Title: Towards Routine Molecular Diagnostics: Multiplex SYBR Green Real-Time PCR for Rapid Molecular Screening of Recurrent Translocations in Acute Myeloid Leukemia

Table S1: Sequences and positions of the Europe Against Cancer (EAC) Primers

EAC code	Target gene	Sequence	Primer Length (bp)	Accession Number	5′–3′ position	Amplicon length (bp)
ENF701	RUNX1 (21q22)	CACCTACCACAGAG CCATCAAA	22	D43969	1005–1026	97bp
ENR761	*RUNX1T1* (8q22)	ATCCACAGGTGAGT CTGGCATT	22	D14289	318–297	
ENF903 (bcr1)	*PML* (15q22)	TCTTCCTGCCCAAC AGCAA	19	M73778	1690–1708	129bp
ENF905 (bcr3)	*PML* (15q22)	CCGATGGCTTCGAC GAGTT	19	M73778	1198–1216	147bp
ENR962	*RARα* (17q21)	GCTTGTAGATGCGG GGTAGAG	21	X06538	485–465	
ENF803	*CBFB* (16q22)	CATTAGCACAACAG GCCTTTGA	22	L20298	389–410	
ENR862 (Exon 34, A)	*MYH11* (16p13)	AGGGCCCGCTTGGA CTT	17	D10667	1952–1936	118bp

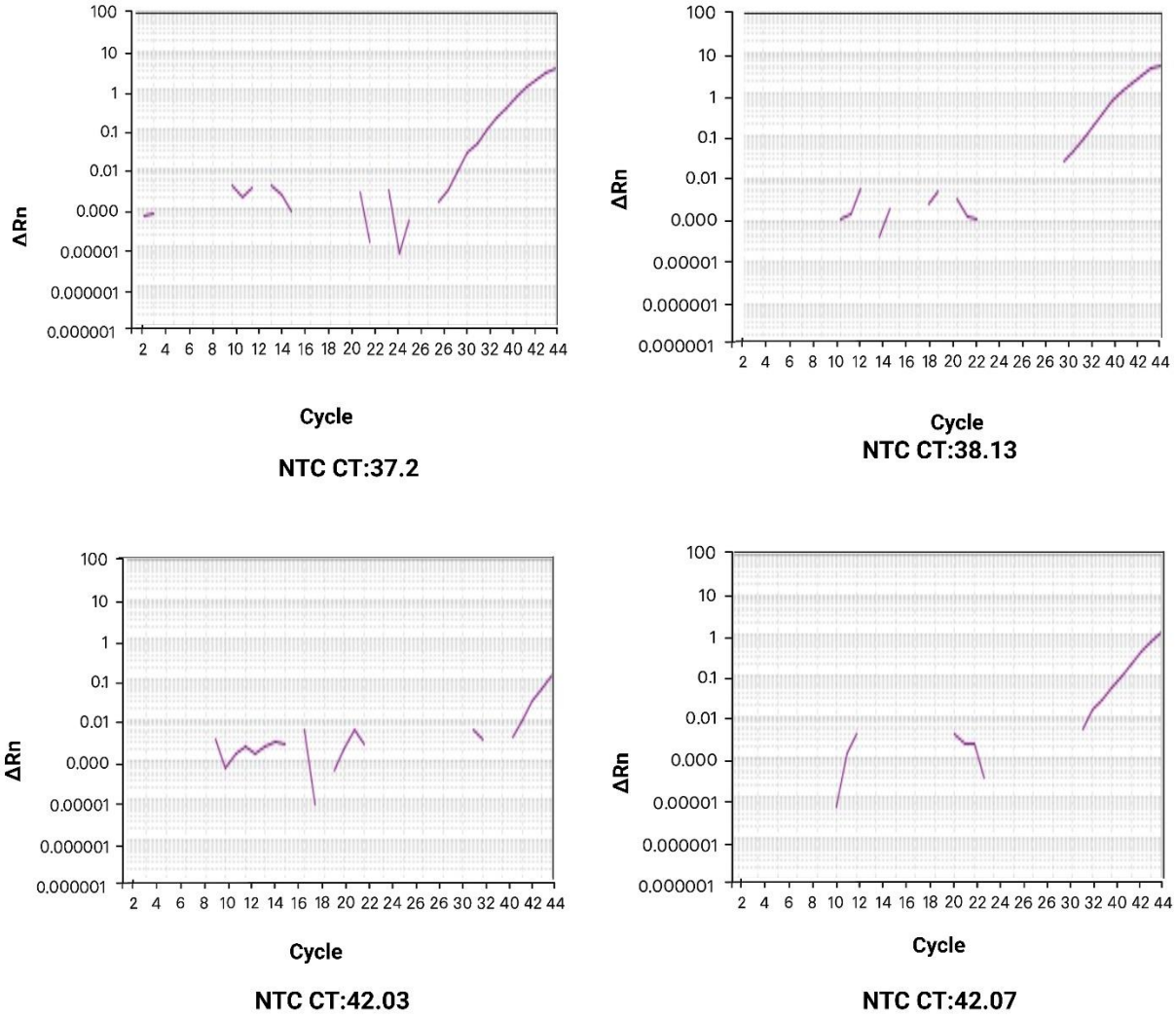

Figure S1. Amplification plot of non-template controls (NTCs) in the multiplex assay. In each run, 3–4 NTC replicates were performed to monitor background amplification and potential contamination. The amplification plots demonstrated no detectable false positivity, with Ct values consistently greater than 35. Based on these findings, 35 cycles were selected as the optimal cutoff to maximize assay sensitivity while minimizing nonspecific or artifactual amplification.

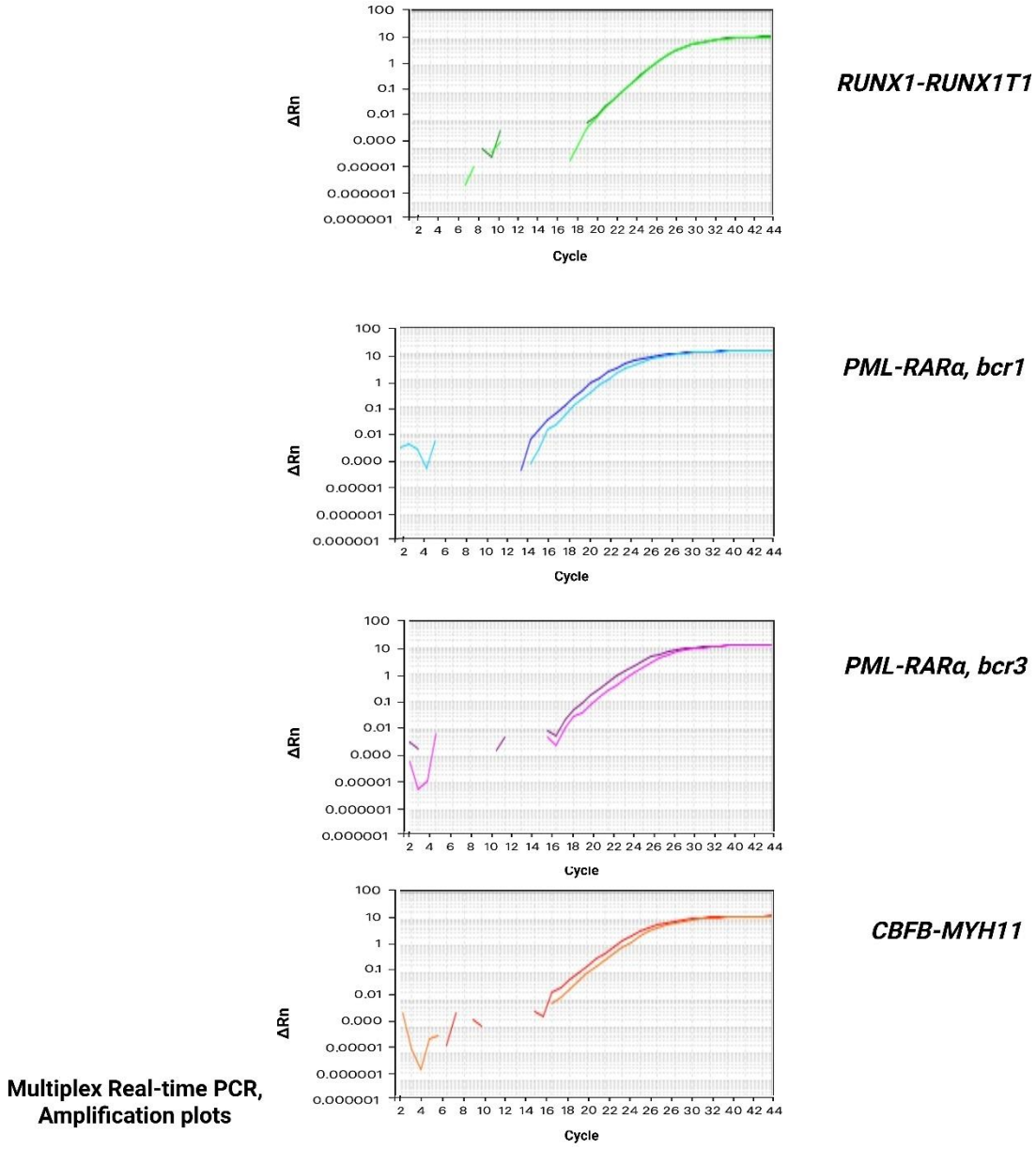

Figure S2. Amplification plot of the multiplex SYBR Green real-time PCR assay. Each run included four successfully amplified positive controls representing each target (*RUNX1–RUNX1T1, PML–RARα bcr1, PML–RARα bcr3, and CBFB–MYH11 type A*). In parallel, the same positive samples were amplified in singleplex reactions under identical cycling conditions. The amplification curves obtained in the multiplex assay showed comparable Ct values and profiles to their corresponding singleplex reactions, confirming equal amplification efficiency and the absence of primer interference.

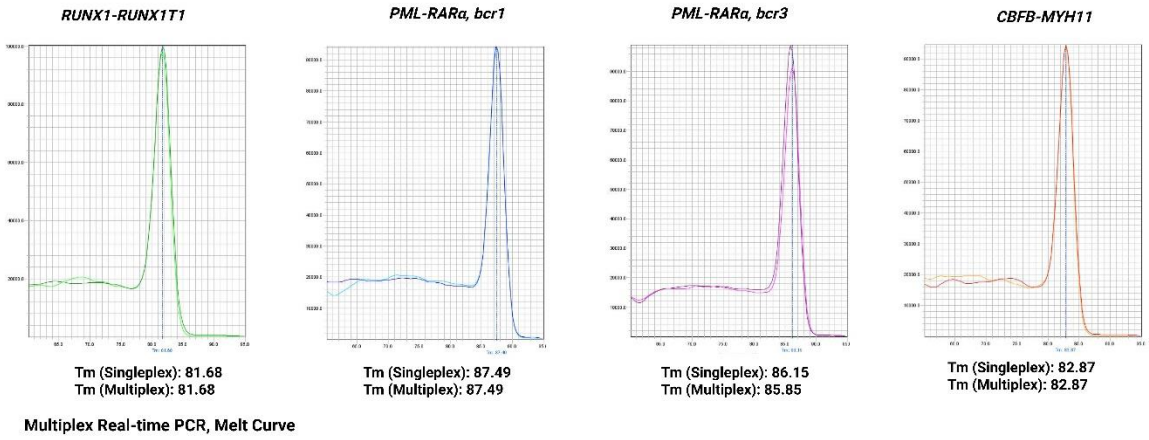

Figure S3. Melting curve analysis of the multiplex assay. Four positive controls for each target were included in every run to confirm target-specific amplification. Parallel singleplex reactions yielded identical melting profiles. The multiplex assay demonstrated distinct and reproducible melting peaks corresponding to each translocation, with Tm values identical or minimally different from those obtained in the singleplex assays.