SUPPLEMENTARY APPENDIX

Monitoring multiple myeloma by next-generation sequencing of V(D)J rearrangements from circulating myeloma cells and cell-free myeloma DNA

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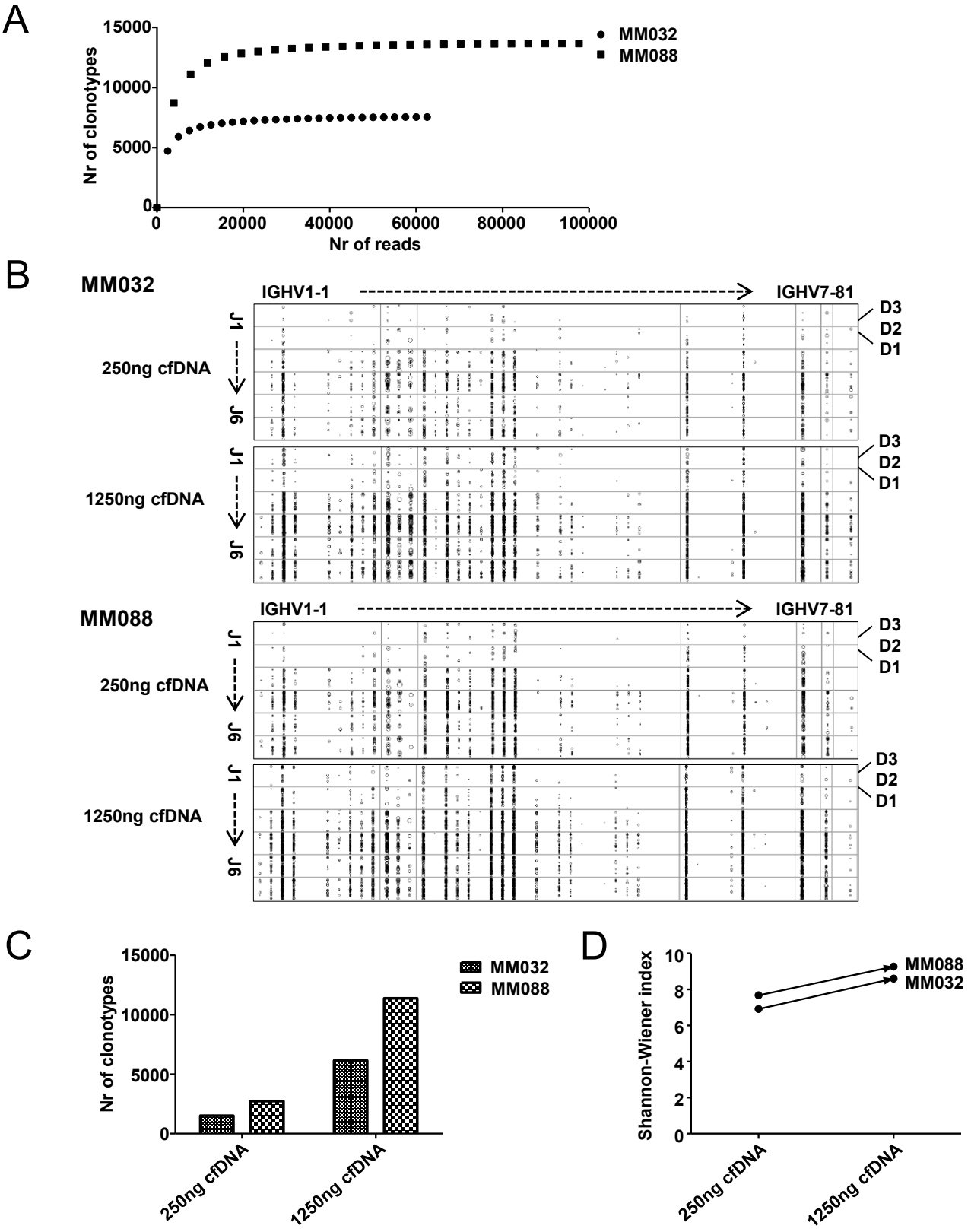
Suppl. Figure 1



Suppl. Figure 1: Estimation of B-lineage repertoire coverage of the V(D)J sequencing approach.
A: Repertoire coverage as a function of sequencing depth. Saturation curve of clonotype repertoires of exemplary patient samples MM032 and MM088 sequenced with a theoretical sequencing depth of 80 000 reads from PCR-amplified cell-free DNA (cfDNA) with 250ng DNA input. Each data point in the curve was generated by averaging over 100 replicate subsamplings. For each subsampling a corresponding number of reads was randomly selected from the sample library. Data was plotted using GraphPad Prism 5. **B:** V(D)J repertoire plots from PCR-amplified cfDNA (250ng versus 1250ng PCR DNA input) of exemplary patients MM032 and MM088. Every dot represents a specific V(D)J rearrangement of the immunoglobulin repertoire. The size of each dot represents the proportion of the clonotype in the repertoire. The plot was generated using R statistical software tools. **C:** Number of unique clonotypes per repertoire as a function of different PCR input cfDNA amounts (250ng versus 1250ng) in exemplary patients MM032 and MM088. **D:** Shannon-Wiener diversity index of V(D)J repertoires with different input cfDNA amounts (250ng versus 1250ng) in exemplary patients MM032 and MM088. Shannon-Wiener indices were calculated as previously described (Akyüz et.al, Int. J. of Cancer 2016) and plotted with GraphPad Prism 5.

Suppl. Table 1: Myeloma cohort blood sampling time points

Code	Blood sample analyzed
MM023	leukocytes at SD plasma at SD and PR
MM031	leukocytes at PD and CR plasma at baseline, PD, vgPR and CR
MM032	leukocytes at baseline and PD plasma at baseline, vgPR and PD
MM048	leukocytes at baseline plasma at baseline, CR and PR
MM050	leukocytes at baseline and PR plasma at vgPR and PR
MM056	leukocytes at vgPR and PR plasma at vgPR and PR
MM059	leukocytes at vgPR plasma at vgPR
MM060	leukocytes at CR plasma at CR
MM062	leukocytes at SD plasma at baseline and SD
MM082	leukocytes at PR plasma at PD
MM085	leukocytes at vgPR (1st line) plasma at vgPR (1st line and 2nd line)
MM087	leukocytes at PR plasma at PR
MM088	leukocytes at PR and PD plasma at PR and PD
MM090	leukocytes at baseline and vgPR (maintenance) plasma at baseline, vgPR (1st line) and vgPR (maintenance)
MM095	leukocytes at baseline and CR plasma at CR
MM098	leukocytes at PD (1st line), PD (r/r) and SD plasma at PD (1st line), PD (r/r) and SD
MM120	leukocytes at SD and PR plasma at baseline and PR
MM122	leukocytes at vgPR plasma at baseline and vgPR
MM123	leukocytes at baseline and vgPR plasma at baseline and vgPR
MM125	leukocytes at vgPR plasma at baseline and vgPR
MM155	leukocytes at baseline plasma at vgPR
MM170	plasma at PR
MM174	plasma at SD
MM094	Excluded from analysis
MM116	Excluded from analysis
MM131	Excluded from analysis
MM132	Excluded from analysis

Remission at sampling time point according to the International Myeloma Working Group uniform response criteria for multiple myeloma.
Abbreviations: CR = complete response, vgPR = very good partial response, PR = partial response, SD = stable disease, PD = progressive disease

Suppl. Table 2: Evaluation of V(D)J sequencing sensitivity

No of spiked DG75 cells per 75 000 leukocytes	DG75 VDJ clonotype detection rate (> 1 read)
0.075	33% (n = 3)
0.75	85.7% (n = 7)
7.5	100% (n = 7)
75	100% (n = 7)
750	100% (n = 7)
7 500	100% (n = 4)
75 000	100% (n = 7)

Spiking of different amounts of Burkitt lymphoma cell line DG75 into leukocytes from buffy coat. 500ng input DNA (corresponding to 75 000 leukocyte genomes) was used for the amplification of IGH and sequencing depth was set to 80 000 reads per sample. The experiments were repeated n times. Sequencing was considered positive if more than one DG75 clonotypic VDJ read was identified.