InVivo Biosystems

Reference Guide

The Zebrafish Embryonic Genotyper (ZEG) by wFluidx

*Patent pending

wFluidx
know your fish

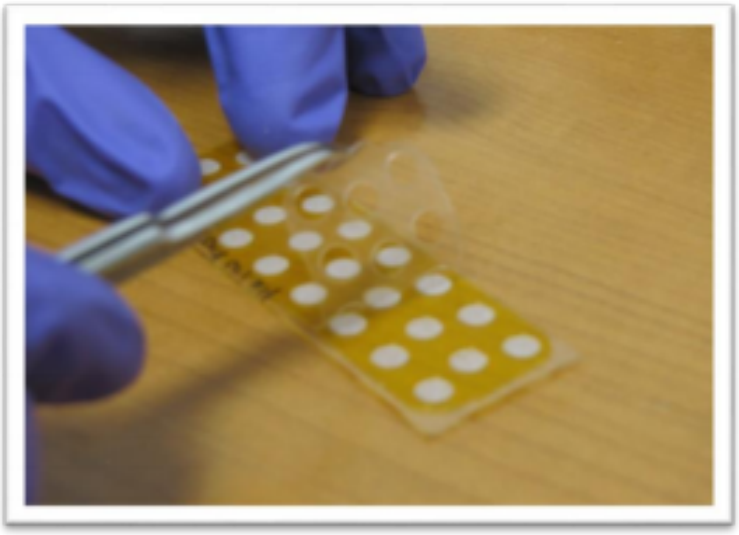
InVivo Biosystems
Formerly NemaMetrix

For questions, contact **support@invivobiosystems.com**

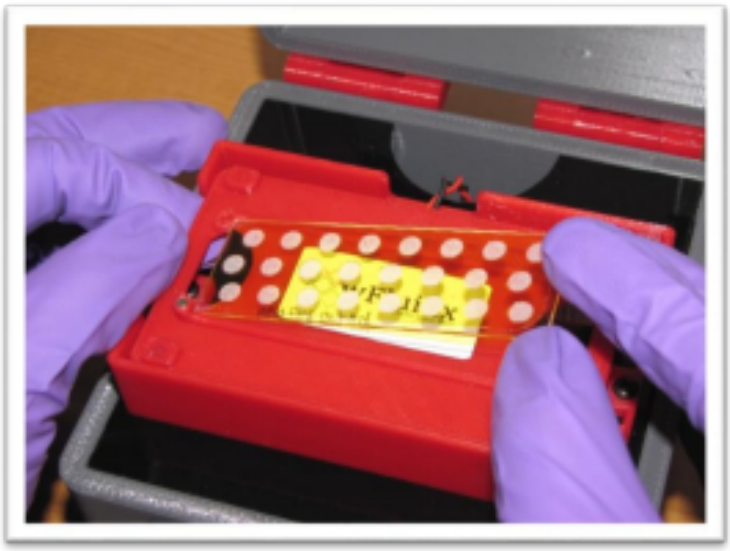
Instructions

1. Remove the ZEG chip from its protective case. Avoid touching circular chambers on the top surface of the chip. Note: The ZEG chip has a smooth side, and the side with circular chambers (surrounded by the yellow colored laminate). When storing multiple unused chips in the protective case, always have smooth side mate the smooth side of the neighboring chip. Similarly, the side with the circular chambers should mate with similar side of the neighboring chip.

2. Remove the clear protective liner from the top surface of the chip. The top surface will be sticky to touch. This hydrophobic layer keeps the droplet samples isolated from one another. This side will be facing up when loaded onto the platform in the next step.



3. Load the chip into the chip holding platform with the roughened chamber circles facing upward. When loading the chip, place the chip in with one side in first, followed by rest of the chip as shown in figure on the right. Be careful not to overstress the springs suspending the platform through excessive compression or stretching (the protective red guard will inhibit some of these stresses). Holding the platform steady with your other hand could be useful in this case.



4. Ensure that the chip is securely held flat by the platform; within the ridges.

5. Load one embryo onto a chamber in a 12 µL volume using our custom cut wide bore pipette tip (provided). Accuracy (+/-1µL) in the total fluid volume on each chamber is important for genotyping sensitivity and embryo survival. Repeat this process for remaining chambers. For this step, we recommend operating the ZEG in an environment that has minimal room air drafts. Areas with high air flow - such as would be found below or near a fan or heating vent - can cause excessive evaporation of the sample leading to reduced performance; and lower genotyping sensitivity.

6. Place the platform lid on the platform. The lid will temporarily affix itself to the platform by magnetic forces and also firmly hold the chip on the platform. In addition, the lid would inhibit loss of media (due to evaporation) from the loaded chambers during DNA extraction.

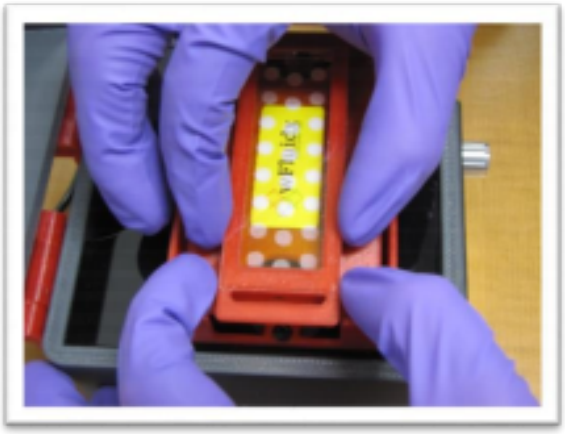
7. Turn on the base unit by pressing the power button (labeled ON/OFF). The base unit powers a vibration motor attached to the chip holding platform. By default your base unit should already be set to the appropriate voltage (as displayed on the LED screen). To ensure your base unit is set to the right voltage, please follow these steps:

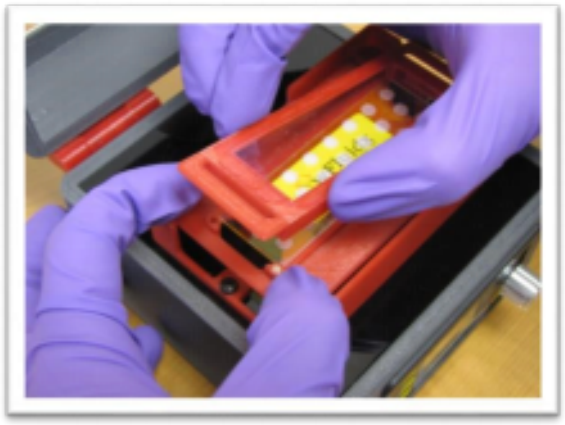
- All base units have serial numbers, starting with 'ZP'. This serial number is located near the vibrating platform at the top right corner.
  - If the serial number of your base unit is between ZP-001 and ZP-041 (inclusive), please ensure the voltage is set to 1.4 V.
  - If the serial number of your base unit is outside the above range then please ensure the voltage is set to 1.8 V.
- If you notice that the voltage is not incorrect or you are unable to locate the serial number then please notify us at support@ivivobiosystems.com

When the base unit is powered ON the platform should be vibrating. A gentle touch on the platform can confirm this. This is the DNA extraction process and should run continuously for 7.5 minutes. During this process, genetic material is being removed from the embryos.

8. Upon completion of the 7.5 minutes, press the ON/OFF button to stop the vibration unit.

9. Carefully remove the platform lid by holding the platform with one hand and gently lifting one side of the cover with the other hand. This ensures that the springs are not over extended, and no excessive vibration of the platform is generated during lid removal.





10. Carefully remove at least 10 µL sample (media around the embryo) from a chamber.

NOTE: Please use single channel pipettes for maximum control and maximum genotyping sensitivity and embryo survivability. Avoid touching the embryo with pipette tip during media collection, as this can damage the embryo. This sample contains PCR amplifiable genetic material from the embryo and should be stored directly in PCR tubes for follow up PCR. Immediately (within a few seconds of sample collection) replace the media with 10-20µL of fresh E3 buffer (for transferring the embryo later to a well plate/petri dish) or relocate the embryo by a transfer pipette to a well plate/petri dish where it has enough media to be viable. Repeat this for remaining chambers.

In step 10 it is important that you collect as much as volume as you can for maximum genotyping sensitivity. For example if you notice that even after collecting 10 µL you still have some collectable volume left in the chamber, then please do not hesitate to collect the remaining volume or adjust the volume setting of your pipette to collect all the fluid in one collection step. Also before collecting the volume from a chamber, try to do a little mixing with the same pipette by doing two cycles of draw/dispense. Both of these methods can likely improve genetic material collection and maximize genotyping sensitivity.

11. Remove the chip from the platform by pressing up on the bottom surface of the chip through the holes in platform. The base unit can now be powered off and the base unit lid can be closed to protect the platform if no further embryos need to genotyped.
Note: It is also possible to unload the chip first, place it on work bench against a contrasting background, and then collect samples and unload the embryos (as mentioned in step 10). This may be required in cases when embryos are less visible against the background color of the platform.