



HLA-C group 1 and 2 KIR ligand genotyping PCR protocol.

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This protocol describes PCR components and conditions for HLA-C group 1 and group 2 genotyping approach based on that published previously (See reference below). This PCR-SSP technique allows for the identification of HLA-C group 1 and group 2 alleles through two independent reactions each. In addition, primers specific for confounding group 1 and group 2 bearing HLA-B alleles allow for these to be identified through two additional PCRs. Internal control primers specific for a 796 bp HLA-DRB1 fragment are included in all reactions. Amplified fragments are documented after 3% agarose gel electrophoresis.

Oligonucleotide primers used:

ID	Sense	Specificity	Sequence	bp	%GC	Tm	Amplicon
HLA-CP1	Fwd	HLA-C generic	5'- GCCGCGAGTCCRAGAGG -3'	17	73.5	60.1	–
HLA-C1P2	Rev	HLA-C group 1	5'- GCGCAGGTTCCGCAGGC -3'	17	76.5	63.5	129 bp
HLA-C1P3			5'- GTTGTAGTAGCCGCGCAGG -3'	19	63.2	58.7	141 bp
HLA-C2P4	Rev	HLA-C group 2	5'- CGCGCAGTTTCCGCAGGT -3'	18	66.7	61.9	130 bp
HLA-C2P5			5'- GTTGTAGTAGCCGCGCAGT -3'	19	57.9	57.8	141 bp
HLA-BP6	Fwd	C1 bearing HLA-B	5'- CCATGAGGTATTTCTACACCT -3'	21	42.9	51.4	156 bp
HLA-BP7	Rev		5'- CCTCCTGCTCCACCCAC -3'	17	70.6	58.2	
HLA-BP8	Fwd	C2 bearing HLA-B	5'- GCCGCGAGTCCGAGAGG -3'	17	76.5	61.3	425 bp
HLA-BP9	Rev		5'- GCCATACATCGTCTGCCAA -3'	19	52.6	55.3	
HLA-DRBF	Fwd	HLA-DRB1	5'- TGCCAAGTGGAGCACCCAA -3'	19	57.9	60.0	796 bp
HLA-DRBR	Rev		5'- GCATCTTGCTCTGTGCAGAT -3'	20	50	55.6	

PCR Components

	<i>C_i</i>	P1+P2	P1+P3	P1+P4	P1+P5	P6+P7	P8+P9	<i>C_f</i>
dH ₂ O	–	11.5 µL	11.5 µL	11.5 µL	11.5 µL	11.5 µL	11.5 µL	–
PCR Buffer	10 x	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	1 x
MgCl ₂	50 mM	0.4 µL	0.4 µL	0.4 µL	0.4 µL	0.4 µL	0.4 µL	1 mM
4x dNTPs	10 mM	0.4 µL	0.4 µL	0.4 µL	0.4 µL	0.4 µL	0.4 µL	200 µM
HLA-DRB Oligos	5 µM	1.5 µL	1.5 µL	1.5 µL	1.5 µL	1.5 µL	1.5 µL	375 nM
Target Oligos	10 µM	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	1 µM
Taq Vivantis	5 UI/µl	0.2 µL	0.2 µL	0.2 µL	0.2 µL	0.2 µL	0.2 µL	0.05 UI
DNA	100 ng/µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	10 ng/µL
<i>V_f</i>	–	20.0 µL	20.0 µL	20.0 µL	20.0 µL	20.0 µL	20.0 µL	
Oligo combination	–	C1-A	C1-B	C2-A	C2-B	B1	B2	
Amplicon size	–	129 bp	141 bp	130 bp	141 bp	156 bp	425 bp	





Forward primer map (only first HLA-C alleles shown)

cDNA	110	120	130	140	150	160	170	180	190	200
C*01:02:01	CATCCGTGTC	CCGGCCTGGC	CGCGGAGAGC	CCCGCTTCAT	CTCAGTGGGC	TACGTGGACG	ACACGCAGTT	CGTGCGGTTT	GACAGCGACG	CCGCGAGTCC
C*01:02:02	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:03	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:04	-----	-C-	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:05	-----	-----	-----	-----	-----	-----	-----	A-	-----	-----
C*01:02:06	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:07	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:08	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:09	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:11	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:12	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:13	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:14	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:15	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:03	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:04	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:05	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:06	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:07	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:08	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:09	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:11	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:12	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:13	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:14	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:15	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Reverse primer map (only first HLA-C alleles shown)

cDNA	HLA-C group 1 Rev	HLA-C group 2 Rev	310	320	330	340	350	360	370	380	390	400
C*01:02:01	AGCCTGCGGA	ACCTGCGCGG	CTACTACAAC	CAGAGCGAGG	CCG	GGTCTCA	CACCCCTCCAG	TGGATGTGTG	GCTGCGACCT	GGGGCCCGAC	GGGCGCCTCC	
C*01:02:02	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:03	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:04	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:05	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:06	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:07	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:08	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:09	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:11	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:12	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:13	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:14	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:02:15	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:03	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:04	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:05	-----	-----	-----	-----	-----	-----	-----	A-----C-	-----	-----	-----	-----
C*01:06	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:07	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:08	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:09	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:11	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:12	-----	-----	-----	-----	-----	-----	-----	-----	A-----	-----	-----	-----
C*01:13	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:14	A-----	-A-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C*01:15	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----



PCR Conditions

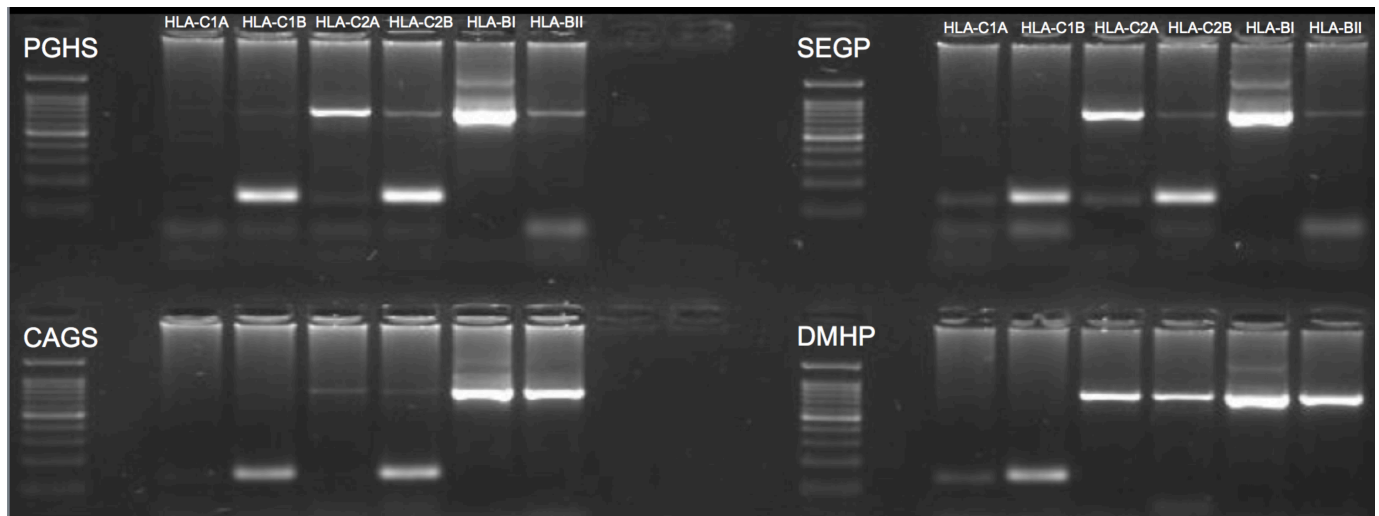
(Total elapsed time: 2 hr 35 min in Axygen TC1 & TC2 cycler /Public/HLA-C ECA)

	Den		Ann	Ext	Den	Ann	Ext	Den	Ann	Ext	F. Ext	
Temp	96°	96°	70°	72°	96°	65°	72°	96°	58°	72°	72°	RT
Time	1'	25"	45"	30"	25"	45"	30"	25"	1'	2'	5	∞
	4 cycles				26 cycles			5 cycles				

Electrophoresis conditions

Add 5 µL of orange loading buffer to each of the six PCR reactions corresponding to each DNA sample tested, mix by pipetting and load 20 µL into a 3% agarose gel well and run at 5 VDC/cm (120 VDC for 50 minutes in a 25 cm gel).

HLA-C genotyping PCR-SSP amplicon patterns for four of our local reference DNA's.



Interpretation: PGHS: C1,C2 SEGP: C1,C2 CAGS: C1,C2 DMHP: C1,-
 None positive for C1 / C2 bearing HLA-B alleles.



Notes

1. Clean workbench with 0.1% NaOCl and 70% Ethanol before and after setting up the PCR.
2. Prepare PCR reactions on ice to prevent evaporation of small liquid volumes.
3. Vortex all reagents (except DNA and Taq DNA polymerase) before dispensing into master mix.
4. Vortex master mix after adding all required reagents then dispense into each tube.
5. Vortex all PCR reactions after adding reagents and DNA. Spin briefly and load into cyclor.

References

1. Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. in “Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage.” Hum Reprod. 2008 Apr; 23(4):972-6.

Revision history

- 1.0 Original document.

