Multiplexing the Homogeneous MassEXTEND™ Assay

Introduction

SEQUENOM® has developed new protocols optimized for multiplexing the homogeneous MassEXTEND (hME) assay. This application note is based on an internal, optimization study conducted by SEQUENOM. Results from 12-plexed assays are discussed, however the optimized protocols are applicable to any multiplex-level up to 15-plex.

We present design parameters to be used with the MassARRAY™ Assay Design 2.0 (Assay Design) software to design assays for optimal multiplexing performance. For the PCR and hME reaction, we provide optimized reagent concentrations and thermal cycling parameters. Additionally, we include a procedure to adjust the concentrations of individual hME primers in a primer mix before using them in actual assay runs—this is critical to the performance of multiplexed reactions.

Overview

The hME assay is a simple and robust method for the analysis of single nucleotide polymorphisms (SNPs). The speed and accuracy of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a solution for high-throughput genotyping. The hME assay is based on the annealing of an oligonucleotide primer (hME primer) adjacent to the SNP of interest. The addition of a DNA polymerase along with a mixture of terminator nucleotides allows extension of the hME primer through the polymorphic site and generates allele-specific extension products, each having a unique molecular mass. The resultant masses of the extension products are then analyzed by MALDI-TOF MS and a genotype is assigned in real time (Figure 1) [1-2].

Performing multiple PCR and hME reactions in a single well (multiplexing) is a way to further increase the throughput and reduce cost per genotype. SEQUENOM has optimized individual reagent concentrations and thermal cycling conditions to multiplex PCR and hME reactions for best MALDI-TOF-MS-based genotyping results (patent pending).
Methods

Assay Design

When designing high-plexed hME assays, we recommend using Assay Design 2.0—software that automatically designs PCR and hME primers for each SNP to be investigated. It uses novel multiplexing algorithms developed to take full advantage of the MassARRAY platform while minimizing chances for overlapping peaks in spectra. The program is also designed to consider potential unwanted intra- and inter-primer interactions in order to avoid non-template extensions. Also the new “replex” function allows previously-designed assays to be combined together while retaining their original primer designs.

Figure 2 shows screen captures of the Assay Design settings to use for optimal design efficiency. Except for the SNP Group, Assay Group, and Multiplexing Level, the exact settings shown in the figure should be used. The SNP Group, Assay Group, and Multiplexing Level are determined by the user.

Note If you want to replex previously designed assays, select Replex under Assay Design.

For more information on Assay Design 2.0 see MassARRAY Assay Design Software User’s Guide.

Figure 2. Assay Design 2.0 Settings Optimized for Multiplexing the hME Assay

Note: Use these settings (except for SNP Group, Assay Group, and Multiplexing Level) exactly as shown. The main Assay Design window is on the left. Two subordinate dialog boxes are shown to the right of the main window; the open-head arrows indicate which button in the main window opens each dialog box. The close-head arrows call your attention to settings that are not the normal defaults for Assay Design.
Prior to the hME reaction, the genomic DNA must be amplified using the polymerase chain reaction (PCR). The use of a 10-mer tag (5'-ACGTTGGATG-3'), referred to as "hME-10," on the 5' ends of each PCR primer provides significant improvement in overall hME performance. The tags increase the masses of unused PCR primers so they fall outside the mass range of analytical peaks. They also help balance amplification. Additionally, we optimized the PCR cocktail composition to make multiplexing more robust.

To prepare and process the PCR, perform the following steps:

1. Prepare a PCR cocktail as described in Table 1.

**TABLE 1. PCR Cocktail for Multiplexing**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure water</td>
<td>0.920 µL</td>
<td>NA</td>
</tr>
<tr>
<td>Genomic DNA (2 ng/µL)</td>
<td>1.000 µL</td>
<td>2 ng/rxn</td>
</tr>
<tr>
<td>HotStar Taq® PCR buffer* containing 15 mM MgCl₂ (10X)</td>
<td>0.625 µL</td>
<td>1.25X/1.875 mM MgCl₂²</td>
</tr>
<tr>
<td>Fresh dNTPs (25 mM)**</td>
<td>0.100 µL</td>
<td>500 µM each</td>
</tr>
<tr>
<td>Forward PCR primers† (500 nM each)</td>
<td>1.000 µL</td>
<td>100 nM each</td>
</tr>
<tr>
<td>Reverse PCR primers† (500 nM each)</td>
<td>1.000 µL</td>
<td>100 nM each</td>
</tr>
<tr>
<td>MgCl₂ (25 mM)</td>
<td>0.325 µL</td>
<td>1.625 mM²</td>
</tr>
<tr>
<td>HotStar Taq (5U/µL) QIAGEN Inc.</td>
<td>0.030 µL</td>
<td>0.15 U/rxn</td>
</tr>
<tr>
<td>Total</td>
<td>5.000 µL</td>
<td></td>
</tr>
</tbody>
</table>

* The PCR buffer concentration should not exceed 1.25X. Higher salt concentrations have negative effects at the hME level.
** Maximum of 5 freeze/thaws.
† Containing a 10-mer tag: hME-10 (5'-ACGTTGGATG-3').
‡ 3.5 mM MgCl₂ total.

**NOTE**: Do not use Q solution. It has negative effects on MALDI-TOF MS analysis.

2. Cycle the PCR as follows in a standard thermal cycler:
   - 95° C for 15 minutes
   - 95° C for 20 seconds
   - 56° C for 30 seconds
   - 72° C for 1 minute
   - 72° C for 3 minutes
   - 4° C forever
   - 45 cycles

**SAP Treatment**

Use SAP (shrimp alkaline phosphatase) enzyme to dephosphorylate unincorporated dNTPs from the amplification reaction as usual.


**Adjusting MassEXTEND Primer Mixes**

The peaks in the mass spectrum for a multiplexed reaction may not have comparable heights. Variations in peak height may stem from 1) inconsistent oligonucleotide quality, 2) inconsistent oligonucleotide concentration, and 3) different desorption/ionization behavior in MALDI.

For best multiplexing results, we recommend the concentrations of hME primers be adjusted to even out peak heights (intensities) in the mass spectrum. This adjustment must be done prior to preparing the hME reaction cocktail and processing the hME reaction (“hME Reaction” on page 5).

**Note** Adjusting MassEXTEND primer mixes requires the use of a SpectroCHIP® bioarray. Adjusting MassEXTEND primer mixes is critical to successful multiplexing. An assay with a very low primer peak will systematically fail when applied to samples as part of a multiplex.

Perform the following steps to adjust MassEXTEND primer mixes:

1. For each multiplex, prepare a mixture of the required MassEXTEND primers (referred to as a primer mix). The final concentration of each primer in the primer mix must be 9 µM.

   Consider how much primer mix you will need. Each single hME reaction (i.e. a single well in a 384-well microplate) requires 1 µL primer mix.

   **Note**: When ordering MassEXTEND primers from your oligonucleotide supplier it may be useful to consider at what plex-level you will use the primers and ask for the primers to be supplied at a certain concentration. For example, ordering primers for a 12-plex at 108 µM makes preparing primer mixes much easier. You can simply mix equal volumes of each 108 µM primer. Each primer will have a concentration of 9 µM in the final primer mix. Similarly, for a 10-plex, order MassEXTEND primers at 90 µM.
2. Pipette 1 µL of the primer mix into a well of a microplate and add 24 µL nanopure water to obtain a 360 nM dilution of the primer mix (referred to as a primer mix sample).

3. Repeat steps 1 and 2 for each multiplex, to generate a microplate containing primer mix samples for all of the multiplexes.

4. Add 3 mg Clean Resin to each well of the microtiter plate (MTP) using the dimple plate.

**Note:** Do not add any water. The existing dilutions of the primer mix samples are appropriate.

5. Dispense the primer mix samples to a SpectroCHIP using standard dispensing conditions for hME reaction products.

For instructions on operating the Nanodispenser, see the “Dispensing MassEXTEND Reaction Products onto SpectroCHIPS” chapter in MassARRAY Nano-dispenser User’s Guide.

**Note:** If the entire SpectroCHIP is not used, you may keep it for future use in adjusting MassEXTEND primer mixes. Use only those pads on the SpectroCHIP that have not been used before; you cannot reuse previously spotted pads. Store SpectroCHIPS—in their original packaging—in a desiccator. SpectroCHIPS may be stored for one week maximum.

6. Acquire spectra from the SpectroCHIP using MassARRAY Typer software (Typer) 3.0.1 or higher.

For instructions on acquiring spectra, see the “SpectroACQUIRE” chapter in MassARRAY Typer User’s Guide.

Use the assay definitions (in Typer) for the actual multiplexes. Each well on the SpectroCHIP will yield no-calls because there is no analyte, only unextended MassEXTEND primers. A peak should appear at the expected mass for each MassEXTEND primer in the mix.

**Note:** At this point, there is a good opportunity to “quality-check” the MassEXTEND primers and the primer mixes. There should be a peak at the expected mass of each primer. A missing peak generally indicates poor primer quality or a primer missing from the mix. An unexpected peak generally indicates poor primer quality or the addition of an unnecessary primer to the mix.

7. Check whether the primer peaks in each mass spectrum have comparable heights. If all peaks are at least 50% the height of the highest peak, they are acceptable. If any peak is less than 50% the height of the highest peak, add more of that primer. Use the guidelines as shown in Figure 3 for primer addition. Once the concentrations of the primers have been adjusted in the primer mix, use the adjusted primer mix in actual assay runs.

**Note:** Adjust the original primer mix, not the primer mix sample in the microplate.

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**Figure 3. Guidelines for Adjusting MassEXTEND Primer Concentrations in Multiplexed Mixes**

The following diagram is a simplified representation of a spectrum. It provides general guidelines on adjusting primer concentrations in the primer mix to even out primer peak heights.

![Diagram](https://via.placeholder.com/150)

Primer B has the highest peak. The diagram indicates how much to add of each primer based on its peak height relative to the highest peak. For example, if you used 100 µL of each primer initially, add 40 µL more primer A, 60 µL more primer C, and 80 µL more primer D to the original primer mix.

**Note:** Each primer may behave differently and may have to be adjusted differently.
**hME Reaction**

Once the hME primer mixes have been adjusted (see “Adjusting MassEXTEND Primer Mixes” on page 3), prepare hME reaction cocktail, add the cocktail to the SAP-treated PCR products, and thermocycle.

1. Prepare hME reaction cocktail as described in Table 2.

**TABLE 2. hME reaction cocktail for multiplexing**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Final Concentration (in 9 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure water</td>
<td>0.76 µL</td>
<td>NA</td>
</tr>
<tr>
<td>Appropriate hME EXTEND Mix (containing buffer and d/ddNTPs)</td>
<td>0.20 µL</td>
<td>50 µM each d/ddNTPs</td>
</tr>
<tr>
<td>Adjusted MassEXTEND primer mix (~9 µM each primer, adjusted)*</td>
<td>1.00 µL</td>
<td>~1 µM each*</td>
</tr>
<tr>
<td>Thermo Sequenase™ (32 U/µL)</td>
<td>0.04 µL</td>
<td>1.25 U/rxn</td>
</tr>
</tbody>
</table>

* The MassEXTEND primer mix must have been adjusted, if necessary, as described in “Adjusting MassEXTEND Primer Mixes” on page 3. Note that the primers in an adjusted mix may not be at 9 µM each. Each starts out at 9 µM, however, the addition of extra amounts of some primers to adjust the mix will change the concentrations. In turn, the final concentration may not be exactly 1 µM.

2. Add 2 µL of hME reaction cocktail to the SAP-treated PCR products as usual.

3. Cycle the hME reaction as follows in a standard thermal cycler.

- 94°C for 2 minutes
- 94°C for 5 seconds
- 52°C for 5 seconds
- 72°C for 5 seconds
- 4°C forever

*100 cycles has been shown to further improve performance (75 cycles is the minimum requirement).

**Desalting**

Dilute and add 6 mg Clean Resin to the hME reaction products for conditioning. Note that this is twice the amount used in the standard hME protocol. Use the manual cleanup procedure, not the automated one (see “Cleaning up the hME Reaction Products” in MassARRAY Liquid Handler User’s Guide). The manual procedure involves the use of a dimple plate to add Clean Resin to sample microplates. The Liquid Handler is used only to add water to sample microplates.

**Note** The standard Clean Resin Kit includes a 3 mg dimple plate. To add 6 mg Clean Resin you must use the dimple plate twice. A 6 mg dimple plate is available (see “Materials” for ordering information).

**Dispensing to a SpectroCHIP**

Use a nanodispenser to dispense reaction product onto a 384-well SpectroCHIP as usual. See the “Dispensing MassEXTEND Reaction Products onto SpectroCHIPS” chapter in MassARRAY Nanodispenser User’s Guide for instructions.

**Note** Because 6 mg Clean Resin was added to the reaction products, you may have to adjust the load offset of the dispensing tips (i.e., distance the tips dip into plate wells) to account for the increased amount of Clean Resin. Be sure to follow the instructions in the Nanodispenser’s manual regarding determining the load offset.

**Acquiring Spectra (MALDI-TOF MS)**

Using Typer 3.0.1 or higher, acquire spectra from the SpectroCHIP as usual. See the “SpectroACQUIRE” chapter in MassARRAY Typer User’s Guide.
Results

Assay Design

One thousand validated SNPs were selected randomly and processed through ProxSNP and PreXTEND. Assays for the SNPs passing ProxSNP and PreXTEND were designed using Assay Design 2.0. The design parameters were set as depicted in Figure 2. Out of 964 assays designed, 864 were combined into 12-plexes (96% design efficiency, 90% 12-plex efficiency). The design results are shown in Table 3.

Multiplexing MassEXTEND

Seven of the 12-plexes designed were processed following the procedure described in “Methods”. Seven individual DNA samples and one negative control were analyzed in 6 replicates (336 reactions, 4032 assays). Figure 4 shows a comparison of success and accuracy rates obtained using:

- Previous experimental conditions (as described in MassARRAY Liquid Handler User’s Guide v1 r6) without tags
- New experimental conditions without tags
- New conditions with tags (hME-10 tag)

Success and accuracy rates were defined by the average percentage of calls made in real-time by Typer 3.0.1.

Accuracy refers to reproducibility compared to previous genotype data obtained from independent, uniplexed, hME reactions done in quadruplicate. Using the protocol proposed in this application note, results show that it is now possible to perform high-level multiplexing producing over 90% successful genotypes with 99.7% accuracy (automated Typer calls, first pass). Performance significantly dropped when the previous experimental conditions were used. Also the positive effects of using the hME-10 tag were demonstrated. Following real-time analysis, the genotypes can be validated by clustering signal-to-noise ratios [3] and, where appropriate, assays can be evaluated by Hardy-Weinberg equilibrium analyses [4]. These type of “post-RT” analyses can be used to correct and/or reject likely miscalls and identify problematic assays.

<table>
<thead>
<tr>
<th>TABLE 3. Multiplexing hME assays (Design Results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pass ProxSNP*</td>
</tr>
<tr>
<td>Pass PreXTEND*</td>
</tr>
<tr>
<td>Assays designed</td>
</tr>
<tr>
<td>Assays 12-plexed</td>
</tr>
<tr>
<td>12-Plex efficiency**</td>
</tr>
</tbody>
</table>

* ProxSNP and PreXTEND are part of the eXTEND suite of programs available through SEQUENOM’s RealSNP® Assay Database (www.realsnp.com). ProxSNP checks SNP syntax, performs a BLAST genome search, and checks for other SNPs in the vicinity of the SNP in question—“pass” means the SNP is correctly formatted and a match was found in the genome. PreXTEND searches the genome for matches to the PCR primers—“pass” means the primers match the genome in only one location. ProxSNP and PreXTEND are scheduled to be available late 3rd quarter 2003. E-mail Meghan Lane for information (mlane@sequenom.com).

** This is a ratio of successfully designed 12-plexes to the number of 12-plexes numerically possible. In this case, out of 80 possible 12-plexes, 72 were successfully designed. (There are 80 12-plexes possible out of the 964 assays designed; 864 of those assays were successfully grouped into 72 12-plexes.)

† These percentages compare design results to the original 1000 assays that passed ProxSNP.
Figure 5 shows multiplexed reaction performances using the new experimental conditions described in this application note (i.e., new PCR/hME conditions with hME-10 tags). Individual 12-plex first-pass success rates varied from 84 to 96% with accuracies from 98.7 to 100%. The overall average first-pass call rate and accuracy obtained (90 and 99.7%, respectively) are in agreement with a previous study conducted, using the MassARRAY system, by the Whitehead Institute Center for Genome Research performed at lower plex-levels. They estimated their accuracy rate to be 99.6% [4].

Viewing our results as a whole, the first-pass accuracy is estimated to be 99.7% (Figure 5, All 12-Plexes). Inaccuracies in a multiplex can be attributed to particular, individual assays. Filtering out weak assays improves accuracy. For instance, 12-plex #2 produced 6 errors. These errors were generated by only two problematic assays. Filtering these two assays out of the data for 12-plex #2 improved calls from 90 to 97% and accuracy from 98.7 to 99.5% (Figure 5, 12-Plex #2).

Performance variability between multiplexes is attributed to unpredictable behavior of individual assays when combined at higher plex-levels. Out of the 84 assays used in this study, 10 exhibited significantly weaker extension rates as compared to the uniplexing format. These assays provided lower calling rates and were more prone to generate errors. Filtering out these 10 assays from the data improved calls from 90 to 97% and accuracy from 99.7 to 99.8% (Figure 5, All 12-Plexes).

Note We found that such assays with weaker extension rates do not behave randomly and, therefore, problematic assays can be detected and filtered out for further reactions/analysis.

Typer’s automated calling algorithm encounters greater challenges as plex-level increases. Higher plex-levels generate spectra of higher complexity. The increased amounts of analytes in multiplexes may lead to lower signal-to-noise ratios because there is increased competition between analytes. This effect can be at least partly alleviated by using higher concentrations of

**Figure 5. Performance of New Conditions**

These are averages of six replicates performed on seven previously genotyped genomic DNA samples. Individual 12-plex performance is shown along with the average performance of all seven 12-plexes. For each 12-plex there are two pairs of bars (left pair = first-pass, real-time results; right pair = weak-assay-filtered results). Within each pair, the left bar represents calls and the right bar represents accuracy. **Calls:** The average percentage of successful calls. **Accuracy:** The average percentage of reproducible calls compared to previous genotype data obtained from independent, uniplexed, hME reactions done in quadruplicate. Standard deviation is indicated at the top of each bar. These results were obtained by Typer 3.0.1. Note: As an example, the reference SNP IDs and oligonucleotide sequences for one of these 12-plexes (#7) are listed in the “Materials” section of this application note.
specific hME primers (see “Adjusting MassEXTEND Primer Mixes” on page 3). For best results, you should always make sure your MassARRAY instruments receive all routine maintenance and optimization.

**Note**  The spectra baselines may not appear perfectly flat. This is a normal characteristic of spectra for high-level multiplexes.

In addition to weak primer extension in particular assays, errors can also stem from biased amplification of some SNPs—an effect observed in uniplexing and enhanced in high-level multiplexing. Coupled with high-mass assays, some heterozygous SNPs were called homozygous because a second allele peak was not detectable. Among the 4,032 genotypes obtained, 16 errors were attributable to only 8 assays. 12-plex #2 produced 6 errors generated by only two problematic assays (due to biased amplification). As mentioned earlier, problematic assays may be detectable through cluster and Hardy-Weinberg equilibrium analyses.

**Note** Typer 3.0.1 takes longer to process multiplexes than uniplexes. Calling occurs real-time as spectra are acquired. Because multiple calls are made on each pad, you may notice the MassARRAY Analyzer (mass spectrometer) dwells on each SpectroCHIP pad longer than it does when processing uniplexes. A new version of Typer is forthcoming that greatly improves processing time.

**Conclusion**

Multiplexing the hME assay provides

- Higher throughput
- Lower cost per genotype

In this application note, we present a generic hME protocol for high-level multiplexing. The conditions described were found to be the most robust, offering the best performance over the majority of assay combinations tested. It is possible to fine-tune the protocol to individual reactions; however, that would limit its versatility.

Using the conditions described, it was possible to combine up to 12 assays with over 90% success—both in the number successfully combined into 12-plexes and the number of genotypes (calls made by Typer, first pass). While 12-plexes are discussed, the protocol presented here is applicable to any multiplex-level up to 15-plex.

**Contributing Authors**

Martin Beaulieu, Ph.D. and Paul Hong

**References**


**Materials**

**Primers**

Oligonucleotide sequences and reference SNP IDs for 12-plex #7 are listed in Table 4. The criteria for the selection of SNPs were based on minor allele frequency of at least 20% and availability of at least 400 bp of flanking sequences.

**Genomic DNA**

Seven individual genomic DNAs were isolated from 10 mL of buffy coat samples using the PUREGENE® kit (Gentra Systems, Inc.). The samples were purchased from the San Bernardino, CA blood bank. Ethnicity and sex are unknown.

**Instruments**

- MassARRAY™ Liquid Handler (SEQUENOM catalog #11230)
- MassARRAY™ Nanodispenser (SEQUENOM part #10024 or #10026)
- MassARRAY™ Analyzer (SEQUENOM catalog #00450)
**Software**

- MassARRAY™ Typer 3.0.1 or higher (SEQUENOM catalog #11406)
- MassARRAY™ Assay Design 2.0 (SEQUENOM catalog #11452)
- ProxSNP and PreXTEND (available late 3rd quarter 2003 through SEQUENOM’s RealSNP® Assay Database (www.realsnp.com))

**Consumables**

**PCR**

- HotStar Taq® (QIAGEN)
- HotStar Taq® PCR buffer (QIAGEN)
- dNTPs (Obtain from oligonucleotide supplier)
- PCR primers (Obtain from oligonucleotide supplier)

**hME**

- MassEXTEND™ Starter Kit (SEQUENOM catalog #10030)
- Homogeneous MassEXTEND™ Mix (SEQUENOM catalog #10035-10051)
- MassEXTEND™ primers (Obtain from oligonucleotide supplier)
- Thermo Sequenase™ (SEQUENOM catalog #10052)
- Clean Resin (SEQUENOM catalog #10053)
- Clean Kit (SEQUENOM catalog #11220)
- Shrimp Alkaline Phosphatase (SAP) (SEQUENOM catalog #10002)

**SpectroCHIP® Bioarrays**

- 384-well SpectroCHIP® (SEQUENOM catalog #00601)

**Clean Resin Dimple Plate**

- 6 mg Dimple Plate (SEQUENOM catalog #11235)

**TABLE 4. Reference SNP IDs and oligonucleotide sequences for 12-plex #7 (the terminator mix used is ACT)**

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>2nd PCR Primer</th>
<th>1st PCR Primer</th>
<th>AMP (bp)</th>
<th>MassEXTEND Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>170447</td>
<td>ACGTTGGATGAAGACCCACACCTCTCCATG</td>
<td>ACGTTGGATGGCTGGATGGTGTAAAGGG</td>
<td>109</td>
<td>CTCAAGCCCTGCCACCC</td>
</tr>
<tr>
<td>108843</td>
<td>ACGTTGGATGAATCATCTGGAATGACACACG</td>
<td>ACGTTGGATGACAGTGAACCTACTGATTAGG</td>
<td>94</td>
<td>GCCATCAACACCGCCGCC</td>
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<tr>
<td>35856</td>
<td>ACGTTGGATGACTTGAGGAAACCAGCCAGAC</td>
<td>ACGTTGGATGGGCTTTGACCTACGATGTG</td>
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<td>CCAGGGCAAGGCTCTCT</td>
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<tr>
<td>39524</td>
<td>ACGTTGGATGGGTATCTTGGAAGACAGC</td>
<td>ACGTTGGATGACAGTGAACCTACTGATTAGG</td>
<td>100</td>
<td>GTTTGAGACTGATGCA</td>
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<tr>
<td>33234</td>
<td>ACGTTGGATGAAAGGTCACAAATACGCTCTC</td>
<td>ACGTTGGATGGGCTTTGACCTACGATGTG</td>
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<td>AATACGCCCCTTGCTCT</td>
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<tr>
<td>174529</td>
<td>ACGTTGGATGGAATGCTGACGCCTTGAGG</td>
<td>ACGTTGGATGGCCTCGATTCTTGCACGAG</td>
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<td>CTGTTGGGCCACCCCAGG</td>
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<tr>
<td>90951</td>
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<td>AGATGGGCGGAGGCCAGAG</td>
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<tr>
<td>174550</td>
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<td>193915</td>
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<td>201422</td>
<td>ACGTTGGATGAGAATGCTACAACACCCG</td>
<td>ACGTTGGATGGGCCTTTGACCTACGATGTG</td>
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<td>TTTTGAGACTGATGCA</td>
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<td>171012</td>
<td>ACGTTGGATGGAATGCTGACGCCTTGAGG</td>
<td>ACGTTGGATGACAGCCTACCTTCTTCC</td>
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<td>AGTACGTTGGGCCACCCCAGG</td>
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<tr>
<td>180870</td>
<td>ACGTTGGATGACTGACAGAGATCCCTTGGCC</td>
<td>ACGTTGGATGACAGCCTACCTTCTTCC</td>
<td>106</td>
<td>TTATATATATCTCACCCAGACAG</td>
</tr>
</tbody>
</table>

Note: Boldfaced portions of the PCR primers are the hME-10 tags.
Ordering Information

To order, or obtain information about, SEQUENOM products contact us at (877) 4GENOME (443-6663) toll-free in the United States.

Additional contact information, including international contact information, is listed at the bottom of this page.

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The optimized conditions provided herein are covered by a pending US patent.

Manufacture or remanufacture of SpectroCHIP solid supports used in conjunction with the MassARRAY System and/or detection of a nucleic acid sequence by mass spectrometry, (and other aspects as claimed) is covered by one or more claims of United States Patent Nos.: 6,569,385; 6,500,621; 6,300,076; 6,258,538, and foreign equivalents. Other U.S. and foreign patents pending.

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PUREGENE is a registered trademark of Gentra Systems, Inc.

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