GENDX
personalizing diagnostics

Improving genotyping capabilities in a close family cohort by expansion of a qPCR marker set for chimerism monitoring.

Introduction

Quantitative PCR not only allows quick and easy monitoring of chimeric mixtures after transplantation, it can also accurately detect the presence of genetic material as low as 0.05%. The detection of adverse transplant events is thereby highly superior in comparison to STR analysis.

Further development of chimerism monitoring by qPCR can be necessary in terms of the genotyping capabilities, especially for family members of the first and second degree due to their similarity in genetic make-up. This can be realized by increasing the number of available markers.
We have KMRtype®/KMRtrack® reagents available to perform chimerism monitoring by qPCR. The marker set is a combination of Celera (AlleleSEQR) and GenDx developed assays. Apart from that we also have the AlleleSEQR markers available for monitoring. Now we have expanded our KMRtype®/KMRtrack® marker set to a total of 39 markers. The location of the different markers is schematically represented in Figure 1, indicating the 10 newly integrated markers in blue.

Materials and Methods

The expanded marker set was tested by screening gDNA samples of a close family cohort consisting of 4 grandparents, 2 parents and 12 children (Pedigree 884 from Coriell Institute for Medical Research, Figure 2). The genotyping capabilities of the marker set expansion was assessed by determining the family member combinations that were distinguishable and resulted in informative markers. The KMRtype® protocol was applied for genotyping, using a multiplexed qPCR system encompassing 3 markers in a single reaction (GenDx). All experiments were performed using the ViiA7 qPCR system (Thermo Fisher).

Results

An overview of all family member combinations and the number of informative markers found with the previous KMRtype/KMRtrack, AlleleSEQR and expanded KMRtype/KMRtrack marker sets is depicted in Table 1A, 1B and 1C respectively. The chance of finding an informative marker is highest for the expanded marker set. When testing the close family cohort with the expanded marker set, each possible combination resulted in at least 1 informative marker. The majority of combinations (96%) resulted in more than 2 informative markers. Most importantly, Table 1 shows that the expanded set is the only marker set that has an informative marker for each family member combination.
As compared to the previous KMRtype/KMRtrack set, the expanded set reduced the number of combinations having 1 informative marker by 59% (from 29 to 12) while the average number of informative markers increased by 50% (from 4 to 6). In Table 2 the informative markers for the first and second degree relationships are represented. This again shows that the extended KMRtype/KMRtrack set is the only set with an informative marker for all combinations.

Conclusions

With the expanded marker set not only the number of combinations with 1 informative marker was reduced, now all family member combinations were distinguishable. It can therefore be concluded that the genotyping capabilities of KMRtype®/KMRtrack® significantly improved with the addition of chimerism monitoring markers and is now also superior to the AlleleSEQR marker set.

Figure 1. Location of KMRtype/KMRtrack markers.

The marker positions on the chromosomes are graphically illustrated. The 10 added markers are indicated in blue.

Figure 2. Family relations between the different Caucasian family members of pedigree 884.

For this study gDNA from this cohort was obtained from the Coriell Institute for Medical Research.

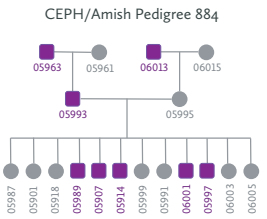

Table 1. Number of informative markers obtained with the different marker sets; expanded KMRtype/KMRtrack set shows an informative marker for each combination (C).

On the horizontal axis the donor is shown, while the vertical axis shows the recipient. Couples that have one informative marker (grey) and couples that have no informative marker (black) are indicated. A. Previous KMRtype/KMRtrack set of 29 markers. B. AlleleSEQR set of 34 markers. C. Superior expanded KMRtype/KMRtrack set of 39 markers, where every combination shows an informative marker.

A

Recipient \ Donor	Grandparents				Parents		Children										
	05963	05961	06013	06015	05993	05995	05987	05901	05918	05989	05907	05914	05999	05991	06001	05997	06003	06005
05963		7	10	5	4	8	7	8	5	8	7	9	7	9	8	4	6	7
05961	3		9	3	4	6	6	7	5	8	6	8	4	5	6	5	6	6
06013	2	5		5	4	5	5	6	5	4	3	6	4	5	4	4	6	6
06015	4	6	12		6	5	7	9	6	9	8	9	6	7	8	5	7	7
05993	1	5	9	4		5	5	6	2	4	3	6	3	5	4	1	3	3
05995	3	5	8	1	3		3	5	3	4	3	5	2	4	3	1	4	3
05987	1	4	7	2	2	2		2	2	5	3	5	2	4	2	2	3	2
05901	1	3	6	3	1	3	1		2	3	3	3	1	3	2	2	2	0
05918	1	3	7	3	0	4	4	4		4	2	5	1	2	4	1	1	2
05989	3	6	6	5	2	4	6	5	4		3	3	2	5	4	1	4	2
05907	2	4	5	4	1	3	4	5	2	3		5	1	3	2	1	3	3
05914	2	4	6	3	1	3	4	3	3	1	3		2	3	2	0	3	1
05999	3	4	7	3	2	3	4	5	2	4	2	6		2	4	2	3	2
05991	2	2	6	1	1	2	3	4	1	4	2	4	0		3	1	2	2
06001	2	4	5	3	1	2	2	4	2	3	0	4	2	3		1	3	2
05997	2	7	10	4	2	4	6	7	3	4	4	5	4	6	5		4	4
06003	1	5	9	3	1	4	4	4	0	4	3	5	3	4	5	1		2
06005	3	6	10	4	2	4	4	4	3	3	4	5	2	5	4	2	3	

B

Recipient \ Donor	Grandparents				Parents		Children										
	05963	05961	06013	06015	05993	05995	05987	05901	05918	05989	05907	05914	05999	05991	06001	05997	06003	06005
05963		8	10	4	7	12	9	11	9	8	9	9	10	11	8	8	7	11
05961	2		8	3	3	9	7	6	4	6	6	6	4	6	5	6	4	6
06013	4	8		5	7	10	8	9	9	6	8	7	9	8	5	7	7	8
06015	4	9	11		9	8	10	10	8	9	10	9	9	9	7	8	6	9
05993	2	4	8	4		8	5	6	3	3	4	5	4	4	2	4	2	4
05995	5	8	9	1	6		3	3	3	6	4	4	4	2	2	1	2	2
05987	3	7	8	4	4	4		3	3	5	4	5	4	2	3	2	1	2
05901	5	6	9	4	5	4	3		2	7	6	5	3	2	3	3	3	2
05918	4	5	9	3	3	5	4	3		5	5	4	3	3	3	4	2	3
05989	2	6	6	3	2	7	5	7	4		3	3	4	5	2	3	2	5
05907	2	5	7	3	2	4	3	5	3	2		2	3	3	1	0	1	3
05914	3	6	7	3	4	5	5	5	3	3	3		4	5	2	2	2	5
05999	4	4	9	3	3	5	4	3	3	2	4	4		4	3	3	3	1
05991	6	7	9	4	4	4	3	3	3	6	5	6	4		3	1	3	2
06001	5	8	8	4	4	4	6	6	5	5	5	5	6	3		3	3	3
05997	4	8	9	4	5	4	4	5	5	5	3	4	5	4	2		2	4
06003	4	7	10	3	4	6	4	6	4	5	5	5	4	4	3	3		4
06005	6	7	9	4	4	4	3	3	3	6	5	6	4	0	1	3	2	

C

Recipient \ Donor	Grandparents				Parents		Children										
	05963	05961	06013	06015	05993	05995	05987	05901	05918	05989	05907	05914	05999	05991	06001	05997	06003	06005
05963		10	12	7	6	15	13	13	9	10	11	12	11	15	11	8	10	13
05961	3		11	4	5	11	10	10	7	9	9	10	6	9	8	8	8	10
06013	3	9		7	7	11	11	12	10	7	7	9	9	11	7	8	10	12
06015	5	9	14		9	10	13	13	9	12	13	12	10	12	11	10	11	12
05993	1	7	11	6		10	9	9	4	4	5	7	5	9	5	3	5	7
05995	4	7	9	1	4		4	5	3	5	4	5	3	4	3	2	5	3
05987	1	5	8	3	2	3		2	2	5	3	5	2	4	2	2	3	2
05901	2	5	9	4	2	5	3		2	4	5	4	2	4	3	4	4	1
05918	2	5	10	4	1	7	7	5		5	5	6	2	4	5	4	3	4
05989	3	8	8	7	2	9	10	8	6		5	5	4	5	5	3	6	6
05907	2	6	6	6	1	6	6	7	4	3		5	3	5	2	1	4	5
05914	3	7	8	5	2	7	8	6	5	2	5		5	6	2	2	5	4
05999	3	5	9	4	2	6	6	6	2	4	4	7		4	5	4	4	4
05991	3	4	8	2	2	3	4	4	1	5	3	4	1		3	2	3	2
06001	3	7	7	5	2	6	6	7	4	4	2	4	5	6		3	5	5
05997	2	9	11	6	2	7	8	9	5	4	4	5	6	8	5		5	6
06003	1	6	10	4	1	7	6	6	1	4	4	5	4	6	5	2		4
06005	4	8	12	5	3	5	5	4	3	4	5	5	3	5	4	2	4	

Table 2. Number of informative markers obtained in first degree or second degree relationships for each marker set.

Only the expanded KMRtype/KMRtrack set shows an informative marker for all combinations, independent of relationship degree.

	Relationship					
	First degree			Second degree		
Informative markers	0	1	>1	0	1	>1
Previous KMRtype/KMRtrack markers	5	14	287	1	15	290
AlleleSEQR markers	3	6	297	0	2	304
Expanded KMRtype/KMRtrack markers	0	5	301	0	7	299

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