Dig Probe Synthesis
(NIBB clones June '05, or large scale prep)

DNA Prep-

-Digest 5ug DNA in a 50ul reaction (Not I for NIBB clones, unless internal site exists)
-run 2ul on check gel
-add 50ul H2O
-Ph/CHCl3 extract in 100ul volumes, spin 5' at 4°C
-add 250ul 100% EtOH, 10ul DEPC treated NaOAc
-incubate at -20° C 30', then spin 10-15' at 4°C
-resuspend pellet in 20ul DEPC H2O

Probe Synthesis-

Master Mix	25ul rxn X (n) reactions
5X Txn buffer	5ul
100mM DTT	2.5ul
2.5mM Dig mix	5ul
DEPC H2O	8.75ul
Mix above components, aliquot, then add:	
DNA	1.25ul
Rnasin	0.5ul
T7	2ul

-Incubate 2hrs at 37°C
-Add 1ul Dnase, incubate 30' at 37°C
-run 1ul on check gel
-(Optional) Add 25ul 2X Bicarbonate, 20' at 60°C to hydrolyze probes
-Add 50-75ul DEPC H2O to bring volume to 100ul
-Add 50ul NH4Acetate, 500ul EtOH
-Incubate at -20°C for 30'
-Spin 10-15' at 4°C
-Resuspend in 100ul DEPC H2O
-Run 1ul on 1% gel

Test probes at 1:10 (if low concentration on gel), 1:25, 1:50 and 1:100