**Annealing Procedure**

*(Updated 10/20/21 by KM)*

* Ensure that no one is using the annealer (check for signs on them and check if the heat is on) and if anyone else is planning to anneal that day.
* DO NOT USE POP LID EPPENDORFS! They will open and your samples will be lost.
* Turn on annealer and allow it to reach 85 or 90 °C (5 mins at 90, 10 mins at 85). The heat light will turn off when it reaches the correct temperature.
* Vortex samples for 3 seconds, then spin for 10 seconds.
* When annealer reaches desired temperature, place samples in, replace lid, and set a timer for 5 mins (90 degrees) or 10 mins (85 degrees).
* After time is up, leave samples in annealer (Do NOT remove lid), turn off the heat, and record the time. Leave samples for 3-5 hours to cool back down to RT. It’s important that the DNA cools slowly so that it folds correctly. Keep track of thermometer temperature.
* Vortex and spin after removing samples. Record time and place at 4 °C overnight to equilibrate.