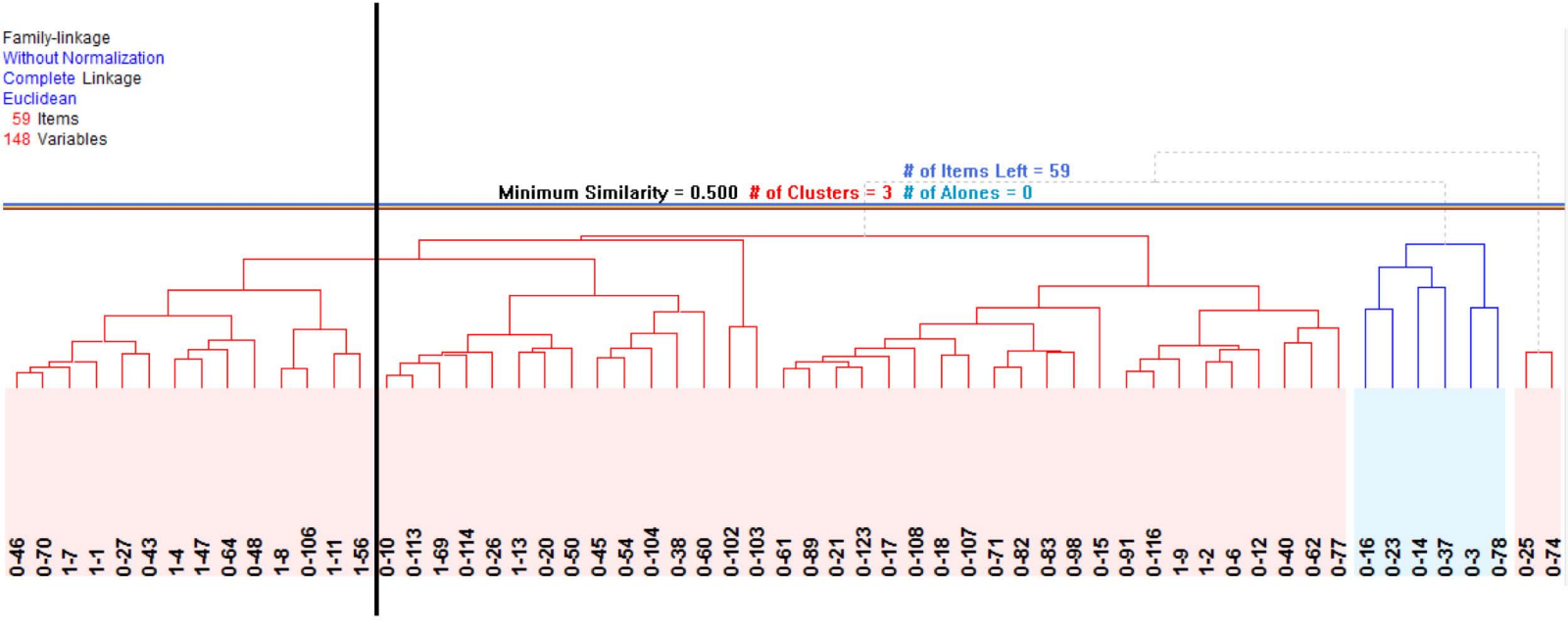
Supplementary Data file 1

Reaction conditions consisted of an initial 94 °C for 3 min followed by 32 cycles of 94°C for 45s, 50°C for 60s and 72°C for 90 s, and a final extension of 72 °C for 10 min. An agarose gel confirmed the presence of product (band at ~465 base pairs) in successfully amplified samples. The remainder of the PCR product (~45 µl) of each sample was mixed thoroughly with 25 µl Agencourt AMPure XP magnetic beads and was incubated at room temperature for 5 min. Beads were subsequently separated from the solution by placing the tubes in a magnetic bead separator for 2 min. After discarding the cleared solution, the beads were washed twice by resuspending the beads in 200 µl freshly prepared 80 % ethanol, incubating the tubes for 30 s in the magnetic bead separator and subsequently discarding the cleared solution. The pellet was subsequently dried for 15 min and resuspended in 52.5 µl 10 mM Tris HCl pH 8.5 buffer. Fifty microliters of the cleared-up solution is subsequently transferred to a new tube. The DNA concentration of each sample was determined using a Qubit® 2.0 fluorometer (www.invitrogen.com/qubit), and the remainder of the sample was stored at -20 °C until library normalization. Library normalization was done the day before running samples on the MiSeq by making 2 nM dilutions of each sample. Samples were pooled together by combining 5 µl of each diluted sample. Ten microliters of the sample pool and 10 µl 0.2 M NaOH were subsequently combined and incubated for 5 min to denature the sample DNA. To this, 980 µl of the HT1 buffer from the MiSeq 2x300 kit was subsequently added. A denatured diluted PhiX solution was made by combining 2 µl of a 10 nM PhiX library with 3 µl 10 mM Tris HCl pH 8.5 buffer with 0.1 % Tween 20. This 5 µl mixture was mixed with 5 µl 0.2 M NaOH and incubated for 5 min at room temperature. This 10 µl mixture was subsequently mixed with 990 µl HT1 buffer. One hundred and fifty microliters of the diluted sample pool was combined with 50 µl of the diluted PhiX solution and was further diluted by adding 800 µl HT1 buffer. Six hundred microliters of the prepared library was loaded into the sample loading reservoir of the MiSeq 2x300 cartridge.


Family-linkage
Without Normalization
Complete Linkage
Euclidean
59 Items
148 Variables
# of Items Left = 59
Minimum Similarity = 0.500 # of Clusters = 3 # of Alones = 0
0-46 0-70 1-7 1-1 0-27 0-43 1-4 1-47 0-64 0-48 1-8 0-106 1-11 1-56 0-10 0-113 1-69 0-114 0-26 1-13 0-20 0-50 0-45 0-54 0-104 0-38 0-60 0-102 0-103 0-61 0-89 0-21 0-123 0-17 0-108 0-18 0-107 0-71 0-82 0-83 0-98 0-15 0-91 0-116 1-9 1-2 0-6 0-12 0-40 0-62 0-77 0-16 0-23 0-14 0-37 0-3 0-78 0-25 0-74