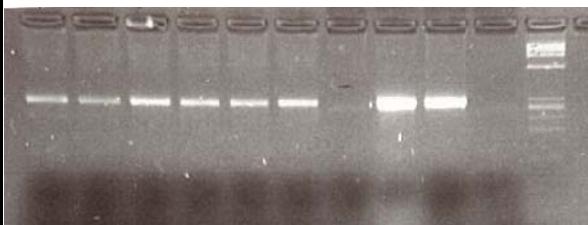
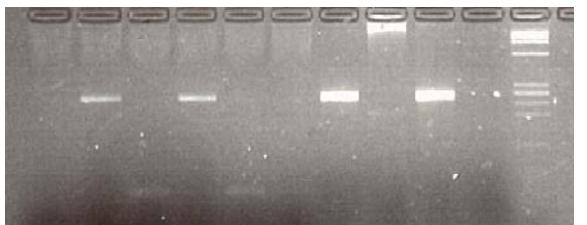


| | | | | | |
|-------------|-----------------------------|-------|-------|-------------|-------------|
| Gene symbol | <i>Kcnq1</i> ^{dfk} | | | | |
| primer | Sequence(5'-3') | | Size | target gene | accession |
| rKcnq1-72 | CACACCCTCTGGGAAGACAC | 259bp | Kcnq1 | NM_032073.1 | NM_032073.1 |
| rKcnq1-74 | GCTGAGGGACCATGAGGATA | | | | |
| rKcnq1-75 | GAGCGGCTTGTCTTCTTCAACTT | | | Kcnq1 | |
| rKcnq1-76 | AAACAAACACATATGGTAAACATGC | | | | |
| comment | | | | | |

| PCR condition | | | | | | | |
|----------------|---|----------------|----------|--------|-----------|-------|----|
| Taq polymerase | BIOTAQTM HS DNA Polymerase (Bioline Reagents Ltd., UK) | | | | | | |
| Thermal cycler | PC-808(ASTEC) | | | | | | |
| PCR buffer | Ampdirect Plus | | | | | | |
| | | first denature | denature | anneal | extension | cycle | |
| PCR | °C | 94 | 94 | 65 | 72 | 35 | 72 |
| | min | 3 | 0.5 | 1 | 0.75 | | 3 |
| comment | PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd,UK) and dried. 1.5mm FTA disc was removed from the bloody-stained region on FTA®card. Untreated sample discs were placed directly in 15 μL PCR mixture containig 1 × Ampdirect®Plus, 0.2 μ M each primer and 0.4 units of BIOTAQTM HS DNA Polymerase. | | | | | | |

| | | | | | | |
|---|---------------------------------------|--|--|--|--|--|
| Electrophoresis ;4% agarose gel 300V, 30min | | | | | | |
| rKcnq1 -72&74(Wild) | | | rKcnq1 -75&76(Mutant) | | | |
| 1 2 3 4 5 6 dfk/dfk +/+ NC M | | | 1 2 3 4 5 6 dfk/dfk +/+ NC M | | | |
|  | | |  | | | |
| Strain harboring mutation | WTC- <i>Kcnq1</i> ^{dfk} /Kyo | | | | | |