Generating cDNA Templates for in vitro Transcription by RT-PCR

For many GEISHA entries (those with GEISHA IDs ending in ".uapcr"), antisense RNA probes were transcribed from a cDNA template that was generated by RT-PCR. For these entries, the primer sequences for generating the cDNA template are shown immediately below the GEISHA ID in the Entry information. The predicted PCR product length is shown to the right of the reverse prime primer sequence. A file containing all primer sequences is available for download.

Primer sequences are designed using standard primer prediction software (e.g. MacVector), and mRNA sequences from NCBI. Primers are designed to generate a 700-1500nt amplicon, unless a shorter amplicon is dictated by the length of the mRNA sequence available and/or the nucleotide profile of the mRNA (high GC content, for example).

Each reverse primer contains the T3 polymerase binding site sequence at the 5' end. T3 RNA polymerase is then used for the in vitro transcription reaction. Some primers contain additional endonuclease restriction enzyme sites to enable cloning of the amplicon, as follows:

```
Add to the 5'end of the forward primer
5'-TTATAAAAGCTTGCGGCCGCAGAATAT-3'          Tm = +27.9
   PslI  HindIII   NotI
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```
Add to the 5'end of the Reverse primer
5'-GCTCTAGAATTAACCCTCACTAAAGG-3'           Tm = +22.9
   XbaI       T3 RNA Polymerase
```

Some primers contain only the T3 RNA polymerase binding site on the reverse primer, as follows:

```
Add to the 5' end of the Reverse primer
5'-ATTAACCCTCACTAAAGG-3'              Tm = +22.9
      T3 RNA Polymerase
```

To generate cDNA templates, we use a pooled RNA stock consisting of an equal mass of RNA isolated from embryos at multiple stages of development (pregastrula, stage 4, stage 12, Stage 18, stages 23-25). The primers are used in a standard RT-PCR reaction to generate the cDNA template. Following column purification, 200ng of the amplicon is used in a standard in vitro transcription reaction for generating Digoxygenin labeled antisense RNA probes.