

Table 3.1: Volumes and concentrations of the reagents used for the optimisation PCR reactions in 0.2 mL tubes.

REAGENTS	VOLUME (μL)	CONCENTRATION
SensiFast mix	10	2x
DNA template	1	*
dH ₂ O	11	0
	22	
Forward primer	2	10 μM
Reverse primer	2	10 μM
Total	26	

Table 3.2: Temperature and duration of the three step pre-amplification program used for the optimisation PCR reactions.

PROCESS	TEMPERATURE	DURATION
Hold	95°C	5 minutes
Amplification (40 cycles)		
Denaturation	95°C	10 seconds
Annealing	55°C – 69°C	5 – 10 seconds
Elongation	72°C	10 seconds
HRM	80°C - 93°C	Increasing by 0.1°C every 2 seconds

Table 3.3: Volumes and concentrations of the reagents used for the case sample PCR reactions in 0.1 mL tubes

REAGENTS	VOLUME (μL)	CONCENTRATION (μM)
SensiFast mix	5	1x
Forward primer	1	10
Reverse primer	1	10
dH ₂ O (dependent on DNA concentration)	Variable	0
	12	-
Case DNA template	Variable	Variable
Total	13	-

Table 4.1: Optimisation annealing temperatures and duration for exons 1-16 of the *KCNQ1* gene.

Exon	Annealing temperature (°C)	Duration of annealing temperature (s)
1a	-	-
1b	-	-
2	66	10
3	67	10
4	67	10
5	-	-
6	69	5
7	66	5
8	66	5
9	66	5
10	69	10
11	66	5
12	62	10
13	66	10
14	62	5
15	69	5
16	-	-

Table 4.2: Annealing temperatures and duration for attempting to optimise exon 1a of the *KCNQ1* gene.

Temperature (°C)	Time (s)	Melt curve analysis
52	10	One broad peak at 87°C
53	10	Two broad peaks at 83°C and 86°C
54	10	One broad peak at 87°C
56	10	Three peaks: 81°C, 83°C, and 86°C
57	10	Messy peaks: 83°C and 86°C
58	10	Two broad peaks, 83°C and 86°C
59	10	Slight peak at 83°C, thick peak at 85°C -86°C
60	10	Multiple peaks, maybe 86°C
61	10	Slight peak 83°C and 86°C
62	10	Multiple peaks 84°C, 86°C, 87°C
63	10	Two broad peaks 83°C and 86°C
64	10	Peak forming
65	10	No clear peaks
66	10	Peak 86°C - 87°C
67	10	Multiple peaks 84°C, 86°C, 87°C
68	10	Peak 86° -87°C
69	10	Multiple peaks 86°C -87°C

Table 4.3: Annealing temperatures and duration for attempting to optimise exon 1b of the *KCNQ1* gene.

Temperature (°C)	Time (s)	Melt curve analysis
52	10	Multiple peaks: 81°C, 83°C and 86°C
53	10	Multiple peaks: 81°C, 83°C and 86°C
54	10	Multiple peaks, predominant peak at 86°C
55	10	Unspecific binding, peak at 86°C
56	10	Two peaks appearing, 82.5°C and 85.5°C
57	10	Two peaks remain, slight improvement
58	10	Improvement of peaks 81°C and 86°C
59	10	Two peaks 84.5°C and 86°C
60	10	Slight improvement, the 86°C curve has a double peak
61	10	Double peak 84-85°C and 86°C
62	10	Double peak 84-85°C and 86°C
63	10	Three peaks on a broad curve: 83°C, 84°C and 86°C
64	10	Improved peak at 86°C
65	10	Broad peak 83°C
66	10	No clear peaks 5s = peak at 85°C
67	10	No clear peaks
68	10	Low df/dt
69	10	No clear peaks

Table 4.4: The relevant case numbers sent in for Sanger sequencing for exons one to sixteen.

Exons	Case samples (S) sent for sequencing of possible variants	Control samples (C) sent for comparative sequencing	Total amount of samples
1.a	-	-	0
1.b	-	-	0
2	S23 S1 S3 S20 S29	C5 C8	7
3	S4 S7 S2 S27 S23 S43 S46	C4	8
4	S20 S38 S53	C1 C4	5
5	-	-	0
6	S4 S21 S46 S66	C18	5
7	S1 S19 S46 S66	C5 C16	6
8	S4 S3 S11	C13	4
9	S53 S22 S42	C6 C8	5
10	S1 S16 S29	C2 C19	5
11	S50 S23 S30 S65 S66	C2 C12	7
12	S1 S9 S22 S66	C7 C16	6
13	S5 S1 S20 S8 S34	C1 C2 C7 C19	9
14	S2 S4 S6 S8 S9 S49	C4	7
15	S6 S33	C7	3
16	-	-	0

A dash (-) indicates that no sample was sent for sequencing.

Table A: The exon and amplicon lengths for exons 1-17.

Exon	Forward (F) / Reverse (R)	Primer (5'-3')	Exon length (bp)	Amplicon length (bp)	Reference
1.2	F	CTCGCCTTCGCTGCAGCTC	494	334	(Splawski <i>et al.</i> , 1998)
	R	GCGCGGGTCTAGGCTCACC			(Splawski <i>et al.</i> , 1998)
1.3	F	CGCCGCGCCCCCAGTTGC		244	(Splawski <i>et al.</i> , 1998)
	R	CAGAGCTCCCCACACCAG			(Splawski <i>et al.</i> , 1998)
2	F	ATGGGCAGAGGCCGTGATGCTGAC	91	91	(Splawski <i>et al.</i> , 1998)
	R	ATCCAGCCATGCCCTCAGATGC			(Splawski <i>et al.</i> , 1998)
3	F	GTTCAAACAGGTTGCAGGGTCTGA	127	256	(Splawski <i>et al.</i> , 1998)
	R	CTTCCTGGTCTGGAAACCTGG			(Splawski <i>et al.</i> , 1998)
4	F	GATCACGAAAAGCTCCCC	79	212	Designed
	R	TGCGGGGGAGCTTGTGGCACAG			(Splawski <i>et al.</i> , 1998)
5	F	TCAGCCCCACACCATCTCCTTC / (CCCACACCATCTCCTTC)	97	213	(Splawski <i>et al.</i> , 1998)
	R	CTGGGCCCCTACCCTAACCC			(Splawski <i>et al.</i> , 1998)
6	F	TCCTGGAGCCCGACACTGTGTGT	141	238	(Splawski <i>et al.</i> , 1998)
	R	TGTCCTGCCCACTCCTCAGCCT			(Splawski <i>et al.</i> , 1998)

Table A (continued): The exon and amplicon lengths for exons 1-17.

Exon	Forward (F) / Reverse (R)	Primer (5'-3')	Exon length (bp)	Amplicon length (bp)	Reference
7	F	TGGCTGACCACTTCCCTCT	111	195	(Splawski <i>et al.</i> , 1998)
	R	CCCCAGGACCCCAGCTGTCCAA			(Splawski <i>et al.</i> , 1998)
8	F	GCTGGCAGTGGCCTGTGTGGA	96	191	(Splawski <i>et al.</i> , 1998)
	R	AACAGTGACCAAAATGACAGTGAC			(Splawski <i>et al.</i> , 1998)
9	F	TGGCTCAGCAGGTGACAGC	123	186	(Splawski <i>et al.</i> , 1998)
	R	TGGTGGCAGGTGGGCTACT			(Splawski <i>et al.</i> , 1998)
10	F	GCCTGGCAGACGATGTCCA	142	216	(Splawski <i>et al.</i> , 1998)
	R	CAACTGCCTGAGGGGTTCT			(Splawski <i>et al.</i> , 1998)
11	F	CTGTCCCCACACTTTCTCCCT	121	195	(Splawski <i>et al.</i> , 1998)
	R	TGAGCTCCAGTCCCCTCCAG			(Splawski <i>et al.</i> , 1998)
12	F	TGGCCACTCACAATCTCCT	76	222	(Splawski <i>et al.</i> , 1998)
	R	GCCTTGACACCCTCCACTA			(Splawski <i>et al.</i> , 1998)
13	F	GGCACAGGGAGGAGAAGTG	95	216	(Splawski <i>et al.</i> , 1998)
	R	CGGCACCGCTGATCATGCA			(Splawski <i>et al.</i> , 1998)

Table A (continued): The exon and amplicon lengths for exons 1-17.

Exon	Forward (F) / Reverse (R)	Primer (5'-3')	Exon length (bp)	Amplicon length (bp)	Reference
14	F	TCCCACAGACGACAG	47	134	Designed
	R	GACACAGAAACCCACC			(Splawski <i>et al.</i> , 1998)
15	F	GGCCCTGATTTGGGTGTTTTA	62	135	(Splawski <i>et al.</i> , 1998)
	R	GGACGCTAACCAGAACCAC			(Splawski <i>et al.</i> , 1998)
16	F	CACCACTGACTCTCTCGTCT	1344	297	(Splawski <i>et al.</i> , 1998)
	R	CCATCCCCCAGCCCCATC			(Splawski <i>et al.</i> , 1998)

APPENDIX C

Table C: The concentration and absorbance results for the DNA extractions of case post-mortem blood samples.

Case	Concentration (ng/uL)	260/280	260/230	Age	Demographics
1	35.977	1.858	2.784	35yrs	WM
2	54.324	1.912	2.617	19yrs	BM
3	124.39	1.910	2.440	5 months	BM
4	100.42	1.992	2.453	Newborn	WM
5	27.752	1.889	2.967	35yrs	BF
6	75.336	1.867	2.062	1 month	BF
7	80.409	1.944	2.499	20yrs	AF
8	30.540	1.902	2.200	41yrs	BM
9	38.986	1.987	2.099	5months	BM
10	43.199	1.832	2.544	35yrs	BM
11	46.854	1.920	2.284	29yrs	BM
12	50.909	1.978	2.480	31yrs	BM
13	114.34	1.918	2.046	4months	BM
14	39.073	2.019	2.744	27yrs	WM
15	35.926	1.848	2.244	24yr	WM
16	27.788	2.233	2.303	25	BF
17	61.615	1.828	1.868	30yr	BM
18	118.12	1.932	1.957	2 months	WM
19	72.448	1.967	2.316	32yrs	WM
20	307.46	1.908	2.364	16yrs	BM
21	68.050	1.941	2.268	35yr	BM
22	75.870	1.915	2.184	18yrs	WF
23	80.552	1.901	2.443	4yr	BM
24	68.026	1.872	2.371	38yr	BM
25	89.732	1.959	2.240	7months	WM
26	74.234	2.020	1.922	9months	BF

Table C (continued): The concentration and absorbance results for the DNA extractions of case post-mortem blood samples.

Case	Concentration (ng/uL)	260/280	260/230	Comments	Demographics
27	55.575	1.983	2.266	36yrs	AM
28	42.132	1.903	1.506	27 yrs	WM
29	31.829	1.990	2.164	26yrs	BM
30	149.17	1.924	2.292	4yrs	WF
31	55.250	1.890	1.225	23yrs	WF
32	38.362	1.895	1.322	23yrs	BM
33	11.704	1.866	1.105	27yrs	BM
34	59.974	1.913	1.882	39yrs	WM
35	43.697	1.899	1.610	17yrs	BF
36	48.872	1.865	2.492	30yrs	BM
37	88.381	1.932	2.087	33yrs	BM
38	39.048	1.918	1.906	41yrs	BM
39	115.10	1.936	2.560	21 months	BF
40	50.304	1.883	1.536	23yrs	BM
41	165.01	1.940	2.151	7 weeks	WF
42	97.350	1.873	1.655	5 months	BM
43	47.970	1.878	2.362	28yrs	BM
44	154.23	1.809	0.625	6 weeks	BM
45	115.40	1.886	1.889	30 yrs	BM
46	200.71	1.941	2.015	2 months	BM
47	61.438	1.816	2.095	29yrs	BF
48	60.051	1.919	2.020	35yrs	BF
49	31.199	2.000	1.363	17yrs	BM
50	53.500	1.982	1.915	4 months	BF
51	121.210	1.838	1.153	21yrs	BF
52	68.719	1.883	2.180	39yrs	BF
53	18.506	2.464	2.557	24yrs	BM

Table C (continued): The concentration and absorbance results for the DNA extractions of case post-mortem blood samples.

Case	Concentration (ng/uL)	260/280	260/230	Age	Demographics
54	62.016	1.854	2.017	38yrs	BF
55	117.23	1.937	2.145	4 months	BM
56	27.363	1.863	2.217	35 yrs	BM
57	50.426	1.940	2.203	14 days	BF
58	26.057	1.828	1.973	35yrs	BM
59	37.629	1.833	1.892	32yrs	BF
60	57.666	1.838	2.172	50yrs	BM
61	63.081	1.875	2.205	13yrs	WF
62	38.922	1.825	1.827	44yrs	WM
63	27.152	1.857	2.562	29yrs	BM
64	40.099	1.872	1.720	29yrs	BM
65	45.577	2.048	2.237	28yrs	BM
66	209.87	1.908	1.512	1 day old	BM
Average	72.274	1,916	2,08		

APPENDIX D

Table D: The concentration and absorbance results for the DNA extractions of control and optimisation whole-blood samples.

Control	Concentration (ug/mL)	260/280	260/230
1	20.474	1.844	3.562
2	57.523	2.012	2.498
3	129.88	1.924	2.547
4	151.73	1.883	2.346
5	120.80	1.960	2.195
6	27.317	1.967	2.154
7	74.253	1.871	2.770
8	51.179	1.906	2.583
9	105.01	1.876	1.895
10	165.17	1.931	1.902
11	78.073	1.930	1.566
12	74.837	1.885	1.719
13	17.556	1.728	3.331
14	51.713	1.858	1.647
15	39.025	2.001	2.888
16	24.023	2.078	2.196
17	431.51	1.849	1.856
18	268.61	1.915	2.341
19	3.790	3.177	0.556
Average	99.604	1.98	2.24
Optimisation DNA			
1	23.199	1.867	2.031
2	49.120	2.12	1.35
3	15.052	1.964	2.479
4	29.124	1.98	2.063
Average	29.124	1.983	1.981

APPENDIX E

Table E.1: The annealing temperatures and duration for attempting to optimise exon 5 of the *KCNQ1* gene.

Temperature (°C)	Time (s)	Melt curve analysis
50	10	No clear peaks
51	10	No clear peaks
52	10	No clear peaks
53	10	No clear peaks
54	10	No clear peaks
55	10	No clear peaks
56	10	No clear peaks
57	10	A few peaks forming at 81°C, 84°C, and 85°C
58	10	Two peaks forming at 82°C and 85°C
59	10	A larger peak at 85°C, smaller peak at 82°C still present
60	10	The peak at 85°C is improving, smaller peak at 82°C still present
61	10	Broad peak forming at 85°C, and the peak at 82°C
62	10	One peak at 85°C, smaller peak at 82°C and nonspecific binding at 83°C - 83°C
63	10	Slight improvement of 85°C peak, peak at 82°C improving, still some non-specific binding
64	10	Improvement of the peak at 85°C, other peak is smaller but still visible
65	10	The 85°C peak, with smaller peaks at 82°C and 83°C - 84°C
66	10	The 85°C looks optimised, but other peaks still present at 82°C and 83°C - 84°C
67	10	Multiple peaks
68	10	Multiple peaks
69	10	Multiple peaks

Table E.2: The annealing temperatures and duration for attempting to optimise exon 16 of the *KCNQ1* gene.

Temperature (°C)	Time (s)	Melt curve analysis
50	10	Thick peak at 83°C
51	10	Single large peak with two smaller peaks 82°C and 83°C
52	10	Thick peak at 82°C - 83°C
53	10	Thick peak at 82°C
54	10	Broad peak at 82°C with slight improvement
55	10	Broad peak at 82°C with slight improvement
56	10	No change from observation at 56°C
57	10	Messy peaks at 82°C - 83°C
58	10	Broad peak at 82°C - 83°C. The left side has become more defined
59	10	Multiple peaks at 82°C, 84°C and 86°C
60	10	Broad peak at 83°C
61	10	No clear peaks
62	10	No clear peaks
63	10	No clear peaks
64	10	No clear peaks
65	10	No clear peaks, maybe 84°C
66	10	Many small peaks at 82°C, 84°C and 86°C, could be non-specific binding
67	10	Low dF/dT, no peaks
68	10	Low dF/dT, no peaks
69	10	Low dF/dT, no peaks