Novel Research Algorithm for Detecting Partial and Complete Loss of Human Leukocyte Antigen

Svetlana Argounova, Bernie Valdez, Hanna Bennett, Farshad Alishahi, Anna Chikova.
One Lambda Inc., part of Thermo Fisher Scientific, West Hills, CA, USA
Farshad.Alishahi@thermofisher.com

Abstract

Loss of heterozygosity in human leukocyte antigens (HLA) is frequently associated with relapses of hematologic malignancies treated by allogeneic hematopoietic stem cell transplantation. Loss of HLA was also described as an immune-escape mechanism in multiple published studies of solid tumors. Thus, identifying loss of heterozygosity (LOH) may impact choice of treatment. One Lambda LABType products provide HLA typing for up to 11 different loci. Though these products are used for HLA genotyping in transplant diagnostic practices, we evaluated the feasibility of applying LABType technology in combination with novel data analysis approaches to detect complete or partial loss of HLA alleles in DNA samples. Homozygous DNA samples well-characterized for all 11 HLA loci were mixed to mimic different ratios of HLA alleles. Resulting mixes were analyzed with LABType products for several loci. Two pilot algorithms were developed to evaluate data from non-transplant and transplant related scenarios. For non-transplant scenarios potentially relevant to cancer immunotherapy, two HLA alleles are expected for each locus, and minor allelic imbalance may be an indication of HLA loss in a fraction of tumor cells. Assay allowed to distinguish 50% DNA mix imitating normal heterozygous samples from 60 to 40% ratio. Feasibility to use LABType in paraffin embedded tissues was also evaluated demonstrating sufficient amplification of selected exons using modified DNA extraction procedure. For mismatched or haplo-matched stem cell transplants scenario, more than two HLA alleles may be detectable in a sample. We evaluated mixtures of 3 DNAs imitating haplo-matched stem cell transplant samples. Experiment demonstrated ability to detect loss of minor allele at 2% of total “patient” fraction in the sample. Thus, the pilot HLA analysis algorithm was able to detect presence of minor allele starting from 1% in DNA mixture.

Materials and Methods

To imitate HLA loss in solid tumor tissues, we tested two homozygous DNA samples 9040 and 9058 and their mixes in several different ratios imitating possible loss of one or another allele (Table 1) with One Lambda LABType products for several loci – A, B, C, and DRB1.

Table1. Homozygous DNA ratios mimicking LOH

Sample Mixture	Ratio						
9040/9058 A*01:01/A*02:01	20:80	30:70	40:60	50:50	60:40	70:30	80:20

We also tested different ratios of homozygous and heterozygous DNA mixtures to mimic both chimerism and LOH expected for HSCT (Table 2).

Table 2. Homozygous and heterozygous DNA mixtures mimicking chimerism with LOH (represented by minor allele - allele unique for patient)

Sample	Typing	Allele (Unique for Patient and Donor or Shared)	Represent	Minor Allele	Chimerism (Patient vs Donor)		
9040	B*49:01:01	Shared	Patient	100%	10%	20%	50%
9058	B*45:01:01	Unique for patient					
E20113	B*49:01:01 B*15:01:01	Shared Unique for donor	Donor				
9040	B*49:01:01	Shared	Patient	10%			
9058	B*45:01:01	Unique for patient					
E20113	B*49:01:01 B*15:01:01	Shared Unique for donor	Donor				
9040	B*49:01:01	Shared	Patient	20%			
9058	B*45:01:01	Unique for patient					
E20113	B*49:01:01 B*15:01:01	Shared Unique for donor	Donor				

We also used individual DNA samples to monitor background signals for each allele. Total DNA input per reaction is reflecting amount of DNA mix used for amplification with primers for each LABType product.

Results

We tested the RSSOA1 CWD kit using different DNA inputs ranging from 1 ng to 20 ng. The 1 ng input showed significantly lower TMFI (trimmed mean fluorescence intensity) positive signals for unique markers (microspheres), although the normalized values did not differ significantly. Although the 1 ng input resulted in increased 95% confidential intervals, the kit was still able to detect allelic loss at this input level. Difference in allelic ratio is detectable with 20% difference between tested DNA samples as evident in both scenarios of 40% and 60% of 9040 DNA in mix. Our new analysis method relies on data generated by beads unique for each allele. Data from beads unique for each allele were analyzed to generate allele specific scores demonstrated on Figure 1. This scenario imitates loss of HLA allele by only a fraction of tumor cells.

RSSO CWD kits provide higher level resolution and include more beads and thus can provide more unique markers per allele than RSSO classic kits. Nevertheless, low resolution products may provide sufficient number of informative markers for reliable allelic imbalance detection when specific combination of alleles provides sufficient number of unique markers.

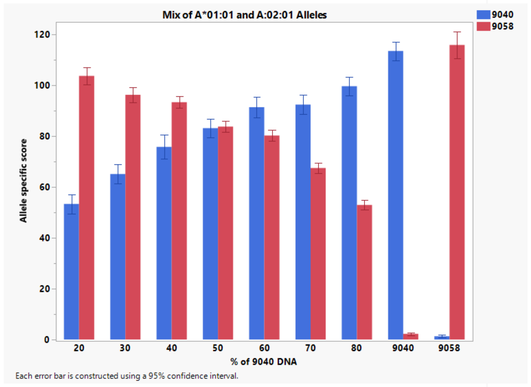


Figure 1. Data analysis for two homozygous DNA mix test
DNA mixes were analyzed by RSSOW1A product. Total DNA input was 20 ng per reaction. X axis displays percent of 9040 DNA sample in the mix. Y axis displays allele specific score calculated by new analysis algorithm with 95% confidence interval for each value

While for loss of HLA analysis performed in solid tumor, sensitivity of the assay to partial allelic imbalance may be important for early detection of allelic loss, scenario of stem cell transplantation is more complex. For haplo-matched and mis-matched stem cell transplant, DNA samples obtained from the patient may include 3 or even 4 alleles for the same HLA locus, and allelic ratios are impacted by different levels of chimerism. Since certain level of chimerism is expected for real life HSCT samples, we evaluated our analysis with DNA mixes imitating different level of chimerism. For this purpose, we mixed two homozygous DNA samples representing patient alleles and added another heterozygous DNA sharing alleles with one of them, thus imitating a haplo-matching transplant scenario. Combining these DNA samples in different ratios, we mimicked different levels of chimerism and different level of allelic loss. Example of experiment design with such DNA mixes is shown in Table 2.

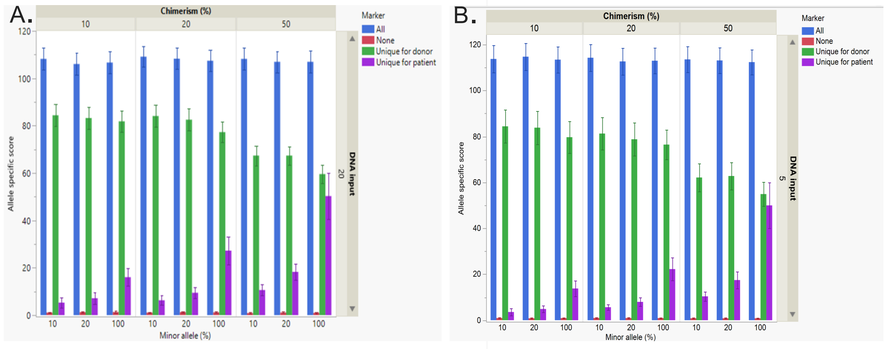
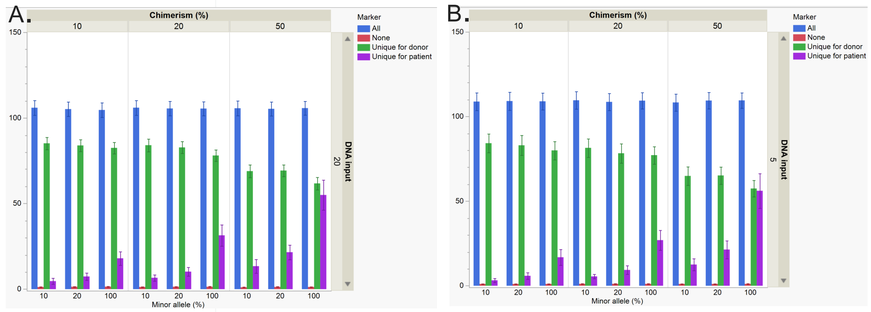


Figure 2. Detecting loss of HLA in DNA mixes representing different levels of chimerism*
B-CWD Lot 6 kit was used. (**A.**) 20 ng total DNA input and (**B.**) 5 ng total DNA input per reaction
*Chimerism values in the graph show the fractions of the “patient’s” DNA**.**

The RSSOW1B CWD kit was tested using DNA inputs ranging from 1 to 20 ng, with various DNA mixtures mimicking LOH (from normal heterozygous containing 100% of unique patient allele to 10% of it remaining) and chimerism (10–50% of the patients’ alleles – both unique one and common with donor allele). Surprisingly, we did not see significant difference in quantification accuracy between total DNA inputs of 5 ng and 20 ng per reaction (Fig.2). Even with 1 ng of total DNA input, loss of HLA was detectable due to sufficient number of informative markers provided by B locus CWD kit. In addition to unique allele specific markers, we included into analysis beads specific for all alleles in the mix and beads representing background as an average negative for all tested DNAs.



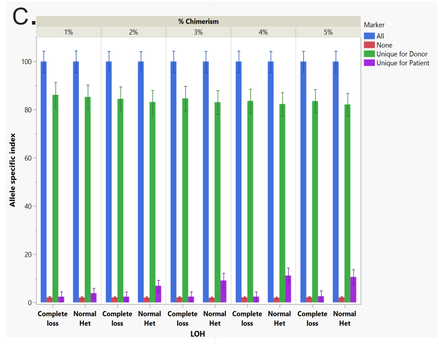


Figure 3. Combined analysis of B CWD and DRB1 CWD kits data
DNA mixes tested with (**A.**) 5 ng and (**B.**)20 ng per reaction.
Mixes were representing range from 10 to 50% of patient DNA per sample. Presence of unique patient allele was ranging from normal homozygous (100%) to 10% from original and complete loss (**C.**) where 100 ng per reaction in scenario of 1 – 5% chimerism.

Increased number of alleles in chimeric mixture expected for haplo-transplants reduces number of allele specific markers available even with latest lots of CWD kits. To evaluate feasibility to increase number of useable markers in haplo-transplant samples, we explored combined analysis of multiple LABType products tested with single DNA mix. Such approach can be useable to enhance data analysis for limiting allelic combination in chimeric samples or when RSSO classic products are used.
Figure 3 shows combined analysis of B CWD and DRB1 CWD products tested with DNA mixes imitating various level of chimerism and HLA loss. We also evaluated scenario of complete loss of donor specific allele for lower chimerism samples (Fig. 3C) – we tested the range from 1 to 5% of total patient’s DNA in the mix, when normal heterozygous ratio is present and when donor-specific allele is lost. Scenario shown on Figure 3C was tested with 100 ng DNA per reaction to ensure reliable quantification

Conclusions

Preliminary data suggest feasibility of developing new method detecting complete or partial loss of HLA alleles using well established LABType technology. Feasibility data suggested that our new analysis may detect not only complete but also partial loss of HLA (from 20% of allelic imbalance) with DNA input starting from 1 ng
This analysis method is experimental and has not been reviewed or approved by the FDA or other regulatory agencies. The new algorithm intended for research use only and is not commercially available at this time.

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