

# INTERLABORATORY INCONSISTENCY IN KIR GENE CONTENT ASSIGNMENT – AN UPDATE



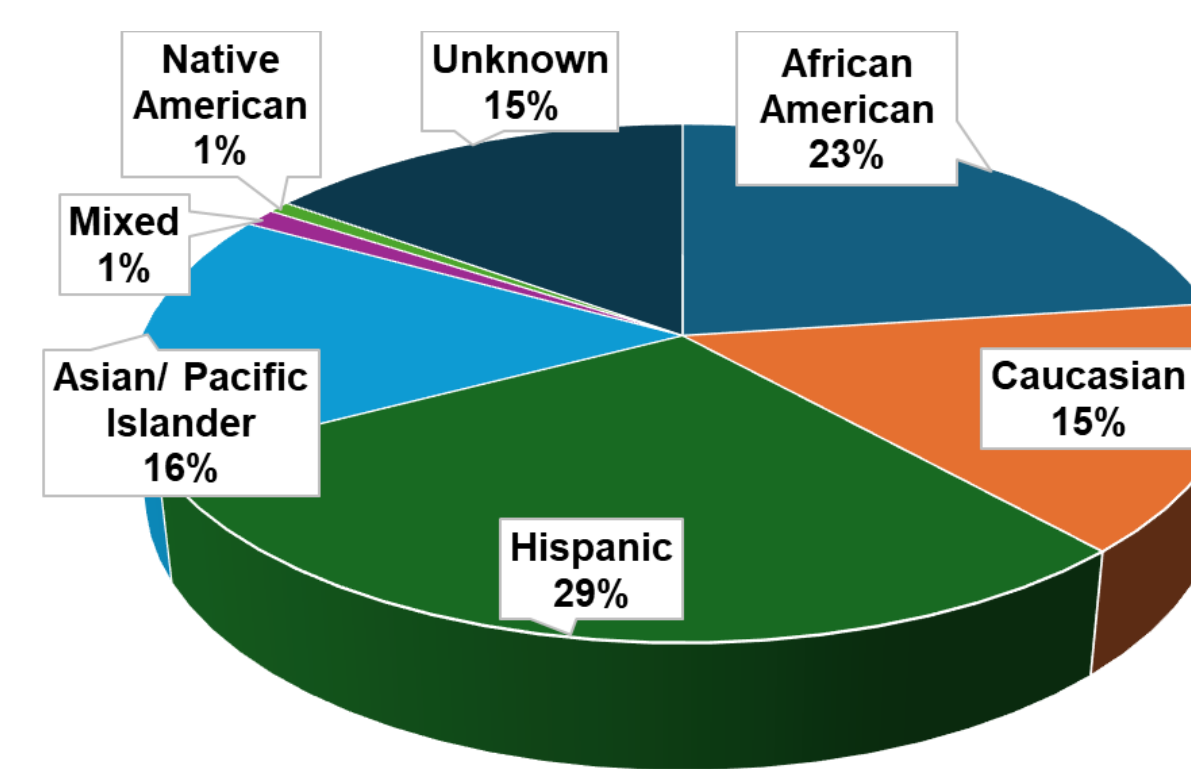
AF Locke, D Gjertson, EF Reed, and Q Zhang  
UCLA Immunogenetics Center, Los Angeles, CA

## Introduction and Method

Killer-cell immunoglobulin-like receptors (KIRs) play an important role in the regulation of NK cell alloreactivity following allogeneic hematopoietic stem cell transplantation (aHSCT). As such, accuracy in the assignment of KIR gene content is essential. In this study, we provide an update on interlaboratory variability in KIR genotyping among labs using different methodologies and reagents.

In 2005, the UCLA Immunogenetics Center developed an external proficiency testing (EPT) program for KIR genotyping. Participants in the survey examine 6 DNA samples two times per year. Data collected include KIR genotypes, typing method, and kit information.

**Figure 1: Breakdown of Ethnic populations for KIR samples examined from 2005 – 2025.**



## Results

To date, 266 DNA samples were tested from donors of various ethnic backgrounds. Among them, a total of 57 individual KIR genes were examined. **Figure 1** illustrates the ethnic makeup of the KIR samples, with the highest percentage of samples coming from donors of Hispanic descent (29%), followed by African Americans (23%), and Asian/Pacific Islanders (16%).

Tables 1a and 1b list each of the 57 KIR genotypes examined from 2005 - 2025, the number of times each was tested, and the ethnicity of the donor DNA. In some cases, the same donor DNA was tested more than once. Repeat samples are noted with an asterisk. At least 7 genotypes (highlighted in yellow) were examined  $\geq 10$  times over the last 20 years. They represent some of the more common KIR genotypes that are observed across multiple ethnic populations.

**Table 1a: List of KIR genotypes examined from 2005 – 2025.**

| Genotype | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 3DS1 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 2DS5 | 2DP1 | 3DP1 | # times tested | AFA | Cauc | Hisp | AAPI | Mixed | A/AN | Unk |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----------------|-----|------|------|------|-------|------|-----|
| 1        | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 8              |     | 3    | 3    |      |       |      | 2   |
| 2        | POS  | NEG  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 13             | 1   | 1    | 5    | 5    |       |      | 1   |
| 3        | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 2              |     |      |      | 2    |       |      |     |
| 4        | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | POS  | NEG  | POS  | POS  | 4              |     | 1    | 1    | 2    |       |      |     |
| 5        | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 3              |     |      | 3    |      |       |      |     |
| 6        | POS  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | 2              |     |      |      |      |       |      | 2   |
| 7        | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | 1              | 1   |      |      |      |       |      |     |
| 8        | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 13             | 5   | 3    | 1    | 2    | 1     |      | 1   |
| 9        | POS  | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | NEG  | POS  | NEG  | POS  | POS  | 9              |     | 2    | 4    | 1    |       |      | 2   |
| 10       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 3              |     |      |      | *3   |       |      |     |
| 11       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | NEG  | POS  | NEG  | POS  | POS  | 3              | 3   |      |      |      |       |      |     |
| 12       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 2              | 1   |      |      |      |       |      | 1   |
| 13       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | NEG  | POS  | NEG  | POS  | POS  | 2              |     |      |      |      |       |      | *2  |
| 14       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 2              |     |      | 2    |      |       |      |     |
| 15       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | 9              | 2   | 6    |      |      | 1     |      |     |
| 16       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 4              |     |      | 2    |      |       |      | 2   |
| 17       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 3              | 2   |      | 1    |      |       |      |     |
| 18       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | NEG  | POS  | 1              |     |      |      |      |       |      | 1   |
| 19       | NEG  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | NEG  | POS  | 4              |     | 2    |      |      |       |      | 2   |
| 20       | POS  | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | 6              |     |      | 4    |      |       |      | 2   |
| 21       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 14             | 1   | 1    | 4    | 7    |       |      | 1   |
| 22       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 11             | 5   | 2    | 2    | 1    |       |      | 1   |
| 23       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 12             | 1   | 1    |      | 5    |       |      | 5   |
| 24       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 7              | 6   |      |      | 5    |       |      | 1   |
| 25       | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | 6              |     |      | 5    |      | 1     |      |     |
| 26       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | 5              | 5   |      |      |      |       |      |     |
| 27       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 2              | *2  |      |      |      |       |      |     |
| 28       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | 3              |     |      | 3    |      |       |      |     |
| 29       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | 2              |     |      |      |      |       |      | *2  |

POS = presence of gene; NEG = absence of gene

**Table 1b: List of KIR genotypes examined from 2005 – 2025.**

| Genotype | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 3DS1 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 2DS5 | 2DP1 | 3DP1 | # times tested | AFA | Cauc | Hisp | AAPI | Mixed | A/AN | Unk |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----------------|-----|------|------|------|-------|------|-----|
| 30       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | 9              | 5   |      |      | 3    | 1     |      |     |
| 31       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | 3              |     |      |      | 1    |       |      | 2   |
| 32       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | 7              | 1   | 2    |      | 3    |       |      | 1   |
| 33       | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | 7              |     | 3    | 2    | 2    |       |      |     |
| 34       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | 5              |     | 4    |      | 1    |       |      |     |
| 35       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | 9              | 1   | 2    | 3    | 2    |       |      | 1   |
| 36       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | 10             |     | 3    | 3    | 2    |       |      | 2   |
| 37       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | 4              | 1   | 2    |      | 2    |       |      | 1   |
| 38       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | 11             | 2   | 2    | 6    |      |       |      | 1   |
| 39       | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | 2              |     |      |      |      |       |      | *2  |
| 40       | NEG  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | 6              | 1   |      | 3    |      |       |      | 2   |
| 41       | NEG  | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS            | 4   |      |      | 4    |       |      |     |
| 42       | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | 1              |     |      |      |      |       |      | 1   |
| 43       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | 1              | 1   |      |      |      |       |      |     |
| 44       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | POS  | 3              | 3   |      |      |      |       |      |     |
| 45       | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | NEG  | POS  | NEG  | POS  | NEG  | NEG  | POS  | 2              | 2   |      |      |      |       |      |     |
| 46       | POS  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | POS  | 2              | 1   |      |      | 1    |       |      |     |
| 47       | POS  | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | 2              |     |      |      | *2   |       |      |     |
| 48       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | POS  | POS  | 2              |     |      | 1    | 1    |       |      |     |
| 49       | POS  | NEG  | POS  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | 3              | 3   |      |      |      |       |      |     |
| 50       | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | NEG  | POS  | POS  | POS  | POS  | 3              | 2   |      | 1    |      |       |      |     |
| 51       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | POS  | POS  | 5              | 1   | 2    | 1    | 1    |       |      |     |
| 52       | POS  | NEG  | POS  | POS  | POS  | NEG  | POS  | POS  | NEG  | POS  | NEG  | POS  | NEG  | NEG  | POS  | POS  | 1              |     |      | 1    |      |       |      |     |
| 53       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | POS  | 1              | 1   |      |      |      |       |      |     |
| 54       | POS  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | 3              |     |      | 1    | 2    |       |      |     |
| 55       | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | POS  | NEG  | POS  | POS  | 1              |     |      |      |      |       |      | 1   |
| 56       | POS  | POS  | NEG  | POS  | POS  | POS  | POS  | POS  | NEG  | NEG  | NEG  | NEG  | POS  | POS  | POS  | POS  | 1              | 1   |      |      |      |       |      |     |
| 57       | NEG  | POS  | NEG  | POS  | NEG  | POS  | POS  | POS  | NEG  | NEG  | NEG  | POS  | NEG  | NEG  | POS  | POS  | 2              |     |      | 2    |      |       |      |     |

AFA = African American; AAPI = Asian American/Pacific Islander; A/AN = American Indian/Alaska Native

**Table 2: Consensus vs. non-consensus across KIR samples (n = 266)**

| ERA       | 90% Consensus |     | Total |
|-----------|---------------|-----|-------|
|           | No            | Yes |       |
| 2005 - 09 | 5             | 27  | 32    |
|           | 16%           | 84% |       |
| 2010-25   | 9             | 225 | 234   |
|           | 4%            | 96% |       |
| Total     | 14            | 252 | 266   |
|           | 5%            | 95% |       |

Fisher's exact test P = 0.015

In the early years of the program, 2005 – 2009, 5 out of 32 (16%) DNA samples tested had at least 1 KIR gene reported with a consensus of < 90% (Table 2). At the time, only 2 typing methods were available for KIR genotyping, SSP (sequence-specific primer-based typing) and SSO (sequence-specific oligonucleotide probe-based typing). As reagents improved and new methodologies such as real-time PCR (RT-PCR) and NGS (next generation sequencing) were introduced, discrepancies in the reporting of the presence/absence of KIR genes decreased among labs.

From 2010 – 2025, only 9 out of 234 (4%) samples tested had a consensus of < 90% for a single KIR gene. In total, KIR2DS3 had the most discrepancies (n = 5) which resulted in consensus to fall below 90%, followed by 2DL3 (n = 3), 3DL1 (n = 2), and 3DS1 (n = 2).

In most cases, discrepancies were observed across kit types, with labs reporting a gene positive by one specific kit type and negative by another. In the case of sample KDNA#0238, for example, 51 labs reported KIR2DS3 as negative, 9 labs reported it as positive, and 2 labs reported ambiguous results (Table 3). Interestingly, all 9 labs reporting 2DS3-POS tested the samples using RT-PCR kits from the same vendor. The 2 labs reporting ambiguous results reported 2DS3-NEG by SSO/SSP and 2DS3-POS by RT-PCR.

In the case of KDNA#0045, the discrepancies were due to the presence of 2 new variants; 2 labs indicated a new KIR3DL1 allele was present, and 5 labs indicated a new KIR2DS4 allele was present (Table 3). The new KIR3DL1 allele was described as differing from 3DL1\*007 at codon 88 (CCC → GCC, Pro → Ala) (D0 domain) and at codon 166 (CTT → TTT, Leu → Phe) (D1 domain). The new KIR2DS4 variant was described as differing from 2DS4\*003 by 2 amino acid substitutions, one at codon 52 (ATT → ACT, Ile → Thr) and the other at codon 129 (AGC → AGA, Ser → Arg).

**Table 3: KIR gene discrepancies (< 90% agreement), 2005 - 2025**

| KDNA        | # Labs | 2DL1    | 2DL2    | 2DL3    | 2DL4    | 2DL5           | 3DL1    | 3DL2    | 3DL3    | 3DS1    | 2DS1    | 2DS2    | 2DS3    | 2DS4         | 2DS5             | 2DP1    | 3DP1             | Race             | Year  |      |
|-------------|--------|---------|---------|---------|---------|----------------|---------|---------|---------|---------|---------|---------|---------|--------------|------------------|---------|------------------|------------------|-------|------|
| #0021       | 44     | POS[44] | NEG[44] | POS[44] | POS[41] | NEG[41]        | POS[44] | POS[43] | POS[42] | POS[27] | NEG[17] | NEG[40] | NEG[44] | POS[43]      | NEG[43]          | POS[39] | POS[39]          | Hisp             | 2005  |      |
| #0023       | 44     | POS[43] | NEG[44] | POS[44] | POS[41] | POS[30]+ B[13] | POS[44] | POS[19] | NEG[23] | POS[42] | NEG[43] | NEG[40] | NEG[43] | NEG[44]      | POS[44]          | NEG[43] | POS[38]          | POS[39]          | Black | 2005 |
| #0033 (#21) | 51     | POS[51] | NEG[50] | POS[51] | POS[48] | NEG[51]        | POS[51] | POS[51] | POS[50] | NEG[37] | POS[14] | NEG[51] | NEG[51] | NEG[51]      | POS[22]+ DEL[29] | NEG[51] | POS[48]          | POS[24]+ DEL[23] | Hisp  | 2006 |
| #0036       | 51     | POS[51] | POS[50] | NEG[16] | POS[48] | POS+ B[23]     | POS[51] | POS[51] | POS[50] | NEG[50] | NEG[49] | POS[51] | POS[51] | FULL DEL[14] | NEG[51]          | POS[48] | POS[24]+ DEL[23] | Cauc             | 2006  |      |
| #0045       | 53     | POS[53] | POS[51] | NEG[51] | POS[51] | POS[30]+ B[23] | POS[45] | NEG[6]  | New[2]  | POS[53] | POS[52] | NEG     | POS[53] | POS[53]      |                  |         |                  |                  |       |      |