XIII. Appendix F

Polymerase chain reaction cycling conditions

The following cycling conditions have been used for PCR amplifications described in this study:

- Cycle A – Standard amplification:
  1 × (3', 95°C); 35 × (30", 95°C – 45", T_a – 1' per 1000 bp amplicon, $T_{DNA\ polymerase}$); 1 × (7', $T_{DNA\ polymerase}$)
- Cycle B – Mutation analysis of all *hKIAA1202* exons apart from 'Exon 8/1a', 'Exon 8/1b' and 'Exon 8/2':
  1 × (1'30, 96°C); 3 × (25", 96°C – 45", 65°C – 30", 72°C); 20 × (25", 96°C – 45", 60°C – 30", 72°C); 7 × (25", 96°C – 50", 55°C – 45", 72°C); 10 × (25", 96°C – 1', 55°C – 1'30", 72°C); 1 × (10', 72°C)
- Cycle C – Mutation analysis of *hKIAA1202* exons 'Exon 8/1a', 'Exon 8/1b' and 'Exon 8/2':
  1 × (3', 95°C); 40 × (20", 95°C – 45", 58.5°C – 30", 72°C); 1 × (7', 72°C)
- Cycle D – First-round 5' and 3' RACE:
  1 × (3', 95°C – 5', 75°C – 2', 50°C – 5', 72°C); 5 × (20", 94°C – 1'30", 72°C); 5 × (20", 94°C – 45", 70°C – 1'30", 72°C); 30 × (20", 94°C – 45", 58°C – 1'30", 72°C); 1 × (3', 72°C)
- Cycle E – Suppression PCR:
  1 × (3', 95°C); 25 × (20", 94°C – 45", 57°C – 3', 72°C); 1 × (7', 72°C)
- Cycle F – *In vitro* mutagenesis:
  1 × (30", 95°C); 12 × (30", 95°C – 1', 55°C – 12'30", 68°C)
- Cycle G – DNA sequencing:
  1 × (3', 96°C); 25 × (10", 96°C – 5", T_a – 4', 60°C)
- Cycle H – Long-range PCR:
  1 × (3', 95°C); 15 × (20", 95°C – 45", 56°C – 1', 68°C); 20 × (20", 95°C – 45", 56°C – 1'*, 68°C); 1 × (7', 68°C)

*+20"/cycle

- Cycle I – Touchdown PCR:

  1 × (3', 95°C); 10 × (20", 95°C – 45", 60°C* – 45", 72°C); 30 × (20", 95°C – 45", 55°C – 45", 72°C); 1 × (7', 72°C)

  *-0.5°C/cycle

- Cycle J – *hKIAA1202* expression analysis in lymphoblastoid cells, T_a 62/60°C

  1 × (3', 94°C); 5 × (20", 94°C – 20", 62°C – 40", 72°C); 40 × (20", 94°C – 20", 60°C – 40", 72°C); 1 × (7', 72°C)

- Cycle K – *hKIAA1202* expression analysis in lymphoblastoid cells, T_a 60/58°C

  1 × (3', 94°C); 5 × (20", 94°C – 20", 60°C – 40", 72°C); 40 × (20", 94°C – 20", 58°C – 40", 72°C); 1 × (7', 72°C)