DNA preparation for Sequenom MALDI-TOF based human SNP genotyping using the *prep*GEM™ Storage card blood kit.

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Introducton

Completed sequences of the human genome have provided biomedical researchers with opportunities to investigate genetic diversity in academic, clinical and commercial areas. Single nucleotide polymorphisms (SNPs) are generally accepted as the most important markers owing to their abundance and simplicity; thus rendering straightforward assay design and scoring (1). They are useful in large studies such as linkage analysis, pharmacogenetics and genome-wide association studies. A platform for simultaneous, accurate analysis of multiple SNPs from a single sample is imperative.

Sequenom's iPLEX Gold and MassARRAY matrix-assisted laser desorption / ionization time of flight (MALDI-TOF) mass spectrometry (MS) technology allows for high throughput, accurate and real-time scoring of up to 40 SNPs from DNA (or cDNA) of an individual (2). The method is based on annealing of an oligonucleotide primer (hME primer) adjacent to the SNP of interest. Addition of a terminator nucleotide allows extension of the primer through the SNP site and generates allele-specific extension products; each with unique molecular mass that are then analyzed and assigned genotype using MS in real time (2). Owing to the sensitivity of the reaction and instrument, any inhibitors from upstream reactions can lead to generation of false data or no data.

Since a large number of samples is required to generate significant data, reagent costs can become prohibitory for research. Thus, a low cost and efficient DNA extraction method is required that produces good quality DNA template for downstream analysis.

This paper describes DNA preparation using *prep*GEM and a downstream post-PCR cleanup kit for Sequenom MALDI-TOF MS genotyping.

Methods

Preparation of cards and punches

A Pin-prick blood of an anonymized healthy individual was spotted onto a Whatman FTA classic card. The card was allowed to air dry and was stored for one week at room temperature in dry and dark conditions. Two 1.2 mm^2 punches were made using a Harris micro punch.

prepGEM Storage card blood DNA extraction

1. 100 µl of water was added to the punches. Samples were briefly vortexed and placed at room temperature for 15 minutes.
2. The tubes were vortexed a second time and the water was aspirated with pipette.
3. The punches were resuspended in:

   44 µl water
   5 µl ZyGEM MAGENTA 10x buffer
   1 µl prepGEM

4. Tubes were placed in a thermal cycler and heated at 75°C for 15 minutes followed 95°C for 5 minutes.
5. Tubes were centrifuged at 16,000 x *g* for 5 minutes.
6. The supernatant was decanted into new tubes.
7. Extracted genomic DNA was quantified using Invitrogen Quant-iT ™ dsDNA PicoGreen.

Primer design

PCR and hME primers were designed for a multiplex reaction of 10 SNPs using Sequenom's MassARRAY assay design v4.0 (Table 1).

NUMBER	SNP NUMBER	PCR PRIMER	hME PRIMER
1	rs61742245	F-ACGTTGGATGTTAGTGCTCTCGCTCTACGC R-ACGTTGGATGAAGACGCGCGAACAGCTGAT	CGCGCGGTAATCCCGGT
2	rs11676382	F-ACGTTGGATGGCCGCAGGTAAGTTCACAAC R-ACGTTGGATGTCTAGAGTTACTCTCCCCAG	AGGGGAAAGTTACCAAG
3	rs1799853	F-ACGTTGGATGCAGTGATATGGAGTAGGGTC R-ACGTTGGATGCTGCGGAATTTTGGGATGG	AAGAGGAGCATTGAGGAC
4	rs17708472	F-ACGTTGGATGGCCCGGCCCTTAAGTAATTC R-ACGTTGGATGCCCAGTCTCTGATGCAAAAC	ACCGAGTGAACCGTTATAC
5	rs339097	F-ACGTTGGATGTCTGTCTTTCCCCTTTAGCC R-ACGTTGGATGCCTTGGATTCTGAATCTGGC	CTGAATCTGGCCAATACTTA
6	rs28371686	F-ACGTTGGATGACATGCCCTACACAGATGCT R-ACGTTGGATGTGTCACAGGTCACTGCATGG	CGCGGTCCAGAGATACATTGA
7	rs2242480	F-ACGTTGGATGTGCTAAGGTTTCACCTCCTC R-ACGTTGGATGGCAGGAGGAAATTGATGCAG	ACCCAATAAGGTGAGTGGATG
8	rs9332131	F-ACGTTGGATGACATGAACAACCCTCAGGAC R-ACGTTGGATGCAAGCAGTCACATAACTAAGC	AGCTTTTGTTTACATTTTACCT
9	rs7294	F-ACGTTGGATGAAAAAAGAGCGAGCGTGTGG R-ACGTTGGATGTTCTAGATTACCCCCTCCTC	TTACCCCCTCCTCCTGCCATACCC
10	rs3814637	F-ACGTTGGATGCGACAATACTTACACAAAGCC R-ACGTTGGATGAGAGAACTGGAAATAACCTC	CTCATTAGGAAATTTAGAACAAATA

Table 1: PCR and hME primers designed using Sequenom's MassARRAY assay design v4.0.

PCR, cleanup and Sequenom analysis

100 µl multiplex PCR reaction was carried out using Qiagen Hot Star Plus *Taq* DNA polymerase according to the protocol described in ref. (2). Cycling conditions were as follows: 95°C, 5 min; (95°C, 30 sec; 56°C, 30 sec; 72°C, 30 sec) x 45; 72°C, 3 min; 4°C, infinite. Successful amplification of DNA was confirmed by visualization on Invitrogen 2% E-gels. The remaining 80 µl of the amplified sample was divided in two equal volumes; one was tested on the Sequenom platform as per the manufacturer's procedure without and the other with post-PCR purification using silica columns.

Results and Discussion

Extracted genomic DNA quantity measured using PicoGreen was found to be 238.6 pg/µl. Ten SNP multiplex PCR amplification products were visualized using an Invitrogen 2% E-gel (Figure 1).

The PCR amplicons without PCR cleanup provided a "bad spectrum" on the Sequenom platform and so no genotype calls were made by the MassARRAY TYPER 4.0 software (Figure 2A). In contrast, when the PCR amplicon from the same PCR reaction was purified using the Qiagen QIAQuick PCR purification kit, successful allele calls were made for all ten SNPs (Figure 2B).

The purpose of the post-PCR cleanup rather than a pre-PCR cleanup is to reduce sample loss and potential amplification bias. The sensitivity of the instrument makes it sensitive to residual inhibitors from the nucelic acid extraction (for example cell debris and denatured proteins that might not be removed during the Sequenom sample cleanup procedure). These can interfere with the MS analysis. Post PCR cleanup is therefore recommended.

Conclusions

The ZyGEM extraction kit provides a simple, less error-prone, low cost and rapid DNA extraction method. It yields genomic DNA that, performs well in polymerase-based reactions such as PCR. Residual inhibitors from the extraction steps should be removed using post-PCR cleanup to provide high quality genotype data with the Sequenom platform.

References

1. Niels Storm, Brigitte Darnhofer-Patel, et al. (2003). Methods in Molecular Biology: MALDI-TOF Mass Spectrometry Based SNP Genotyping. Totowa, Humana Press Inc.
2. Martina Bradic´, João Costa, et al. (2011). Methods in Molecular Biology: Genotyping with Sequenom, Springer Science+Business Media.

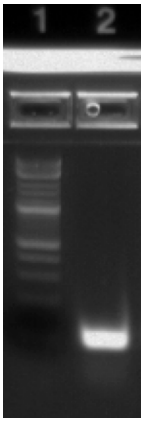

Figure 1:

Ten SNP multiplex amplification products amplified from the DNA extracted with prepGEM Storage card blood and analysed on 2% Invitrogen E-gel (20 µl of PCR product run without post-PCR cleanup).

Lane 1: 2 log 10 kb DNA ladder.

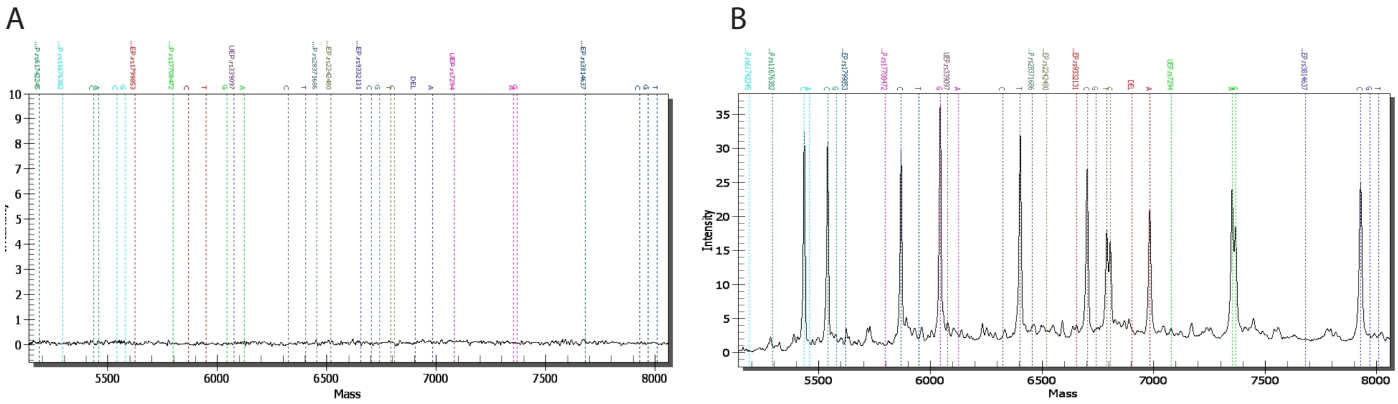

Figure 2: Sequenom MALDI-TOF multiplex genotype analysis of 10 SNPs without post-PCR cleanup (A) and with PCR cleanup using silica columns (B).