Killer-cell Immunoglobulin-like Receptor (KIR) genotyping by PCR-SSP

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This protocol describes the genotyping procedure of the 17 Killer-cell Immunoglobulin-like Receptor (KIR) by Polymerase Chain Reaction with sequence specific primers (PCR-SSP). Natural killer (NK) cell function is regulated by different types of membrane-bound receptors of which killer-cell immunoglobulin-like receptors (KIRs) are the most complex and diverse. KIR are encoded by 17 different genes located within the leukocyte receptor complex (19q13.4). The frequency with which KIR gene features are present in different human populations differs.

Oligonucleotide primers

Name	Sequence	bp	Position	% GC	Tm (°C)	DNA (bp)	RNA (bp)	Ref	Cross-hybridisation	Allele drop-out
F2DL1b	AACTTCTCCATCAGTCGCATGACG	24	E4(158)	50	67.1	1733	201	1	2DL3, 2DP1, 2DS2, 3DL3	
R2DL1b	GAGCTGCAGGACAAGGTCACAT	22	E5(225)	55	67.3					
F2DL2	CAATGTTGGTCAGATGTCAG	22	E4(128)	45	59.5	1850	345	1		
R2DL2	GCCCTGCAGAGAACCTACA	19	E5(243)	58	64.5			2		
F2DL3	TCCTTCATCGCTGGTGCTG	19	E7(344)	58	65.3	815	216	1		
R2DL3	GGCAGGAGACAACTTTGGATCA	22	E9(416)	50	65.3			1		
F2DL4	AGCGCTGTGGTGCCTCA	17	E3(20)	65	67.6	1442	813	1	3DL1*027, 3DL2*008/12/16	
R2DL4	TTCCTCACCTGTGACAGAAACAG	23	E5(291)	48	65			1		
F2DL5	TTCTTCTTTCTCCTTCATTGCTGC	24	E7(342)	42	64.3	950	309	1		
R2DL5	GGTACATGGGAGCTAGCAAC	20	E9(445)	55	63.1			1		2DL5B*004
F2DS1a	TCTCCATCAGTCGCATGAA	19	E4(165)	53	61.8	1839	303	2	2DS3	
F2DS1b	TCTCCATCAGTCGCATGAG	19	E4(165)	53	62			2	2DL1*004/7/10	*00202/00302
R2DS1	CATCTGGAGGTCCCTCCA	18	E5(266)	56	63.3			1		
F2DS2	TGCACAGAGAGGGGAAGTA	19	E4(140)	53	63.1	1775	222	2		
R2DS2	CACGCTCTCTCCTGCCAA	18	E5(214)	61	64.8			2		
F2DS3	TCACTCCCCCTATCAGTTT	19	E4(185)	47	60.8	1794	243	2		
R2DS3	GCATCTGTAGGTTCCTCCT	19	E5(266)	53	61.6			2		
F2DS4	AGTGACCCTCTGGACATG	18	E4(194)	56	61.5	1870	270	1		
R2DS4	GACGGAAACAAGCAGTGGA	19	E5(288)	53	63.3			1		
F2DS5	AGAGAGGGGACGTTTAACC	19	E4(142)	53	61.7	1795	267	2		
R2DS5	TTCCCTGGATAGATGGTAC	19	E5(231)	47	58.6			1	2DL1/2/3/5, 2DS1-4, 2DP1, 3DL1/S1	
F2DP1	CCAGCACACACAGGGACG	18	E3(71)	67	65.8	1768	285	1		
R2DP1	AGGTCCCTGCCAGGTCTTC	19	E4(166)	63	66.5			1		
F3DL1	TCCATCGGTCCCATGATGCT	20	E4(160)	55	58.6	1652	118	1	Revised on 16/Jul/2014	
R3DL1	CTGAGAGAGAAGGTTTCTCATATG	24	E5(200)	42	52.5			1	Revised on 16/Jul/2014	
F3DS1	GGCACCCAGCAACCCCA	18	E3(87)	71	61.7	1737	249	1	Revised on 16/Jul/2014	
R3DS1	CAAGGGCACGCATCATGGA	18	E4(164)	58	58.4			1	Revised on 16/Jul/2014	
F3DL2	TCATGCTGTACAAAGAAGACAGAAG	25	E3(45)	40	63.7	1713	204	1		
R3DL2	ATGACTGTCTCTCCTGATTTCAG	23	E4(113)	43	62.5			1		
F3DL3	CTCTCTGCCTGGCCCG	16	E3(15)	75	65.1	1621	309	1		
R3DL3	ACCAACATTGCAGGATGACC	20	E4(118)	50	63.8			1		
F3DP1	CCCTGGACATCGTGATTACAG	21	E4(196)	52	62.9	1575	12	1		
R3DP1	CTGAGAGAGAAGGTTTCCCAC	21	E5(200)	52	62.6			1		

PCR components

	2DL3 2DL4 2DL5 2DS4 2DP1 3DL3	2DL1	2DL2	3DP1	2DS2	2DS1	2DS5	2DS3	3DL2	3DL1	3DS1
dH_20	7.525	7.525	7.525	7.45	7.525	7.525	7.525	7.525	7.4	8.05	7.15
10x Buffer	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$MgCl_2$	0.375	0.375	0.375	0.5	0.375	0.375	0.375	0.375	0.5	0.75	0.75
dNTPs	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Taq	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Primers *Cf*	800 nM	800 nM	800 nM	800 nM	100 nM	800 nM	800 nM	800 nM	800 nM	100 nM	100 nM
Total DNA	200 ng	200 ng	100 ng	200 ng	100 ng	100 ng	200 ng	200 ng	200 ng	100 ng	100 ng
Program	KIR-62	KIR-62B	KIR-62A	KIR-60	KIR-60	KIR-60	KIR-58B	KIR-58	KIR-58B	KIR-58	KIR-58
TC-1			✔						✔		
TC-2	✔	✔			✔	✔				✔	✔

PCR conditions

KIR-66	Temperature (°C)	95º	95º	66º	72º	95º	62º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	15			15			1	1	1

KIR-62	Temperature (°C)	95º	95º	62º	72º	95º	58º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	15			15			1	1	1

KIR-62A (TC-1)	Temperature (°C)	95º	95º	62º	72º	95º	58º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	20			15			1	1	1

KIR-62B (TC-2)	Temperature (°C)	95º	95º	62º	72º	95º	58º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	15			20			1	1	1

KIR-60	Temperature (°C)	95º	95º	60º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	35			1	1	1

KIR-58B	Temperature (°C)	95º	95º	58º	72º	95º	54º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	20			15			1	1	1

KIR-58	Temperature (°C)	95º	95º	58º	72º	72º	5º	RT
	Time	3’	30”	30”	1.5’	3’	5’	∞
	Cycles	1	35			1	1	1

Notes

1. The PCR reactions have been optimized for Vivantis™ reagents (Cat. # PL1202), with 50 mM $MgCl_2$ and 5 IU/µL Taq DNA polymerase.

2. dNTP working dilutions were at 10 mM for each dNTP.

3. Primers (with the exception of 3DS1) were prepared at 10 µM final concentration. The KIR2DL2, 2DS2, 2DS1, 3DL1 and 3DS1 primers are used at a lower final concentration than the rest of primers

4. The DNA work solutions must be stored at refrigerator temperatures (between 4ºC and 0ºC) and not frozen. These work solutions must be vortexed and briefly centrifuged (2 seconds) before use.

5. PCR reactions must be prepared in an icebox or *Eppendorf Ice Coolers* to avoid unspecific priming and initiation of reaction and loss of reagents due to evaporation.

6. The PCR workbench must be cleaned with 70% ethanol before and after use. The reagents, PCR conditions, thermocycler and the electrophoresis conditions (voltage, time and agarose concentration) must be documented in the corresponding logbook.

References

1. Alvarado-Hernández DL, Hernández-Ramírez D, Noyola DE, García-Sepúlveda CA. KIR gene diversity in Mexican mestizos of San Luis Potosí. Immunogenetics. 2011 Sep;63(9):561-75. doi: 10.1007/s00251-011-0540-x. Epub 2011 Jun 4. PMID: 21638211

2. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, Tyan D, Lanier LL, Parham P. Human diversity in killer cell inhibitory receptor genes. Immunity. 1997 Dec;7(6):753-63. PMID: 9430221

Revision history

1.0 Original document.

6.0 Changes to document format only.