Guidelines for Thawing the Sequel® Sequencing Kit 2.0

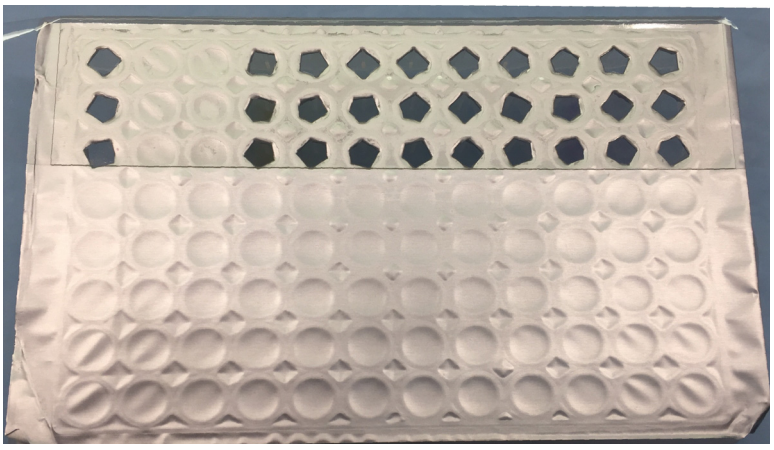
User Bulletin

This Bulletin describes PacBio's best practices for thawing the Sequel Sequencing Kit 2.0. It is highly recommend to follow this procedure to completely dissolve any solids that may be present in the wells of the deep well plate.

Prepare Reagents

Prepare the following reagents accordingly:

1. Remove the OS Enzyme from the sequencing package and place in ice until ready to use. When ready to use, spin down the tube for 5 seconds and replace the cap with a tube septum.
2. Spin down the SMRT Cell Oil tube for 5 seconds and replace the cap with a tube septum.
3. When using the sequencing plate for the first time:
   - Remove the plate from the mylar bag
   - Place the plate in a room temperature (~22 ºC) water bath covered with foil to protect the plate from light for 60 minutes.
   - Remove the plate from the water bath and invert, tap, and observe the plate to look for any remaining frozen materials. Repeat this 5 times to ensure sufficient observation. If wells remain frozen, return to the water bath for additional time.
   - Once thawed, mix by vortexing at 1000 rpm for 1 minute.
   - Spin down the plate for 1 minute at 150 rcf to ensure that the reagents are in the bottom of the deep-well plate.
   - Before loading on the instrument, wipe any moisture or contaminants from the top of the plate.
   - After use, if a part of the plate is unused, ensure that the plate is kept at 4ºC and protected from light. Any accompanying unused OS enzyme should also be stored at 4ºC.
4. When using the sequencing plate for a second time:
   - Keep the plate at 4ºC and protected from light.
   - Cover the used wells (we recommend a removable glue plate seal - see below). Do not cover the unused wells as this may prevent piercing in the subsequent use.



   - Mix by vortexing at 1000 rpm for 1 minute.
   - Spin down the plate for 1 minute at 150 rcf to ensure that the reagents are in the bottom of the deep-well plate.
   - Remove the glue plate seal. It is important that the unused wells are not occluded by any foil seal other than that with which it is shipped.
   - Before loading on the instrument, wipe any moisture or contaminants from the top of the plate.

Prepare the Sample Plate

1. Prepare samples for loading following the procedure outlined in the Binding Calculator or SMRT Link Sample Setup.
2. Transfer samples to a 96-well plate (Eppendorf) and seal the plate with a heat seal foil for 2.5 seconds at 172ºC.
3. Spin down the sample plate for 1 minute at 150 rcf.
4. Leave on ice until ready to load on the instrument.

Note: Only the Sequel Sample Plate heat seal foil should be used on the sample plate. Other seals can cause run failures. Also, because the heat sealing necessarily compromises some of the structure of the sample plate, reusing sample plates is not recommended.

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