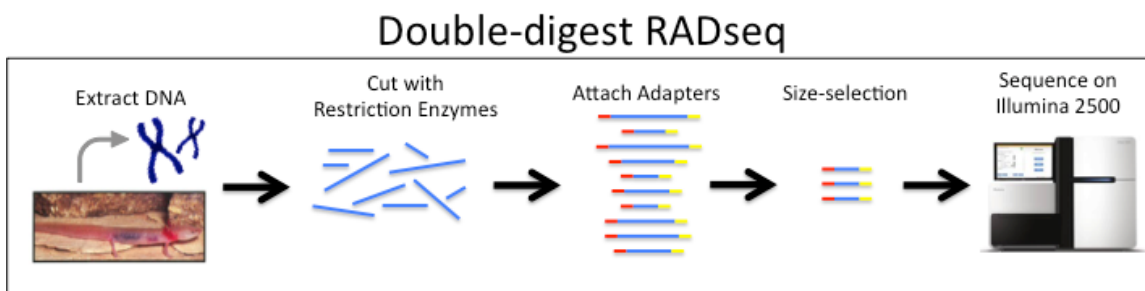




Estimating effective population sizes for central Texas cave and spring salamanders (*Eurycea*)

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In late February and early March, we selected 40 samples to be sequenced to accompany the ~72 sequences already in hand. Our sampling for this project now includes every described species of central Texas *Eurycea*, and several known but undescribed species. We then prepared samples for double-digest restriction-site associated DNA sequencing (ddRADseq). Briefly, we extracted DNA from each tissue sample, and then ‘cut’ the DNA with specific restriction enzymes. Then, we attached adapters to the ends of the DNA. Each individual received a unique combination of adapters so that we can now tell which sequence belongs to which individual. We then selected a narrow size range using gel electrophoresis. This enabled us to sequence more fragments (larger size range) or fewer fragments (narrow size range). We targeted around 10K genomic loci per individual. These samples were then pooled together and sequenced on an illumina 2500 machine at the BYU DNA Sequencing Center.



The subsequent data files include several billion base pairs of DNA! We are now in the initial phases of analyzing these data. We have already generated DNA sequences for 72 individuals, so our dataset now includes 112 samples from over 40 populations of Texas *Eurycea*. Analyzing this much data is computationally intensive, and can take several days or even weeks for certain analyses. We think it will be worth the wait, as we will be able to estimate effective population sizes for dozens of populations of federally Threatened or Endangered cave and spring salamanders.