Methods for DNA and RNA Isolation from Stool

Total Nucleic Acid was extracted via the Chemagic MSM I with the Chemagic DNA Blood Kit-96 from Perkin Elmer. This kit combines a chemical and mechanical lysis with magnetic bead-based purification.

Prior to extraction on the MSM-I, TE buffer, Lysozyme, Proteinase K, and RLT Buffer with beta-mercaptoethanol were added to each stool sample. The stool lysate solution was vortexed to mix. Samples were then placed on the MSM I unit. The following steps were automated on the MSM I.

M-PVA Magnetic Beads were added to the stool lysate solution and vortexed to mix. The bead-bound Total Nucleic Acid was then removed from solution via a 96-rod magnetic head and washed in three Ethanol-based wash buffers. The beads were then washed in a final water wash buffer. Finally, the beads were dipped in elution buffer to re-suspend the DNA sample in solution. The beads were then removed from solution, leaving purified TNA eluate.

The eluate was then split into two equal volumes, one meant for DNA the other for RNA. SUPERase-IN solution was added to the DNA samples, the reaction was cleaned up using AMPure XP SPRI beads. DNase was added to the RNA samples, and the reaction was cleaned up using AMPure XP SPRI beads.

DNA samples were quantified using a fluorescence-based PicoGreen assay.

RNA samples were quantified using a fluorescence-based RiboGreen assay. RNA quality was assessed via smear analysis on the Caliper LabChip GX.