Genomic DNA Extraction from Cultured Cells on a 96-well Plate

1. [A: Extraction without detaching cells from a plate] When the cells are ready (>70% confluent), wash the plates twice with PBS. Using the multichannel pipettor, add 50 ul of Lysis Buffer [10 mM Tris pH7.5, 10 mM EDTA pH8.0, 10 mM NaCl, 0.5% N-Lauroylsarcosine, and 1 mg/ml Proteinase K (added fresh)] per well.
   [B: Extraction with detaching cells from a plate] When the cells are ready (>70% confluent), wash the cells with PBS and detach the cells with Trypsin/EDTA, Accutase, or other enzymes. Using the multichannel pipettor, aliquot 50 ul of Lysis Buffer per well on a 96-well plate. Using the multichannel pipettor, add up to 15 ul of the cell suspension in the enzyme solution to Lysis Buffer and mix.
2. Incubate the plates O/N @55-60˚C in a plastic container with water.
3. Add ice-cold 100 ul of 75 mM NaCl in EtOH solution (add 150 ul of 5 M NaCl per 10 ml EtOH, kept @-80˚C) and mix well.
4. Allow the plate to rest on the bench @ room temperature for 2 hours. The DNA adheres to the plastic bottom of the well, so check the DNA is precipitated using a microscope.
5. Invert the plate to discard the solution (the DNA will remain adhered to the plate). Using the multichannel pipettor, add 100 ul 70% EtOH to wash well. Invert the plate to discard the 70% EtOH and repeat the wash 2 times. After the last wash, the plate was inverted and slammed down onto a sheet of paper to remove the residual ethanol.
6. Put a sheet of paper on the plate to avoid dust, and allow the plate to air-dry for 30-45 minutes.
7. Add 30 ul TE per well to dissolve the DNA and spec the concentration of the DNA in a couple of wells to check successful DNA extraction.