

# Beyond Gender: Determining Sex-Linked Markers for Donor Registries by Genomic Sequencing

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## Background

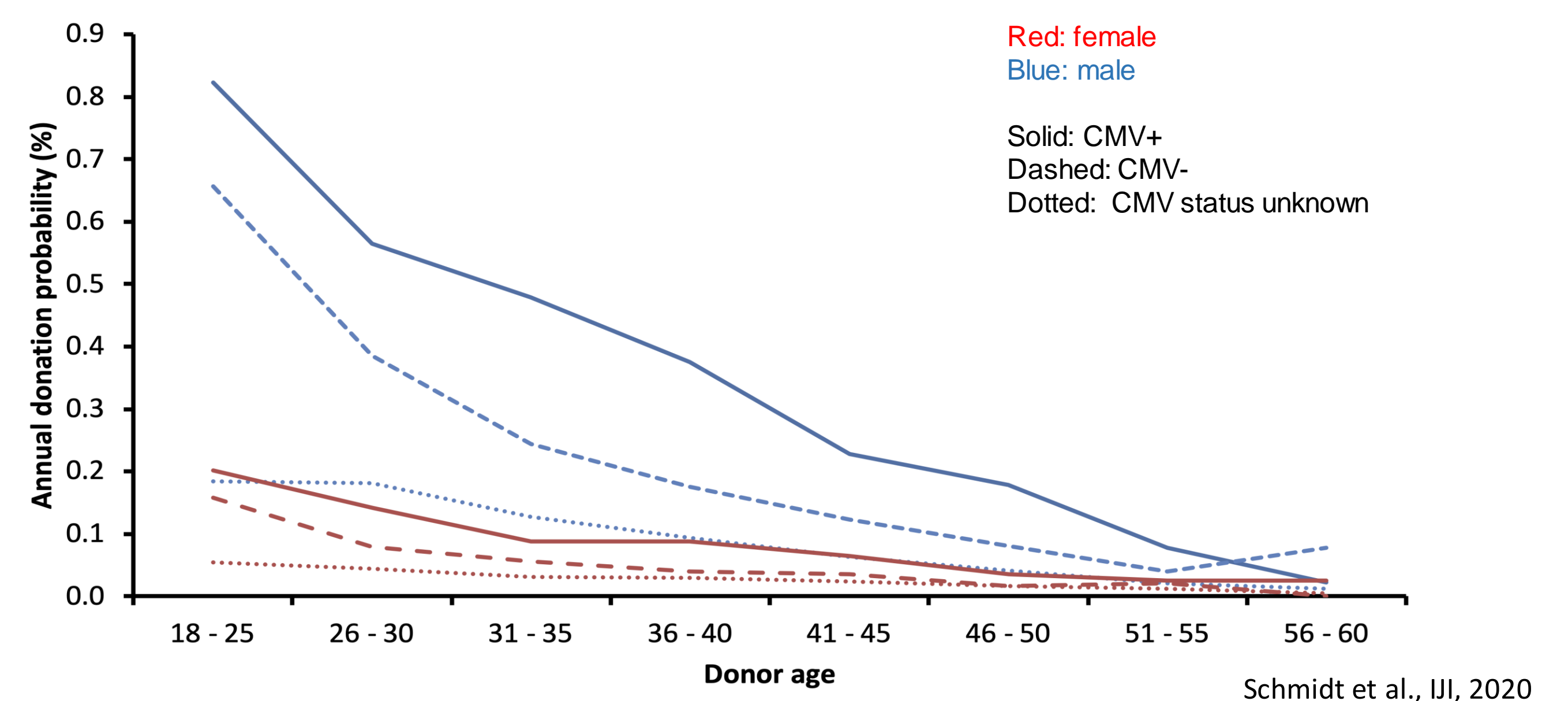
- HLA compatibility and donor age are the primary determinants in selecting optimal unrelated donors for allogeneic hematopoietic stem cell transplantation (HSCT).

- The immunological effects of genetic sex, may also contribute to transplant outcome. For instance, a female donor for a male patient has been associated with increased risk for GvHD

## Donor registries' role

- Registries of potential donors for HSCT record gender at recruitment.
- Gender does not always reflect the immunologically relevant sex but also social norms or roles.
- To address this gap, DKMS implemented determination of genetic sex in the high-throughput workflow for donor HLA genotyping.
- This allows for accurate and independent use of gender in communication and genetic sex in donor selection.

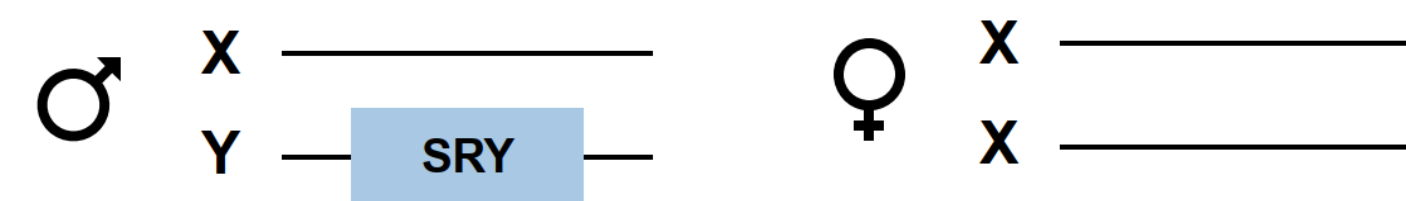
## Transplant centers' choice



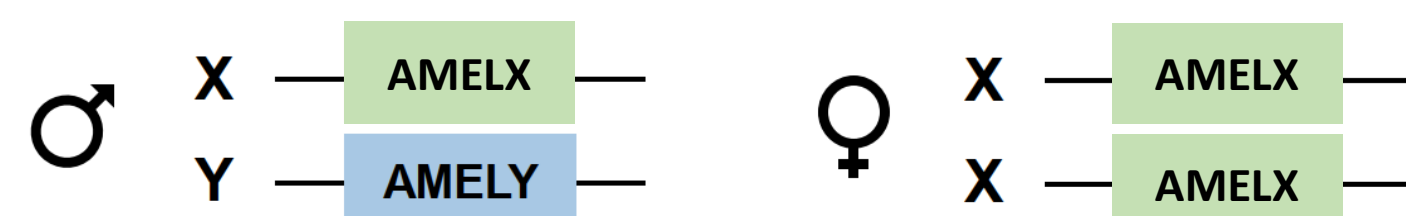
## Workflow for the Identification of Y-Chromosome

We developed a redundant approach based on SRY, AMELX, and AMELY genes to determine genetic sex. This approach was added to our efficient high-throughput HLA-typing workflow at the DKMS Life Science Lab.

- The SRY (Sex-determining Region of Y) gene is located on the Y-Chromosome and its amplification indicates a male genotype.

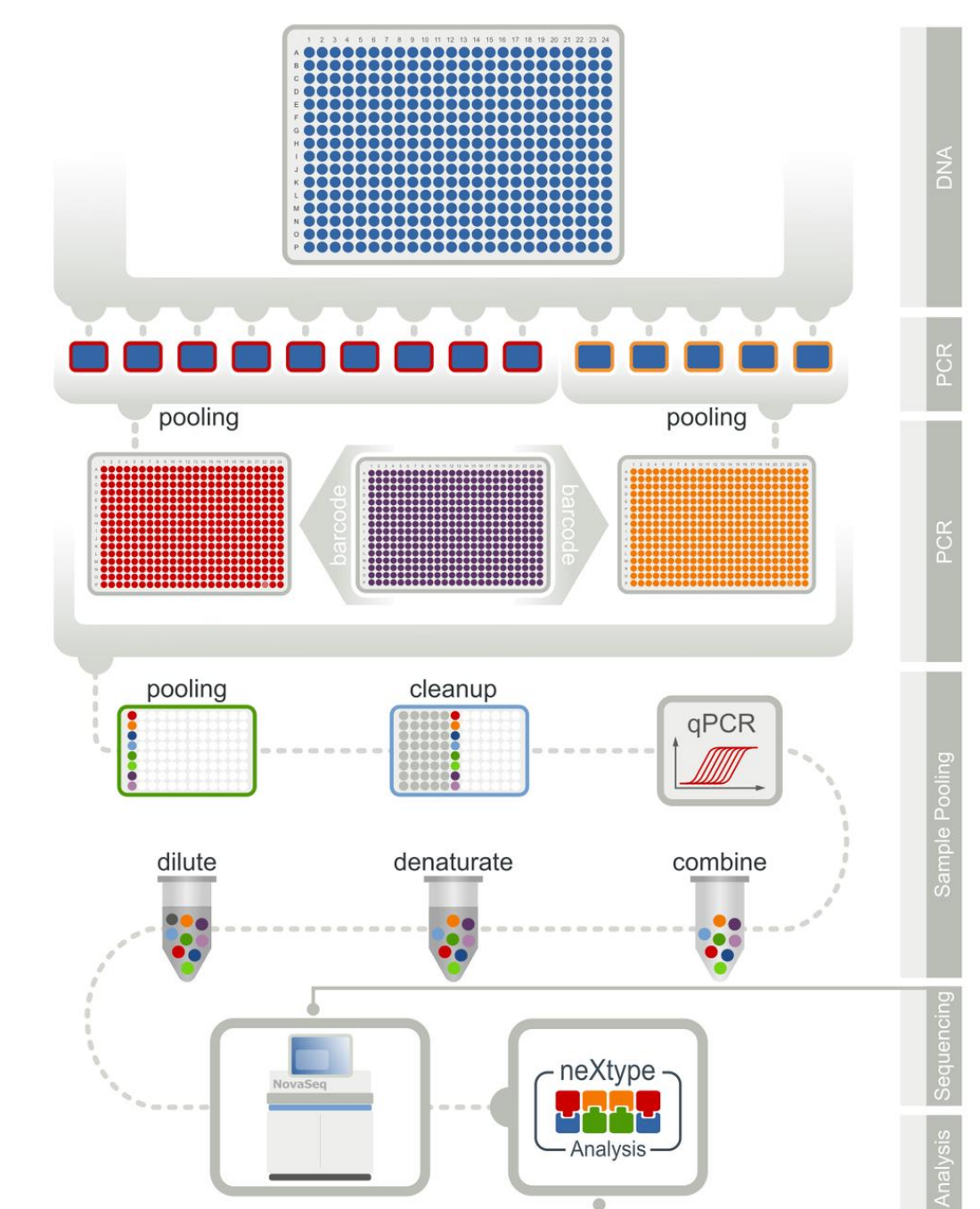


- The amelogenin (AMEL) locus consists of two homologous genes:
  - AMELX, located on the X-Chromosome
  - AMELY, located on the Y-Chromosome



AMELX and AMELY are amplified with identical primer pairs but can be distinguished by short deletions within the amplicon.

## Typing workflow



After the initial multiplex-PCR of all three genes, products are pooled with the HLA amplicons followed by barcoding PCR and sequencing on a NovaSeq instrument. Data is analyzed with an extended version of neXtype.

## Data analysis

Y-Chromosome status is determined based on genotyping results of AMELX, AMELY, and SRY, following the algorithm outlined below.

For data exchange with donor registries, results are categorized as *male* or *female*. This standardized classification ensures compatibility with existing reporting fields and facilitates integration in established registry-to-registry communications.

AMELX	AMELY	SRY	Example (as seen in neXtype)	Y-Chromosome ?
positive	positive	positive	Gemacht: 356 Readpaare (96.2% aller zugeordneten) Im Ergebnis: 356 Readpaare (100% aller gemachten) Befund: SRY 205 205 205 0 AMELX 87 87 87 0 AMELY 64 64 64 0	Yes
positive	negative	negative	Gemacht: 230 Readpaare (99.6% aller zugeordneten) Im Ergebnis: 230 Readpaare (100% aller gemachten) Befund: SRY 0 0 0 0 AMELX 230 230 230 0	No
positive	negative	positive		repeat with independent primers
positive	positive	negative		
negative	any	any		

## Conclusion

Genetic sex determination improves donor data quality and complements sociocultural gender in communication.

- Reduces inconsistencies during donor selection
- Enables a smoother, more reliable matching process
- High-throughput workflow: > 3,800 samples/day (> 83,000/month)

Current status of implementation:

- Ramp-up of result delivery from DKMS Life Science Lab to DKMS Donor Centers started April 9<sup>th</sup>, 2026

