Guidelines for Thawing the Sequel® Sequencing Kit 2.1

User Bulletin

This Bulletin describes PacBio's best practices for thawing the Sequel Sequencing Kit 2.1. It is highly recommended to follow this procedure to completely dissolve any solids which may be present in the wells of the deep well plate.

The 8-reaction Sequel Sequencing Kit 2.1 includes two tubes of OS Enzyme to ensure compatibility with the longer movie times (20 hours) enabled by the Sequel SMRT® Cell LR. When running 20 hour movies, only 4 reactions may be run at a time. One tube of OS Enzyme should be used for the first 4 rows of the sequencing plate and the second tube should be used for the last 4. When using standard Sequel SMRT Cells (with movies ≤10 hours), a single tube of OS Enzyme may be used for a complete, continuous 8-SMRT Cell run.

Prepare Reagents

Prepare the following reagents accordingly:

1. Remove the OS Enzyme from the sequencing package and place on ice until ready to use. When ready to use, spin down the tube for 5 seconds and replace the cap with a tube septum. Retain the cap if you plan to reuse the OS Enzyme in a subsequent run.
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 5 times to ensure sufficient observation and mixing. If necessary, return to the water bath for additional time.
   - Once thawed, mix on a shaker plate at 1000 rpm for 1 minute.
   - Spin down the plate for 1 minute at 150 rcf to ensure that the reagents are at 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, cover the used wells (we recommend a removable glue plate seal cut to cover only the used wells - see below). **Do not cover the unused wells as this may prevent piercing.**
   - Store at 4ºC and **protect from light**.
   - Unused OS Enzyme should be stored at 4°C. Note that there is potential for evaporation through the septa of the OS Enzyme tube. Therefore, it should be stored with the original cap in place. Discard the tube if it has been used for ≥4 runs or stored with the septa for 80 hours or more. Use the second tube of OS Enzyme for subsequent runs on the remainder of the plate.
4. When using the sequencing plate for a second time:
   - Repeat steps 1, 2, and 3. If necessary, use a new tube of OS Enzyme.
   - It is important that the unused wells are not occluded by **any seal** other than that with which it was shipped. Remove the glue plate seal before placing the plate on the instrument if any portion of it occludes the unused wells.

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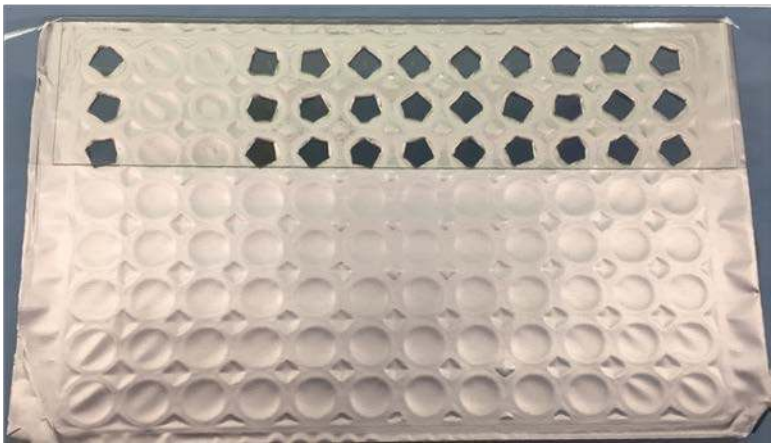

Prepare the Sample Plate

1. Prepare samples for loading following the procedure outlined in SMRT Link Sample Setup.
2. Transfer samples to a 96-well plate 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.

Revision History (Description)	Version	Date
Initial release for Sequencing Kit 2.1	01	February 2018