Touchdown PCR Protocol

There is a protocol published in *Nature Protocols*, [2] which works very well and is a great reference to start off with for touchdown PCR.

The suggested cycling program has two phases. The first phase of touchdown programming uses a Tm that is approximately 10°C above the calculated Tm. The temperature is reduced by 1°C every successive cycle until the calculated Tm range is reached. This is done for a total of 10–15 cycles.

Phase 2 follows generic PCR amplification of up to 20–25 cycles using the final annealing temperature reached in the touchdown phase (Table 1).

Table 1: An example of a touchdown PCR protocol based on a Tm of 57°C.

Step	Temperature (°C)	Time (minutes)	Stage and number of cycles
1. Initial denature	95	3:00	
2. Denature	95	0:30	Stage 1
3. Anneal	67 (Tm +10)	0:45	10 cycles
4. Extensions	72	0:45	
5. Denature	95	0:30	Stage 2
6. Anneal	Last anneal temp -1	0:45	15-20 cycles
7. Extension	72	0:45	
8. Final Extension	72	15:00	

The cycles and temperature drop during the touchdown phase can be adjusted from 1–3 cycles per 1-3°C drop in temperature if non-specific products are still observed or if the yield is low. You can also set the final annealing temperature 1–2°C below the calculated Tm.

Make two reactions for Gene (annealing temperature 60 C) and the Vector (annealing temperature 62 C)