***in situ* Hybridization Probe Synthesis**

To generate probes for use visualizing mRNA in Gryllus.

General Notes:

1) Amplify your target gene.
2) Assemble the following reaction in a 1.5mL eppendorf tube, using your target gene as template and either T7 or Sp6-T7 primers for sense and antisense probes. Seal tube lids with parafilm.

Reagent	1x (µl)
10x RNA polymerase buffer	2
Dig labeling mix	2
RNAse inhibitor	1
RNA Polymerase	2
500ng template	x
MQ H_2O	To 20µl

3) Incubate 3-4h at 37°C.
4) Add 30µl TE, 5µl 3M NaOAC, and 2.5volumes (125µl) of cold 100%EtOH.
5) Incubate on ice at least 20min.
6) Spin 15min at max speed at 4°C in a microfuge.
7) Aspirate and discard supernatant.
8) Add 500µl; cold 75% EtOH in H_2O.
9) Invert several times to mix.
10) Spin 5min at max speed at 4°C in a microfuge.
11) Aspirate and discard supernatant.
12) Air dry 2-3 (and certainly under 5) minutes.
13) Dissolve in 20µl TE. Do not pipette or vortex.
14) Dilute 1:10 in TE to check reaction by measuring the concentration on a spectrophotometer and running in gel electrophoresis.
    *Wash box and comb, and change running buffer before making gel to decrease RNAses.
15) Adjust to 100ng/µl in hybridization solution. Store at -20°C.