This work details methods for generating and cloning immortal cell lines from *Xenopus* embryos. It describes the application of these techniques to the development and characterization of four distinct, novel cell lines from the important vertebrate model organism, *Xenopus tropicalis.* Three of these lines represent the first reported diploid cell lines from this species. The cell lines can be grown in conventional media without a CO2 incubator. They are readily manipulated by transfection and will be useful for producing gene-edited derivatives. These cell lines will be of wide practicality in mapping pathways of *X. tropicalis* cell biology and development.