GENDX

personalizing diagnostics

Replacement of Pre-Transplant DNA with Cell Line DNA in Real-Time PCR Chimerism Monitoring Experiments

Quantitative PCR (qPCR) can be used to determine the chimeric status of a recipient after stem cell transplantation. With this method genetic markers, differentiating between the recipient and donor, are monitored over time in post-transplant samples. The great advantage of qPCR over Short Tandem Repeat (STR) based chimerism monitoring is the high sensitivity and therefore the possibility to detect relapse early. However, a pre-transplant DNA sample needs to be included as a reference in every monitoring experiment. This presents difficulties when the pre-transplant DNA is limiting. Here, we demonstrate the possibility to replace the pre-transplant DNA with cell line DNA in the KMRtype/KMRtrack workflow from GenDx and present some important considerations.

Typing of Coriell Cell lines

The Coriell Institute for Medical Research (Camden, New Jersey, USA) generates high-quality, well-characterized genomic DNA samples from cell lines. To test if these commercially available DNA samples can be used to replace the pre-transplant DNA, we typed a panel of 14 Coriell DNA samples for the presence or absence of all 39 KMRtype/KMRtrack markers (Figure 1).

A consistent outcome with Coriell cell line DNA and pre-transplant DNA

We created artificial chimeric DNA samples with different ratios of recipient and donor DNA of known genotype, representing series of post-transplant DNA samples. We determined the percentage of chimerism by applying informative assays, using corresponding pre-transplant or Coriell cell line DNA (Figure 2). KMRengine software can be used to manage cell line data and calculate chimerism percentages using cell lines in parallel to the pre-sample DNA (KMRengine will not calculate percentages above 100%. To get an accurate ratio between cell line and pre-transplant sample outcome, all chimerism percentages need to be between 0.05% and 99%). The chimerism percentage in series A (Figure 2A, B) was approximately 2-fold lower when the cell line NA10851 was used instead of the actual pre-sample. This ±2-fold difference was consistent for all DNA mixtures in series A. The chimerism percentage in series B (Figure 2C, D) was consistently ±0.65-fold different when the cell line NA10851 was used. This shows that the ratio between cell line and pre-transplant sample outcome can differ but will be constant over a series of post-transplant samples. A cell line selected to replace a pre-transplant sample will have a preferred ratio close to 1 (equivalent outcome in chimerism percentage).

Finding a cell line that is similar to a pre-transplant sample

We investigated if we could find suitable cell lines (ratio close to 1) for a number of pre-transplant samples in which KMR045 was informative. We determined the difference in chimerism percentage using 3 Coriell cell lines for 7 different pre-transplant samples (Figure 3). We found NA10845 and NA10851 gave similar outcomes as pre-sample 191, 195, 196, 199, 200, 212 (ratio ±1) and NA10861 gave a similar outcome as pre-sample 192 (ratio ±1). NA10861 is homozygous for marker KMR045. NA10845 and NA10851 are heterozygous for KMR045. The data indicate that zygosity is the major factor in determining the applicability of a cell line.

Conclusion

This study shows that it is possible to replace the pre-transplant DNA with cell line DNA in the KMRtype/KMRtrack workflow. The suitability of cell line DNA to replace pre-transplant DNA will depend on the markers that are informative. Figure 1 can be used to identify Coriell cell lines in which the target of a KMRtrack assay is present (homozygous or heterozygous) or absent. Zygosity is the major factor in determining the suitability of a cell line. However, zygosity is likely not the only factor and therefore the applicability of a cell line should always be tested experimentally in parallel to a pre-transplant sample in multiple monitoring experiments.

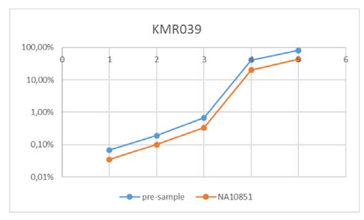
	NA10830	NA10831	NA10842	NA10845	NA10851	NA10852	NA10854	NA10857	NA10858	NA10860	NA10861	NA12547	NA12560	NA17201
KMR004			1	2							1			
KMR009	1		1			1	1	1	2				1	
KMR010	1	2	1		1						1		1	
KMR011	1		1		1	2	1			1				
KMR013				1		1			1	1	1			1
KMR014	1		2	1	1						1	1		
KMR016	1		2		1			1		1				1
KMR017	1			1	1			1	1	1		1	1	1
KMR019	1	1	1	1	1		1	1				1		1
KMR020	2			1	2	1		1	1				1	1
KMR028			1		1	1	1	1	2	1	1	1	1	1
KMR029	1	1		1	2		1	1	1	2	1		1	1
KMR030			1	1	1	1	1	1	1		1	1	2	2
KMR031		2	1	2	1	1	2	1		1	1	1	1	
KMR033	2	1	1	1	1	1	1	1	2	1	1			1
KMR034	2	2	1	1	2		1	1	1	1		1	1	1
KMR035	1		1	2	2			1			1		2	1
KMR036								2	2			1		
KMR037	1	2	1	2		2	2		1	1		1	1	1
KMR038	1				1				1	1	2		2	1
KMR039	1	1	1	1	2	2		2			1		1	
KMR040	1		1			2	1						1	1
KMR041		1	1	2	2			1		1	1		1	
KMR042				1						1			2	2
KMR043	1		1		1		1	1	1	1				2
KMR044		1		1	1	2		1		1			1	1
KMR045	2		1	1	1	1		1	1		2		1	
KMR046	1		1			1		2	1	1	1		1	
KMR047	1	1			1	1		1						
KMR048			1	1		1		1			1	1	1	
KMR049			1		1	1		1	1	1			1	
KMR050					1	1		1			1		1	
KMR051		2	1	2	2		1	1						1
KMR052		1	1			2		2			1	1		
KMR053	1		1	1	1	1								
KMR054	1		1	1	1			1	1	1		1		1
KMR055	2	2	1	2	1	1	2	2	1					2
KMR056						2						2	1	
KMR057	1				1	1			1					1

Figure 1. KMRtrack assay status in Coriell cell line DNA. Green indicates that the Coriell cell line DNA is heterozygous for the marker (1 gene copy in genome), blue indicates that the Coriell cell line DNA is homozygous for the marker (2 gene copies in genome), blank indicates negative for the marker and grey indicates unknown.

A

KMR039	pre-sample	NA10851	ratio
post-sample A1	**0,07%**	**0,04%**	1,92
post-sample A2	**0,19%**	**0,10%**	*1,90*
post-sample A3	**0,67%**	**0,33%**	*2,04*
post-sample A4	**41%**	**20%**	*2,05*
post-sample A5	**81%**	**43%**	*1,90*

B



C

KMR048	pre-sample	NA10851	ratio
post-sample B1	**0,12%**	**0,18%**	0,67
post-sample B2	**0,25%**	**0,36%**	*0,68*
post-sample B3	**0,69%**	**1,20%**	*0,58*
post-sample B4	**40%**	**64%**	*0,63*
post-sample B5	**92%**	**128%**	*0,71*

D

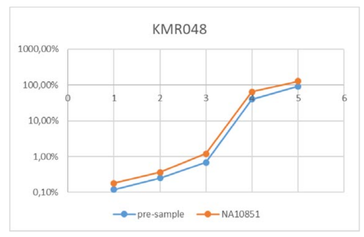


Figure 2. Chimerism percentage for 5 subsequent post-transplant samples, independently measured on different days, calculated by using either the pre-transplant sample (blue) or a Coriell DNA sample (orange). 5 DNA mixtures from series A (A, B) and 5 DNA mixtures from series B (C, D).

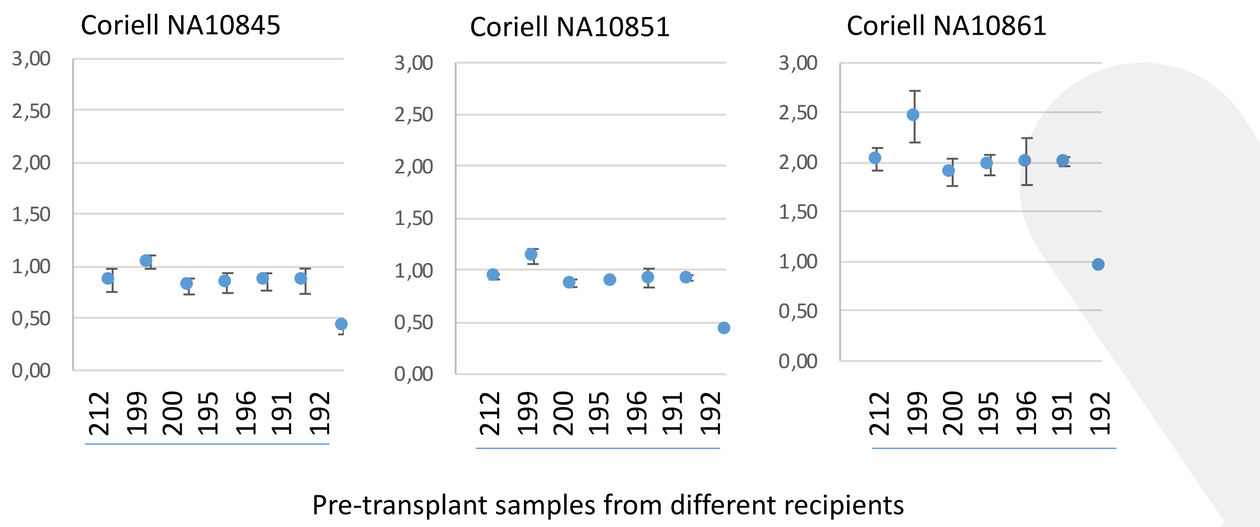


Figure 3. Difference in chimerism percentage using multiple Coriell cell lines for 7 different pre-transplant samples, mean of 3 independent experiments. NA10861 and pre-sample 192 are homozygous for KMR045, all others are heterozygous for KMR045.

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