

Whole Transcriptome Analysis of Rare Stem Cell Sub-Sets in Human Bone Marrow



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Abstract

Several populations of stem cells reside in the bone marrow (BM). It is well known that hematopoietic stem cells (HSCs) can be defined and isolated. Recent work has demonstrated that there are additional non-hematopoietic stem/progenitor cells that can give rise to ectodermal, mesodermal, and endodermal tissues. These cells are rare; after identification and isolation by means of high speed cell sorting, resulting sample size is often only a few thousand cells or less. Further characterization and functional identification of these fractions is often limited by low cell numbers. To study the gene expression of the rare populations, a method was used to amplify total RNA from the isolated/sorted stem cell populations. The whole transcriptome amplification technology from Sigma-Aldrich, TransPlex[®] was tested using input RNA from decreasing numbers of purified stem cells.

Using a panel of seven markers (CD105, CD73, CD34, CD90, CD117, CD133, CD45), four populations of Stem Cells were identified and sorted on a Beckman Coulter MoFlo high speed sorter. Even with sort rates around 20,000 cells/second, total cell yield was low. HSCs were isolated and further characterized in terms of CD38 expression, as well as size. Endothelial and Mesenchymal progenitors were also identified and sorted. Aliquots of 3000, 1000, 300, 100, 30 and 10 cells each were collected in RNA purification buffer. These samples were frozen and stored for subsequent RNA purification and amplification.

Whole transcriptome TransPlex[®] amplified "Heme Small" 1000-, 100-, and 10-cell samples were tested using beta-actin, and GAPDH qPCR primers. All transcripts are present in each amplified sample. Array analysis studies are in progress to determine whether consistent profiles of gene expression can be detected among the 1000-, 100-, and 10-cell samples.

Background

Most RNA experiments start with millions of cells. We were interested in getting data from some of the rare cell populations that we can sort. We had the opportunity to evaluate Sigma's RNA amplification technology and determine how low it could go.

Recent work in the characterization and isolation of stem cells from various tissue sources presented an occasion in which we could provide samples of several populations of interest. Using a combination of seven markers, four populations of stem cells would be sorted on the Beckman-Coulter MoFlo[™], high speed cell sorter. Aliquots of 3000, 1000, 300, 100, 30, and 10 cells were prepared and subjected Sigma's TransPlex[®] amplification technology.

The MoFlo[™] gives us the ability to sort complex gating strategies quickly with above average yields. Even so, with extremely rare populations such as mesenchymal stem cells (less than 0.1% of total cells) collecting adequate samples is time consuming. With sort rates exceeding 20,000 cells per second, a 500 million cell sort can take several hours.

We performed two sorts to collect samples for RNA amplification. **Table 1** summarizes the samples collected. The second sort focused on the collection of Hematopoietic stem cells, utilizing CD38 to help define a resting population that would most likely have smaller amounts of RNA.

Table 1: Sample log

First Sort:		Heme Small CD38 low	
		Heme Large CD38 high	
Heme Small	SH		3,000 (x3)
Heme Total	HT		1,000 (x3)
Endothelial	E		300 (x6)
Mesenchyme	M		100 (x6)
			30 (x12)
3000, 1000, 300, 100			10 (x12)
Total 16 tubes		Total 84 tubes	
Sorted 2/6/08		Sorted 3/3/08	

We started our analysis on the "Heme small" samples for several reasons:

- 1) The "Heme small" and "Heme total" samples were represented in both sample collection run (we had a lot of these samples).
- 2) This was a well defined sub-set of stem cells and there were several genes of interest that we could pursue in follow-up studies.
- 3) The "Heme small" population represented a population of resting stem cells. These cells would have little RNA to begin with and if we could get the system to work on this population, we predicted good results with other populations.

Conclusions

Amplified RNA was analyzed by qPCR (MJ Opticon 2) instrument, using gene primer sets for 18s, beta-Actin, and GAPDH. **Figure 1** indicates the presence of these common/housekeeping genes. The C(t) values from the graphs indicate that the genes are present and shows an indication that the copy number of genes is consistent in the 1000, 100, and 10 cell samples. C(t) data compiled in **Table 2**.

150ng of total RNA was loaded per lane on TAE 1% agarose shown in **Figure 2**. Left to Right: Marker, 10 cell, 100 cell, 1000 cell. Marker(bp) of 2000, 1500, 750, 500, 300, 150, 50 indicate that the product of amplification are around 300 to 50 base pair, well within our range for good qPCR and micro Array analysis.

This initial data is a good indicator that we can proceed with more specific gene Arrays. Our goal is to determine if we can use this method to detect consistent gene expression profiles within populations that we study, keeping in mind that cell numbers may be low; and that cells of interest may not yield large quantities of RNA.

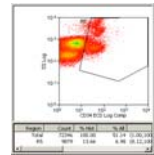


University of Pittsburgh Cancer Institute Cytometry Facility High-Speed Cell Sorter with Custom Biohazard Safety Cabinet

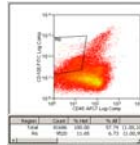
Populations of Interest

Hematopoietic Progenitors
CD45+ CD34+

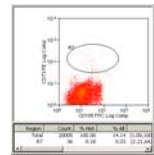
Additionally characterized by Side Scatter and CD38 expression



Endothelial Progenitors
CD45- CD105+



Mesenchymal Progenitors
CD34- CD45- CD105+ CD73+



Gating Strategy for Cell Sorting

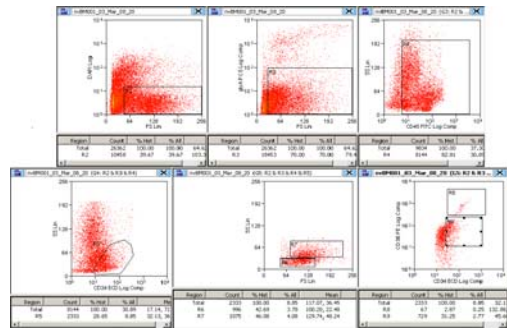


Figure 1

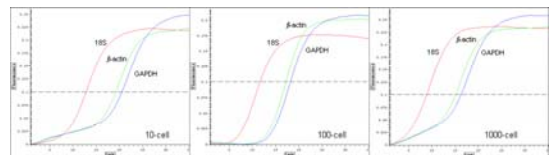


Fig 1: Total RNA was amplified from sorted 10 cell, 100 cell and 1000 cell "Heme Small" populations using Sigma's WTA 27M RNA amplification technology. Amplified RNA was analyzed using qPCR(MJ Opticon 2), using primer sets for 18s, b-Actin, and GAPDH.

Table 2:

C(t) vales for the 10, 100, and 1000 "Heme Small" sorted populations.

	18S C(t)	b-actin C(t)	GAPDH C(t)
10	12.82	19.23	20.5
100	11.63	17.2	18.16
1000	8.89	15.1	16.26

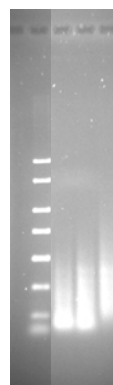


Figure 2:

150 ngs of total RNA was loaded per lane on TAE 1% Agarose. Left to right: Marker, 10 cell, 100 cell and 1000 cell of "Heme Small" populations. Marker (bp): 2000, 1500, 750, 500, 300, 150, 50