

# BEC MULTIPLEX

## The Penta BEC Multiplex Primers from Promega: Additional Loci Available for Identity Testing

By Kim Gorman, Zachary Slinker, Scott Johnson and Michelle Beckwith  
PTC Laboratories, Columbia, Missouri, USA

### INTRODUCTION

The Penta BEC Multiplex Primers provide additional power to resolve difficult cases. The Penta B and Penta E loci are highly polymorphic. Although less polymorphic, the Penta C locus is very helpful in many cases. Often paternity cases with mutations, single-parent paternity cases and family reconstructions cannot be sufficiently resolved with standard STR kits. In the past, RFLP testing was often used to resolve these types of cases, but RFLP testing is no longer used by many facilities.

The Penta BEC multiplex is a robust system that amplifies under the same conditions as the PowerPlex® Systems. It is a quick and easy addition in cases where more extensive testing is required. The multiplex is labeled with fluorescein and is compatible with both capillary electrophoresis and FMBIO® platforms. Allele calls are easy, and microvariants are extremely rare or nonexistent. Using the internal lane standard 600 BIO (ILS 600 BIO), allele calls can be made based on the size of the amplicon.

In this validation study, DNA from unrelated Caucasian, African American and Hispanic individuals was tested using the Penta BEC multiplex. Databases were constructed, and frequency tables were generated.

### MATERIALS AND METHODS

DNA isolated using the DNA IQ™ System<sup>(a)</sup> was used in all amplifications. Amplifications were analyzed using the FMBIO® platform. Allelic ladder was provided by Promega Corporation as prototype material or was generated in-house. The Promega ILS 600 BIO was used to size the amplicons. All DNA used was previously amplified and analyzed using the Promega PowerPlex® 16 BIO System<sup>(b-e)</sup>. To ensure integrity of the allele calls, the Penta E locus was cross-checked between the two systems.

The primer sets for the Penta B, Penta E and Penta C loci were provided by Promega Corporation. The BEC multiplex was tested under the same amplification and preparation conditions as used for the PowerPlex® 16 BIO System and CSF1PO, TPOX, TH01, vWA Multiplex (Fluorescein)<sup>(b,f)</sup>. Two nanograms of DNA were added to each amplification reaction, and the products were prepared by adding 2µl of amplified product to 1.5µl of Bromophenol Blue Loading Solution and 0.5µl ILS 600 BIO. Denaturation was carried out by heating the mixture at 96°C for 5 minutes. Amplicons were held on ice after denaturation and before loading. Post-amplification electrophoresis was carried out using a 5% Long Ranger® polyacrylamide gel run at 52 watts for approximately 1 hour 45 minutes. The gels were then scanned on an FMBIO® II Fluorescence Imaging System and analyzed using the FMBIO® Analysis 8.0 software. Allele calls were made using STaRCa11™ version 3.0.

*PTC Laboratories has used the Penta BEC triplex on 33 cases that were not satisfactorily resolved with the PowerPlex® 16 BIO System alone. The addition of the Penta BEC triplex was sufficient to resolve 28 of the 33 cases.*

## RESULTS AND DISCUSSION

Since Penta E is not a new locus, this discussion will focus on the Penta B and C loci.

Caucasian, African American and Hispanic databases were constructed for the Penta B (7q33) and Penta C (9q13.1) loci. See Tables 1 and 2. Penta B passed an exact test for Hardy Weinberg equilibrium for all three racial groups. Penta C passed the exact test for Hardy Weinberg equilibrium for the African American and Hispanic groups but showed an excess of homozygous 11 alleles for the Caucasian group.

The first and second groups of Caucasians typed were primarily from the state of Missouri. The total in the Missouri groups was approximately 300 individuals. Since the majority of the Caucasian participants were from the same area of the country, it was possible there could be some degree of population substructure resulting in disequilibrium. The next group of individuals typed consisted of random Caucasians from throughout the country. Unfortunately, the same results were obtained with the more diverse group. Both Penta C Caucasian databases show an excess of homozygous 11 phenotypes. In both databases, the individuals homozygous for allele 11 were amplified with a different primer set to confirm that there was not a primer site mutation causing allele dropout.

## PENTA BEC MULTIPLEX PRIMERS

The Penta BEC multiplex primers can be used under the same conditions as the PowerPlex® 16 BIO System. Reaction volumes, cycling conditions and ILS 600 BIO are the same for both systems, making it convenient. Since it is rare for a Penta locus to have microvariants, the allele calls are straightforward. In most cases, allele

**Table 1. Penta B Allele Frequencies.**

Size	African American		Caucasian		Hispanic	
	# of Alleles	Frequency	# of Alleles	Frequency	# of Alleles	Frequency
5	12	0.02166	0	0.00819*	0	0.01543*
6	4	0.00902*	0	0.00819*	0	0.01543*
7	19	0.03430	3	0.00819*	0	0.01543*
8	48	0.08664	19	0.03115	6	0.01852
9	79	0.14260	12	0.01967	10	0.03086
10	99	0.17870	43	0.07049	23	0.07099
11	75	0.13538	78	0.12787	38	0.11728
12	60	0.10830	88	0.14426	69	0.21296
13	40	0.07220	84	0.13770	45	0.13889
14	41	0.07401	112	0.18361	68	0.20988
15	27	0.04874	61	0.10000	30	0.09259
16	26	0.04693	31	0.05082	16	0.04938
17	12	0.02166	27	0.04426	7	0.02160
18	3	0.00902*	7	0.01148	5	0.01543
19	4	0.00902*	17	0.02787	2	0.01543*
20	3	0.00902*	9	0.01475	1	0.01543*
21	0	0.00902*	3	0.00819*	1	0.01543*
22	0	0.00902*	6	0.00984	1	0.01543*
23	0	0.00902*	4	0.00819*	0	0.01543*
24	1	0.00902*	1	0.00819*	0	0.01543*
26	0	0.00902*	1	0.00819*	0	0.01543*
26.2	0	0.00902*	1	0.00819*	0	0.01543*
27	0	0.00902*	0	0.00819*	1	0.01543*
28	0	0.00902*	0	0.00819*	1	0.01543*
29	0	0.00902*	1	0.00819*	0	0.01543*
30	1	0.00902*	1	0.00819*	0	0.01543*
38	0	0.00902*	1	0.00819*	0	0.01543*
<b>Total</b>	<b>554</b>		<b>610</b>		<b>324</b>	
<b>Penta B Power of Exclusion</b>						
African American	0.484338					
Caucasian	0.745515					
Hispanic	0.650381					
*Minimum allele frequency as recommended by the NRC II.						
No mutation rates are available at this time.						

calls can be made visually using an allelic ladder. The ILS 600 BIO makes it possible to make allele calls by using STaRCaLL™ or Genotyper® software.

The observed alleles for Penta B vary from 5 to 38 repeats. Penta B is extremely polymorphic and is helpful in resolving cases. The Penta C locus has common alleles that vary from 5 to 17 repeats. Penta C is less polymorphic than Pentas B or E but is still helpful in many cases.

## PRACTICAL APPLICATION

In our laboratory, approximately 1 in 25 paternity cases cannot be resolved using the PowerPlex® 16 BIO System alone. To date, PTC Laboratories has used the Penta BEC multiplex on 33 cases that were not satisfactorily resolved with the PowerPlex® 16 BIO System alone. The addition of the Penta BEC multiplex was sufficient to resolve 28 of the 33 cases. In the past, all 33 of the unresolved cases would have required additional RFLP testing before the case was released.

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**Table 2. Penta C Allele Frequencies.**

Size	African American		Caucasian		Hispanic	
	# of Alleles	Frequency	# of Alleles	Frequency	# of Alleles	Frequency
5	14	0.02527	5	0.01299	9	0.02744
7	10	0.01805	2	0.00812	0	0.01524*
8	28	0.05054	4	0.00812	18	0.05488
9	87	0.15704	67	0.13149	81	0.24695
10	37	0.06679	17	0.06006	24	0.07317
11	167	0.30144	172	0.44643	92	0.28049
12	133	0.24007	92	0.20617	73	0.22256
13	62	0.11191	58	0.11688	29	0.08841
14	12	0.02166	4	0.01461	1	0.01524*
15	3	0.00903	3	0.00812	1	0.01524*
17	1	0.00903	0	0.00812	0	0.01524*
<b>Total</b>	<b>554</b>		<b>424</b>		<b>328</b>	
<b>Penta C Power of Exclusion</b>						
Hispanic	0.631102					
African American	0.530053					
There is an excess of individuals homozygous for allele 11 in the Penta C Caucasian database.						
*Minimum allele frequency as recommended by the NRC II.						
No mutation rates are available at this time.						

With the addition of the Penta BEC multiplex primers, it is possible to amplify the previously extracted DNA and resolve most cases in a day or two instead of 2 weeks.

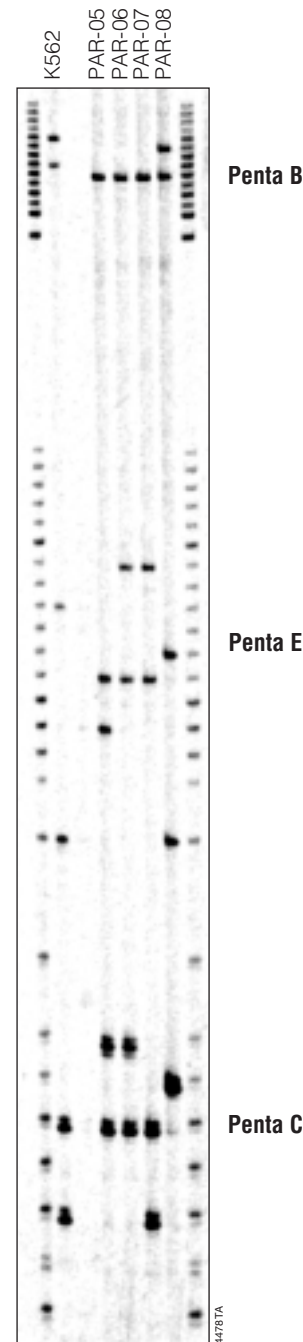
When used with the PowerPlex® 16 BIO or PowerPlex® 2.1 System<sup>(b,c,f)</sup>, the Penta BEC multiplex allows for a cross-check of the Penta E locus to confirm that the same DNA was amplified. When used with other systems, the Penta BEC multiplex adds the additional power of the Penta E locus.

The Penta BEC multiplex is an excellent robust system that is easy to use, compatible with other PowerPlex® Systems and very helpful in resolving difficult cases.

**REFERENCES:**

- *Penta BEC Multiplex Primers System Technical Manual #TMD019*, Promega Corporation.
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- Basten, C.J., Exact test for Hardy Weinberg equilibrium provided by North Carolina State University Raleigh, North Carolina, USA.
- *FMBIO® User's Manual*, Hitachi Corporation.

Editor's note: The Penta BEC Multiplex Primers<sup>(c)</sup> (Cat.# X225X) are available from Promega by custom order. A Penta BEC allelic ladder is not available from Promega at this time. Please contact Promega for additional information.



**Figure 1. The DNA samples submitted by the CAP Paternity Testing Program, PAR-B 2003, were amplified with the BEC multiplex primers. Lane 1 and 8, BEC allelic ladder; lane 2, K562 DNA; lane 3, amplification blank; lane 4, mother; lane 5, child; lane 6, alleged father 1; lane 7 alleged father 2.**