KIR Genotyping using a Real Time Assay and FLX 454

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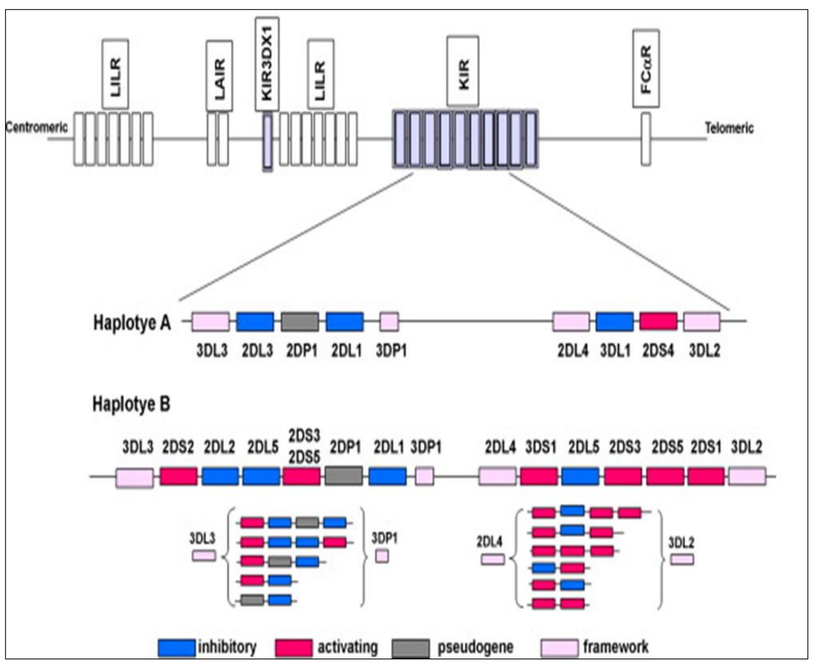
Background:

- Killer immunoglobulin-like receptor (KIRs) are cell surface receptors expressed on Natural killer (NK) cells.
- KIRs regulate the function of NK cells by interacting with HLA Class I ligands.
- KIR and HLA interactions are associated with malignant, infectious and autoimmune diseases.
- KIR can be grouped into two haplotypes – A and B.

Introduction:

- KIR are a highly polymorphic family of 16 genes on chromosome 19q13.4.
- KIR genes are identified as both inhibitory and activating.
- KIR proteins are characterized as 2D and 3D (with two or three extracellular immunoglobulin-like domains) with long(L) and short (S) cytoplasmic tails.



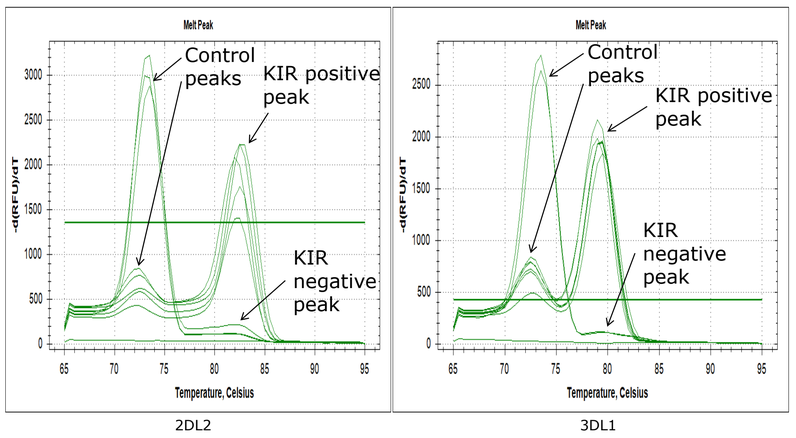
Gene organization of KIRs on chromosome 19 and genes present in haplotype A and B. (Adapted from S.Kulkarni et al. 2008)

Method:

- Previously published primers sets were evaluated for genotyping the KIR loci with the real-time assay using SYBR green. 15 primer sets were selected for validation.
- 24 samples from the UCLA KIR panel were selected for assay validation and subsequently 86 samples from the Swiss HIV Cohort study were genotyped.
- The process was automated on a 384 well plate which allowed four KIR genes for up to 94 samples to be tested on a single plate.
- A positive internal control GALC was included.
- Melt curve analysis was performed after real-time PCR.
- For FLX Sequencing, RNA was extracted from previously genotyped cells lines. New primers were designed to amplify the KIR genes: 2D L1-5, S1-5, P1 and 3D L1-3, S1, P1. Amplified products were sequenced on the long read 454 FLX platform.

Results:

- 100% concordance was achieved for all 15 KIR genes on 24 samples.
- Each KIR amplicon had a characteristic melting temperature (Tm) ranging between 79-85°C.
- GALC had Tm=75°C allowing easy discrimination between KIR and positive control peaks.
- Melting peaks were analysed to determine the presence/absence of KIR genes.



Melt peaks obtained from 2DL2 and 3DL1 representing curves from positive and negative samples.

Results analysed from 86 samples from Swiss HIV Cohort study.

KIR profile										
Haplotype A			Haplotype B							
2DL3	3DL1	2DS4	2DS2	2DL2	2DL5	3DS1	2DS3	2DS5	2DS1	
1	1	1	1	1	0	0	0	0	0	
1	1	1	1	1	0	0	0	0	0	
0	1	0	1	1	1	1	1	0	1	
1	1	Failed	1	1	1	1	1	1	1	
1	1	1	1	1	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	
1	0	1	1	1	1	1	1	1	1	
1	1	1	0	0	1	1	1	1	1	
1	1	1	1	1	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	
1	1	1	1	1	1	1	1	1	1	
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0	1	1	1	1	1	1	1	1	1	
1	1	1	0	0	0	0	0	0	0	
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1	1	1	1	0	1	1	1	0	1	
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1	1	1	0	0	0	0	0	0	0	
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1	1	1	0	0	0	0	0	0	0	
0	1	1	1	1	1	Failed	1	1	1	
0	1	1	1	1	1	1	1	1	1	
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1	1	1	0	0	0	0	0	0	0	
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1	1	1	0	0	0	0	0	0	0	
1	0	0	1	1	1	1	0	1	1	
1	1	1	1	1	1	0	1	0	0	
1	1	1	0	0	0	0	0	0	0	
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1	1	1	1	1	1	1	0	1	1	
1	1	1	0	0	0	0	0	0	0	
0	1	1	1	1	1	1	1	1	1	
0	1	1	1	1	1	0	1	0	Failed	
1	1	1	0	0	0	0	0	0	0	
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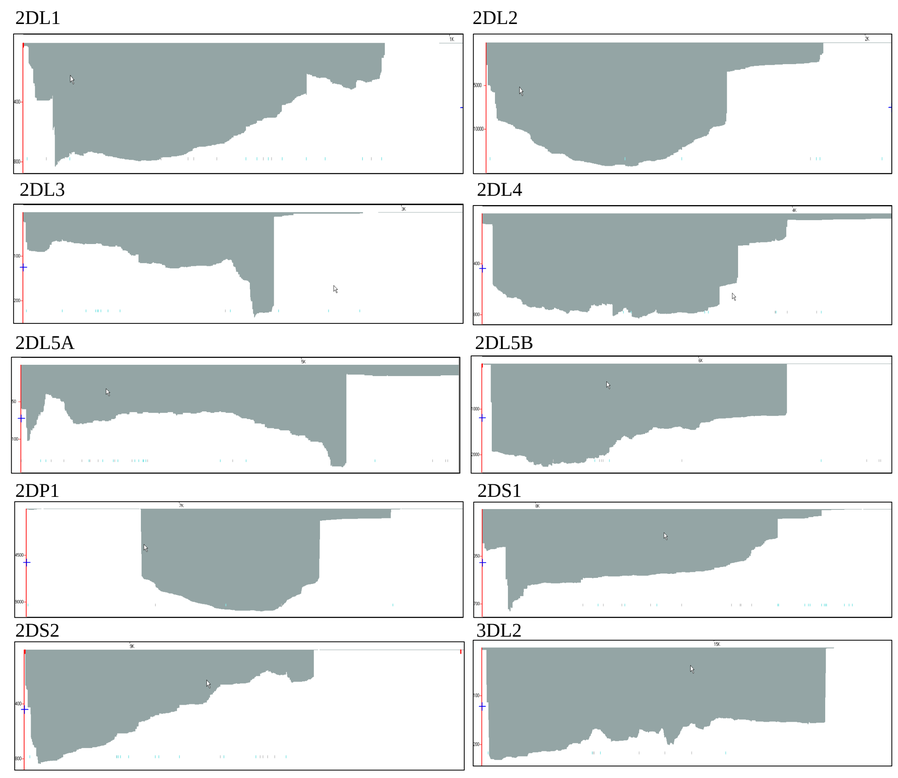
1	1	1	1	1	1	1	1	0	1	
1	1	1	0	0	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	
0	1	1	1	1	1	1	0	1	0	

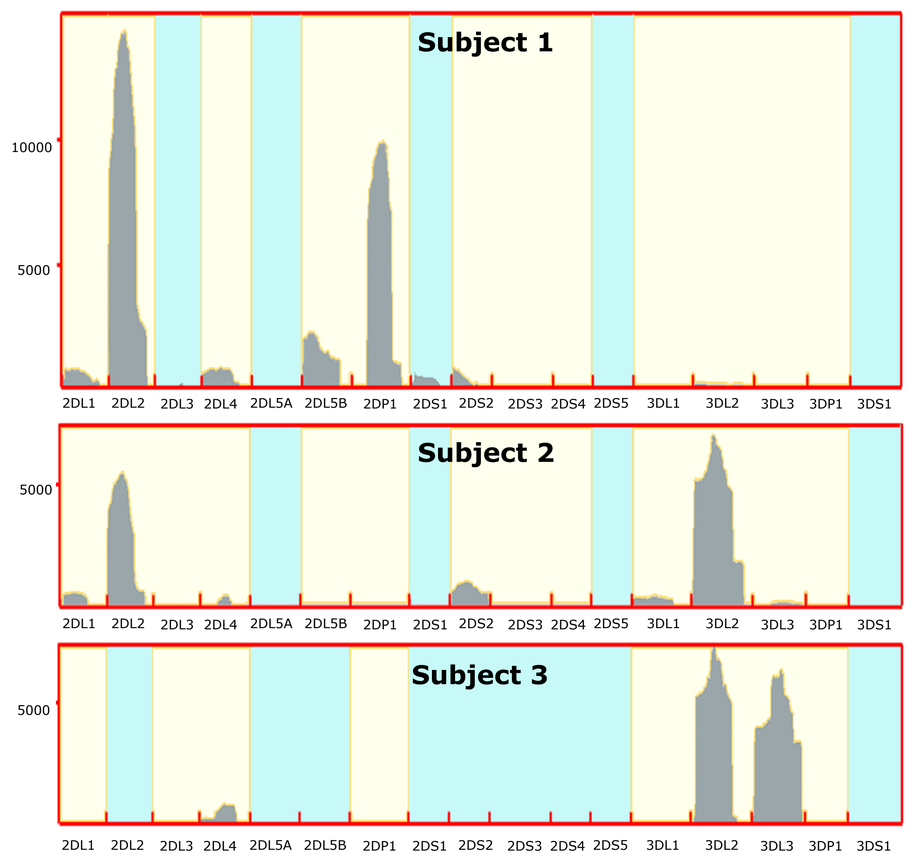
Genotype	Genotype occurrence
AA	29
BB	1
AB	56

Haplotype	Haplotype Frequency
A	66.28%
B	33.72%

Coverage obtained from FLX Sequencing :



Example coverage plots for genes captured by these primers. Primers failed to capture known genes 3DP1, 2DS3 and 2DS4. Gene absence could either be due to the gene not being expressed, primer bias or poor alignment.



Sequence coverage of the KIR genes captured by the primers for three cell-lines.

Conclusion 1:

Real-Time KIR Genotyping using SYBr Green is-

- A simple and time-efficient technique that requires minimal post-PCR processing.
- Fast with analysis based on Ct values and melting temperatures.
- Able to easily determine presence/absence of KIR genes.
- A valid method to determine KIR haplotypes.

Conclusion 2 :

The primers designed to amplify KIR coding sequences from RNA samples have captured most of the KIR genes. Further analysis is required to call alleles and understand why some genes were not captured and potential differences in expression between subjects.

Acknowledgements

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