

Online Resource 2. Conditions for site directed mutagenesis and PCR reactions.

PCR #	Template	Forward primer	Reverse primer	Hot start	Temperatures (°C) and time (seconds) for Denaturation, Annealing, Extension	Cycles	Final extension
Site-directed	3F4Prnp	3F4-WT-F	3F4-WT-R	95°C, 2min	Temp: 95, 60, 68 Time: 60, 50, 360	18	68°C, 7min
	PrP-myc	D177NF	D177NR				
	PrP-myc	E199KF	E199KR				
	PrP-myc	V209IF	V209IR				
A	WTPrnp or 3F4Prnp	EcoPrPF	MycPrPR2	94°C, 5min	Temp: 94, 60, 72 Time: 60, 60, 60	35	72°C, 10min
B	WTPrnp or 3F4Prnp	MycPrPF2	BamPrPR	94°C, 14min	Temp: 95, 55, 72 Time: 30, 30, 60	45	72°C, 1min
C	PCR products A and B				Temp: 94, 50, 72 Time: 30, 1500, 300	10	
D	PCR product C	EcoPrPF	BamPrPR	94°C, 5min	Temp: 94, 60, 72 Time: 60, 60, 60	35	72°C, 10min