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Introduction

BGL currently offers the Agilent 8x60K ISCA oligonucleotide array from either Oxford Gene Technology or BlueGnome™. EZ1 extracted DNA was shown to be the method most suitable for use on a BAC array platform whereas the standard Qiagen Autopure LS™ system compromised whole blood protocol was shown to be unsuitable. This meant all samples referred for array CGH or possible future array CGH received two Qiagen EZ1 extractions and a single extraction using the compromised whole blood protocol as EZ1 extractions have low DNA yields, insufficient if several molecular tests were required. This complexity of the decision process for DNA extraction meant that DNA from some samples was extracted using the wrong method. The DNA QC pass rate for EZ1 extracted DNA was 92.5% and the array pass rate, 93% (overall pass rate 88%) which lead to several requests for repeat samples. EZ1 extracted DNA frequently produced artefactual calls on an oligonucleotide array platform which increased the amount of follow up work and therefore reporting times.

In order to increase the array success rate and to consolidate extractions across the department so that a single DNA extraction method was used for all referrals an alternative DNA extraction method from the Autopure LS™ system called the 2PC compromised whole blood protocol was tested. The compromised whole blood protocol adds both the protein precipitation buffer and cell lysis buffer at the same time while the 2PC method separates this step into two separate steps with an incubation period and centrifugation step in between.

Methods

56 samples were selected from blood samples referred for array CGH only. All samples received two EZ1 DNA extractions and a single DNA extraction using the Autopure LS™ system 2PC protocol. All blood samples were frozen prior to DNA extraction until enough samples were received for a single run on the genra. Each DNA sample was assessed by spectrophotometric measurement using the NanoDrop 1000. The OD260/280 ratio should exceed 1.8 and the OD260/230 ratio should be greater than 2.0 for a sample to be considered suitable for aCGH. It was noted after several arrays that the quality of the arrays had greatly improved so the QC criteria for the 2PC extractions was reduced to include any sample with a OD260/280 ratio exceeding 1.5 and a OD263/230 ratio exceeding 1.7. All 2PC extracted DNA was analysed on a 1% agarose gel to check the integrity of the DNA. All samples extracted by the 2PC method that passed the QC criteria and the reduced QC criteria were used for array CGH in preference to the EZ1 extracted DNA. Array pass rate was assessed as a DLRS value of <0.20 and a standard deviation of <0.17.

Results

Table 1: Comparison of mean quality data for EZ1 and Autopure LS™ extracted DNA

DNA extraction method	Mean conc. ng/µl	Mean OD260/280	Mean OD260/230
EZ1	127	1.83	4.21
2PC compromised whole blood	317	1.88	1.99
*Compromised whole blood	316	1.85	1.71

* Quality data was taken from archived data.

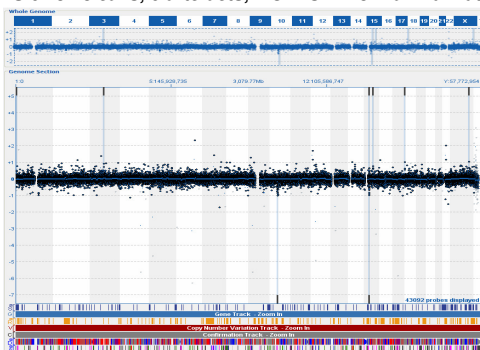
Table 2: Comparison of QC pass rate of EZ1 and 2PC extracted DNA

	EZ1 Extracted DNA	2PC extracted DNA
No. samples passed DNA QC	25	36
% of samples passed QC	67%	64%
No. samples passed reduced criteria DNA QC	-	53
% samples passed reduced criteria DNA QC	-	95%
No. of samples passed array QC	-	100%
Total No. of samples	49*	56

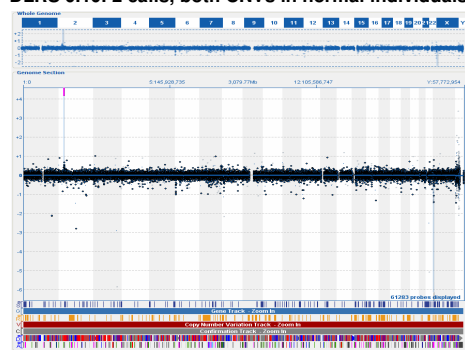
*Some samples had insufficient volume for three separate extractions. 2PC extraction prioritised.

Figure 1: Typical images from arrays analysed on cytore v3.4.3 from EZ1 and 2PC extracted DNA

A: EZ1 extracted DNA
 DLRS 0.13. 8 calls, 6 artefacts, 2 CNVs in normal individuals



B: 2PC extracted DNA
 DLRS 0.10. 2 calls, both CNVs in normal individuals



Conclusions

- By separating the cell lysis and protein precipitation steps the DNA extracted using the 2PC protocol increased DNA QC pass rate to 95% and array pass rate to 100%. This is in line with expected pass rates for other diagnostic genetic tests.
- The quality of the array data has improved with fewer artefactual calls reducing the analysis time for each case and reducing the amount of follow up work required to confirm array findings.
- As of 21st March 2011, all blood samples with a sufficient volume received for constitutional cytogenetic and molecular genetic testing across the department are extracted using the 2PC method. This means that the whole DNA extraction process is simplified to a single DNA extraction method, reducing the cost to the department in staff time and consumables. It also means that clinicians requiring array CGH testing on stored DNA where possible future array CGH testing was not mentioned on the original referral will not have to re-bleed a patient if the DNA was extracted by a method unsuitable for array CGH.

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