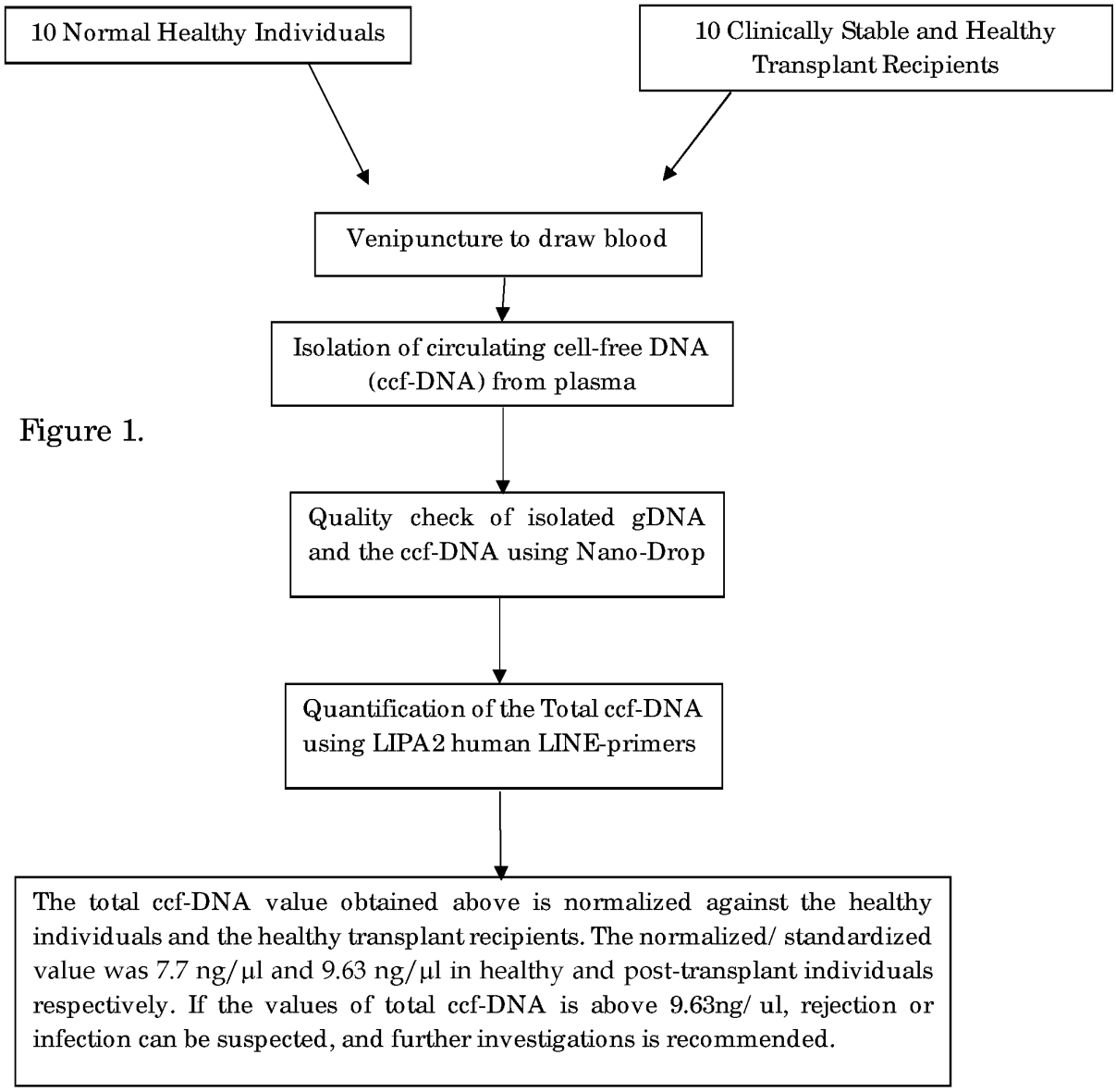
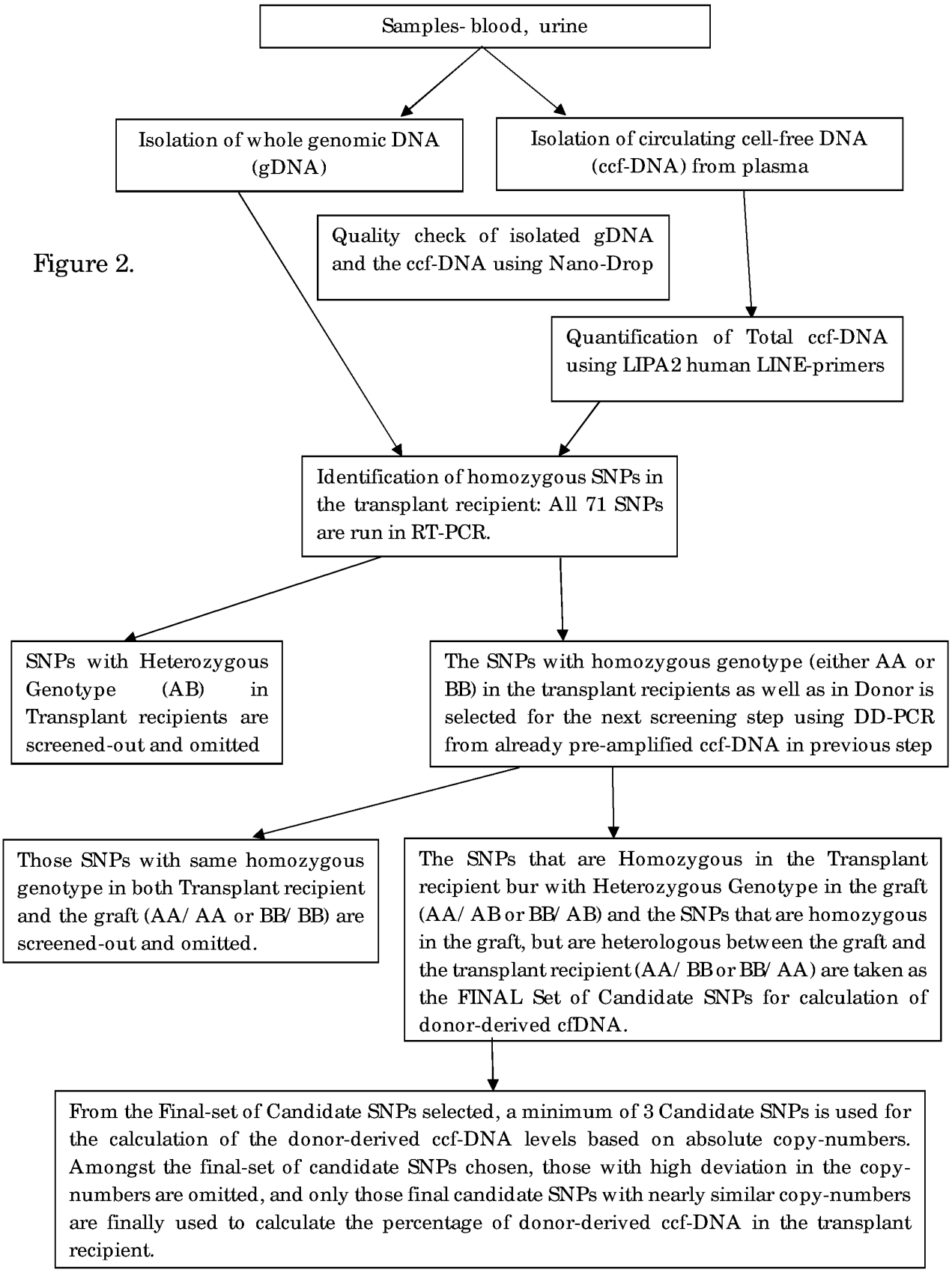

10 Normal Healthy Individuals
10 Clinically Stable and Healthy Transplant Recipients
Venipuncture to draw blood
Isolation of circulating cell-free DNA (ccf-DNA) from plasma
Quality check of isolated gDNA and the ccf-DNA using Nano-Drop
Quantification of the Total ccf-DNA using LIPA2 human LINE-primers
The total ccf-DNA value obtained above is normalized against the healthy individuals and the healthy transplant recipients. The normalized/ standardized value was 7.7 ng/µl and 9.63 ng/µl in healthy and post-transplant individuals respectively. If the values of total ccf-DNA is above 9.63ng/ ul, rejection or infection can be suspected, and further investigations is recommended.

Figure 1.

Figure 2.


Samples- blood, urine
Isolation of whole genomic DNA (gDNA)
Isolation of circulating cell-free DNA (ccf-DNA) from plasma
Quality check of isolated gDNA and the ccf-DNA using Nano-Drop
Quantification of Total ccf-DNA using LIPA2 human LINE-primers
Identification of homozygous SNPs in the transplant recipient: All 71 SNPs are run in RT-PCR.
SNPs with Heterozygous Genotype (AB) in Transplant recipients are screened-out and omitted
The SNPs with homozygous genotype (either AA or BB) in the transplant recipients as well as in Donor is selected for the next screening step using DD-PCR from already pre-amplified ccf-DNA in previous step
Those SNPs with same homozygous genotype in both Transplant recipient and the graft (AA/ AA or BB/ BB) are screened-out and omitted.
The SNPs that are Homozygous in the Transplant recipient bur with Heterozygous Genotype in the graft (AA/ AB or BB/ AB) and the SNPs that are homozygous in the graft, but are heterologous between the graft and the transplant recipient (AA/ BB or BB/ AA) are taken as the FINAL Set of Candidate SNPs for calculation of donor-derived cfDNA.
From the Final-set of Candidate SNPs selected, a minimum of 3 Candidate SNPs is used for the calculation of the donor-derived ccf-DNA levels based on absolute copy-numbers. Amongst the final-set of candidate SNPs chosen, those with high deviation in the copy-numbers are omitted, and only those final candidate SNPs with nearly similar copy-numbers are finally used to calculate the percentage of donor-derived ccf-DNA in the transplant recipient.