A Rapid, Sample-Safe, Evaporative Technique for Preparation of DNA and Oligonucleotide Samples

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The difficulties of concentration of oligonucleotides and especially tagged oligonucleotides are well documented in the literature[1]. Adverse conditions can damage the sample, and in some cases totally degrade it. When sourcing a concentration method for their microtitre plates containing oligonucleotides (oligos), researchers at Wellcome Trust Centre for Human Genetics (WTCHG) were careful to choose an evaporator system that would not only provide fast drying, but also take good care of their samples.

Concentration and Microarray Production

The Microarray team at WTCHG run a high throughput custom microarray platform studying mouse and rat gene expression. For these experiments, total RNA is harvested from tissue or cells, amplified, labelled and hybridized onto oligo printed slides. The experiments show the differential expression between a control and the study sample. It is in preparing the oligo printed slides that a problem arises.

The oligo printed slides are typically prepared using 70mer oligos stored in over thirty 384 well plates. The 384 well plates contain oligos resuspended in a volatile phosphate buffer, the buffer causes an unknown volume of water to evaporated during the Microarray printing process. This does not affect the printing process, but may affect subsequent printing, as the oligos are at a higher and unknown concentration than before. The WTCHG considered the optimum method of ensuring a known concentration, is to dry the plates between each printing run, and to resuspend the oligos to a known concentration when required. The oligo plates are stored dry in between runs.

WTCHG had an elderly vacuum concentrator which could accept only two plates at a time, and took up to 3 hours to dry them. Therefore it took two weeks to dry all the plates after each run. To preserve the integrity of the plates awaiting drying these were frozen at –18°C, and thawed when the concentrator was available. It is well documented in the literature that freeze thaw cycles can be detrimental to oligo quality and can cause degradation. WTCHG began to search for a new high throughput concentrator which could keep up with their microarray production. The system they selected was the Genevac EZ-2 personal evaporator (Figure 1). The EZ-2 can take eight plates per run, and dries them in just over an hour, meaning that all the plates for a microarray printing run can be dried within one day. Samples waiting drying are kept in the fridge at +4°C, eliminating the need to freeze, and preventing damage to samples.

Figure 1 – EZ-2 personal concentrator

In this article researchers at WTCHG report upon that they have more confidence in the quality of their oligos, and believe that they are of higher quality, now that they have introduced state of the art concentration processes. These findings support the work of Knight[2] who discusses the importance of correct drying for best results within MALDI target production.

High Density Microarray Preparation

Another team at WTCHG are studying hereditable disease by analysing Single-Nucleotide Polymorphisms (SNPs). SNPs are one-letter changes in the DNA sequence, which vary from individual to individual. Many have no effect; others cause differences in countless characteristics, and may increase the risk of certain diseases. When it has been established that a particular pattern of SNPs is associated with a disease, a microarray of those SNPs can be used to determine whether an individual is at risk of developing that illness. In the procedure for the detection of the disease the genomic DNA of the individual is hybridized to the SNP array and will bind with greater frequency with the SNPs associated with that person, the array is then visualized by fluorescence. The presence of the SNPs in the genomic DNA indicates that the person is susceptible to the disease.

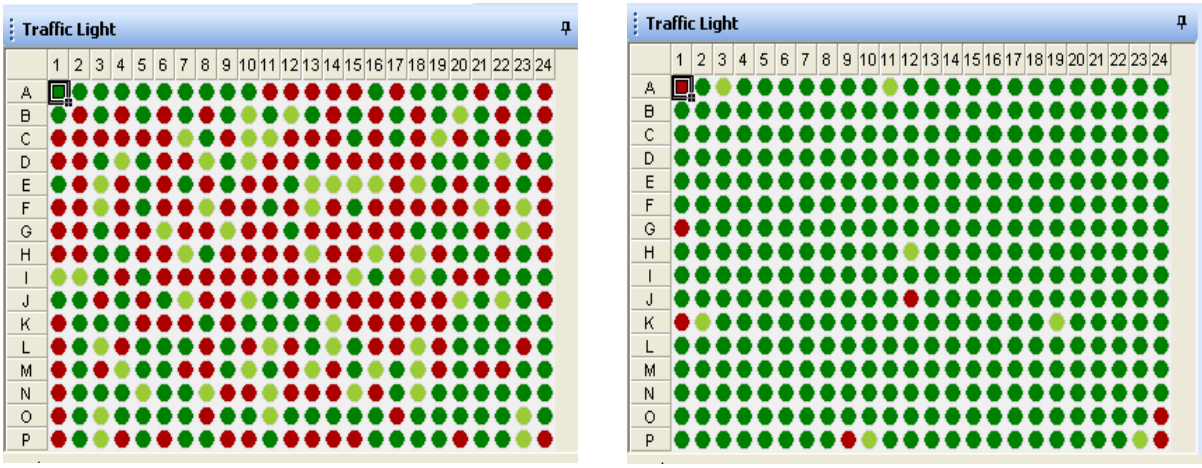
In the identification of the disease threat it is clearly imperative that the researcher has confidence in the quality of the array, this integrity of product is dependant on the way the detection array is synthesized and constructed. One of the key steps in this process is achieving a concentration of the cDNA in the array that will give a signal when visualizing. As this study will detail, the technique used during concentration of the sample may be damaging, contributing to inaccuracies and/or low yields.

To study SNPs, WTCHG operate a state of the art Sequenom MassARRAY® SNP Genotyping system. MassARRAY® is a high throughput, high fidelity system for SNP analysis within genotyping studies which performs all steps of the assay and analysis in the one system. The technology employed requires very small volumes of sample, specifically 5µl, and containing as little as 2.5ng of material. The details of the Sequenom MassARRAY® method can be found at www.sequenom.com .

The Sequenom system is very specific in its requirements for sample preparation. To achieve the required volume of 5µl some samples needed to be concentrated, this used to be achieved by air drying the samples over a number of days. Using the Genevac EZ-2 personal evaporator these samples are concentrated aseptically within the hour, saving time and reducing risk of contamination.

Methods using the EZ-2 evaporator were compared to the previous standard method where plates were air dried over a period of time. Plates containing identical samples were dried using each method and then analysed using the MassEXTEND reaction – details available via **www.sequenom.com**. The duplex pass rate for each well is represented in the traffic light diagram in Figure 2 (a), where dark green shows good data, light green mid quality data, and red shows poor or no data. The results of a 2-plex assay looking for two different SNPs within the same plate are shown in Figure 2 (b). The number of "no calls" – i.e. no data, has significantly reduced on both assays. The quality of results achieved following drying on the EZ-2 evaporator are clearly better than achievable with simply air drying in this case.

Figure 2(a) - Sequenom Sample Quality analysis following air drying (left) and drying on EZ-2 evaporator (right).



Key: Dark Green – high sample data quality
Light Green – medium sample data quality
Red – poor sample quality or no data

Figure 2 (b) – Dual SNP analysis following air drying (left) and drying on EZ-2 evaporator (right)

Air Drying

Genotype	Assay 1 (%)
G	0.30
T	33.07
GT	7.03
no calls	59.64

Genotype	Assay 2 (%)
G	27.60
T	5.99
GT	20.83
no calls	45.57

Dried in EZ-2

Genotype	Assay 1 (%)
G	0.78
T	77.60
GT	19.79
no calls	1.82

Genotype	Assay 2 (%)
G	44.53
T	8.07
GT	35.68
no calls	11.72

Summary

When working with DNA and oligo samples great care needs to be taken at every stage to ensure that degradation does not occur, and the highly potent samples are not contaminated. Use of state of the art concentration systems can significantly speed up concentration rates, saving researchers time, and also eliminate potential sources of damage to samples.

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