ZEG protocol

Materials:
ZEG and chips
Embryos (48hpf-7dpf) in dish of E3
E3
Transfer pipets
P-20 and tips
Scissors/razor blade to cut tips
Labeled PCR tubes
96 well plate for recovery of embryos
Optional: multichannel and tips; Tricaine

Procedure:

1. Cut off a P-20 tip to enlarge the bore to accommodate an embryo.
2. Set P-20 to 15 ul and aspirate an embryo into the tip.
3. Pipette embryo onto ZEG chip. Repeat until the chip is full.
4. Vibrate chip for 7.5 minutes.
5. Carefully remove E3 to a clean, labeled PCR tube. Use either a P-20 or P-10 multichannel if you're extra careful (keep pipette tip more vertical).
6. Add new E3 to embryos on the chip to keep them hydrated.
7. Remove embryos to appropriately labeled wells in a 96-well plate with disposable pipette tips.
8. Do PCR using 5ul of the material in the PCR tube. There is no need to heat it or do further manipulation - just use the 5ul directly in the PCR. Best results are obtained using Phire polymerase, #F126 from ThermoFisher, an amplicon smaller than 300bp and 40 cycles of PCR.

PCR tips:

- Optimize your PCR procedure before you use the ZEG
    - Especially, if historically you had trouble with PCR with fin clips
- Phire Polymerase #F126 works the best
- 40 cycles of PCR
- If you use 15ul for each embryo on the chip, you get back at least ~12ul and you could do probably 3 PCRs with that, using 4ul each, and make it work
- Fish inside the chorion won't work. So if you must do ZEG before they hatch, manually dechorionated them first, or wait until they hatch.
- Don’t dilute or extract ZEG samples at all. There's not a lot of material in there (which is kind of the point, since you don't want to harm the embryo). Use 5ul of ZEG material straight up in my PCR. Do not do a thermopol isolation on ZEG samples either.
- Don't need mineral oil for PCR but you do need it for HRMA
- Also, the polymerase/mix you use is critical. We have gotten the best results with LC Green from BioFireIn our experience HRMA works better with a dye optimized for that purpose, such as LC Green from BioFire (we love this and it works so much better than other dyes! You do need to optimize the annealing temp; it's usually a bit higher, which makes the reaction run faster anyway!) or using BioRad's HRMA master mix.