

# Colony lysis procedure for riboprobe synthesis

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This protocol starts with a single bacterial colony and makes PCR generated template for *in vitro* transcription reactions. The PCR reaction can also be used on plasmid DNA at 5ng/ul to generate probe template directly instead of digestion, etc.

## Colony Lysis

### Colony Lysis Buffer

1% Triton X-100  
20mM Tris\*Cl pH 8.0  
2mM EDTA pH 8.0

Pick 6 single colonies (leave some on the plate for growth later) and place each into 25ul of Colony Lysis Buffer in a 96well PCR plate. The best technique is to smear the cells onto the side of the well. Seal plate with sealing mat or a foil sealer. (NOTE: When using clone specific primers you can pick a single colony since the primers will serve as the quality control.)

Heat in the PCR machine at 95°C for 10min, cool on ice. The DNA is now ready for PCR.

## PCR Reaction

To generate probe template, perform PCR with *clone* specific primers (preferred) or *promoter* specific primers (T3, T7, SP6), typically using a 25ul volume PCR. Below is the procedure for using promoter primers on BMAP clones (pT7T3D-PAC vector). Adjust to the established PCR conditions for clone specific primers (i.e. conditions used for primer design).

Qiagen Tag Buffer	2.5ul
10mM dNTPs	0.5ul
10uM T3 primer	1.25ul
10uM T7 primer	1.25ul
Qiagen Taq Poly.	0.125ul
H <sub>2</sub> O	18.375ul
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<b>Colony Lysate</b>	<b>1ul</b>
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Total reaction vol.	25ul

PCR conditions:    94°C 3min    1cycle  
                          94°C 40sec    -----  
                          48°C 40sec                --35cycles  
                          72°C 2min    -----  
                          72°C 5min    1cycle

Run 5ul on a 1.2% agarose gel. Select one of the six that represents the expected product and amplified well. Usually all 6 or at least 4 of the six agree in size so those are the ones to pick from.

### **In vitro transcription**

Use 10ul-12.5ul of the PCR product directly in a standard 20ul in vitro transcription reaction to produce DIG labeled probe (Lab/Roche protocol). Probe is column purified, quantified and diluted to a 20X concentration for storage at -80C (whole-mount protocol).