SUPPLEMENTARY APPENDIX

Factor VIII cross-matches to the human proteome reduce the predicted inhibitor risk in missense mutation hemophilia A

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Supplemental Information

Supplemental Table S1. Breakdown of calculations required to identify novel peptide-MHC surfaces.

Description	Count	Calculation	Total
Step 1: calculations required for a single residue			
15-mers spanning residue	15		15
Positions 15-mer can bind to MHC	7	15 × 7	105
HLA alleles	25	105 × 25	2,625
Step 2: calculations required for endogenous FVIII (using total from **step 1**)			
Reported *F8* missense mutations	956	2,625 × 956	2,509,500
Step 3: calculations required for tFVIII (using total from **step 1**)			
Locations associated with *F8* mutations	605	2,625 × 605	1,588,125
Step 4: summation			
Sum totals from **step 2** and **step 3**		2,509,500 + 1,588,125	4,097,625
Step 5: proteome scanning			
Two different types of calculation are required: a) matching 9-mer cores of risk-associated peptides (from **step 4**) to proteome 9-mers (see Figure 4A); and b) calculating the MHC binding cores and binding strengths of matched peptides (see Figure 4B). We do not keep track of the exact number of calculations performed, so here we estimate the lower bounds.			
a) Proteome matching:			
Risk-associated peptides	≫12,189		
Non-identical proteome 9-mers	11,272,502	12,189 × 11,272,502	137,400,526,878
b) MHC binding:			
F8 mutation/HLA allele combinations with reduced risk after scanning	4,302		
Rough estimate of ratio of non-binders to binders	10	4,302 × 10	43,020

Note that the number of calculations at step 2 is higher than the number at step 3 because a single location may be associated with more than one reported missense mutation, e.g. K48E, K48T and K48N.

Supplemental Table S2. Percentage of risk-associated F8 missense mutations for different HLA alleles, before and after proteome scanning

HLA allele	risk (%) with 1000 nM threshold		risk (%) with 500 nM threshold	
	before	after	before	after
DRB1*01:01	86	48	78	41
DRB1*03:01	34	25	24	16
DRB1*04:01	62	38	47	25
DRB1*04:04	66	38	53	26
DRB1*04:05	60	35	49	28
DRB1*07:01	70	46	60	39
DRB1*08:02	36	23	16	10
DRB1*09:01	66	41	50	25
DRB1*11:01	58	37	43	24
DRB1*13:02	34	25	26	16
DRB1*15:01	64	36	49	25
DRB3*01:01	35	25	24	17
DRB4*01:01	60	33	39	16
DRB5*01:01	58	40	48	31
DPA1*01-DPB1*04:01	41	27	33	23
DPA1*01:03-DPB1*02:01	46	29	36	21
DPA1*02:01-DPB1*01:01	59	34	47	25
DPA1*02:01-DPB1*05:01	30	18	17	8
DPA1*03:01-DPB1*04:02	52	33	41	22
DQA1*01:01-DQB1*05:01	27	21	19	15
DQA1*01:02-DQB1*06:02	54	34	36	20
DQA1*03:01-DQB1*03:02	21	15	10	6
DQA1*04:01-DQB1*04:02	21	13	11	6
DQA1*05:01-DQB1*02:01	36	18	24	12
DQA1*05:01-DQB1*03:01	58	32	40	20

Supplemental Figure S1. MHC-binding strengths of *F8* peptides predicted to form novel pMHC surfaces with and without proteome scanning.

Heatmap showing the predicted occurrence of novel pMHC surfaces and binding strengths for 25 HLA-DR/DP/DQ alleles (*y* axis) covering the complete set of missense mutations in the Factor VIII Gene (F8) Variant Database (*x* axis). Black and grey squares indicate *F8* missense mutation/HLA allele combinations that are not predicted to form a novel pMHC surface. Otherwise the temperature color scale indicates the predicted binding strength of the strongest binding peptide with a novel pMHC surface for each remaining *F8* missense mutation/HLA allele combination. (A) MHC-binding strengths of *F8* peptides predicted to form novel pMHC surfaces without proteome scanning. (B) MHC-binding strengths of *F8* peptides predicted to form novel pMHC surfaces with proteome scanning. Grey squares indicate *F8* missense mutation/HLA allele combinations that are no longer predicted to form a novel pMHC surface after cross-matches to the proteome are taken into account.

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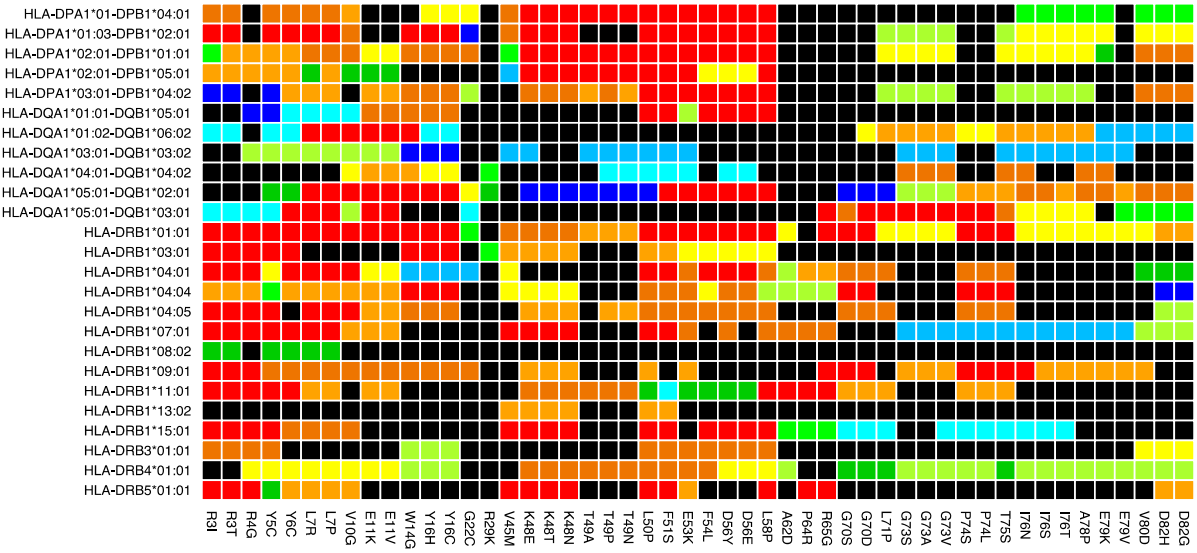

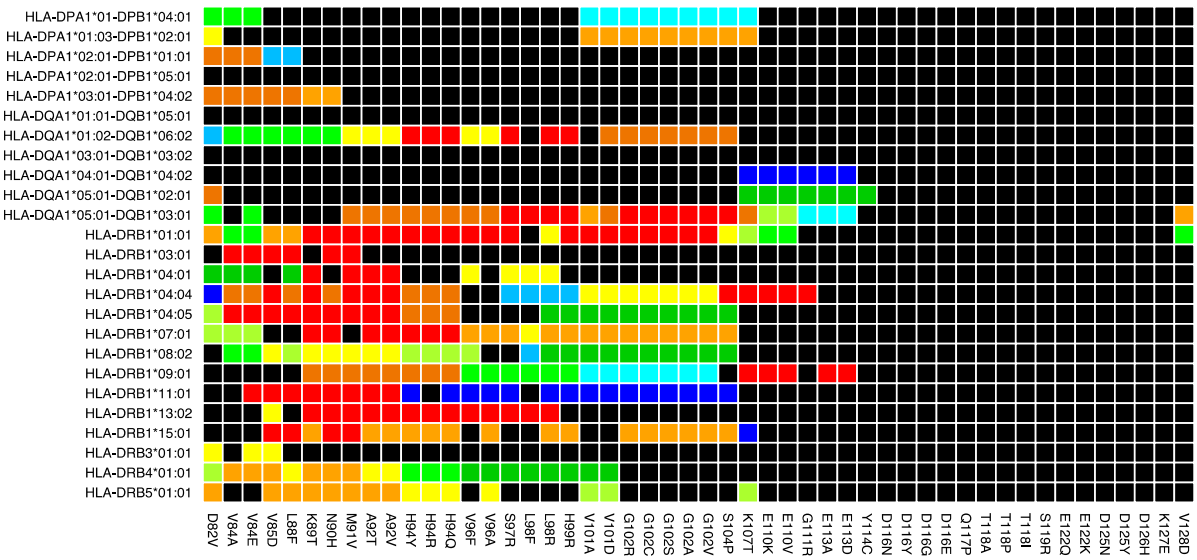

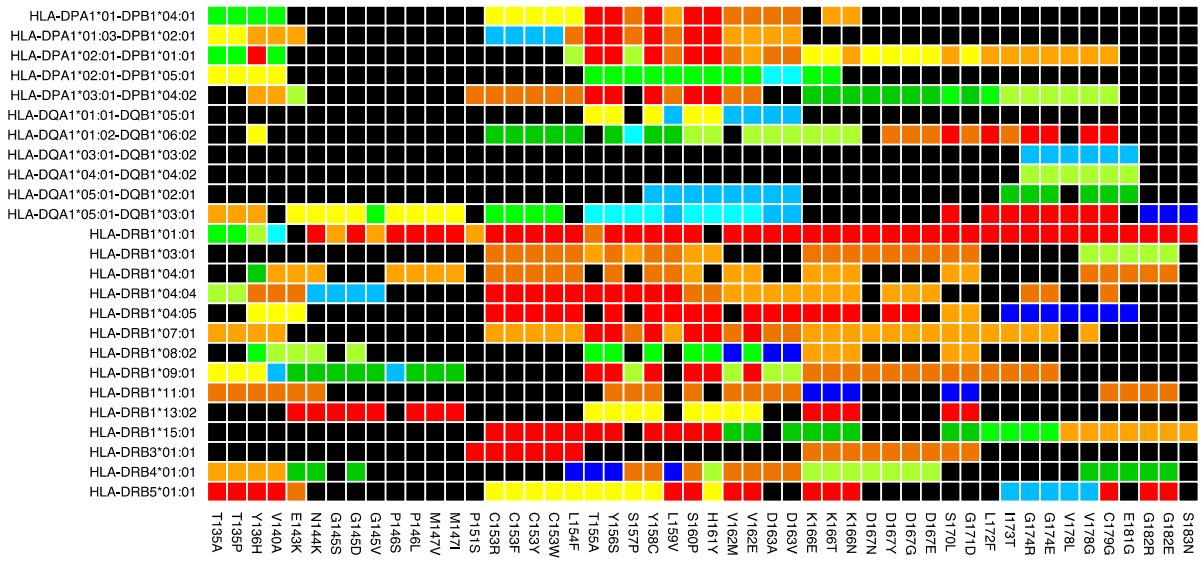
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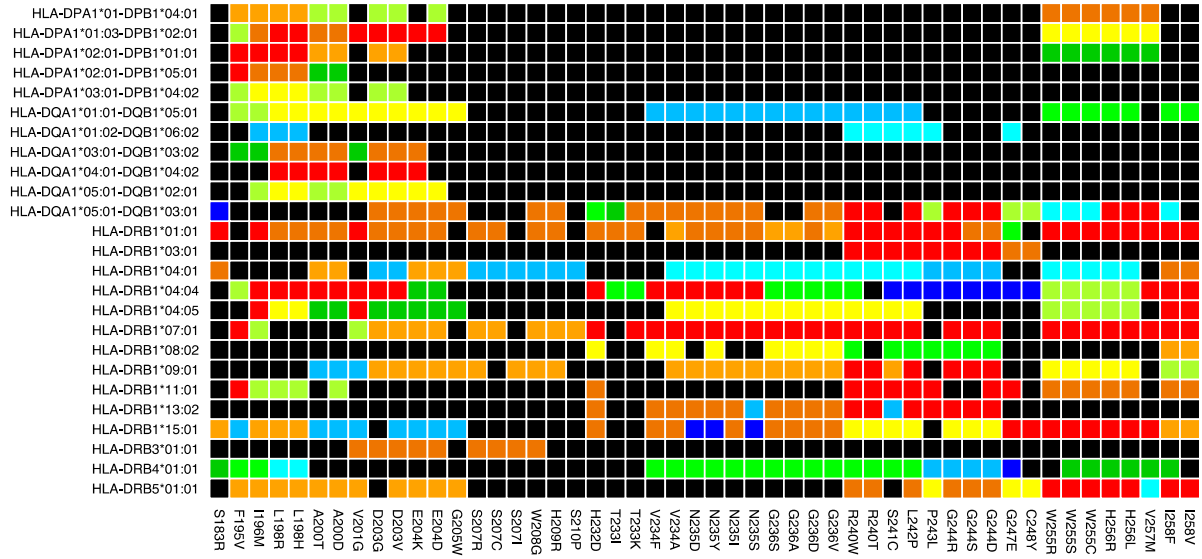
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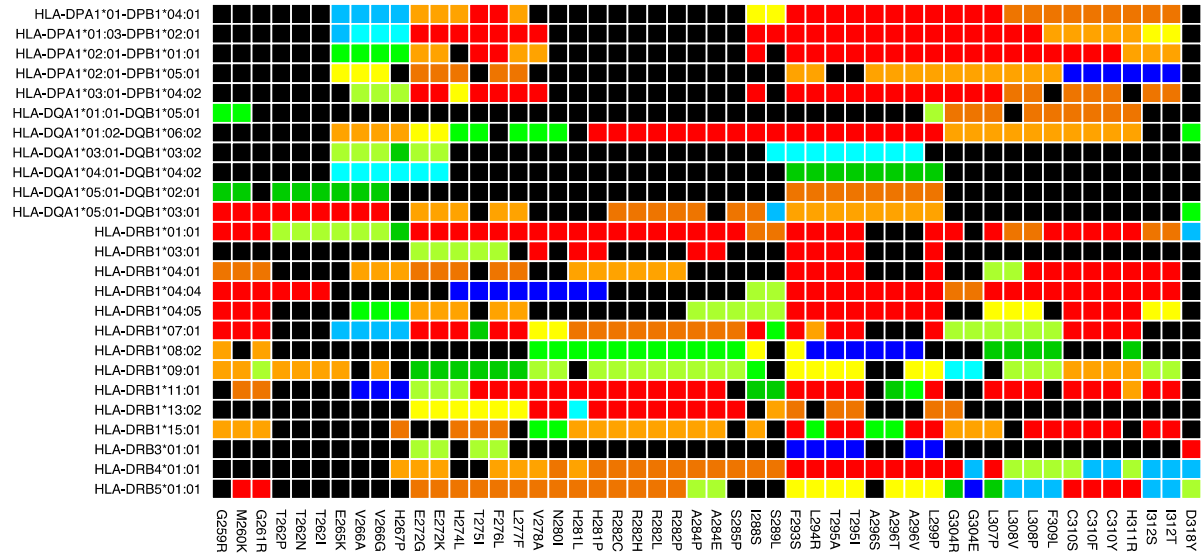
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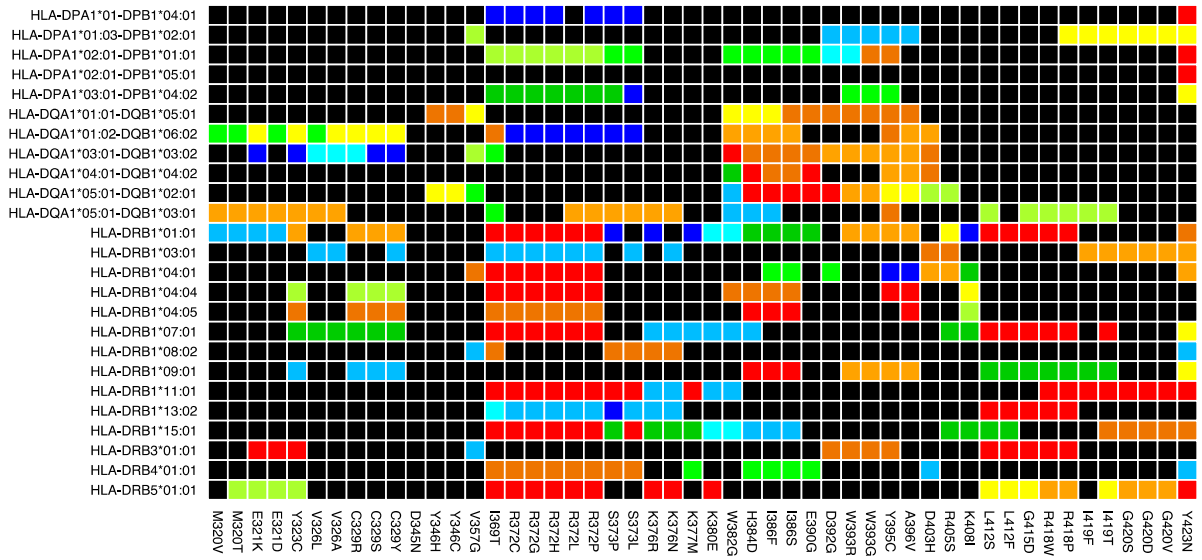
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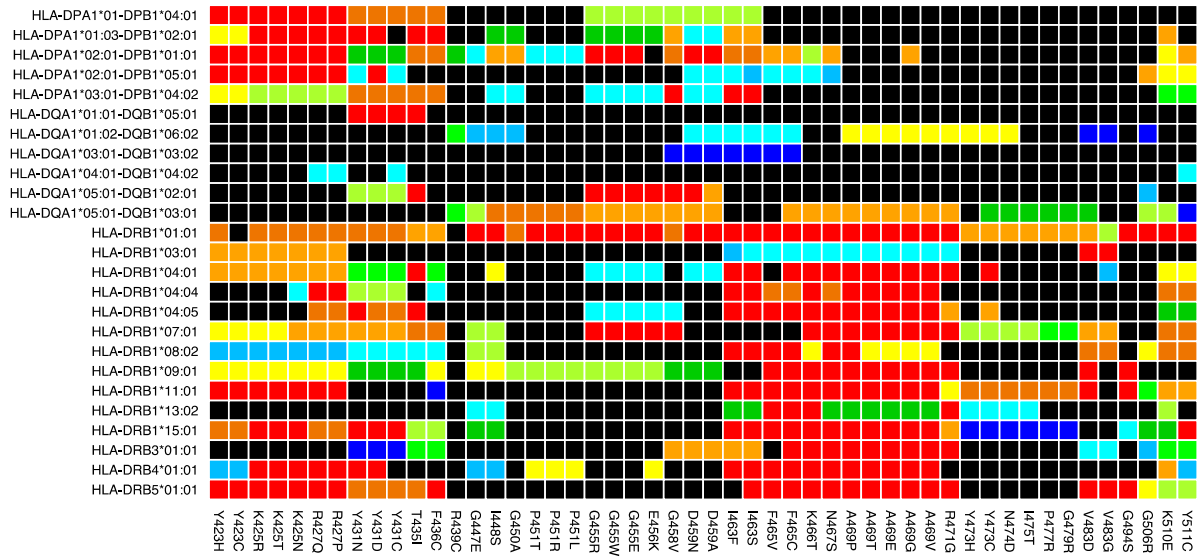
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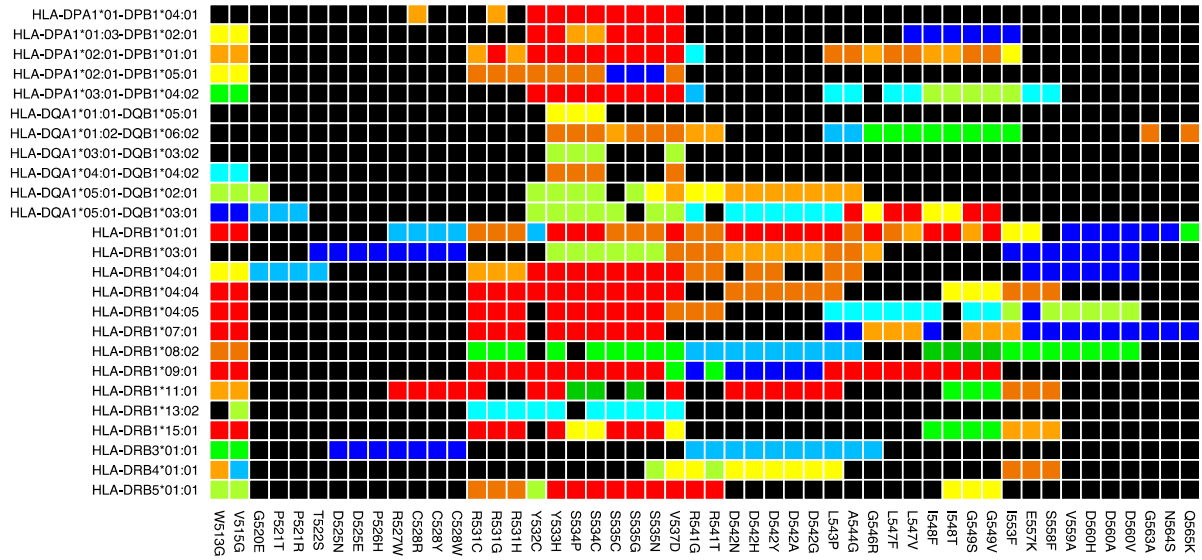
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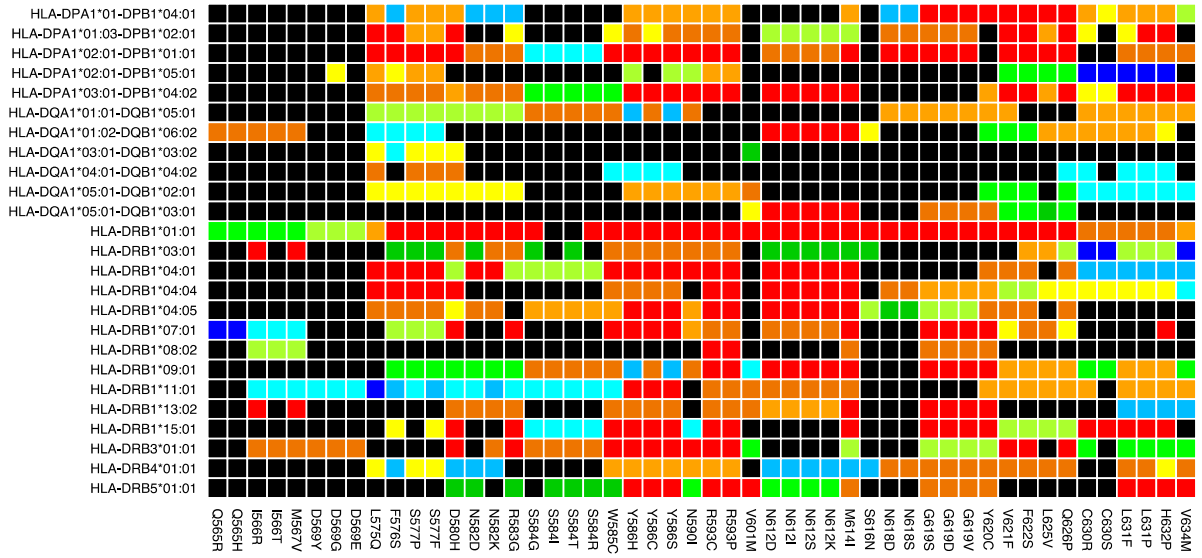
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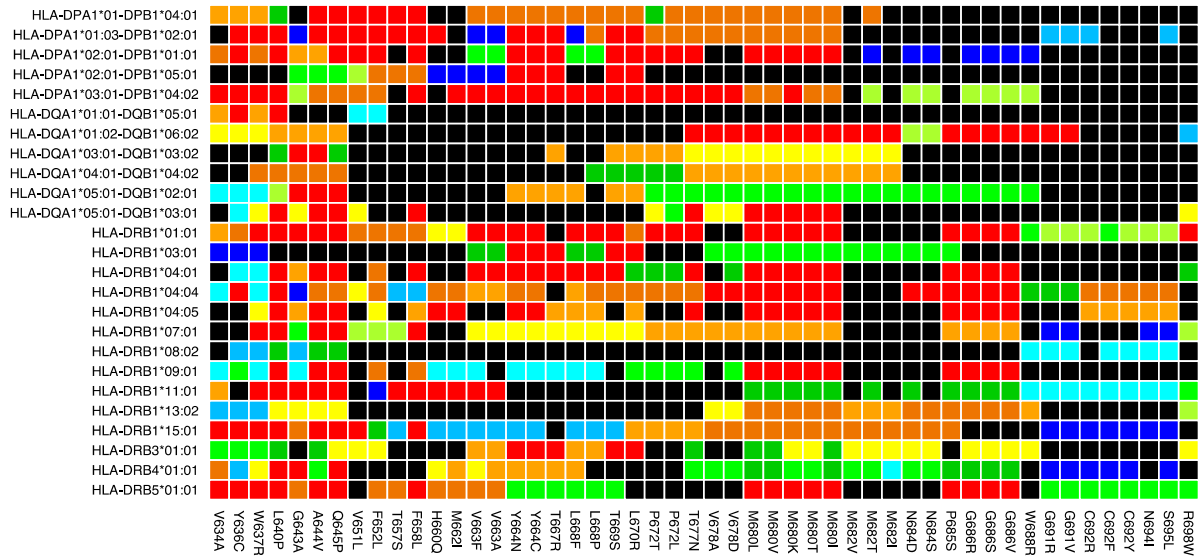
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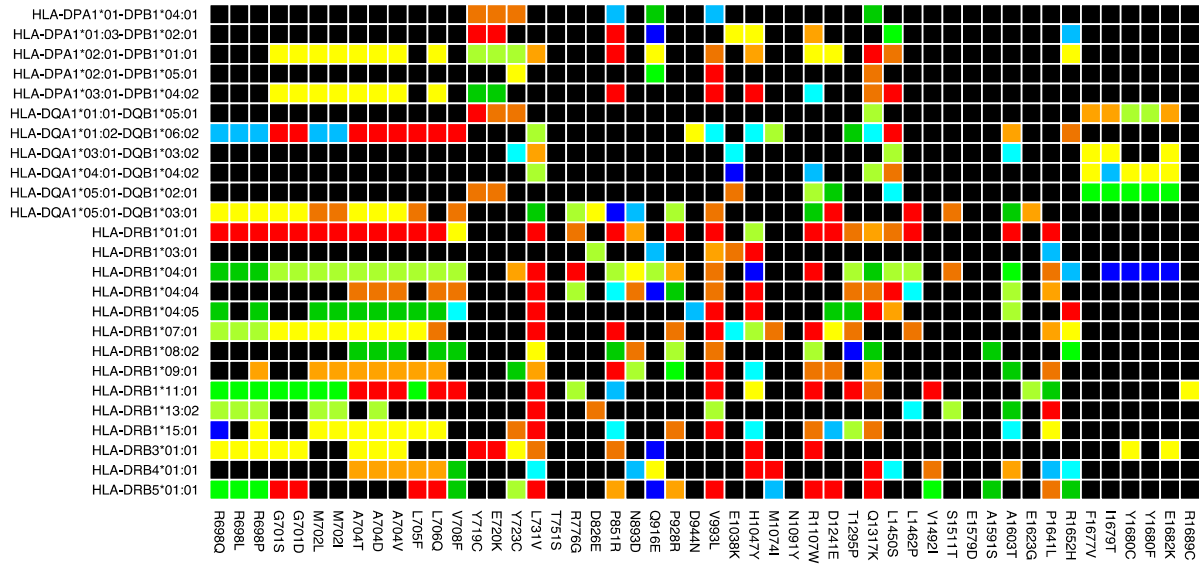
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Y723C
L731V
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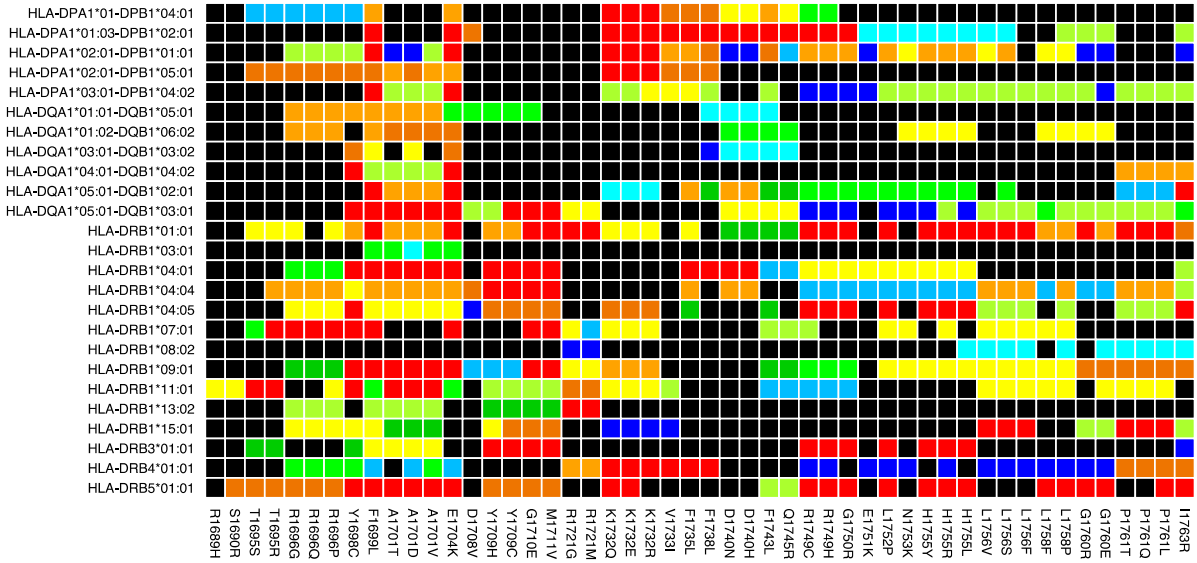
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D1740H
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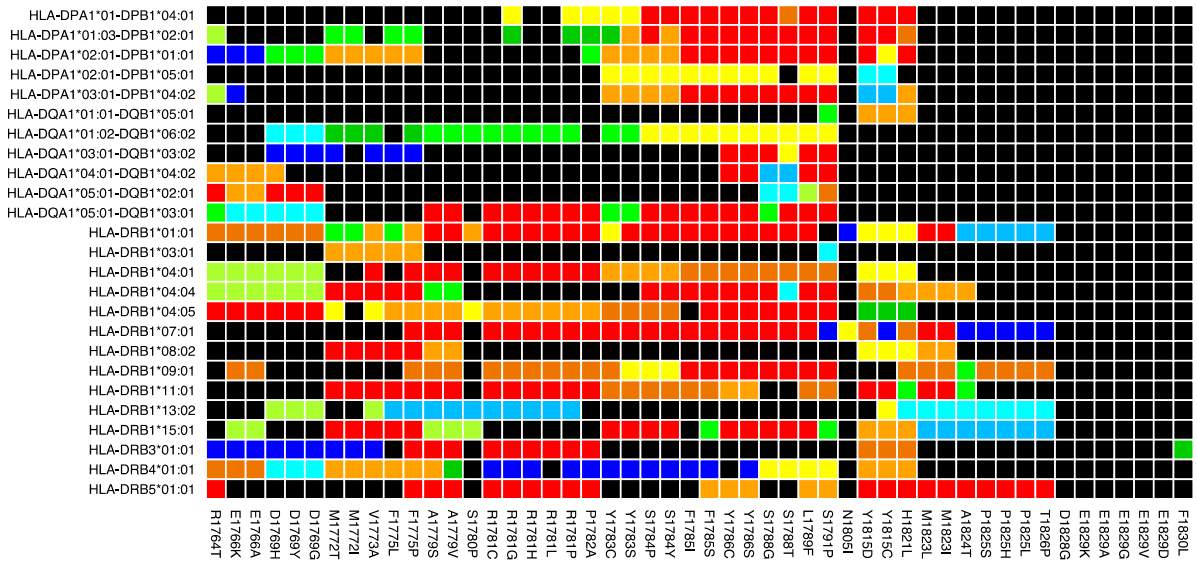
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F1775P
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A1779V
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R1781L
R1781P
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F1785I
F1785S
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S1791P
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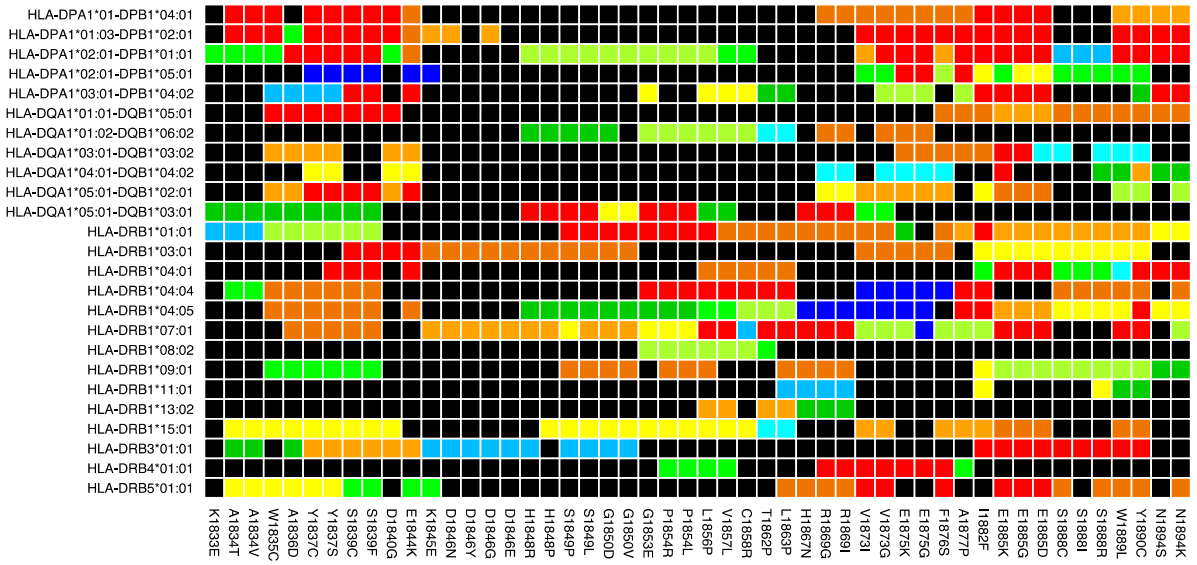
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K1845E
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D1846G
D1846E
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H1848P
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S1849L
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G1850V
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L1856P
V1857L
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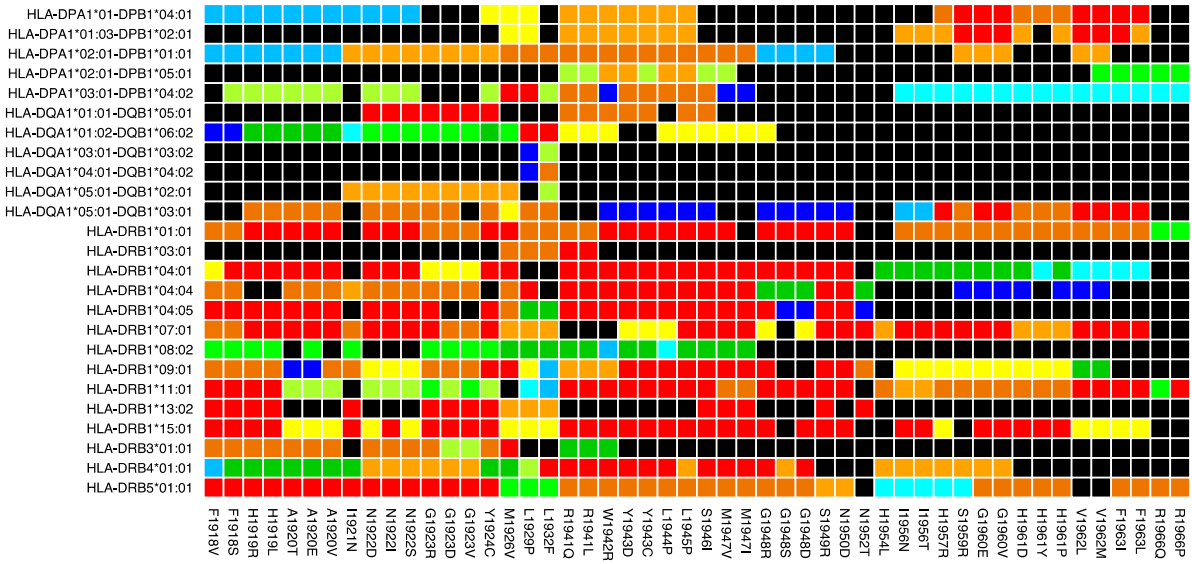
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G1923V
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R1941L
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Y1943C
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L1945P
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M1947V
M1947I
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G1948S
G1948D
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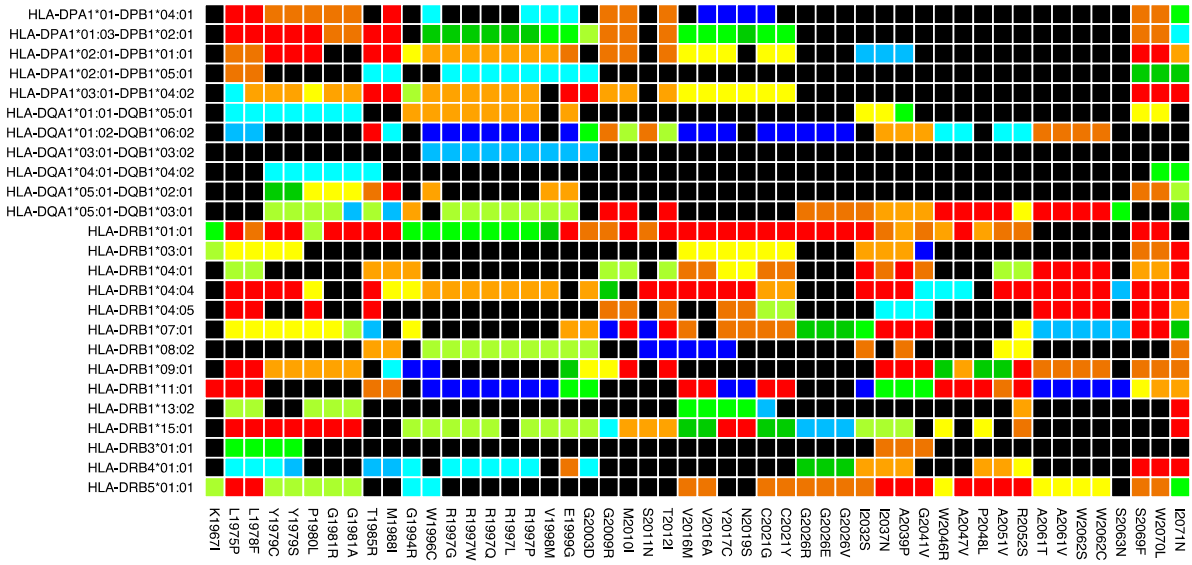
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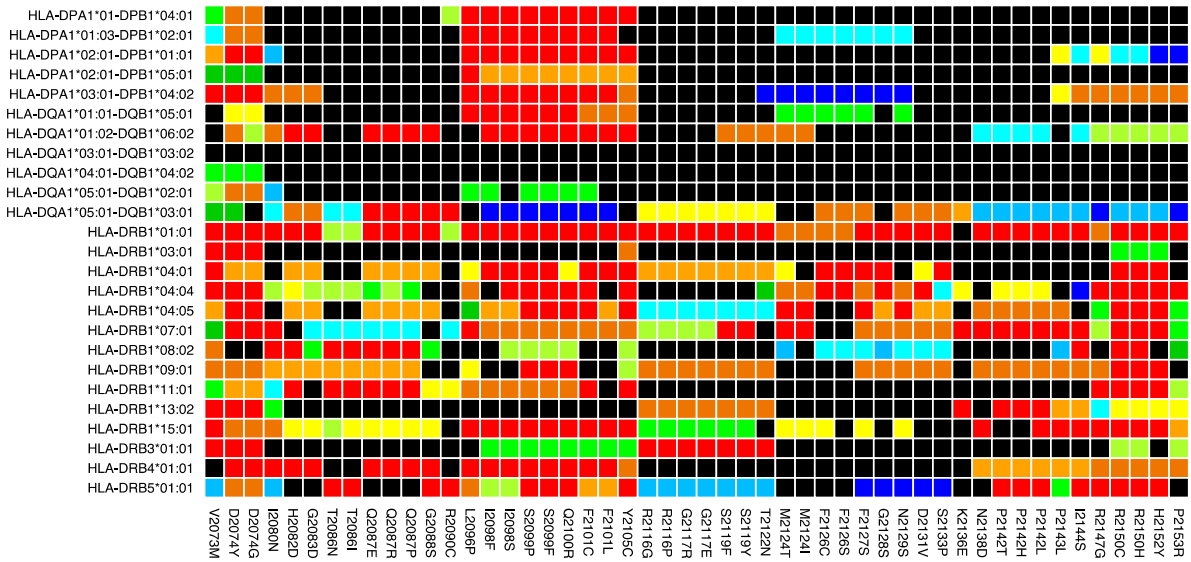
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D2074G
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T2086I
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S2099P
S2099F
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F2101C
F2101L
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S2119F
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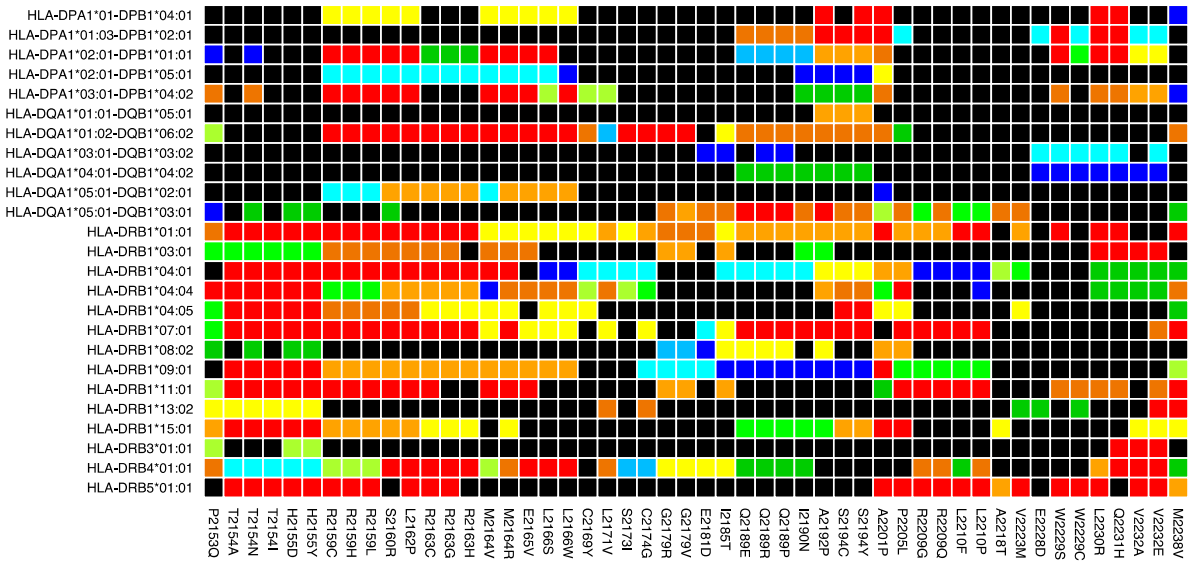
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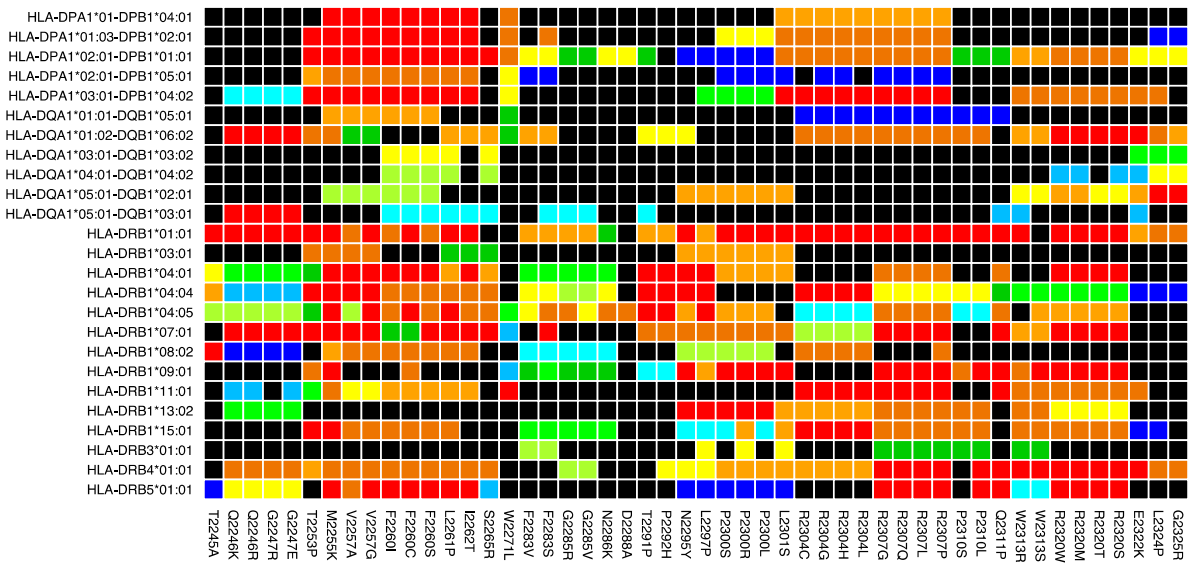
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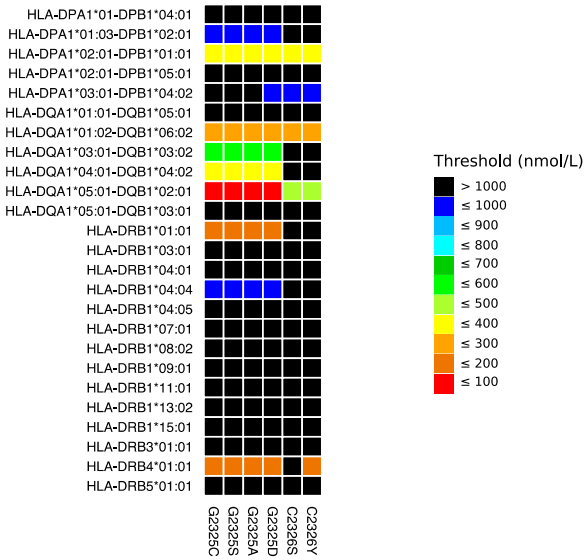
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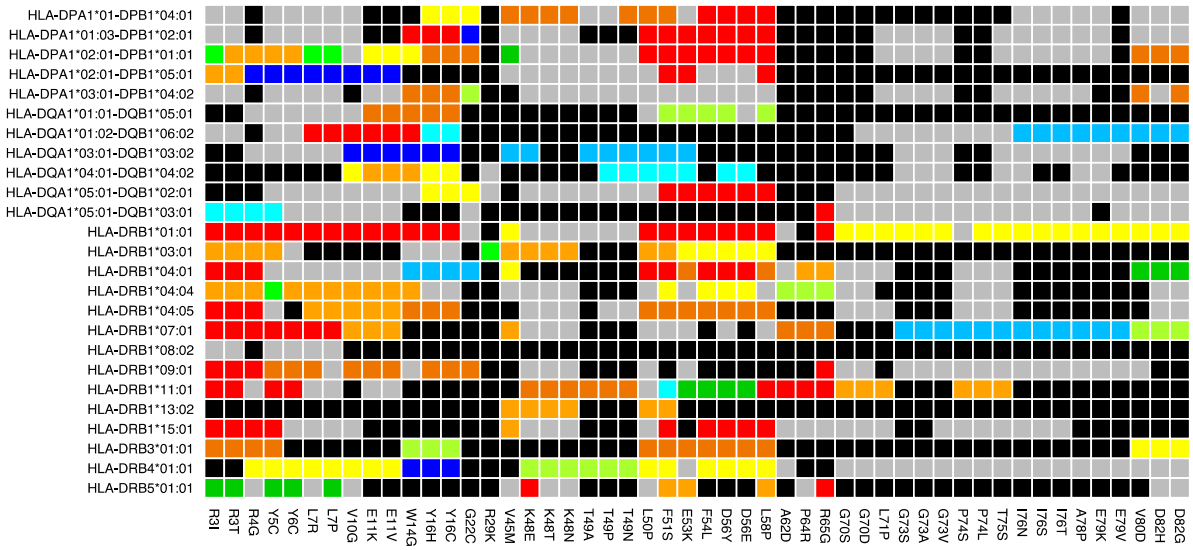
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
G2325C
G2325S
G2325A
G2325D
C2326S
C2326Y
Threshold (nmol/L)
> 1000
≤ 1000
≤ 900
≤ 800
≤ 700
≤ 600
≤ 500
≤ 400
≤ 300
≤ 200
≤ 100

B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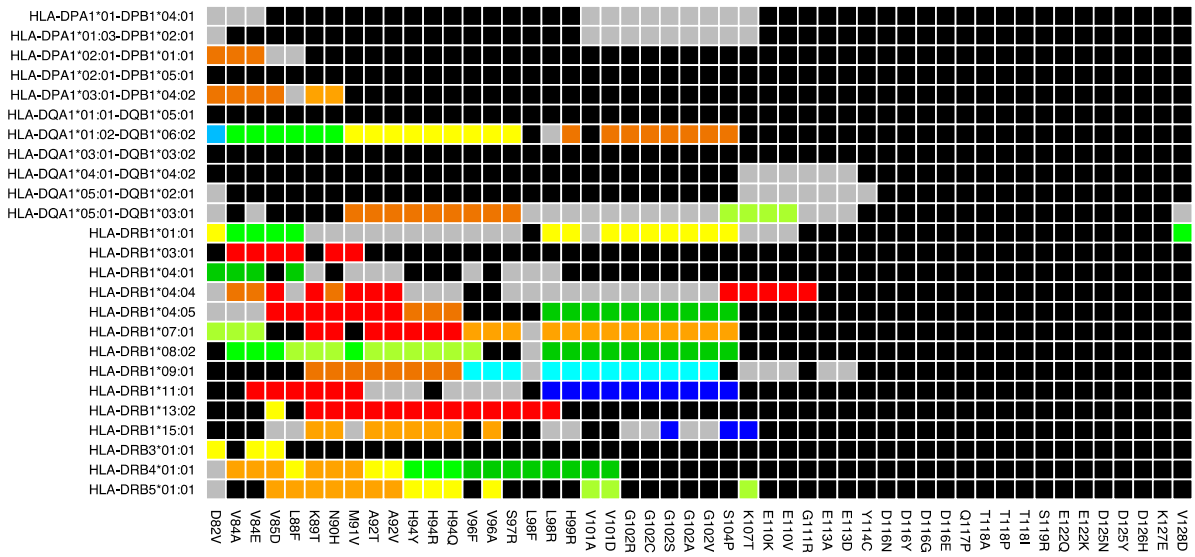
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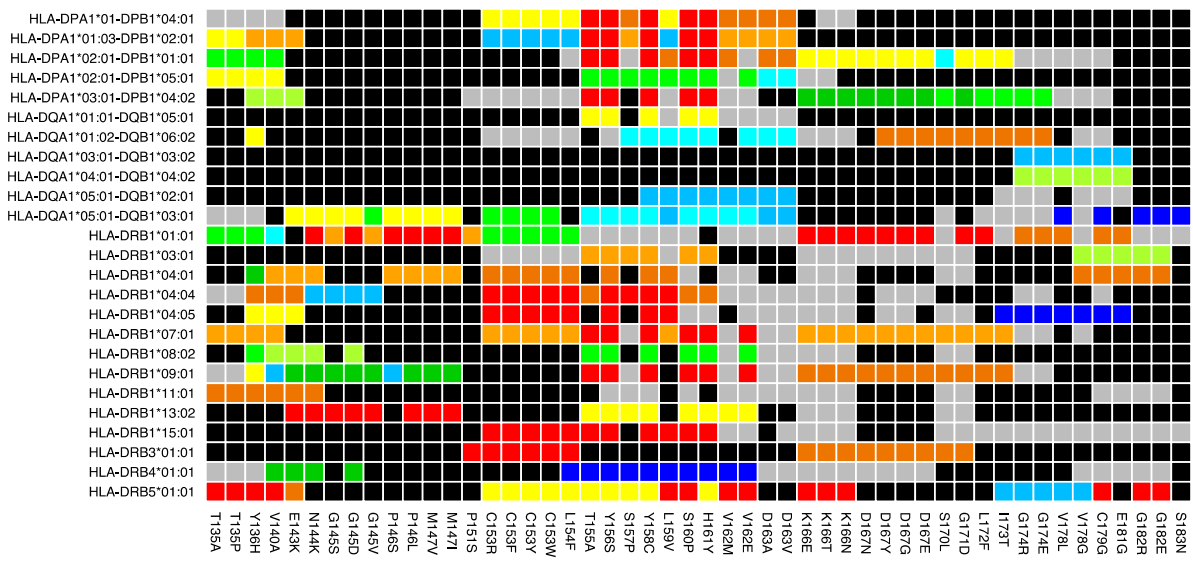
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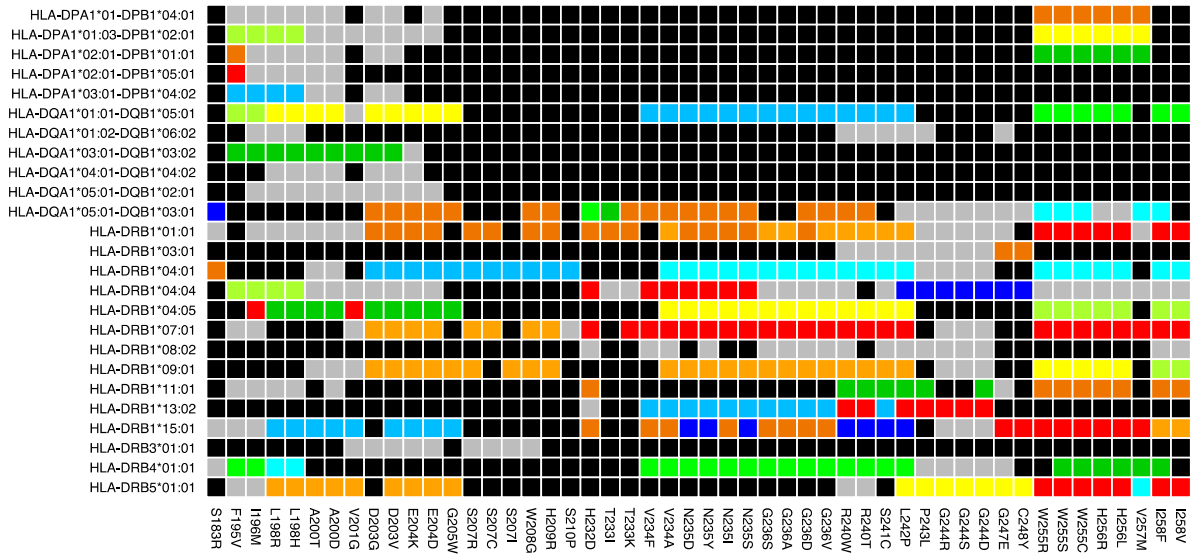
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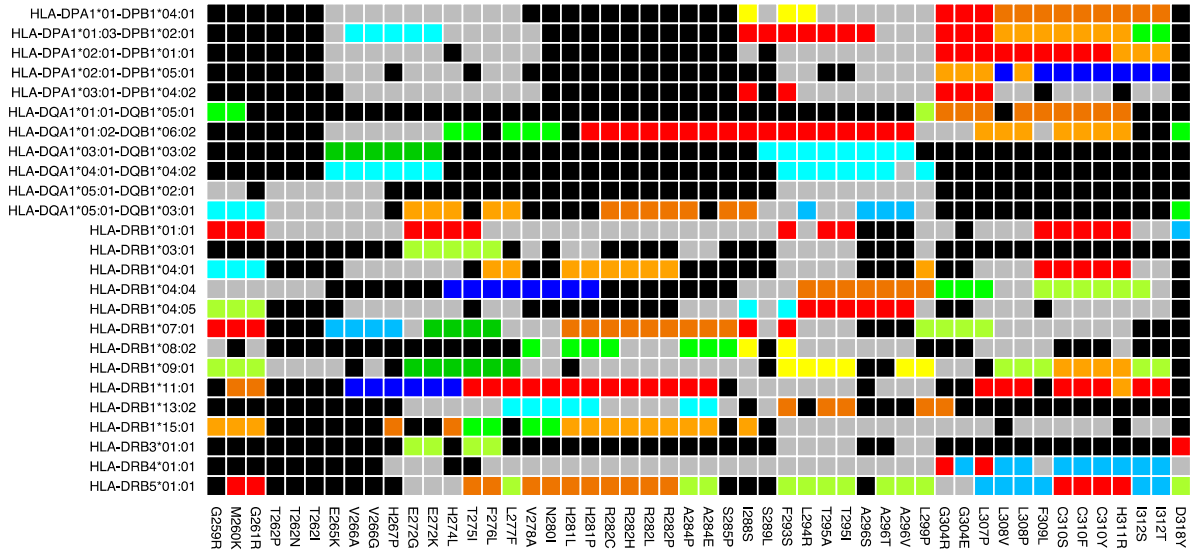
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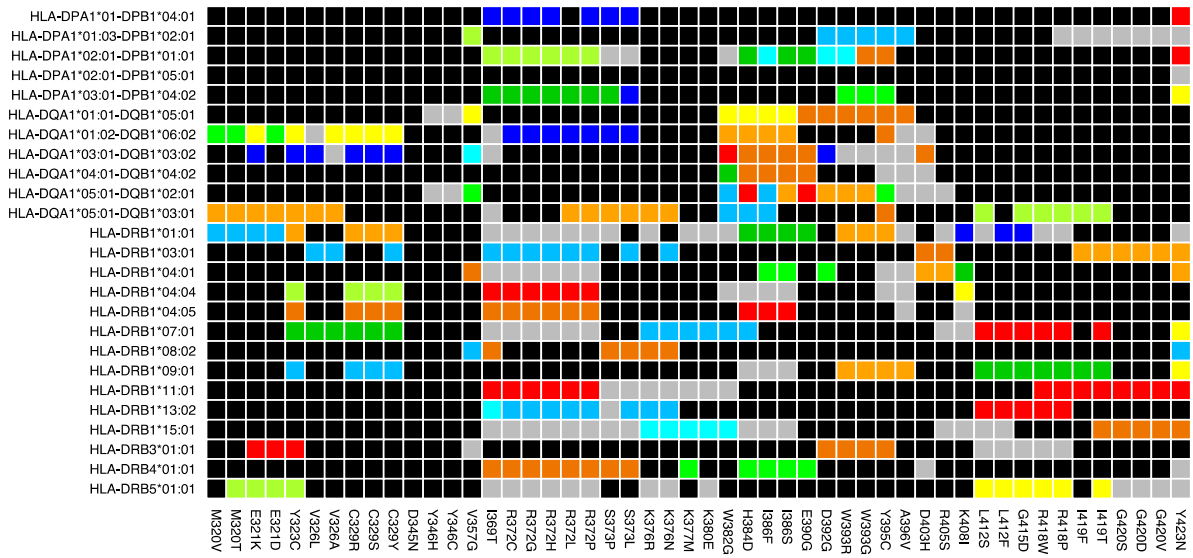
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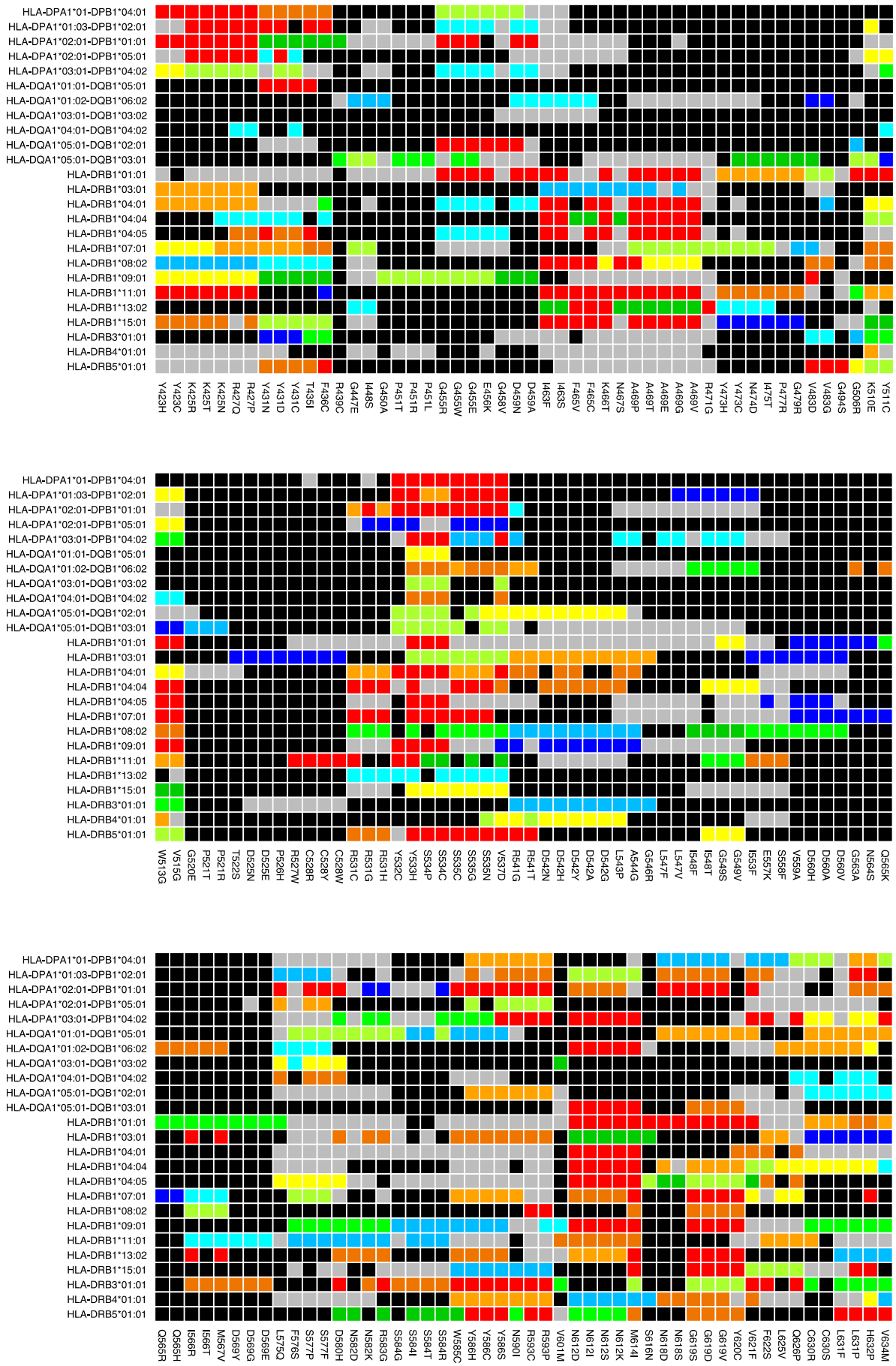
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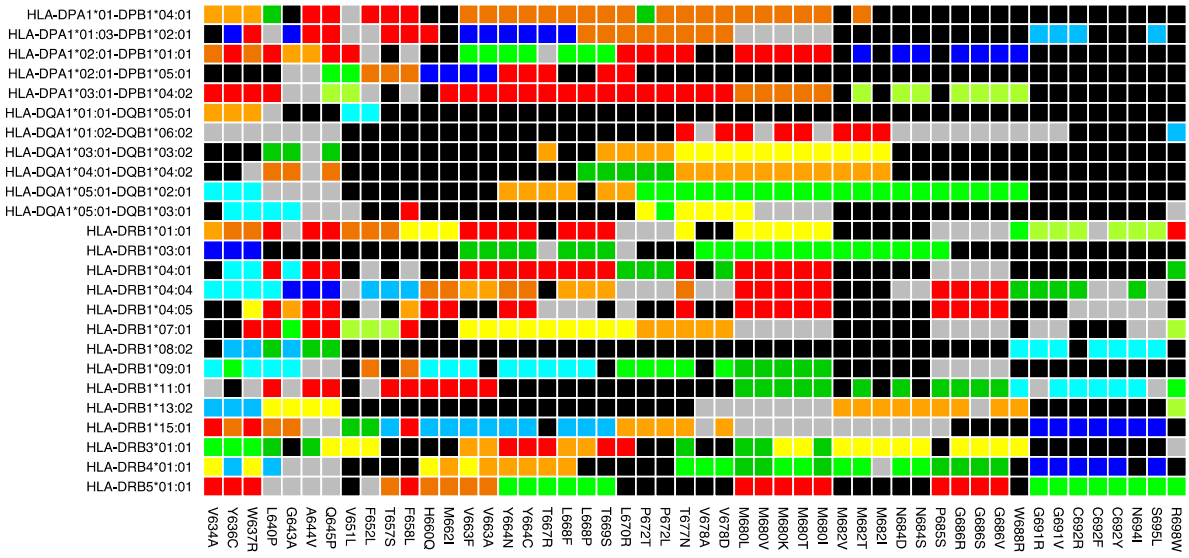
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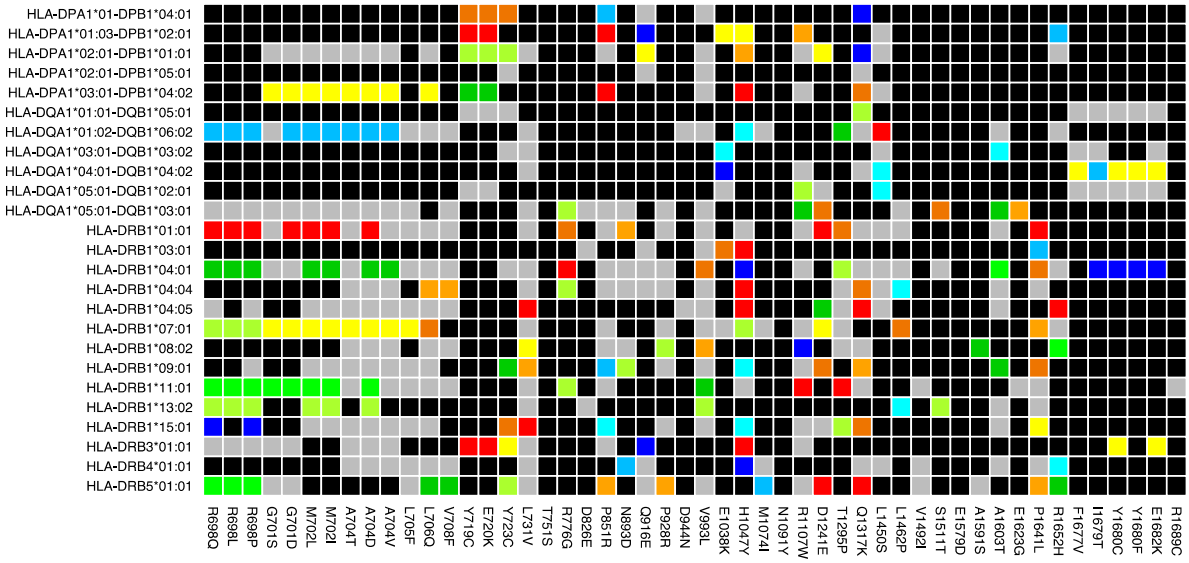
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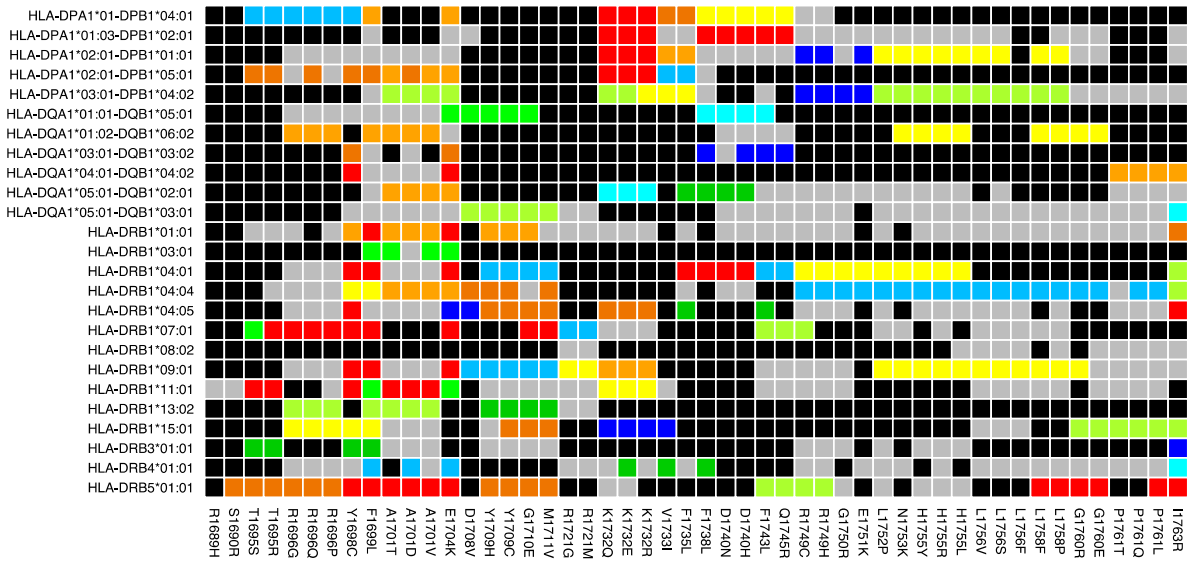
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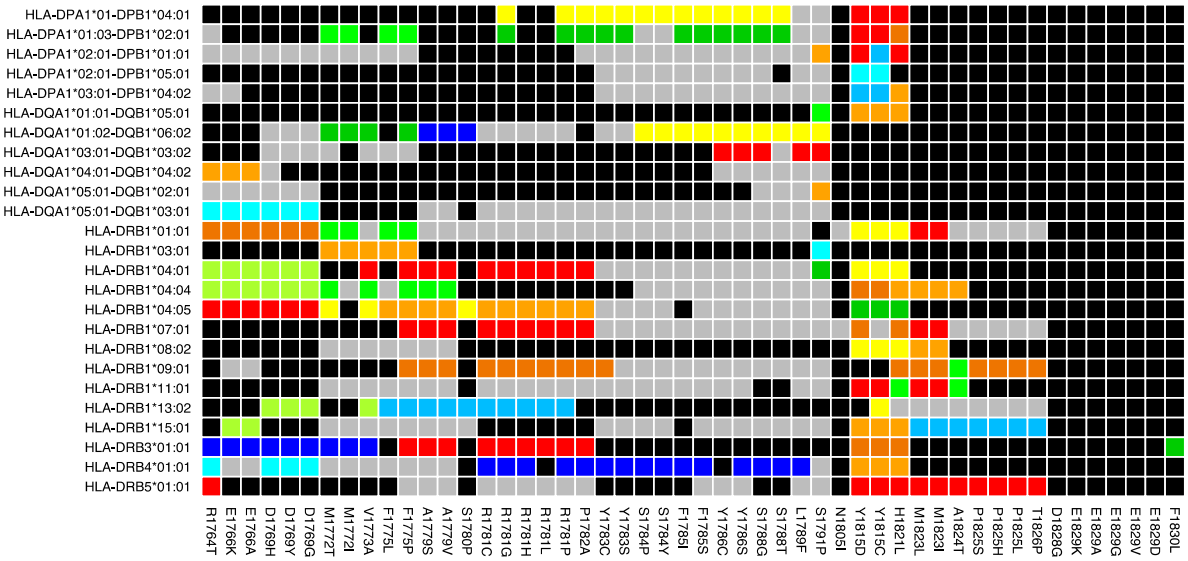
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HLA-DQA1*05:01-DQB1*03:01
HLA-DRB1*01:01
HLA-DRB1*03:01
HLA-DRB1*04:01
HLA-DRB1*04:04
HLA-DRB1*04:05
HLA-DRB1*07:01
HLA-DRB1*08:02
HLA-DRB1*09:01
HLA-DRB1*11:01
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HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
R1764T
E1766K
E1766A
D1769H
D1769Y
D1769G
M1772T
M1772I
V1773A
F1775L
F1775P
A1779S
A1779V
S1780P
R1781C
R1781G
R1781H
R1781L
R1781P
P1782A
Y1783C
Y1783S
S1784P
S1784Y
F1785I
F1785S
Y1786C
Y1786S
S1788G
S1788T
L1789F
S1791P
N1805I
Y1815D
Y1815C
H1821L
M1823L
M1823I
A1824T
P1825S
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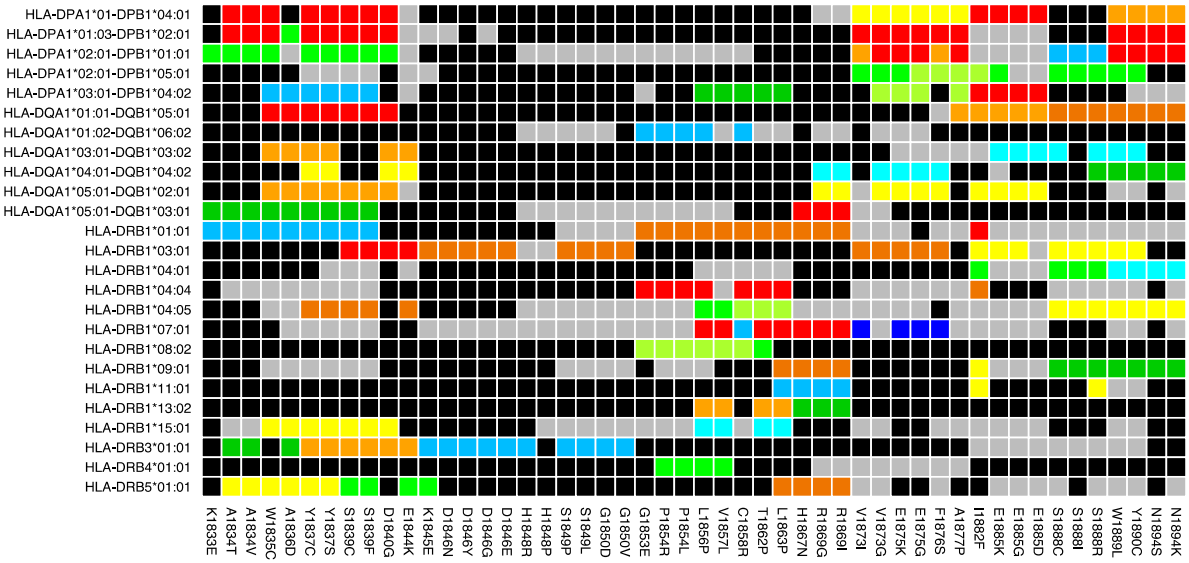
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HLA-DRB1*03:01
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HLA-DRB1*04:05
HLA-DRB1*07:01
HLA-DRB1*08:02
HLA-DRB1*09:01
HLA-DRB1*11:01
HLA-DRB1*13:02
HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
K1833E
A1834T
A1834V
W1835C
A1836D
Y1837C
Y1837S
S1839C
S1839F
D1840G
E1844K
K1845E
D1846N
D1846Y
D1846G
D1846E
R1848R
H1848P
S1849P
S1849L
G1850D
G1850V
G1853E
P1854R
P1854L
L1856P
V1857L
C1858R
T1862P
L1863P
H1867N
R1869G
R1869I
V1873I
V1873G
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F1876S
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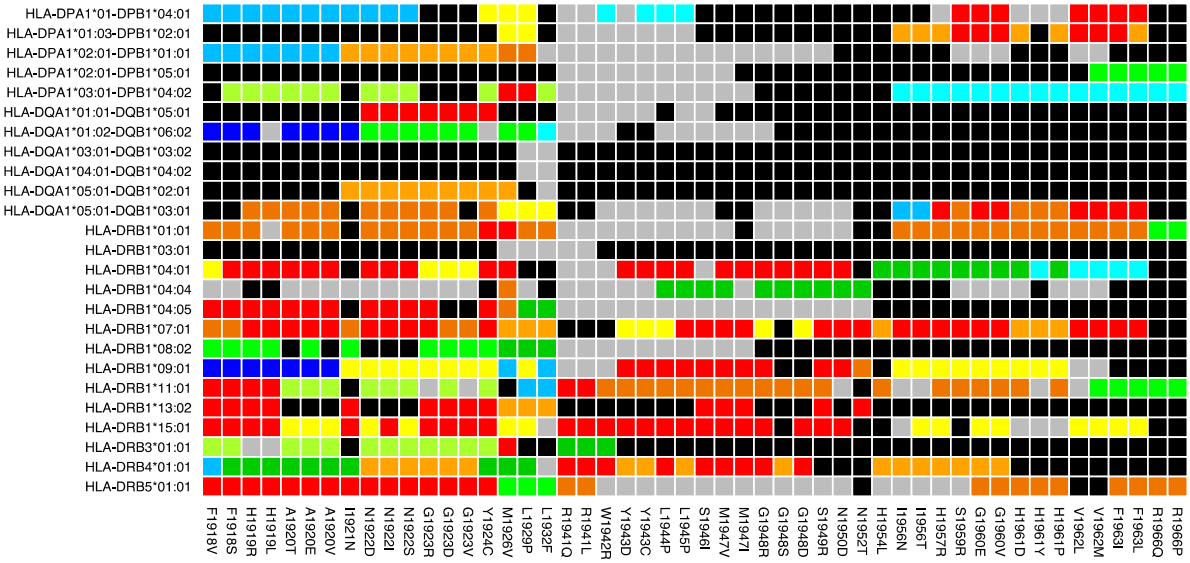
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HLA-DRB1*03:01
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HLA-DRB1*07:01
HLA-DRB1*08:02
HLA-DRB1*09:01
HLA-DRB1*11:01
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HLA-DRB4*01:01
HLA-DRB5*01:01
F1918V
F1918S
H1919R
H1919L
A1920T
A1920E
A1920V
I1921N
N1922D
N1922I
N1922S
G1923R
G1923D
G1923V
Y1924C
M1926V
L1929P
L1932F
R1941Q
R1941L
W1942R
Y1943D
Y1943C
L1944P
L1945P
S1946I
M1947V
M1947I
G1948R
G1948S
G1948D
S1949R
N1950D
N1952T
H1954L
I1956N
I1956T
H1957R
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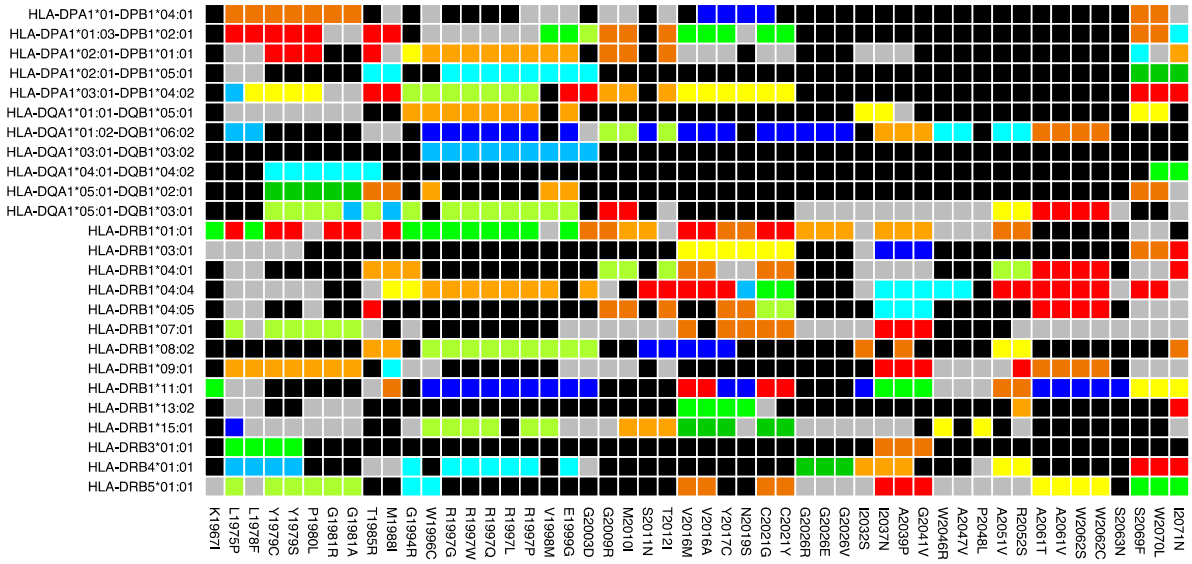
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HLA-DQA1*01:01-DQB1*05:01
HLA-DQA1*01:02-DQB1*06:02
HLA-DQA1*03:01-DQB1*03:02
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HLA-DQA1*05:01-DQB1*03:01
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HLA-DRB1*03:01
HLA-DRB1*04:01
HLA-DRB1*04:04
HLA-DRB1*04:05
HLA-DRB1*07:01
HLA-DRB1*08:02
HLA-DRB1*09:01
HLA-DRB1*11:01
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HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
K19671
L1975P
L1978F
Y1979C
Y1979S
P1980L
G1981R
G1981A
T1985R
M1988I
G1994R
W1996C
R1997G
R1997W
R1997Q
R1997L
R1997P
V1998M
E1999G
G2003D
G2009R
M2010I
S2011N
T2012I
V2016M
V2016A
Y2017C
N2019S
C2021G
C2021Y
G2026R
G2026E
G2026V
I2032S
I2037N
A2039P
G2041V
W2046R
A2047V
P2048L
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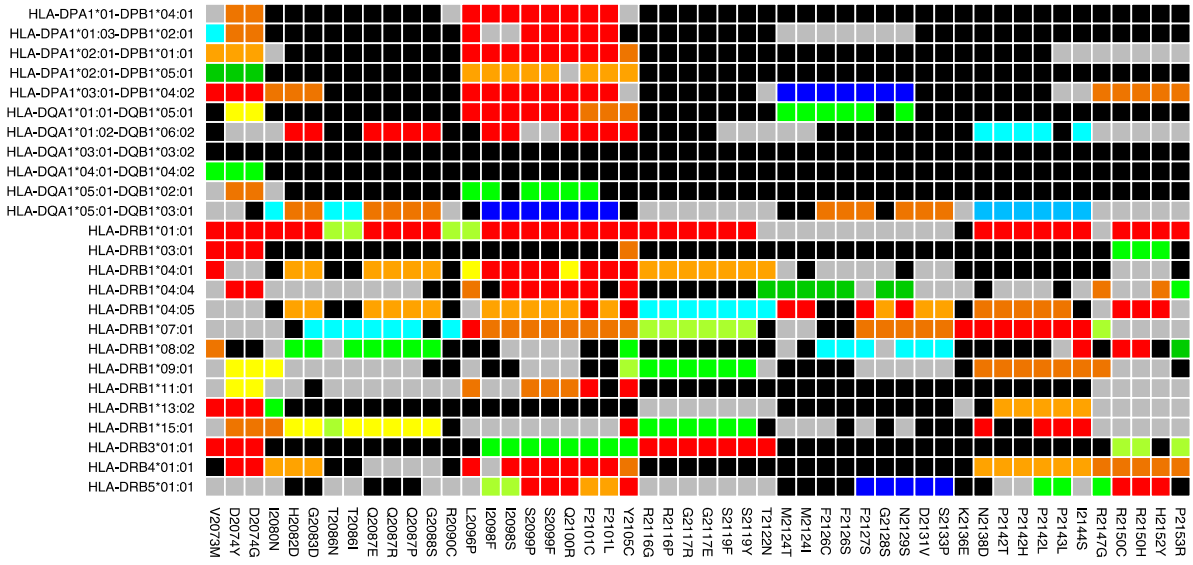
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HLA-DPA1*03:01-DPB1*04:02
HLA-DQA1*01:01-DQB1*05:01
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HLA-DQA1*04:01-DQB1*04:02
HLA-DQA1*05:01-DQB1*02:01
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HLA-DRB1*03:01
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HLA-DRB1*04:04
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HLA-DRB1*08:02
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HLA-DRB1*11:01
HLA-DRB1*13:02
HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
V2073M
D2074Y
D2074G
I2080N
H2082D
G2083D
T2086N
T2086I
Q2087E
Q2087R
Q2087P
G2088S
R2090C
L2096P
I2098F
I2098S
S2099P
S2099F
Q2100R
F2101C
F2101L
Y2105C
R2116G
R2116P
G2117R
G2117E
S2119F
S2119Y
T2122N
M2124T
M2124I
F2126C
F2126S
F2127S
G2128S
N2129S
D2131V
S2133P
K2136E
N2138D
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R2150C
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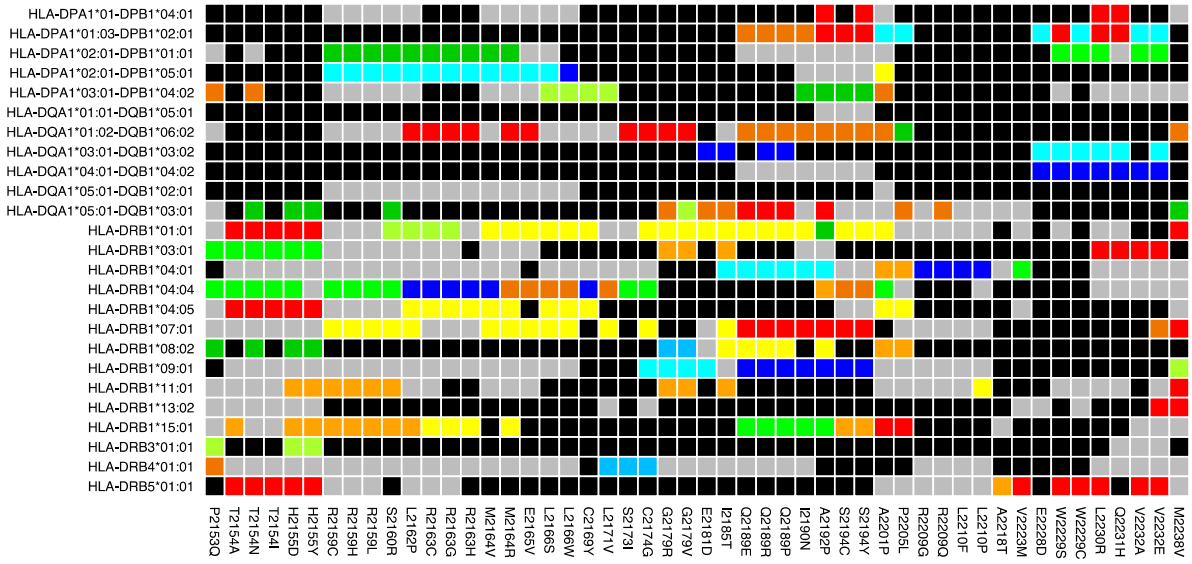
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HLA-DRB1*03:01
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HLA-DRB1*09:01
HLA-DRB1*11:01
HLA-DRB1*13:02
HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
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T2154N
T2154I
H2155D
H2155Y
R2159C
R2159H
R2159L
S2160R
L2162P
R2163C
R2163G
R2163H
M2164V
M2164R
E2165V
L2166S
L2166W
C2169Y
L2171V
S2173I
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G2179R
G2179V
E2181D
I2185T
Q2189E
Q2189R
Q2189P
I2190N
A2192P
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S2194Y
A2201P
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R2209Q
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L2210P
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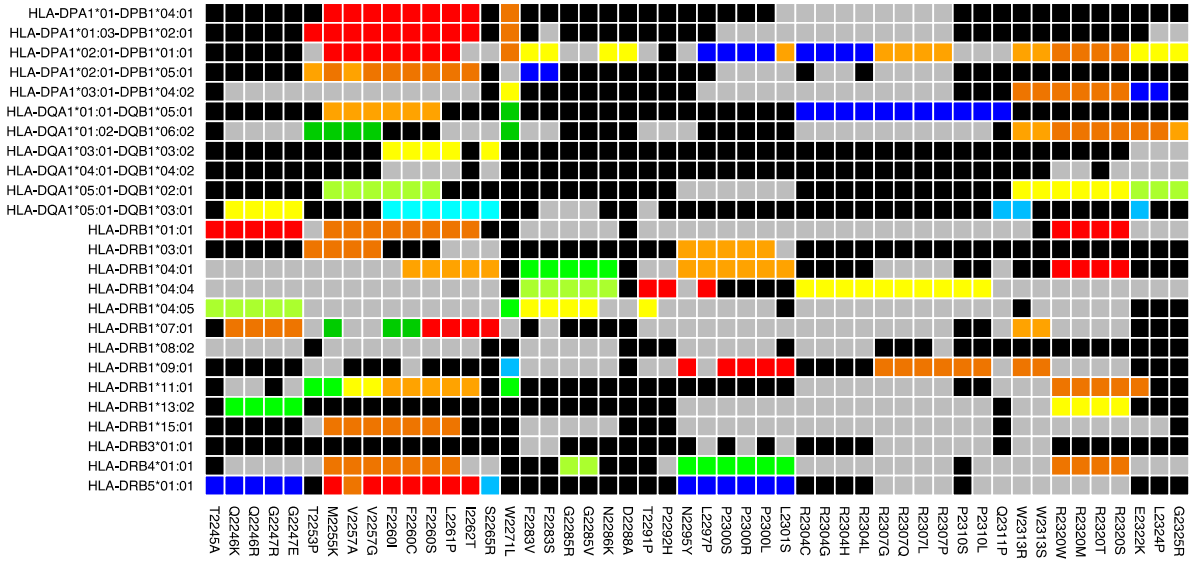
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HLA-DRB1*11:01
HLA-DRB1*13:02
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HLA-DRB4*01:01
HLA-DRB5*01:01
T2245A
Q2246K
Q2246R
Q2247R
G2247E
T2253P
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V2257A
V2257G
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F2260S
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S2265R
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F2283S
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N2286K
D2298A
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L2297P
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P2300R
P2300L
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P2310L
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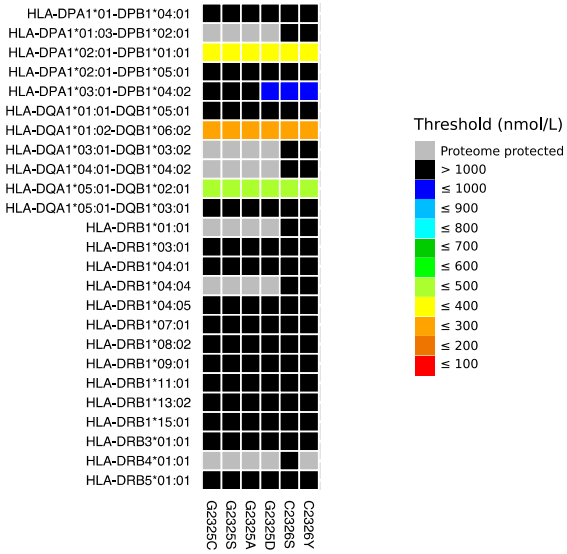
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HLA-DPA1*03:01-DPB1*04:02
HLA-DQA1*01:01-DQB1*05:01
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HLA-DQA1*03:01-DQB1*03:02
HLA-DQA1*04:01-DQB1*04:02
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HLA-DRB1*13:02
HLA-DRB1*15:01
HLA-DRB3*01:01
HLA-DRB4*01:01
HLA-DRB5*01:01
G2325C
G2325S
G2325A
G2325D
C2326S
C2326Y

Threshold (nmol/L)
Proteome protected
> 1000
≤ 1000
≤ 900
≤ 800
≤ 700
≤ 600
≤ 500
≤ 400
≤ 300
≤ 200
≤ 100