**Multiplex PCR Protocol**
Modified from protocol by Giacomo Bernardi

1. Dilute stock primers from 1 mM to 50 uM.
    a. Make and label tubes of 19 uL water.
    b. Add 1 uL stock primer to each tube.

2. Make primer mix:
    a. Each reaction requires 1uL of primer mix containing 0.04 uL of each primer.
    b. For N reactions, mix (N*1.1)uL of primer and add water to make (N*1.1)uL total volume.

3. Add primer mix, master mix, and water to 96 well plate.

4. Add DNA to plate

5. Amplify in thermocycler using desired PCR parameters. For Amphiprion, I have been successful using: 95º/15'; 40x(94ºC/30";57ºC/90";72ºC/60"); 72ºC/7')

6. Mix 26.15uL size standard (ROX 500) with 973.85uL HiDi Formamide to make 1000uL of solution. Add 10uL of this solution to each well of a 96-well plate.

7. Add 0.5uL of PCR product to each well.

8. Seal and send out for analysis or do it yourself!

Notes:
Allow at least 2.5 hours for PCR to complete.