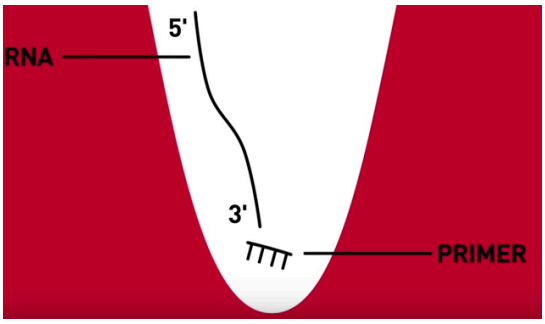
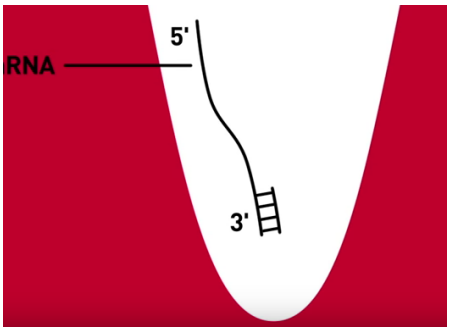
**Two Step RT-PCR – Step 1: Reverse Transcription of RNA using random hexamer primers**

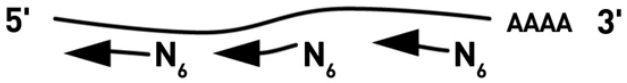
The total RNA from the bees, including RNA from any viruses they carry, needs to be reverse transcribed into cDNA so that we can complete PCR on it.

**Primer annealing**: In a PCR tube, we'll combine random hexamer primers, RNA fragments from our bees, and RT with buffering salts. In the room temperature primer annealing step, each single RNA will have many short primers attach to it.

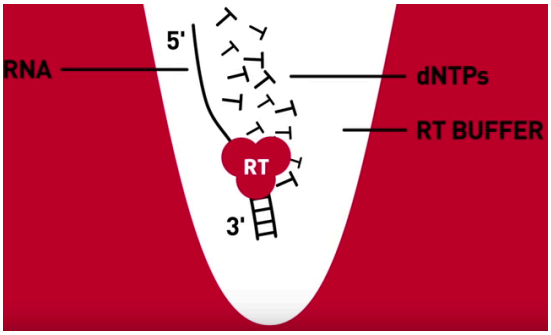


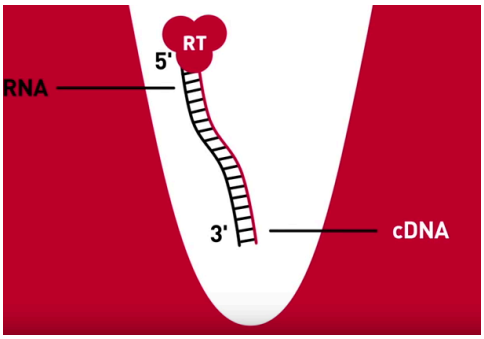




We can zoom in to see that many random hexamer primers, denoted below as $N_6$, will attach to a single RNA to generate full coverage:




**Reverse transcription**: Next, the reaction heats up to 48C, the optimal temperature for the Taqman reverse transcriptase enzyme. The RT enzyme adds dNTPs to complement the RNA strand, building a new DNA strand.




This RNA/DNA hybrid can then be used in PCR or stored at -20 or -80 for use in a later experiment.

Images screen captured from Thermo Fisher Scientific video https://youtu.be/0MJIbrS4fbQ