TECHNICAL BULLETIN

PowerPlex® 16 and PowerPlex® ES Monoplex Systems

Instructions for Use of Products
DC6551, DC6561, DC6571, DC6581, DC6591, DC6601, DC6611, DC6621, DC6631, DC6641, DC6651, DC6661, DC6671, DC6681, DC6691 and DC6751

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PowerPlex® 16 and PowerPlex® ES Monoplex Systems

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 Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The PowerPlex® 16 and PowerPlex® ES Monoplex Systems contain primer pairs that have the same sequence as those used in the PowerPlex® 16 (Cat.# DC6531), PowerPlex® 16 BIO (Cat.# DC6541), PowerPlex® ESX 16 Fast System (Cat.# DC1611), PowerPlex® ESI 16 Fast System (Cat.# DC1621), PowerPlex® ESX 17 Fast System (Cat.# DC1711) and PowerPlex® ESI 17 Fast System (Cat.# DC1721). Refer to the *PowerPlex® 16 System Technical Manual* #TMD012, for locus-specific information, allele size range, allelic ladder components and genotypes of commonly used standard DNA templates. Refer to the *PowerPlex® ESX 17 Fast System Technical Manual* #TMD040 for SE33 locus-specific information. Technical Manuals are available upon request or at: **www.promega.com/protocols/**

The PowerPlex® 16 and PowerPlex® ES Monoplex Systems were developed for human identification applications including forensic analysis, relationship testing and research use.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerPlex® 16 Monoplex System, Penta E (Fluorescein)[a,b]	**100 reactions**	**DC6591**
PowerPlex® 16 Monoplex System, Penta D (JOE)[a,b]	**100 reactions**	**DC6651**
PowerPlex® ES Monoplex System, SE33 (JOE)[a]	**100 reactions**	**DC6751**

Not For Medical Diagnostic Use. Each system includes:

- 150µl Internal Lane Standard
- 300µl Gold ST★R Buffer
- 250µl 10X Primer Pair Mix
- 70µl Allelic Ladder

PRODUCT*	SIZE	CAT.#
PowerPlex® 16 Monoplex System, D3S1358 (Fluorescein)[a]	**100 reactions**	**DC6551**
PowerPlex® 16 Monoplex System, TH01 (Fluorescein)[a]	**100 reactions**	**DC6561**
PowerPlex® 16 Monoplex System, D21S11 (Fluorescein)[a]	**100 reactions**	**DC6571**
PowerPlex® 16 Monoplex System, D18S51 (Fluorescein)[a]	**100 reactions**	**DC6581**
PowerPlex® 16 Monoplex System, D5S818 (JOE)[a]	**100 reactions**	**DC6601**
PowerPlex® 16 Monoplex System, D13S317 (JOE)[a]	**100 reactions**	**DC6611**
PowerPlex® 16 Monoplex System, D7S820 (JOE)[a]	**100 reactions**	**DC6621**
PowerPlex® 16 Monoplex System, D16S539 (JOE)[a]	**100 reactions**	**DC6631**
PowerPlex® 16 Monoplex System, CSF1PO (JOE)[a]	**100 reactions**	**DC6641**
PowerPlex® 16 Monoplex System, vWA (TMR)[a]	**100 reactions**	**DC6661**
PowerPlex® 16 Monoplex System, D8S1179 (TMR)[a]	**100 reactions**	**DC6671**
PowerPlex® 16 Monoplex System, TPOX (TMR)[a]	**100 reactions**	**DC6681**
PowerPlex® 16 Monoplex System, FGA (TMR)[a]	**100 reactions**	**DC6691**

Not For Medical Diagnostic Use. Each system includes:

- 150µl Internal Lane Standard
- 300µl Gold ST★R Buffer
- 250µl 10X Primer Pair Mix

Storage Conditions: Store all components at –20°C. The fluorescent 10X Primer Pair Mix is light-sensitive; therefore minimize light exposure and store in the dark.

*Items listed are available from Promega as "special order" items. A minimum order is not required, but items may not be immediately available for shipment. Contact Promega Customer Service for more information.

3. Amplification Protocols

Follow the protocols in the *PowerPlex® 16 System Technical Manual* #TMD012 for the PowerPlex® 16 Monoplex Systems and in the *PowerPlex® ESX 17 Fast System Technical Manual* #TMD040 for the PowerPlex® ES Monoplex System. However, follow the directions below for the amount of AmpliTaq Gold® DNA polymerase to use per 25µl reaction. We highly recommend the use of gloves and aerosol-resistant pipette tips.

1. Prepare the amplification mix as directed in Tables 1 and 2.
2. Add template DNA as directed in Tables 1 and 2. For optimal amplification results, we recommend 0.5–2ng of DNA per reaction.

Table 1. Amplification Mix for a Single Sample.

PCR Component	Volume Per Sample
Nuclease-Free Water	17.4µl
Gold ST★R 10X Buffer	2.5ul
10X Primer Pair	2.5µl
AmpliTaq Gold® DNA polymerase[1]	0.1µl (0.5 units)
Total amplification mix volume	**22.5µl**
Template DNA[2] (to be added)	2.5µl
Total Reaction Volume	**25.0µl**

[1]Assumes the AmpliTaq Gold® DNA polymerase is 5u/µl. If the enzyme concentration is different, adjust the volume of enzyme used accordingly.
[2]Assumes the template DNA volume is 2.5µl.

Table 2. Preparation of Amplification Mix for Multiple Samples.

PCR Component	Volume Per Sample	×	Number of Reactions	=	Final Volume (µl)
Nuclease-Free Water	µl				
Gold ST★R 10X Buffer	2.5µl				
10X Primer Pair	2.5µl				
AmpliTaq Gold®					
DNA polymerase[1]	0.1µl (0.5 units)				
Total amplification mix volume[2]	**µl**				
Template DNA (to be added)	µl				
Total Reaction Volume	**25.0µl**				

[1]Assumes the AmpliTaq Gold® DNA polymerase is 5u/µl. If the enzyme concentration is different, adjust the volume of enzyme used accordingly.
[2]The volume of template DNA plus the volume of amplification mix should equal 25.0µl.

4. Detection Methods

Note: Allelic ladders are only included with the PowerPlex® 16 Monoplex System, Penta E (Fluorescein), PowerPlex® 16 Monoplex System, Penta D (JOE) and PowerPlex® ES Monoplex System, SE33 (JOE). Allelic ladders for the other systems are available by custom order. Allelic ladder options are included in Tables 3 and 4.

4.A. Detection Using the ABI PRISM® 310, 3100 or 3100-*Avant* Genetic Analyzer and the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer

Specific directions for detection using the ABI PRISM® 310, 3100 or 3100-*Avant* Genetic Analyzers and the Applied Biosystems 3130 or 3130*xl* are given in the *PowerPlex® 16 System Technical Manual* #TMD012 and the *PowerPlex® ESX 17 Fast System Technical Manual* #TMD040. Please follow these protocols when using the PowerPlex® Monoplex Systems. For additional questions, contact Promega Technical Services.

Table 3. Allelic Ladder Options for the ABI PRISM® 310, 3100 and 3100-*Avant* Genetic Analyzers and the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer.

PowerPlex® 16/ES Monoplex Loci	Allelic Ladder Options[1,2]
D18S51, D21S11, TH01, D3S1358, FGA, D8S1179, vWA	PowerPlex® 16
Penta E	Penta E or PowerPlex® 16
Penta D	Penta D or PowerPlex® 16
CSF1PO, D16S539, D7S820, D13S317, D5S818, TPOX	PowerPlex® 16
SE33	SE33

[1]The PowerTyper™ Macros (Cat.# DG3470) can be used for data analysis.
[2]All allelic ladders are available by custom order.

4.B. Detection Using the Hitachi FMBIO® II Fluorescence Imaging System

For detection using the Hitachi FMBIO® II Fluorescence Imaging System, see the *PowerPlex® 16 BIO System Technical Manual* #TMD016. The filter set used for detection of the PowerPlex® 16 and PowerPlex® ES Monoplex loci is shown in Table 4.

Table 4. Allelic Ladder Options for the Hitachi FMBIO® II Fluorescence Imaging System. The filter set used for detection of each PowerPlex® 16 or PowerPlex® ES Monoplex locus is shown in bold in the "Filters Needed for Detection" Column.

PowerPlex® 16/ES Monoplex Locus	Allelic Ladder Options[1]	Filters Needed for Detection (nm)	PowerPlex® Filter Set
D18S51, D21S11, TH01, D3S1358	PowerPlex® 2.1	**505**, 585, 650	1.1/2.1
	PowerPlex® 16 BIO[2]	**505**, 577, 598, 665	16 BIO
CSF1PO, D16S539, D7S820, D13S317, D5S818	PowerPlex® 16 BIO[2]	505, **577**, 598, 665	16 BIO
	JOE Ladder Mix	**585**, 650	1.1/2.1
		577, 665	16 BIO
FGA, TPOX, D8S1179, vWA	PowerPlex® 2.1	505, **585**, 650	1.1/2.1
		505, **577**, 665	16 BIO[3]
SE33	SE33 Allelic Ladder	**585**, 650	1.1/2.1
		577, 665	16 BIO
Penta E	PowerPlex® 2.1	**505**, 585, 650	1.1/2.1
	PowerPlex® BIO	**505**, 577 598, 665	16 BIO
	Penta E Allelic Ladder	**505**, 650	1.1/2.1
		505, 665	16 BIO
Penta D	PowerPlex® BIO	505, **577** 598, 665	16 BIO
	JOE Ladder Mix	**585**, 650	1.1/2.1
		577, 665	16 BIO
	Penta D Allelic Ladder	**585**, 650	1.1/2.1
		577, 665	16 BIO

[1]All allelic ladders are available by custom order.
[2]The Matrix 16 BIO is required for use with the PowerPlex® 16 BIO Allelic Ladder Mix. The Internal Lane Standard 600, labeled with CXR, can be detected with the 665nm filter.
[3]When using the PowerPlex® 16 BIO filter set for TMR-labeled loci, do not include the data from the 598nm filter in the FMBIO® Analysis Project or do not scan using the 598nm filter. Both TMR and CXR will be detected with the 598nm filter making color separation impossible. The TMR-labeled loci will be detected in the 577nm scan (JOE channel).

5. Summary of Changes

The following changes were made to the 8/15 revision of this document:

1. Replaced mention of discontinued products with current products.
2. Removed expired disclaimers.
3. Moved document into new format.

[a] The purchase of this product does not convey a license to use AmpliTaq Gold® DNA polymerase. You should purchase AmpliTaq Gold® DNA polymerase licensed for the forensic and human identity field directly from your authorized enzyme supplier.

[b] U.S. Pat. No. 6,238,863, Chinese Pat. No. ZL99802696.4, European Pat. No. 1058727, Japanese Pat. No. 4494630 and other patents pending.