OPTIMIZED Genomic DNA purification protocol using Promega kit

(suitable for cell culture plates and PBMC)

For full CELL CULTURE PLATE:

BEFORE START:

1. Add 1 ml of Lysis Buffer to the entire plate
2. Leave the Lysis Buffer for at least 5 minutes and then detach the cell lysate with the help of a scraper
3. Transfer the lysate into a clean 2ml tube and store it at -20° C

For PBMCs:

BEFORE START:

1. Centrifuge the tube containing the PBMC at full speed (14000 rpm or more) for 5 minutes to pellet
2. Remove or discard the supernatant
3. Add 1 ml or appropriate volume of PBS and resuspend the pellet to wash properly
4. Centrifuge the tube containing the PBMC at full speed (14000 rpm or more) for 5 minutes to pellet
5. Remove or discard the supernatant
6. Repeat twice steps 3 to 5 to wash properly
7. Add 1 ml of Lysis Buffer
8. Leave the Lysis Buffer for at least 5 minutes and then vortex the tube
9. Keep the lysate at -20°C until extraction

GENOMIC DNA PURIFICATION

BEFORE START:

1. Switch on the heating block to 60°C
2. Prepare Nuclease Free Water with appropriate volume of RNAse A (8 ul every 1 ml of H2O) for the final elutions steps
3. Decide the amount of lysate to process according to its concentration (If it's too dense, add more Lysis Buffer), usually 350 ul of Lysate are enough
4. Transfer a proper amount of lysate in a new/clean tube and add Lysis Buffer until reaching appropriate volume (up to 700 ul)

THEN:

1. Transfer each sample lysate from the tube to a separate Wizard® SV Minicolumn Assembly and spin the Assembly at 13,000 × g for 3 minutes
2. **CAREFULLY CHECK THAT ALL THE LYSATE HAS PASSED THROUGH THE MEMBRANE DOWN TO THE COLUMN** (if not, add more Lysis Buffer to the assembly and spin again)
3. Remove minicolumn from the Assembly and discard the liquid in the Collection Tube. Replace the minicolumn into the Collection Tube
4. Add 650µl of Column Wash Solution (CWA; with 95% ethanol added) to each assembly Centrifuge at 13,000 × g for 1 minute. Discard the liquid from the Collection Tube. Repeat this step for a total of 4 washes
5. Discard the liquid from the Collection Tube and reassemble the minicolumn assembly Centrifuge for 2 minutes at 13,000 × g to dry the binding matrix
6. Transfer the Wizard® SV Minicolumn to a new 1.5ml tube (**Cut the Caps**!!!)
7. **FIRST ELUTION STEP**: add 125µl of 60°C Nuclease-Free Water with RNAse A solution. Incubate for 2 minutes at room temperature
8. Centrifuge the minicolumn/elution tube assembly at 13,000 × g for 1 minute.
9. **DO NOT DISCARD THE LIQUID!!!**
10. **SECOND ELUTION STEP:** repeat steps 7 and 8 once more (total elution volume will be 250 ul)
11. Remove the minicolumn and store the purified DNA at –20 to –70°C
12. Assess the obtained DNA concentration (Nanodrop or equivalent)