 1:100 dilution (from Step 2.a) to 198µl of the PowerSeq® Quant MS 1X Dilution Buffer.
 - d. Mix the dilution thoroughly by pipetting 10 times.
3. Based on the DNA quantification results obtained, normalize the DNA library concentrations for pooling. In order to allow a final pool concentration of 4nM when accounting for low concentration samples or NTCs we recommend using the following procedure to set the dilution target for libraries requiring normalization:
 - a. Count the total number of libraries, including negative controls, that you plan to sequence.
 - b. Count the number of libraries in the pool with concentration greater than 1nM, including positive controls.
 - c. Calculate the dilution target concentration using the following equation:

$$\text{Adjusted Dilution Target for Normalized Libraries} = \frac{\text{Total Number of Libraries in Pool}}{\text{Number of Libraries Greater than 1nM in Pool}} \times 4\text{nM}$$

- d. Dilute each library with Resuspension Buffer or 10mM Tris-HCl (pH 8.5) to the adjusted dilution target.
 - e. Libraries with concentrations below the dilution target are not diluted prior to addition to the pool. Dilute NTC reactions in the same volumes as the lowest concentration sample library, or pool undiluted if the lowest concentration sample library is below the adjusted dilution target.
4. Pool equal volumes of the diluted libraries prepared in Step 3. Vortex the pooled libraries for 10 seconds to mix.

5.D. Denature and Dilute Libraries for Sequencing

Denature and dilute libraries following the protocol provided. For additional information, refer to the MiSeq® System Denature and Dilute Libraries Guide Document#15039740, v10.

1. Thaw the MiSeq® v3 reagent cartridge following the instructions provided in the MiSeq® System Guide #15027617.
2. Prepare a fresh dilution of 0.2N NaOH. Add 10µl of 2N NaOH to 90µl of Nuclease-Free Water. Dilution should be used within 12 hours. Vortex the dilution to mix thoroughly.
Note: The NaOH stock may concentrate over time. Higher concentrations of NaOH can have deleterious effects on sequencing.
3. Thaw the HT1 hybridization buffer (HT1 buffer) at room temperature. Store the HT1 buffer at +2°C to +8°C until needed to dilute the denatured libraries.
4. Denature the libraries by combining components in a microcentrifuge tube as indicated below:

Component	Volume Per Sample
Pooled 4nM libraries (from Step 4, Section 5.C)	5µl
0.2N NaOH	5µl
total volume	10µl

5. Briefly vortex and incubate for 5 minutes at room temperature.
6. Add 990µl of chilled HT1 buffer to the tube containing the denatured library. Mix by tube inversion or pipetting.
Note: Library concentration is now 20pM.
7. Dilute and denature the PhiX Control v3 by combining components in a microcentrifuge tube as indicated below:

Component	Volume Per Sample
PhiX Control v3 (10nM)	2µl
Resuspension Buffer or Tris-HCl, 10mM (pH 8.5)	3µl
0.2N NaOH	5µl
total volume	10µl

8. Briefly vortex and incubate for 5 minutes at room temperature.
9. Add 990µl of chilled HT1 buffer to the tube containing the denatured PhiX control. Mix by tube inversion or pipetting. **Note:** PhiX Control concentration is now 20pM.

- Prepare the sequencing dilution by combining components in a microcentrifuge tube as indicated below:

Component	Volume Per Sample
HyB Buffer	195µl
20pM pooled and denatured libraries (from Step 6, Section 5.D)	365µl
20pM denatured PhiX Control (from Step 9, Section 5.D)	40µl
total volume	600µl

- Mix the sequencing dilution by tube inversion or pipetting.
Note: If the sequencing dilution will not be immediately added to the cartridge for sequencing, place the dilution on ice until ready to add to the cartridge.

5.E. Instructions for Loading Sample Libraries onto the MiSeq® v3 Reagent Cartridge

- Mix thawed reagent cartridge by inverting 10 times.
- Use a new 1ml pipette tip to pierce the foil seal over the well labeled “Load Samples.”
Note: Do not pierce any other well positions.
- Pipet all 600µl of the prepared sample libraries into the “Load Samples” well. Avoid touching the seal while dispensing.

5.F. Creating a Sample Plate and a Sample Sheet in Illumina Experiment Manager

Create a sample plate and a sample sheet using the protocol provided. For additional information, refer to the Illumina Experiment Manager User Guide, Part# 15031335.

- Open the Illumina Experiment Manager software (v1.18.1).
- Select the Create Sample Plate option.
- On the ‘Index Adapters Selection’ screen, select **IDT-ILMN TruSeq DNA UD Indexes** (96 Indexes), followed by **Next**.
- On the ‘Plate Parameters’ screen, type a plate name in the ‘Unique Plate Name’ field.
- Select the number **2** in the ‘Index Reads’ field for the indexes for the samples on this plate, followed by **Next**.
- In the ‘Table’ tab of the ‘Plate Samples’ screen, enter a Sample ID for each sample.
- In the ‘Index Well’ column, select the appropriate index for each Sample ID based on the IDT for Illumina TruSeq UD Indexes (96 Indexes, 96 Samples) PlateLayout.
- Select **Finish**, and save the plate file in the desired folder.
- Select **Create Sample Sheet** to open the Sample Sheet Wizard.
- On the ‘Instrument Selection’ screen, select **MiSeq** followed by **Next**.

5.F. Creating a Sample Plate and a Sample Sheet in Illumina Experiment Manager (continued)

11. On the 'MiSeq Application Selection' screen, select **Other** and **FASTQ Only** followed by **Next**.
12. On the 'Workflow Parameters' screen, make the following selections in the 'FASTQ Only Run Settings':
 - a. Enter the barcode number of the MiSeq® v3 reagent cartridge in the 'Reagent Cartridge Barcode' field.
 - b. Select **TruSeq DNA PCR-Free** from the drop-down menu for 'Library Prep Workflow.'
 - c. Select **IDT-ILMN TruSeq DNA UD Indexes (96 Indexes)** from the drop-down menu for 'Index Adapters.'
 - d. Select **2** for the number of Index Reads.
 - e. If desired, enter an Experiment Name.
 - f. If desired, enter an Investigator Name.
 - g. Select the appropriate date.
 - h. For the *Read Type* select either:
 - i. **Paired End** and enter 301 in the fields 'Cycles Read 1' and 'Cycles Read 2', **or**
 - ii. **Single Read** and enter 301 in the field 'Cycles Read 1'.

Note: We do not recommend reducing the number of cycles to less than 301 because fewer cycles could lead to incomplete sequencing of some STRs.
13. On the 'Workflow Parameters' screen in the FASTQ Only Workflow-Specific Setting Section, ensure that only **Use Adapter Trimming** and **Use Adapter Trimming Read 2** are selected.
14. Select **Next**.
15. On the 'Sample Selection' screen, make the following selections:.
 - a. In the 'Sample Plate' section, choose **Select Plate**, and navigate to the sample plate file created in Step 9.
 - b. Select **Open**.
 - c. Choose **Select All** followed by **Add Selected Samples**. The samples appear in the 'Samples to include in sample sheet' section.
16. Select **Finish**.

5.G. Starting a MiSeq Sequencing Run

1. Perform DNA sequencing using the MiSeq® Reagent Kit v3 and MiSeq® Reagent Kit v3 Cartridge as directed by the manufacturer:

For the MiSeq® instruments, refer to the MiSeq® System Guide, Document# 15027617 v05, Material# 20000262, Cat.# SY-411-9001DOC, August 2019.

For the MiSeq FGx™, refer to the MiSeq FGx™ Instrument Reference Guide, Part# 15050524 Rev. C, Cat.# SY-411-9001DOC, February 2015.

6. Sequencing Analysis

Analyze with commercial software such as SoftGenetics GeneMarker®HTS software.

6.A. Instructions for Analysis with GeneMarker®HTS Software from SoftGenetics

1. Open the SoftGenetics GeneMarker®HTS software.
2. Under “Project Folder:” navigate to the folder where the analysis results are to be exported.
3. Under “Reference Path:” and “Motif Path:” default boxes can be checked.
4. Under the “Panel:” pulldown menu select **Promega_PowerSeq_46GY**.
5. At the bottom of the screen select **Add** and navigate to the folder where the FASTQ files to be analyzed are located. Select all of the FASTQ files to be analyzed.
6. On the right hand side under “Sequencer:” make sure “Illumina” is marked.
7. Under “Filter Settings” default settings are acceptable. Internal validation should be done to establish thresholds.
8. Select **OK** to begin analysis.
9. When the analysis is complete for each file, the file name font color will change from gray to black.
10. Select each file in the ‘Project Viewer’ one at a time. The file will open in the STR Results window showing histograms of alleles at each locus and a table at the bottom.
11. Filter settings may be adjusted for each sample separately.
12. Select **Export** from the top menu. In the window that pops up, navigate to the folder where the “.csv” files are to be saved and save the file.

6.B. DYS393 Artifact

The forward primer for the DYS393 locus extends into the repeat structure and uses a degenerate base (A,C) at the first position in the repeat. Do not interpret this single nucleotide variant as a true sequence variant.

7. References

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8. Troubleshooting

8.A. Amplification and Library Detection

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com E-mail: genetic@promega.com

Symptoms	Possible Causes and Comments
Low coverage	<p>The PowerSeq® 5X Master Mix was not mixed well before use. Mix the 5X Master Mix using a vortex mixer for 15 seconds before dispensing into the PCR amplification mix.</p> <p>An air bubble formed at the bottom of the reaction tube or plate. Use a pipette to remove the air bubble or centrifuge the reactions briefly before thermal cycling.</p> <p>Thermal cycler, plate or tube problems. Review the thermal cycling protocol in Section 4. We have not tested other reaction tubes, plates or cyclers. Calibrate the thermal cycler heating block if necessary.</p> <p>Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerSeq® 46GY 5X Primer Pair Mix for 15 seconds before use.</p> <p>Samples were not denatured completely. Denature samples with 0.2N NaOH and incubate for 5 minutes at room temperature.</p> <p>Do not vortex the libraries once they are single-stranded.</p>
Low or no coverage for the positive control reaction	<p>Improper storage of the 2800M Control DNA. Ensure that the Preparing and Plating protocol is strictly followed.</p>
Extra alleles detected in multiple loci	<p>Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.</p>
Imbalanced locus-to-locus coverage	<p>Miscellaneous balance problems. At first use, thaw the 5X Primer Pair Mix and 5X Master Mix completely. Vortex the 5X Primer Pair Mix and 5X Master Mix for 15 seconds before use; do not centrifuge the 5X Primer Pair Mix and 5X Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely.</p> <p>PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.</p> <p>Instructions for library preparation were not followed. Ensure that each step of the workflow was performed as described.</p> <p>Purification steps with the Sample Purification Beads were not adequately mixed, or the ratio of beads was incorrect.</p>

8.B. Amplification of Extracted DNA

The following information is specific to amplification of extracted DNA. For information about general amplification and detection, see Section 8.A.

Symptoms

Low coverage

Possible Causes and Comments

Impure template DNA. Because a small amount of template is used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample.

Insufficient template. Use the recommended amount of template DNA if available.

High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+ , Na^+ , Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE⁻⁴ buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA), TE⁻⁴ buffer with 20 μ g/ml glycogen or Nuclease-Free Water. Faint or absent peaks may be seen more often when using the maximum template volume or reduced amplification reaction volume.

The reaction volume was too low. This system is optimized for a final reaction volume of 25 μ l. Decreasing the reaction volume may result in suboptimal performance.

Extra alleles detected in multiple loci

Artifacts of STR amplification. Amplification of excess amounts of purified DNA can result in a higher number of artifact peaks. Use the recommended amount of template DNA. Amplification of excess amounts of purified DNA also may result in over-amplification.

Imbalanced locus-to-locus coverage

Excessive amount of DNA. Amplification of >1ng of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles.

Degraded DNA sample. DNA template was degraded, and larger loci showed diminished reads.

The reaction volume was too low. This system is optimized for a final reaction volume of 25 μ l to overcome inhibitors present in DNA samples. Decreasing the reaction volume can result in suboptimal performance.

8.B. Amplification of Extracted DNA (continued)

Symptoms	Possible Causes and Comments
Imbalanced locus-to-locus coverage (continued)	<p>Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance. Imbalance may be seen more often when using the maximum template volume or a reduced amplification volume.</p> <p>Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.</p>

9. Appendix

9.A. Direct-Amplification Reagents and Cycle Number

	Preprocessing	Additional Reagent During Amplification	Suggested Cycle Number¹
Lytic storage cards	None	5X AmpSolution™ Reagent (Cat.# DM1231)	26 cycles
Swabs	SwabSolution™ Kit (Cat.# DC8271) ²	5X AmpSolution™ Reagent (Cat.# DM1231)	26 cycles
Nonlytic storage cards	PunchSolution™ Kit (Cat.# DC9271) ²	5X AmpSolution™ Reagent (Cat.# DM1231)	26 cycles

¹Internal validation is recommended to optimize cycle number based on sample type.

²Refer to SwabSolution (TMD037) and PunchSolution™ Kit (TMD038) Technical Manuals for preprocessing swabs and nonlytic storage cards and amplification using AmpSolution™ Reagent.

9.B. Locus-Specific Information Tables

Table 1. The PowerSeq® 46GY System Locus-Specific Information.

Locus Name	Chromosomal Location¹	Repeat Sequence² 5'→3'
Amelogenin3	Xp22.1–22.3 and Y	N.A.
CSF1PO	5q33.1 (149.436Mb)	AGAT
D10S1248	10q26.3 (130.567Mb)	GGAA
D12S391	12p12 (12.341Mb)	AGAT/AGAC Complex
D13S317	13q31.1 (81.62Mb)	TATC
D16S539	16q24.1 (84.944Mb)	GATA
D18S51	18q21.33 (59.1Mb)	AGAA
D19S433	19q12 (35.109Mb)	AAGG Complex
D1S1656	1q42 (228.972Mb)	TAGA Complex
D21S11	21q21.1 (19.476Mb)	TCTA Complex
D22S1045	22q12.3 (35.779Mb)	ATT
D2S1338	2q35 (218.705Mb)	TGCC/TTCC
D2S441	2p14 (68.214Mb)	TCTA
D3S1358	3p21.31 (45.557Mb)	TCTA Complex
D5S818	5q23.2 (123.139Mb)	AGAT
D7S820	7q21.11 (83.433Mb)	GATA
D8S1179	8q24.13 (125.976Mb)	TCTA Complex
DYS19	Y	TAGA
DYS385a/b	Y	GAAA
DYS389I/II	Y	(TCTG) (TCTA)
DYS390	Y	(TCTA) (TCTG)
DYS391	Y	TCTA
DYS392	Y	TAT
DYS393	Y	AGAT
DYS437	Y	TCTA
DYS438	Y	TTTTTC
DYS439	Y	AGAT
DYS448	Y	AGAGAT

(Table continued next page.)

9.B. Locus-Specific Information Tables (continued)

Table 1. The PowerSeq® 46GY System Locus-Specific Information (continued).

Locus Name	Chromosomal Location¹	Repeat Sequence² 5'→3'
DYS456	Y	AGAT
DYS458	Y	GAAA
DYS481	Y	CTT
DYS533	Y	ATCT
DYS549	Y	GATA
DYS570	Y	TTTC
DYS576	Y	AAAG
DYS635	Y	TSTA compound
DYS643	Y	CTTTT
FGA	4q28 (155.866Mb)	TTTC Complex
Penta D	21q22.3 (43.88Mb)	AAAGA
Penta E	15q26.2 (95.175Mb)	AAAGA
TH01	11p15.5 (2.149Mb)	AATG
TPOX	2p25.3 (1.472Mb)	AATG
vWA	12p13.31 (5.963Mb)	TCTA Complex
Y-GATA-H4	Y	TAGA

¹Information about the chromosomal location of these loci can be found in references 28, 29 and 30 and at:

<https://strbase.nist.gov/chrom.htm>

²The August 1997 report (31,32) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, “1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used”.

³Amelogenin is not an STR.

N.A. = Not applicable

Table 2. The PowerSeq® 46GY System Allele Determinations for 2800M Control DNA.

Locus Name	2800M	Locus Name	2800M
Amelogenin	X,Y	DYS393	13
CSF1PO	12, 12	DYS437	14
D10S1248	13, 15	DYS438	9
D12S391	18, 23	DYS439	12
D13S317	9, 11	DYS448	19
D16S539	9, 13	DYS456	17
D18S51	16, 18	DYS458	17
D19S433	13, 14	DYS481	22
D1S1656	12, 13	DYS533	12
D21S11	29, 31.2	DYS549	13
D22S1045	16, 16	DYS570	17
D2S1338	22, 25	DYS576	18
D2S441	10, 14	DYS635	21
D3S1358	17, 18	DYS643	10
D5S818	12, 12 ¹	FGA	20, 23
D7S820	8, 11	Penta D	12, 13
D8S1179	14, 15	Penta E	7, 14
DYS19	14	TH01	6, 9.3
DYS385a/b	13, 16	TPOX	11, 11
DYS389I	14	vWA	16, 19
DYS389II	31	Y-GATA-H4	11
DYS390	24		
DYS391	10		
DYS392	13		

¹Flanking region in one allele contains A→G variant at position chr5:123775612 (GRCh38.p12). SNP reference ID rs25768.



9.C. Related Products

Product	Size	Cat.#
PowerQuant® System	200 reactions	PQ5002
	800 reactions	PQ5008
Plexor® HY System	200 reactions	DC1001
	800 reactions	DC1000
PowerPlex® Fusion System	200 reactions	DC2402
	800 reactions	DC2408
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320

Not for Medical Diagnostic Use.

Accessory Products, Reagents

Product	Size	Cat.#
PowerSeq® Quant MS System	500 reactions	PS5000
CXR Reference Dye	100µl	C5411
QuantiFluor® dsDNA System	1ml	E2670
	100µl	E2671
QuantiFluor® ONE dsDNA System	100 reactions	E4871
	500 reactions	E4870
PunchSolution™ Kit*	100 preps	DC9271
SwabSolution™ Kit*	100 preps	DC8271
5X AmpSolution™ Reagent*	100 preps	DM1231
2800M Control DNA (10ng/µl)*	25µl	DD7101
2800M Control DNA (0.25ng/µl)*	500µl	DD7251
Water, Amplification Grade*	6,250µl	DW0991
Tris-HCl, Molecular Biology Grade (Tris-Hydrochloride)	100g	H5121
	500g	H5123
	2,500g	H5125
Proteinase K (Lyophilized)	100mg	V3021
Proteinase K (PK) Solution	4ml	MC5005
Nuclease-Free Water	50ml	P1193
	150ml	P1195
	500ml	P1197

All products in this table (unless otherwise indicated) are For Research Use Only. Not for Use in Diagnostic Procedures.

*Not for Medical Use.

Accessory Products, Instrumentation

Product	Size	Cat.#
Quantus™ Fluorometer	1 each	E6150
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	Fully Loaded	GM3500
	Luminescence or Fluorescence	GM3510

10. Summary of Changes

The following changes were made to the 3/22 revision of this Technical Manual:

1. Added Note with web link in Section 5.A.
2. Updated concentration for Step 7 in Section 5.D.
3. Changed [™] to [®] for PowerSeq in the document.
4. Updated cover page.

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