PREPARATORY PCR AMPLIFICATION FOR CLONING

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).	0.25
Template DNA (plasmid)*	(10-200 ng)*
	25 ul

* ***Use approximately 2ul miniprep DNA per 25ul reaction.***

PCR Cycling Program (on Hybaid OmnE with "block" control)

95°C X 2′	X 1 cycle
92°C X 20"	
55°C X 30"	X 10-15 cycles
70°C X 30-60'' (or 1kb/min)	
70°C X 5′	X 1 cycle

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

Note: 1) **Lower 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) To amplify a fragment from a RT-cDNA library, one may have to use touchdown PCR or gradient PCR protocol.