

Materials and Methods:

DNA Extraction

KingFisher:

The extraction of DNA from whole blood samples is performed with the Mag-Bind Blood & Tissue Kit (Omega Bio-Tek, Norcross, GA) on the KingFisher Flex Purification System (ThermoFisher Scientific, Waltham, MA) using the '*SOP1113_Blood*' protocol in 96-well format, developed internally and described below. AL Buffer (232 μ L) and Proteinase K Solution (16 μ L) are added to 200 μ L of input material then mixed for 10 min at 70°C for cell lysis. HDQ binding buffer (320 μ L) and RNase A/Magnetic Bead mixture (18 μ L) are then added to lysate and mixed for 10 min to remove RNA and bind the DNA to beads. The bound DNA is then collected and washed in VHB Buffer (500 μ L) for 3 min, VHB Buffer (500 μ L) a second time for 1 min, and SPM Buffer (500 μ L) for 1 min. The beads are dried for 5 min before eluting purified DNA in 150 μ L Elution Buffer for 5 min at 50°C.

QIASymphony:

The extraction of DNA from whole blood samples is performed with the QIASymphony DSP DNA Midi Kit (Qiagen, Germantown, MD) on the QIASymphony SP (Qiagen, Germantown, MD) using the protocol '*Blood_400_V7_DSP*' for 450 μ L input, yielding a final DNA eluant volume of 200 μ L.

DNA-Staging

DNA analytes are transferred into 0.5 mL FluidX tubes (Brooks Life Sciences, Chelmsford, MA), then briefly centrifuged to collect contents in order to estimate sample volume using non-contact VolumeCheck100 instrument (BioMicroLab, Concord, CA). Fluorescence-based solution assay for dsDNA quantitation is performed using the Quant-iT PicoGreen dsDNA assay kit (Life Technologies, Grand Island, NY) in which 4 μ L of sample is added to 196 μ L of 1:200 dilution of PicoGreen in 1X TE in a black-bottom fluotrac plate (Greiner Bio-One, Monroe, NC). Following a 5 min incubation for intercalation, results are measured using an InfiniTE spectrofluorometer (Tecan, Morrisville, NC) microplate reader (excitation: 485 nm, emission: 535 nm). The resulting fluorescence values (RFU) are used to interpolate the concentration from a standard curve, generated with Lambda DNA (ThermoFisher Scientific, Waltham, MA) with 10 known concentration points (0, 1.48, 2.966, 5.93, 11.865, 23.73, 31.64, 42.1875, 50.25, 75.0 ng/ μ L), run in triplicate, using the same 1:200 dilution of PicoGreen reagent in 1X TE. If results must be extrapolated outside the range of the standard curve, the sample is diluted with 1X TE and repeated on the assay.