PowerPlex™ 16 "Frequently Asked Questions"

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This article provides the answers to a number of questions about Promega's new *GenePrint*® PowerPlex™ 16 System[a,b].

Q: What is the PowerPlex™ 16 System?

The PowerPlex™ 16 System allows the coamplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin) in a single reaction. The amplified STRs are detected using the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA Sequencer.

Included with the PowerPlex™ 16 System is 9947A DNA, a human genomic DNA that is used as control DNA for the STR product line. Also available are the Matrix FL-JOE-TMR-CXR, developed to generate a matrix for color separation using 4 fluorescent dyes on the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA Sequencer, and the PowerTyper™16 Macro, created to facilitate analysis of the data generated by allowing automatic assignment of genotypes using the ABI Genotyper® 2.0 or 2.5 software.

Q: What is the optimum amount of template to add?

We recommend using 0.5–1ng of template DNA in each reaction. When using more template DNA, an imbalance in peak heights from locus-to-locus can be seen. Generally, the smaller loci show greater amplification yield than the larger loci. Reducing the number of cycles in the amplification program by 2–4 can improve locus-to-locus balance, however to obtain better balance, a re-amplification using less template is recommended.

The volume of template added can also be a concern. We recommend that the template volume be no more than 20% of the final reaction volume. This is because there may be inhibitors of the amplification reaction within the sample. For example, if the sample is suspended in TE (pH 7.5), the added volume may change the pH of the reaction, creating non optimal conditions for *Taq* DNA polymerase function. In addition, EDTA present in the TE buffer will chelate the magnesium necessary for polymerase activity. Both instances will result in a failed amplification.

Q: Can I use FTA™ paper with this system?

We have not thoroughly investigated the use of FTA™ paper with this system; however, in initial experiments, we have found an imbalance, with the Penta E and Penta D loci being lower than the others. Further studies have yet to be performed.

Q: Are there any special considerations for the thermal cycler used with the PowerPlex™ 16 System?

The PowerPlex™ 16 System has been optimized on the GeneAmp® Model 9600 Thermal Cycler. Protocols for using the Perkin-Elmer Model 480, the GeneAmp® Model 9700 or the GeneAmp® System 2400 Thermal Cyclers are also included in the Technical Manual provided with the system (TMD012). Small differences in balance among loci or fluorophores are observed when using different thermal cyclers or detection instrumentation. The detection instrumentation itself can play a significant role in locus-to-locus balance. We have seen variation between ABI PRISM® 310 Genetic Analyzer instruments that has resulted in differences in the relative efficiency of detection of various fluorescent dyes. The three colors of the PowerPlex™ 16 System loci may be balanced on one instrument and not balanced on a second instrument even when using the same amplification product. In addition, the peak height display (i.e., relative fluorescent units (RFU)) of all alleles using the same amplified product may be lower on one ABI PRISM® 310 Genetic Analyzer than on another.

Q: Why use deionized formamide?

Formamide must be used with the ABI PRISM® 310 Genetic Analyzer to keep the sample denatured prior to injection. The quality of the formamide used is critical. Use deionized formamide with a conductivity less than 100µS/cm. Formamide with greater conductivity may contain ions that compete with DNA during injection, resulting in lower peak heights and reduced sensitivity. Thus, it can appear that the amplification failed. In some instances using a resin to deionize the formamide can actually increase conductivity, leading to lower peak heights. Amresco formamide has a QC specification of 100µS. The lower conductivity of the Amresco formamide gives greater sensitivity as evidenced by increased peak height RFU.

Q: Is there any specific information to include when setting up sample sheets?

For the PowerTyper™ 16 Macro to determine the samples that contain allelic ladder, it is imperative that the sample info column contain the word "ladder". If this is omitted, the PowerTyper™ 16 Macro will show the error message: "Could not complete the 'Run Macro' command because no dye/lanes are selected." This information can be added or modified after importing into Genotyper®. This is done by selecting "Show Dye/Lanes Window" under "Views," highlighting the sample and adding "ladder" to the "Sample Info" field.

Q: What injection time is best?

We recommend an injection time of between 2 and 5 seconds. This should be optimized for each individual instrument. Generally, 3 seconds is a good initial timeframe to start with when optimizing. Peak heights should be around 1,000 RFU for a heterozygous locus amplified using 0.5–1ng template.

Q: See an elevated baseline in one or all of my samples.

An elevated baseline may be the result of using a matrix created on another instrument, using a matrix made on the same instrument after service, or from a matrix

made using different dyes. Generally, we recommend generating a new matrix with the appropriate dyes. The Matrix FL-JOE-TMR-CXR (Cat.# DG2860), is required for matrix standardization when using the PowerPlex™ 16 System.

Elevated baseline may also be caused by extremely high peak heights, greater than 2,000 RFU. To minimize this, change the analysis parameters in the GeneScan® Analysis Software, increasing the minimum cutoff to greater than 50 RFU. Alternatively, amplify using less DNA.

Q: I see red background streaks in my ABI 377 gel.

The red background streaks or smears, also known as "red rain", appear when the plates are positioned away from the laser window or as a result of dirt or oil buildup on the plates. Wash the plates in 2N NaOH. If this does not alleviate the problem, soak the plates in 2N HCl to remove any buildup then rinse thoroughly in deionized water.

Q: My 310 runs are inconsistent from sample-to-sample.

Sample-to-sample variation may be due to inconsistent pumping of the polymer in the capillary. This can be an indication that the syringe needs to be replaced.

Q: I see a constant low peak in every sample I run.

A peak that is seen in every sample could indicate that the block on the ABI 310 needs to be cleaned. We clean the block using autoclaved water and make up all buffers in autoclaved water to minimize the potential for bacterial growth within the block. We also recommend changing the tubes that are used for the buffer, water and waste at the same time the block is cleaned.

Q: See extra peaks within my CXR ladder.

This can be caused by bleed-through of the TMR dye into the CXR. This will occur when the sample peak heights are very high. This can also occur as a result of differences in the balance of each dye between instruments. As such, it may be difficult to eliminate all of the bleed-through peaks. This can be seen particularly with amelogenin.

Q: When using PowerTyper™ 16 Macro for the first time, I get the error message "not enough memory"?

When first loading Genotyper® software, it may be necessary to allocate more memory. To do this, close the program and highlight the Genotyper® program file. Under the Apple, select "get info" and highlight "memory". Additional memory can be allocated for the program.

Q: There is no data for the internal lane standard (ILS) 600 when analyzing my data in PowerTyper™ 16 Macro.

When using Genotyper® 2.5, it may be necessary to change preferences under "edit" to import the blue, green, yellow and red colors.

Q: I see peaks within my data, but PowerTyper™ 16 Macro does not call them.

This can happen when the peak heights are below the peak amplitude thresholds value set in the analysis parameters.

Q: PowerTyper™ 16 Macro does not call the alleles in the allelic ladder.

If the internal lane standard is not correctly defined, it will miscall the alleles within the allelic ladder. If this is the case, go back into the GeneScan® Analysis Software and redefine the ILS, reapply to the samples and reanalyze with the newly defined size standard.

CE PowerTyper™ 16 spikes can also be identified as alleles by the Macro. CE spikes are usually detected as a peak in all of the dye colors within the sample. When this occurs, use a different injection of the allelic ladder.

Q: Why are off-ladder alleles called?

There are at least two reasons why this occurs. Migration of samples may change slightly over the course of a CE run with many samples. This may be due to changes in temperature or in the CE column over time. The base pair size for the alleles is based on the first allelic ladder imported into the Genotyper® software. This is common when using the first injection on a new column for a ladder sample. Running more than one allelic ladder within a set of samples is recommended; the samples can be reanalyzed using a different ladder injection. For the ABI 377, off-ladder alleles can be caused by streaking within a gel. Thus, it is important to run at least two ladder lanes in each gel.

Off-ladder peaks may also be caused by the assignment of incorrect fragment sizes to the ILS. Confirm that the internal lane standard fragments are assigned correctly. Redefine the internal lane standard fragments and reanalyze the sample using GeneScan® Software.

Q: What are the Matching Probability, Paternity Index and Power of Exclusion of the PowerPlex™ 16 System?

Population statistics for the loci contained in the PowerPlex™ 16 System are being developed as part of a collaboration with The Bode Technology Group (Springfield, VA). Generation of these data includes analysis of over two hundred individuals from each of the three major racial and ethnic groups in the United States. Preliminary data has been used to generate the following population statistics.

The matching probability increases with the number of STR loci that are amplified. The PowerPlex™ 16 System has a matching probability ranging from 1 in 1.83×10^{17} for Caucasian-Americans to 1 in 1.42×10^{18} for African-Americans.

As with the matching probability, the typical paternity index (PI) increases with the number of STR loci that are amplified. The PI for the PowerPlex™ 16 ranges from 1,520,000 for Caucasian-Americans to 5,220,000 for Hispanic Americans.

The Power of Exclusion also increases with the number of STR loci that are amplified. For the PowerPlex™ 16 this number ranges from 0.9999983 for Hispanic-Americans to 0.9999998 for Asian Americans.

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(a,b) Please refer to the patent and disclaimer statements on page 2.