1.2 Order of setup and incubation

Before anything else, write up, check and double check your protocol. I always write it out completely (see sample protocol below) in order to not make a stupid mistake. Get your DNA ready, fill and check the H2O bath temperature (37øC) and label clean 1.5 ml microfuge tubes taking care to avoid touching internal surfaces including cap -- some people even wear gloves. Then assemble on ice a "master mix" of all reagents except DNA in the order listed (a through g plus H2O as needed, see sample protocol). Aliquot master mix to the bottom of each microfuge tube, add DNA using a new yellow tip for each tube, directly into the bottom of the tube, vortex gently and place in incubation at 37øC for 1 hour. The temp optimum is very sharp. I always make sure the bath is full and tubes immersed to their necks, and the top of the bath covered in order to minimize the temp gradient and evaporation -- which can be a problem for transcription volumes of 10 ul at this temp for an hour. At the end of the hour transfer to ice or freeze at -80. If a higher concentration of RNA is needed at the end of 1 hour, spin transcript down in microfuge (15 sec), add .2 ul SP6 polymerase, continue incubation an additional 1 hour.