

Colony PCR

This protocol is designed to quickly screen for plasmid inserts directly from *E. coli* colonies. The plasmid should be high copy number such as pUC18 pUC 19, or pBluescript, etc. Even though blue/white screening can be used to determine if inserts are present, this technique can be used to determine insert size and/or orientation in the vector. Alternately, the presence of an insert and its size can be determined by growing each colony in liquid, the plasmid purified by a boiling or alkaline preparation protocol, digestion of the plasmid with restriction enzyme(s) that excises the insert, followed by separation by agarose gel electrophoresis.

Typical colony PCR reaction

Mix together the following on ice; always adding enzyme last. For multiple samples, make a large master mix and aliquot 50 μ l in each PCR tube (also on ice).

38 μ l	sterile distilled water
5 μ l	10X PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0), 1.0% Triton X 100)
3 μ l	25 mM MgCl ₂
1 μ l	10 mM dNTPs (10 mM each dATP, dTTP, dGTP, dCTP)
1 μ l	20 μ M forward primer
1 μ l	20 μ l reverse primer
0.2-1 μ l	<i>Taq</i> polymerase
50 μ l	total volume

To each cold PCR tube containing the PCR reaction, add a small amount of colony. To do this, use a fine yellow pipette tip attached to a pipetter (set at 30 μ l to avoid addition of air into the PCR reaction) and pipette up and down to mix. The amount of cells should be small, just a touch will do, the small amount required to fill the end of the opening is sufficient. Sufficient mixing will result in complete cell lysis and high yields.

PCR conditions

		What happens?
1 cycle	5 min at 95°C	-initial cell breakage and DNA denaturation
30-40 cycles	1 min at 95°C	-DNA denatures into single strands
	1.5 min at 54°C	-primers anneal to ssDNA template (temp depends on primers)
	1 min at 72°C	-primers are extended from 3'-end by <i>Taq</i> (1 min/kb)
1 cycle	5 min at 72°C	-final extension to make sure all products are full length (72°C is optimal for <i>Taq</i> polymerase)