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Molecular analysis of single circulating tumour cells following long-term storage of clinical samples

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ABBREVIATIONS

CTC: circulating tumor cell; **TC**: tumour cell; **WBC**: white blood cell; **WGA:** whole genome amplification; **SD:** standard deviation

ABSTRACT

The CellSearch® semi-automated CTC enrichment and staining system has been established as the "gold standard" for CTC enumeration with CellSearch® CTC counts recognized by the FDA as prognostic for a number of cancers. We and others have gone on to show that molecular analysis of CellSearch® CTCs isolated shortly after CellSearch® enrichment provides another valuable layer of information that has potential clinical utility including predicting response to treatment. Although CellSearch® CTCs can be readily isolated after enrichment, the process of analysing a single CellSearch® patient sample, which may contain many CTCs, is both time consuming and costly. Here we describe a simple process that will allow storage of all CellSearch® enriched cells in glycerol at -20ºC for up to 2 years without any measureable loss in the ability to retrieve single cells or in the genome integrity of the isolated cells. To establish the suitability of long term glycerol storage for single cell molecular analysis we isolated individual CellSearch® enriched cells by DEPArray™ either shortly after CellSearch® enrichment or following storage of matched enriched cells in glycerol at -20ºC. All isolated cells were subjected to whole genome amplification (WGA) and the efficacy of single cell WGA was evaluated by multiplex PCR to generate a Genome Integrity Index (GII). The GII results from 409 single cells obtained from both "spike in" controls and clinical samples showed no statistical difference between values obtained pre and post-glycerol storage and that there is no further loss in integrity when DEPArray™ isolated cells are then stored at -80ºC for up to 2 years. In summary, we have established simple yet effective „stop off" points along the CTC workflow

enabling CTC banking and facilitating selection of suitable samples for intensive analysis once patient outcomes are known.

1. INTRODUCTION

Molecular analysis of tumours has led to the identification of mutational drivers of cancer and the development of targeted therapies, which when used to treat stratified patient cohorts have been shown to lead to significant improved response rates (Sharma et al., 2007; Sosman et al., 2012; Vogel et al., 2002). The molecular characterisation of tumours also has the potential to give insight into mechanisms of resistance to therapies and tumour heterogeneity (Burrell & Swanton, 2014; Carter et al., 2017; Casassent et al., 2017; Jamal-Hanjani et al., 2015; Navin et al., 2015). Circulating tumour cells (CTCs) found in the blood of cancer patients are being evaluated for clinical utility as a source of liquid biopsy that facilitates molecular characterisation of the patient‟s disease (Carter et al., 2017; Diaz & Bardelli, 2014; Fusi et al., 2013; Krebs et al., 2014; Rothwell et al., 2016). Use of a simple blood collection and CTC analysis has the added benefit of ease of sample collection as well as the possibility of repeat sampling. CTC number is associated with prognosis, response to chemotherapy and identification of disease recurrence in several types of cancer including lung, colorectal, breast and prostate (Attard et al., 2011; Cohen et al., 2008; Cristofanilli et al., 2004; Crosbie et al., 2016; de Bono et al., 2008; Krebs et al., 2011; Miller et al., 2010; Rack et al., 2014; Smerage et al., 2014; Vargas & Harris, 2016). Recently, CTCs have been used to infer the genetic status of the patient's tumour and to identify a molecular profile linked to response to therapy (Carter et al., 2017; Lowes et al., 2015; Neal & Lilja, 2016). Downstream molecular analysis of isolated CTCs can also give greater insight into intratumour heterogeneity and identify specific signalling pathways that could be explored in the clinical setting.

However, detailed analysis of CTCs presents difficulties; the paucity of CTCs in blood, associated enriching processes and downstream molecular analyses are technically challenging and expensive. A variety of technologies have been developed to enrich CTCs that commonly rely on cell surface antigen expression or on physical properties including cell size and deformability that distinguish CTCs from blood cells (Alix-Panabieres & Pantel, 2014; Alix-Panabieres & Pantel, 2016; Chudziak et al., 2016; Ferreira et al., 2016; Hyun et al., 2016; Pantel, 2016). CellSearch® is a FDA approved epitope dependent platform capable of CTC enrichment and enumeration. To enable single cell analysis enriched samples require further manipulation on platforms such as the DEPArray™ (Silicon Biosystems), a microfluidic system that combines high resolution imaging with the dielectrophoretic

movement of fluorochrome labelled cells enabling selection as well as isolation of individual cells. We and others demonstrated the utility of a combined CellSearch® and DEPArray™ workflow to enable molecular analysis of CTCs (Carter et al. 2017; De Luca et al., 2016; Hodgkinson et al., 2014). Although, effective, the isolation of CTCs using the combined CellSearch® and DEPArray™ approach is both time consuming and costly making it impractical for routine analysis of all CellSearch® enriched samples. Furthermore, it is often not clear which clinical samples will warrant CTC isolation and detailed molecular analysis. For example, in the course of a project or clinical trial it may be that all pre-treatment samples are collected but only a sub-set will be of interest based on a patient‟s response to treatment.

To make the CellSearch® and DEPArray™ approach more flexible and compatible with clinical trials, we have established a simple yet robust workflow (Figure 1) which allows banking of either the entire CellSearch® enriched sample prior to cell isolation, or individual CTCs after DEPArray™ single cell isolation. We show that banked samples can be stored for over a year without any adverse impact on single cell whole genome amplification (WGA) and subsequent molecular analysis.

2. MATERIAL AND METHODS

2.1. Blood Collection

Blood samples were collected in CellSave® vacutainers and transferred to the Clinical and Experimental Pharmacology laboratory for processing within four days of blood draw. All samples were collected following receipt of informed consent in compliance with the approved by the local Research Ethics Committee (REC reference 07/H1014/96; REC reference 13/LO/1546; MCRC Biobank Research Tissue Bank Ethics (ref: 07/H1003/161+5)).

2.2. Cell culture

Human colon carcinoma cell line LS174-T was obtained from the ATCC collection and maintained as recommended. Briefly, LS174-T cells were maintained in RPMI media, supplemented with 10% (v/v) fetal calf serum (FCS), penicillin (100 U/mL) and streptomycin (100 U/mL). Cells were maintained in a humid atmosphere at 37°C with 5% CO2. Cell survival and counting were determined by a trypan blue exclusion assay.

One thousand tumour cells (TCs) were spiked into a healthy normal volunteer (HNV) 7.5 mL of blood collected in CellSave tubes.

2.3. CellSearch® Enrichment

Circulating tumour cells enumeration was performed on a CellSearch® system (Menarini Silicon Biosystems, Inc) using the CellSearch® Epithelial Cell Kit (Menarini Silicon Biosystems, Inc). In brief, 7.5 mL of blood were collected in a CellSave preservative vacutainer and stored at room temperature (RT) for up to 96 hours. Afterwards the blood samples were processed into the CellTracks® AutoPrep® system, the CellSearch® Circulating Tumour Cell Kit and the CellTracks® Analyser II (Menarini Silicon Biosystems, Inc). During the automated CellSearch® process, TCs or CTCs were immunomagnetically captured by anti-epithelial cell adhesion molecule (EpCAM)-antibody coated ferrofluid and detected by immunofluorescent staining of pan-cytokeratins and 4',6-diamidino-2-phenylindole (DAPI), as well as by negativity for the leukocyte specific antigen CD45. TCs and CTCs were identified as DAPI+/CK+/CD45- cells, while white blood cells (WBCs) as DAPI+/CK-/CD45+. After scanning, the cartridges were removed from the MagNest™ device and stored at 4°C in the dark until further processing.

2.4. Glycerol storage

Following CellSearch® enrichment samples with cell counting results between 120 and 200 cells, were resuspended, and half of the volume of the CellSearch® cartridge was stored at -20oC in 50% glycerol prior to isolation using DEPArray™. If the cell count was ≥ 200, one third of the resuspended cell solution was stored at 4ºC, one third at -20ºC in glycerol and the remaining isolated by DEPArray™ as the following description.

2.5. DEPArray™ isolation

CTCs and WBCs pre-stained with antibodies to CD45 and pan-CK and DAPI stained were aspirated from the CellSearch® cartridge used for the CTC enumeration, and single cells were isolated using the DEPArray™ system (Silicon Biosystems) as per manufacturer's instructions. After CTC enrichment, from samples with CTCs counts superior to 5 and inferior to 120, individual or pooled CTCs and WBCs were isolated by DEPArray™ and immediately stored at -80ºC. If the cell count was superior, the samples were prepared as described in the previous section (2.4 Glycerol Storage). Individual and pools of TCs, CTCs and control WBCs were isolated from samples pre and post-glycerol storage. Cells showing positive staining for pan-cytokeratin (CK), undetectable CD45 labeling and positive nuclear staining were classified as TCs or CTCs, and WBCs were detected as DAPI+/CK-/CD45+. All were subjected to whole genome amplification.

For calculation of the number of cells loaded onto the DEPArray™, a correction of the CellSearch® count for each sample evaluated pre- and post-glycerol storage was performed. This involved reducing the CellSearch® count by 30% to account for loss of sample associated with the dead volume in the DEPArray™ cartridge. The number of aliquots for each sample was then also corrected for to give a final "Expected DEPArray count" for CTC and total cell counts (Table ST1).

2.6. Whole Genome Amplification

Whole genome amplification of single or pools of TCs, CTCs and WBCs was performed using the Ampli1™ WGA kit versions 1 and 2 (Silicon Biosystems), following manufacturer's instructions to obtain a 50 µL of WGA product. The Ampli1™ WGA procedure (Silicon Biosystem, Inc.) were performed in a single tube according to manufacturer's protocol. This whole genome amplification method is based on adaptor-ligation-mediated amplification. After cells lysis, the genomic DNA was digested with *Mse*I restriction enzyme to generate sticky ends fragments, followed by ligation of a single adaptor on both overhangs of each fragment, and a fill-in reaction to complement the sequence of the adaptor. The resultant WGA PCR product (50 µL) was produced by amplification of the entire genome library with one single high specific PCR primer corresponding to the adaptor.

2.7. Genome Integrity Index (GII)

The efficacy of WGA was evaluated by a multiplex quality-control PCR (GII-PCR). Concisely, a four amplicon multiplex PCR to assess fragments of various lengths in different chromosomes (12p:91bp; 5p:108-166bp; 17p:299bp; 6p:614bp), was performed and visualized on a 1.5% (w/v) agarose gel. This quality control step allowed us to establish a Genome Integrity Index (GII) of 0-4 for each sample.

It has been observed that the number of bands amplified (0-4) is associated with the integrity of the starting material, with cells with good quality DNA presenting 3 or 4 PCR bands, whilst cells with degraded DNA present fewer bands (Polzer et al., 2014). For the mean GII values the GII value for each individual single cell was determined and used to determine the mean for the entire sample. Human genomic DNA was used as positive control.

2.8. Statistical Analysis

Tests to evaluate the normality of each group of data were performed (D´Agostino-Pearson and Shapiro-Wilk) and accordingly statistical tests were further performed. Comparisons within and

across groups were assessed by Mann-Whitney, t-test, Kruskal-Wallis, Wilcoxon and Friedman´s test (with Dunn´s correction). *p* values < 0.05 were classified as significant.

3. RESULTS

We and others have previously demonstrated the potential clinical benefit of molecular characterisation of CTCs following single cell isolation from patient blood samples (Carter et al., 2017; Diaz & Bardelli, 2014; Fusi et al., 2013; Rothwell et al., 2016). To facilitate single CTC isolation and analysis, we have established a simple yet robust workflow, which allows banking of either the entire CellSearch® sample prior to cell isolation, or individual CTCs after DEPArray™ single cell isolation (Figure 1). To address the stability of stored samples, we first compared the effect of long-term storage at -80°C on CTCs which had undergone CellSearch® enrichment, followed by DEPArray™ isolation. We examined CTCs from 22 small cell lung cancer (SCLC) patient samples that had undergone CellSearch® enrichment, followed by DEPArray™ isolation (Figure 1; Table ST1). Following storage at -80°C from 11 days (0.4 months) to 37 months DEPArray™ CTCs were subjected to WGA using Ampli1™ followed by a GII-PCR quality check for each cell (Figures 2A. and 2B.). Comparable GII values were seen in all isolated single cells, regardless of time stored at -80°C and no observable or statistical drop in GII was seen over time (Figure 2C. and 2D.). These data demonstrate that isolated single CTCs can be reliably stored at -80°C for up to 37 months with no significant detrimental effect on the genetic integrity of the cells.

To extend the flexibility of our sample processing workflow (Figure 1), we next tested whether cells could be stored post CellSearch® enrichment but prior to DEPArray™ single cell isolation. To allow storage at -20°C and also avoid cellular damage due to freezing, we tested storing samples in high concentration of glycerol at -20°C. Primary experiments used the cell line LS174-T spiked into HNV blood (1000 cells ml-1 blood) which were then enriched using the CellSearch® platform. Following enrichment all cells were harvested from the CellSearch® MagNest™ and half of the cells immediately isolated on the DEPArray® (Figure 3A.). The remaining cells were suspended in glycerol and transferred to -20°C for 24 months prior to DEPArray™ isolation (Figures 3B.). Individual isolated LS-174 cells underwent Ampli1™ WGA and the resulting products were subjected to GII-PCR to determine the GII of the cell line cells isolated pre and post-glycerol storage (Figures 3A. and 3B.). No significant difference in the GII of the individual recovered cells pre and post-glycerol storage was seen (Figure 3C.), indicating long-term storage of CellSearch® enriched samples in glycerol has no detrimental effect on the DNA integrity of the cells.

Next tested long-term glycerol storage of clinical samples. To this end we collected CTCs from SCLC patients by CellSearch® and following harvesting aliquots of each enriched sample were assessed immediately on the DEPArray™ for single CTC isolation, with the remainder of the sample being stored in glycerol at -20ºC. For the first patient the glycerol sample was stored at -20ºC for 17 months prior to DEPArray™ isolation (Patient 23; Table ST2) and there was no observable or statistically significant difference seen for post-glycerol stored cells when compared to cells isolated immediately after CellSearch® enrichment (Figure 4C). To confirm these initial findings we expanded our analysis to an additional 10 patients covering three cancer types (SCLC, NSCLC and prostate cancer) (Table ST2). For all patient samples, CTCs were enriched by CellSearch® with one aliquot of the enriched sample ran immediately on the DEPArray™ and the remaining sample stored in glycerol at -20ºC for up to 18 months prior to DEPArray™ isolation (Table ST2). Analysis of the samples showed that the mean GII post-glycerol storage remained high across all storage time points, up to 18 months post-CellSearch® enrichment (Figure 5A.). Pairwise analysis of the samples pre- and post-glycerol storage showed no detrimental effect of storage across all 11 patient samples with the post-glycerol samples showing a slight but statistically insignificant improvement in DNA integrity (Figure 5B). Analysis of the effect of storage at set time intervals of 0-6 months, 6-12 months and 12-18 months found there was no statistically significant change in the mean GII of all CTCs in clinical samples across all time points (Supplementary Figure S1).

To address whether glycerol storage could have a detrimental effect on total cell numbers we compared the total cell numbers for all 11 clinical samples following CellSearch® enumeration pre- and post-glycerol storage. No significant differences in total cell counts were observed between the CellSearch® and both pre- and post-glycerol storage results (Friedman´s test with Dunn´s correction, $p>0.05$) (Figure 6).

4. DISCUSSION

Both CTCs and circulating free DNA (cfDNA) can be readily obtained from a simple peripheral blood draw and together represent a “liquid biopsy” that holds considerable potential for providing biomarkers that can improve cancer patient management (Carter et al., 2017; Diaz & Bardelli, 2014; Fusi et al., 2013; Krebs et al., 2014; Rothwell et al., 2017). The isolation and characterisation of single tumour cells from the circulation can give an insight into the heterogeneity and genetic status of tumours, which has been associated with prognosis (Carter et al., 2017; Casassent et al., 2017; Navin et al., 2015). However, despite the promising clinical significance, the costs associated with isolation

and molecular characterisation of single CTCs is a significant obstacle. Consequently, pre-isolation storage would be advantageous, enabling careful selection of the samples to be subsequently analysed. Our aim was to establish a workflow that could be readily implemented and would allow the long-term storage of enriched CTCs samples at -20°C in glycerol.

The CellSearch® semi-automated CTC enrichment and staining system has been established as a reliable process that has been adopted worldwide and CellSearch® CTC counts have been recognized by the FDA as prognostic in a number of cancers. Although CellSearch® CTCs can be readily isolated after enrichment, the process of analysing a single CellSearch® patient sample which may contain many CTCs is both time consuming and costly so we have evaluated CTC "stop off" points where CTCs can be banked then retrieved for analysis. Our initial experiments focused on the stability of single CTCs isolated by combined CellSearch® and DEPArray™ (Figures 2, 3 and 4). Storage of isolated single cells from clinical samples at -80°C showed that there was no measurable decline in genome integrity for at least 37 months. These results demonstrate that storage of isolated cells at -80°C provides long-term stability for subsequent genomic analysis.

Having established that isolated cells can be stored for long periods at -80°C we next looked for approaches that can be used to bank CellSearch® enriched cells prior to single cell isolation. For this we identified glycerol as a potential cryoprotective agent given that it has been used in high concentrations (40-50%) for blood cells storage in clinical practice (Henkelman et al., 2015; Morris et al., 2006; Ragoonanan et al., 2013), and that it may be a superior alternative to other cryoprotectants, such as DMSO, which may adversely affect cells by exposure to strong bi-polar compounds (Best, 2015; Henkelman et al., 2015). We first examined storage in glycerol at -20°C using cells from a cell line "spiked" into HNV blood collected using CellSave® blood collection tubes then enriched via CellSearch® and subsequently stored in glycerol at -20°C for 24 months prior to DEPArray™ isolation. Data from this "spike in" experiment showed there was no statistical difference observed based on the GII of the recovered cells pre and post-glycerol storage (Figure 3C). Though these "spike in" experiments were encouraging we were aware of the limitations of using cell lines due to the intrinsic differences, both biologically and physically between cell lines and true CTCs. Therefore, to confirm the clinical utility of this approach, we subsequently extended the glycerol storage at -20°C to 11 clinical samples from three disease types and again compared the GII pre- and post-glycerol storage at -20°C. The results from the clinical samples also confirmed that glycerol storage did not have a detrimental effect on the GII of samples (Figures 4 and 5). Interestingly, paired analysis of the clinical samples showed a minor improvement in the GII of glycerol stored samples compared to freshly processed samples, though this was not statistically significantly (Figure

5B.). These results are from a limited cohort of three different cancers (SCLC, NSCLC and prostate cancer) and the effect of storage of CTCs from other disease types may differ and is part of an ongoing study in the laboratory.

One concern raised by these results was that the storage of enriched samples may lead to the loss of potentially more "fragile" cells, for example cells in the early stages of apoptosis, with only "robust" cells surviving long-term storage, which was then reflected in apparent improved GII values. This bias could be problematic as the number of CTCs in clinical samples is usually low and the loss of cells would decrease our ability to analyse patient samples effectively. However, both the total number of cells and total number of CTCs was found to be unchanged following glycerol storage demonstrating that glycerol storage was not leading to cell loss (Figure 6). Another possible explanation for the improved GII of the glycerol samples is that although the cells enriched by CellSearch® are fixed, it has been shown that glycerol has a cryoprotective effect on cells (Ragoonan et al., 2013). This additional cryoprotection may protect the cells during DEPArray™ manipulation, increasing the integrity of the cell membranes and thereby maintaining the GII of these samples.

In conclusion, the results presented in this study indicate that CellSearch® enriched samples can routinely be stored in glycerol at -20°C, prior to single cell isolation and molecular analysis. This approach greatly increases the utility of CTCs as a liquid biopsy in the clinical setting, where they have potential in addressing questions of tumour response and heterogeneity, as well as giving insight into mechanisms of resistance due to the simplicity of longitudinal collection of bloods.

5. ACKNOWLEDGMENTS

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6. AUTHORS' CONTRIBUTIONS

GB, CD and DGR designed and supervised the project. BM, DGR and GB co-wrote the manuscript, planned, ran the experiments and analysed the data. DB ran all the DEPArray™ isolations. FC, JA and LC provided additional experimental input. LC, FHB and LK provided clinical input including establishing ethical permission and patient consent for blood samples. All authors read and approved the final manuscript.

7. FIGURES

Figure 1. Schematic showing the workflow developed for the isolation, storage and genomic analysis of circulating tumour cells (CTCs) from clinical blood samples. Yellow boxes with blue text indicates stop off points where material can be stored prior to downstream processing.

Figure 2. A. Molecular analysis of isolated SCLC CTCs following short term storage. After CellSearch® enrichment and DEPArray™ isolation cells were stored at -80°C for 11 days. All cells underwent WGA and GII-PCR to determine their Genome Integrity Index (GII). The top panel shows DEPArray images of cells labelled with CK (green) and DAPI (pink) with the scale bar represents 10µm. The bottom panel shows an agarose gel of the GII-PCR products with 0-4 bands generated for four different genomic regions with different sizes (12p:91bp; 5p:108-166bp; 17p:299bp; 6p:614bp). M = 100 bp ladder. **B.** Molecular analysis of CTCs isolated from SCLC patients after enrichment by CellSearch®, isolation by DEPArray™ and storage at -80°C for 25 months. Isolated cells then underwent WGA and GII-PCR to determine GII and data presented using the same format as in panel A. **C.** Graphical representation of the mean GII of CTCs following storage at -80°C for up to 36 months for 21 SCLC patients. Linear regression analysis found no detrimental effect of long term storage on GII (blue trend line) (p=0.8429; R square=0.002011). **D.** Graphical representation of GII mean value grouped according to time stored at -80°C following DEPArray™ isolation. No statistically significant difference was seen across all groups (Mann-Whitney test, p values >0.05; error bars show SD).

Figure 3. A. Cultured LS174T cells were added to HNV blood, enriched by CellSearch® and isolated by DEPArray™. All cells underwent WGA and GII-PCR to determine the GII and data presented using the same format as in Figure 2 panel A. **B.** LS174T cells recovered from the CellSeach® cartridge and stored at -20°C in glycerol for 24 months prior to DEPArray™ isolation. Following WGA, GII-PCR was used to determine GII for each sample and data presented using the same format as in Figure 2 panel A. **C.** A comparison of the GII of individual cells enriched by CellSearch® and DEPArray™ isolated prior to glycerol storage (0 months) and stored at -20°C for 24 months in glycerol before DEPArray™. No statistically significant difference (Mann-Whitney test; p=0.860; error bars show SD) was seen.

Figure 4. A. CellSearch® was used to enrich CTCs from a SCLC patient blood sample and DEPArray™ was used to isolate CTCs from a portion of the enriched sample prior to storage in glycerol at -20°C. All isolated cells were subjected to WGA plus GII-PCR and data presented using the same format as in Figure 2 panel A. **B.** An aliquot of the same CellSearch® enriched cells described in panel A was stored in glycerol at -20°C for 17 months prior to CTC isolation by DEPArray™. All isolated cells were

subjected to WGA plus GII-PCR and data presented using the same format as in Figure 2 panel A. **C.** A comparison of the GII from cells enriched by CellSearch® and DEPArray™ isolated prior to glycerol storage (0 months), and stored at -20°C for 17 months in glycerol prior to DEPArray™ isolation. No statistically significant difference in the GII values was found following long term storage (Mann-Whitney test; p=0.820; error bars show SD).

Figure 5. A. The average GII for 11 clinical samples was established for CellSearch® enriched samples following glycerol storage at -20°C for up to 18 months. Following DEPArray™ isolation cells were subjected to WGA and GII-PCR. Linear regression analysis was applied and showed no detrimental effect of long term storage shown by the trend line (red line) (p=0.4056; R square=0.078). **B.** A comparison of the average GII of CellSearch® cells isolated by DEPArray™ pre and post-glycerol storage at -20°C. Following DEPArray™ isolation each CTC was subjected to WGA and QC-PCR to determine GII. The GII mean was not statistically significant improved in the group of samples stored in glycerol at -20°C.

Figure 6. Total countable events from CellSearch® were compared to the number of total events assessed by DEPArray™, pre and post-glycerol storage at -20°C. No statistical significant difference was seen following analysis of paired samples(Friedman´s test with Dunn´s correction, error bars show SD).

Figure S1. The graph shows pre and post-glycerol storage GII data from 11 clinical samples divided into 3 groups based the length of time samples were stored in glycerol at -20°C. In all cases the pre-glycerol storage samples are labeled 0 months and post-glycerol samples labeling according to time stored in glycerol at -20°C. There was no statistical significant difference (Wilcoxon test; p>0.05) between any of the paired samples although there was a noticeable increase in the mean GII seen for samples stored for in glycerol at -20°C for 0- 6 months.

Table ST1: Characterisation of the 22 clinical samples from small cell lung cancer (SCLC) patients detailing the number of CTCs and total cell count after CellSearch® enumeration. For each patient the time that DEParray isolated CTCs were stored at -80°C prior to WGA is shown in months.

Table ST2: Details of the 11 clinical samples evaluated pre and post-glycerol storage. Details include: type of disease; the number of CTCs; the total cell count after CellSearch® enumeration; the predicted DEParray counts (based on volumes transferred and a 30% of dead volume in the DEParray cartridge); actual DEParray total cell counts; actual DEParray CTC counts (DAPI+/CK+/CD45-) and the time in days of glycerol of storage at -20°C.

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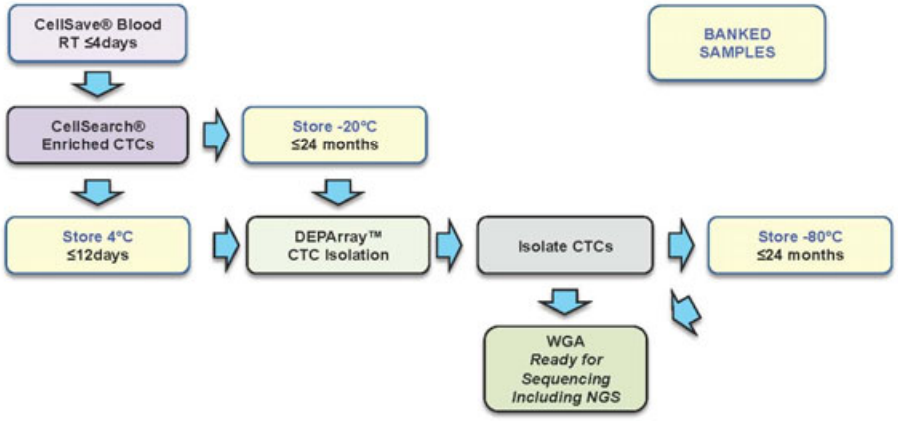
Figure 1.



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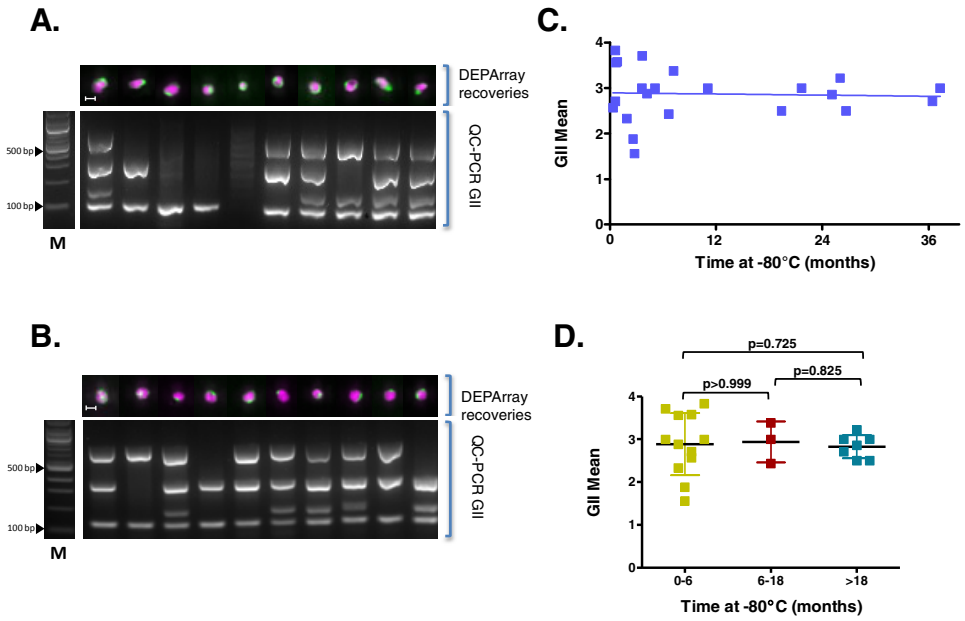

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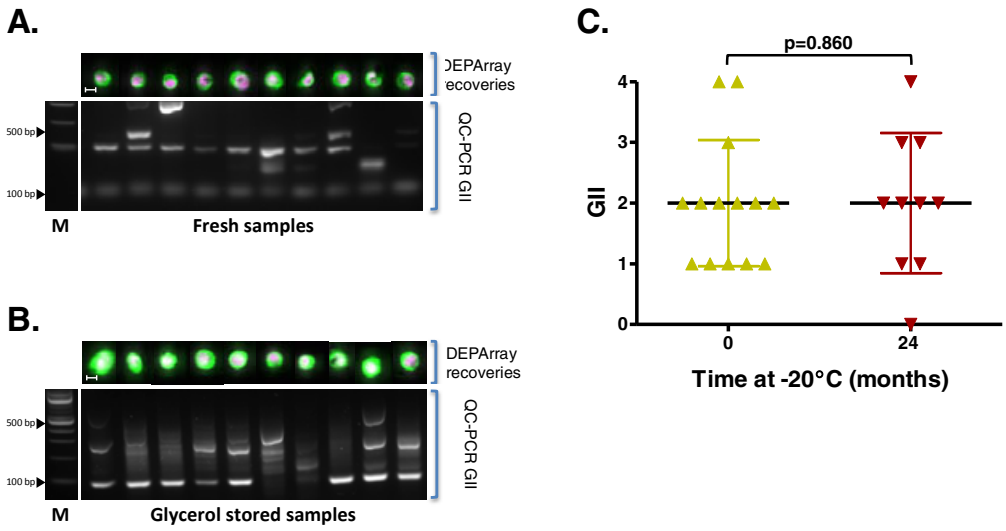

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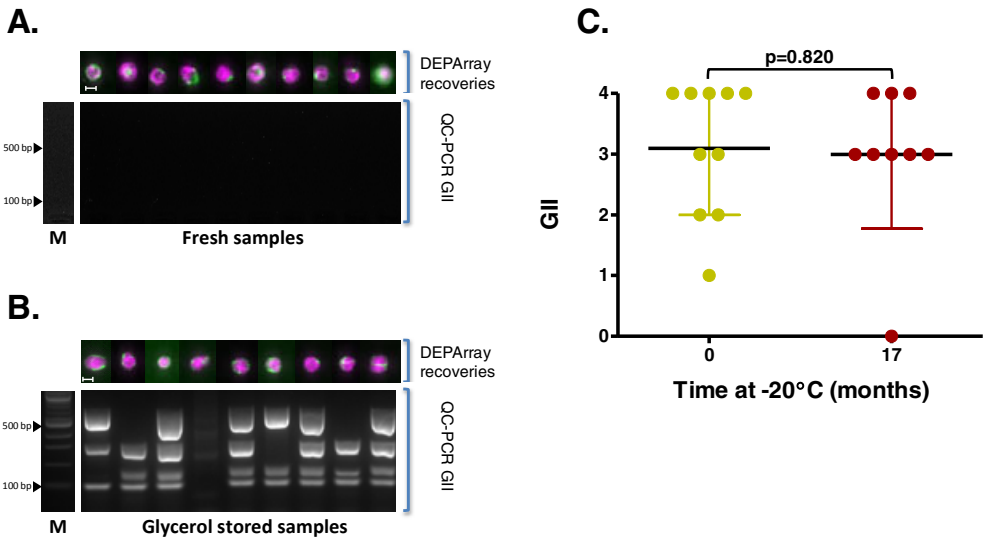

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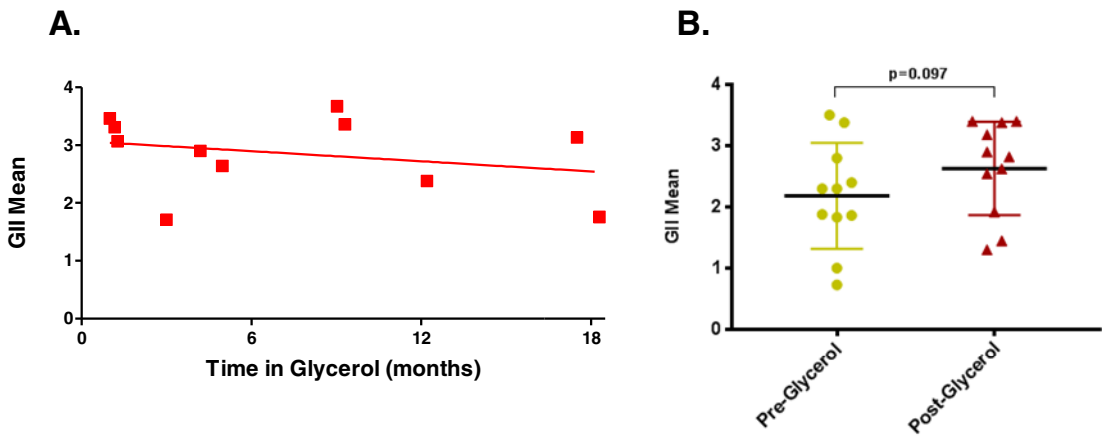

Figure 6.