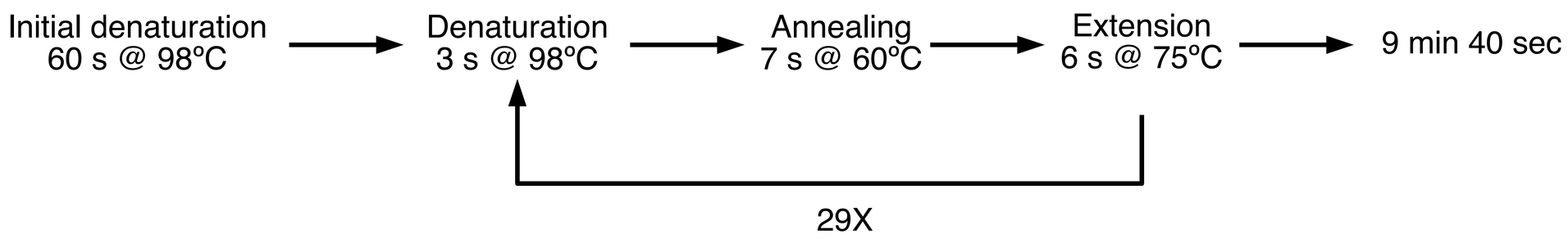
NGPCR Application Sheet
NextGenPCR Sanger Sequencing

Introduction

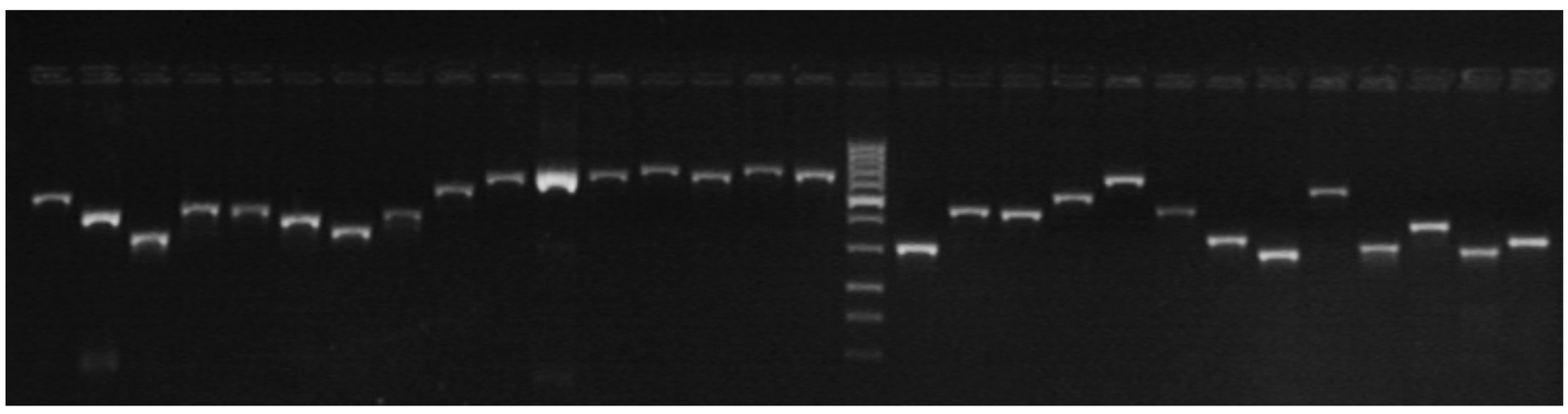
PCR is, in most cases, an inevitable and lengthy step in clinical genetic diagnostics and research. An example of this is the preparation for Sanger sequencing. Using NextGenPCR technology, this application can shrink from a 2-day process to same day results. As an example we analysed 29 fragents of the BRCA1 gene, with a fragment length of approximately 700 bp.

Experimental

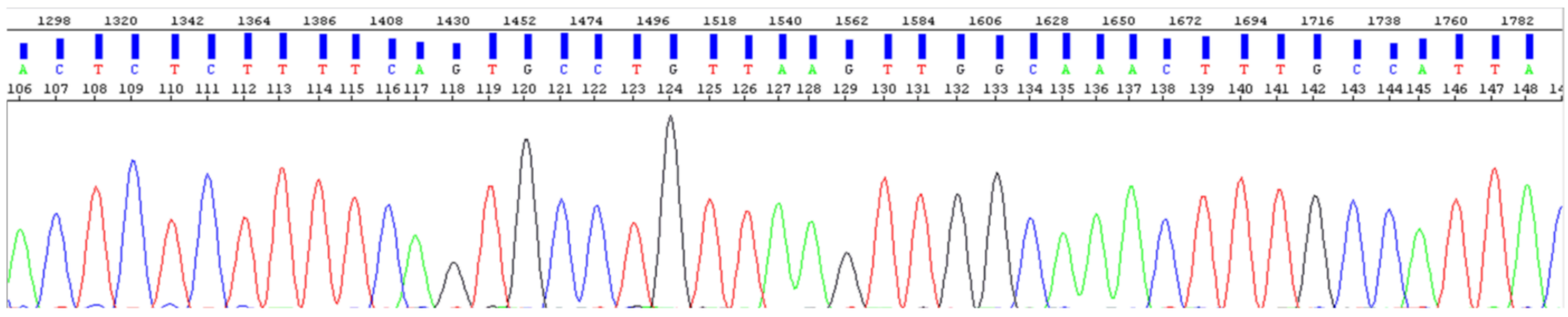
For analysis of the BRCA1 gene, the diagnostic setup of the Erasmus MC is used: 29 primer pairs containing M13 tails for subsequent Sanger sequencing. Amplification was performed in 5 ul reaction volume containing 1XPCR buffer (Kapa Biosystems), 0.3 mM dNTP (Kapa Biosystems), 2 mM MgCl2, 2.5 ng template DNA, 0.5 uM of each primer, 0.125 Units of Kapa 2G Fast HotStart polymerase. The PCR-protocol used on our NextGenPCR thermocycler is shown below.



Results



BRCA1 exon 10



Fragments can be amplified in 30 cycles in less than 10 minutes. Sequencing data look as solid as ever.

Discussion

With both the initial PCR and sequencing reaction performed in our NextGenPCR thermocycler, reductions in time of up to 4 hours become possible. A 2-day process is reduced to same day results.