

Chamber Imaging Pre-PCR

For Sapphire Chip

For Research Use Only. Not for use in diagnostic procedures.

This protocol is intended to describe how to scan the Sapphire Chip between the partitioning step and the PCR step in the Geode during a **Crystal Digital PCR™** run.

1. When Should This Protocol Be Used?

This protocol is used when one want to visualize the Droplet Crystal after its creation (partitioning step in the Geode) but before the PCR takes place and proceed then with the PCR step in the Geode.

2. Materials Needed To Perform This Protocol?

- Sapphire Chip(s)
- Reaction mix containing naica® PCR MIX or naica® multiplex PCR MIX, assay and sample(s) being analysed
- 3-color naica® system or 6-color naica® system, compatible to process Sapphire Chip consumables
- Extra vented PCR caps
- Optional: Petri Dish containing a Precision Wipe to transport the Sapphire Chip(s) if the plastic blister is not available

3. Procedure

1. Load 25 µL of reaction mix into each inlet of the Sapphire Chip(s)
2. Place the Sapphire Chip(s) in the plastic blister or in a Petri Dish containing a Precision Wipe to transfer it to the Geode instrument.
3. Ensure the Geode is ready to operate Sapphire Chip:
 - a. Geode magnetic frame for Sapphire Chip for the thermal plate
 - b. No Geode lid adaptors
4. Place the Sapphire Chip(s) in the Geode.
5. Load the “*Sapphire Protocol Imaging pre-PCR*” program in the Protocol directory (consisting of a partition phase followed by a release).
6. Click Play to launch the program.
7. At the end of the program, place the Sapphire Chip(s) in the plastic blister or in a Petri Dish containing a Precision Wipe to transfer to the respective naica® system scanner, Prism3 or Prism6.
8. Scan the Sapphire Chip(s) using the Prism3 or Prism6 as described in the User Manual.
9. Once the scan is finished, remove the Sapphire Chip(s) from the Prism3 or Prism6 and process it (them, one by one) as follow:
 - a. Delicately disconnect the blue caps from the outlet port and replace them by the vented PCR caps (figure 1).

Note: When disconnecting the blue caps from the outlets, incline the chip a little bit. This allows the Droplet Crystal to move toward the inlet port.

- b. Place the Sapphire Chip(s) back in the plastic blister or in a Petri Dish containing a Precision Wipe.

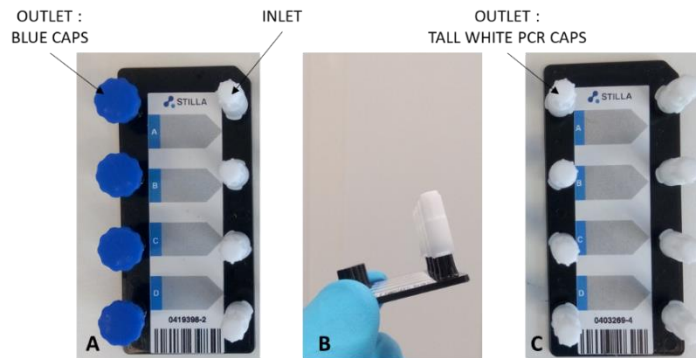


Figure 1 : **A.** Sapphire Chip at the end of the partitioning program. **B.** Sapphire chip tilt after removal of the blue caps. **C.** Blue cap replacement by the vented PCR cap.

10. Transport the Sapphire Chip(s) to the Geode and place it (them) back in.
11. Load the “*Sapphire Protocol Imaging-post PCR*” program in the Program panel
 - a. Edit the number of PCR cycles
 - b. Edit the temperature and duration of the PCR cycles
12. Save the Program in the Scripts directory
13. Go to the Run panel and load the program saved.
14. Click Play to launch the program.
15. At the end of the program, place the Sapphire Chip(s) in the plastic blister or in a Petri Dish containing a Precision Wipe to transfer to the respective naica® system scanner, Prism3 or Prism6.
16. Scan the Sapphire Chip(s) using the Prism3 or Prism6 as described in the User Manual.