

## PWWG Conclusion II : RT-PCR procedure

### • Specific amplification of HLA-G transcripts :

#### Primer set :

G. 257 (forward): 5'-GGA AGA GGA GAC ACG GAA CA (exon 2)

G. 1004 (reverse): 5'-CCT TTT CAA TCT GAG CTC TTC TTT (exon 5-exon 6)

#### PCR conditions:

35 cycles :    1 min to 94°C  
                  1 min 30s to 61°C  
                  1min 30s to 72°C

#### PCR products:

0.77 kb (HLA-G1), 0.49 kb (HLA-G2, HLA-G4), 0.22 kb (HLA-G3)

#### HLA-G probes :

**GR** (ubiquitous): 5'-GGT CTG CAG GTT CAT TCT GTC (exon 2)

**G.526** (selective): 5'-CCA ATG TGG CTG AAC AAA GG (exon 3)

**G.647** (selective): 5'-CCA CCA CCC TGT CTT TGA CT (exon 4)

Hybridization: 60°C (GR); 55°C (G526, G647)

Washing (2XSSC, 0.1% SDS): 55°C

### • Detection of HLA-E, HLA-F and HLA-G transcripts using pan-class I primers :

#### Primer set :

**Pan class I** (forward): 5'-TCC CAC TCC ATG AGG TAT TTC (exon 2)

**Pan class I** (reverse): 5'-TCC AGA AGG CAC CAC CAC AG (exon 4)

#### PCR conditions:

35 cycles: 1 min to 94°C  
                  1 min 30 s to 55°C  
                  2 min to 72°C

HLA probes:

HLA-E (**ER**): 5'-ATCATTTGACTTTTGCTCGGA (exon 3)

HLA-F (**FR**): 5'-GGCGTACCCTGTGGTCCACTC (exon 2)

HLA-G (**GR**): 5'-GGT CTG CAG GTT CAT TCT GTC (exon 2)

Hybrization: 60°C

Washing (2XSSC 0.1% SDS): 62°C

• **Internal control for PCR amplification :**

**β-actin primers are introduced in the same PCR after 19 cycles. Coamplification is performed during 16 cycles.**

primer set :

**β-actin 5' : 5'-TCG TCG TCG ACA ACG GCT CC**

**β-actin 3' : 5' -GAA GCA TTT GCG GTG GAC GA**

PCR product:

1100 bp

probe:

**5'-ATC ATG TTT GAG ACC TTC AAC ACC CCA GCC**