

5-25-16 DNA Isolation

2ad

PTU

S

down
30 sec.,

- DNA isolation for Winthrop 2016 steelhead adults RBCs [with Jon]
 - ✓ Add 100 μ l PBS [From Ambion 10x stock diluted to 1x with nuclease-free water] + 2.5 μ l RBCs to 96-well plates
 - ✓ Make a bulk mix of proteinase K + PBS [100 μ l PBS + 20 μ l prot. K per sample], for two plates: 19.2 ml PBS + 3.84 ml prot. K add 120 μ l to appropriate wells in blue blocks
 - Pipette up and down to mix PBS and RBCs in 96-well plates, transfer all to blue blocks [DO NOT PIPETTE INTO LIQUID ALREADY IN WELLS, SIMPLY SHOOT LIQUID ONTO SIDE OF WELL WALL]
 - Add 200 μ l Buffer AL to each well [MAKE SURE BUFFER AL HAS NOT HAD ETHANOL ADDED]
 - Seal wells with clear caps, put paper towels over caps, ✓ close blocks with lid, hold tightly and shake vigorously for 15 sec.
 - ✓ Centrifuge briefly at 3000 rpm
 - ✓ Incubate blocks on shaker at 56°C for 10 min., centrifuge briefly at 3000 rpm
 - ✓ Carefully remove caps, add 200 μ l 100% EtOH to each sample
 - Seal wells with clear caps (new ones), put paper towels over caps, ✓ close blocks with lid, hold tightly and shake vigorously for 15 sec.
 - ✓ Centrifuge briefly at 3000 rpm
 - Carefully remove caps from one column at a time, remove 625 μ l using Matrix multichannel, dispense 625 μ l into appropriate column [at fastest speed] of DNeasy 96 plate on top of S-block, seal plates with AirPore tape

- Centrifuge 4 min. @ 6000 rpm [turn refrigeration on centrifuge to room temp. so that it doesn't get too warm]
- Transfer DNeasy 96 plate to new S-block, discard liquid in S-blocks and rinse out
- Add 500 μ l Buffer AW1 to each well [make sure EtOH has been added to AW1]
- Seal DNeasy 96 plate with new AirPore Tape, centrifuge 2 min. @ 6000 rpm
- Add 500 μ l Buffer AW2 to each well [make sure EtOH has been added to AW2]
- Centrifuge 15 min. @ 6000 rpm [do not seal], bump temp. in centrifuge to 35°C to evaporate residual EtOH
- Place DNeasy 96 plate in Elution rack, add 200 μ l Buffer AE @ 56°C, seal with AirPore Tape, incubate 1 min. @ room temp.
- Centrifuge 4 min. @ 6000 rpm
- Transfer all eluted DNA to blocks from the DNA lab for heat seal and storage.