WHITEPAPER

Fast and Easy B Cell Isolation for More Sensitive CLL Assays

Introduction

B-Cell Chronic Lymphocytic Leukemia (CLL) is the most common type of leukemia in the western world. CLL affects B cell lymphocytes, and causes malignant cells to proliferate and accumulate in the bone marrow and peripheral blood.[1,2]

These malignant CLL cells are distinguished from normal B lymphocytes by the expression of the CD5 surface marker in addition to CD19, CD20 and CD23.[1] They also carry genetic abnormalities, which can differ among subtypes of CLL and among different leukemic subpopulations in the same individual.[3,4]

CLL is a highly variable disease, and can be divided into a number of subtypes based on the distribution of genetic abnormalities present in the malignant cell population. Since these abnormalities can indicate the course that the disease will take, it is essential that CLL assays are able to identify all underlying mutations with the highest-possible sensitivity and accuracy.[5,6]

Array-Based Assays for CLL

Array-based comparative genomic hybridization (array-CGH) and array-based single-nucleotide polymorphism analysis (array-SNP) are powerful, efficient and increasingly common tools for profiling genetic abnormalities in CLL and other hematological malignancies.[7-9]

Array-based CLL assays have several advantages over older techniques, such as fluorescence in situ hybridization (FISH). For example, it is possible to screen tens of thousands of loci in parallel using arrays, whereas FISH is typically limited to a few probes. Similarly, arrays allow mutations to be detected down to the single-nucleotide level, whereas FISH and conventional cytogenetic techniques can detect only gross chromosomal mutations.

Recent research shows that array-based assays can detect abnormalities missed by FISH.[9-14] However, the sensitivity of these arrays is contingent upon a **purified cell population**.

B Cell Isolation for Increased Array-Based Assay Sensitivity

The purpose of molecular assays for CLL is to detect or quantify a specific DNA sequence or RNA transcript in the malignant B cell population. Therefore, the sensitivity and accuracy of the assay increase with the ratio of B cells to non-B cells in the cell population used for DNA/RNA extraction.

Enriching blood samples for B cells improves the sensitivity of molecular assays because it reduces the effects of normal clone contamination caused by DNA from non-B cells.[15] Furthermore, B cell enrichment allows more appropriate comparisons of log2 ratios among samples, since it eliminates variability attributable to differences in B cell frequency.[9, 16]

For example, Hagenkord & Chang (2009) review array-based assays for hematological malignancies, in particular CLL. The authors compare array-SNP results on a non-enriched sample and on the same sample after B cell enrichment using RosetteSep™, a reagent for cell isolation directly from whole blood. They show that enriching B cells before array-SNP allows aberrations to be detected that would otherwise have been missed. (Figure 1).[9]

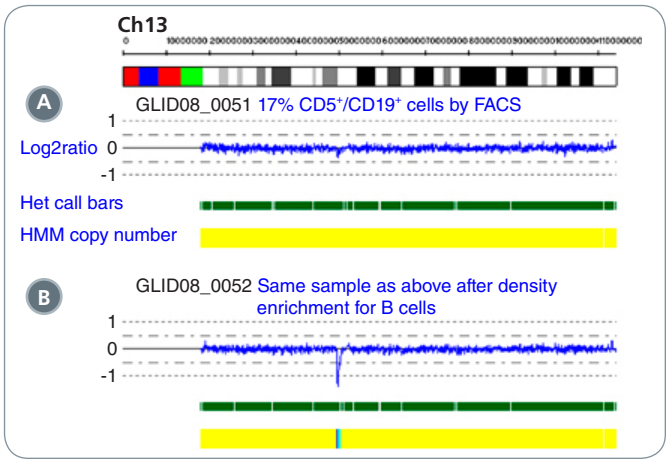

Figure 1. 13q14 regional copy number heterogeneity and efficacy of B-cell enrichment.

(A) SNP array-based karyotype of the 13q14 locus of a CLL sample with 17% $CD5^+/CD19^+$ CLL cells by flow cytometry. (B) Array-based karyotype of the same sample as (A), but enriched for B cells before DNA extraction, revealing a 13q14 aberration that appears partially heterozygously deleted (light blue) and partially homozygously deleted (dark blue). Dark blue indicates copy number of zero, light blue indicates copy number of one and yellow indicates copy number of two. The log2 ratio plot is shown as a smoothed average over 10 SNPs. Figure and caption adapted by permission from Macmillan Publishers Ltd: Leukemia 23: 829-833, copyright 2009.

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713 • INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

In another publication, Hagenkord et al. (2010) use SNP arrays to analyze 6 paired samples that were either enriched for B cells using RosetteSep™ or left un-enriched. The authors find that that array-SNP detected lesions that would have been missed by FISH. They also note the importance of B cell enrichment as a sample preparation step, particularly for samples with a low tumor burden.[16]

> "Virtually all tumor samples contain some 'normal clone contamination' from non-neoplastic cells. This will dampen the signals from the tumor and cause failure of both copy number and genotyping algorithms… In our hands, enrichment for B cells not only evens the playing field with regard to comparing log2 ratios between samples, but also resolves lesions in samples with low tumor burden or small subclones."
>
> – Hagenkord et al. 2010

Patel et al. (2008) compare array-CGH using RosetteSep™-enriched CLL samples to FISH using non-enriched samples.[17] In several cases, array-CGH detected aberrations that were missed by FISH (Table 1).

	ABNORMALITIES DETECTED	
Case Number	FISH	Array-CGH
25	34	35
6	0	2
31	34	37

Table 1. Abnormalities detected by FISH versus array-CGH analysis. Data from Patel et al. 2008.

Furthermore, Patel et al. note that their assay was twice as sensitive as previous studies have reported. They attribute this to enriching B cells prior to DNA extraction.

> "…our clonal detection cutoff value of 25% is two times more sensitive than what has been previously reported. This increase in sensitivity is most likely due to using DNA from an enriched population of cells in our study. Therefore, these results suggest that for CLL samples, enriching for B-cells allows for higher sensitivity for detecting clonal abnormalities using array-CGH analysis."
>
> – Patel et al. 2008

Summary

These selected publications show that B cell isolation can significantly improve the sensitivity and consistency of downstream molecular assays for CLL, and is particularly important when the starting frequency of B cells in a sample is low or unknown.

To facilitate CLL research, STEMCELL Technologies offers several platforms for B cell isolation from whole blood or bone marrow by either immunomagnetic or immunodensity selection. All B cell isolation kits are easy-to-use, cost-effective, and simple to incorporate into existing workflows.

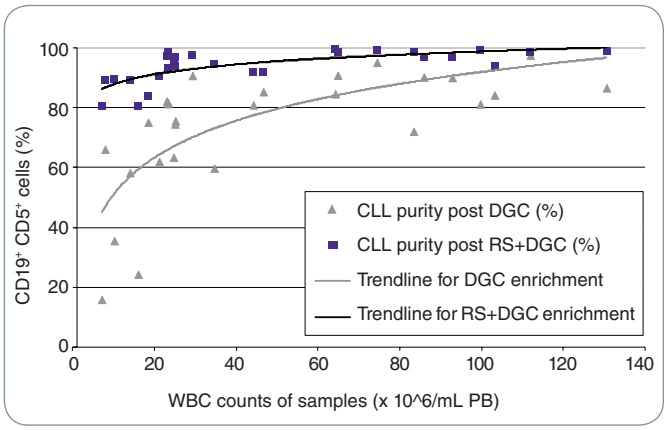

Figure 2.

Purity of $CD5^+CD19^+$ CLL cells in the same sample after enrichment using RosetteSep™ (RS, purple squares) or density gradient centrifugation alone (DGC, grey triangles). Adapted from Essakali et al., 2008, under the terms of the Creative Commons Attribution Licencse.

B Cell Isolation from CLL Samples

STEMCELL Technologies offers several products for B cell isolation from CLL samples, ensuring that an ideal platform is available for every application (Table 2). To complement our existing products, we also offer custom cell separation kits for your specific needs.

	RosetteSep™ Human B Cell Enrichment Cocktail	EasySep™ Human B Cell Enrichment Kit w/o CD43 Depletion	EasySep™ HLA Whole Blood B Cell Positive Selection Kit
SELECTION METHOD	Immunodensity Negative	Immunomagnetic Negative	Immunomagnetic Positive
SURFACE MARKERS USED	CD2, CD3, CD16, CD36, CD56, CD66b (depletion)	CD2, CD3, CD14, CD16, CD56 (depletion)	CD19, CD20
CATALOG #	15064	19154	18454HLA

Table 2. B cell isolation kits for CLL research.

To inquire about a custom cell isolation kit, contact **techsupport@stemcell.com**.

High-Throughput B Cell Isolation Directly from Whole Blood with RosetteSep™ and SepMate™

RosetteSep™ offers rapid, high-throughput cell isolation directly from human peripheral blood samples. Isolate cells from whole blood in as little as 25 minutes by combining RosetteSep™ with SepMate™, the specialized tube for density gradient centrifugation. The RosetteSep™ Human B Cell Enrichment Cocktail is highly published for CLL research, and does not deplete cells expressing CD43, which can be expressed on CLL cells (Figure 2).

The Novel RosetteSep™ and SepMate™ Procedure

Incubate the blood sample with RosetteSep™ for 10 minutes at room temperature.

Add density gradient medium to SepMate™ tube, then rapidly pipet or pour the blood sample directly over the insert.

After a 10-minute centrifugation with the brake on, simply pour highly purified target cells into a new tube.

25 Minutes

"The short purification time, the independence from expensive and time-consuming procedures such as FACS and MCS, and the flexible adjustment of cell yields makes [RosetteSep™] an attractive purification method for a wide spectrum of downstream applications… in which a CLL purity of >90% is desirable.

– Essakali et al. 2008[18]

SAMPLE REQUEST

Request a RosetteSep™ Sample
www.stemcell.com/rosettesepsample

Fast and Easy B Cell Isolation for More Sensitive CLL Assays

Fast and Easy B Cell Isolation with EasySep™

EasySep™ is a fast, easy and column-free system for immunomagnetic cell isolation. Isolate untouched B cells by negative selection, or use positive selection to achieve the highest-possible purity. EasySep™ can also be fully automated using RoboSep™, the instrument for true walk-away cell isolation. RoboSep™ processes up to four samples at once, freeing up technician time and preventing sample cross-contamination.

References

1. Chiorazzi N, et al. N Engl J Med 352: 804-815, 2005
2. Keating, MJ. Semin Oncol 26(5 Suppl 14):107-14, 1999
3. Dierlamm J, et al. Cancer Genet Cytogenet 94(1): 27-35, 1997
4. Kipps, TJ. Blood 112: 1550, 2008
5. Gunnar J, et al. N Engl J Med 323(11): 720-724, 1990
6. Shanafelt TD, et al. Blood 103(4): 1202-1210, 2004
7. Simons A, et al. Hum Mutat 33: 941–948, 2012
8. Vermeesch J, et al. Hum Mutat 33: 906-915, 2012
9. Hagenkord JM and Chang CC. Leukemia 23: 829-833, 2009
10. Gunn SR, et al. J Mol Diagnos 10(5): 442-451, 2008
11. Gunn SR, et al. Leukemia 23: 1011-1017, 2009
12. Pfeifer D, et al. Blood 109: 1202-1210, 2007
13. Lehmann S, et al. Cancer 112(6): 1296-1305, 2008
14. Schwaenen C, et al. PNAS 101(4): 1039-1044, 2004
15. Garnis C, et al. Genomics 85(6): 790-793, 2005
16. Hagenkord JM, et al. J Mol Diagnos 12(2): 184-196, 2010
17. Patel A, et al. Am J Hematol 83: 540-546, 2008
18. Essakali S, et al. BMC Biotech 8(6): doi:10.1186/1472-6750-8-6, 2008

SAMPLE REQUEST
Request an EasySep™ Sample
www.stemcell.com/easysepsample