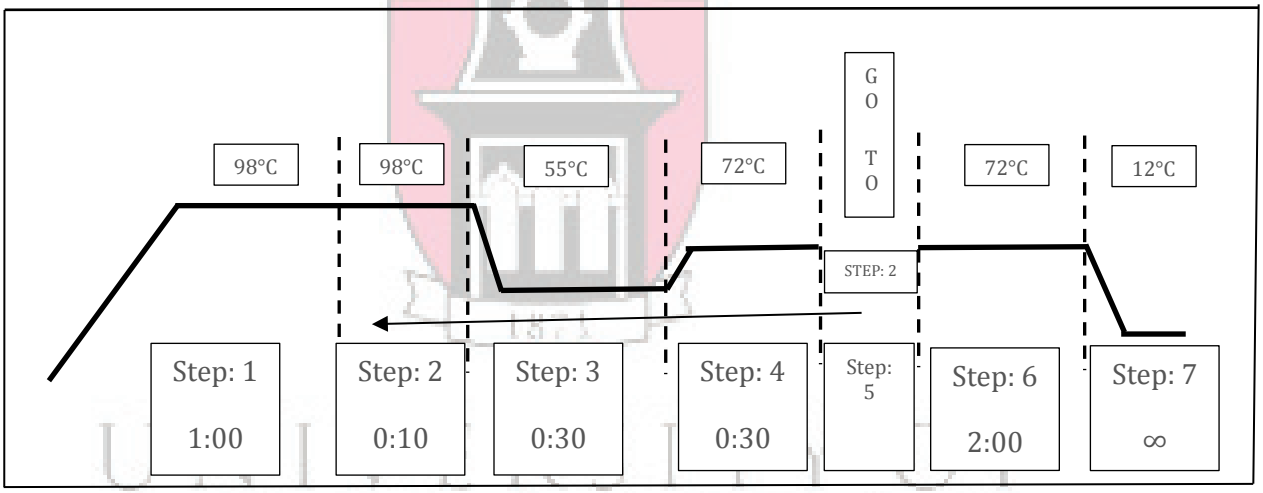
WORKSHOP ON MOLECULAR CLONING
University of Arkansas
Department of Chemistry and Biochemistry
Dr. Fan's Lab

Day: 1 Preparation of the plasmid DNA

- To a PCR tube, add 20 µl PCR premix (contains 2x Q5 polymerase, dNTPs, reaction buffer, water, primer) and 1 µl template.



- Step:1 98°C 60 seconds
- Step:2 98°C (denaturation) 10 seconds
- Step:3 55°C (annealing) 30 seconds
- Step:4 72°C (extension) 30 seconds
- Step:5 Repeat cycle from Step: 2 (25 times)
- Step:6 72°C (final extension) 2 minutes
- Step:7 12°C (hold) infinity

- After the PCR is completed, add 5 µl of 6x loading buffer to 5 µl of the PCR product.
- Load this mixture in the agarose gel.
- Run the gel at 130mV for 20 minutes.
- View the gel using ChemiDoc.

Day: 2 Transformation of Competent Cells

- Thaw the competent Top10 cells on ice completely and pipette 15 µl of these cells into a 14 ml culture tube.
- To this add 1 µl of plasmid, shake gently and place it on ice for 15 minutes.
- Then, heat shock the cells in the 42°C water bath for 30 seconds.
- Place it back on the ice for 2 minutes and add 500 µl of Luria-Bertani media.
- Incubate it for 30 minutes at 37°C.
- After incubation, add 100 µl of this culture to the LB plate and spread it evenly on the plate.
- Label the plate and incubate it at 37°C overnight.

Day: 3 Results

- Check the plate and record the results.