



## Supplementary Materials

**Table S1.** EST-SSR, genomic (G-) SSR and EST-PCR primer pairs: their sequences and annealing temperatures.

Primer pair <sup>1</sup>	Forward and Reverse primer sequence (5'-3')	Annealing temperature (°C)
EST-SSR		
CA23	F: GAGAGGGTTTCGAGGAGGAG R: GTTAGAAACGGGACTGTGAGACG	60
CA112	F: TCCACCCACTTCACAGTTCA R: GTTATTGGGAGGGAATTGGAAAC	49
CA169	F: TAGTGGAGGGTTTGCTTGG R: GTTATCGAACGGAAGGTCAAAGA	52
CA236	F: GTTAAGCTTTAGATGAGTTGATGG R: GTTAACCAGTCCCAGACCCAAAT	54
CA421	F: TCAAATTCAAAGCTAAAATCAA R: GTTAAGGATGATCCGAAGCTCT	58
CA483	F: GTCTCCTCAGGTTCGGTTG R: GAACGGCTCCGAAGACAG	58
CA787	F: TCCTCGTTCTCTCCCTCTCA R: GTTCGCTGAAGTTGGAGTCCTT	60
NA800	F: CAATCCATTCCAAGCATGTG R: GTTCCCTAGACCAAGTGCCACTTA	62
NA961	F: TCAGACATGATTGGGGAGGT R: GTTGGATAATAGAGGGCGGTGGA	56
NA1040	F: GCAACTCCCAGACTTCTCC R: GTTAGTCAGCAGGGTGCACAA	58
G-SSR		
VCC_B3	F: CCTTCGATCTGTTCCCTTGC R: GTTGATGCAATTGAGGTGGAGA	62
VCC_I2	F: AGCGTTTTGAGGCTAACAA R: TAAAAGTCGGCTCGTTGC	62
VCC_I8	F: TTCAGCATTCAATCCATCCA R: GTTCTCTCTCCAATCTCTTCCA	58
VCC_J1	F: CTCATGGGTTCCCATAAGACAA R: TGCAAGTGAGGCAAAAGATG	60
VCC_J3	F: TGATTACATTGCCAGGGTCA R: TGGAAACAACCGGGTTACAT	58
VCC_J9	F: GCGAAGAACTTCCGTAAAA R: GTGAGGGCACAAAGCTCT	62
VCC_K4	F: CCTCCACCCACTTCATTA	62

VCC_S10	R: GCACACAGGTCCAGTTTG F: ATTTGGTGTGAAACCCCTGA R: GTTGCAGCTATATCCGTGTTGT	60
EST-PCR		
CA21	F: TCCGATAACCCTTACCAAGC R: TATACAGCGACACGCCAAAA	52
CA54	F: CCGGTGAACCTCCACTTGT R: AGATACTACTGGGGTGGGG	52
CA227	F: TGGAGACTGGAGTGATGCAA R: TTTGCAAGAACCATGCTGAG	56
CA287	F: AGGGCTTCCCTCAATCACT R: CCTTGTGTTCCCTCCCTTCG	58
CA791	F: AGAGCCAAAAGAAGGGGAAG R: TCAAAATTTCCGGACCAG	56
CA1029	F: GAAGTTTCCGTTCTGCAA R: CTGCAGCTAGGACCGAAGAG	52
CA1423	F: TCATAGCCAATACACTCGAAC R: GCCCCCACCTTAGCAAACCTC	60
NA27	F: CGCTCGCTCCATTGTTTC R: TATGCATGAAGCTTGCCGTA	60

<sup>1</sup>Above markers were developed from EST libraries generated from floral buds of cold acclimated (designated as prefix CA) and non-acclimated (designated as prefix NA) highbush blueberry plants [20].

**Table S2.** Total antioxidant activity (TAA), phenolic (TPC) and flavonoid contents (TFC) of individual wild clones collected from four Canadian provinces: Newfoundland and Labrador (NL), Prince Edward Island (PE), Quebec (QC) and New Brunswick (NB), cultivars (CV): Fundy, Polaris, Patriot, Chippewa, St. Cloud, Northblue (NOB), hybrids: Cross1 and Cross2. GAE = gallic acid equivalents, CE = catechin equivalent, fl = fresh leaf.

Clones/Cultivars/Hybrids	TAA (mg GAE/g fl)	TPC (mg GAE /g of fl)	TFC (mg CE/g fl)
BC1	4.45 ± 0.07	0.18 ± 0.01	6.55 ± 0.39
BC2	4.58 ± 0.07	0.24 ± 0.01	9.99 ± 0.11
BC3	4.03 ± 0.09	0.20 ± 0.02	6.99 ± 0.12
BC4	2.75 ± 0.08	0.14 ± 0.01	3.93 ± 0.13
BC5	4.57 ± 0.05	0.20 ± 0.02	6.03 ± 0.02
BC6	5.82 ± 0.03	0.20 ± 0.01	7.07 ± 0.05
BC7	4.20 ± 0.07	0.11 ± 0.00	3.49 ± 0.03
BC8	3.53 ± 0.12	0.09 ± 0.01	2.00 ± 0.01
BC9	2.45 ± 0.07	0.11 ± 0.01	2.41 ± 0.07
BC10	3.04 ± 0.08	0.13 ± 0.00	4.18 ± 0.01
BC11	4.88 ± 0.05	0.15 ± 0.01	2.92 ± 0.13
BC12	1.14 ± 0.08	0.11 ± 0.01	1.56 ± 0.02
BC13	5.45 ± 0.09	0.14 ± 0.00	2.65 ± 0.12
BC14	2.77 ± 0.05	0.10 ± 0.01	1.37 ± 0.04

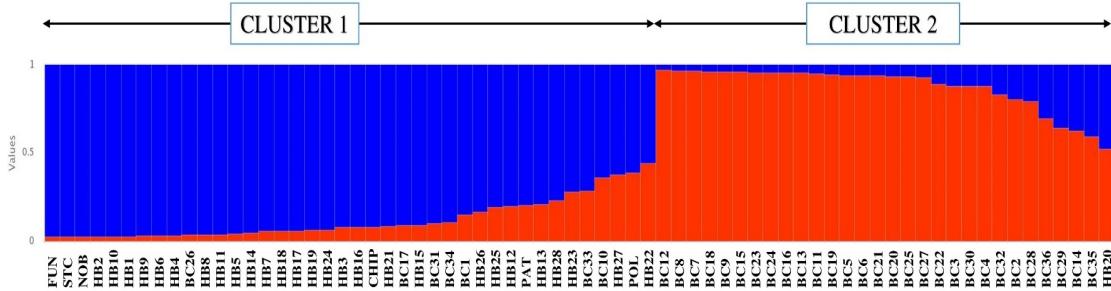
BC15	$1.79 \pm 0.10$	$0.10 \pm 0.01$	$1.22 \pm 0.02$
BC16	$2.85 \pm 0.07$	$0.11 \pm 0.00$	$1.66 \pm 0.09$
BC17	$4.14 \pm 0.08$	$0.10 \pm 0.01$	$1.89 \pm 0.12$
BC18	$4.40 \pm 0.05$	$0.15 \pm 0.00$	$3.77 \pm 0.09$
BC19	$2.86 \pm 0.03$	$0.10 \pm 0.00$	$2.35 \pm 0.17$
BC20	$3.14 \pm 0.08$	$0.09 \pm 0.00$	$1.96 \pm 0.11$
BC21	$2.56 \pm 0.07$	$0.06 \pm 0.01$	$0.81 \pm 0.02$
BC22	$5.82 \pm 0.03$	$0.18 \pm 0.01$	$5.56 \pm 0.04$
BC23	$3.25 \pm 0.08$	$0.07 \pm 0.01$	$1.35 \pm 0.02$
BC24	$4.71 \pm 0.07$	$0.10 \pm 0.01$	$2.15 \pm 0.08$
BC25	$4.62 \pm 0.03$	$0.09 \pm 0.01$	$2.26 \pm 0.02$
BC26	$3.26 \pm 0.07$	$0.09 \pm 0.00$	$1.11 \pm 0.01$
BC27	$1.41 \pm 0.05$	$0.08 \pm 0.00$	$0.89 \pm 0.07$
BC28	$1.14 \pm 0.05$	$0.08 \pm 0.00$	$0.93 \pm 0.03$
BC29	$1.84 \pm 0.05$	$0.08 \pm 0.00$	$1.19 \pm 0.02$
BC30	$3.85 \pm 0.05$	$0.09 \pm 0.00$	$1.43 \pm 0.04$
BC31	$4.82 \pm 0.07$	$0.11 \pm 0.01$	$1.82 \pm 0.04$
BC32	$4.85 \pm 0.05$	$0.11 \pm 0.01$	$1.92 \pm 0.04$
BC33	$4.71 \pm 0.07$	$0.11 \pm 0.00$	$1.59 \pm 0.01$
BC34	$5.13 \pm 0.07$	$0.10 \pm 0.00$	$2.70 \pm 0.05$
BC35	$4.60 \pm 0.05$	$0.10 \pm 0.01$	$1.85 \pm 0.03$
BC36	$4.49 \pm 0.03$	$0.15 \pm 0.01$	$1.45 \pm 0.02$
FUN	$5.12 \pm 0.07$	$0.15 \pm 0.01$	$2.83 \pm 0.02$
PAT	$4.55 \pm 0.05$	$0.13 \pm 0.01$	$1.76 \pm 0.04$
POL	$5.19 \pm 0.03$	$0.16 \pm 0.01$	$1.92 \pm 0.04$
CHIP	$4.73 \pm 0.07$	$0.12 \pm 0.02$	$2.69 \pm 0.02$
STC	$2.48 \pm 0.08$	$0.09 \pm 0.00$	$1.20 \pm 0.02$
NOB	$3.28 \pm 0.05$	$0.10 \pm 0.00$	$0.64 \pm 0.02$
HB1	$1.95 \pm 0.09$	$0.16 \pm 0.01$	$1.04 \pm 0.03$
HB2	$2.75 \pm 0.08$	$0.09 \pm 0.00$	$1.57 \pm 0.02$
HB3	$4.07 \pm 0.10$	$0.07 \pm 0.01$	$1.71 \pm 0.02$
HB4	$3.68 \pm 0.05$	$0.11 \pm 0.00$	$1.40 \pm 0.02$
HB5	$2.94 \pm 0.17$	$0.10 \pm 0.01$	$1.42 \pm 0.02$
HB6	$0.29 \pm 0.10$	$0.13 \pm 0.01$	$1.22 \pm 0.02$
HB7	$2.41 \pm 0.10$	$0.09 \pm 0.00$	$1.23 \pm 0.02$
HB8	$2.00 \pm 0.33$	$0.14 \pm 0.01$	$1.48 \pm 0.06$
HB9	$2.74 \pm 0.08$	$0.10 \pm 0.00$	$1.42 \pm 0.02$
HB10	$0.79 \pm 0.10$	$0.10 \pm 0.00$	$1.32 \pm 0.02$
HB11	$4.48 \pm 0.04$	$0.16 \pm 0.01$	$1.49 \pm 0.02$
HB12	$4.82 \pm 0.09$	$0.09 \pm 0.01$	$1.95 \pm 0.03$
HB13	$3.13 \pm 0.10$	$0.10 \pm 0.02$	$1.27 \pm 0.01$
HB14	$5.11 \pm 0.05$	$0.12 \pm 0.00$	$1.67 \pm 0.02$
HB15	$4.47 \pm 0.07$	$0.09 \pm 0.00$	$1.40 \pm 0.02$
HB16	$2.52 \pm 0.07$	$0.09 \pm 0.00$	$0.91 \pm 0.03$
HB17	$4.79 \pm 0.07$	$0.10 \pm 0.00$	$1.30 \pm 0.04$
HB18	$4.09 \pm 0.03$	$0.10 \pm 0.00$	$1.45 \pm 0.02$
HB19	$2.74 \pm 0.05$	$0.08 \pm 0.00$	$0.87 \pm 0.04$
HB20	$4.69 \pm 0.09$	$0.10 \pm 0.00$	$1.37 \pm 0.02$
HB21	$5.13 \pm 0.05$	$0.10 \pm 0.00$	$1.40 \pm 0.02$
HB22	$4.62 \pm 0.03$	$0.10 \pm 0.00$	$1.27 \pm 0.01$
HB23	$4.01 \pm 0.04$	$0.10 \pm 0.00$	$1.82 \pm 0.02$
HB24	$3.25 \pm 0.08$	$0.09 \pm 0.00$	$1.19 \pm 0.01$
HB25	$2.76 \pm 0.10$	$0.09 \pm 0.00$	$1.22 \pm 0.01$

HB26	$3.66 \pm 0.07$	$0.10 \pm 0.00$	$1.52 \pm 0.03$
HB27	$4.79 \pm 0.09$	$0.09 \pm 0.00$	$1.56 \pm 0.02$
HB28	$4.72 \pm 0.06$	$0.10 \pm 0.00$	$1.74 \pm 0.04$

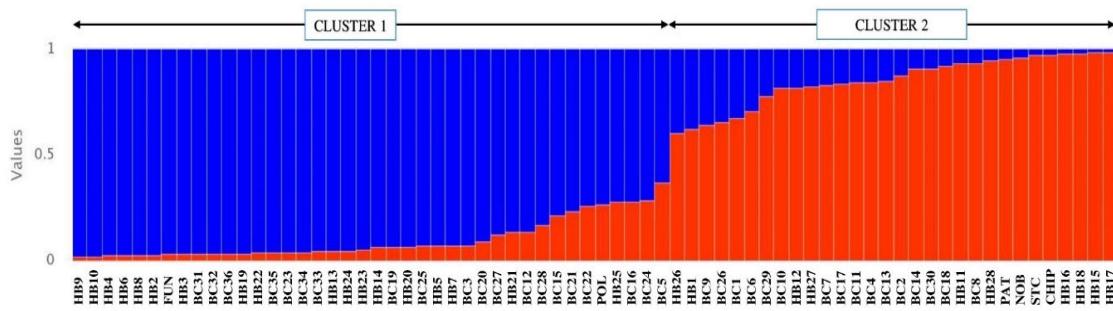
Values are means  $\pm$  SD values of at least three replicates. There was no significant difference among individuals in TAA, TFC and TPC according to the Kruskal-Wallis test.

**Table S3.** Pearson correlation coefficients of antioxidant properties. The respective p-values are shown in brackets. The significance level alpha is 0.05.

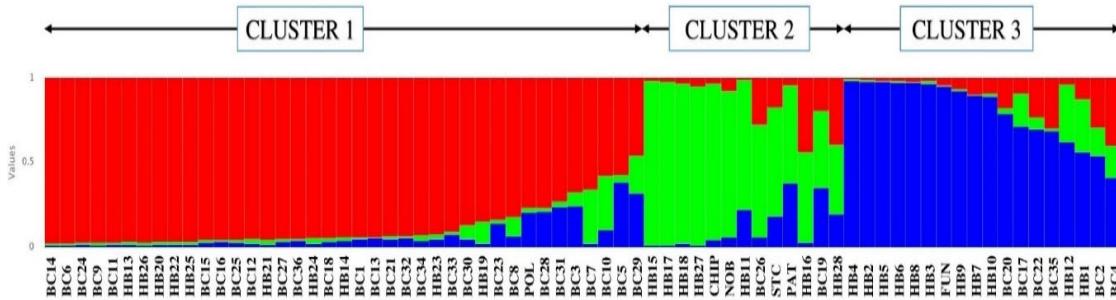
Variables	TAA (mg GAE/g fl)	TFC (mg CE/g fl)	TPC (mg GAE/g fl)
TAA (mg GAE/g fl)	1 (0)	0.387 (0.001)	0.352 (0.003)
TFC (mg CE/g fl)	0.387 (0.001)	1 (0)	0.826 (<0.0001)
TPC (mg GAE/g fl)	0.352 (0.003)	0.826 (<0.0001)	1 (0)



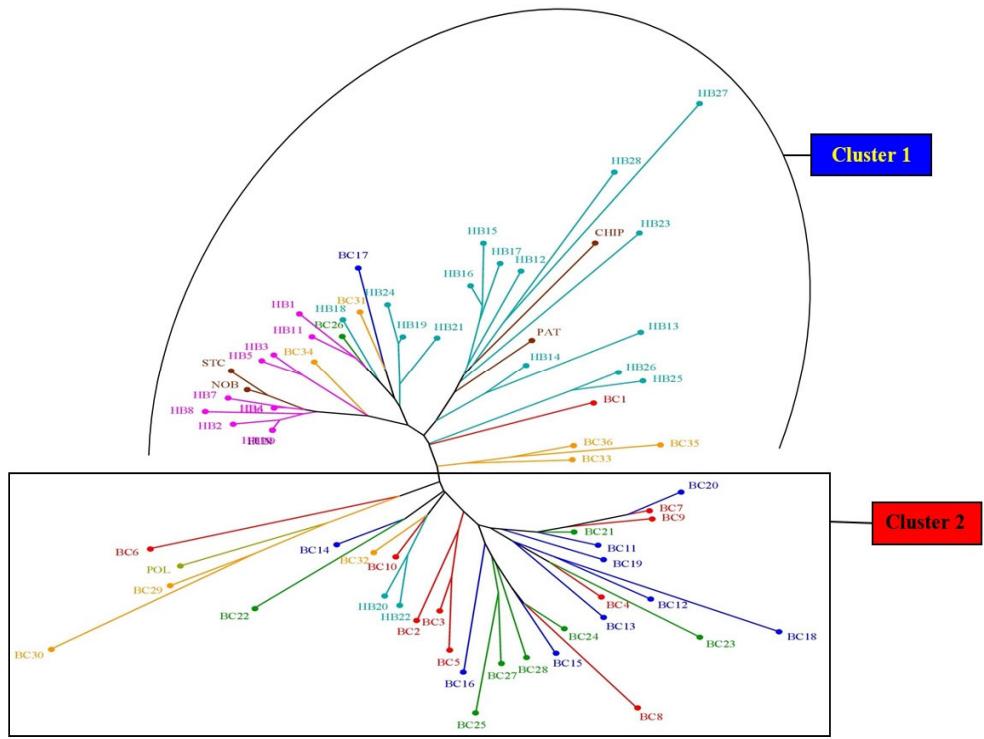
**Figure S1.** Distribution of blueberry genotypes as per STRUCTURE analysis ( $K = 2$ ) based on EST-SSR primer pairs. The genotypes are represented as vertical bars, and the colour represents different clusters (see Tables 1 and 2 for genotype label).



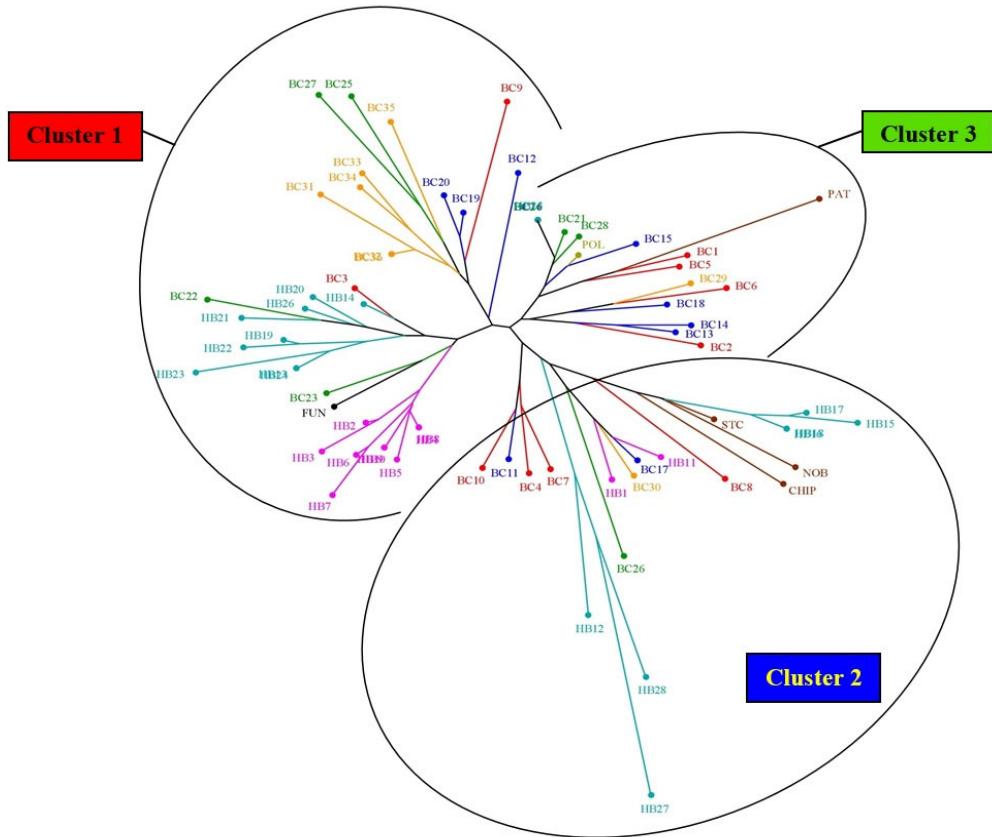
**Figure S2.** Distribution of blueberry genotypes as per STRUCTURE analysis ( $K = 2$ ) based on genomic (G-) SSR primer pairs. The genotypes are represented as vertical bars, and the colour represents different clusters (see Tables 1 and 2 for genotype label).



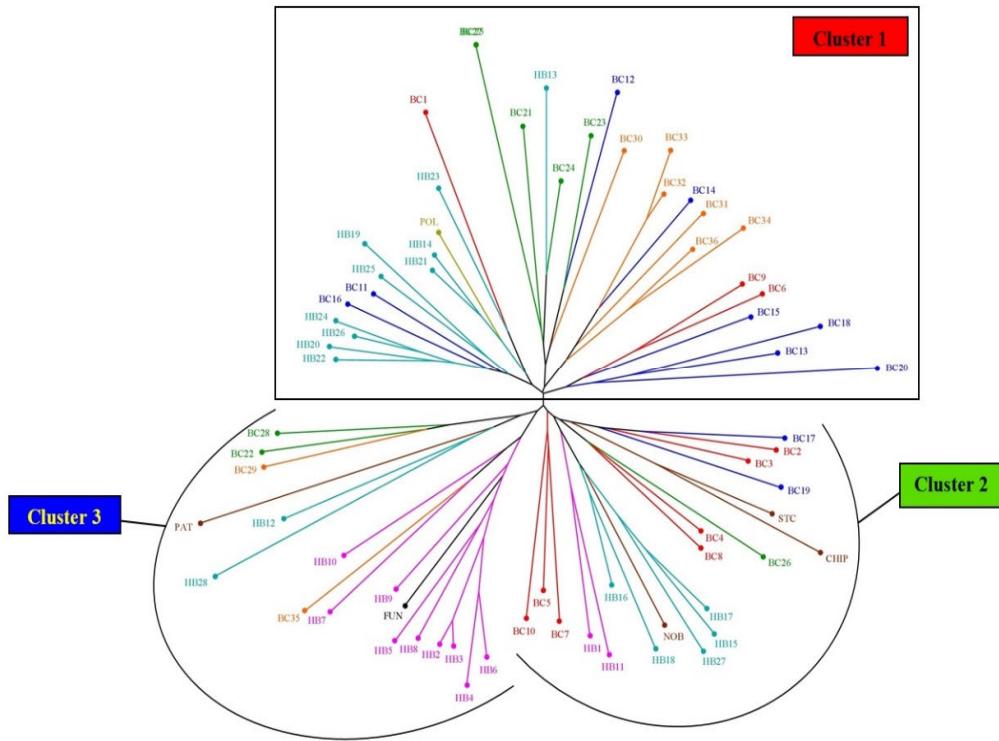
**Figure S3.** Distribution of blueberry genotypes as per STRUCTURE analysis ( $K = 3$ ) based on EST-PCR primer pairs. The genotypes are represented as vertical bars, and the colour represents different clusters (see Tables 1 and 2 for genotype label).



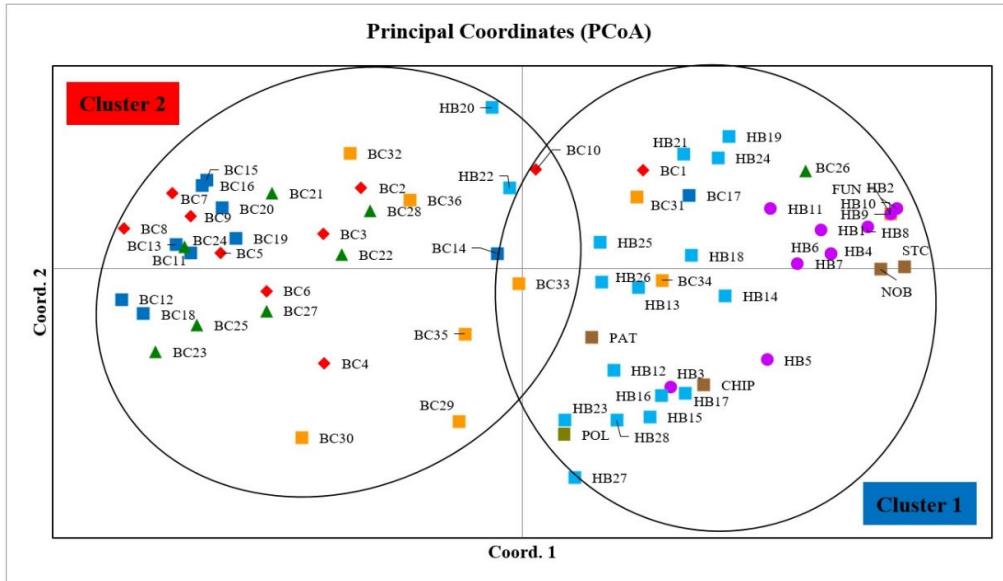
**Figure S4.** Dendrogram produced by using the unweighted neighbour-joining (NJ) method based on genetic dissimilarity produced by EST-SSR markers among blueberry genotypes. The colour of the branches indicates different groups (see Tables 1 and 2 for genotype label).



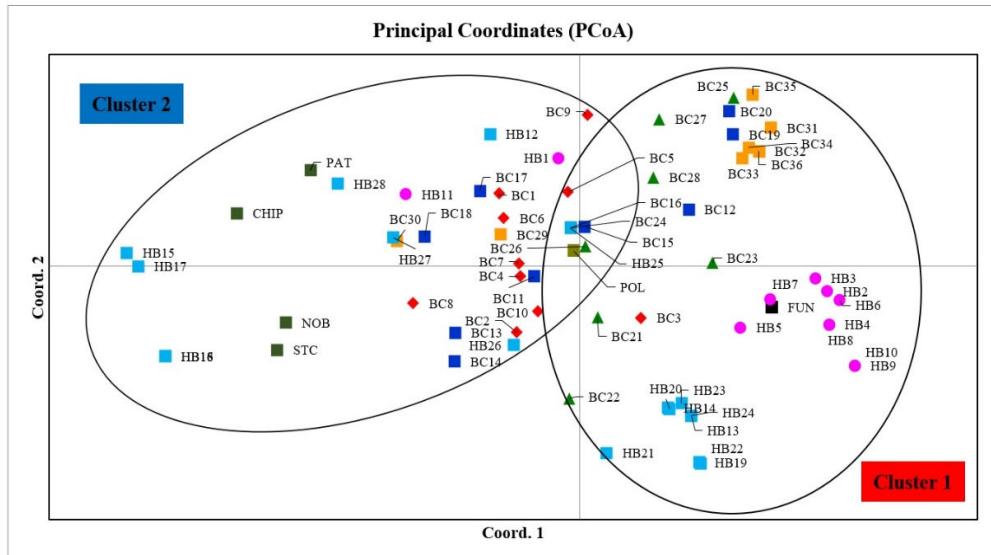
**Figure S5.** Dendrogram produced by using the unweighted neighbour-joining (NJ) method based on genetic dissimilarity produced by genomic (G-) SSR markers among blueberry genotypes. The colour of the branches indicates different groups (see Tables 1 and 2 for genotype label).



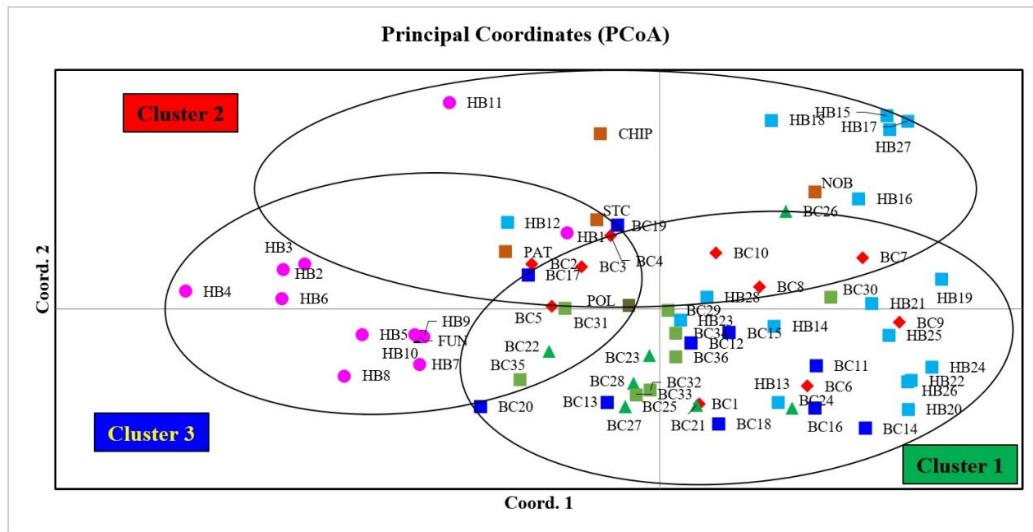
**Figure S6.** Dendrogram produced by using the unweighted neighbour-joining (NJ) method based on genetic dissimilarity produced by EST-PCR markers among blueberry genotypes. The colour of the branches indicates different groups (see Tables 1 and 2 for genotype label).



**Figure S7.** 2D principle coordinate analysis (PCoA) plot of blueberry genotypes using genetic distance matrix produced by EST-SSR markers. The colour and shape of the points indicate different groups (see Tables 1 and 2 for genotype label).



**Figure S8.** 2D principle coordinate analysis (PCoA) plot of blueberry genotypes using genetic distance matrix produced by genomic (G-) SSR markers. The colour and shape of the points indicate different groups (see Tables 1 and 2 for genotype label).



**Figure S9.** 2D principle coordinate analysis (PCoA) plot of blueberry genotypes using genetic distance matrix produced by EST-PCR markers. The colour and shape of the points indicate different groups (see Tables 1 and 2 for genotype label).