MANUAL TOUCHDOWN PCR AMPLIFICATION

by TCH 8/13/01

PCR Reaction Mix

	25 ul Rx
10x Buffer	2.5
dNTPs (10mM each)	3.0
DMSO	1.5
Primer #1 (330ng/ul)	0.5
Primer #2 (33ong/ul)	0.5
ddH_2O	q.s. to 25 ul
Taq DNA polymerase (BRL).	0.5
Template DNA (plasmid)	(10-200 ng)
	25 ul

PCR Cycling Program (on Hybaid OmnE with “block” control)

Stage 1	95°C X 2′	X 1 cycle
Stage 2	92°C X 20" 64°C X 30" 70°C X 30-60’’ (or 1kb/min)	X 4 cycles
Stage 3	92°C X 20" 61°C X 30" 70°C X 30-60’’ (or 1kb/min)	X 4 cycles
Stage 4	92°C X 20" 58°C X 30" 70°C X 30-60’’ (or 1kb/min)	X 4 cycles
Stage 5	92°C X 20" 55°C X 30" 70°C X 30-60’’ (or 1kb/min)	X 25-35 cycles
Stage 6	70°C X 5′	X 1 cycle

Load 5-10ul of the PCR product to a 0.8% agarose gel.

Note: 1) Lower total cycle numbers are preferred because of lower mutation rate;

2) To obtain larger quantity of DNA, one may set up 2 to 4 reactions (25 ul each);

3) This protocol is suitable for amplification of a specific fragment from a cDNA or genomic library, as well as RT-PCR analysis.