

Table IG / TCR multiplex PCR: preferred method of analysis, expected size ranges, and non-specific bands ¹ (update taken from Langerak et al., Leukemia 2012;26;2159-2171)

Multiplex PCR	Preferred method of analysis	Size range (nt)	Non-specific bands (nt)
IGH V _H -J _H	GS and HD both suitable	tube A: 310-360 tube B: 250-295 tube C: 100-170	tube A: ~85 tube B: ~228 ^a tube C: ~211 ^a
IGH D _H -J _H	HD slightly preferred over GS (amplicon variation hampers GS)	tube D: 110-290 (D _H 1/2/4/5/6-J _H) 390-420 (D _H 3-J _H) tube E: 100-130	tube D: ~161 ^e , ~350 ^b tube E: 211 ^c
IGK	GS and HD have complementary value (small CDR3 + amplicon variation hamper GS)	tube A: 120-160 (V _K 1f/6/V _K 7-J _K) 190-210 (V _K 3f-J _K) 260-300 (V _K 2f/V _K 4/V _K 5-J _K) tube B: 210-250 V _K 1f/6/V _K 7-Kde 270-300 (V _K 3f/intron-Kde) 350-390 (V _K 2f/V _K 4/V _K 5-Kde)	tube A: ~217 ^a tube B: ~404 ^a
IGL	HD slightly preferred over GS (small CDR3 hampers GS)	tube A: 140-165	tube A: -
TCRB	GS and HD both suitable	tube A: 240-285 tube B: 240-285 tube C: 170-210 (Dβ2) 285-325 (Dβ1)	tube A: ~213 ^{a,d} , ~273 ^{a,d} tube B: ~93, ~126, ~221 ^{a,d} tube C: ~128, ~337 ^{a,d}
TCRG	GS and HD both suitable	tube A: 145-255 tube B: 80-220	tube A: - tube B: -
TCRD	HD slightly preferred over GS (low template amount + amplicon variation hamper GS)	tube A: 120-280	tube A: ~90, ~123

¹ Update of Table 25 of earlier BIOMED-2 / EuroClonality report (Van Dongen et al., Leukemia 200317:2257-2317)

^a Particularly seen in samples with low numbers of contaminating lymphoid cells

^b Non-specific 350 bp band is the result of cross-annealing of the D_H2 primer to a sequence upstream of J_H4. In GeneScanning this non-specific band does not comigrate with D-J products.

^c 211 bp PCR product represents product from germline D_H7-J_H1 region; when PCR amplification is very efficient, also longer PCR products might be obtained based on primer annealing to downstream J_H genes; e.g. 419 bp (D_H7-J_H2), 1031 bp (D_H7-J_H3), etc.

^d Detection of non-specific band depends on quality of primers (batch-dependent)

^e Detection of non-specific product, additional to the ones published by the EuroClonality/BIOMED-2 group. This non-specific product has been detected by multiple users and analyzed by the Radboud University Medical Centre Nijmegen.