Single Cell PCR for Zebrafish embryos

1. Prepare PCR tubes. One tube per fish to PCR. Add 5 ul of 10X Qiagen PCR buffer plus 5 ul of ProK/NP40 mix (below) to each tube.
    a. ProK/NP40 mix
        i. 1 ul of 20mg/ml ProK
        ii. 1 ul of 10% NP40
        iii. 48 ul of H2O

2. Remove small number of cells from blastula stage embryo using micromanipulation/transplantation apparatus in the Bartel Lab.

3. Put cells into PCR tube with PCR buffer and ProK/NP40.

4. Incubate tubes in 50 degree water bath for 1 hour.

5. Make up PCR mix for your desired product.
    a. PCR mix
        i. 1ul 10mM dNTP mix
        ii. Forward Primer of 0.3ul 20uM
        iii. Reverse Primer of 0.3ul 20uM
        iv. 2ul 25mM MgCl2
        v. H2O up to 50 ul total
        vi. Taq Polymerase (Qiagen) 0.25ul per reaction
        (don't forget you already have 10 ul in your reaction tube to start with)

- add mix to each PCR reaction tube and start PCR.

Set PCR machine for the desired settings according to your primers and product size.

95 degrees 15 min
95 degrees 1 min
60 degrees 1 min (annealing temp should be about 5 degrees lower than primer Tm)
72 degrees 1 min per kb product size
72 degrees 10 min
4 degrees until you can take it out.

Run on an agarose gel and look for the appropriately sized band.