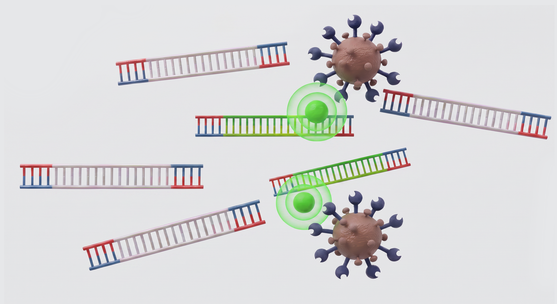
ADAPTATION AND VALIDATION OF HYBRITYPE (ONE LAMBDA – THERMO FISHER) WORKFLOW ON THE MGI SEQUENCING PLATFORM (DNBSEQ-G99)

AUTHORS: LUCAS SILVA[1], LUCAS PEREIRA[1], MARCELO MION[1]. 1 –BIOMETRIX DIAGNÓSTICA, CURITIBA - PR - BRASIL.

OBJECTIVE

Enrichment strategy is a central design choice in targeted next-generation sequencing. PCR-based (amplicon) methods amplify targets using primers annealed to conserved flanking sequences. This approach is fast and well established, but a single polymorphism at a primer-binding site can cause allele-specific dropout, and coverage typically becomes less even across long, highly polymorphic regions. Hybrid capture instead enriches targets by hybridizing sequence-specific, biotinylated probes directly to genomic DNA, independent of primer annealing. This generally yields more uniform coverage across large or structurally complex loci, lower susceptibility to allele dropout, and, as reported in the literature, more robust performance on degraded or low-input DNA such as buccal swabs. These properties have made hybrid capture an increasingly favored strategy for comprehensive HLA and immunogenetic characterization.

HybriType (One Lambda–Thermo Fisher Scientific) applies hybrid capture to HLA and ABO genotyping but was developed and validated only for Illumina sequencing. As MGI platforms gain adoption for their scalability and cost-efficiency, extending validated hybrid-capture assays to MGI is of practical interest for histocompatibility laboratories. This study aimed to adapt the HybriType workflow for compatibility with the MGI DNBSEQ-G99 and to validate its analytical performance (genotyping accuracy and sequencing quality) against the 19th International HLA & Immunogenetics Workshop reference panel.

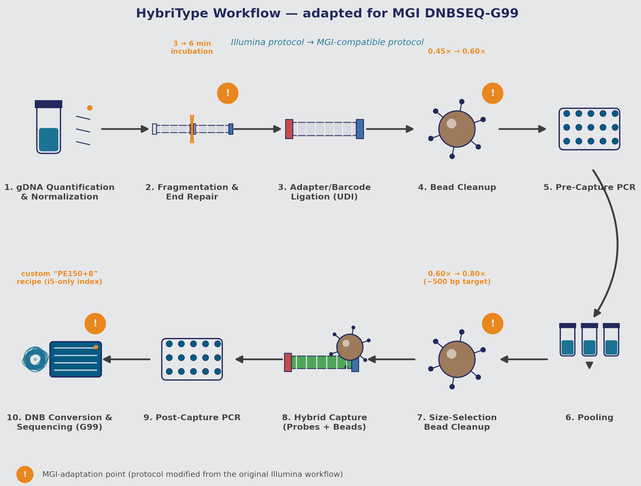


METHODS

HybriType HLA Plus Typing Flex Kit (One Lambda–Thermo Fisher): hybrid capture-based NGS assay covering 11 classical HLA genes, 7 non-classical HLA class I genes, and ABO. All loci were assessed in this study:

CATEGORY	LOCI
Classical HLA (11)	HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1, -DPB1
Non-classical class I (7)	HLA-E, -F, -G, -H, -J, MICA, MICB
Additional	ABO

The illustrated workflow below covers all steps from DNA input to sequencing; orange markers flag the three protocol parameters adjusted for MGI compatibility (detailed in Results):



144 reference samples (19th IHIWS panel) were received as extracted genomic DNA; cell culture of the reference lines was performed by the Fred Hutch BPB Biorepository group. Samples were typed and compared, locus by locus, against the validated AllType NGS assay (11 loci with an established comparator). Sequencing was assessed against DNBSEQ-G99 minimums (≥80M reads, Q30 >85%, split rate >90%).

RESULTS

Adapting HybriType (~900 bp Illumina libraries) to MGI required three changes: fragmentation time 3→6 min; post-ligation bead ratio 0.45×→0.60×; size-selection bead ratio 0.60×→0.80× (target size ~500 bp). A custom recipe ("PE150+8–HybriType"), indexing only from i5, resolved an i7-UMI demultiplexing conflict, reaching 100% demultiplexing.

Genotyping for the 11 classical loci showed 100% concordance with AllType across all 144 samples. In loci without an established comparator (E, F, G, H, J, MICA, MICB, ABO), only 3 isolated SNP discrepancies were seen (2 synonymous, 1 non-synonymous) plus a shared MICA variant in 4 related samples, suggesting a possible novel allele:

METRIC	LOCI
Samples / comparable loci	144 / 11 classical HLA loci
Concordance	100%

Candidate/novel variants observed in loci without a validated comparator:

LOCUS	NUMBER OF SAMPLES	VARIANT	EFFECT
HLA-E	1	Exon 4, C>T	Synonymous
HLA-H	2	Exon 4, 2 SNPs	1 synonymous, 1 non-synonymous (Tyr→His)
HLA-J	1	Exon 4, C>A	Synonymous
MICA	4 (possible related)	Exon 6, G>A	Non-synonymous (Gly→Arg)

Both sequencing runs exceeded DNBSEQ-G99 minimums, and DNB conversion (28–32 ng/μL) was well above the 10 ng/μL minimum:

METRIC	MIN. (G99)	RUN 1	RUN 2
Total reads (M)	≥ 80	104.87	118.47
Q30 (%)	> 85	93.89	96.06
Split rate (%)	> 90	98.23	99.01

HybriType was reliably adapted to the DNBSEQ-G99 without loss of accuracy: the protocol changes resolved library-size and demultiplexing issues, and 100% concordance plus sequencing metrics above spec confirm the workflow's robustness. Loci lacking a validated comparator (E, F, G, H, J, MICA, MICB, ABO) showed only candidate variants, not assay errors

CONCLUSON

HybriType was successfully adapted to the DNBSEQ-G99 through targeted changes to fragmentation, bead-based purification, and demultiplexing. The adapted assay reached 100% concordance across all classical HLA loci in 144 international reference samples, with sequencing quality consistently above platform minimums, supporting its use for comprehensive HLA/ABO typing in routine histocompatibility labs.

BIOMETRIX DIAGNÓSTICA