Single Fly Squish Prep and gDNA Purification -- Full Plate Version

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* This protocol is adapted from previous versions, but some of the language has been modified for the sake of clarity. Read through the entire protocol before starting! *

List of Reagents Needed:

1. 6 mL of Lysis Buffer (50 mM Tris-HCl (pH 8), 100 mM EDTA, 100 mM NaCl, 0.5% SDS)
2. 110 µl of Proteinase K (500 mAU/mL concentration)
3. 9 mL of SPRI Beads
4. 18 mL of 70--80% EtOH
5. 3 mL UltraPure H_20
6. dsDNA Qubit Reagents

Protocol:

Squish Prep (duration ~ 1.5 hours + overnight incubation)

1. Prepare a 100X Master Mix of Squish Buffer in a 15 mL falcon tube:

Reagent	Volume	110X Volume
Lysis Buffer	50 µl (110)	5500 µl
Proteinase K	1 µl (110)	110 µl

2. Pour the master mix into a 50 mL reservoir. It does not need to be on ice.
3. Spin down your plate of flies, making sure that each well contains only one fly and it is at the bottom of the well. Plate the plate on ice, or on a 96-well cold block.
    a. DNAses in the flies will degrade their DNA if they aren't kept on ice.
4. Use a multichannel pipette to dispense 51 µl of squish buffer into each well of a row of flies.
5. Use the multichannel pipette to *gently smash* each fly. Don't smash up and down or you'll ruin your multichannel pipette. Instead, rock it back and forth in all directions. Eject the pipette tips in the wells. Remove the plate from the ice/cold block and visually check to see if you crushed each fly. If the fly is still completely intact, use the pipette tip in that well to crush the fly. Once all flies in that row are crushed, use the multichannel to load the tips back on, and eject all of the squish buffer that may have come up into the tip. Discard the pipette tips and move on to the next row.
    a. To adequately lyse the fly, you only need one direct body hit. The fly doesn't need to be completely macerated for this to work, but make sure you have broken the body to some visibly obvious extent.

6. Once you have crushed all of the flies, seal the plate with either strip caps or a foil seal. Make sure every well is completely sealed, or else the buffer will evaporate over time. Vortex the plate aggressively and spin down in a plate spinner.
7. Place the plate on a plate shaker, and let the lysis run overnight with constant gentle shaking, at room temperature.
8. The next morning, take out your SPRI beads to give them time to reach room temperature. Cold beads dramatically reduce their ability to bind the DNA.
9. Spin the contents of the plate down, and place in a thermal cycler with the following program with the lid set to 105ºC:
    a. 55ºC for 5 minutes
    b. 95ºC for 10 minutes (to deactivate the prot K)
    c. 25ºC hold ∞
10. Wait for the lid to cool slightly. Take your plate out of the thermal cycler and spin down again. Make sure all of the contents are at the bottom of the wells!

You must cleanup your gDNA following the lysis. The high SDS concentration in the lysis buffer will inhibit tagmentation, or any downstream enzymatic processes. DO NOT move into tagmentation until your gDNA is purified!!!

At this point, you can freeze the crude lysate at -20ºC, however you will notice a decreased yield in gDNA after thawing out the plate and performing bead purification. It is strongly recommended that you move directly into the cleanup step following the lysis.

SPRI Bead gDNA Purification from Crude Fly Lysate (duration ~2 hours)

1. Make sure your beads have reached room temperature by this point.
2. Using a multichannel pipette, transfer as much of the crude fly lysate over to a new plate as possible, while avoiding transferring large chunks of fly.
    a. Usually at this point I can get about 40--45 µl of lysate transferred over while avoiding fly bodies.
3. Pour SPRI Beads into a reservoir. Add SPRI Beads in a 1.8:1 bead:lysate ratio, and pipette up and down ~30 times to mix the beads to the lysate completely.
4. Lets the beads bind the gDNA at room temperature for 3--5 minutes.
5. Plate the plate on a plate magnet, and allow the beads to collect on the magnet for about 5 minutes.
6. Aspirate off all of the supernatant and discard.
7. Pour the 70--80% EtOH solution in a reservoir. Wash the beads with 90 µl of EtOH. You can add the EtOH to all of the wells all at once, or go one row at a time. Either way, you'll want the EtOH to sit on top of the beads for about 30 seconds, and not much longer.
8. Aspirate off all of the EtOH and discard.
9. Repeat steps 7 and 8 for a second EtOH wash.
10. After the second wash, reseal the plate and spin it down, bringing any residual EtOH to the bottom of the wells.

11. Aspirate off all of the remaining EtOH and discard. You really don't want any EtOH in the purified gDNA moving forward.
12. Briefly allow the beads to dry, but not to the point of them completely cracking.
13. Take the plate off of the magnet. Pour UltraPure H_2O into a reservoir. Resuspend the bead pellets with 30 µl of UltraPure H_2O by dispensing the water on the side of the well that has the pellet, aspirating back up, and repeating until the beads are fully resuspended.
14. Reseal the plate, vortex it briefly, and spin down *very* briefly. You don't want the beads to fall out of solution, so you can't spin down to hard.
15. Incubate the plate at room temperature for about 5 minutes.
16. Place the plate back on the magnet and allow the beads to re-pellet for a few minutes. Once the eluate is clear, qubit at least four wells at "random." Chose one corner (ie. A1, H12), one edge (ie. A6, D1, H6), and at least two random wells from any row/column that isn't on the edge.
    a. It's also a good idea to qubit any wells you may suspect of being low in concentration.
    b. From this, you should yield anywhere from 3 ng/µl -- 30 ng/µl in the 30 µl eluate.
17. Once you have confirmed your successful purification, while keeping the plate on a magnet, transfer as much of the eluate as possible to a new plate, while avoiding the beads.
    a. I usually am able to transfer about 25 µl of eluate out of the 30 µl you elute with to a new plate while avoiding beads. It's easiest to do this with a few transfers with a p10 multichannel pipette.
    b. DO NOT FREEZE THE BEADS. They will break open and completely contaminate your samples.
18. You are now able to freeze your purified gDNA for later use in library prep. Make sure you are not freezing any beads with the eluate.