GEL ELECTROPHORESIS AND ANALYZATION OF RT-PCR PRODUCTS:

General notes on running gels:

- Use 1% gel for large DNA frag's (>1 Kb) & 2% gel for frag's ≤ 1 Kb for best resolution.
- Gel typically ran at 400 V. 2% @ 20 min. & 1% @15 min.
- Multi-Channel loader loads every other well. Can use same tips to load different samples-just rinse first in gel buffer.
- Check to see that loading dye isn't too viscous. Add dH2O if so.
- Load appropriate DNA ladder at each end of samples.

Running PCR Product Gel:

- Using multi-channel pipetter, load 3 λ loading dye to top inside of each well (just so as to not touch sample). Mix loading dye into samples by tapping plate.
- Load 3 λ of PCR prod. + Load. Dye into each well.
- Load 1Kb and/or 100bp ladder on either side.

Analysis of PCR Product Gel Electrophoresis Results:

- Look for bands of the length the primer pair was suppose to extend
    - Ex. Sca11a-1 primers were to amplify a 1 Kb region so look for a 1 Kb band.
- Look for prominent bands. A prominent band that is shorter than expected for the primer pair may indicate that you do have that candidate gene present but perhaps a splicing event occurred in that cell type so that the amplified region is shorter/longer than expected.
- Lower bands (<100 bp) may be indicative of primer dimmers.
- Lanes w/ smears of bands are questionable. If interested in a band, you must do a gel extraction or stab and then amplify that segment with the same primers.