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DEVELOPMENT OF AN STR PANEL FOR SCREENING OF 1P32.3 DELETION IN MULTIPLE MYELOMA

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INTRODUCTION

Background: Deletion 1p32.3, routinely detected by FISH on CD138+ cells, is an independent poor prognostic factor in multiple myeloma. However, FISH interpretation can sometimes be complicated, and testing of archival paraffin-embedded samples may be hindered due to fragmentation or poor DNA quality. A simple PCR-based test could serve as a useful method in routine practice.
Purpose: To develop a primer system for PCR with capillary electrophoresis of STR loci in 1p32.3 region and validate it on DNA samples from multiple myeloma patients.
Methods: STR loci and primers were selected using the UCSC Genome Browser and Primer-BLAST. The cohort included 44 newly diagnosed multiple myeloma patients; 36 underwent FISH for del(1p32.3) using the *CKS1B/CDKN2C* probe. DNA was extracted from bone marrow CD138+ cells and paired peripheral blood samples.

RESULTS

Four unique primer pairs (Table 1) were designed for the GRCh38/hg38 chr1:50,917,447–51,074,652 region, covering *CDKN2C* and part of *FAF1* genes (Fig.1).The large distances between target loci enabled reliable single tube multiplex PCR. Amplicon lengths were approximately 103 bp, 150 bp, 273 bp, and 324 bp - sufficiently diverse to prevent overlap during capillary electrophoresis. All 44 peripheral blood samples were informative, containing at least one heterozygous STR marker (Fig.2). Allelic loss was detected in CD138+ DNA from seven patients (15.9%). Among six patients with FISH data available, in four 1p32.3 deletions were confirmed by FISH (11% of 36) (Fig.3). No additional FISH-positive cases were found. Discrepancies may be due to copy-neutral loss of heterozygosity, which FISH is unable to identify (Fig.4).

Marker	Primer (Forward / Reverse)	Amplicon Length (bp)	Fluorescent Label
F1/R1	F: cagatcagactgttaacagtggt R: catcttgatctcccctctcgaa	~103 bp	FAM (on F)
F2/R2	F: cctgggtaacagagtgagact R: tggagacagccagtggatca	~150 bp	FAM (on F)
F3/R3	F: caatcctaaatttgtatgcacat R: cagtgaaaccatctaggcatga	~273 bp	FAM (on F)
F4/R4	F: gcagcaatcagagggcctca R: accctggctggtcctgatgt	~324 bp	FAM (on F)

Table 1. Designed STR Primers (4 pairs, single-tube multiplex PCR)

Figure 1. Visualization of the 1p32.3 region targeted for STR panel development. Screenshot from the NCBI Genome Data Viewer (assembly GRCh38/hg38, chr1:50,917,447–51,074,652) showing the Ensembl genes (release 115) in the target region. The interval spans part of the CDKN2C and FAF1 loci, where four STR markers were designed for multiplex PCR analysis.

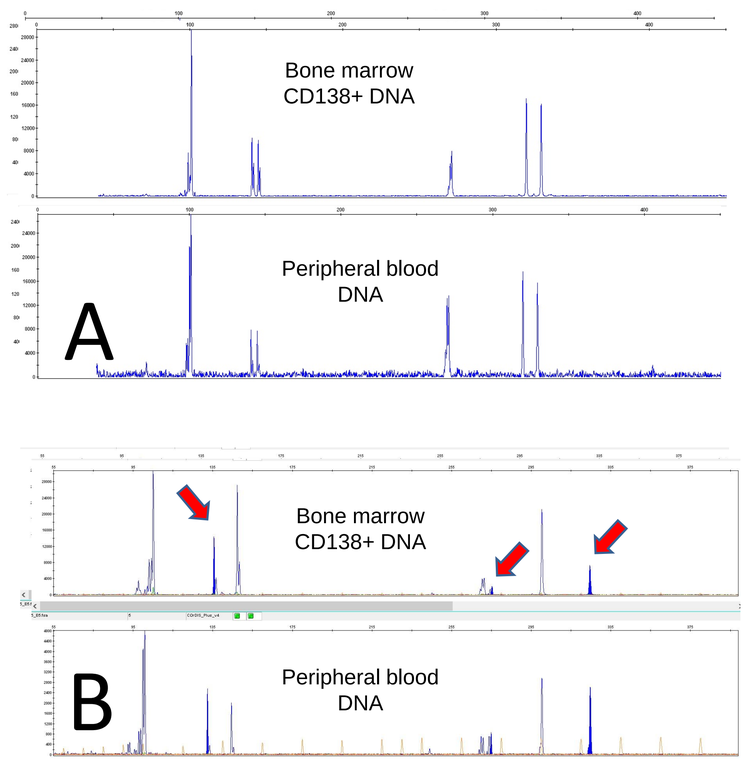

Figure 2. STR-based detection of 1p32.3 LOH in multiple myeloma patients.
(A) CD138+ cells without LOH - heterozygous pattern unchanged compared to the corresponding peripheral blood STR profile (constitutional control) .
(B) CD138+ cells with LOH - allelic imbalance (marked reduction of one allele, red arrows)

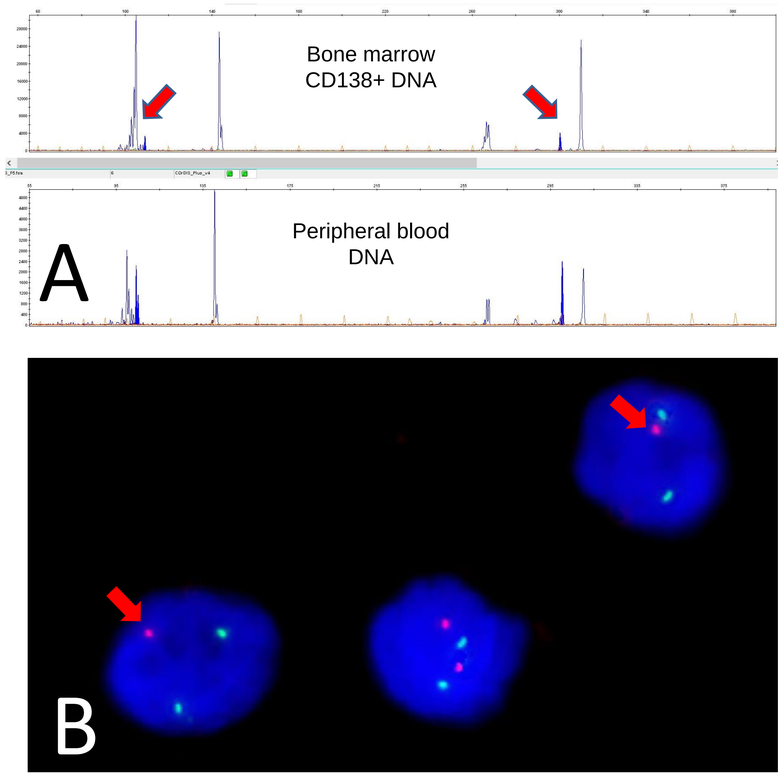

Figure 3. STR panel detects 1p32.3 LOH confirmed as a true deletion by FISH.
(A) Capillary electrophoresis: CD138+ cells show allelic imbalance (reduction of one allele, red arrows) compared to peripheral blood control.
(B) FISH with the CKS1B/CDKN2C probe confirms a physical deletion of the 1p32.3 region in CD138+ cells.

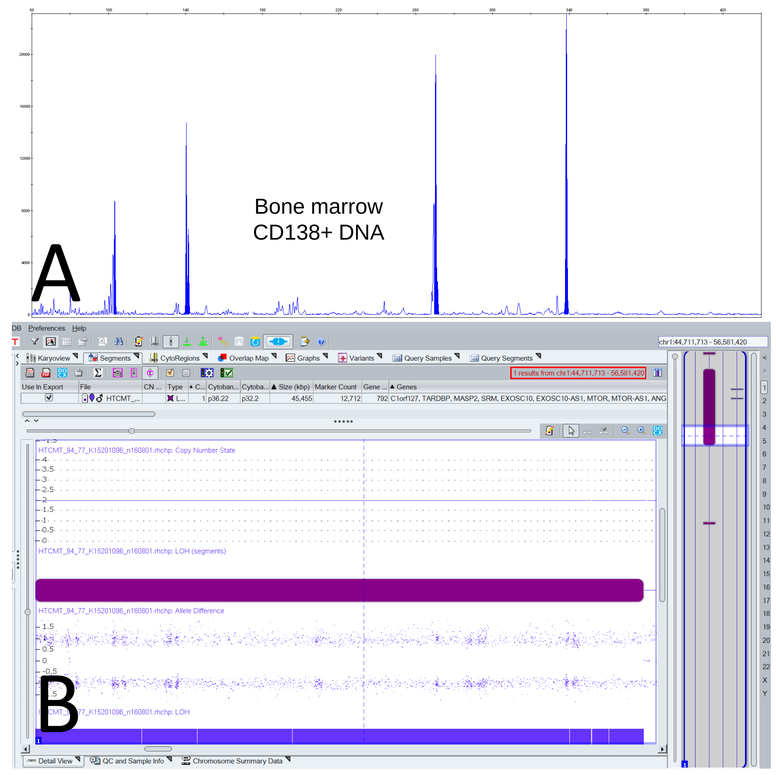

Figure 4. Limitation of the STR screening panel: inability to differentiate between deletion and cnLOH.
(A) Multiplex PCR with capillary electrophoresis shows homozygosity for all four STR markers in a patient sample, indicating LOH at 1p32.3.
(B) SNP array reveals copy-neutral LOH (copy number 2.0, LOH segment present). FISH would be negative, while the STR panel is positive. Thus, STR-positive cases require FISH confirmation to determine whether LOH results from a true deletion (poor prognosis) or cnLOH.

CONCLUSION

The panel we described is appropriate for initial screening of all patients. Detected aberrant variants at 1p32 should be verified as deletions through FISH.

CONTACTS

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