



# Performing DNA Amplification Reactions on Electrowetting Chips

Anita Kalra | Advisors: Jim Sterling/Ali Nadim  
Keck Graduate Institute, REU Program 2005



The miniaturization of biochemical assays is becoming an increasingly popular method of performing analyses with higher throughput, smaller volumes of reagents and lower costs. Microfluidic technologies, both mechanical and electrokinetic, are enabling such miniaturizations. We report on the application of one such technique, “electrowetting,” to DNA hybridization reactions, and the DNA amplification reactions EXPAR and PROXAR. We have successfully performed hybridization on an electrowetting chip by moving and coalescing two droplets containing complementary DNA sequences. One 3 $\mu$ L droplet consisted of a molecular beacon (1 $\mu$ M in 2mM MgCl solution), and the other 3 $\mu$ L droplet consisted of the beacon’s complement (2 $\mu$ M in water). The reaction was detected using fluorescence microscopy. We observed that the MgCl, while necessary to stabilize the hybridized DNA, hindered droplet actuation. We also performed the Exponential Amplification Reaction (EXPAR) on electrowetting chips by merging droplets containing EXPAR enzymes and oligonucleotide sequences. This reaction is susceptible to biomolecular adsorption, in which adhesion to the electrowetting substrate decreases the droplet’s contact angle, inhibiting actuation by electrowetting. We investigated a number of immersions and coatings to prevent biomolecular adsorption, including silicon oil, polyethylene glycol (PEG), and varying Teflon coatings. We characterized biomolecular adsorption for droplets containing EXPAR enzymes and oligonucleotides, and determined that oligonucleotides in water show the least adsorption. In future applications, one can perform PROXAR by electrowetting three or more droplets containing oligonucleotides and PROXAR enzymes. Current research includes characterizing droplet evaporation and investigating the effect of enclosing substrates to minimize evaporation, as well as studying the effects of mixing in accelerating the PROXAR reaction.

Hybridization detected by fluorescence  
3  $\mu$ L DNA droplets



ProxAR Linear Amplification Reaction

