



TECHNICAL MANUAL

# Casework Direct System

Instructions for Use of Products  
**DC4560 and DC4561**

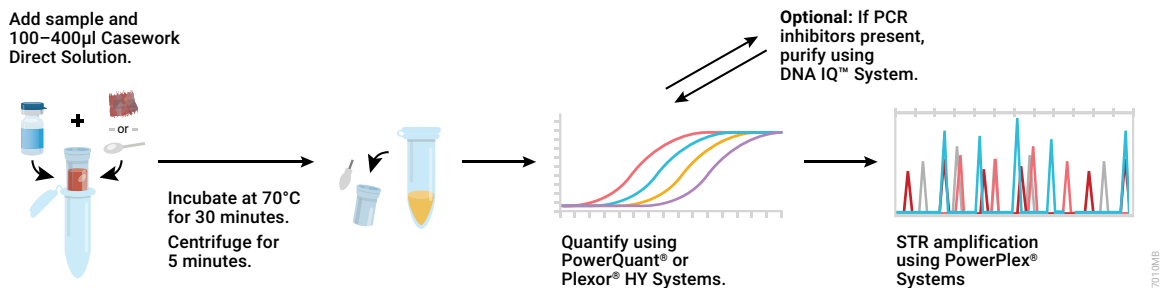
# Casework Direct System

All technical literature is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)  
 Visit the website to verify that you are using the most current version of this Technical Manual.  
 Email Promega Technical Services if you have questions on use of this system: [techserv@promega.com](mailto:techserv@promega.com)

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## 1. Description

The Casework Direct System is used for rapid processing of swabs from casework samples, cuttings of sexual assault swabs or stained clothing prior to quantification of human DNA using either the PowerQuant® System or Plexor® HY System, and amplification of normalized template using PowerPlex® Systems for human STR genotyping. The Casework Direct System generates a lysate with a single incubation step (see Figure 1), significantly reducing sample processing time and manipulations of the sample. For casework samples, no subsequent purification of the Casework Direct Solution Lysate is required prior to STR amplification unless internal PCR control (IPC) data from the PowerQuant® System or Plexor® HY System indicate the presence of possible PCR inhibitors. In this case, sample cleanup can be performed using the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) on the Maxwell® FSC Instrument (Cat.# AS4600) or the Maxwell® RSC 48 Instrument (Cat.# AS8500). Sample processing decisions in Y-screening applications are facilitated by the Y quantification result and [Auto]/[Y] ratio. If desired, the Casework Direct Solution Lysate can be used directly in STR amplification (e.g., for amplification with the PowerPlex® Y23 System if the [Auto]/[Y] ratio indicates very low level male contributor DNA).



**Figure 1. The Casework Direct System workflow.**

The Casework Direct System contains Casework Direct Reagent and 1-Thioglycerol as a reducing agent. In addition, the Casework Direct System contains 5X AmpSolution™ Reagent, which must be added to Plexor® HY System amplification reactions and with certain PowerPlex® Systems depending on sample volume (see Table 1). A developmental validation study on the use of Casework Direct System, Custom, with forensic casework samples was performed and the results are documented in a white paper (1).

**Note:** The formulation of Casework Direct System reagents is identical to the formulation for Casework Direct Kit, Custom, reagents. The developmental validation study (1) with the Casework Direct Kit, Custom (Cat.# AX4560), is therefore applicable to the Casework Direct System (Cat.# DC4560, DC4561).

**Table 1. 5X AmpSolution™ Reagent Requirements for Casework Direct Solution Lysate.**

| <b>STR System</b>                | <b>Maximum Sample Volume Available</b> | <b>Maximum Sample Volume in Absence of 5X AmpSolution™ Reagent</b> | <b>Maximum Sample Volume in Presence of 5X AmpSolution™ Reagent</b> | <b>Volume of 5X AmpSolution™ Reagent</b> |
|----------------------------------|--|--|---|--|
| PowerPlex® ESX Fast and ESI Fast | 17.5µl                                 | N.A.   | 15µl  | 2.5µl                                    |
| PowerPlex® Fusion                | 15µl                                   | 15µl   | N.A. <sup>1</sup>   | N.A.                                     |
| PowerPlex® Fusion 6C             | 15µl                                   | 15µl   | N.A. <sup>1</sup>   | N.A.                                     |
| PowerPlex® Y23 <sup>2</sup>      | 17.5µl                                 | 15µl   | 16.5µl <sup>1</sup>   | N.A.                                     |
| PowerPlex® 21                    | 15µl                                   | 15µl   | N.A. <sup>1</sup>   | N.A.                                     |
| PowerPlex® 35GY                  | 15µl                                   | 15µl   | N.A. <sup>1</sup>   | N.A.                                     |
| PowerPlex® 18E                   | 17.5µl                                 | 17.5µl   | N.A. <sup>1</sup>   | N.A.                                     |

<sup>1</sup>In some cases partial inhibition may be seen with a maximal sample volume of 15µl. This is manifested by increased baseline noise and reduced heights for some larger amplicons. This can be mitigated by adding 1µl of 5X AmpSolution™ Reagent to the 25µl PowerPlex® amplification reaction and reducing the maximum sample volume to 14µl.

<sup>2</sup>For the PowerPlex® Y23 System, you can add sample volumes of 17.5µl to the standard 25µl amplification reaction. However, if using Casework Direct Solution Lysate, you can add up to 15µl of sample. As with the other PowerPlex® Systems mentioned above, partial inhibition may be seen with 15µl of Casework Direct Solution Lysate, which can be mitigated by adding 1µl of 5X AmpSolution™ Reagent to the 25µl PowerPlex® amplification reaction, reducing the sample volume to 14µl. However, due to the protective effect provided by including 1µl of 5X AmpSolution™ Reagent, you can increase the maximum sample volume to 16.5µl.

N.A. = Not applicable.

## 2. Product Components and Storage Conditions

| PRODUCT                       | SIZE         | CAT. #        |
|-------------------------------|--------------|---------------|
| <b>Casework Direct System</b> | <b>100ml</b> | <b>DC4560</b> |

Not for Medical Diagnostic Use. Each kit contains sufficient reagents for 250–1,000 assays depending on volume of reagent used per assay. Includes:

- 100ml Casework Direct Reagent
- 75µl 1-Thioglycerol
- 500µl 5X AmpSolution™ Reagent
- 5 × 1,250µl Water, Amplification Grade

| PRODUCT                       | SIZE        | CAT. #        |
|-------------------------------|-------------|---------------|
| <b>Casework Direct System</b> | <b>10ml</b> | <b>DC4561</b> |

Not for Medical Diagnostic Use. Each kit contains sufficient reagents for 25–100 assays depending on volume of reagent used per assay. Includes:

- 10ml Casework Direct Reagent
- 75µl 1-Thioglycerol
- 500µl 5X AmpSolution™ Reagent
- 2 × 1,250µl Water, Amplification Grade

**Storage Conditions:** Upon arrival, thaw the Casework Direct Reagent completely by placing in a 4°C refrigerator overnight. After thawing, mix thoroughly by gentle inversion and store at +2°C to +10°C. Thaw the 1-Thioglycerol and Water, Amplification Grade, and store at +2°C to +10°C. Thaw the 5X AmpSolution™ Reagent completely (in a 37°C water bath or at ambient temperature), mix by vortexing and store at +2°C to +10°C.

### Notes:

- **Do not** store reagents in the refrigerator door where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.
- The 5X AmpSolution™ Reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the reagent briefly at 37°C, then vortex until clear.

### **3. Processing Swabs and Cuttings from Stained Clothing**

#### **Materials to Be Supplied by the User**

- ClickFit Microtubes (Cat.# V4745)
- CW Spin Baskets (Cat.# AS8101)
- aerosol-resistant pipette tips
- heat block that accommodates 1.5ml tubes and settings of 70°C

#### **Notes:**

- We recommend use of the ClickFit Microtube, 1.5ml (Cat.# V4745), to ensure that the caps will not open during the 70°C incubation.
- Do not use an incubator to incubate tubes. Heat transfer is inefficient and will result in poor performance. Only use a heat block to maintain efficient heat transfer. For long-term storage of samples, we recommend the use of ClickFit tubes.

### 3.A. Preparing Casework Direct Solution

1. Dilute 1-Thioglycerol tenfold in Water, Amplification Grade (for example, add 30µl 1-Thioglycerol to 270µl Water, Amplification Grade). Vortex tube for 10–15 seconds. The diluted 1-Thioglycerol is stable at 4°C for 6 months. **Note:** 1-Thioglycerol is viscous. To facilitate accurate pipetting, warm to room temperature, pipette slowly and avoid pipetting small volumes.
2. Add diluted 1-Thioglycerol to Casework Direct Reagent to make Casework Direct Solution as shown in Table 2.

**Table 2. Casework Direct Solution Mix.**

| Mix Component                  | Volume Per Extraction | × | Number of Extractions | = | Final Volume |
|--------------------------------|-----------------------|---|-----------------------|---|--------------|
| Casework Direct Reagent        | 400µl                 | × |                       | = |              |
| 1-Thioglycerol diluted tenfold | 2µl                   | × |                       | = |              |
|                                |                       |   |                       |   |              |
| Mix Component                  | Volume Per Extraction | × | Number of Extractions | = | Final Volume |
| Casework Direct Reagent        | 300µl                 | × |                       | = |              |
| 1-Thioglycerol diluted tenfold | 1.5µl                 | × |                       | = |              |
|                                |                       |   |                       |   |              |
| Mix Component                  | Volume Per Extraction | × | Number of Extractions | = | Final Volume |
| Casework Direct Reagent        | 200µl                 | × |                       | = |              |
| 1-Thioglycerol diluted tenfold | 1µl                   | × |                       | = |              |
|                                |                       |   |                       |   |              |
| Mix Component                  | Volume Per Extraction | × | Number of Extractions | = | Final Volume |
| Casework Direct Reagent        | 100µl                 | × |                       | = |              |
| 1-Thioglycerol diluted tenfold | 0.5µl                 | × |                       | = |              |

**Note:** The volume of Casework Direct Solution Mix used per sample depends on swab/cutting size. We have successfully tested volumes from 100–400µl. Larger swabs or cuttings require larger volumes of Casework Direct Solution Mix to completely submerge the swab/cutting. **Do not** exceed 400µl; using volumes less than 400µl may compromise lysate recovery from the CW Spin Basket. See Section 8, Troubleshooting for additional information.

### **3.B. Processing of Swab or Stained Clothing Cutting**

1. Set a heat block capable of accepting 1.5ml tubes to 70°C. The heat block must reach 70°C prior to the incubation in Step 5.
2. Place the CW Spin Basket inside a ClickFit Microtube, 1.5ml.
3. Place swab head, cutting of swab, cutting of a sexual assault swab or fabric cutting inside the CW Spin Basket.
4. Add 100–400µl of Casework Direct Solution to each swab head or cutting. Close the lid of the CW Spin Basket/ ClickFit Microtube assembly. Vortex the tube for 5–10 seconds.
5. Place the tube in heat block, and incubate sample at 70°C for 30 minutes.
6. Vortex for 5–10 seconds and then centrifuge at room temperature for 5 minutes at maximum speed in a microcentrifuge. Carefully remove the CW Spin Basket containing the swab. Close the tube.

### **4. Quantification of Human DNA with PowerQuant® System**

The amount of human genomic DNA present in the lysate can be quantitated using the PowerQuant® System. Adding 5X AmpSolution™ Reagent to PowerQuant® amplification reactions is not required for quantification using the PowerQuant® System. Follow the protocol in the *PowerQuant® System Technical Manual #TMD047*, without any changes, using 2µl of lysate per 20µl PowerQuant® System amplification reaction. In addition, the quality of the human DNA in these lysates may be evaluated using the PowerQuant® data. The ratio of the autosomal-to-degradation target quantification results can indicate whether or not the human DNA is degraded, whereas the ratio of the autosomal-to-Y target quantification results can provide information on the ratio of female-to-male DNA in the sample. The performance of the PowerQuant® IPC target can indicate whether PCR inhibitors are present, which are likely to inhibit downstream STR amplification.

If inhibitors are indicated, we recommend that the lysate be cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit following the protocol for liquid samples in the *Maxwell® FSC DNA IQ Casework Kit Technical Manual #TM499* (Section 3.B, Step 4). Use up to a maximum of 400µl of lysate per purification.

**Note:** If the IPC target is flagged in a sample using the PowerQuant® System, then the DNA quantification value will be unreliable in downstream applications. If inhibitors are indicated, then the quantification will likely be an underestimation of the true DNA amount present in the lysate. Samples can be diluted (e.g., tenfold dilution) and requantified to determine a more accurate DNA quantification (i.e., if IPC indicates no inhibition). However, if PCR inhibitors are present in the sample, then sample purification is recommended prior to requantification and STR amplification. Sample workflow will need to be optimized in each laboratory.



## 5. Quantification of Human DNA with Plexor® HY System

The Plexor® HY System requires 5X AmpSolution™ Reagent to tolerate 2µl of lysate per 20µl Plexor® HY System amplification reaction (Table 3). In addition to quantification, the ratio of the autosomal-to-Y target quantification results can provide information on the ratio of female-to-male DNA in the sample, whereas the Plexor® HY IPC target can indicate whether or not PCR inhibitors are present that are likely to inhibit downstream STR amplification.

If inhibitors are indicated, we recommend that the lysates be cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit following the protocol for liquid samples in the *Maxwell® FSC DNA IQ Casework Kit Technical Manual #TM499* (Section 3.B, Step 4). Use up to a maximum of 400µl of lysate per purification.

**Table 3. PCR Amplification Mix for Plexor® HY System with Casework Direct Solution Lysate.**

| PCR Amplification Mix Component  | Volume Per Reaction | ×        | Number of Reactions | =        | Final Volume |
|----------------------------------|---------------------|----------|---------------------|----------|--------------|
| Plexor® HY 2X Master Mix         | 10µl                | ×        |                     | =        |              |
| Plexor® HY 20X Primer/IPC Mix    | 1µl                 | ×        |                     | =        |              |
| 5X AmpSolution™ Reagent          | 4µl                 | ×        |                     | =        |              |
| Water, Amplification Grade       | 3µl                 | ×        |                     | =        |              |
| <b>Total Reaction Mix Volume</b> | <b>18µl</b>         | <b>×</b> |                     | <b>=</b> |              |

Add 2µl of Casework Direct Lysate to 18µl of Plexor® HY System amplification reaction mix.

Perform thermal cycling as described in the technical manual for the Plexor® HY System.

**Note:** If the IPC target is flagged in a sample using the Plexor® HY System, then the DNA quantification value will be unreliable in downstream applications. If inhibitors are indicated, then the quantification will likely be an underestimation of the true DNA amount present in the lysate. Samples can be diluted (e.g., tenfold dilution) and requantified to determine a more accurate DNA quantification (i.e., if IPC indicates no inhibition). However, if PCR inhibitors are present in the sample, then sample purification is recommended prior to requantification and STR amplification. Sample workflow will need to be optimized in each laboratory.

## 6. Amplification with PowerPlex® ESI/ESX Fast Systems

The PowerPlex® ESI Fast and ESX Fast Systems require 5X AmpSolution™ Reagent for efficient amplification in the presence of Casework Direct Solution (see Table 4).

**Table 4. PCR Amplification Mix for PowerPlex® ESI Fast and ESX Fast Systems with Casework Direct Solution Lysate.**

| PCR Amplification Mix Component                            | Volume Per Reaction | x        | Number of Reactions | =        | Final Volume |
|--|---------------------|----------|---------------------|----------|--------------|
| PowerPlex® ESI/ESX Fast 5X Master Mix                      | 5µl                 | x        |                     | =        |              |
| PowerPlex® ESI 16/17 or ESX 16/17 Fast 10X Primer Pair Mix | 2.5µl               | x        |                     | =        |              |
| 5X AmpSolution™ Reagent                                    | 2.5µl               | x        |                     | =        |              |
| <b>total reaction mix volume</b>                           | <b>10µl</b>         | <b>x</b> |                     | <b>=</b> |              |

Add up to 15µl of Casework Direct Solution Lysate to 10µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manuals for the PowerPlex® ESI Fast or ESX Fast System, as appropriate.

## 7. Amplification with PowerPlex® Systems Not Requiring 5X AmpSolution™ Reagent

The PowerPlex® Fusion, PowerPlex® Fusion 6C, PowerPlex® 21 and PowerPlex® Y23 Systems as well as the eight-color PowerPlex® chemistries (PowerPlex® 35GY and PowerPlex® 18E Systems) do not require 5X AmpSolution™ Reagent. Set up the reaction for each PowerPlex® System in the same way according to the corresponding technical manual. PowerPlex® Fusion 6C (Table 5) and PowerPlex® Y23 Systems (Table 7) are shown as examples below.

**Note:** In some cases, partial inhibition may be seen with a maximal sample volume of 15µl. This is manifested by increased baseline noise and reduced heights for some larger amplicons. This can be mitigated by adding 1µl of 5X AmpSolution™ Reagent to the 25µl PowerPlex® amplification reaction and reducing the maximum sample volume to 14µl as shown in Tables 6 and 8 (except for the PowerPlex® Y23 System, where the inclusion of 1µl of 5X AmpSolution™ Reagent increases the maximum sample volume to 16.5µl).

**Table 5. PCR Amplification Mix for PowerPlex® Fusion 6C System.**

| PCR Amplification Mix Component         | Volume Per Reaction | x        | Number of Reactions | =        | Final Volume |
|---|---------------------|----------|---------------------|----------|--------------|
| PowerPlex® Fusion 6C 5X Master Mix      | 5µl                 | x        |                     | =        |              |
| PowerPlex® Fusion 6C 5X Primer Pair Mix | 5µl                 | x        |                     | =        |              |
| <b>total reaction mix volume</b>        | <b>10µl</b>         | <b>x</b> |                     | <b>=</b> |              |

Add up to 15µl of Casework Direct Solution Lysate to 10µl of amplification reaction mix. This setup will be the same for PowerPlex® 21 and PowerPlex® Fusion kits, using 10µl amplification reaction mix.

## 7. Amplification with PowerPlex® Systems Not Requiring 5X AmpSolution™ Reagent (continued)

**Table 6. PCR Amplification Mix for PowerPlex® Fusion 6C System with AmpSolution™ Reagent.**

| PCR Amplification Mix Component         | Volume Per Reaction | x        | Number of Reactions | =        | Final Volume |
|---|---------------------|----------|---------------------|----------|--------------|
| PowerPlex® Fusion 6C 5X Master Mix      | 5µl                 | x        |                     | =        |              |
| PowerPlex® Fusion 6C 5X Primer Pair Mix | 5µl                 | x        |                     | =        |              |
| 5X AmpSolution™ Reagent                 | 1µl                 | x        |                     | =        |              |
| <b>total reaction mix volume</b>        | <b>11µl</b>         | <b>x</b> |                     | <b>=</b> |              |

Add up to 14µl of Casework Direct Solution Lysate to 11µl of amplification reaction mix. This setup will be the same for PowerPlex® 21 and PowerPlex® Fusion kits, using 10µl amplification reaction mix.

**Table 7. PCR Amplification Mix for PowerPlex® Y23 System.**

| PCR Amplification Mix Component    | Volume Per Reaction | x        | Number of Reactions | =        | Final Volume |
|------------------------------------|---------------------|----------|---------------------|----------|--------------|
| PowerPlex® Y23 5X Master Mix       | 5µl                 | x        |                     | =        |              |
| PowerPlex® Y23 10X Primer Pair Mix | 2.5µl               | x        |                     | =        |              |
| Water, Amplification Grade         | 2.5µl               | x        |                     | =        |              |
| <b>total reaction mix volume</b>   | <b>10µl</b>         | <b>x</b> |                     | <b>=</b> |              |

Add up to 15µl of Casework Direct Solution Lysate to 10µl of amplification reaction mix.

**Table 8. PCR Amplification Mix for PowerPlex® Y23 System with AmpSolution™ Reagent.**

| PCR Amplification Mix Component    | Volume Per Reaction | x        | Number of Reactions | =        | Final Volume |
|------------------------------------|---------------------|----------|---------------------|----------|--------------|
| PowerPlex® Y23 5X Master Mix       | 5µl                 | x        |                     | =        |              |
| PowerPlex® Y23 10X Primer Pair Mix | 2.5µl               |          |                     | =        |              |
| 5X AmpSolution™ Reagent            | 1µl                 | x        |                     | =        |              |
| <b>total reaction mix volume</b>   | <b>8.5µl</b>        | <b>x</b> |                     | <b>=</b> |              |

Add up to 16.5µl of Casework Direct Solution Lysate to 8.5µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manuals for the PowerPlex® Fusion, PowerPlex® Fusion 6C, PowerPlex® 21 or PowerPlex® Y23 System, as appropriate.

## 8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). Email: [genetic@promega.com](mailto:genetic@promega.com)

| Symptoms   | Possible Causes and Comments   |
|--|--|
| Very high degradation quantification value (e.g., >10,000ng/μl) for lysate with PowerQuant® System resulting in [Auto]/[Deg] ratio of 0.00 | <p>Concentration of 1-Thioglycerol in Casework Direct Solution Mix too high. Results in artificially raised signal for degradation target in PowerQuant® System that crosses amplification threshold prematurely (giving artificially elevated estimate of DNA concentration). Ensure that you are adding tenfold diluted 1-Thioglycerol to Casework Direct Reagent.</p> <p>When diluting 1-Thioglycerol 10-fold with Water, Amplification Grade, pipet large volumes of 1-Thioglycerol to increase accuracy and ensure that no 1-Thioglycerol is stuck to the outside of the pipette tip, thereby increasing the volume of concentrated 1-Thioglycerol added to the Water, Amplification Grade.</p> |
| Liquid remains in the spin basket following centrifugation.  | Centrifuge for an additional 5 minutes. Lysate recovery from the spin basket can be dependent on the weight of the substrate and reagent. Inconsistent lysate recovery has been observed for volumes <400μl and smaller cutting sample sizes.  |
| Increased baseline noise and peak broadening   | Intermittent and partial inhibition in PowerPlex® Fusion, PowerPlex® Fusion 6C, PowerPlex® 21 and PowerPlex® Y23 Systems when using 15μl of Casework Direct Lysates. This can be mitigated by adding 1μl of 5X AmpSolution™ Reagent to the 25μl PowerPlex® amplification reaction, reducing the maximum sample volume to 14μl for PowerPlex® Fusion, PowerPlex® Fusion 6C and PowerPlex® 21 Systems, as shown in Table 6. In the case of PowerPlex® Y23, adding 1μl of 5X AmpSolution™ Reagent to the 25μl amplification reaction allows for a maximum sample volume of 16.5μl, as shown in Table 8.   |

## 9. Reference

1. Graham, E.K. *et al.* (2018) "Developmental Validation of the Casework Direct Kit, Custom: A Method for the Rapid Processing of Casework Samples." Promega Corporation.

## 10. Related Products

| PRODUCT                           | SIZE          | CAT. # |
|-----------------------------------|---------------|--------|
| Maxwell FSC instrument            | 1 each        | AS4600 |
| Maxwell RSC 48 instrument         | 1 each        | AS4800 |
| Maxwell® FSC DNA IQ™ Casework Kit | 48 preps      | AS1550 |
| PowerPlex® 35GY System*           | 200 reactions | DC3520 |
| PowerPlex® 18E System*            | 200 reactions | DC3620 |
| PowerPlex® Fusion 6C System*      | 50 reactions  | DC2705 |
|                                   | 200 reactions | DC2720 |
| PowerPlex® Fusion System*         | 200 reactions | DC2402 |
|                                   | 800 reactions | DC2408 |
| PowerPlex® ESX 17 Fast System*    | 100 reactions | DC1711 |
|                                   | 400 reactions | DC1710 |
| PowerPlex® ESI 17 Fast System*    | 100 reactions | DC1721 |
|                                   | 400 reactions | DC1720 |
| PowerPlex® ESX 16 Fast System*    | 100 reactions | DC1611 |
|                                   | 400 reactions | DC1610 |
| PowerPlex® ESI 16 Fast System*    | 100 reactions | DC1621 |
|                                   | 400 reactions | DC1620 |
| Plexor® HY System*                | 200 reactions | DC1001 |
|                                   | 800 reactions | DC1000 |
| ClickFit Microtube, 1.5ml         | 100/pack      | V4745  |

\*Not for Medical Diagnostic Use.

## 11. Summary of Changes

The following changes were made to the 1/25 revision of this document:

1. Updated Section 3.A heading.
2. Added PowerPlex® 18E and PowerPlex® 35GY Systems to Table 1 and Section 10.
3. Edited list of PowerPlex® Systems in Section 7.
4. Removed mention of the DNA IQ™ Casework Pro Kit for Maxwell® 16.
5. Updated the cover page and document font.

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