

Supplementary File

Title:

No evidence of association between mutant alleles of the CYP27B1 gene and multiple sclerosis

Authors:

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Materials and Methods

All subjects studied were white non-Hispanics with European ancestry (where known), all affected subjects satisfied standard clinical diagnostic criteria.^{1,2} Written informed consent was obtained from all participants and the study was approved by the respective ethics committee/Institutional Review Board at each center. Basic demographics for the affected individuals are shown in table S1.

Table S1. Case demographics

	Norway	UK	USA
Female / Male Ratio	2.64	2.84	2.98
Mean Age (range) / years	49 (17-82)	45 (17-90)	43 (14-82)
Mean Age At Onset (range) / years	33 (10-68)	31 (5-74)	31 (3-75)
Mean EDSS (range)	4.0 (0-10)	4.3 (0-9)	3.9 (2-7)
Mean MSSS (range)	4.5 (0.4-10.0)	5.2 (0.1-9.9)	5.3 (0.7-9.9)
%Primary Progressive Disease	13	11	5

SNP genotyping was completed using ABI custom TaqMan assays (primer sequences shown in Table S2 and genotyping success rates in Table S3). PCRs were carried out in 384-well plates using Applied Biosystems TaqMan Genotyping Master Mix under manufacturer's conditions on an ABI GeneAmp PCR System 9700. The plates are then read on an ABI 7900HT Sequence Detection System using SDS 2.3 software. Reactions were 5-10 μ l in volume and included 5-10ng of genomic DNA. Details related to each SNP are listed below

rs118204009

In Norway DNA from the known homozygote and heterozygote Bergen VDDR1 subjects were included on every plate. Suspected novel heterozygotes were confirmed by sequencing (see below).

In the UK all individuals were double typed, with and without the DNA from the Bergen VDDR1 subjects. Any samples lying outside the main wild type homozygote cluster were re-sequenced (even if the sample was not a consistent outlier). In total 51 UK and 4 Norwegian samples were resequenced.

In the USA samples were initially typed without including Bergen VDDR1 DNA on plates. Any sample suspected of lying outside the main cluster was retyped with and without DNA from the Bergen VDDR1 subjects.

rs118204011

This variant was not typed in Norway. No known mutant heterozygous or homozygous individuals were available prior to genotyping. In the UK two potentially heterozygous subjects were identified. Resequencing these confirmed just a single heterozygous individual.

Table S2. Taqman Primer sequences

rs118204009	
Forward_Primer_PCR	TGTGCTTTGCAACCTAGACTGTAC
Reverse_Primer_PCR	TCACCCACATGAATGTCTTTGTCT
Reporter_Sequence_Allele1	CTGGAAATTCTCGTGTCCC
Reporter_Sequence_Allele2	TGGAAATTCTCATGTCCC
rs118204011	
Forward_Primer_PCR	CCGGCACCCCGAAGTC
Reverse_Primer_PCR	GCTCAGGGCAGCTGTGAT
Reporter_Sequence_Allele1	CTCTGAGTGGAGTGCTGT
Reporter_Sequence_Allele2	CTGAGTGGAAATGCTGT

Table S3. Genotyping Success rates (proportion of samples typed)

	Norway/%	UK/%	USA/%
rs118204009	97.8	98.8	99.5
rs118204011	-	99.2	99.5

References

- Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., et al., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol.* 13, 227-231.
- McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., et al., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol.* 50, 121-127.

Raw Data

Cluster plots from the above genotyping are shown below

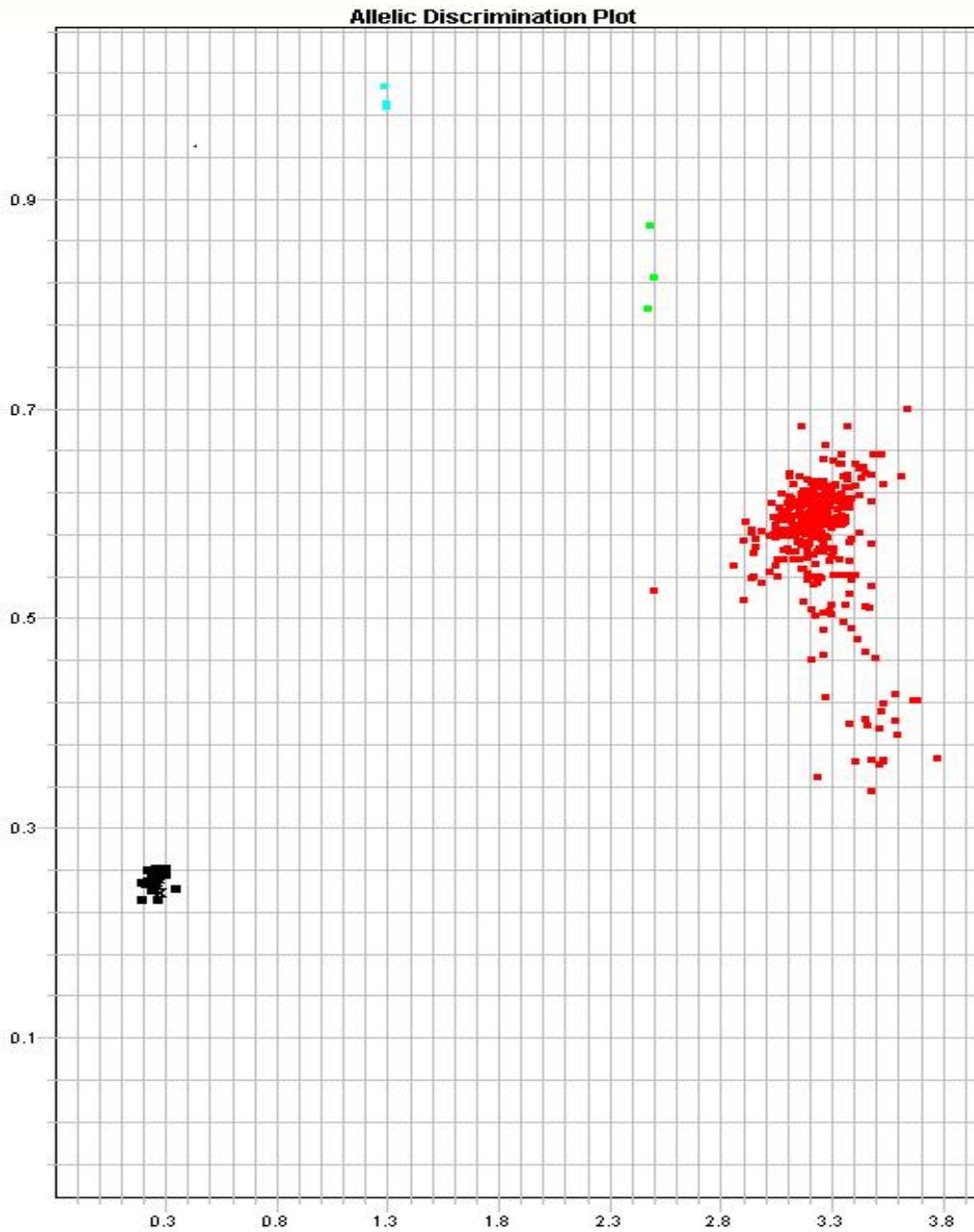
SNP: rs118204009

Plate Name: Norway_1

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



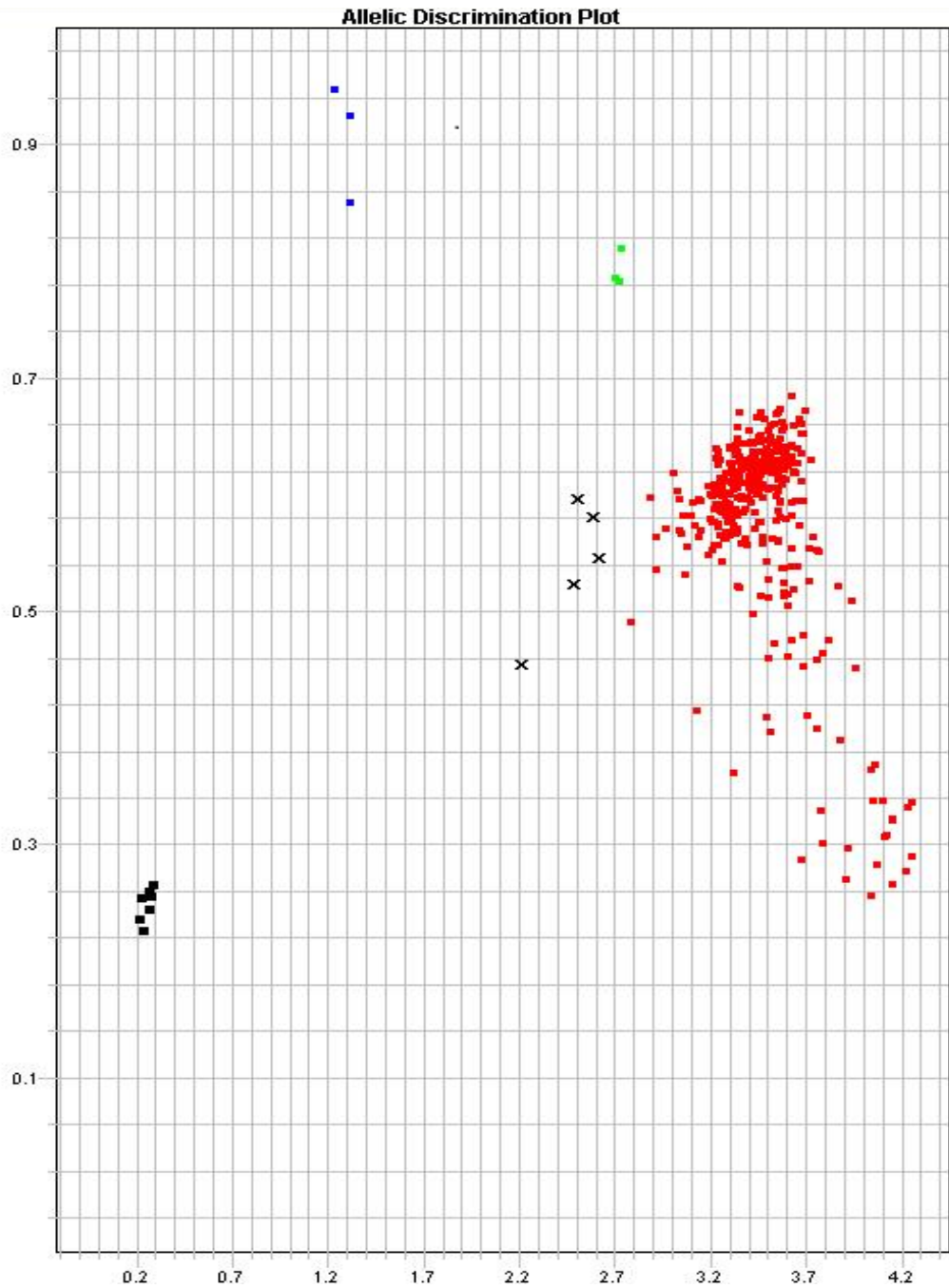
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Plate Name: Norway_2

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



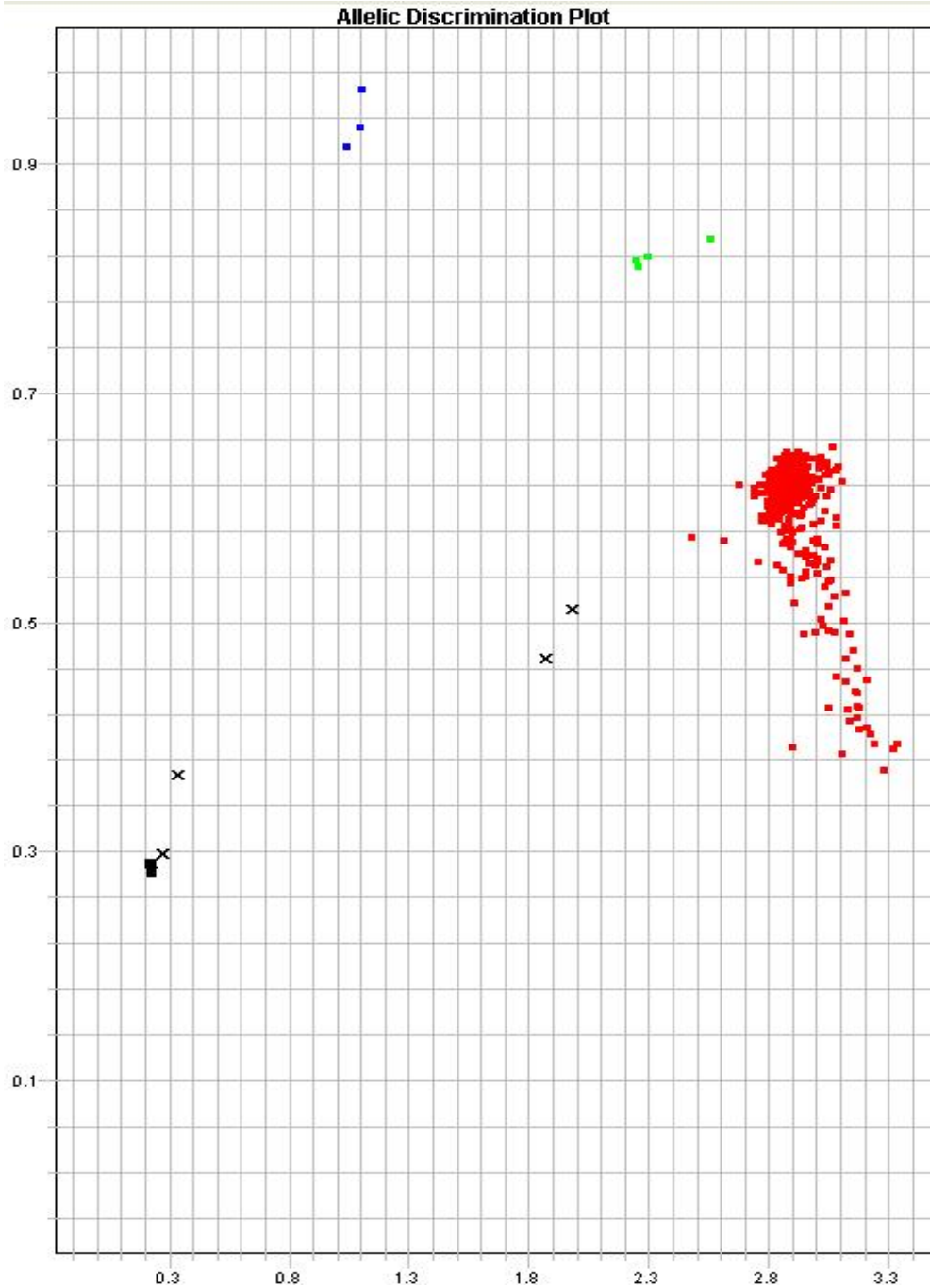
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Plate Name: Norway_3

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 1



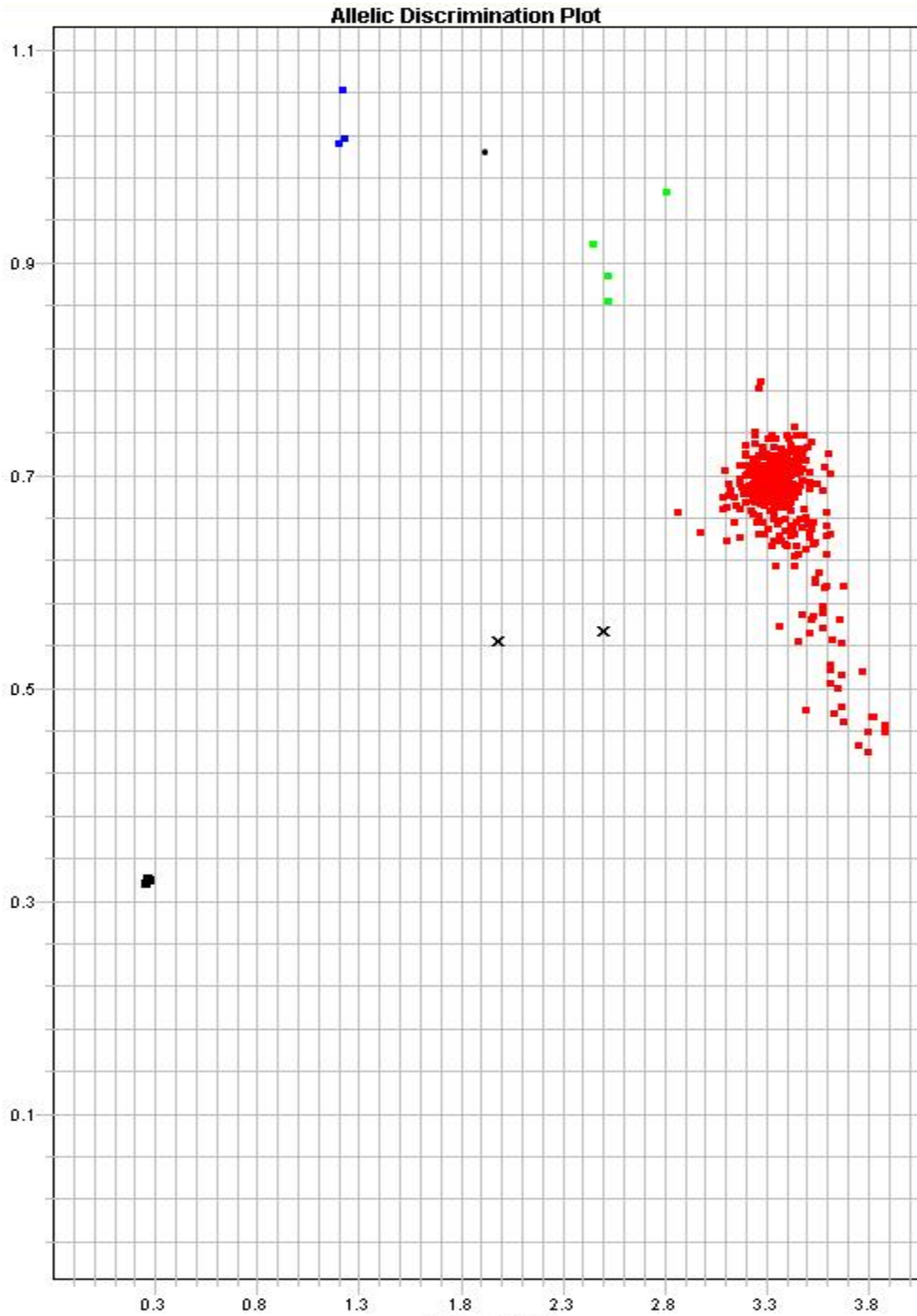
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Plate Name: Norway_4

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 1



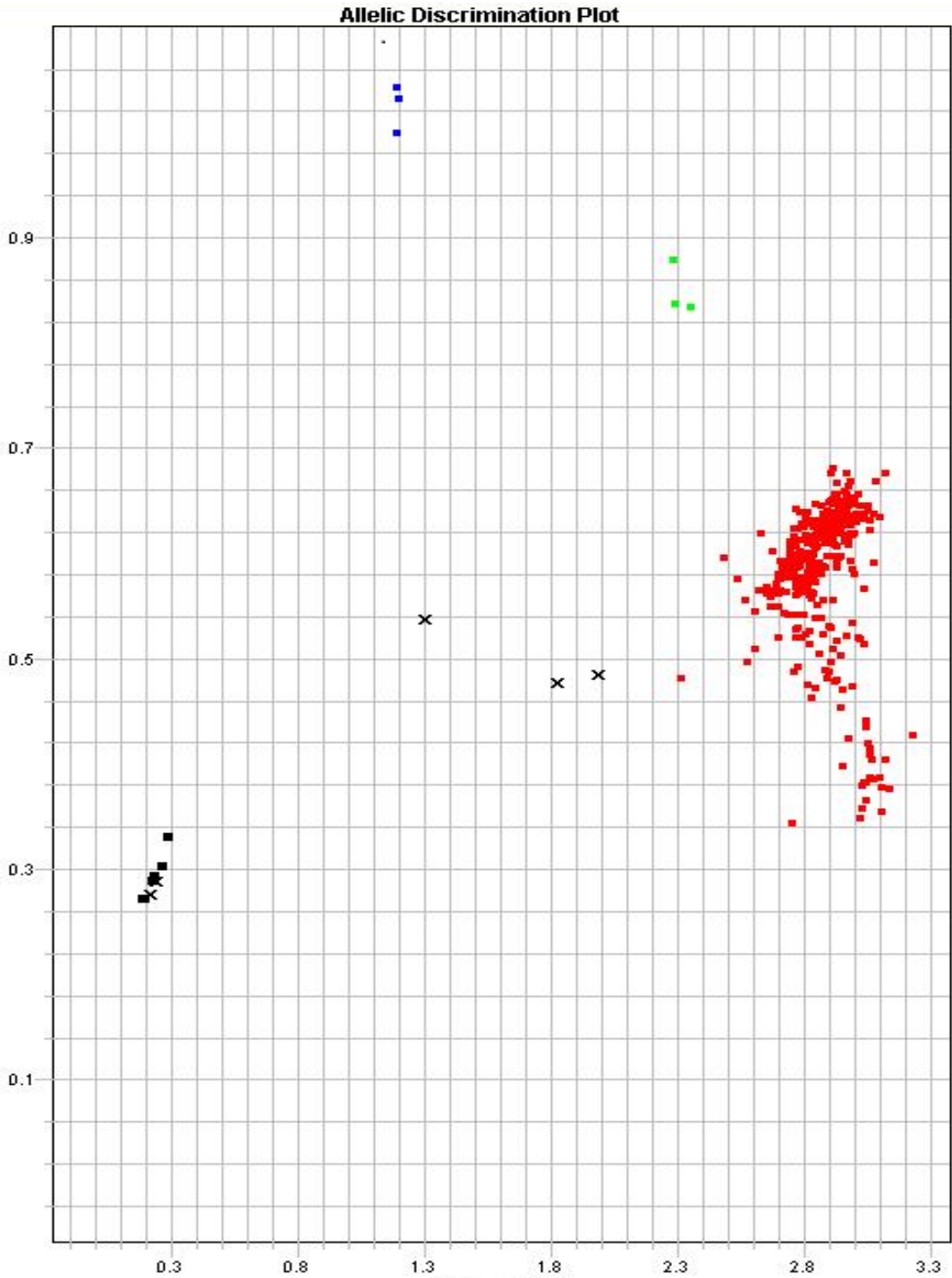
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Plate Name: Norway_5

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



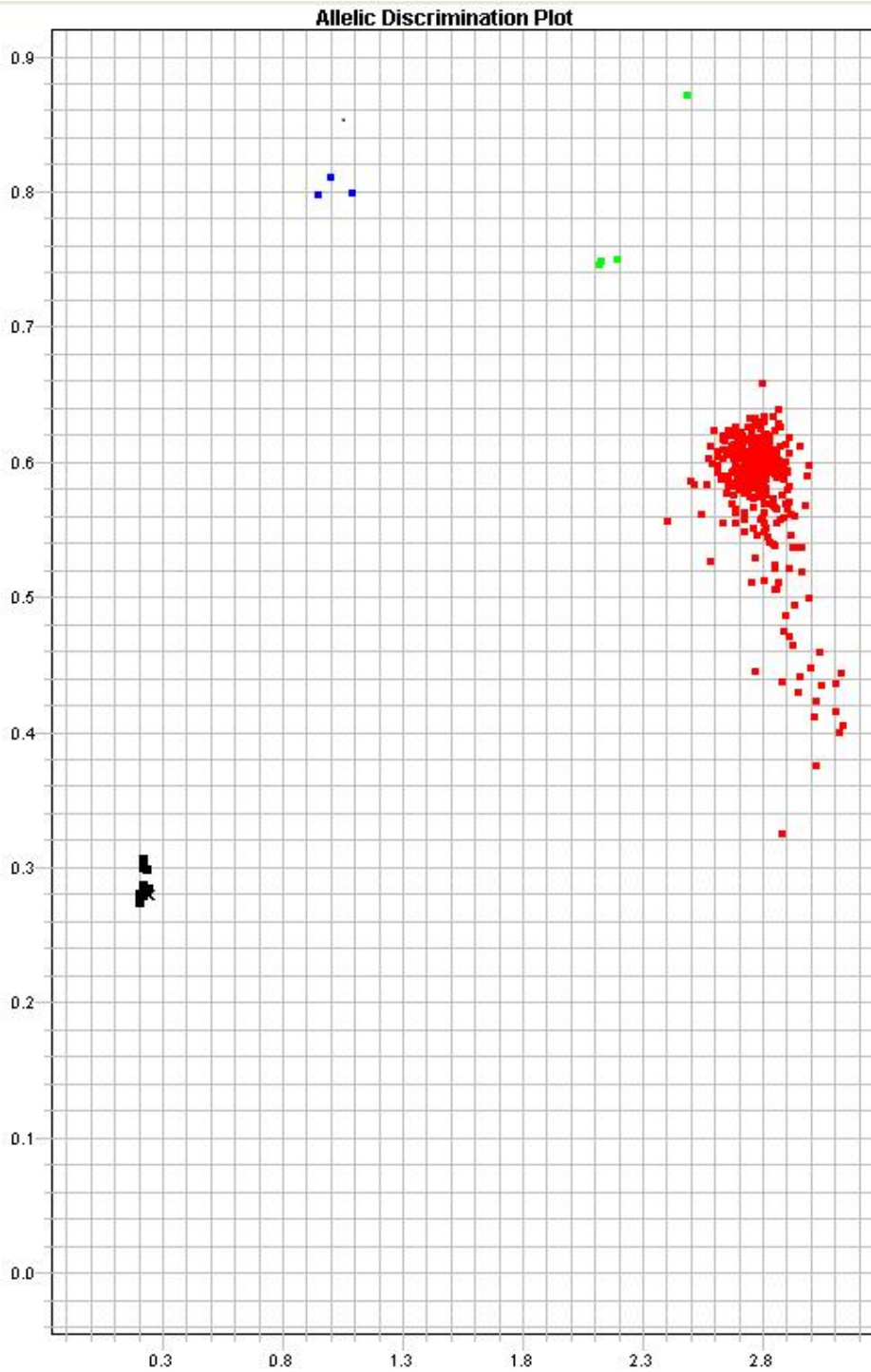
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Plate Name: Norway_6

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 1



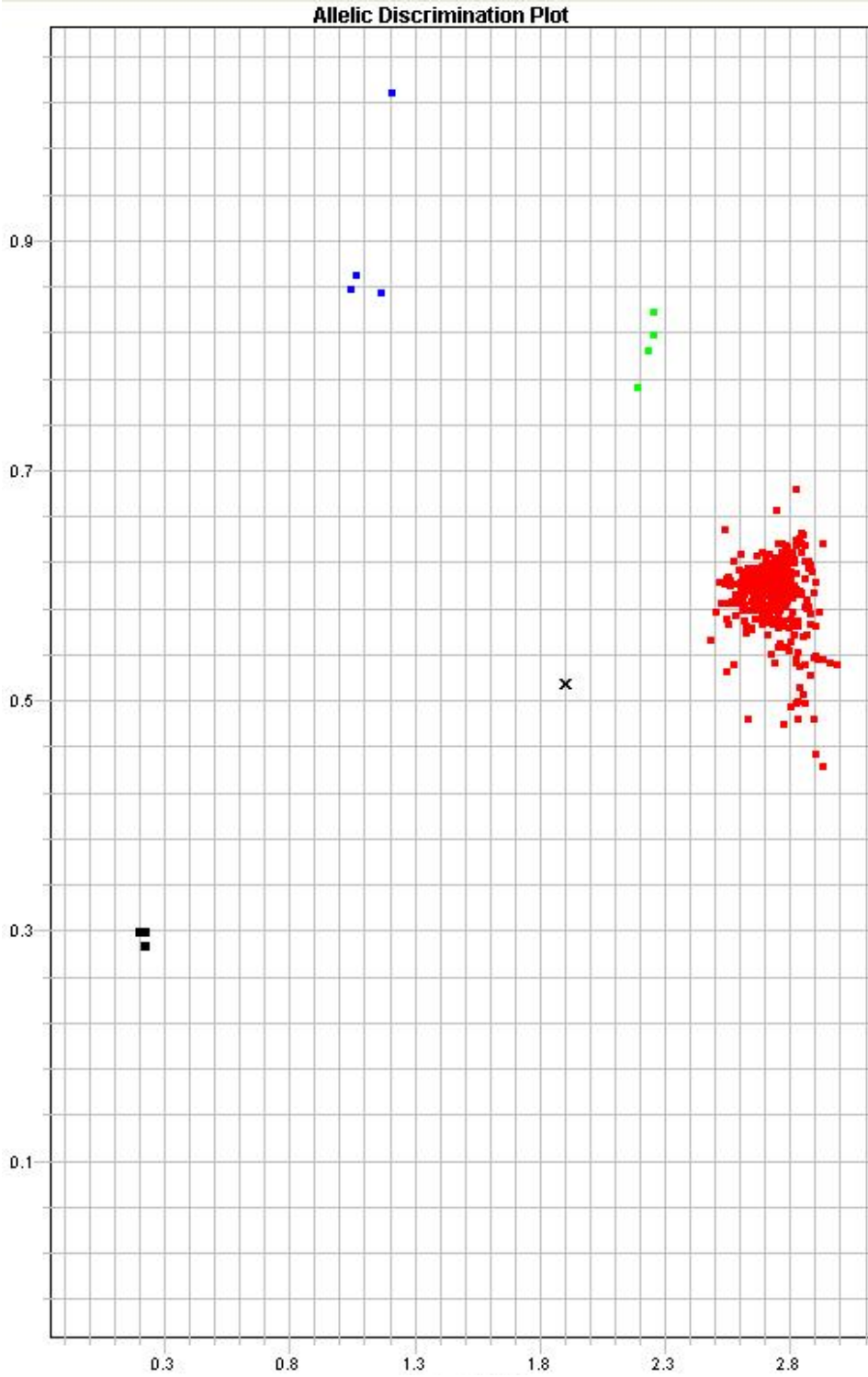
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Plate Name: Norway_7

Homozygote Controls on plate: 4

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



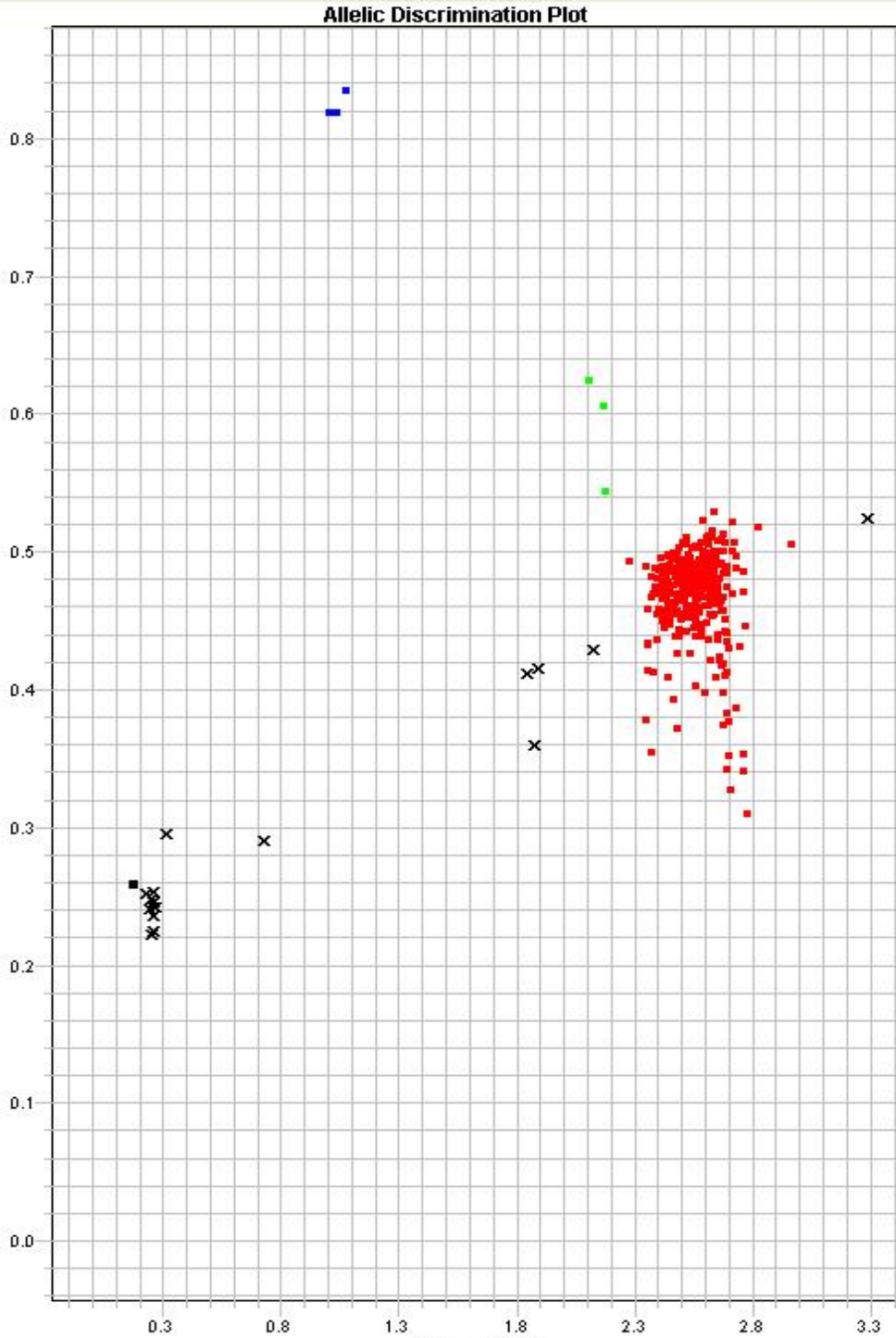
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Plate Name: Norway_8

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



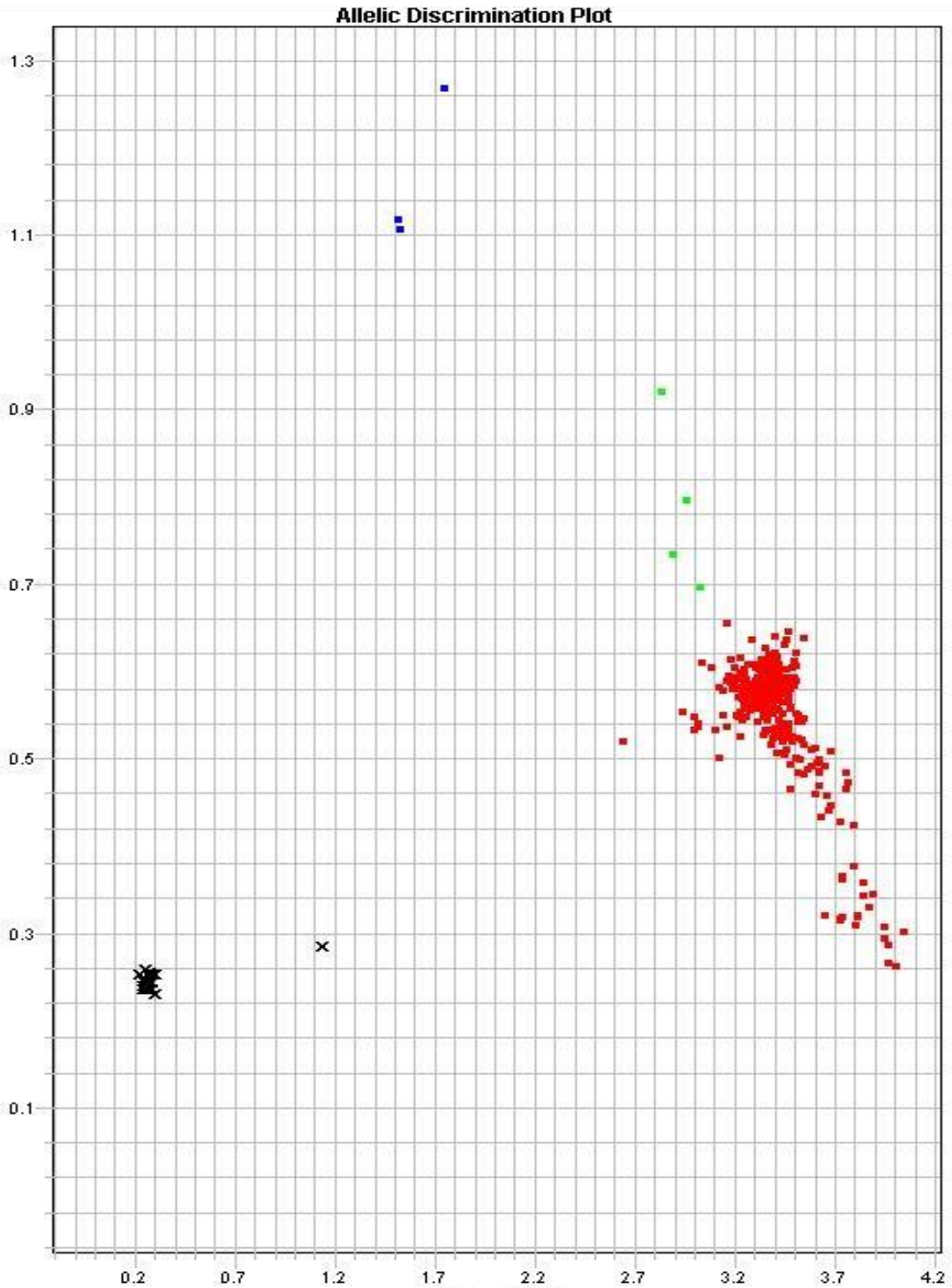
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Plate Name: Norway_9

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 1



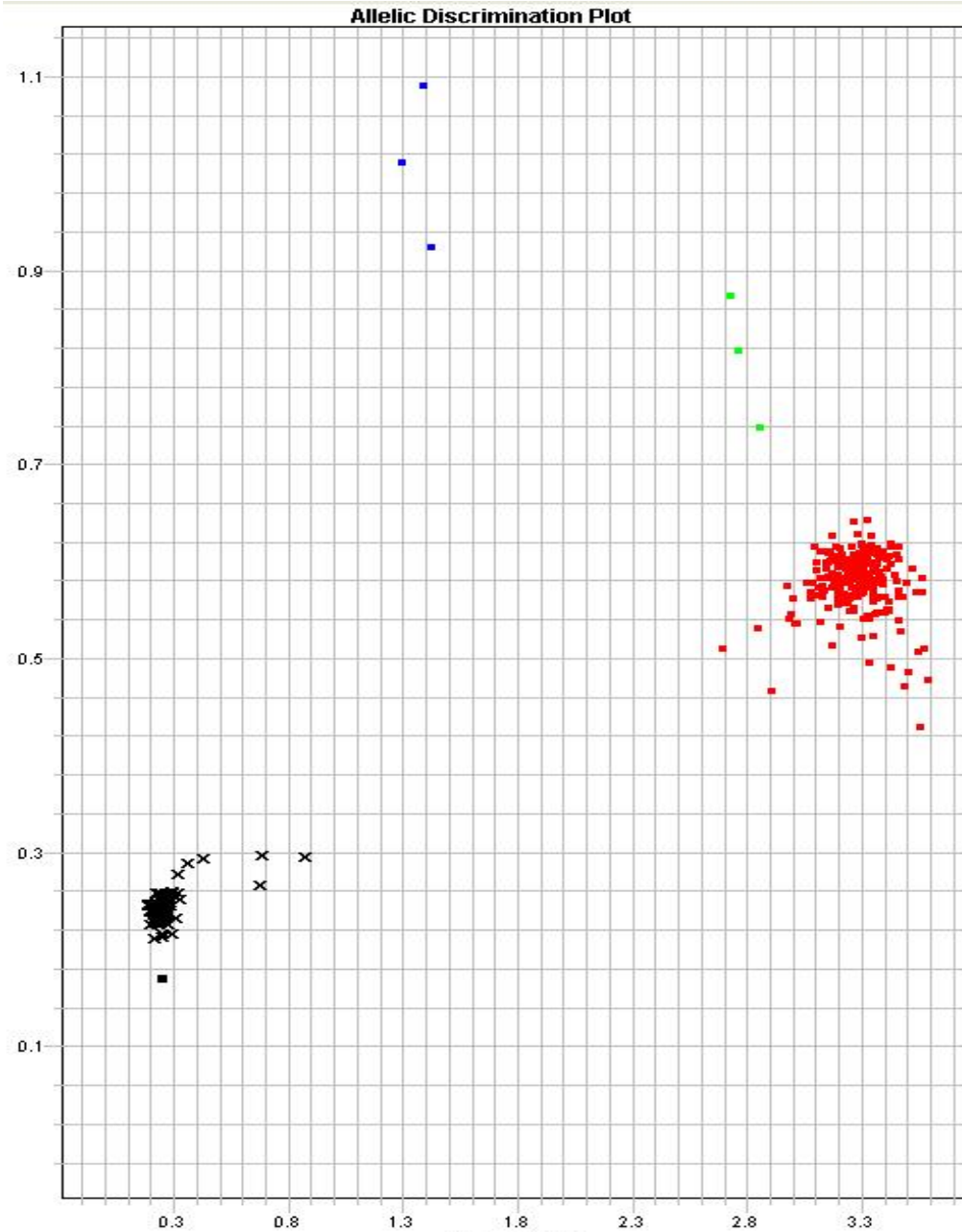
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Plate Name: Norway_10

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



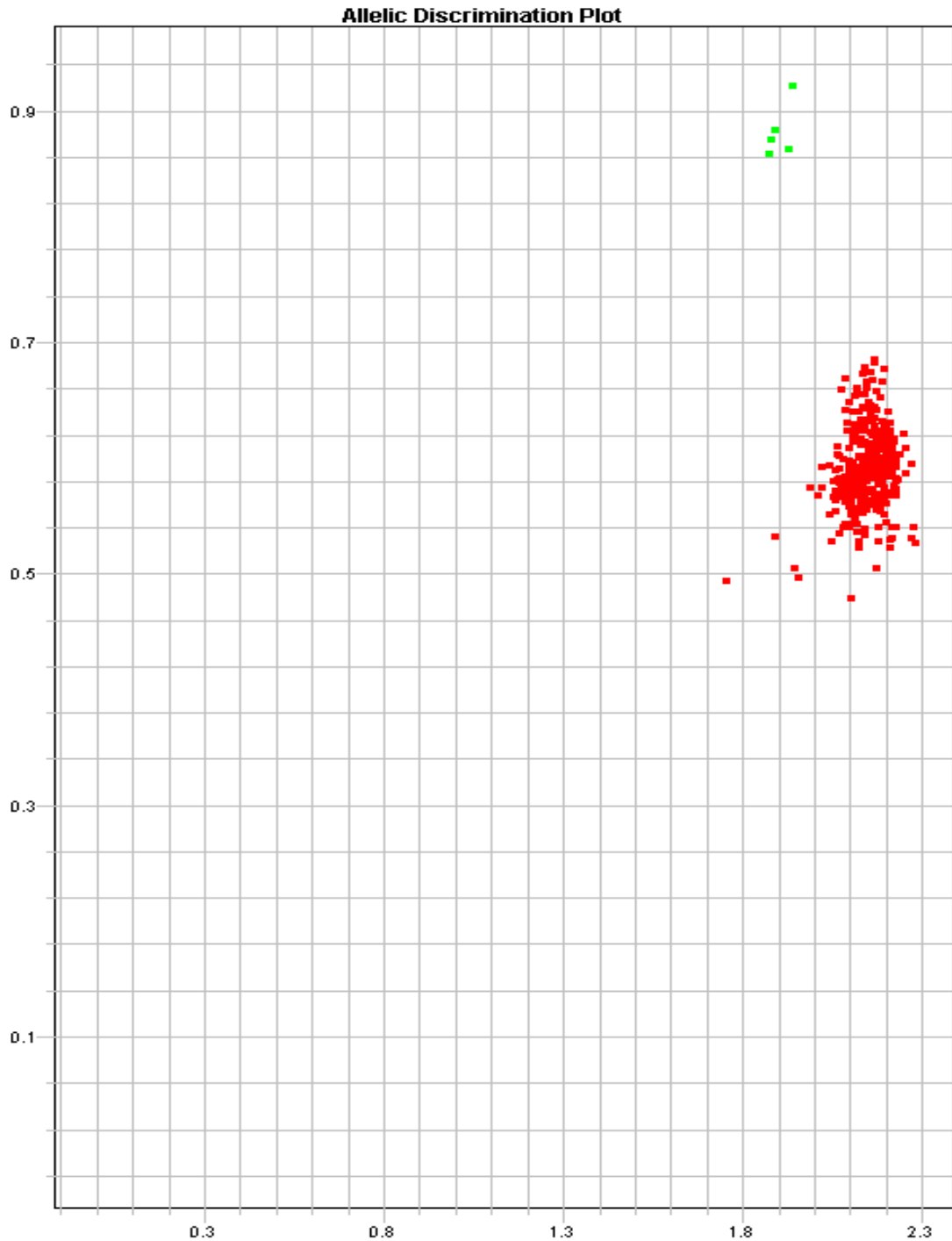
SNP: rs118204009

Plate Name: UK_1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 1



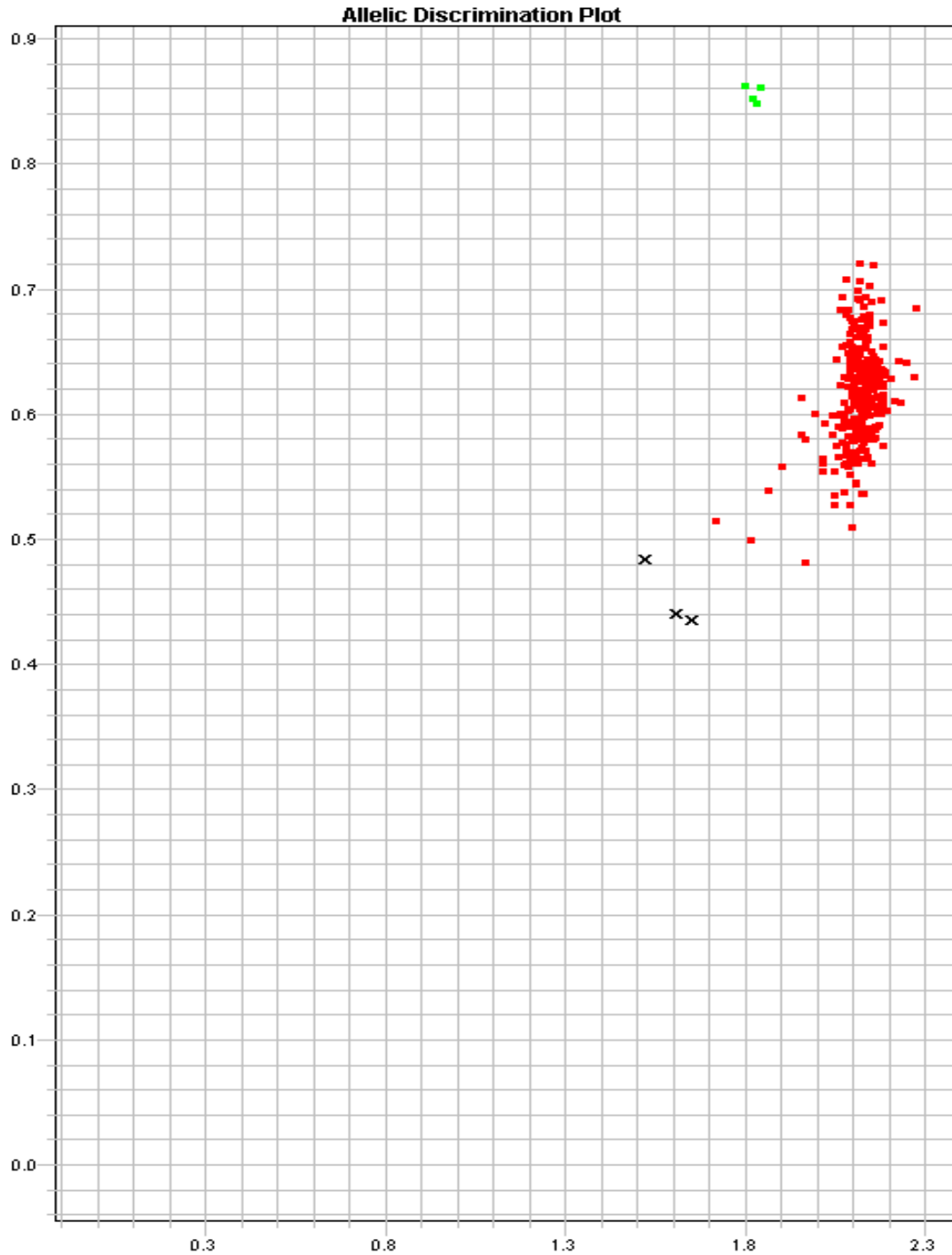
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Plate Name: UK_2

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



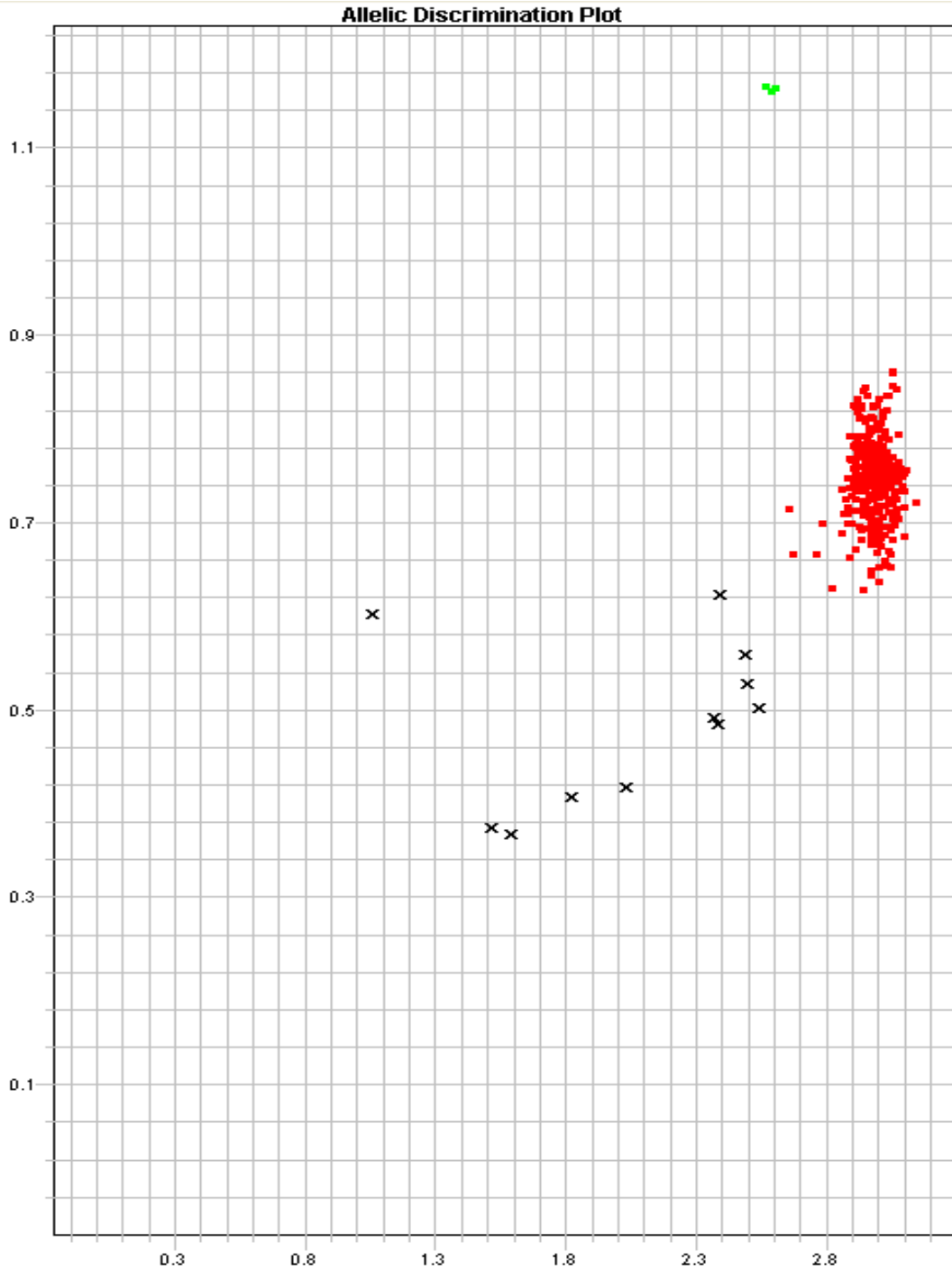
SNP: rs118204009

Plate Name: UK_3

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



SNP: rs118204009

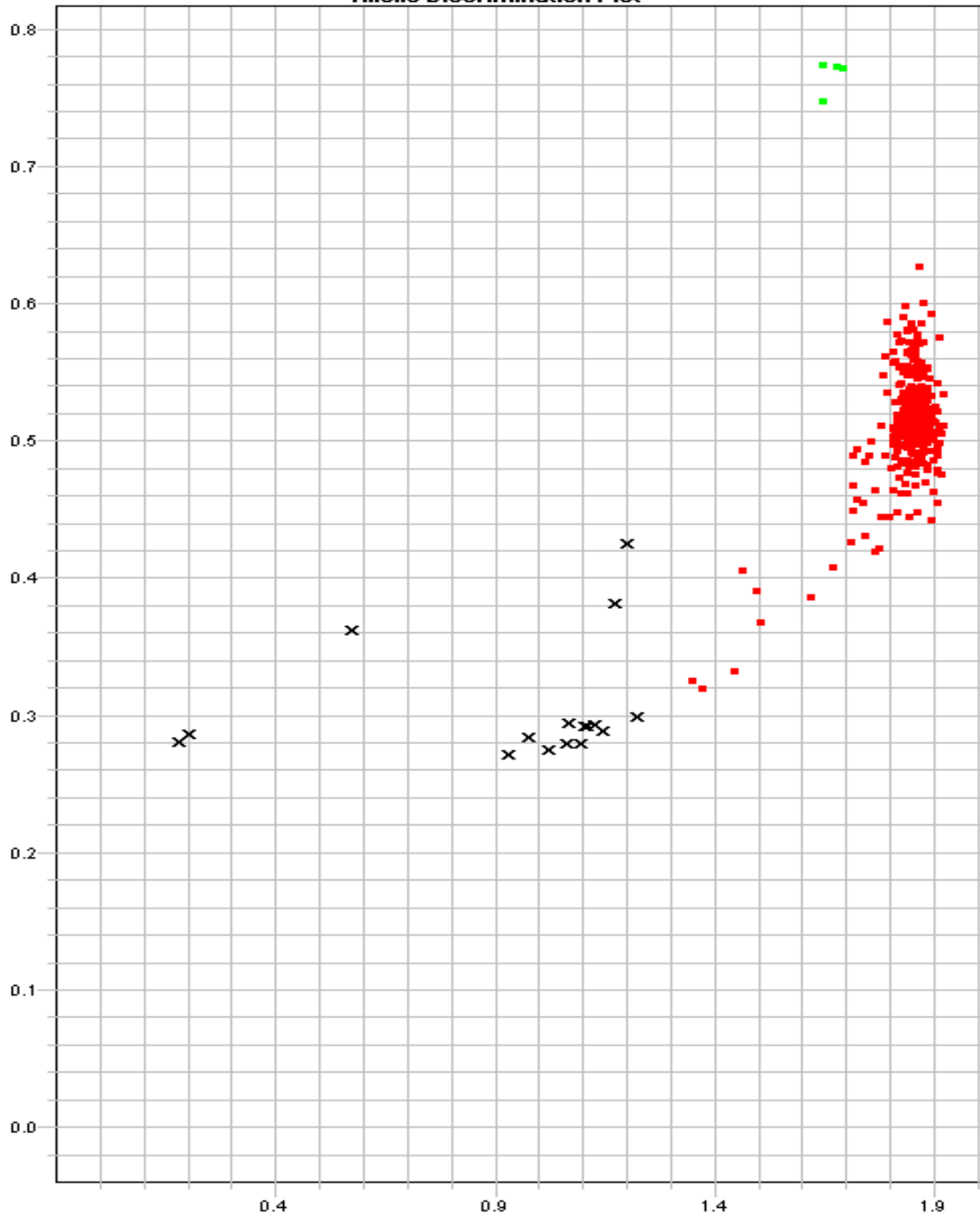
Plate Name: UK_4

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0

Allelic Discrimination Plot



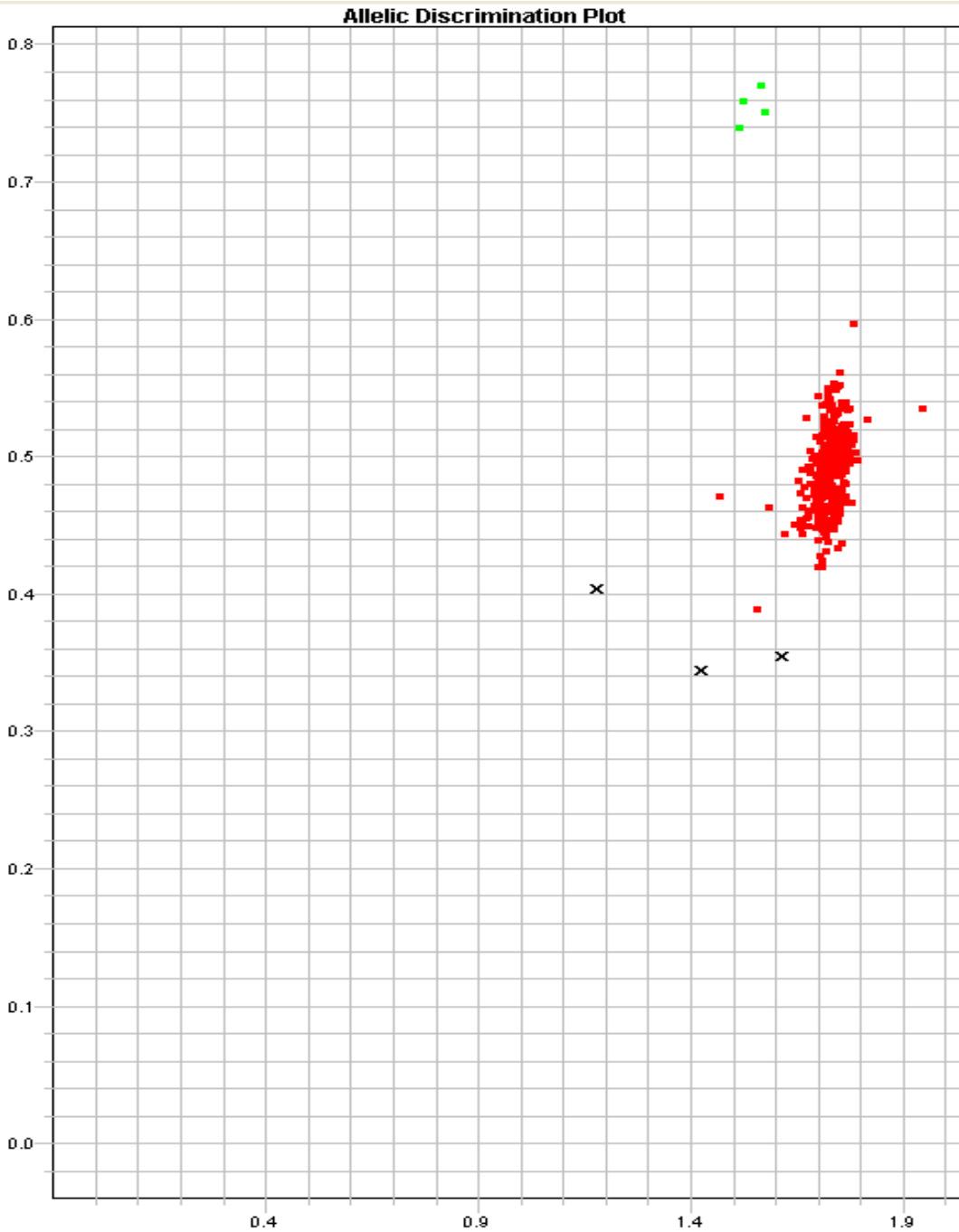
SNP: rs118204009

Plate Name: UK_5

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



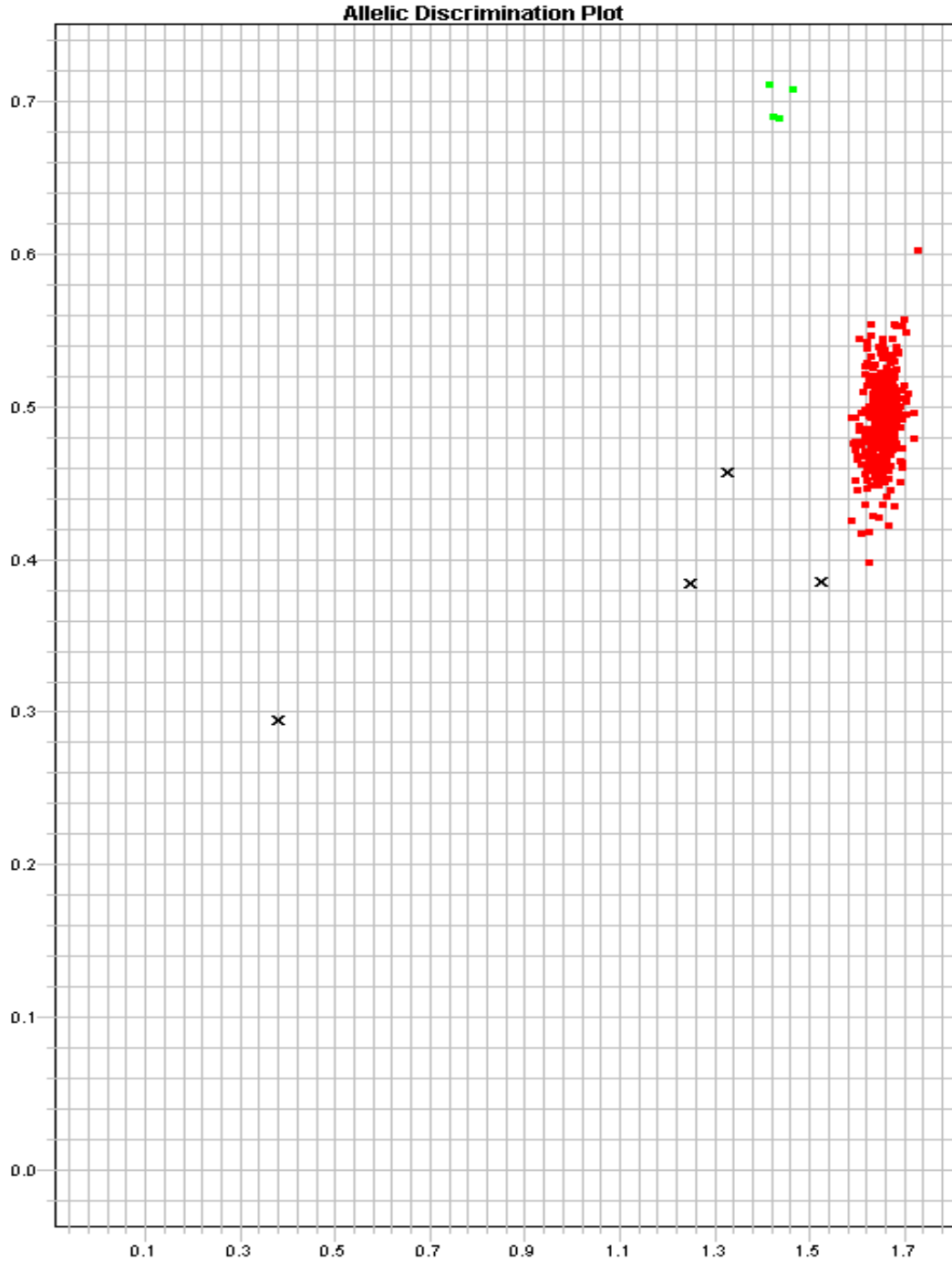
SNP: Rs118204009

Plate Name: UK_6

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



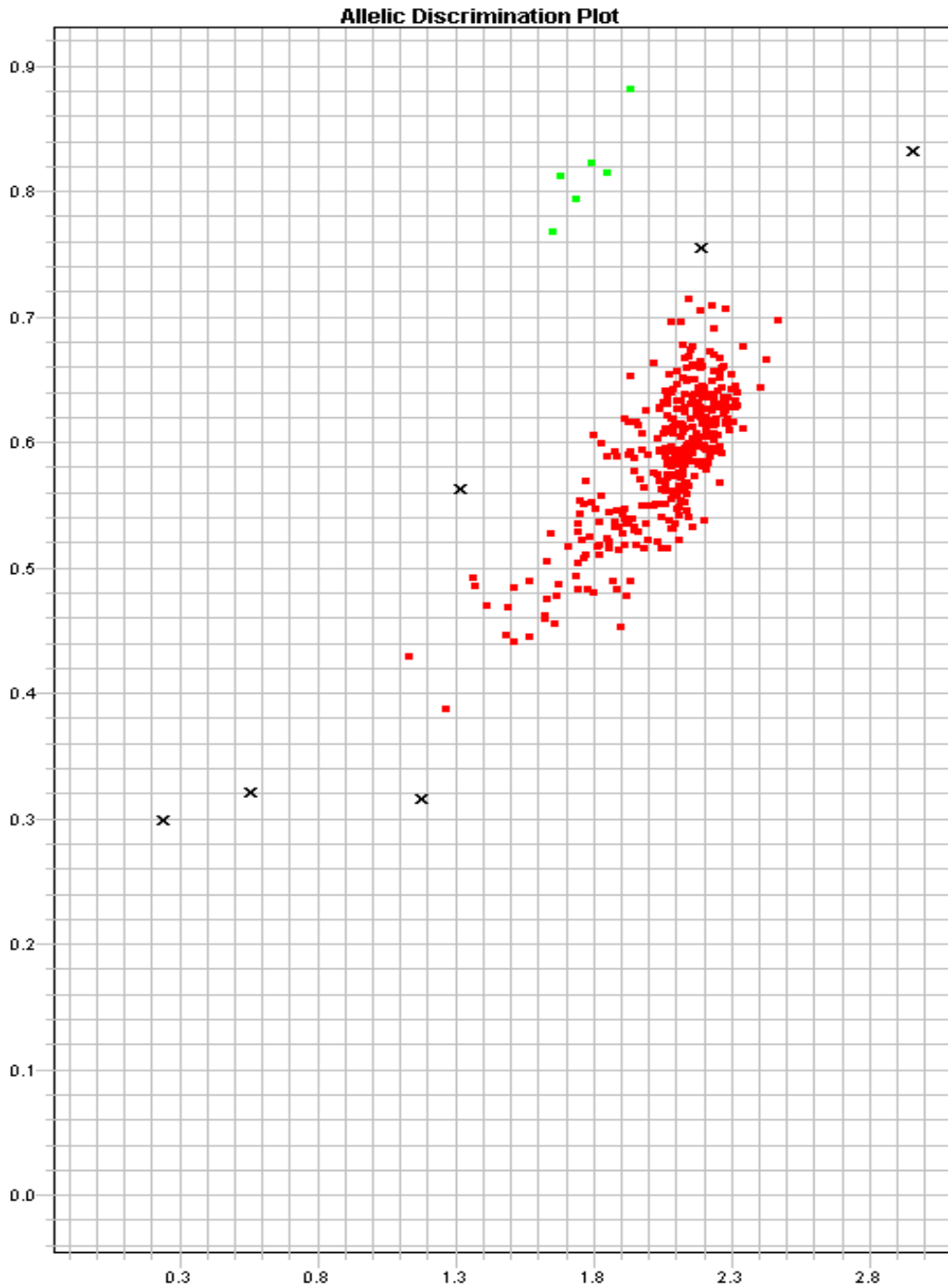
SNP: rs118204009

Plate Name: UK_7

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 2



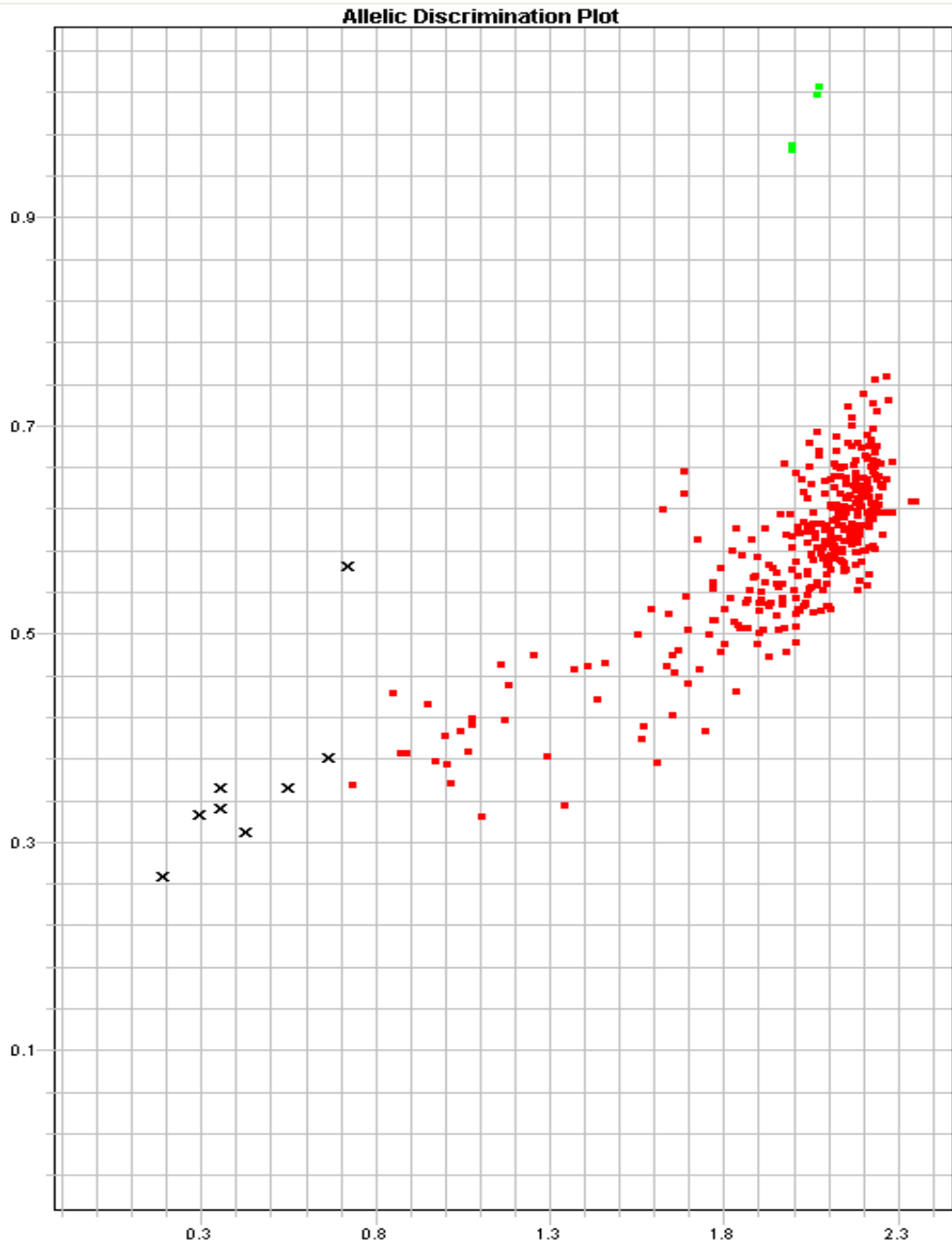
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Plate Name: UK_8

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



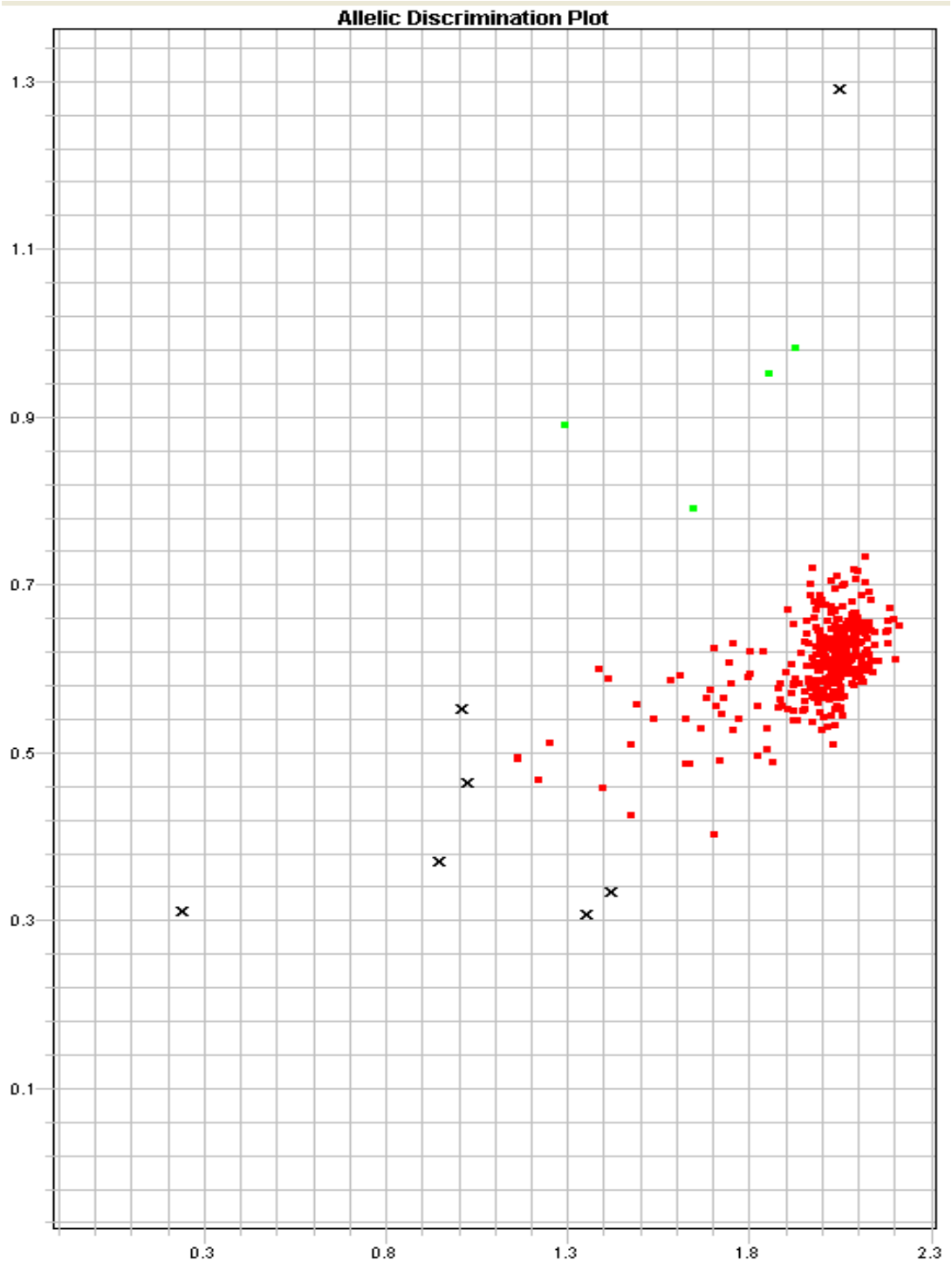
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Plate Name: UK_9

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



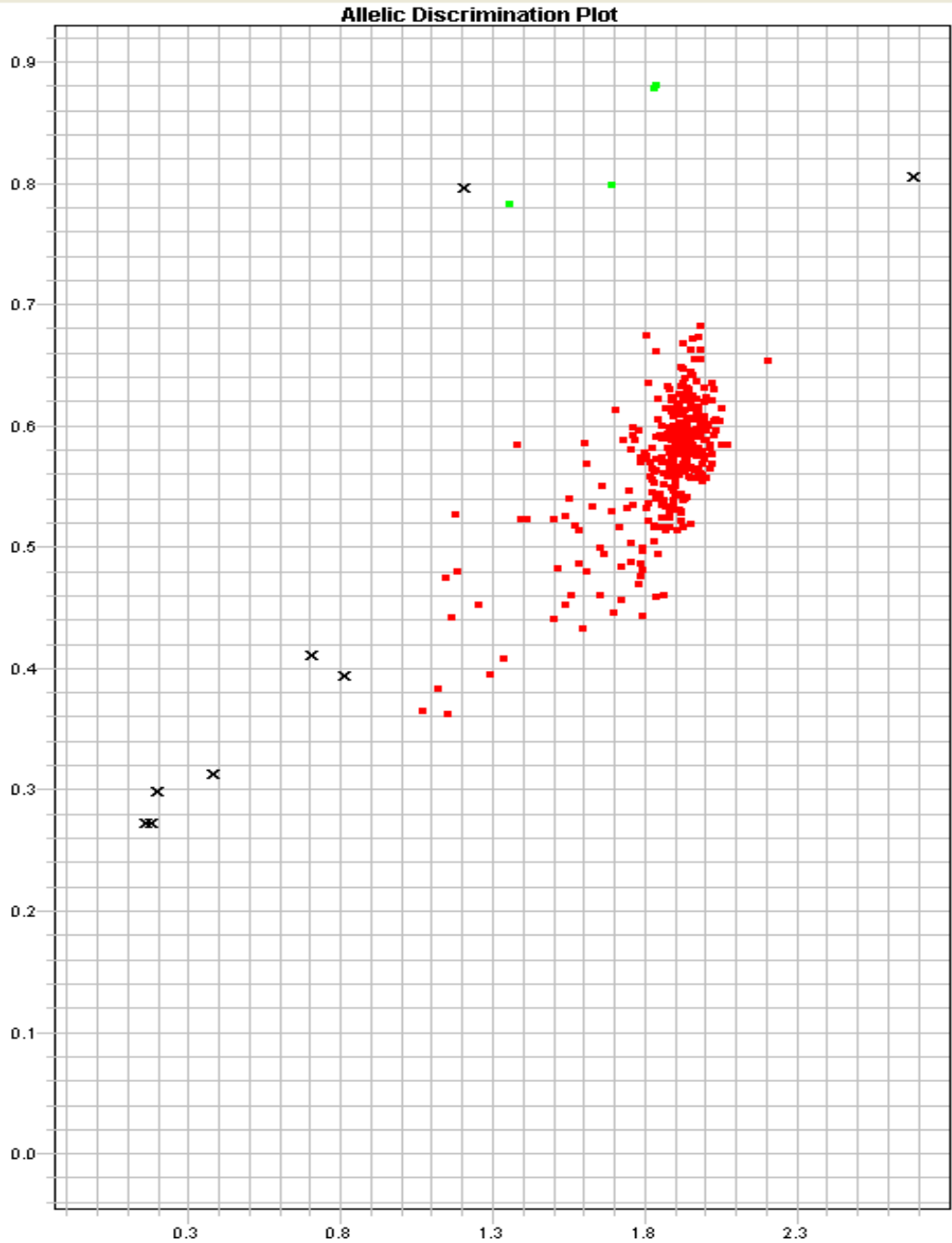
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Plate Name: UK_10

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



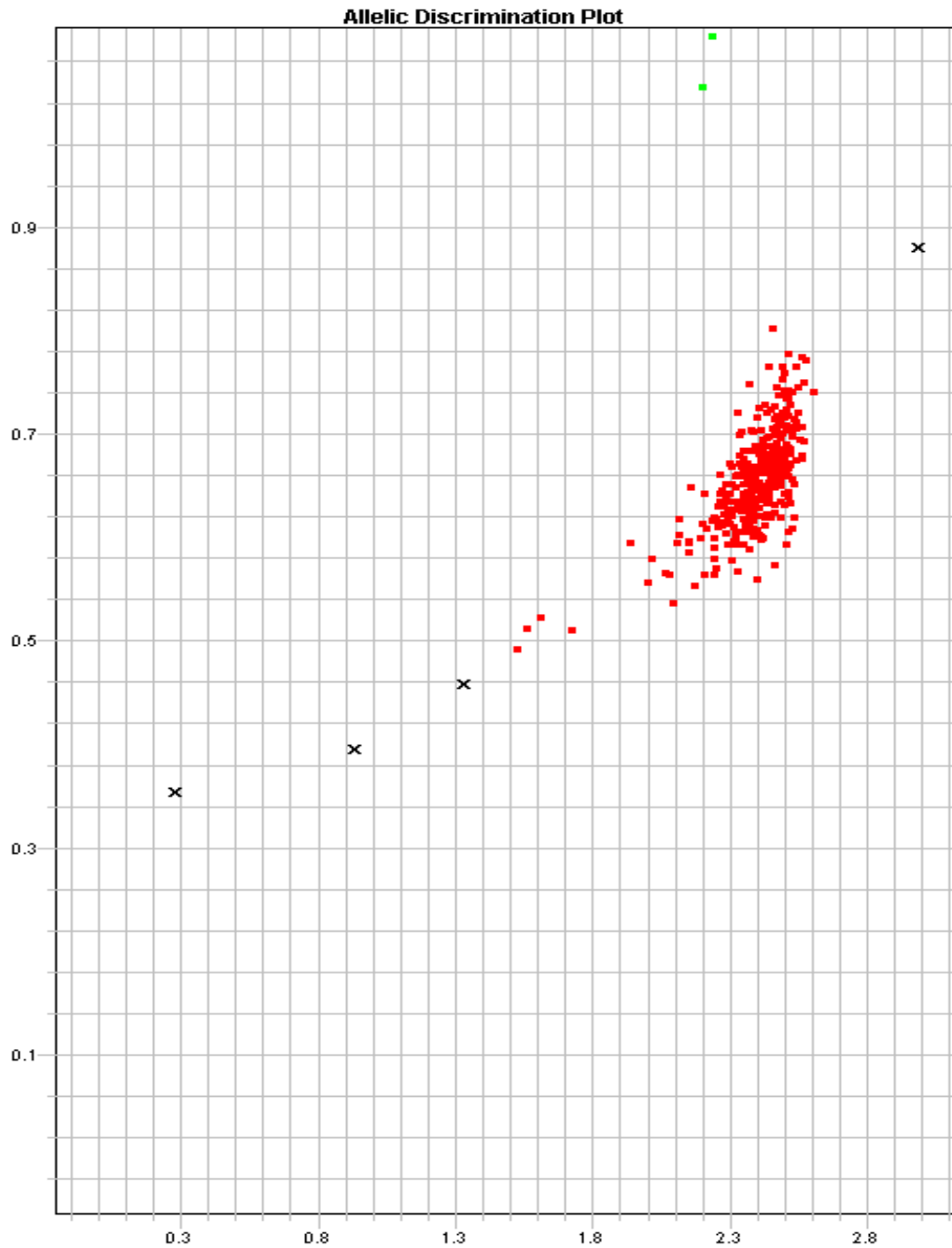
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Plate Name: UK_11

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0



SNP: rs118204009

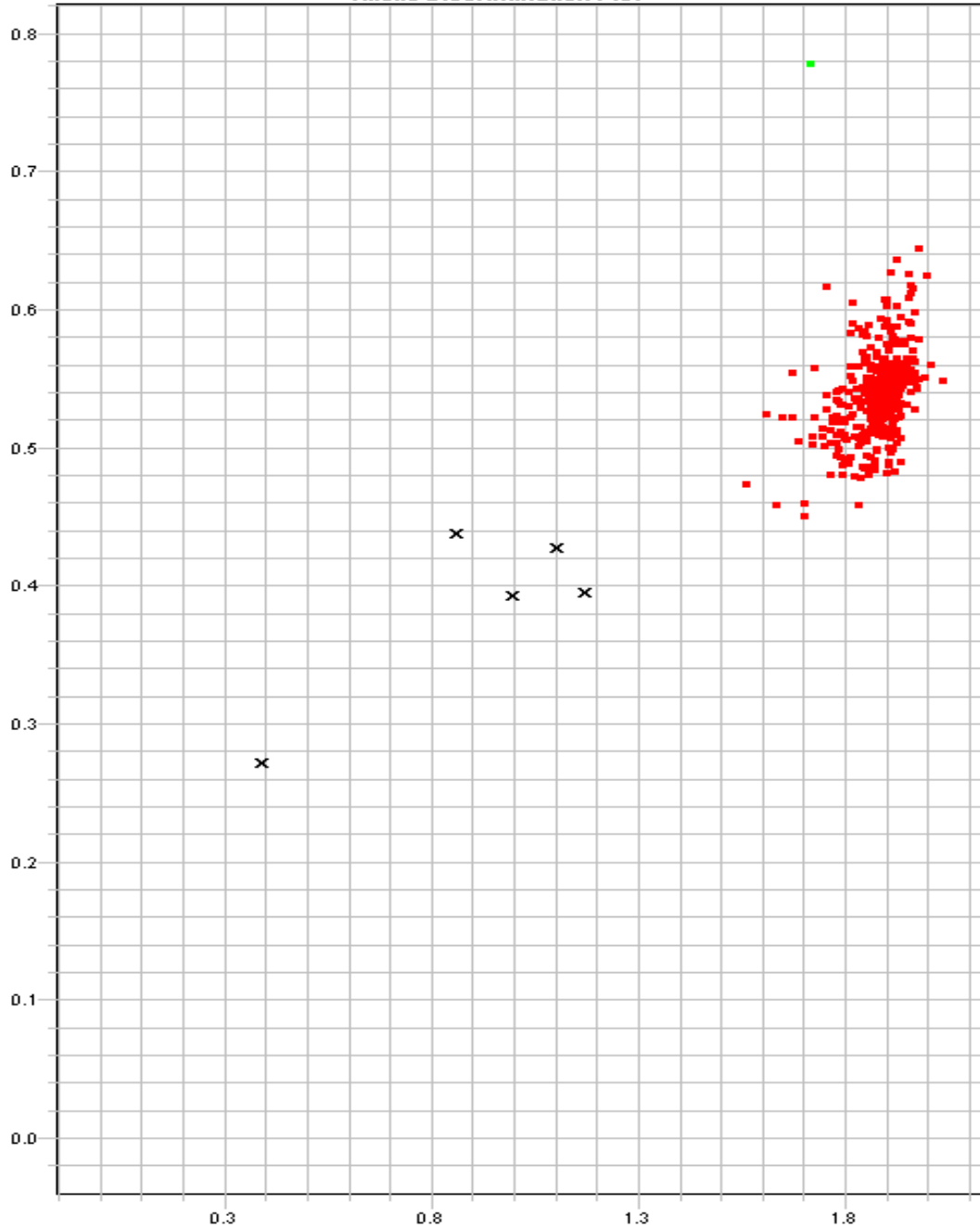
Plate Name: UK_12

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 1

New Heterozygote's on plate: 0

Allelic Discrimination Plot



SNP: rs118204009

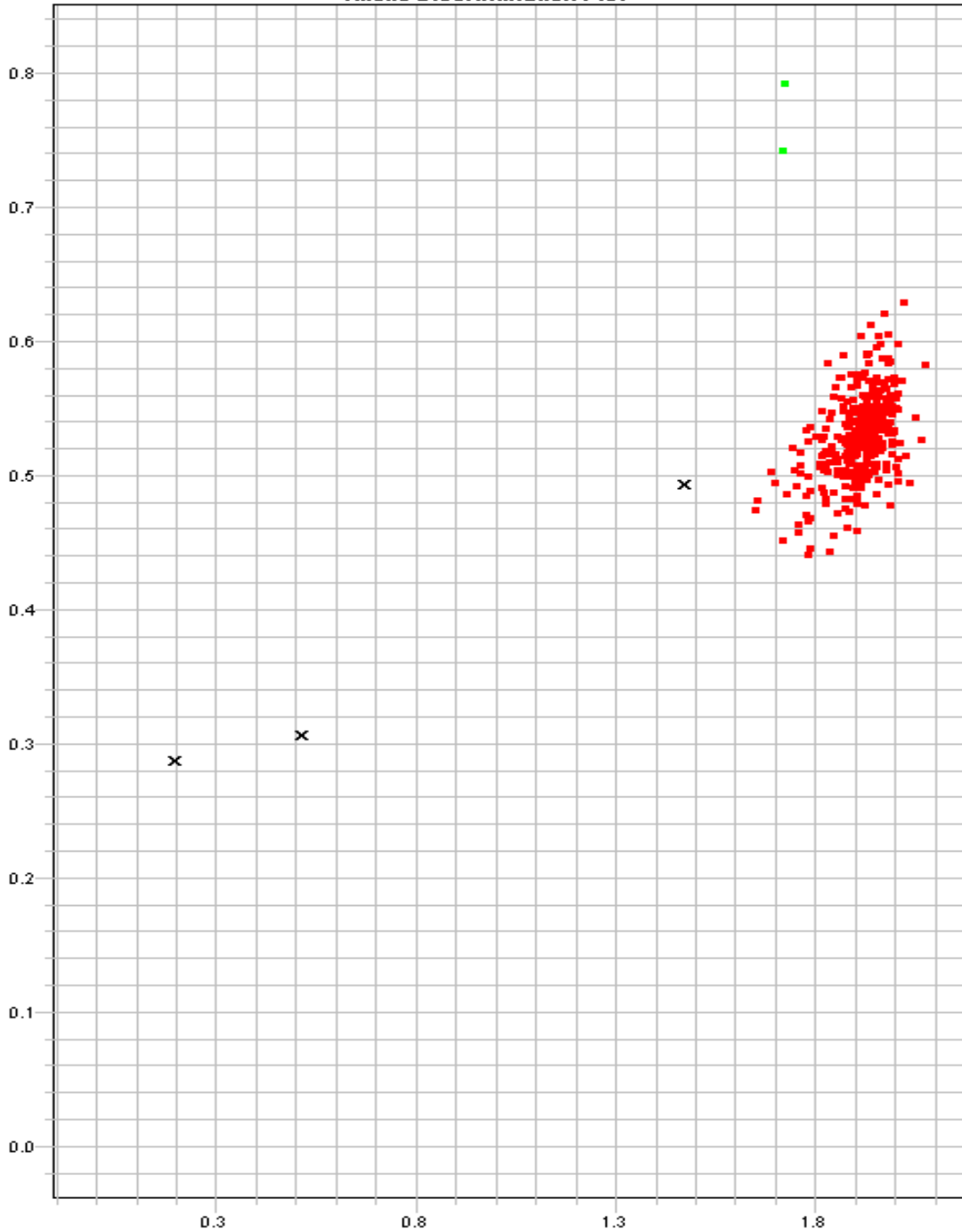
Plate Name: UK_13

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0

Allelic Discrimination Plot



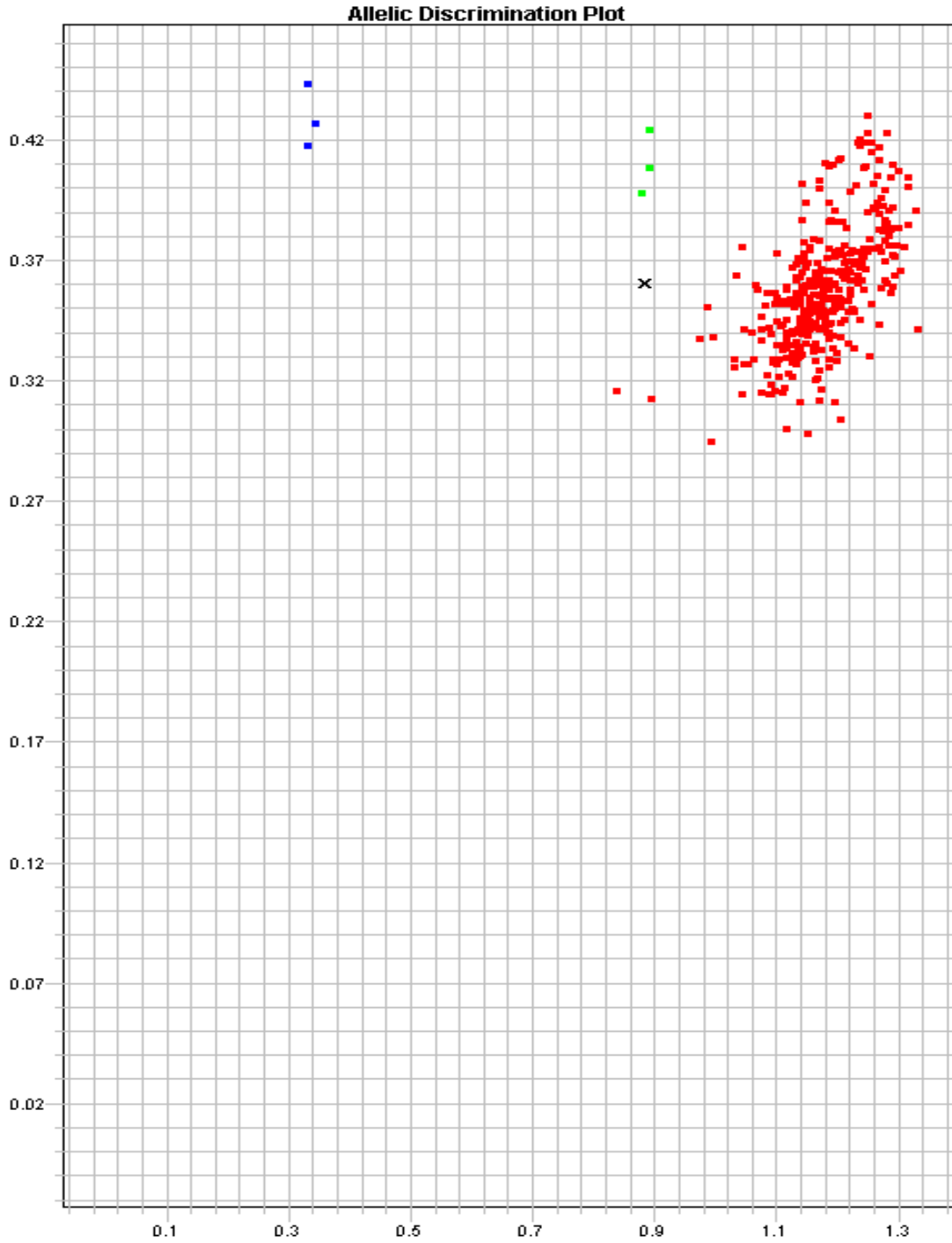
SNP: rs118204009

Plate Name: UK_14

Homozygote Controls on plate: 3

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



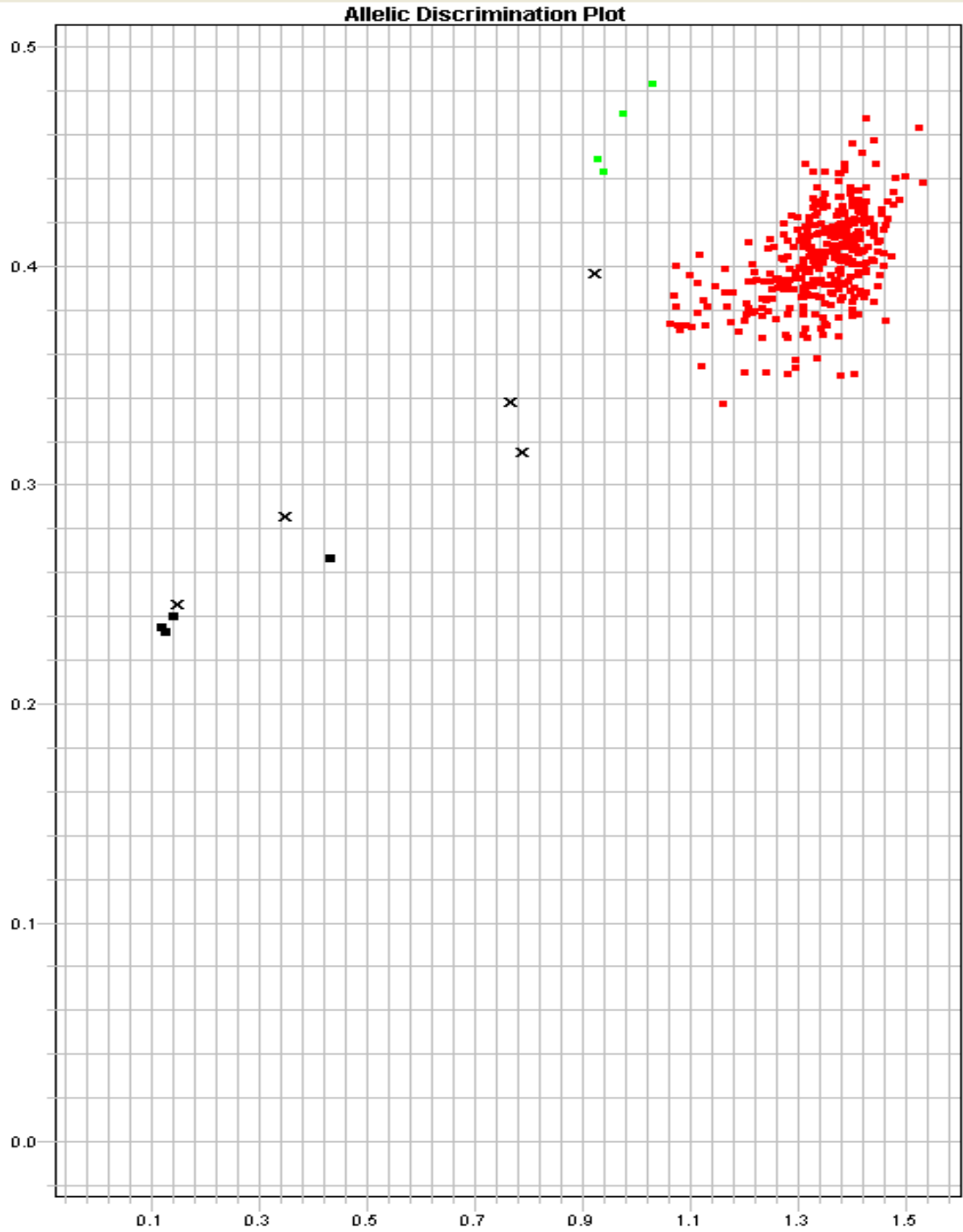
SNP: rs118204009

Plate Name: UK_15

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



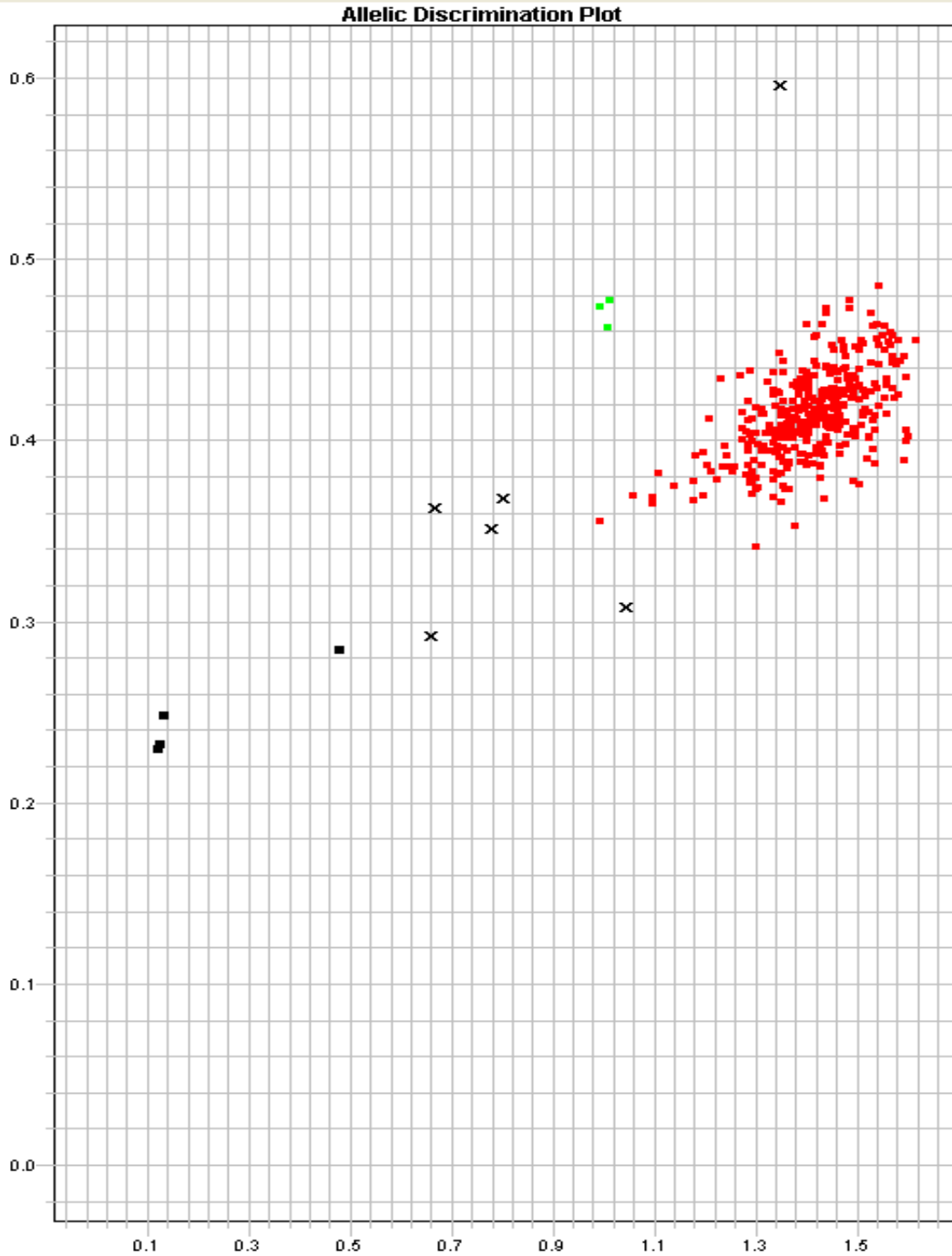
SNP: rs118204009

Plate Name: UK_16

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



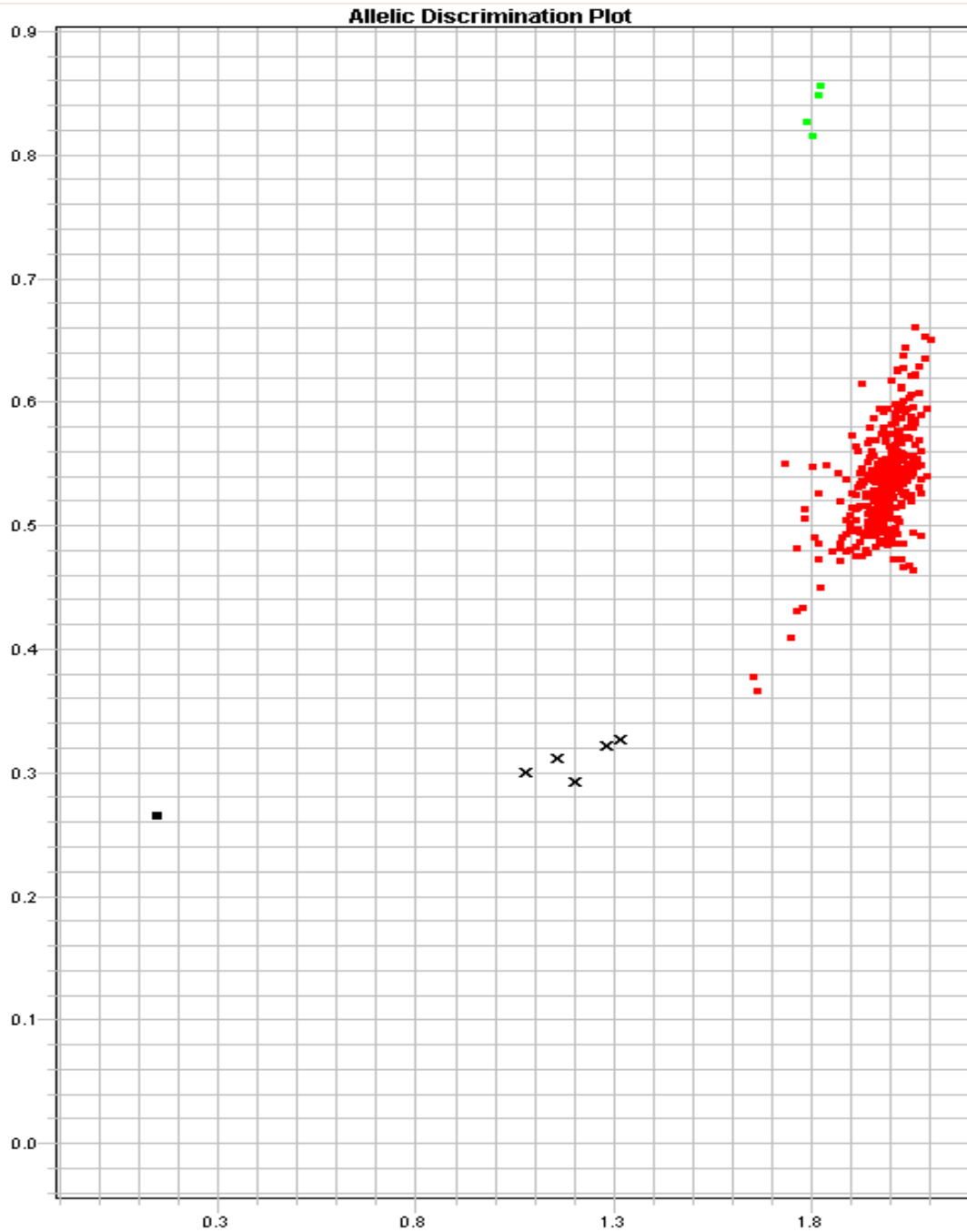
SNP: rs118204009

Plate Name: UK_17

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



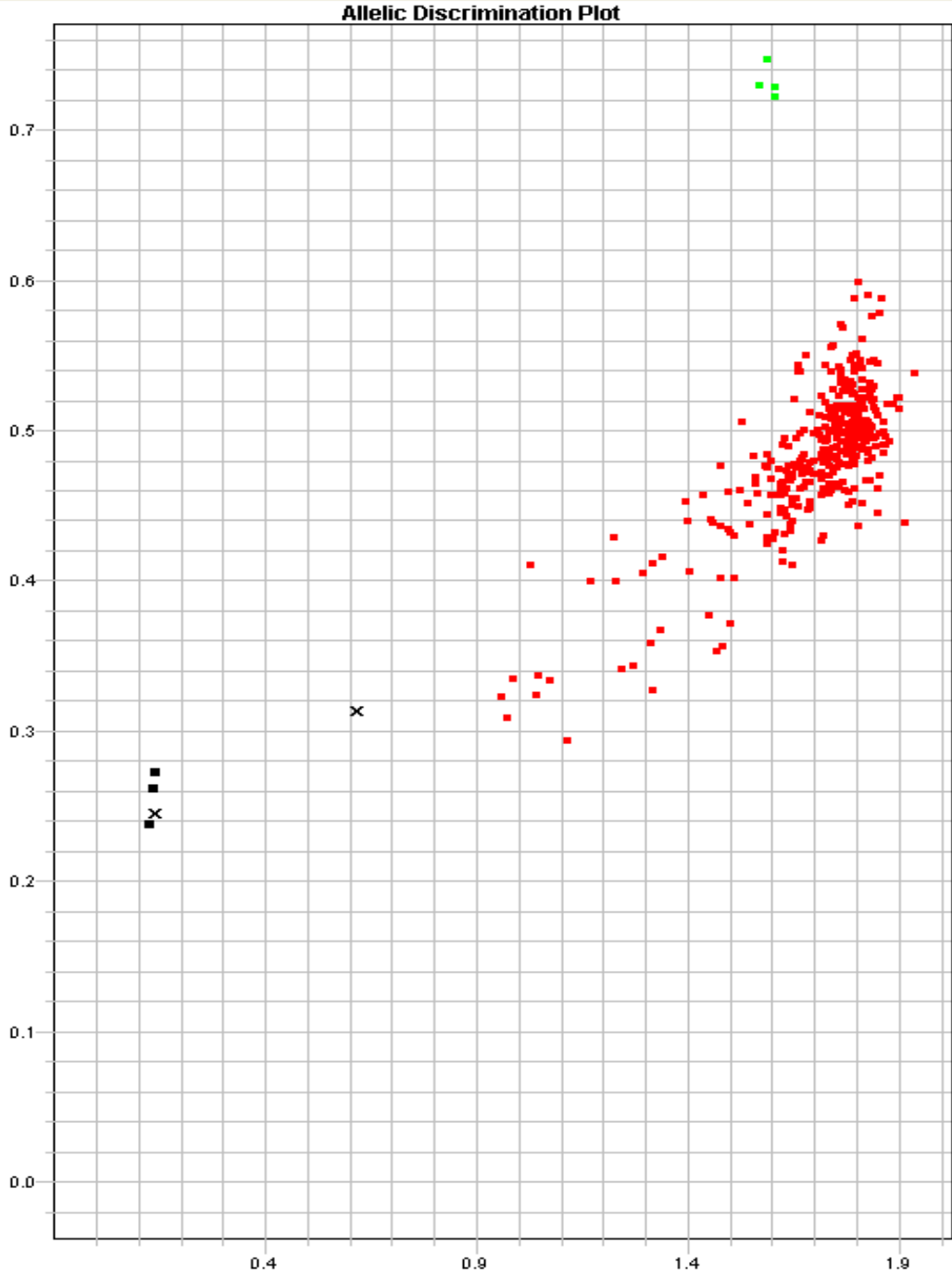
SNP: rs118204009

Plate Name: UK_18

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 4

New Heterozygote's on plate: 0



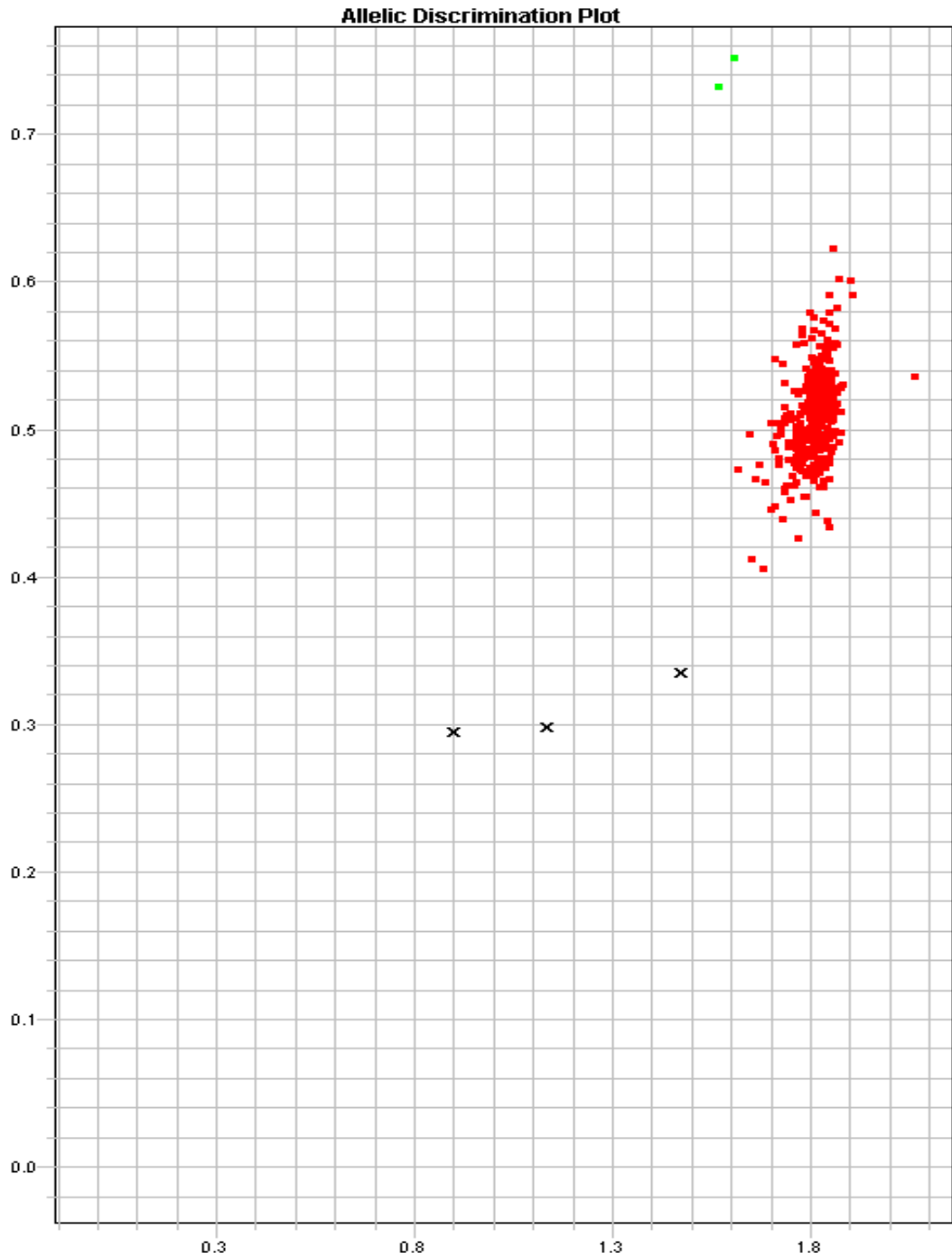
SNP: rs118204009

Plate Name: UK_19

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0



SNP: rs118204009

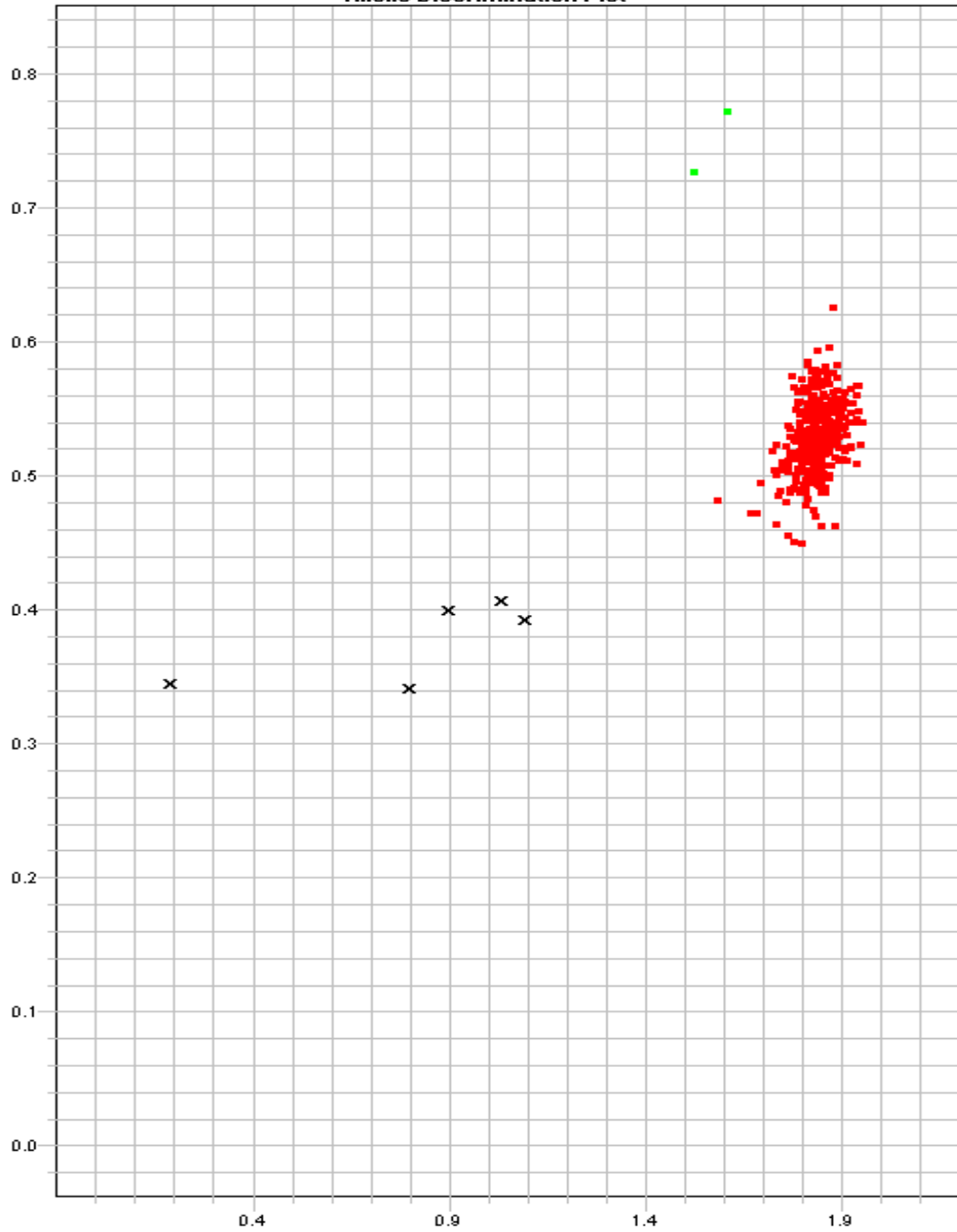
Plate Name: UK_20

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0

Allelic Discrimination Plot



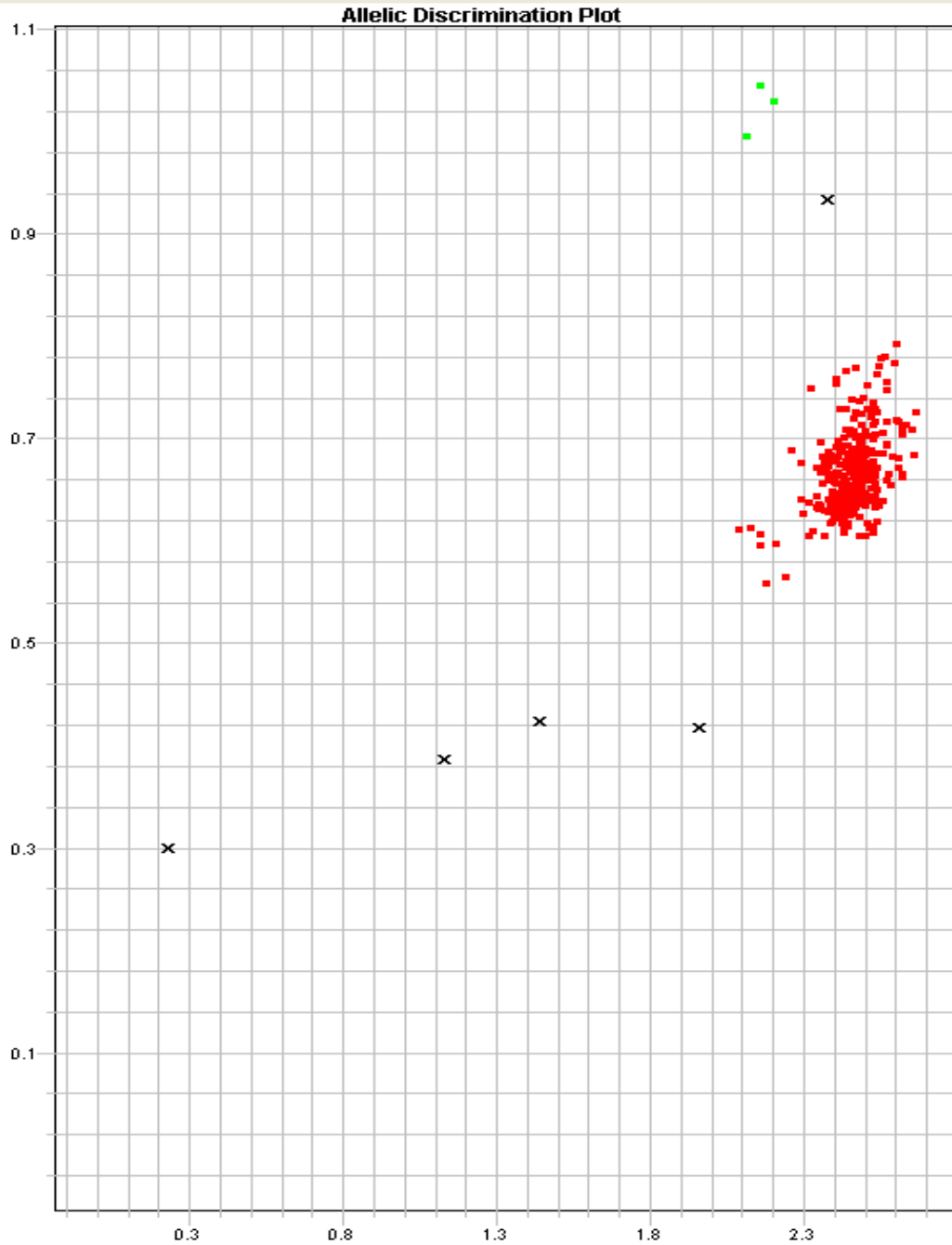
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Plate Name: UK_21

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



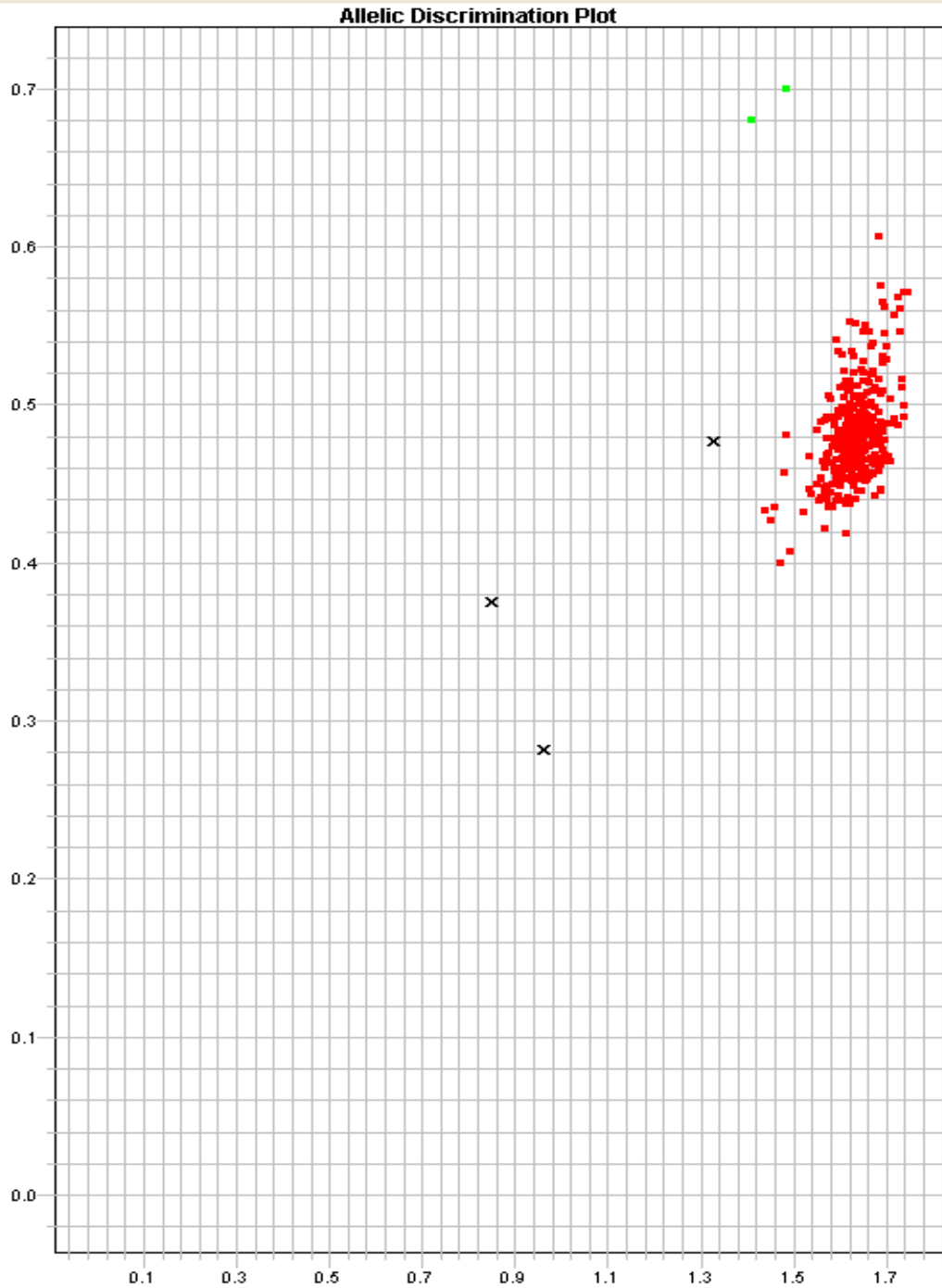
SNP: rs118204009

Plate Name: UK_22

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0



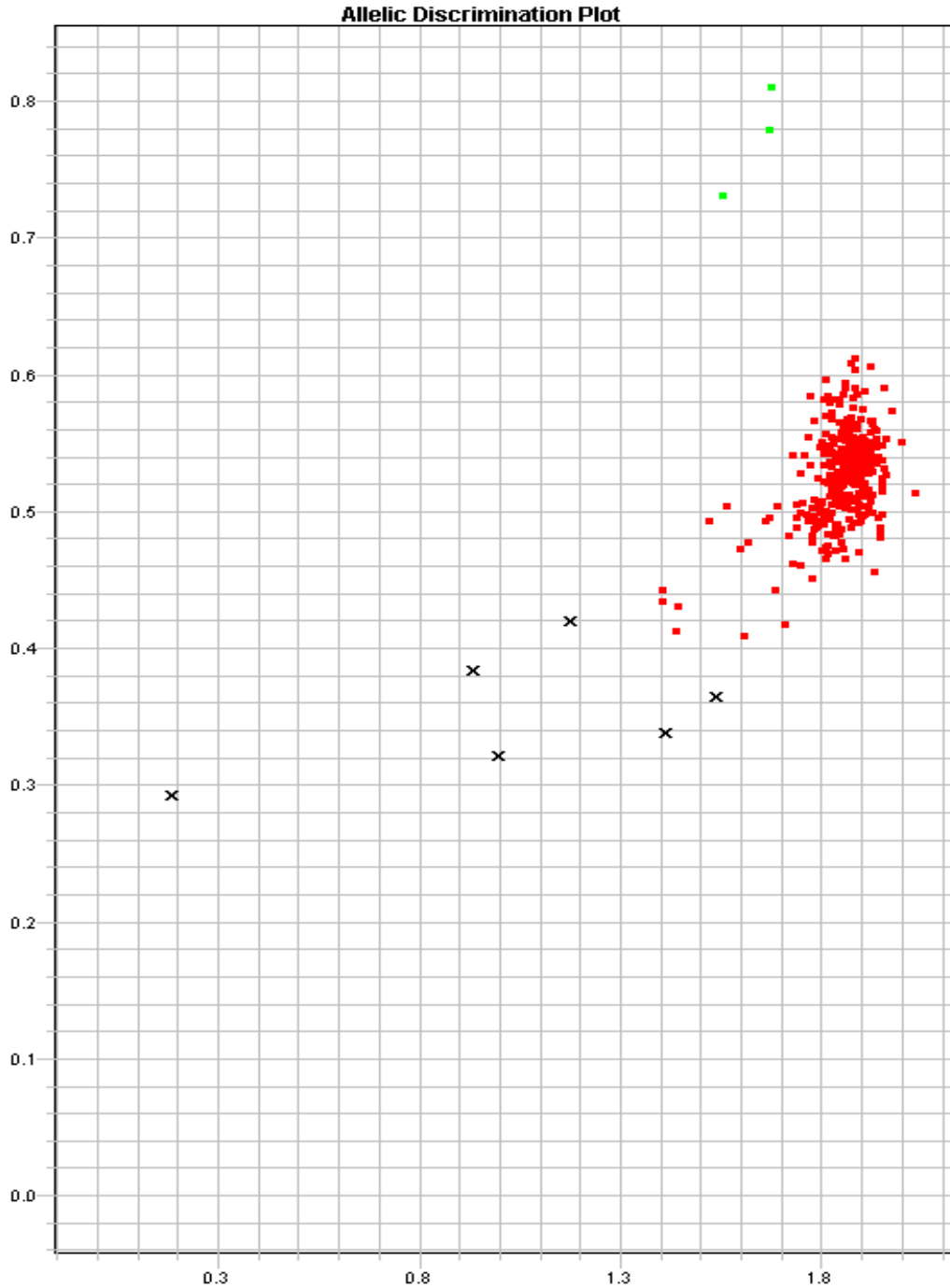
SNP: rs118204009

Plate Name: UK_23

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 3

New Heterozygote's on plate: 0



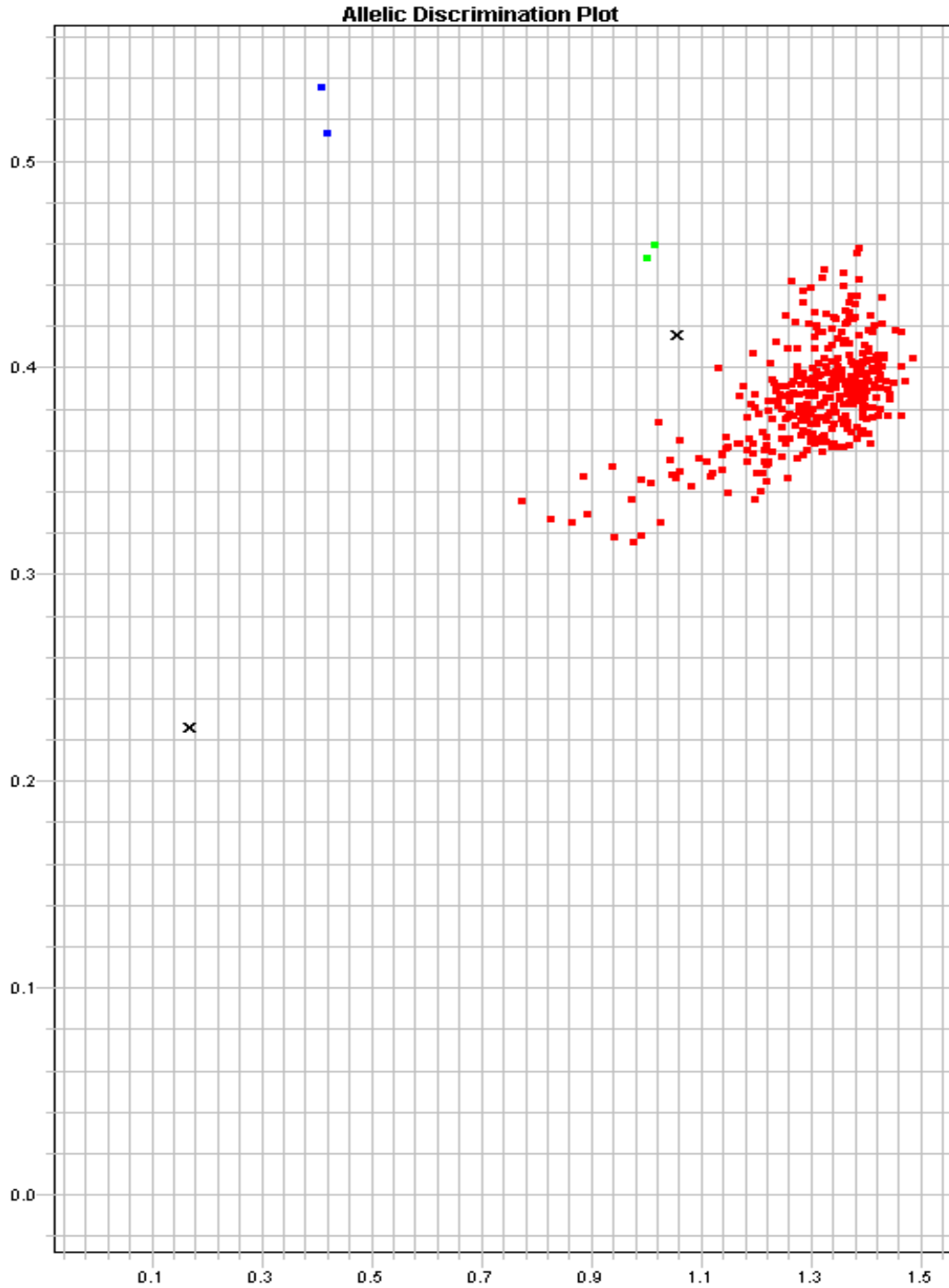
SNP: rs118204009

Plate Name: UK_24

Homozygote Controls on plate: 2

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0



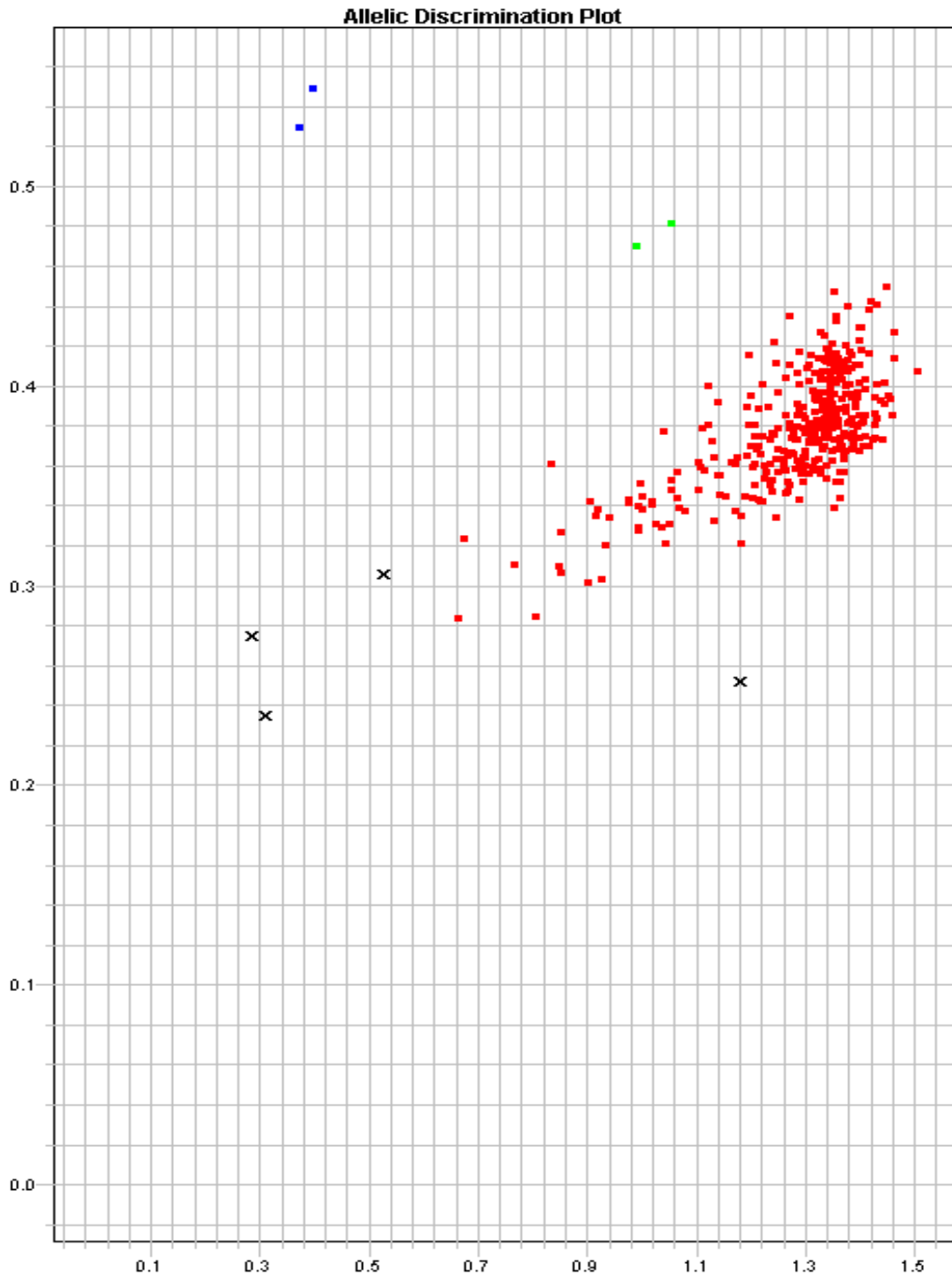
SNP: rs118204009

Plate Name: UK_25

Homozygote Controls on plate: 2

Heterozygote Controls on plate: 2

New Heterozygote's on plate: 0



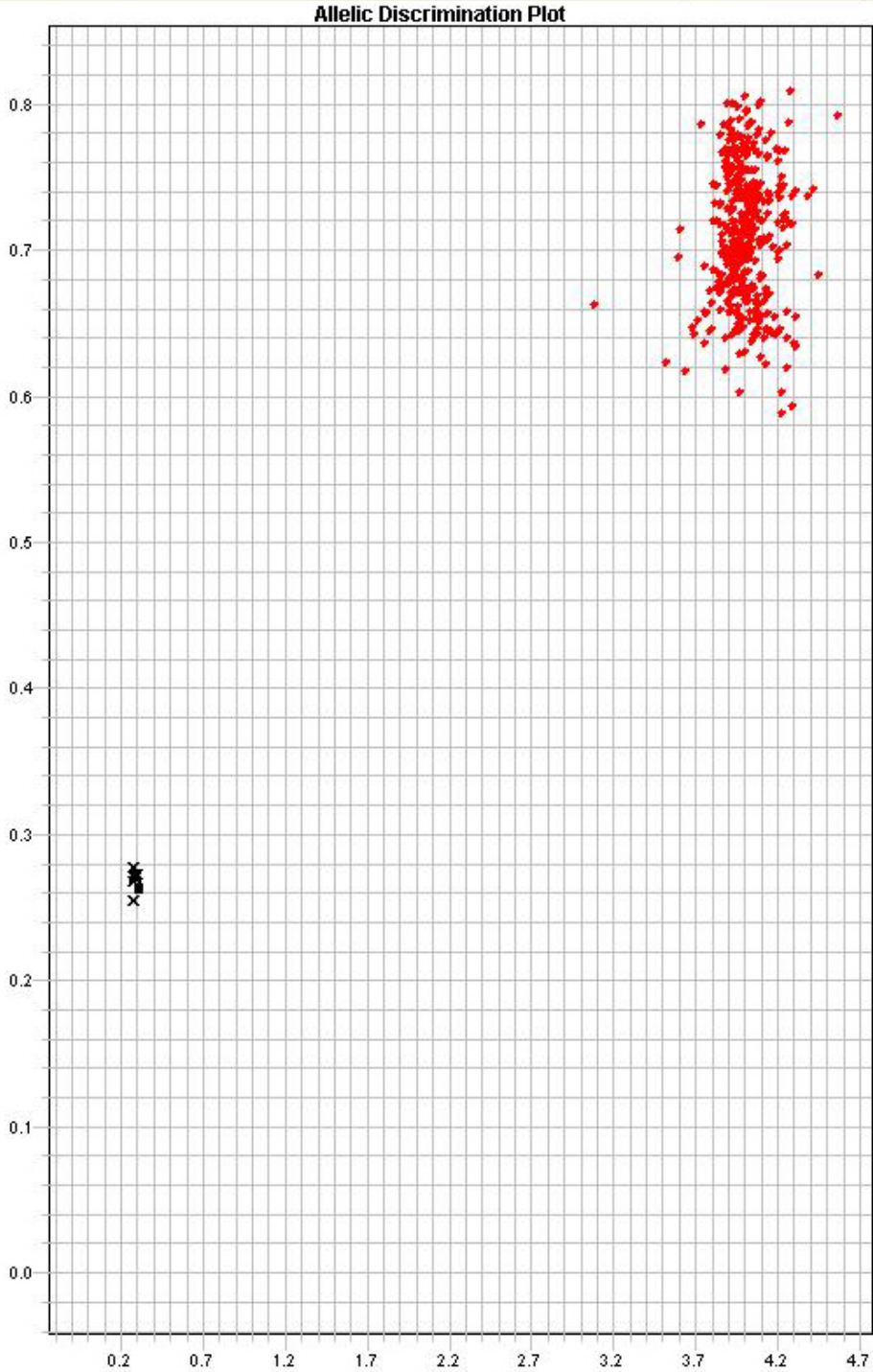
SNP: rs118204009

Plate Name: USA_1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204009

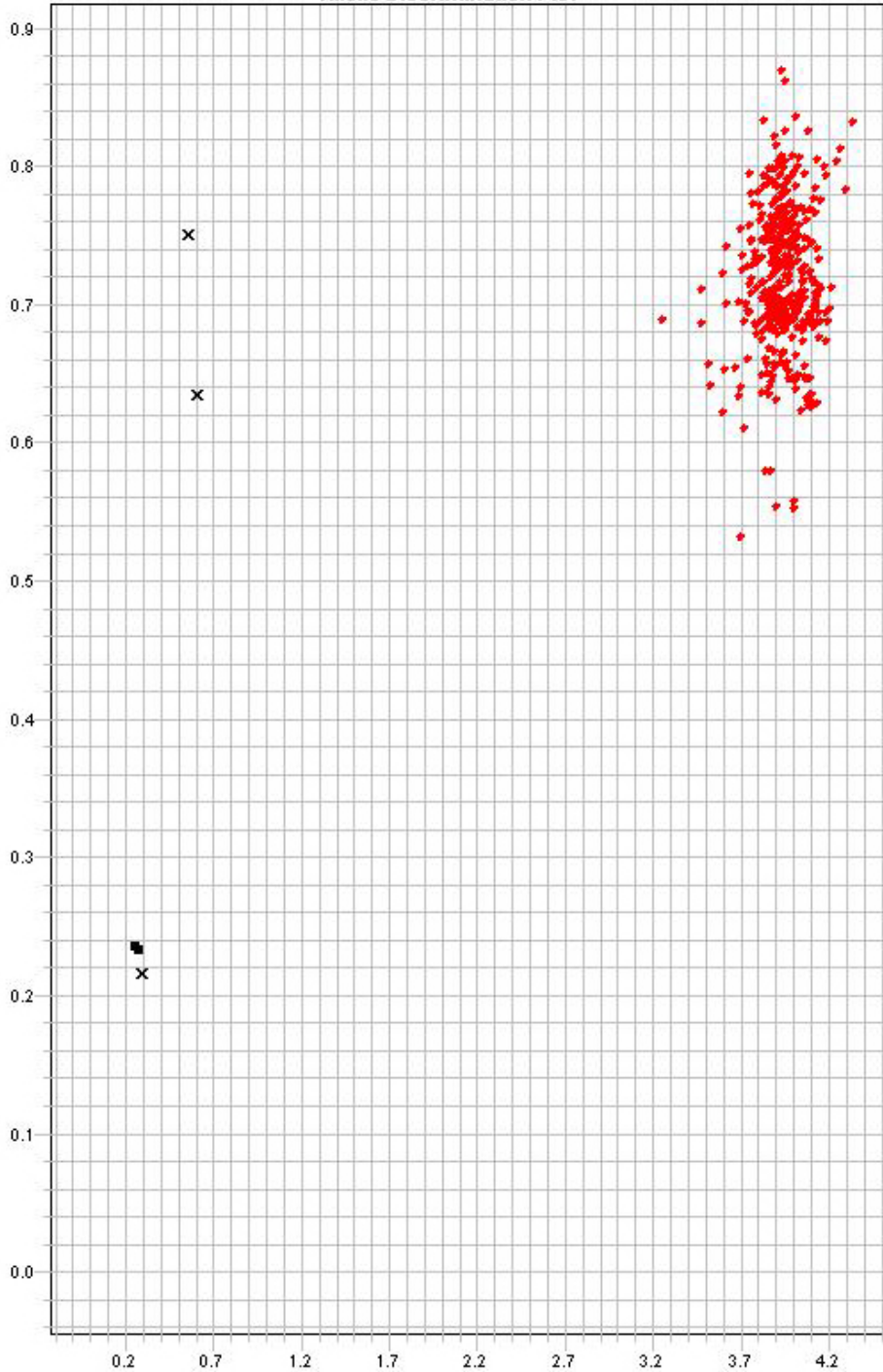
Plate Name: USA_2

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



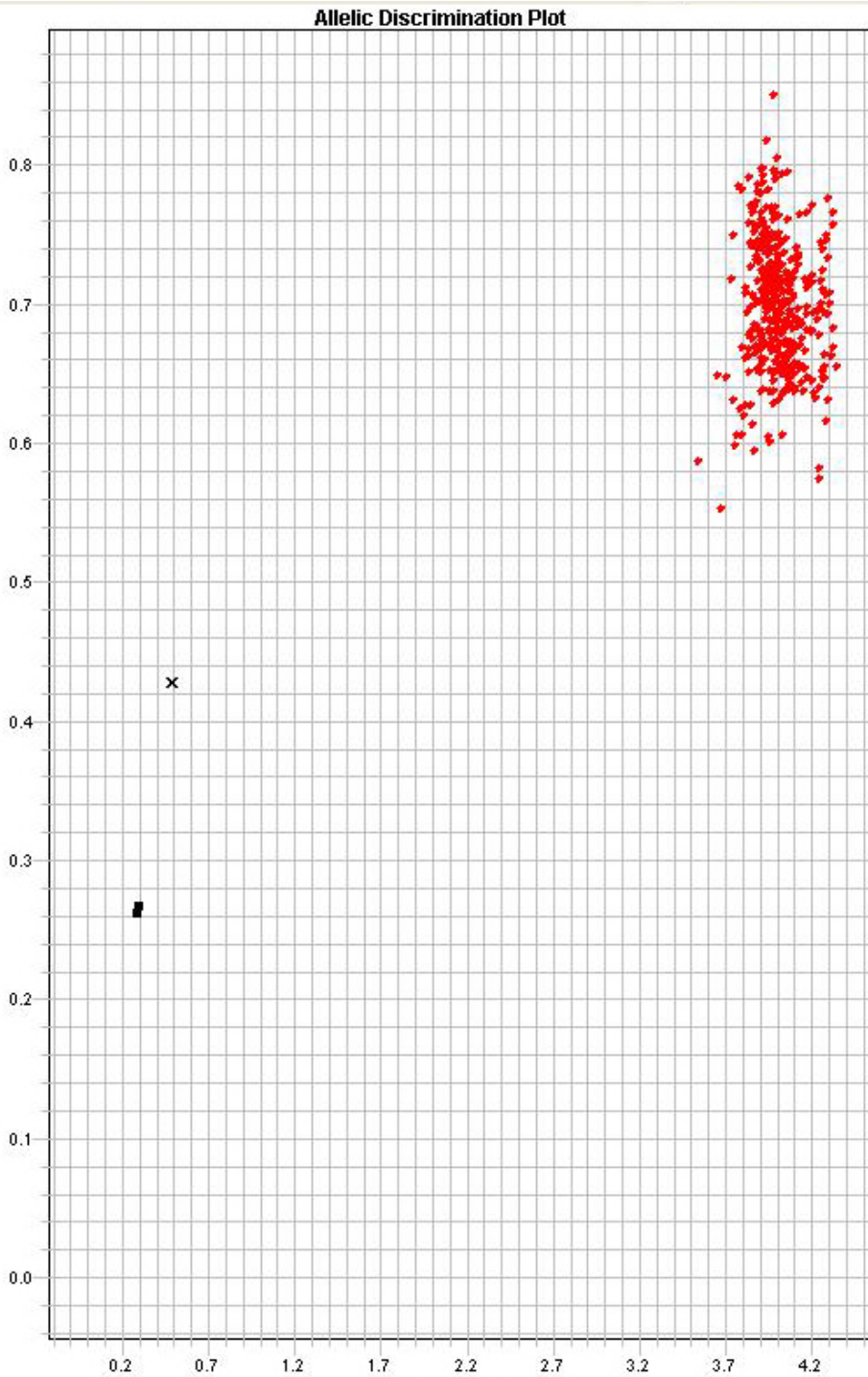
SNP: rs118204009

Plate Name: USA_3

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



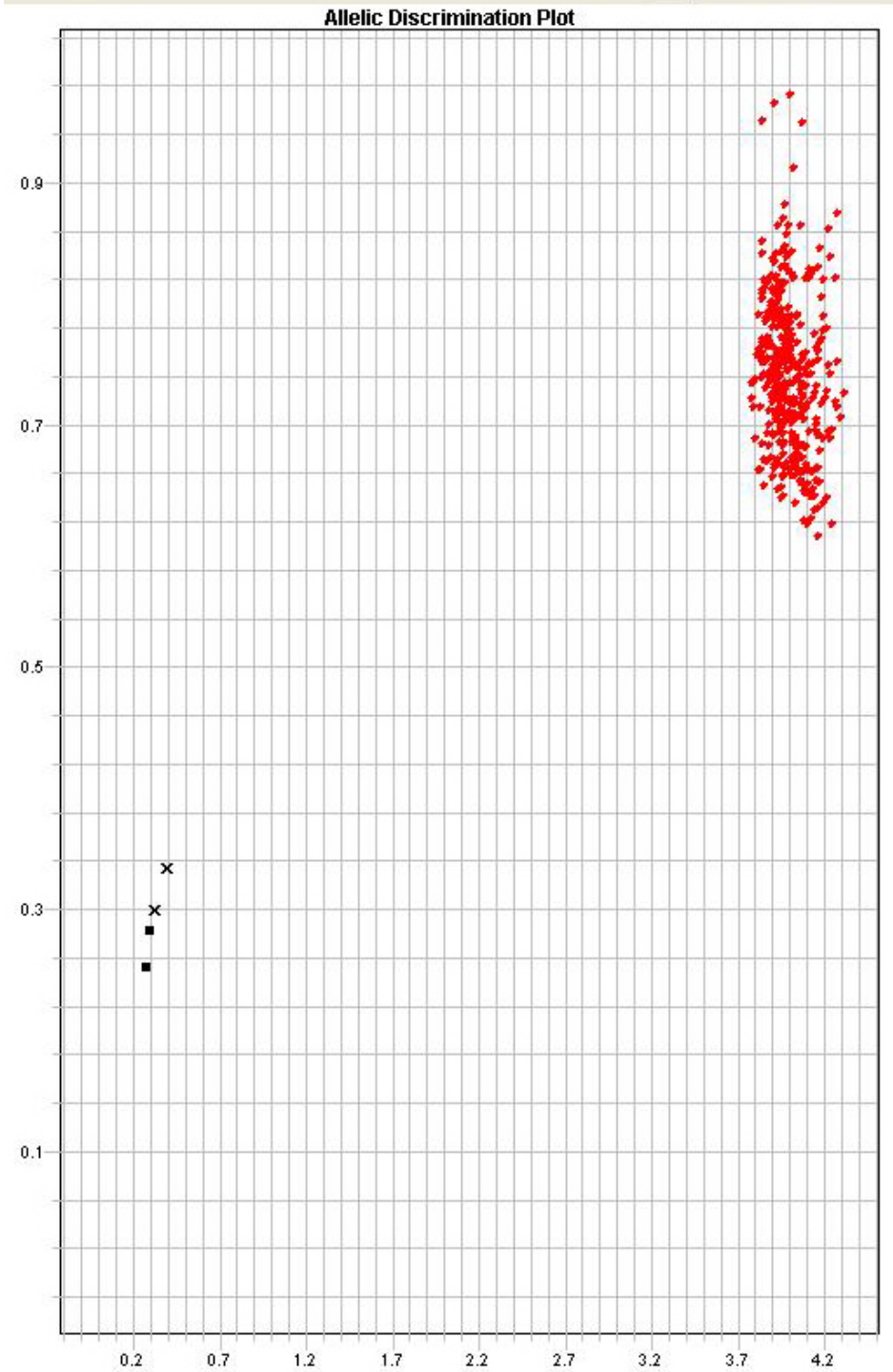
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Plate Name: USA_4

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



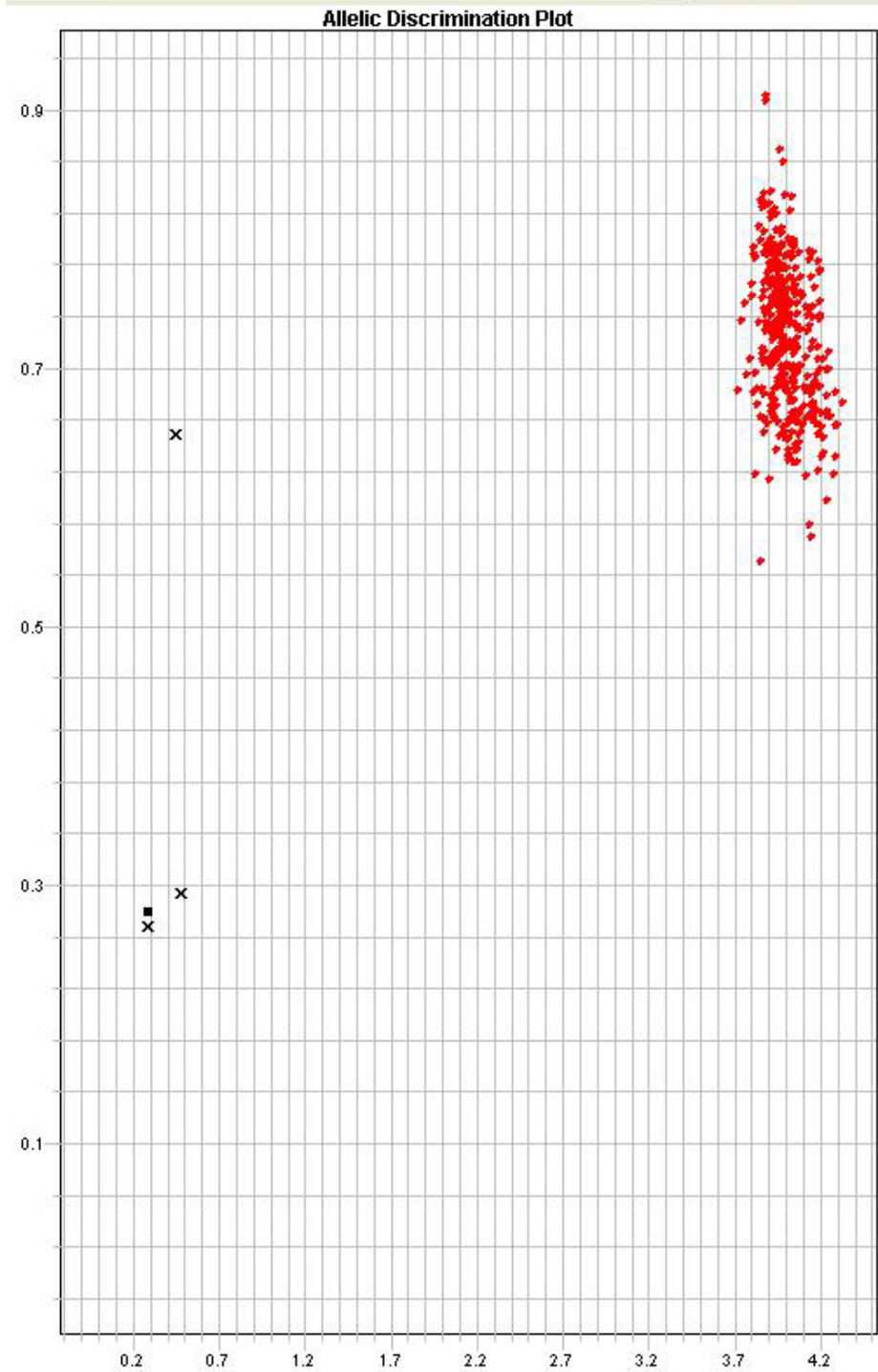
SNP: rs118204009

Plate Name: USA_5

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



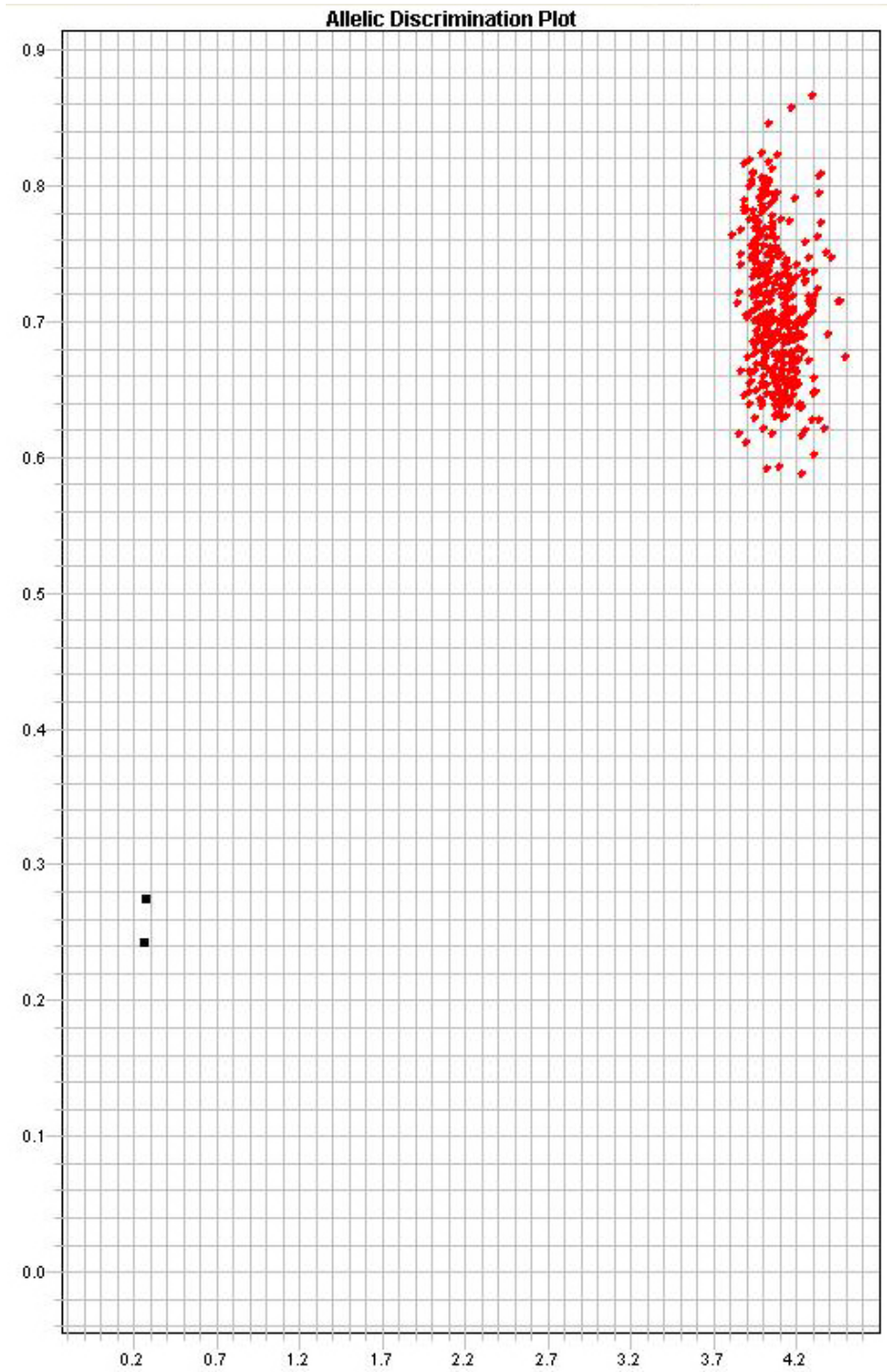
SNP: rs118204009

Plate Name: USA_6

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



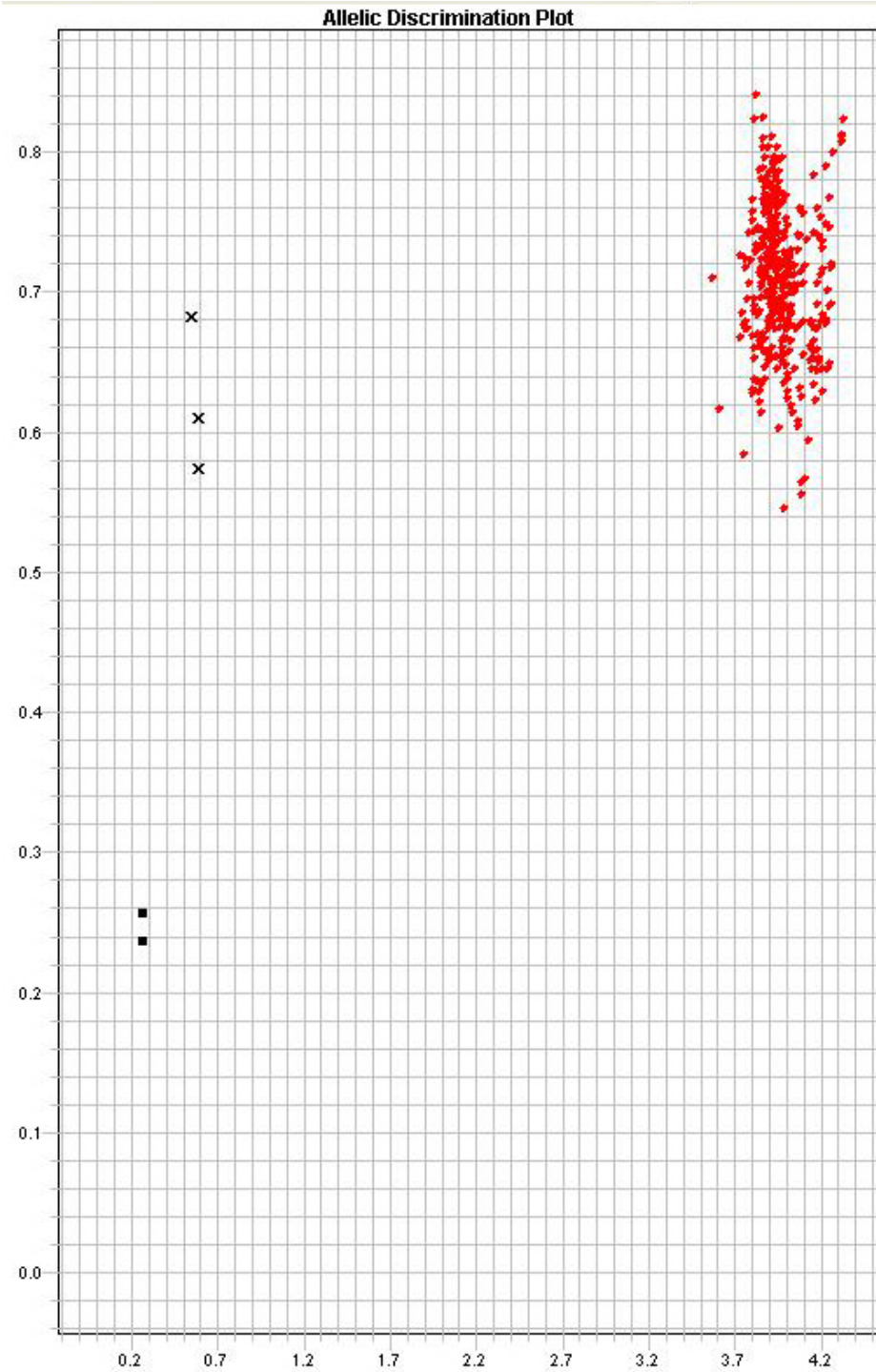
SNP: rs118204009

Plate Name: USA_7

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



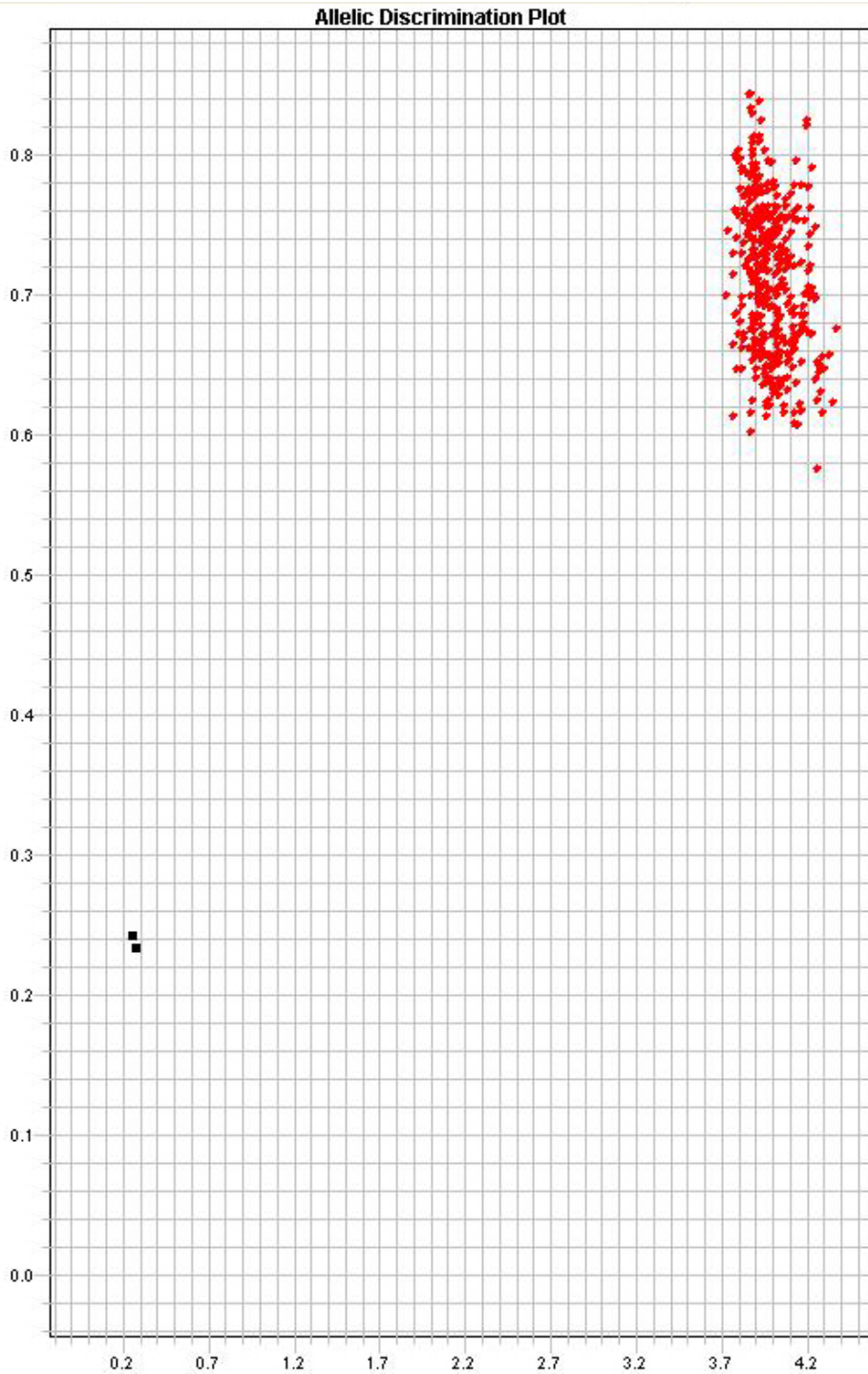
SNP: rs118204009

Plate Name: USA_8

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



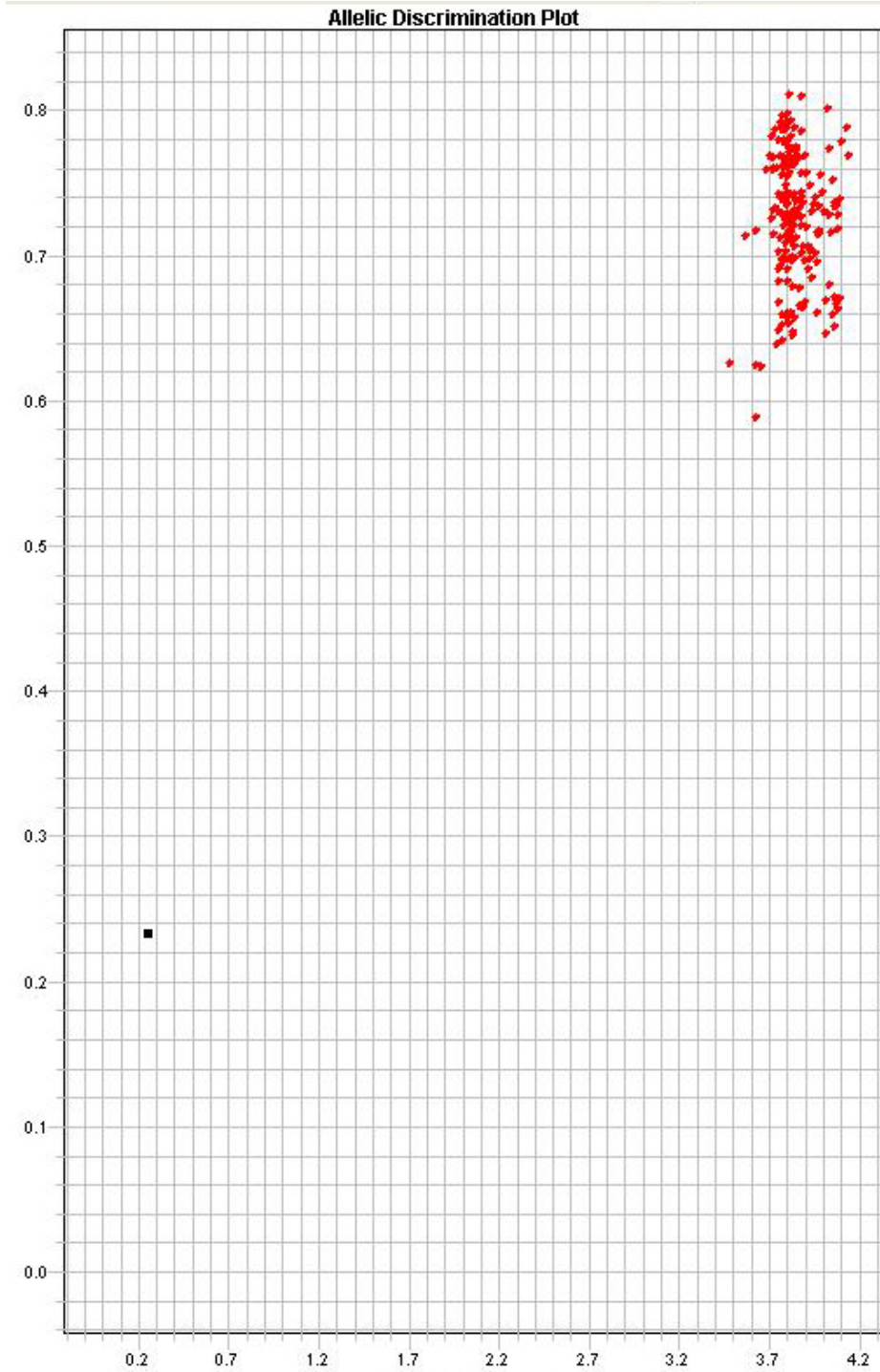
SNP: rs118204009

Plate Name: USA_9

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



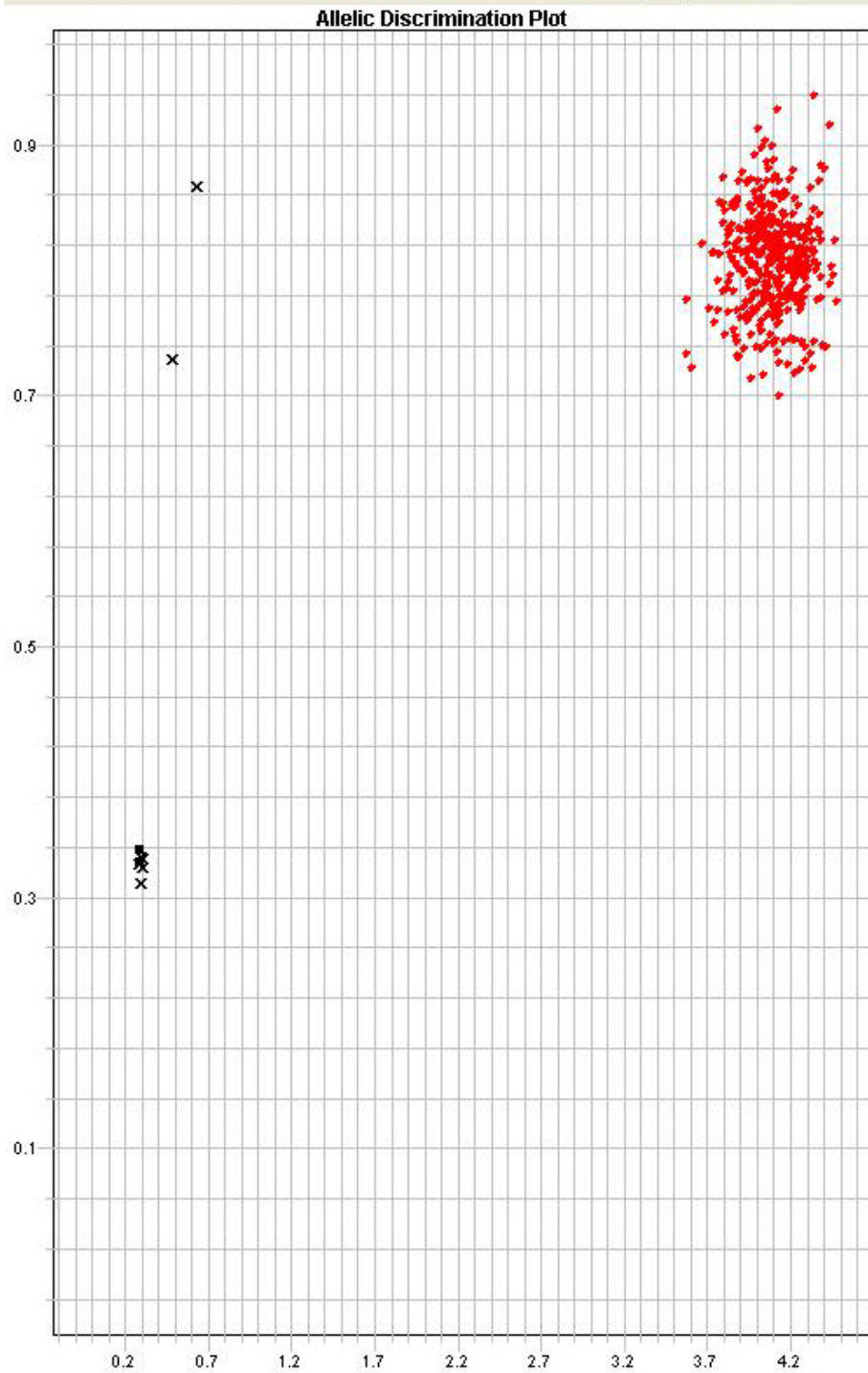
SNP: rs118204009

Plate Name: USA_10

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



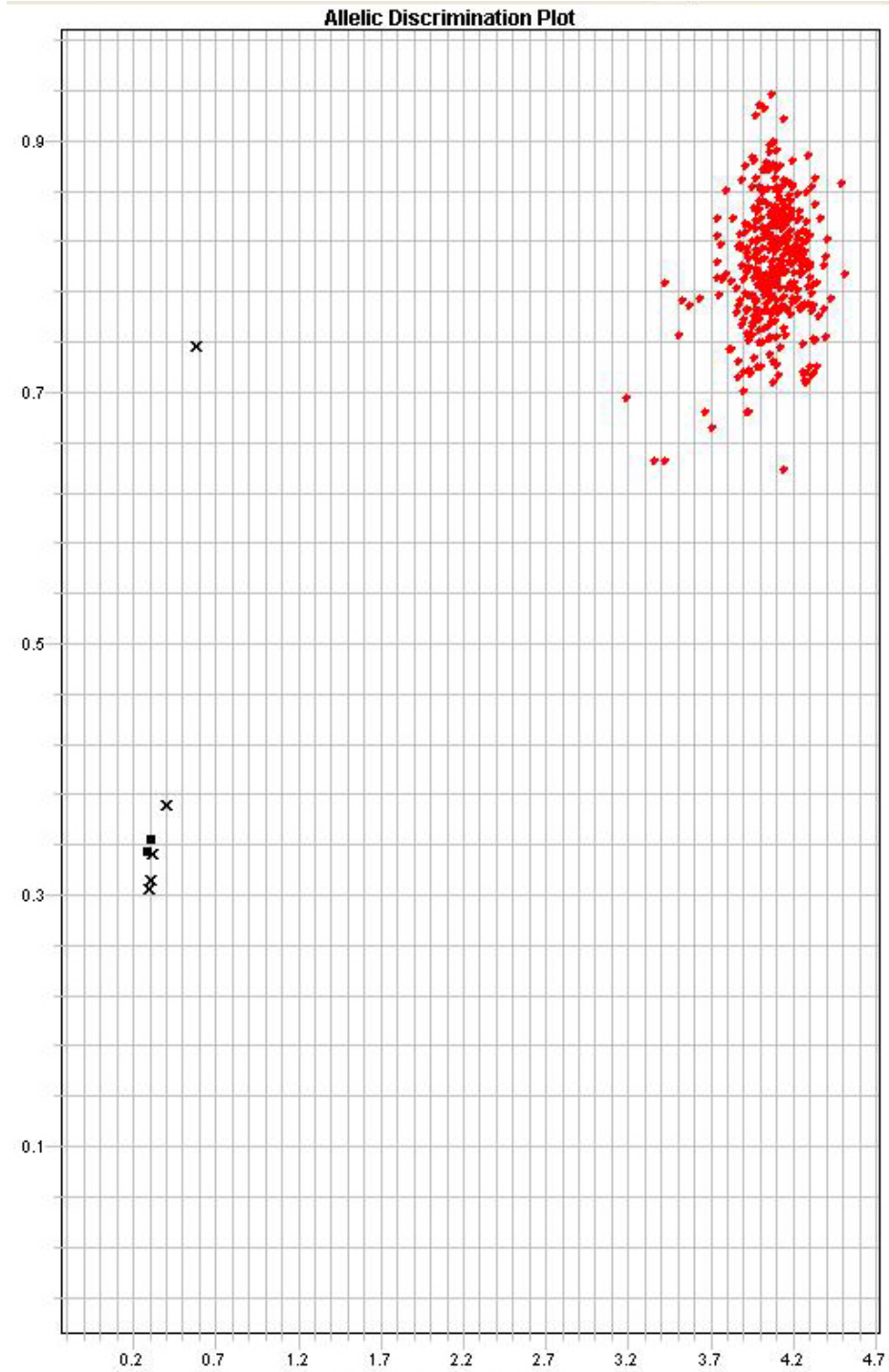
SNP: rs118204009

Plate Name: USA_11

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



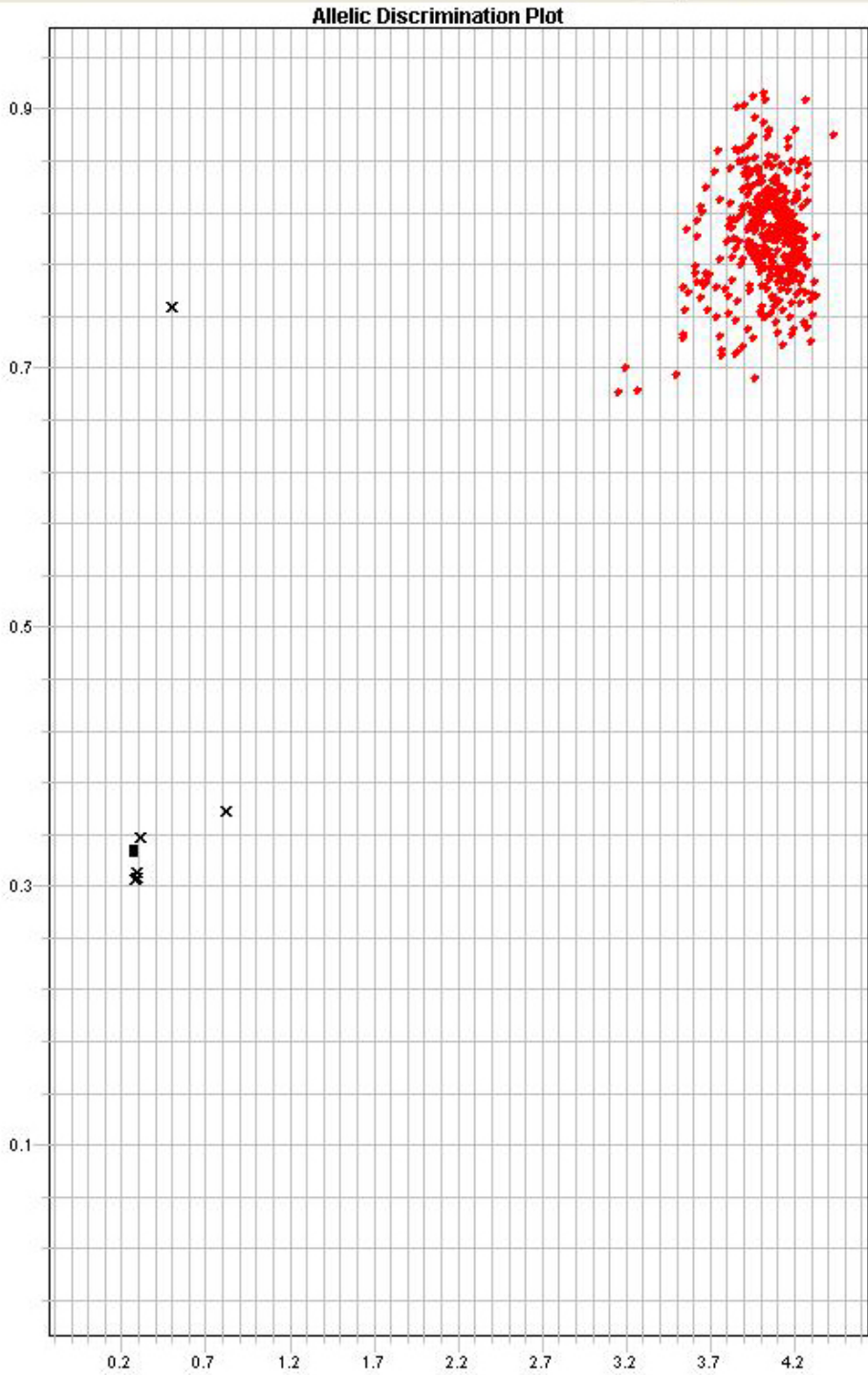
SNP: rs118204009

Plate Name: USA_12

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



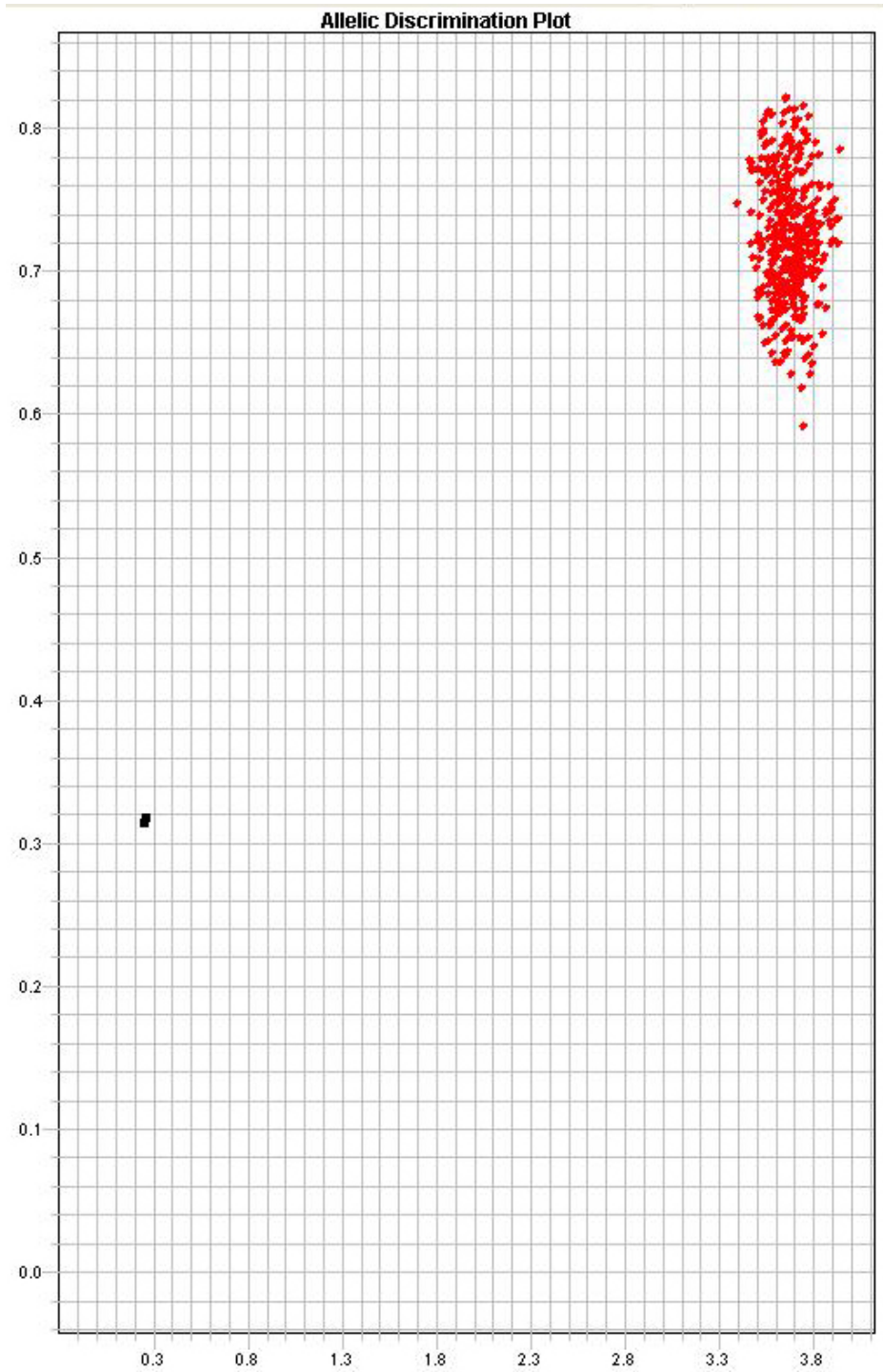
SNP: rs118204009

Plate Name: USA_13

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



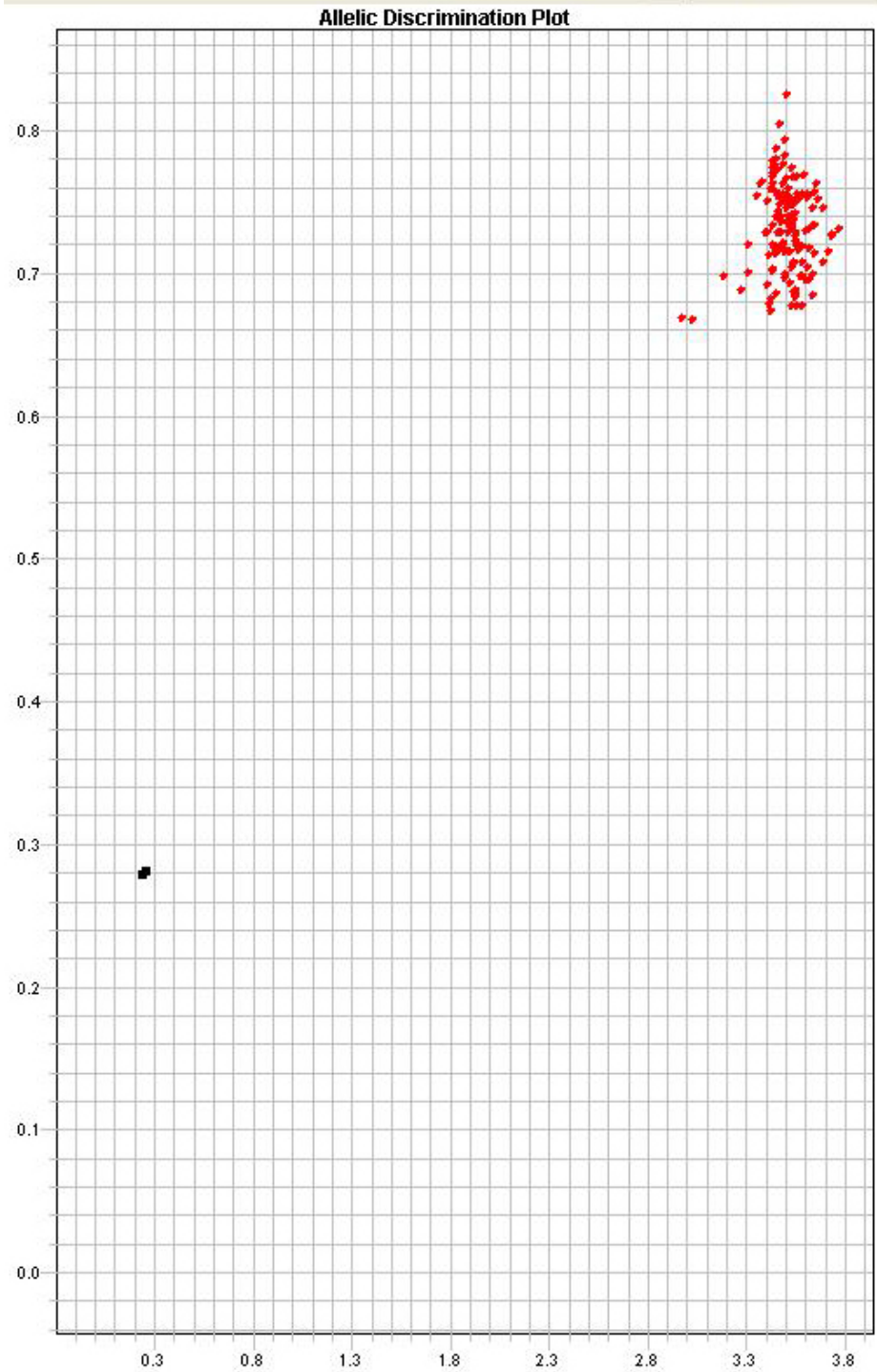
SNP: rs118204009

Plate Name: USA_14

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



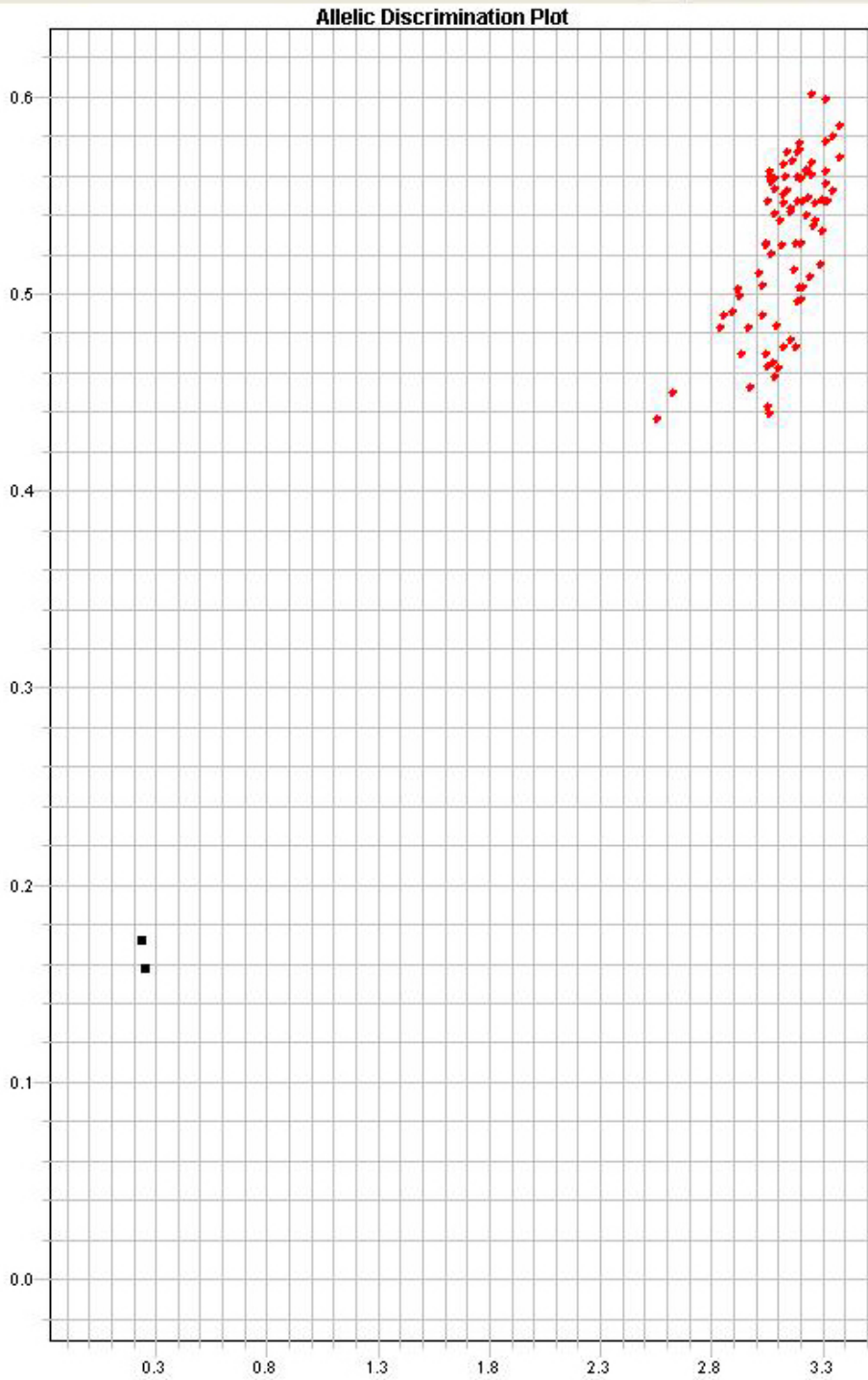
SNP: rs118204009

Plate Name: USA_reruns1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



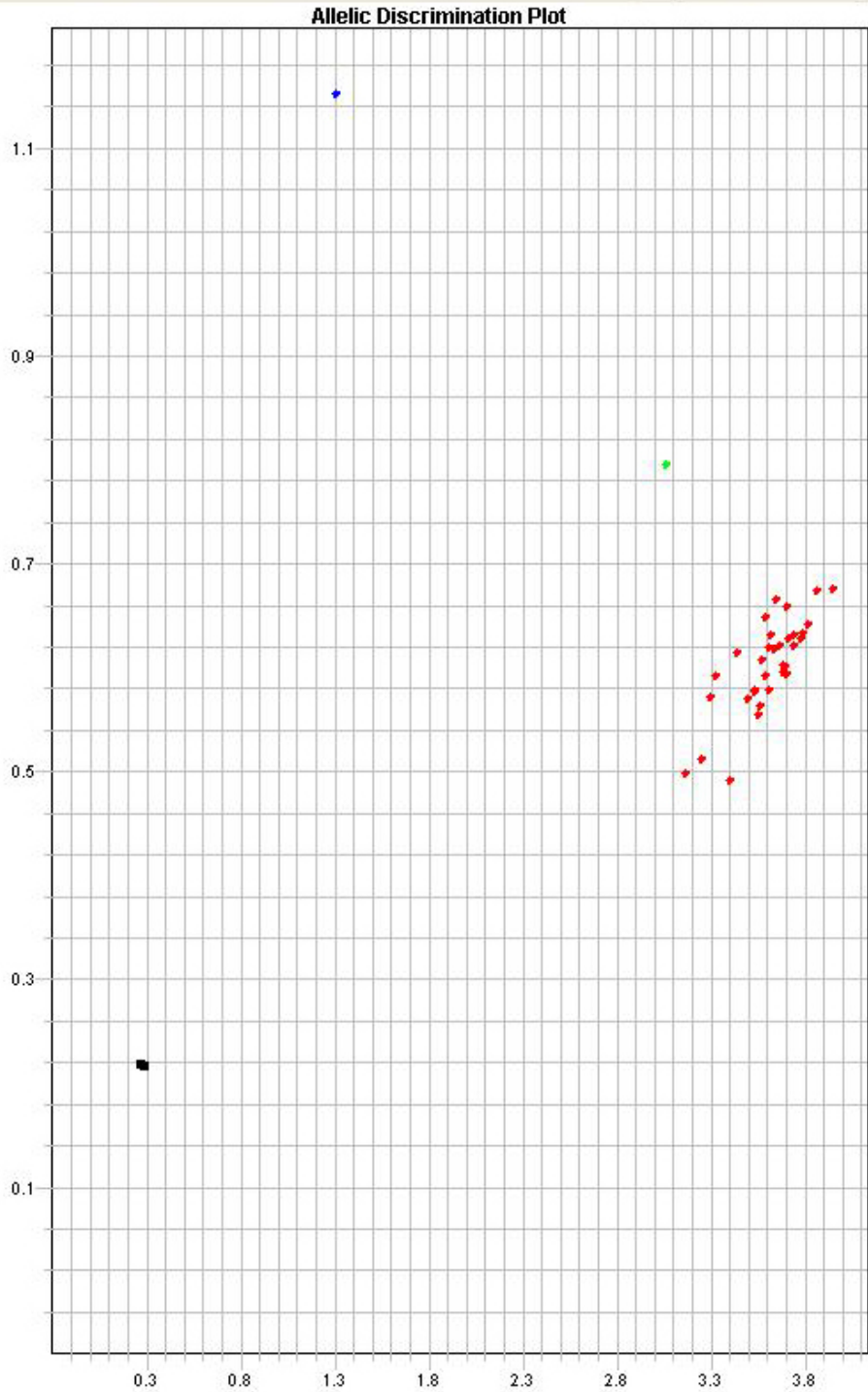
SNP: rs118204009

Plate Name: USA_reruns2

Homozygote Controls on plate: 1

Heterozygote Controls on plate: 1

New Heterozygote's on plate: 0



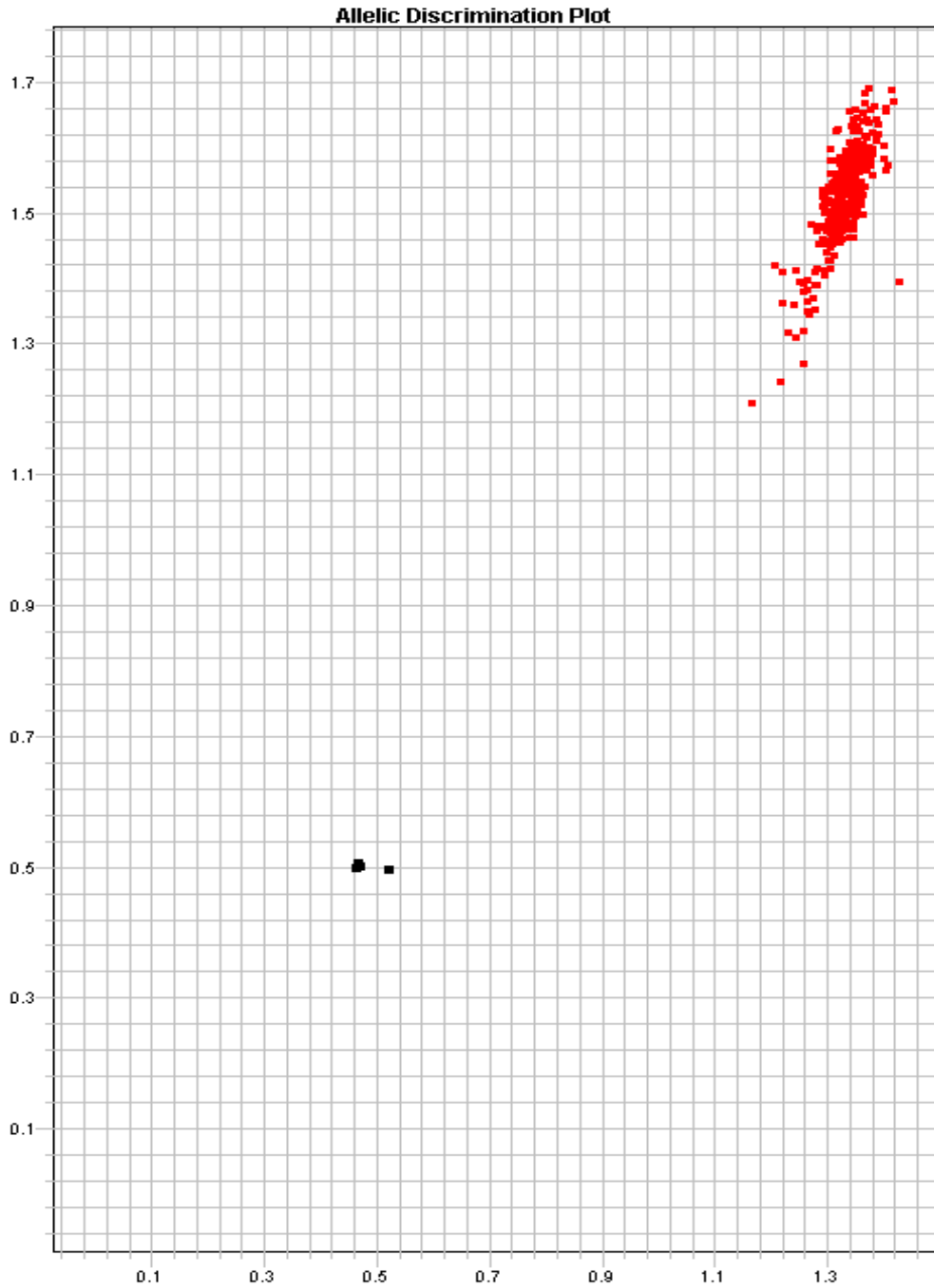
SNP: rs118204011

Plate Name: UK_1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



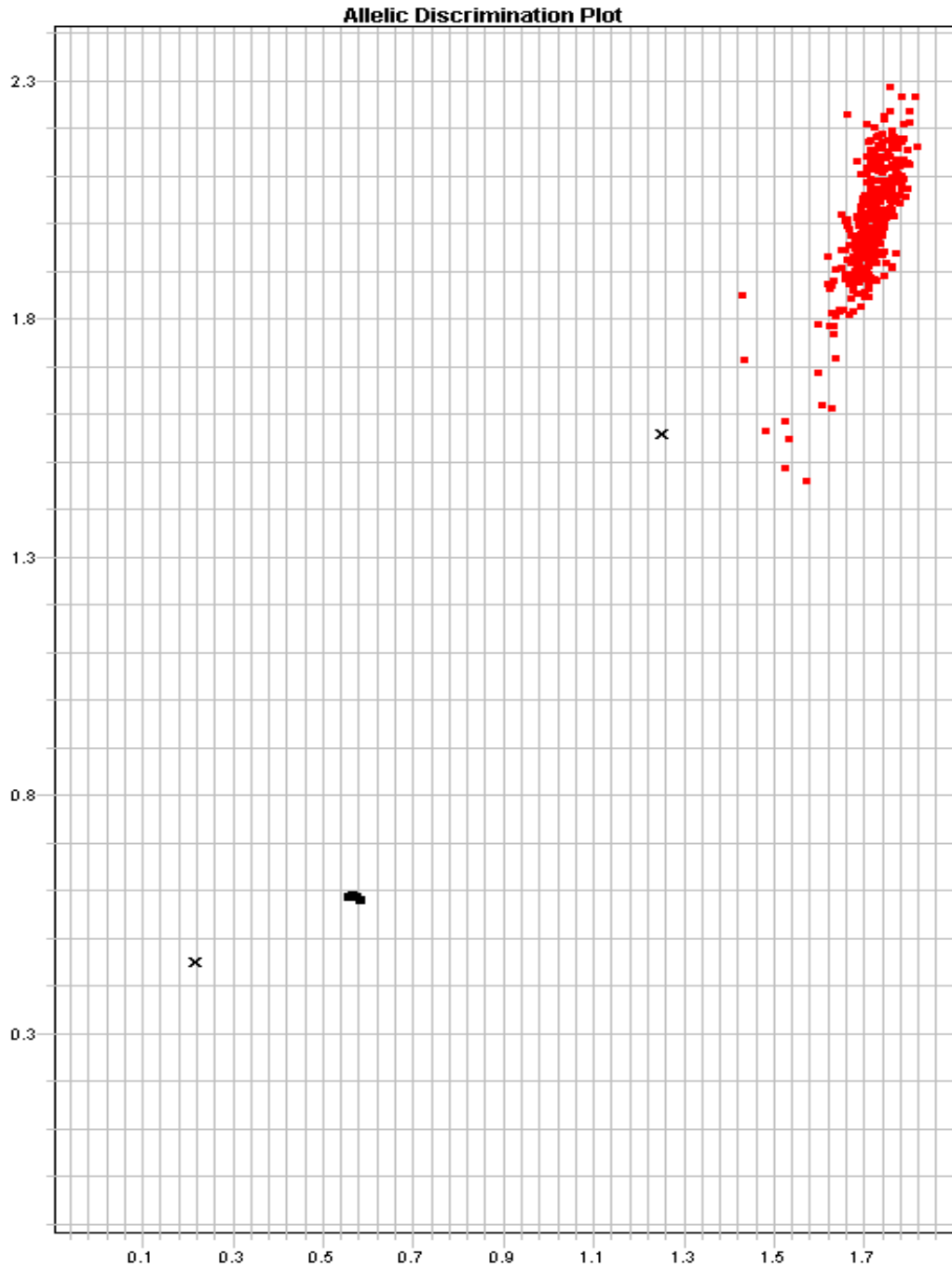
SNP: rs118204011

Plate Name: UK_2

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204011

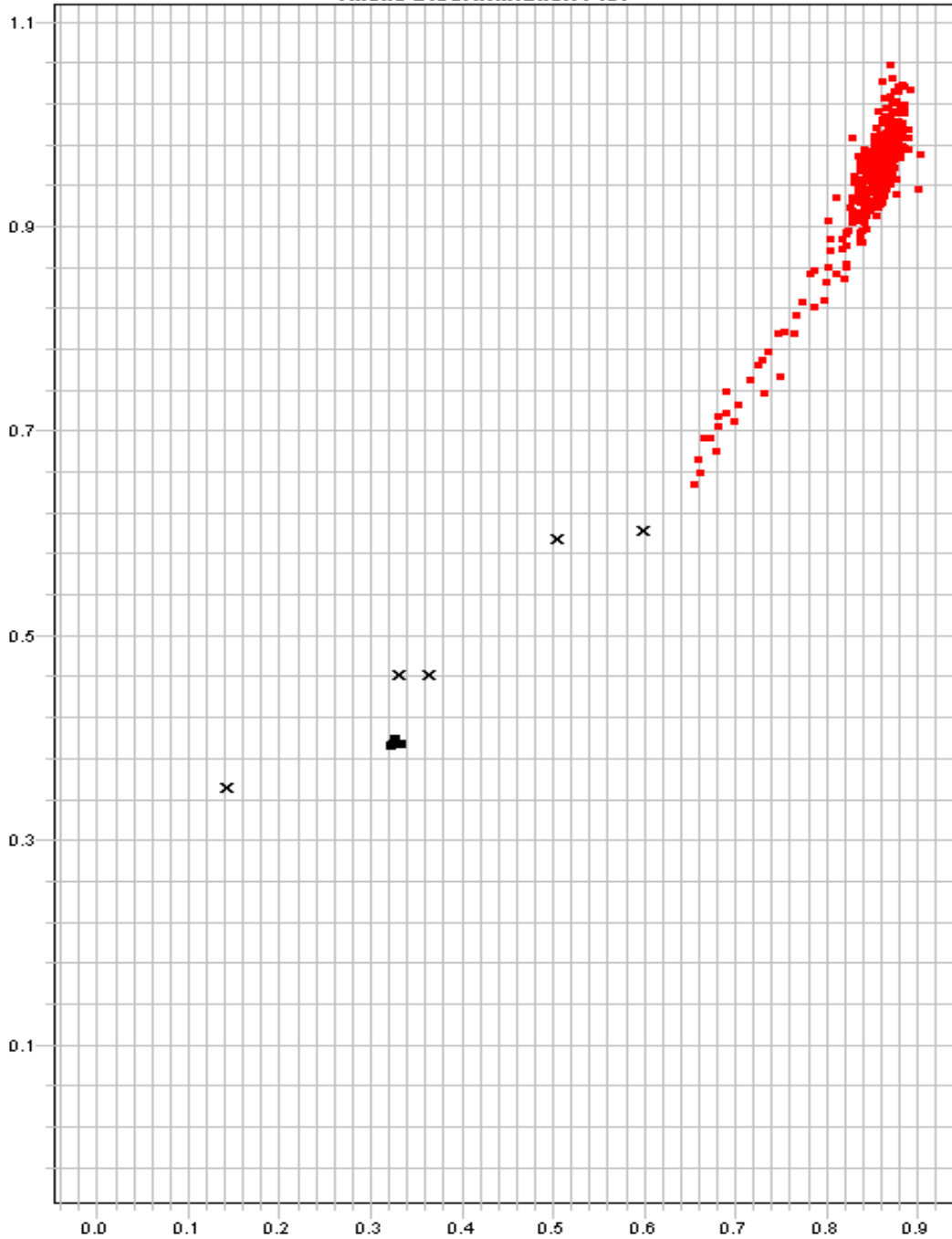
Plate Name: UK_3

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



SNP: rs118204011

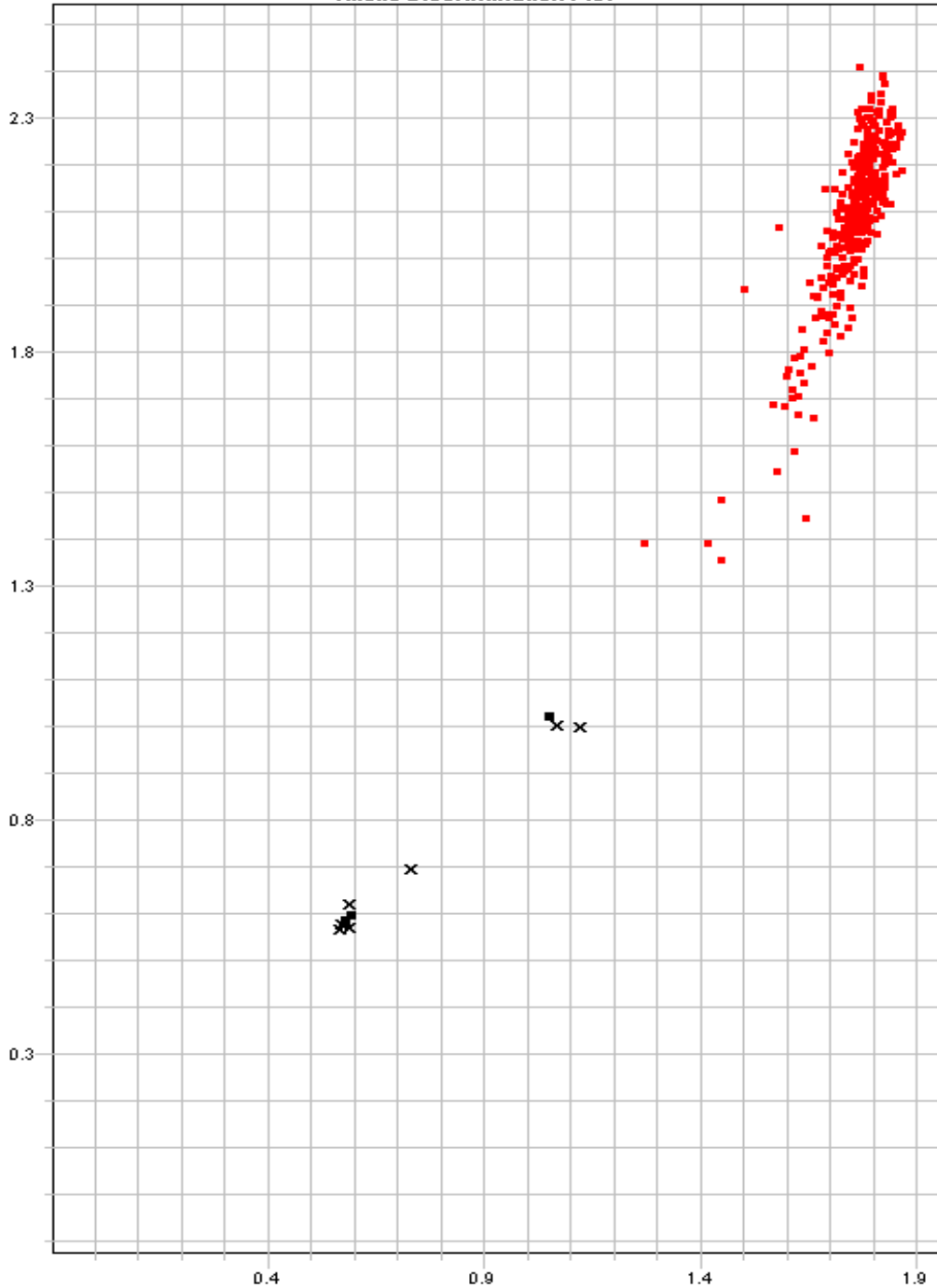
Plate Name: UK_4

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



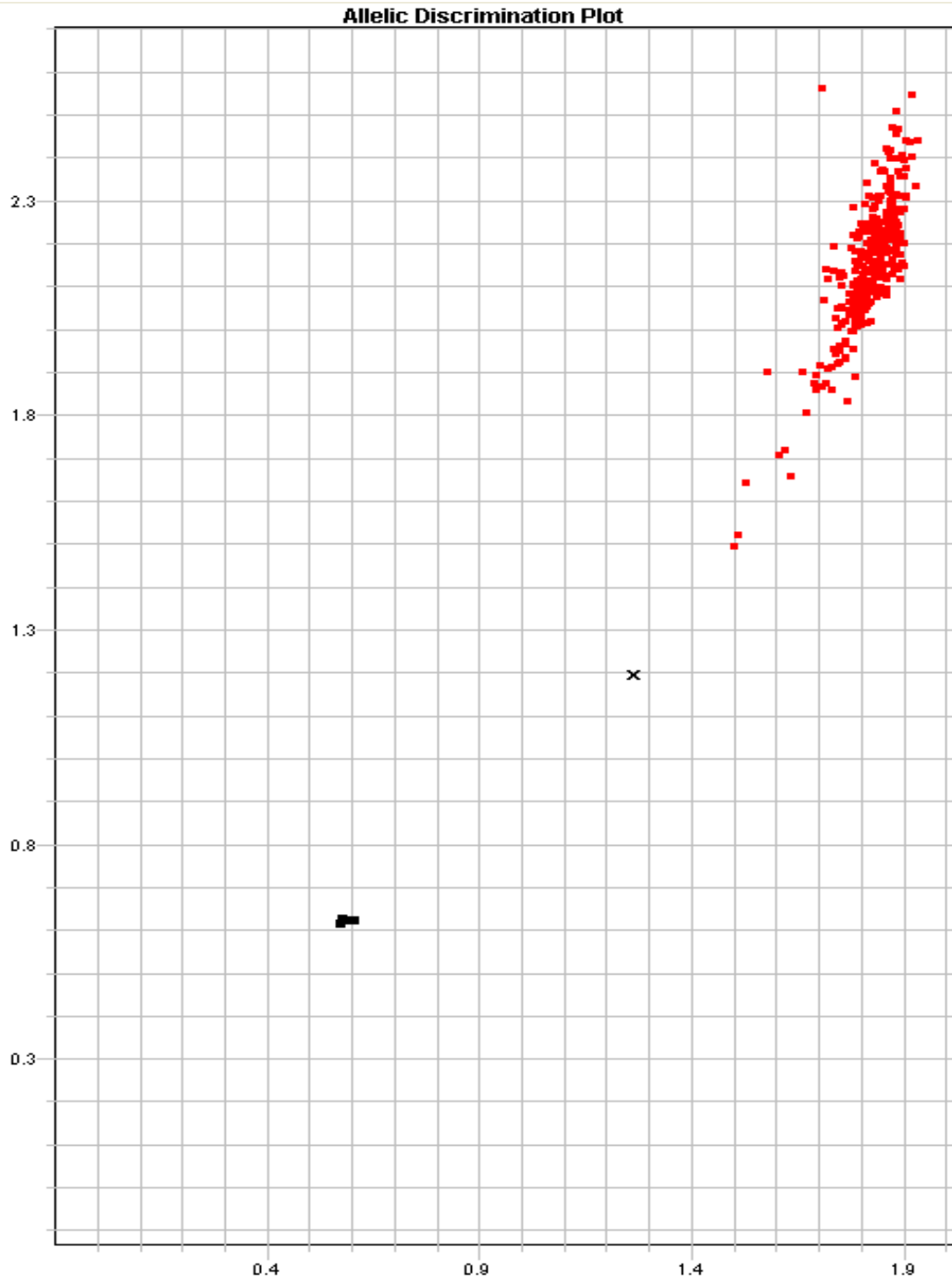
SNP: rs118204011

Plate Name: UK_5

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



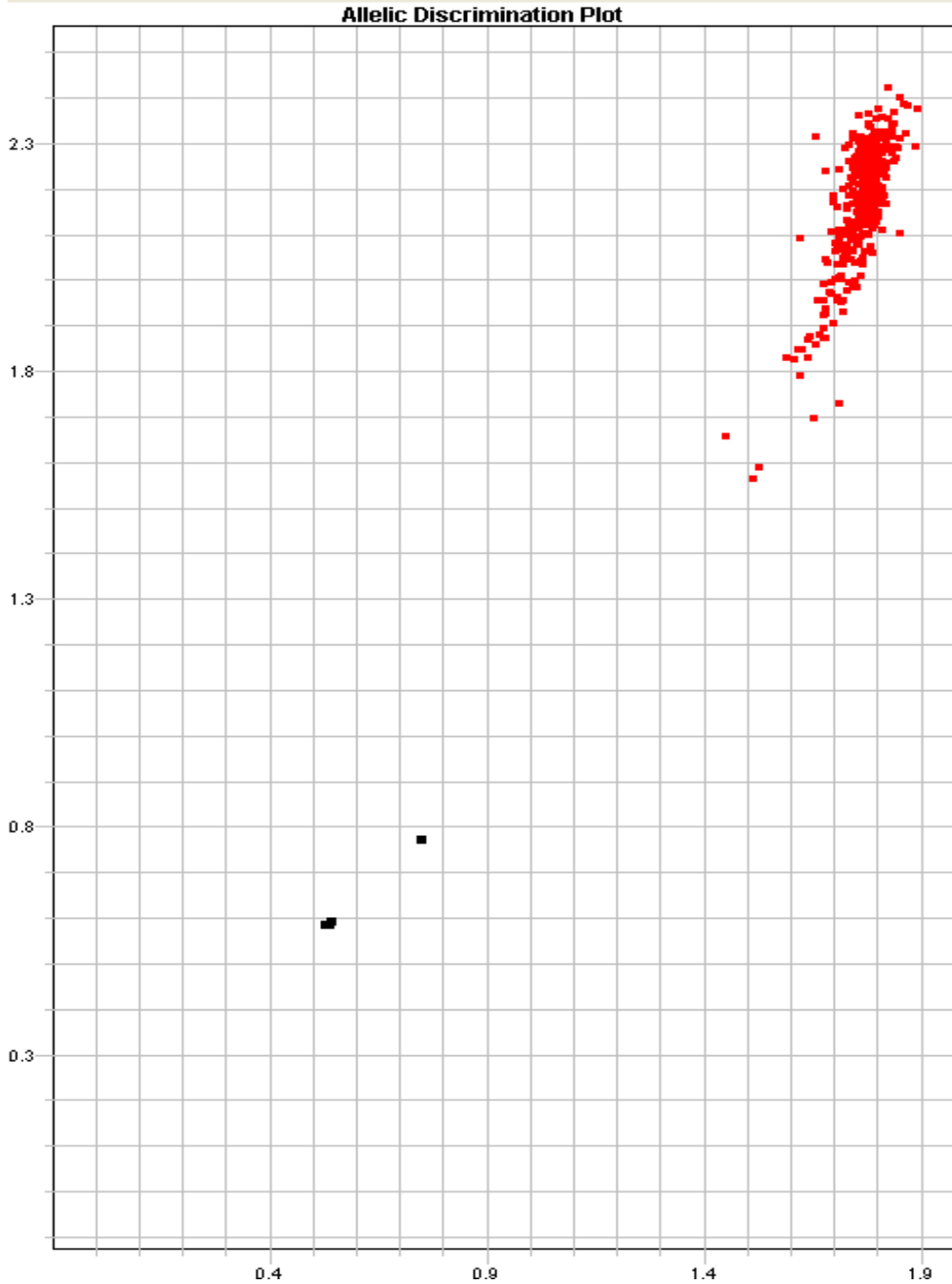
SNP: rs118204011

Plate Name: UK_6

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



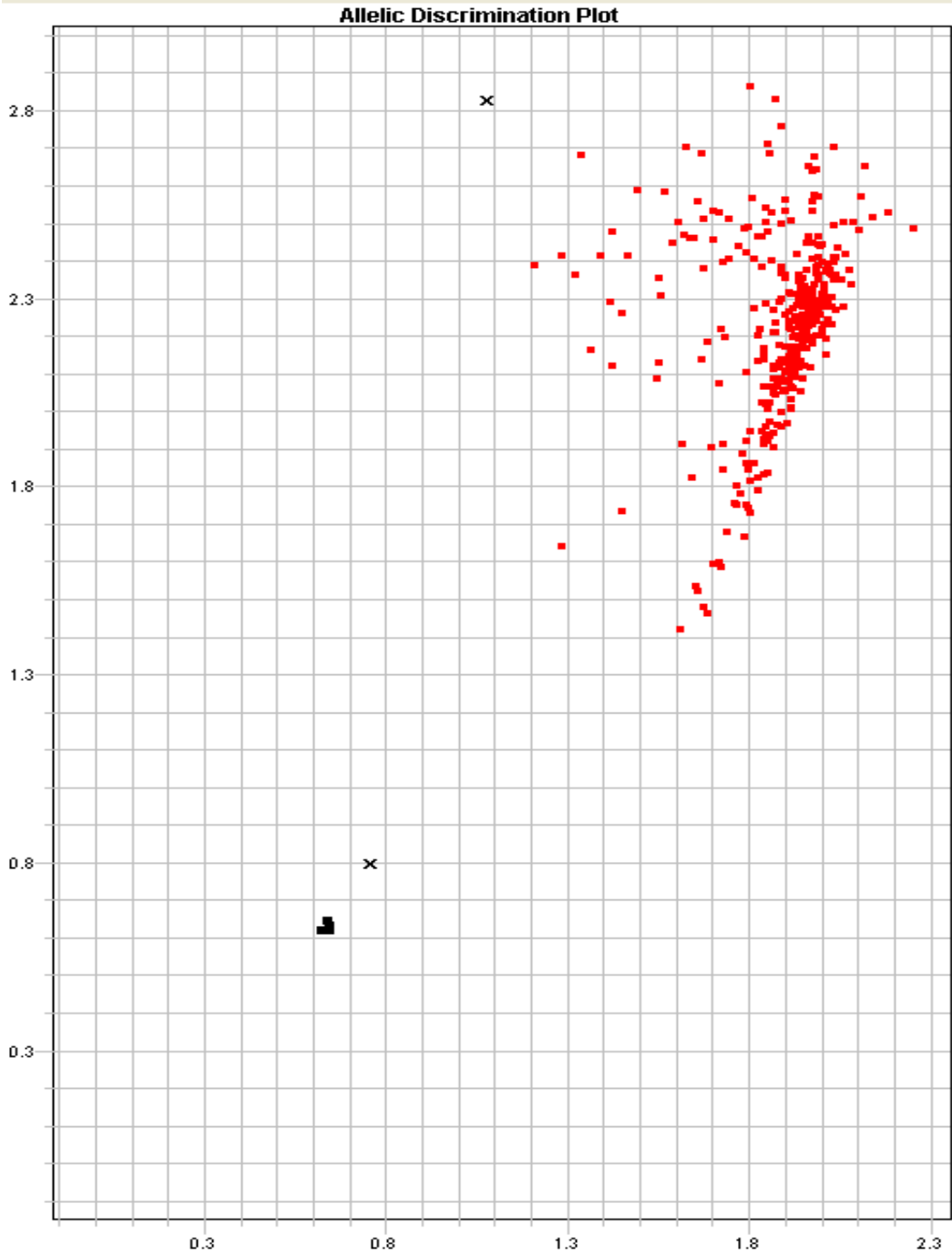
SNP: rs118204011

Plate Name: UK_7

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



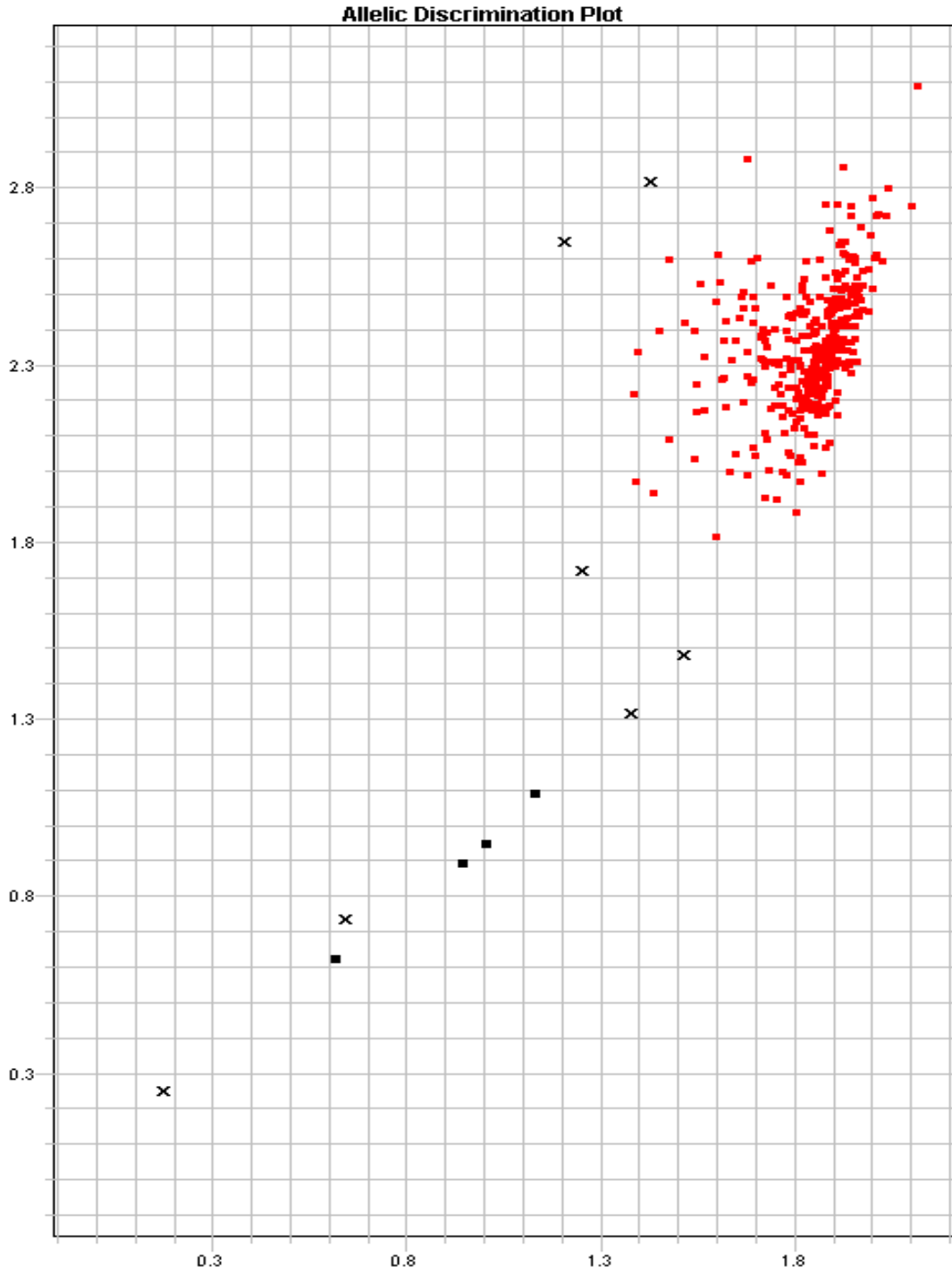
SNP: rs118204011

Plate Name: UK_8

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



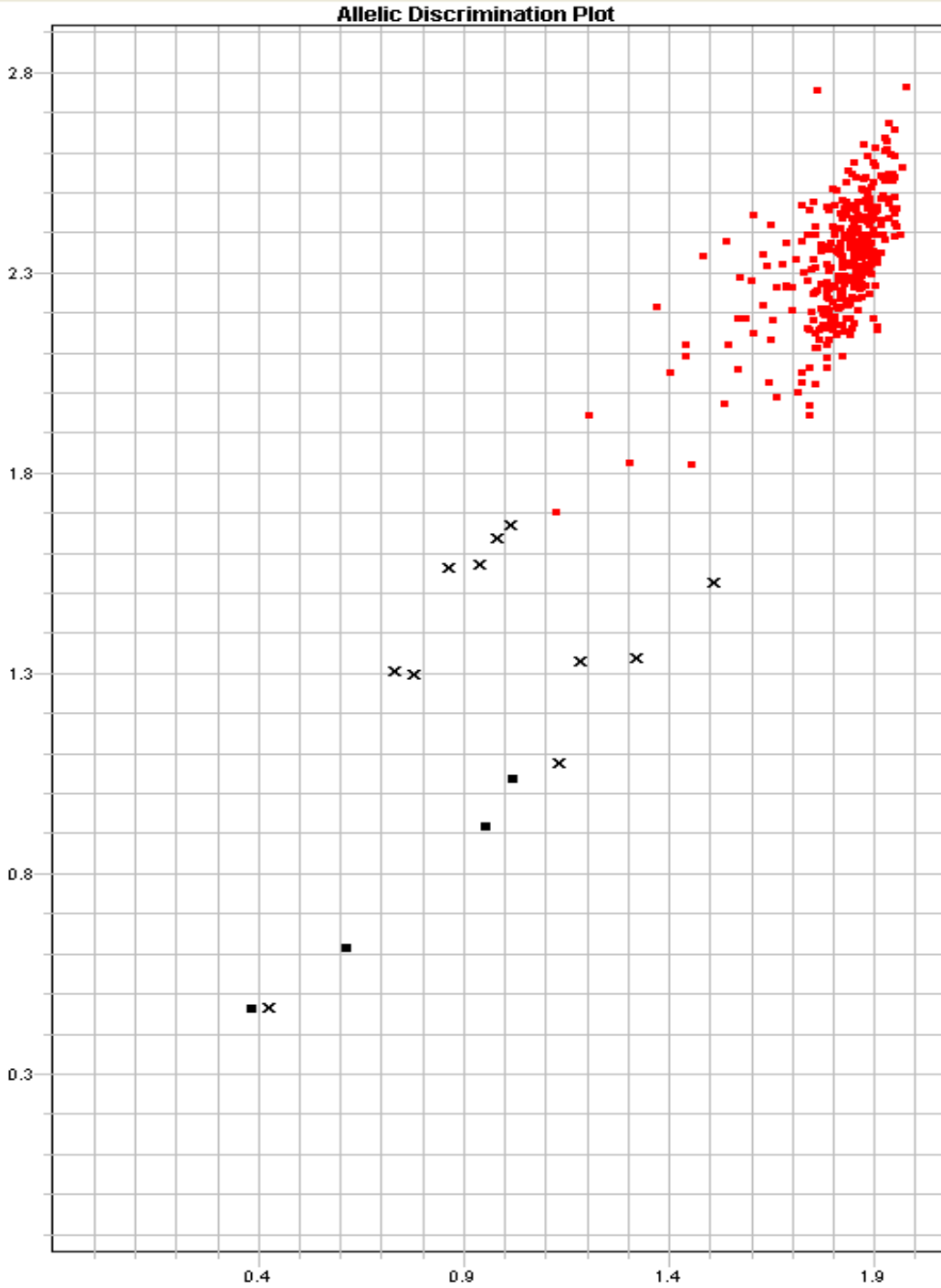
SNP: rs118204011

Plate Name: UK_9

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204011

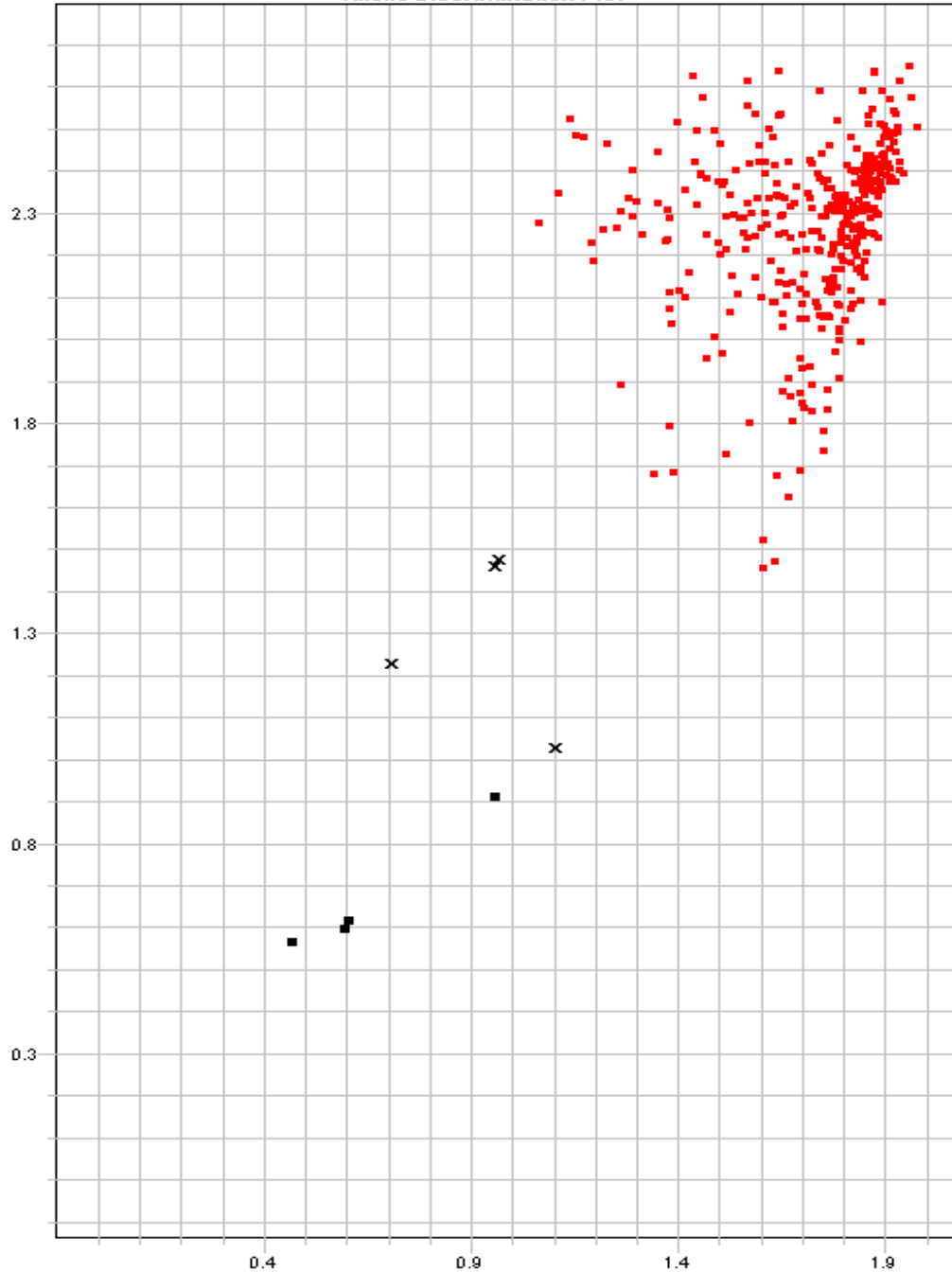
Plate Name: UK_10

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



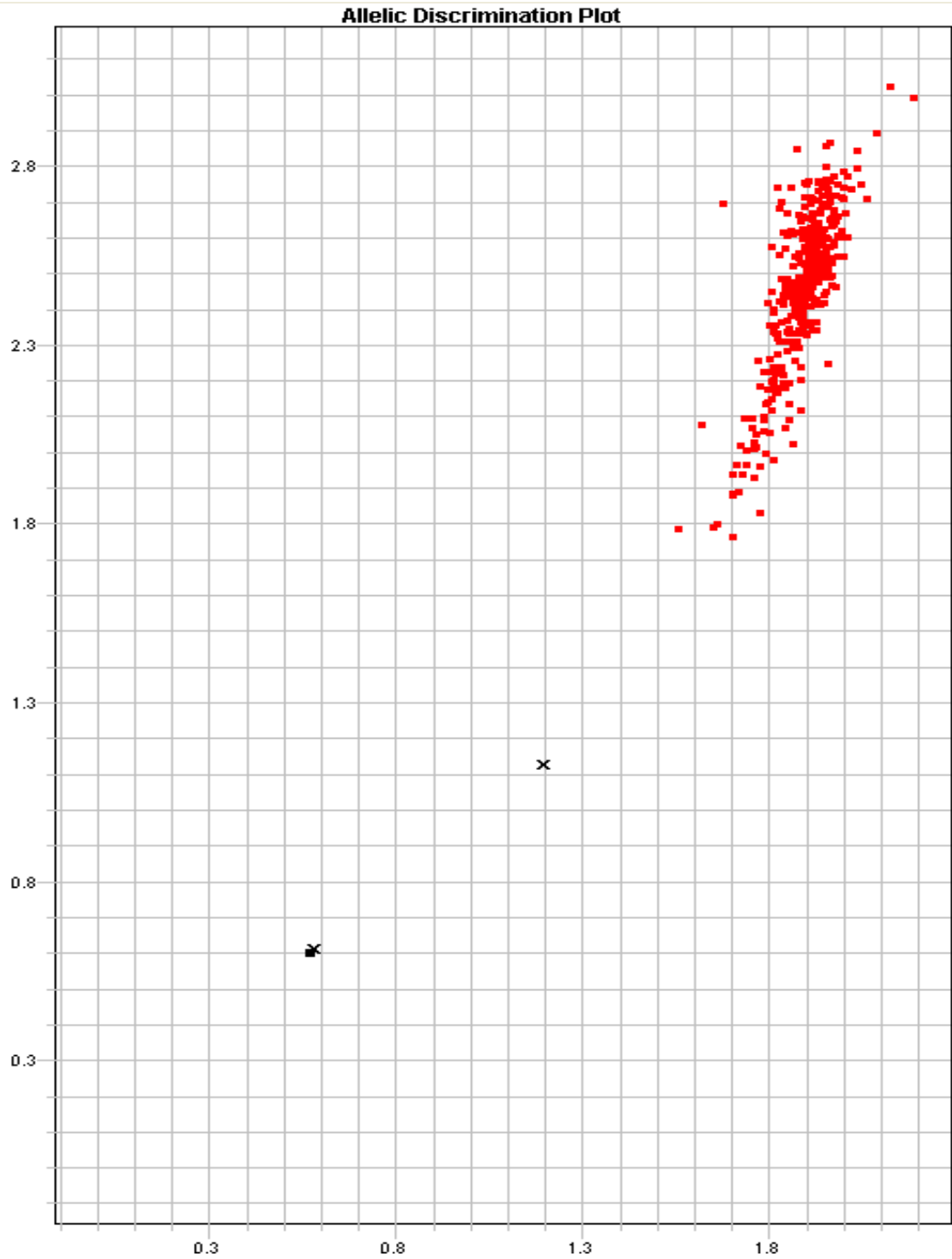
SNP: rs118204011

Plate Name: UK_11

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



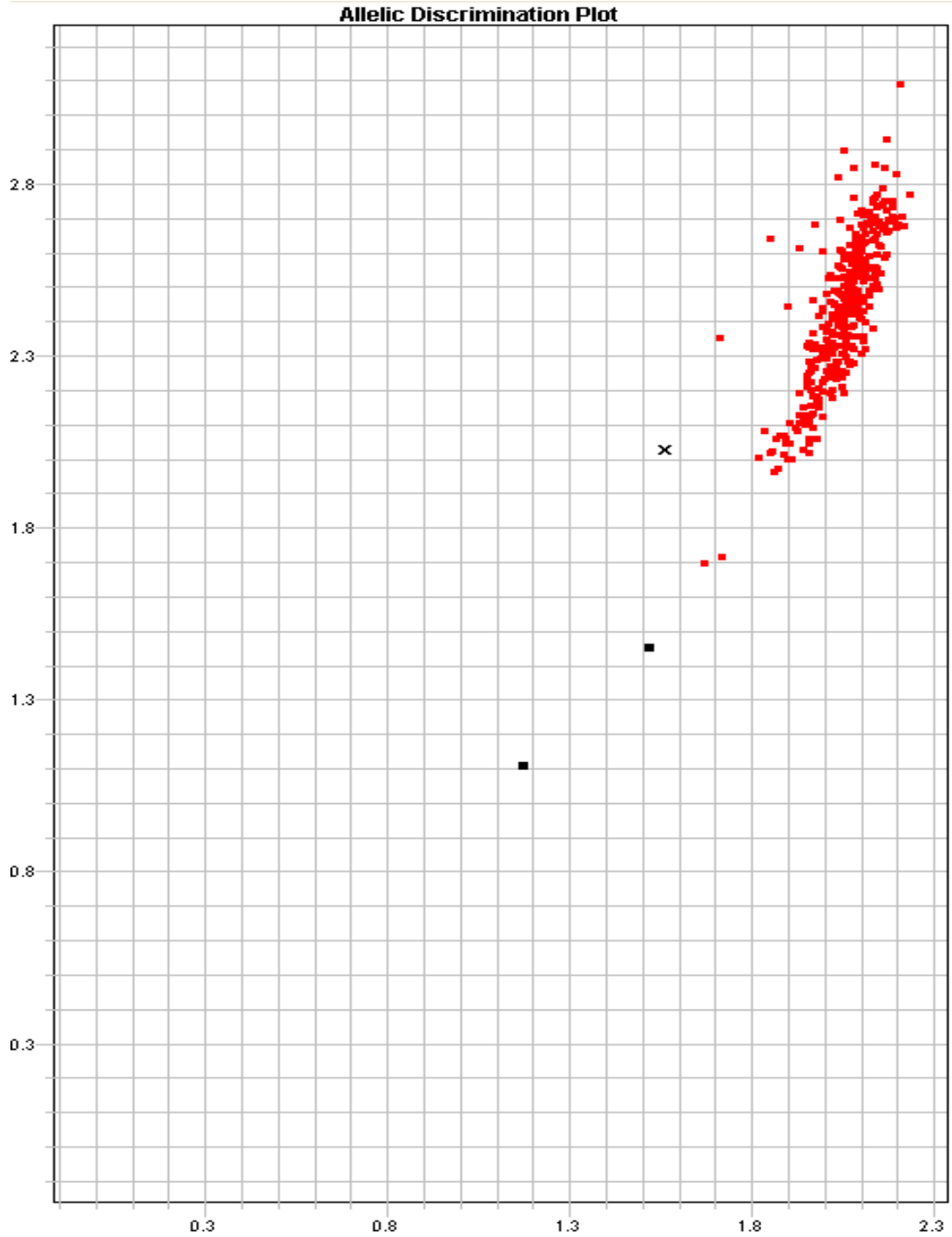
SNP: rs118204011

Plate Name: UK_12

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



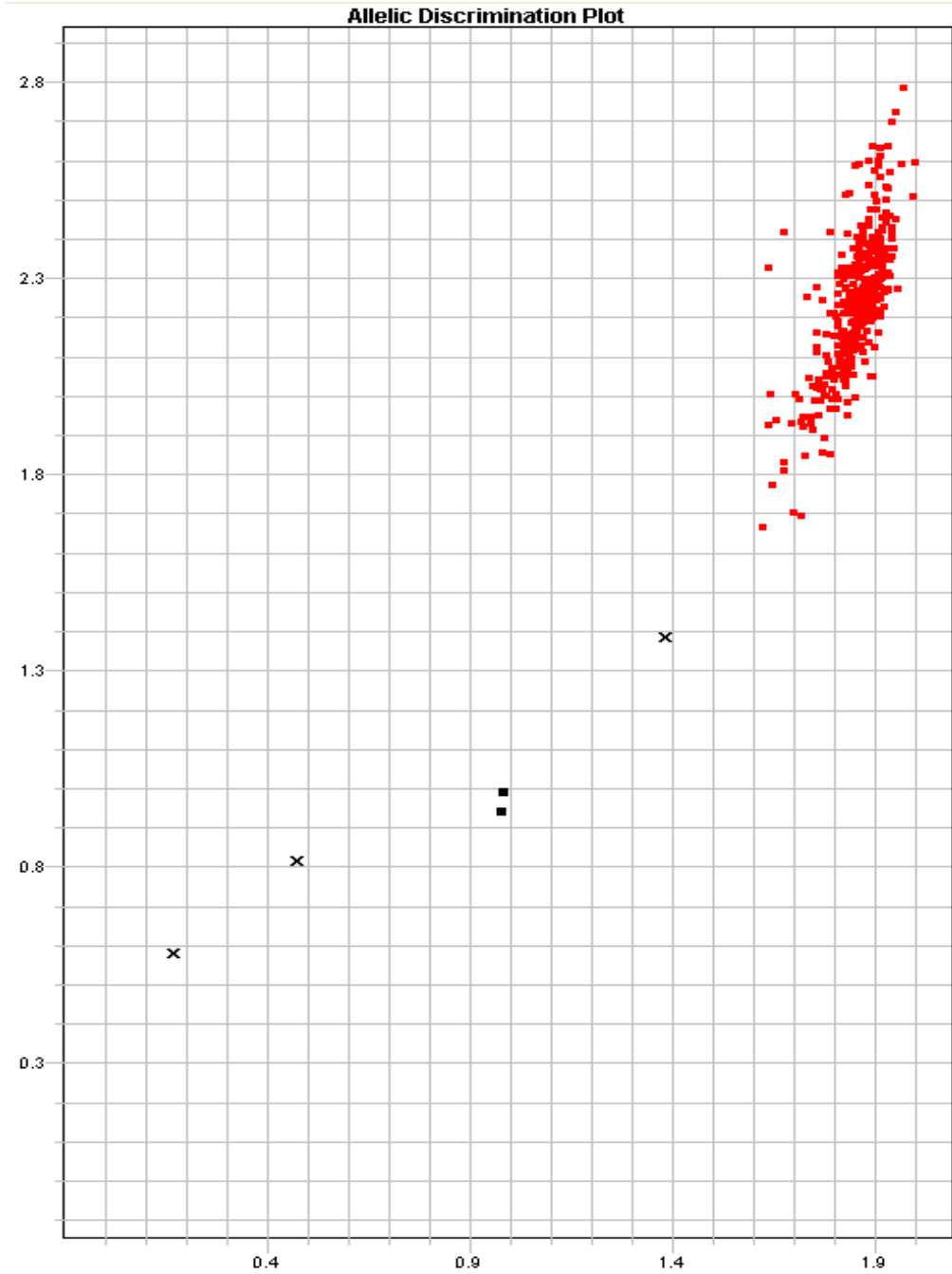
SNP: rs118204011

Plate Name: UK_13

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



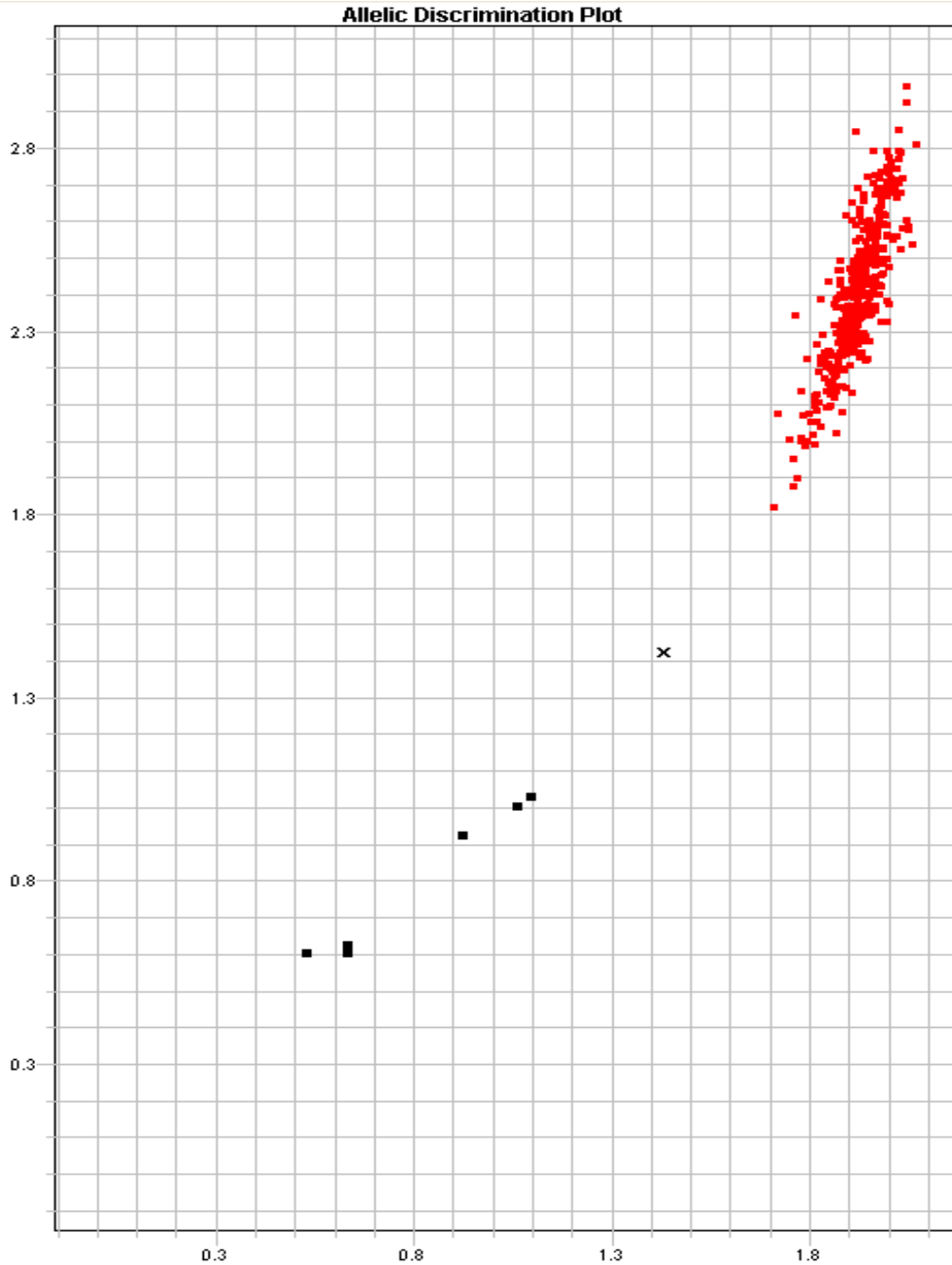
SNP: rs118204011

Plate Name: UK_14

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



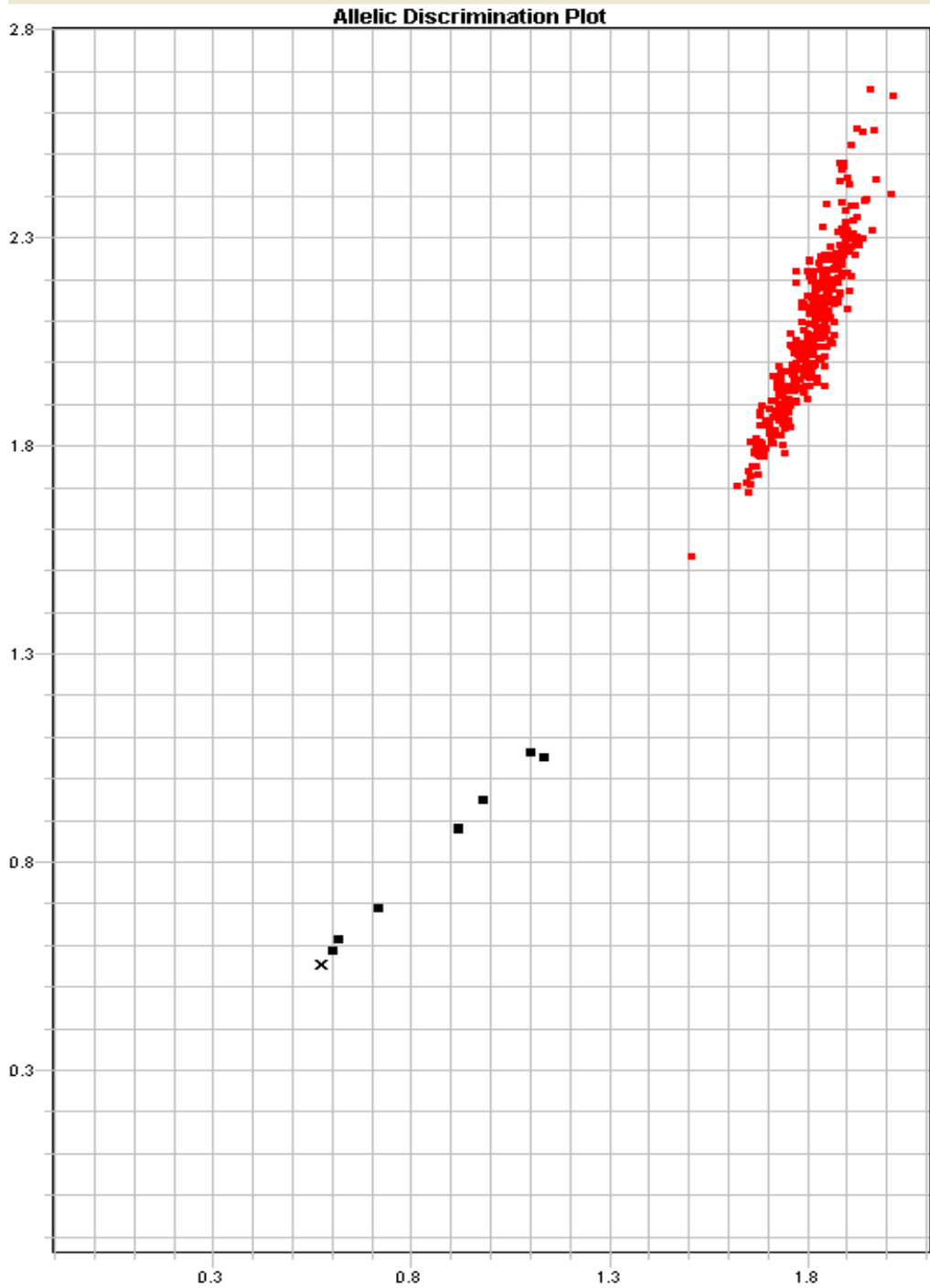
SNP: rs118204011

Plate Name: UK_15

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



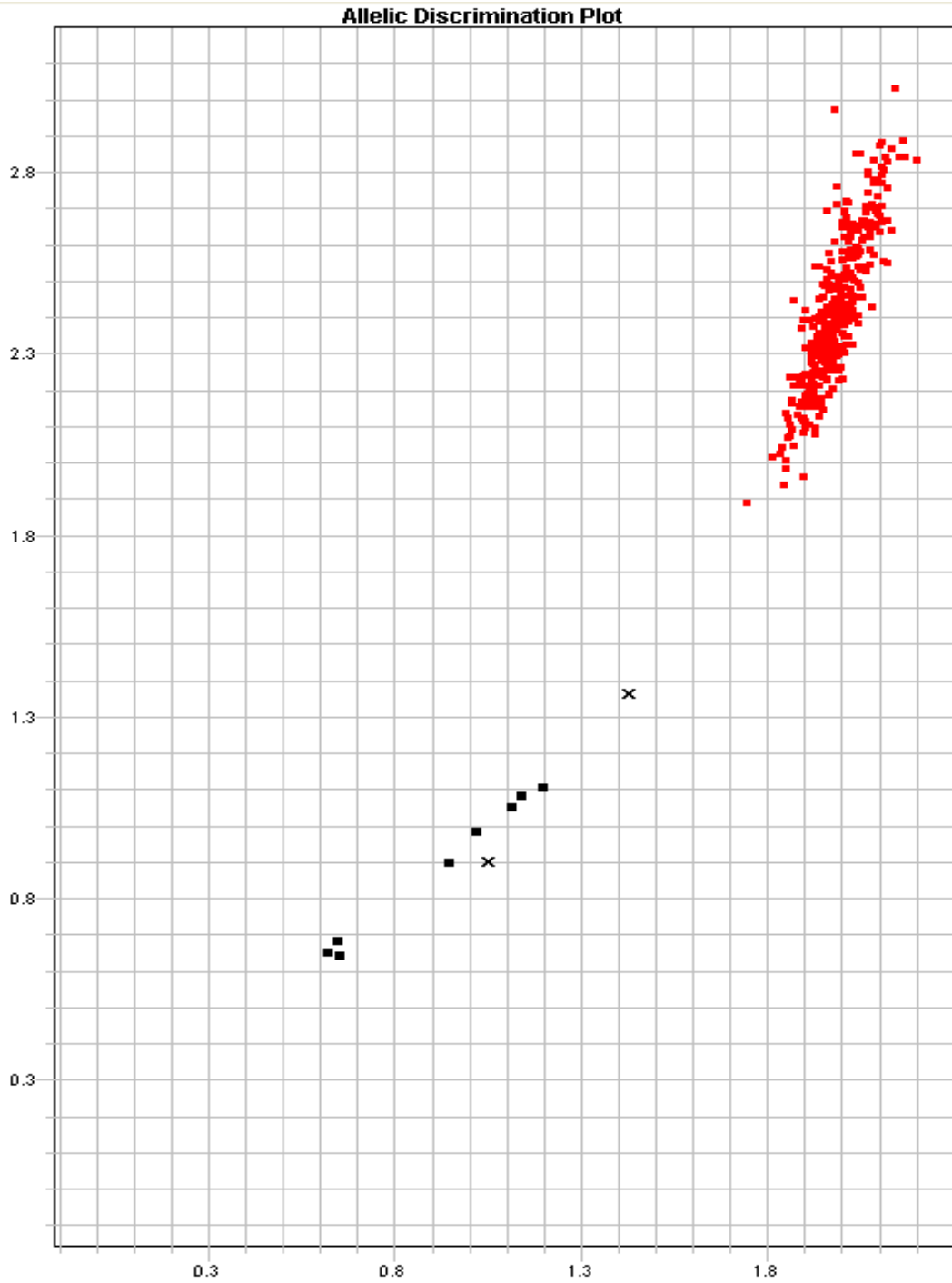
SNP: rs118204011

Plate Name: UK_16

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204011

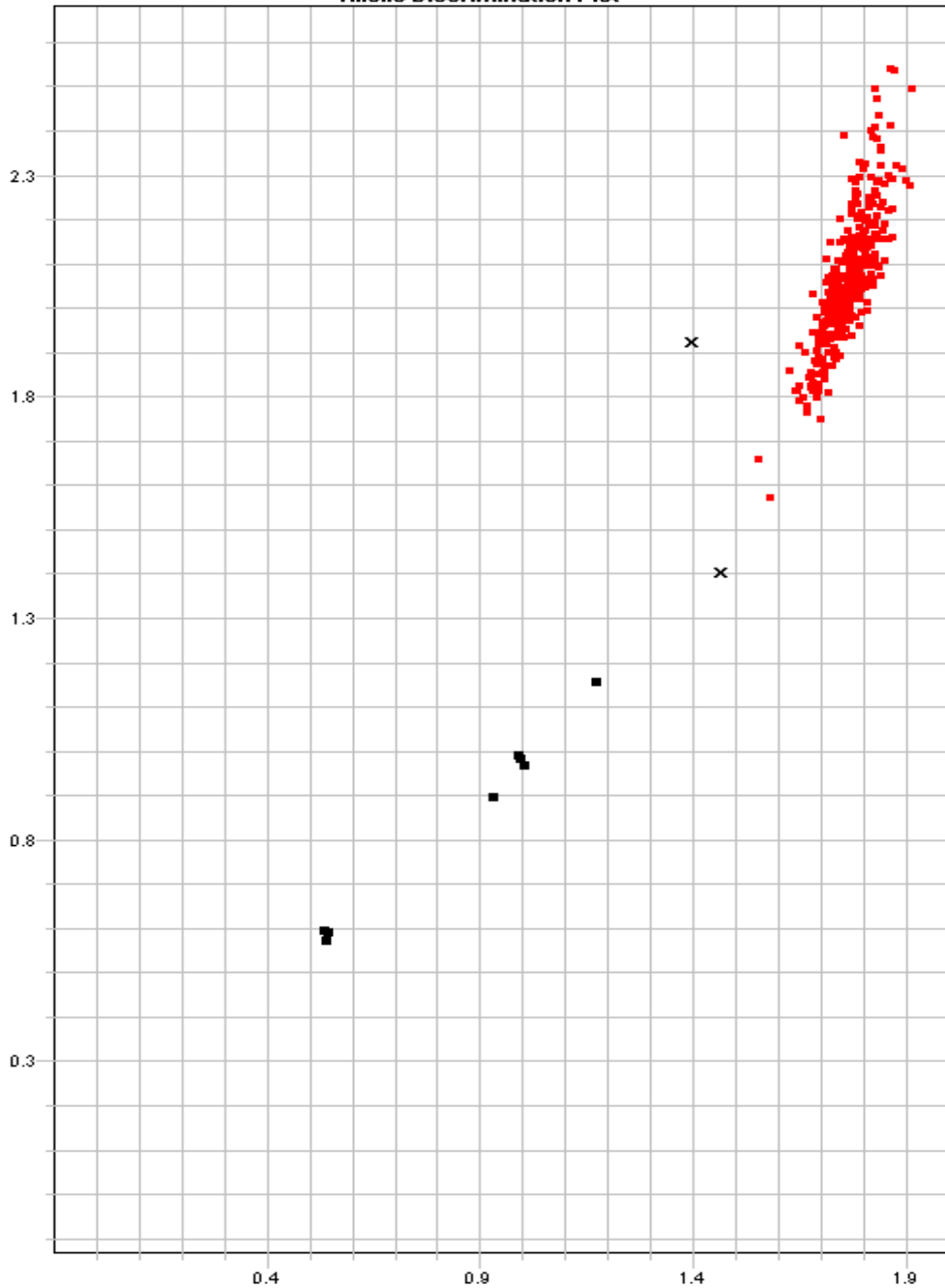
Plate Name: UK_17

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



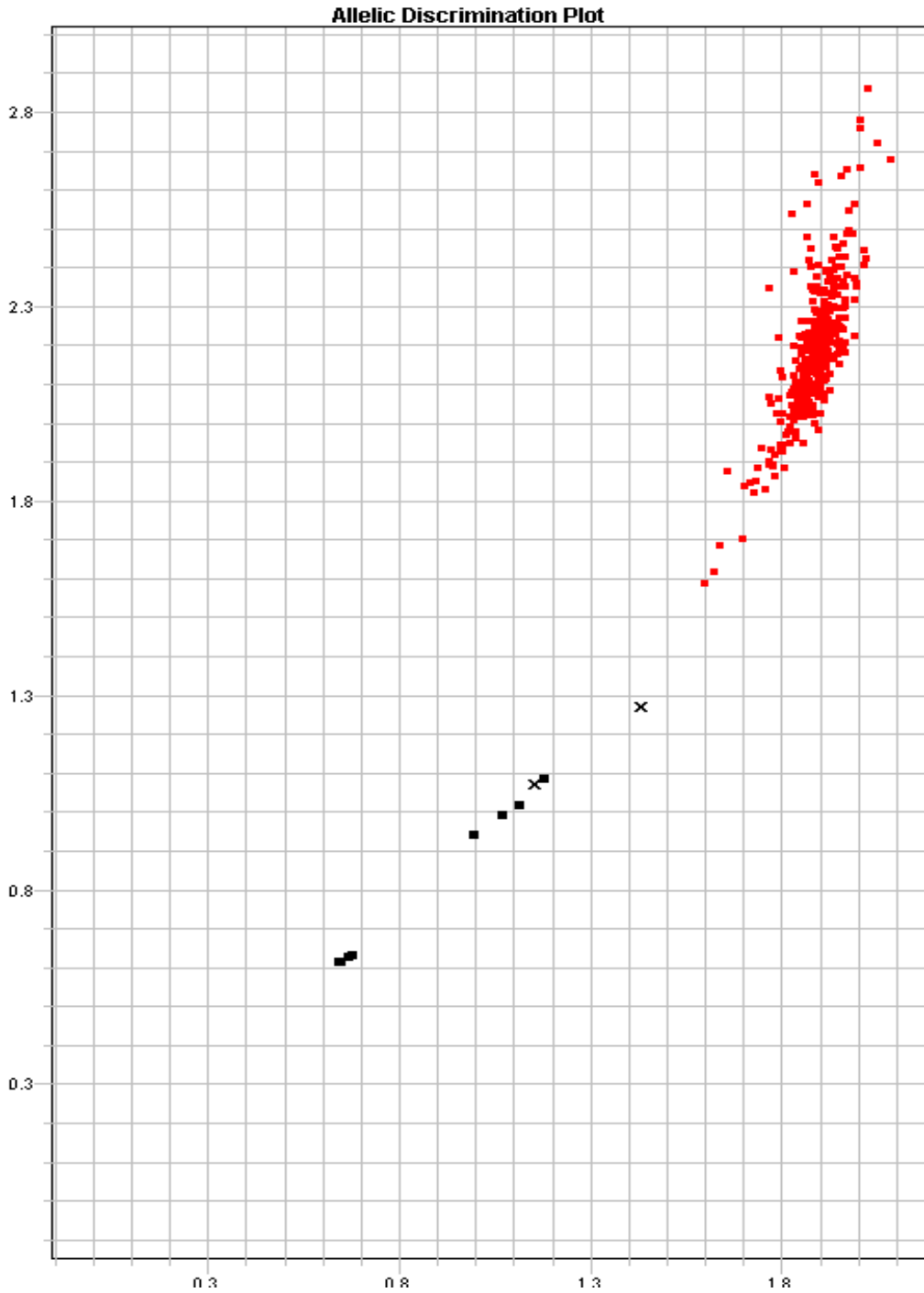
SNP: rs118204011

Plate Name: UK_18

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



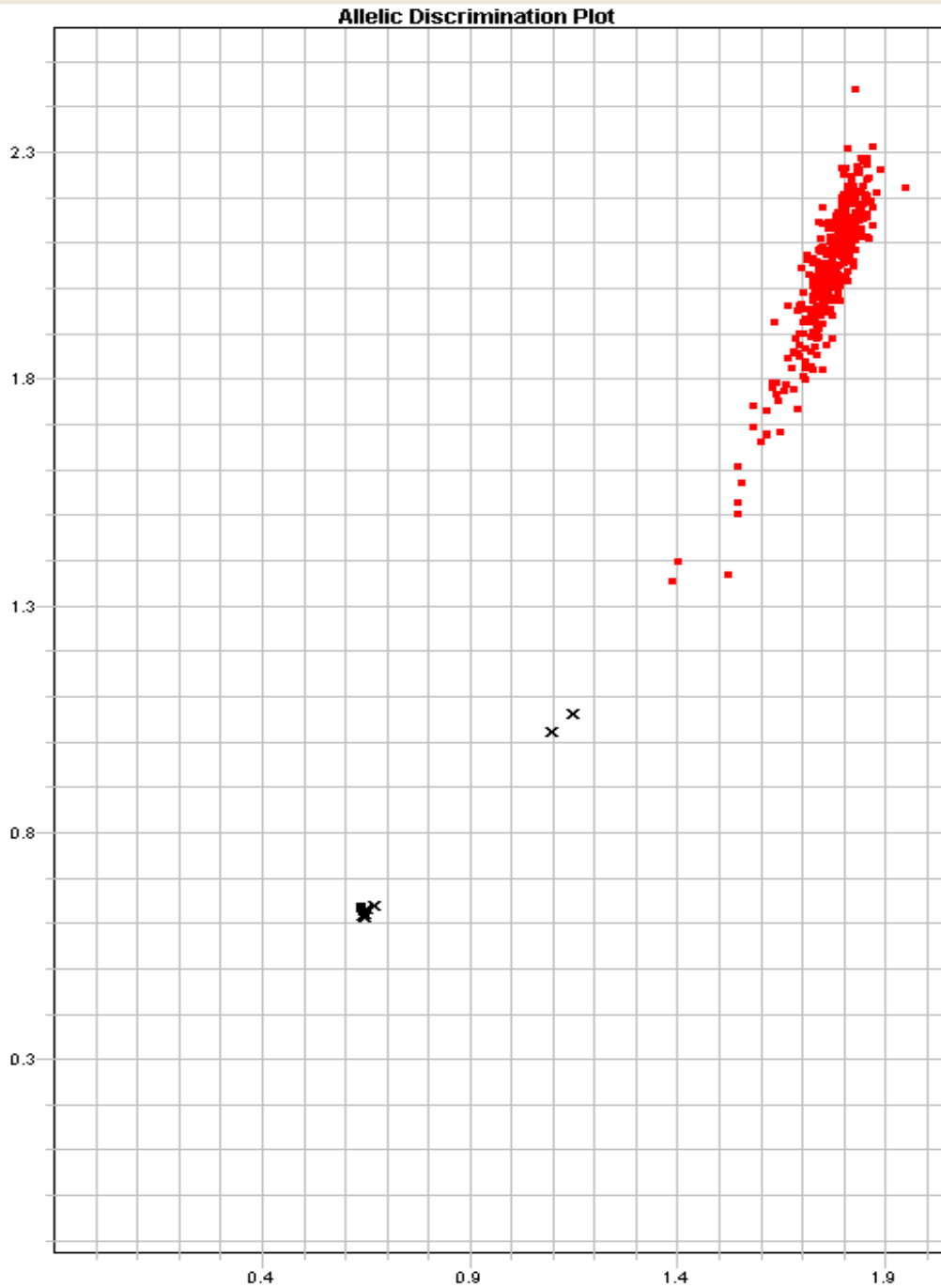
SNP: rs118204011

Plate Name: UK_19

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



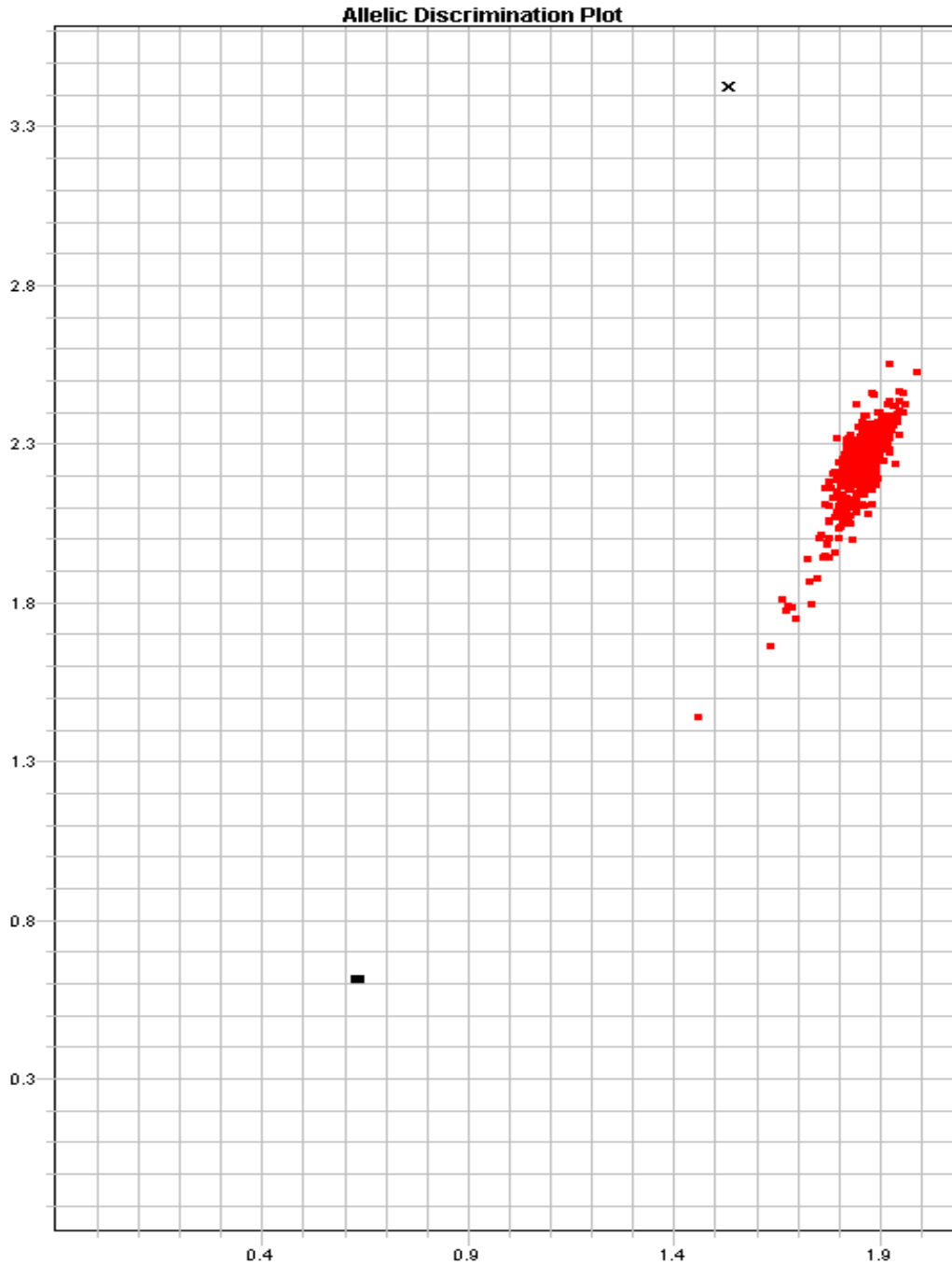
SNP: rs118204011

Plate Name: UK_20

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



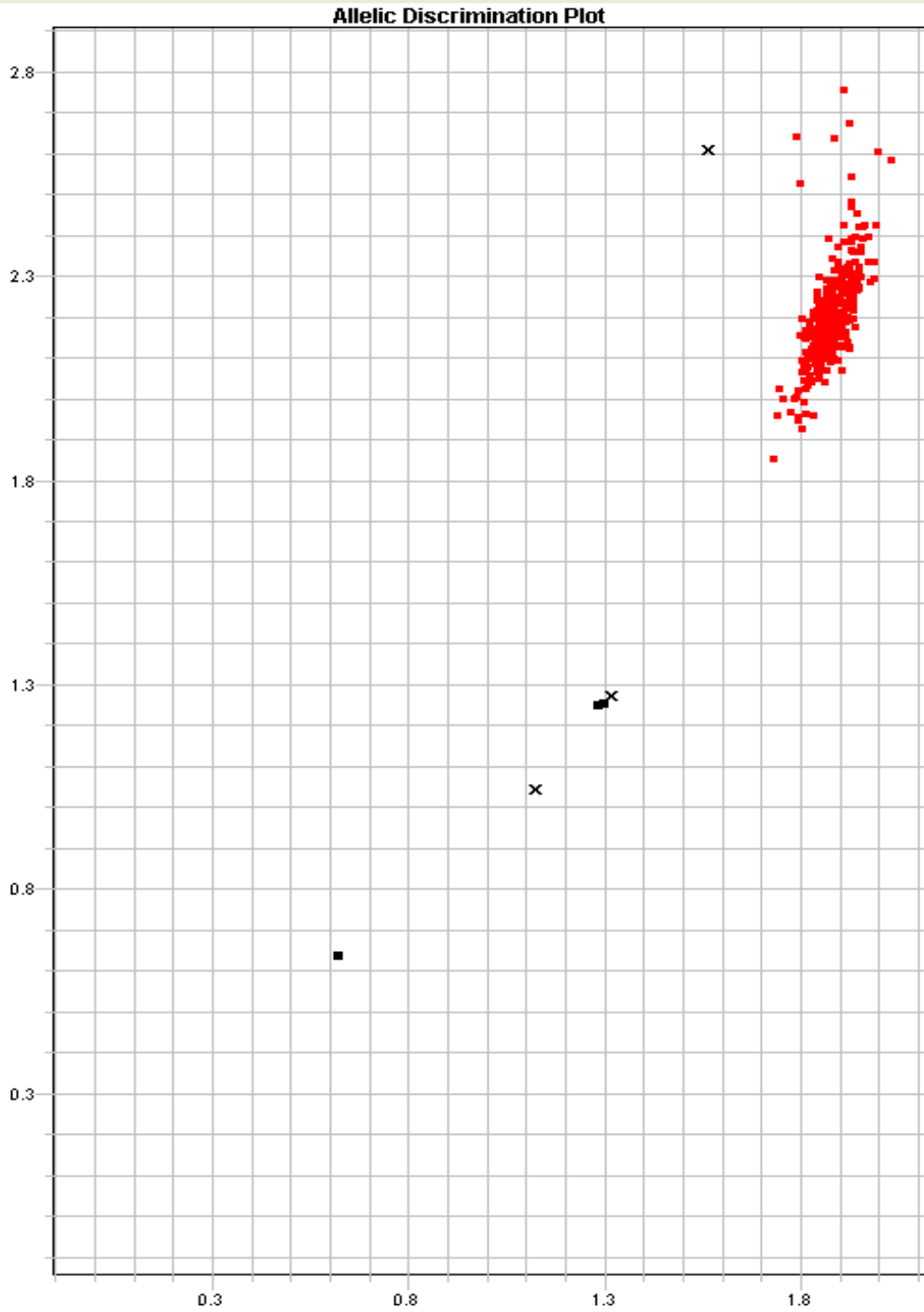
SNP: rs118204011

Plate Name: UK_21

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



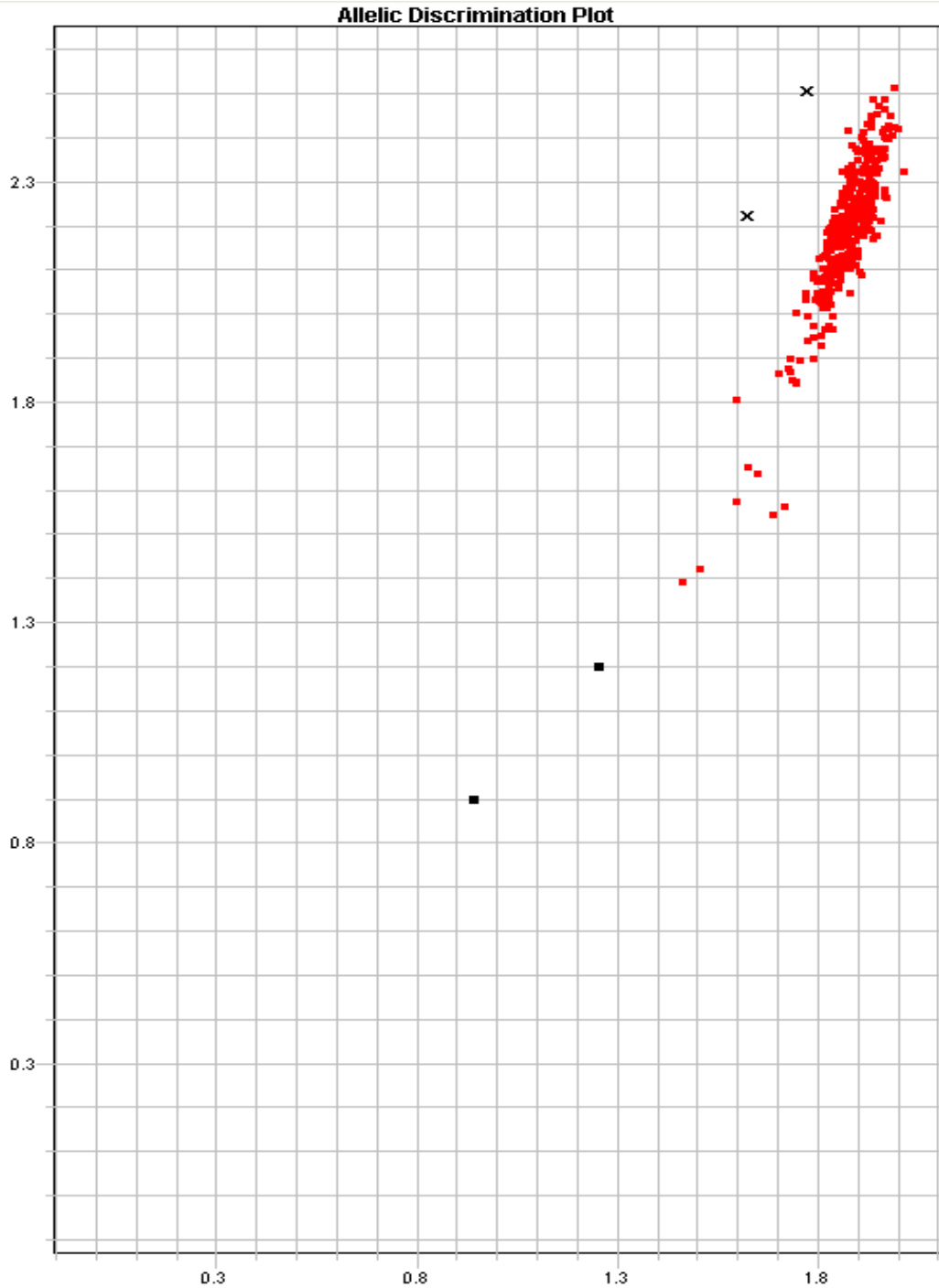
SNP: rs118204011

Plate Name: UK_22

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



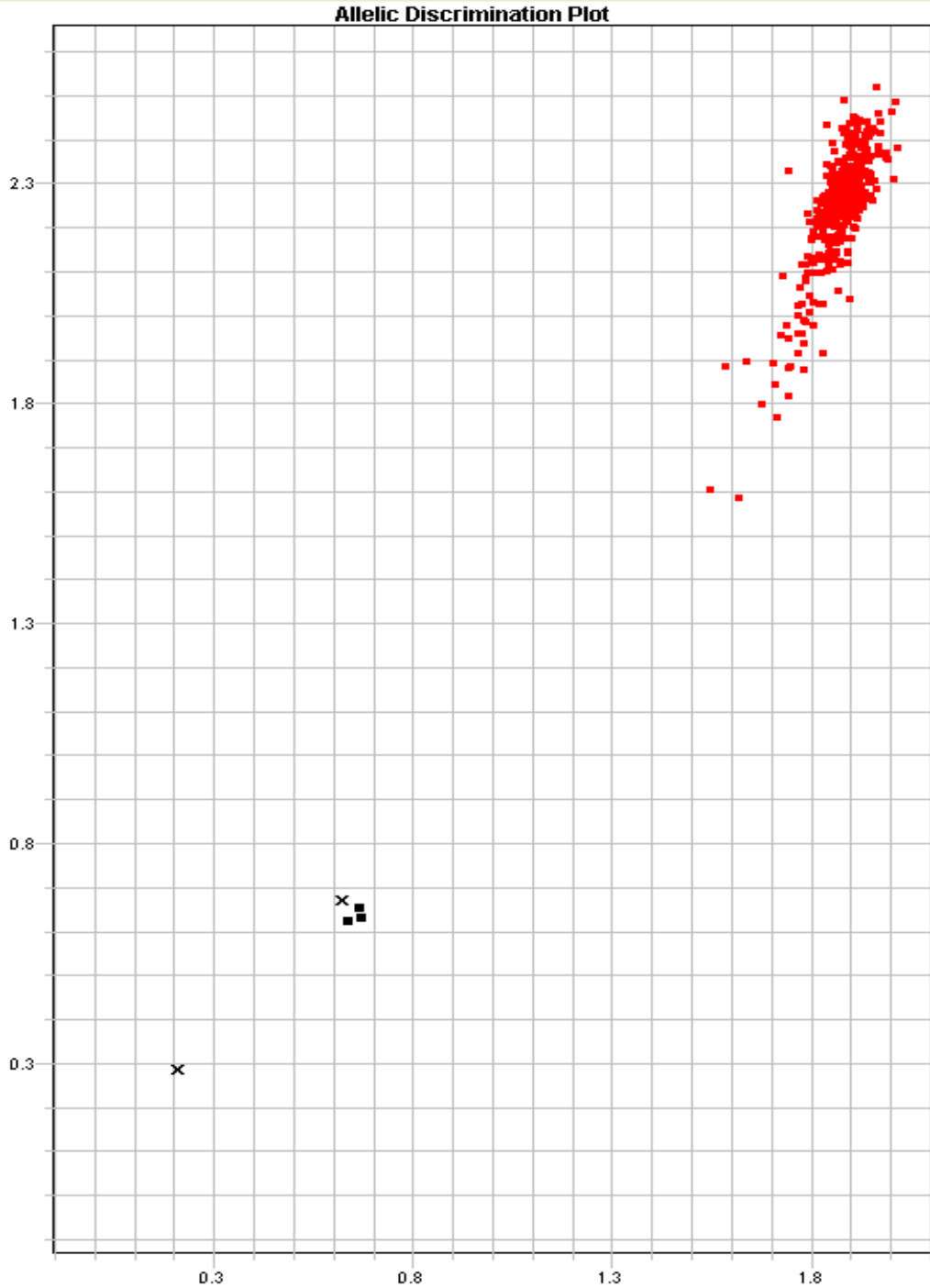
SNP: rs118204011

Plate Name: UK_23

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204011

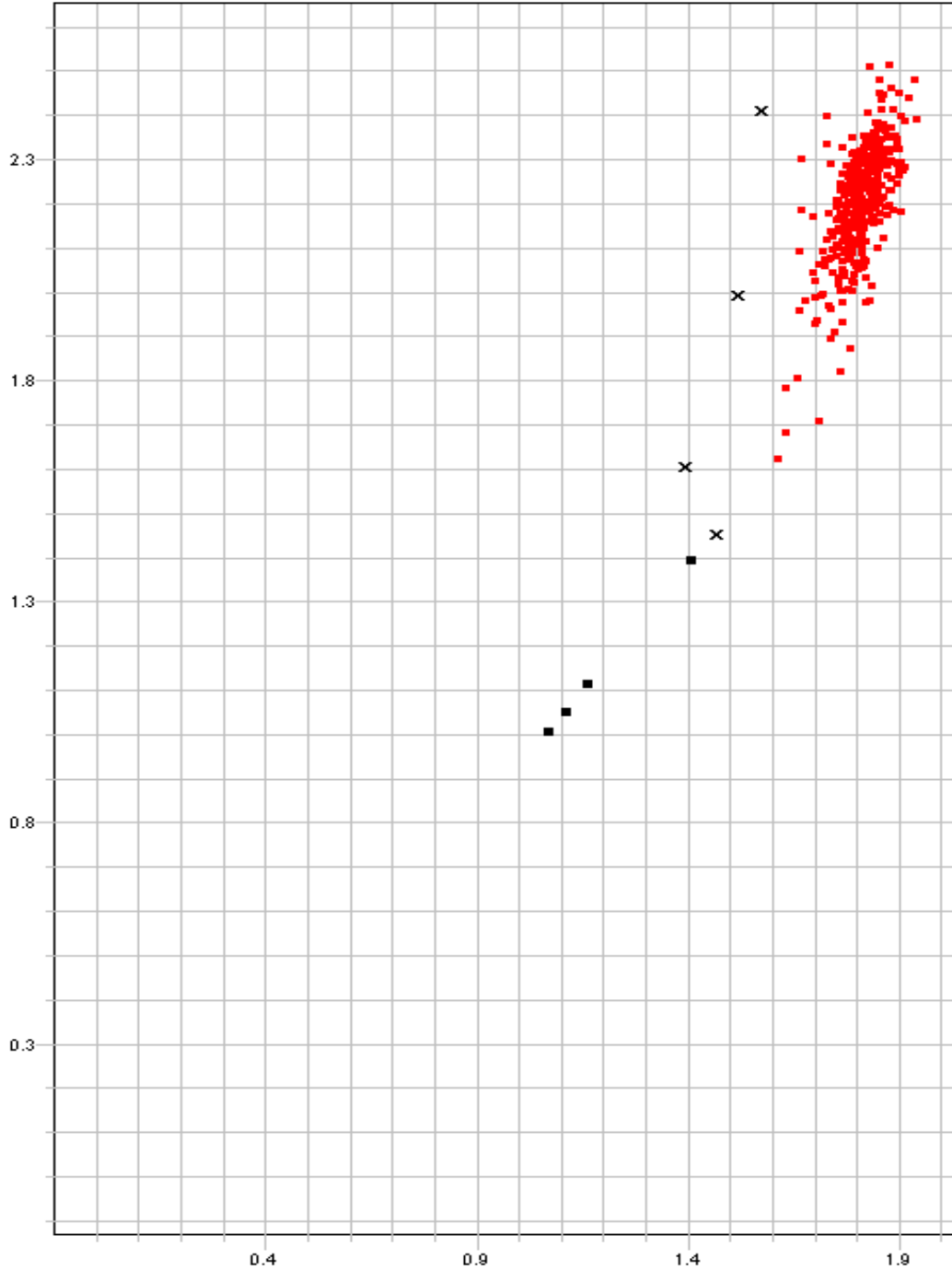
Plate Name: UK_24

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

Allelic Discrimination Plot



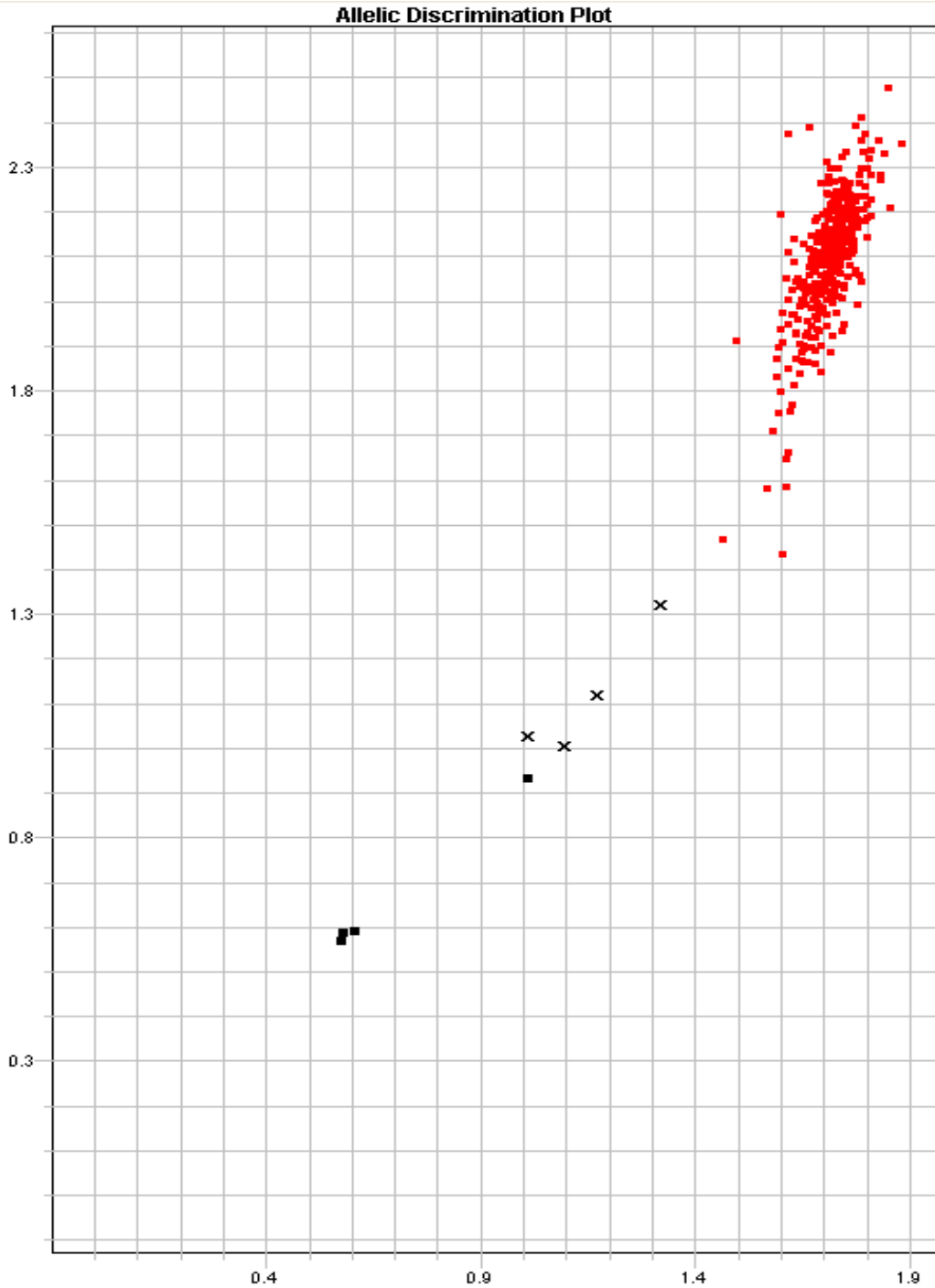
SNP: rs118204011

Plate Name: UK_25

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



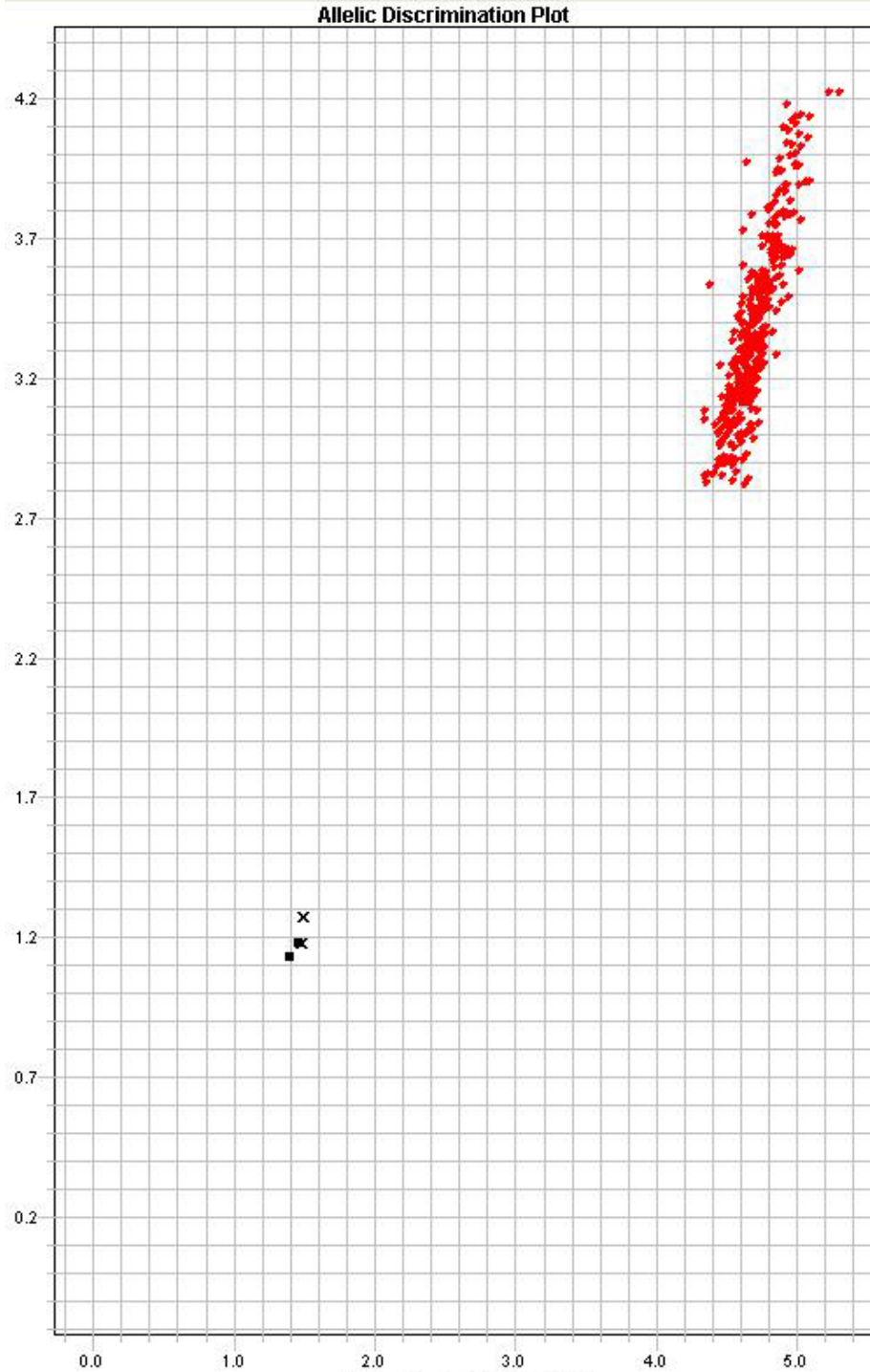
SNP: rs118204011

Plate Name: USA_1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



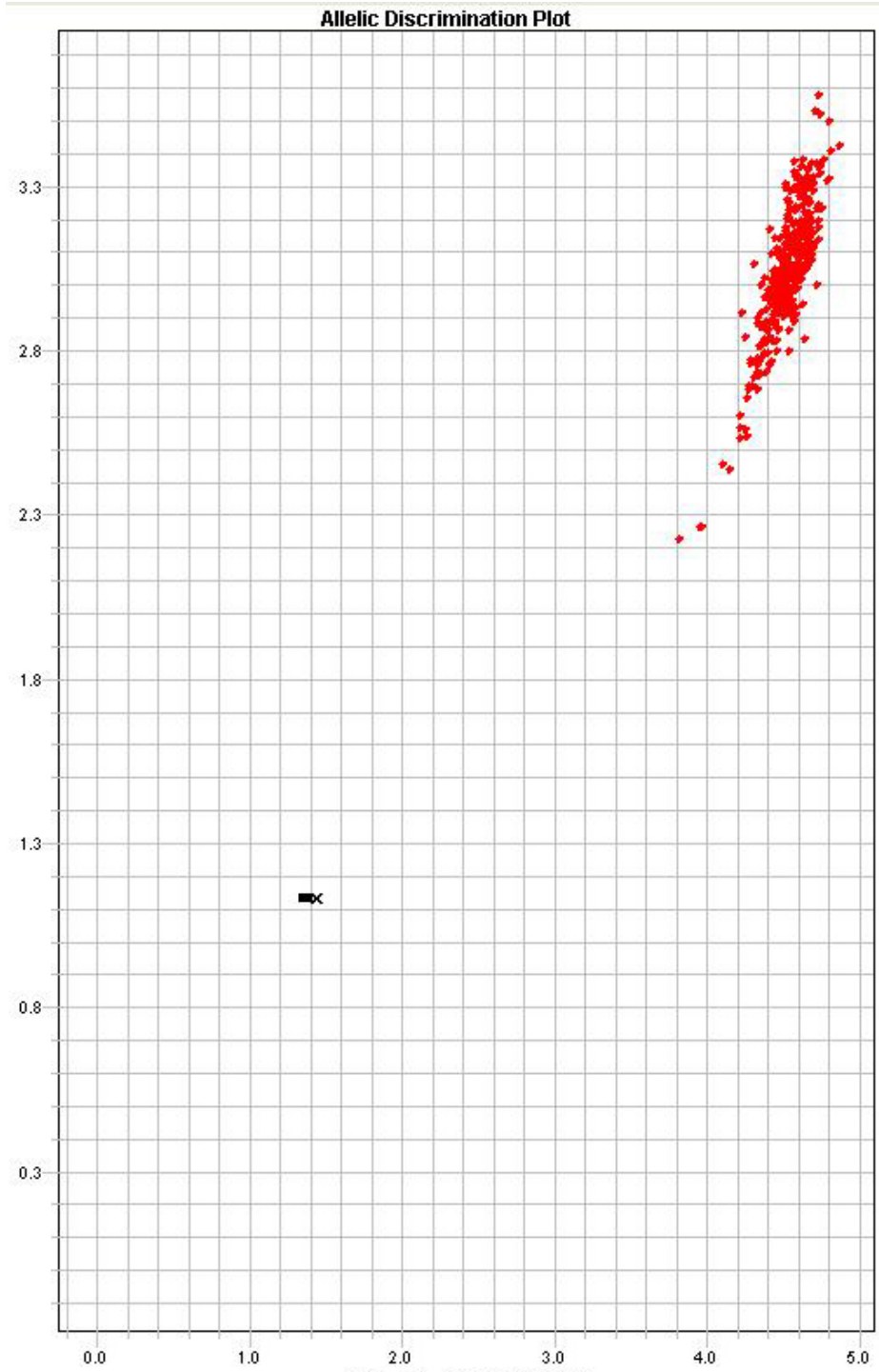
SNP: rs118204011

Plate Name: USA_2

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



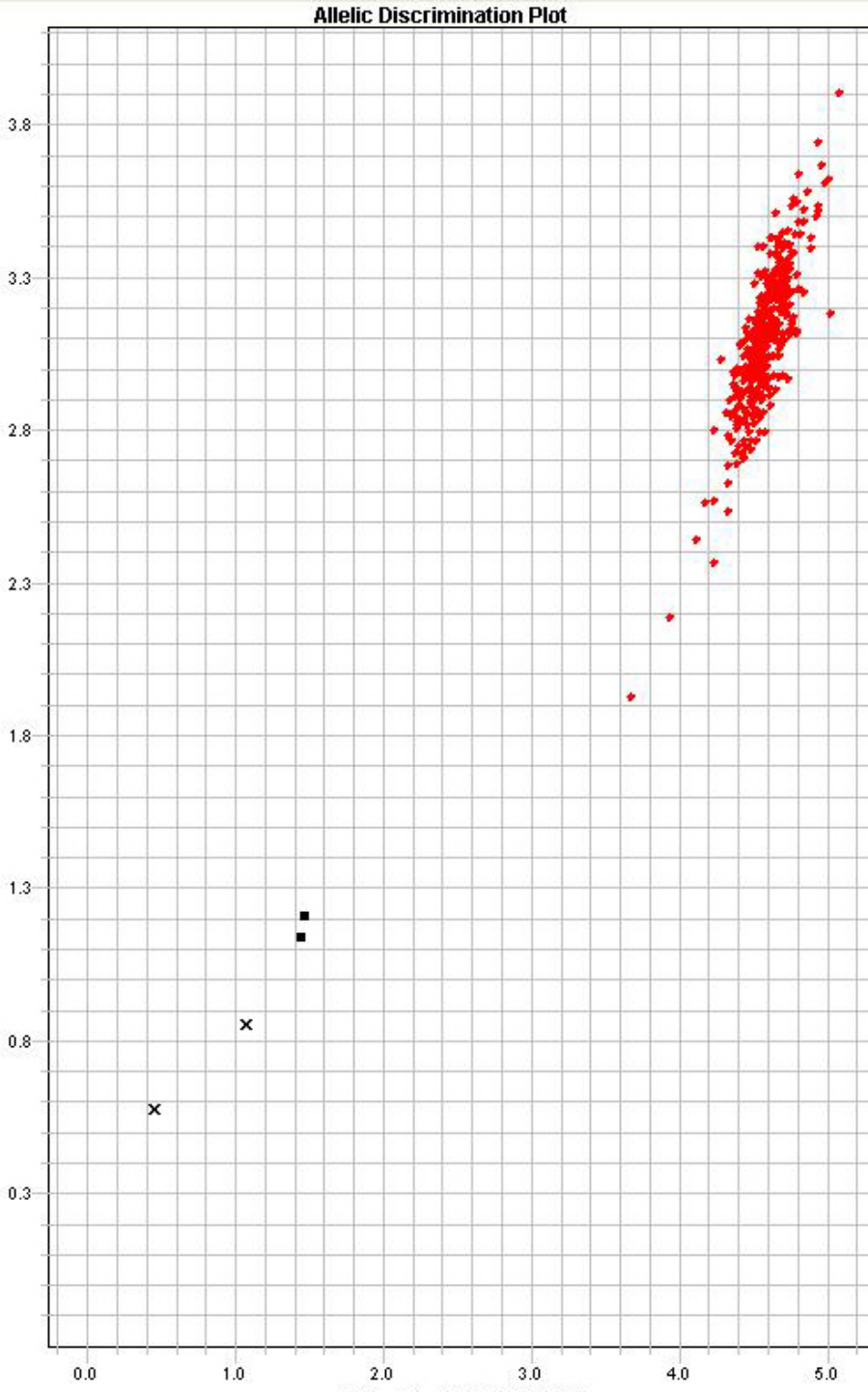
SNP: rs118204011

Plate Name: USA_3

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



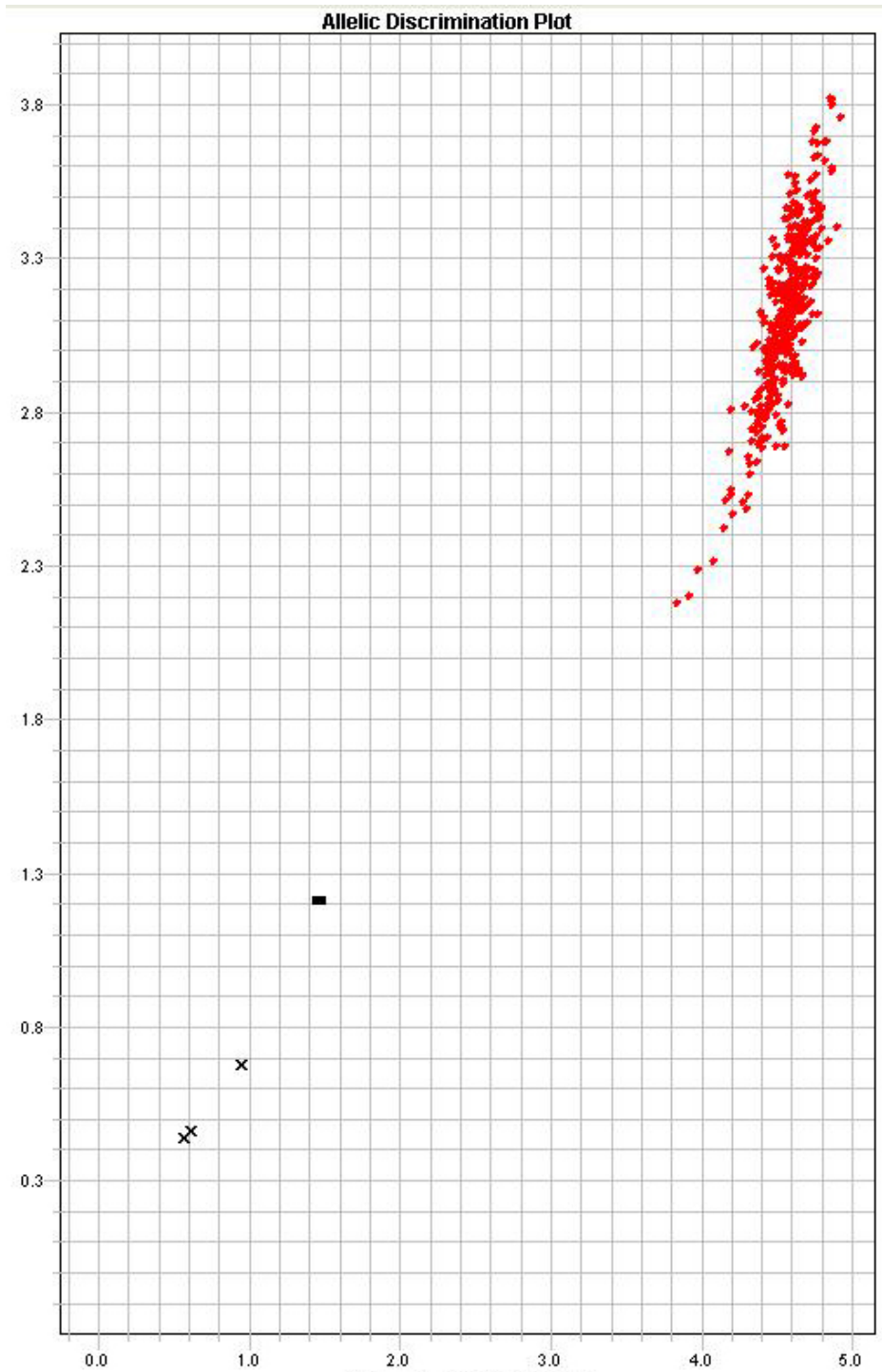
SNP: rs118204011

Plate Name: USA_4

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



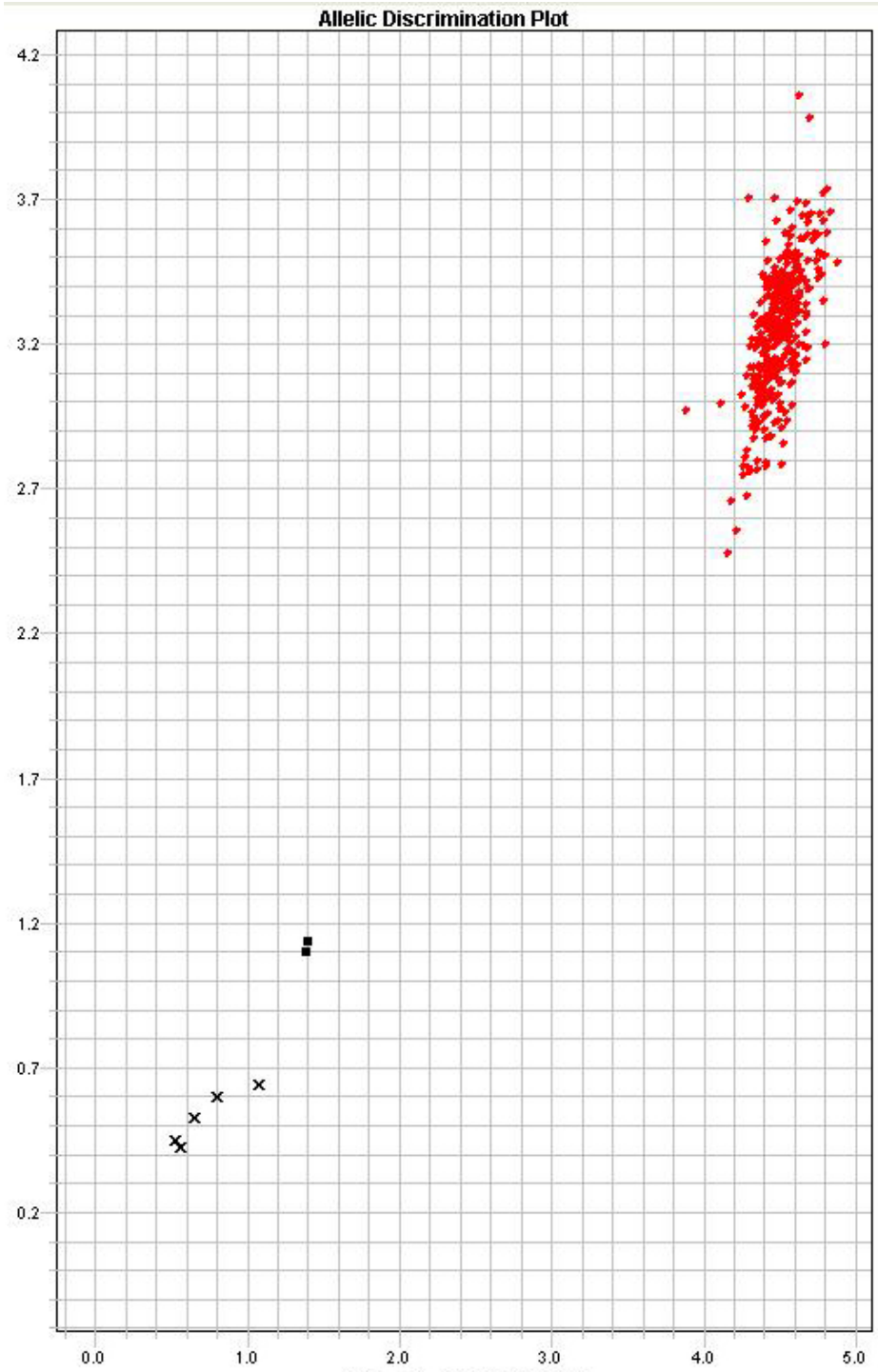
SNP: rs118204011

Plate Name: USA_5

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



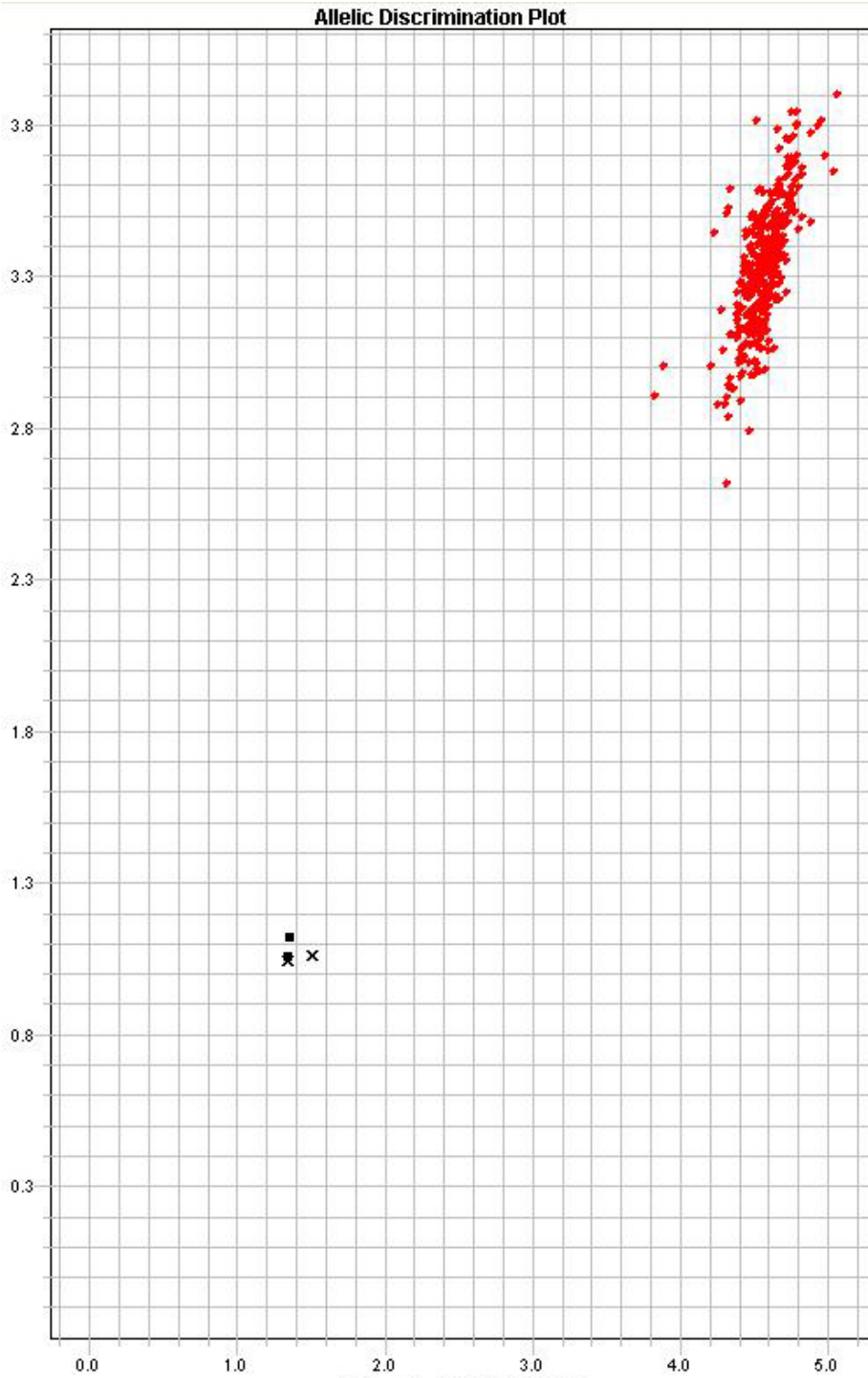
SNP: rs118204011

Plate Name: USA_6

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



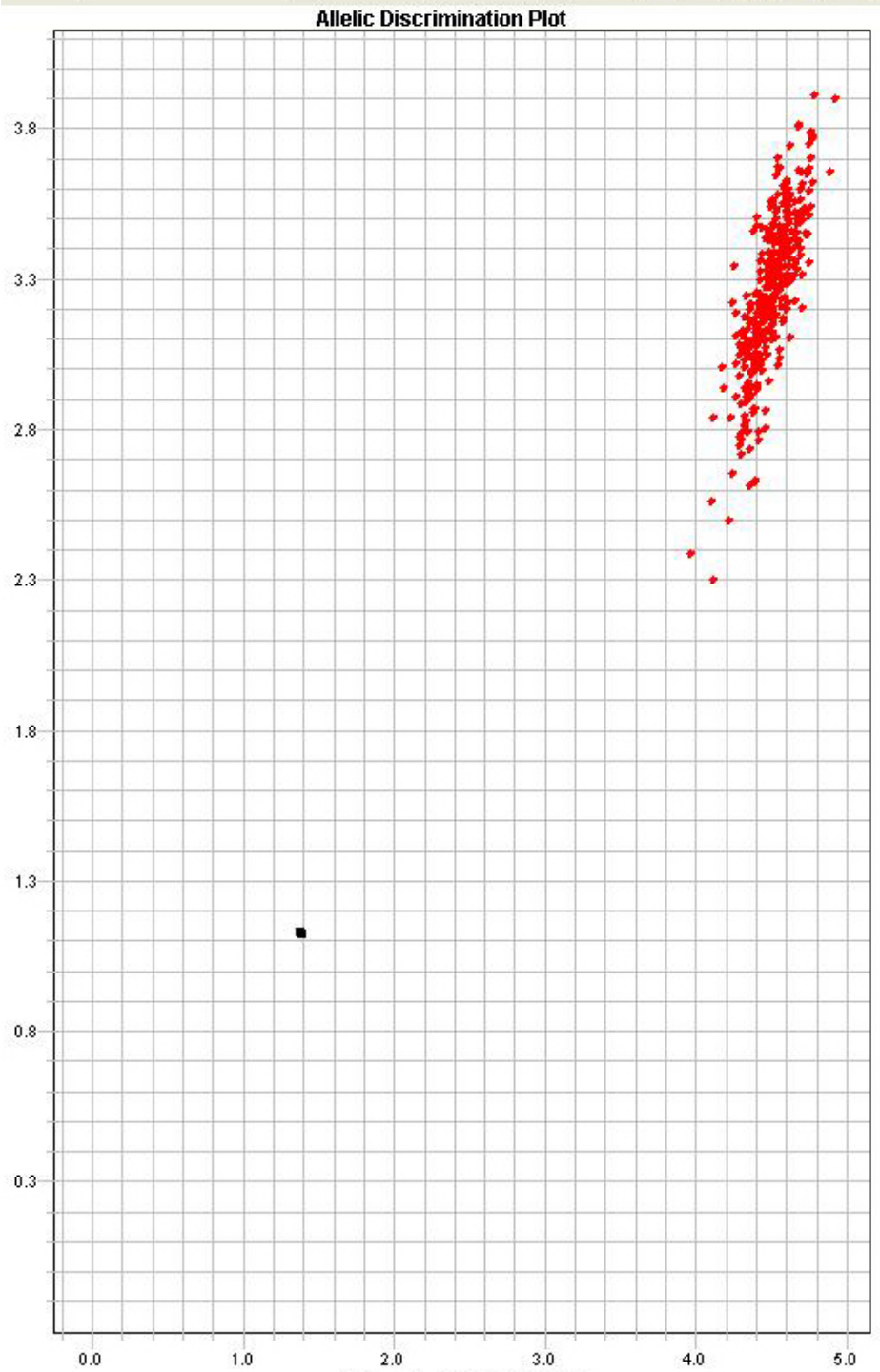
SNP: rs118204011

Plate Name: USA_7

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



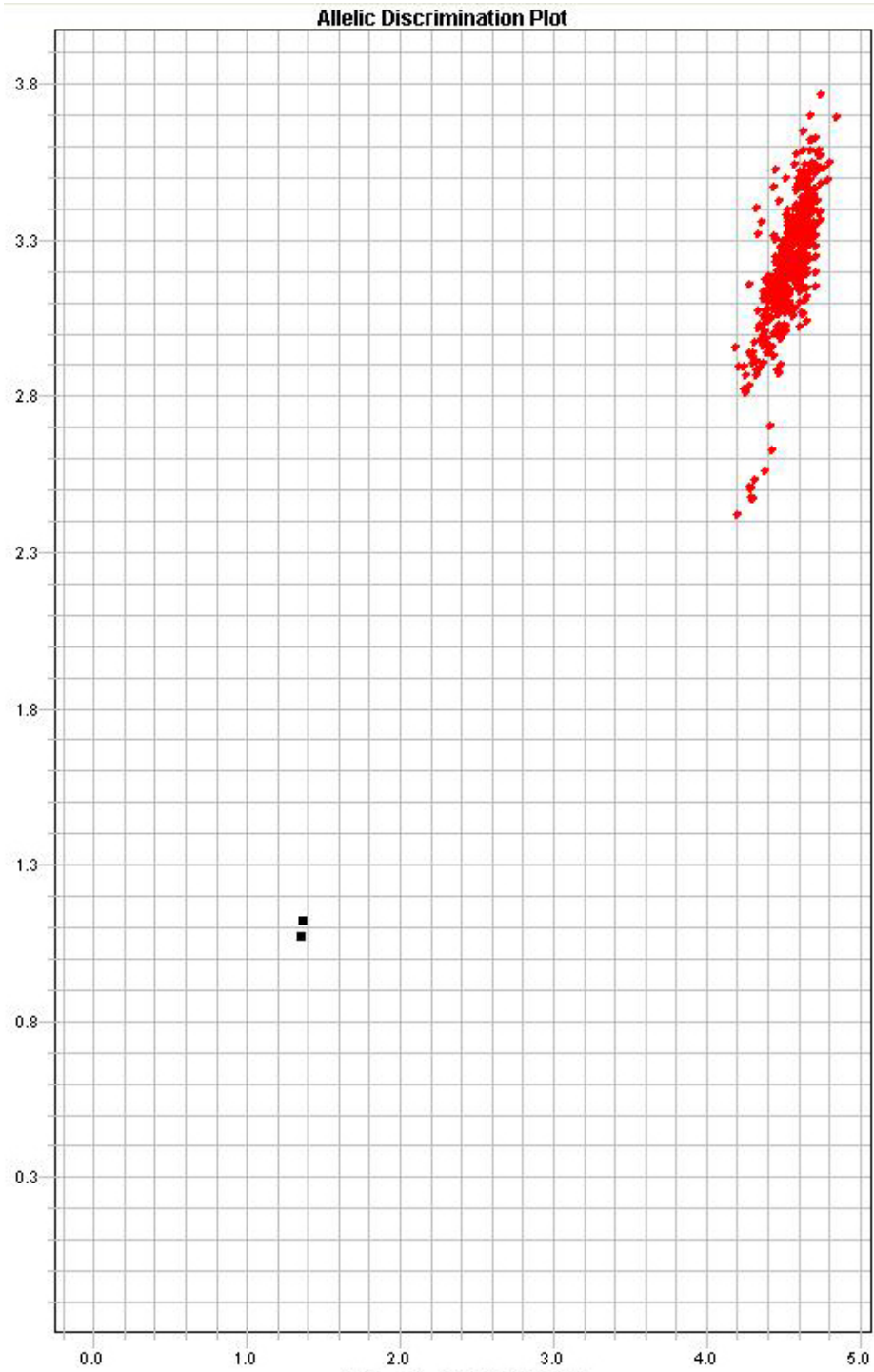
SNP: rs118204011

Plate Name: USA_8

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



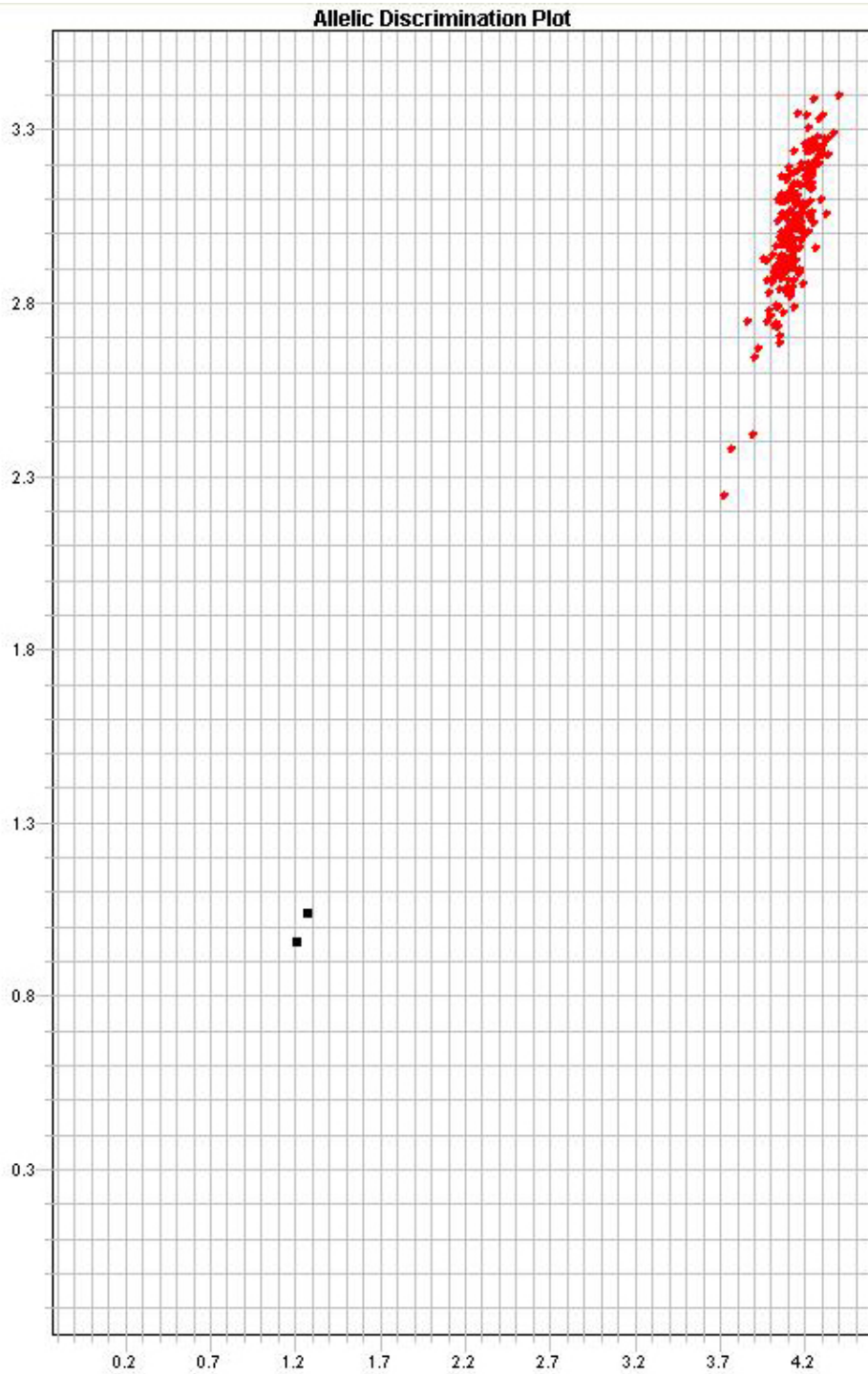
SNP: rs118204011

Plate Name: USA_9

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



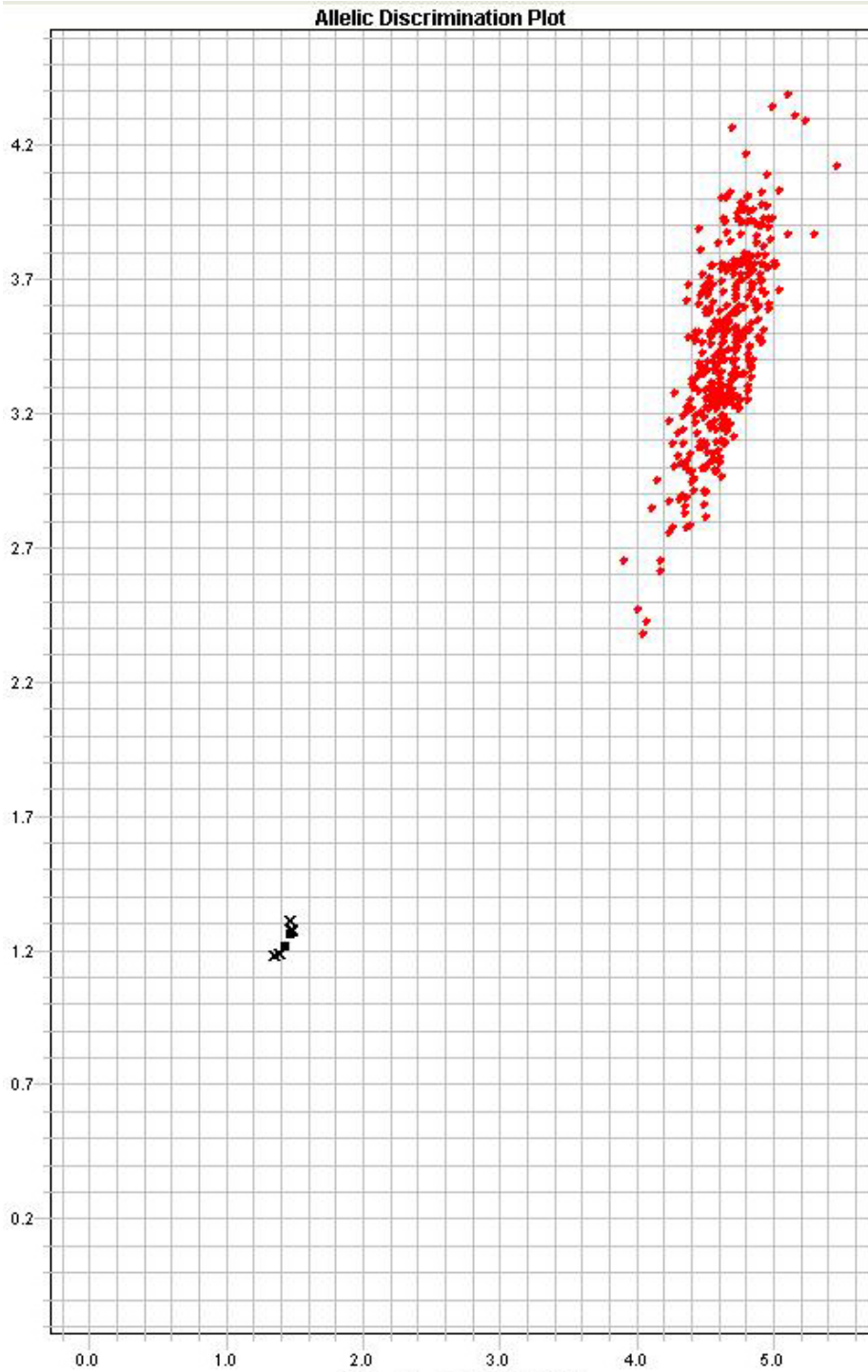
SNP: rs118204011

Plate Name: USA_10

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



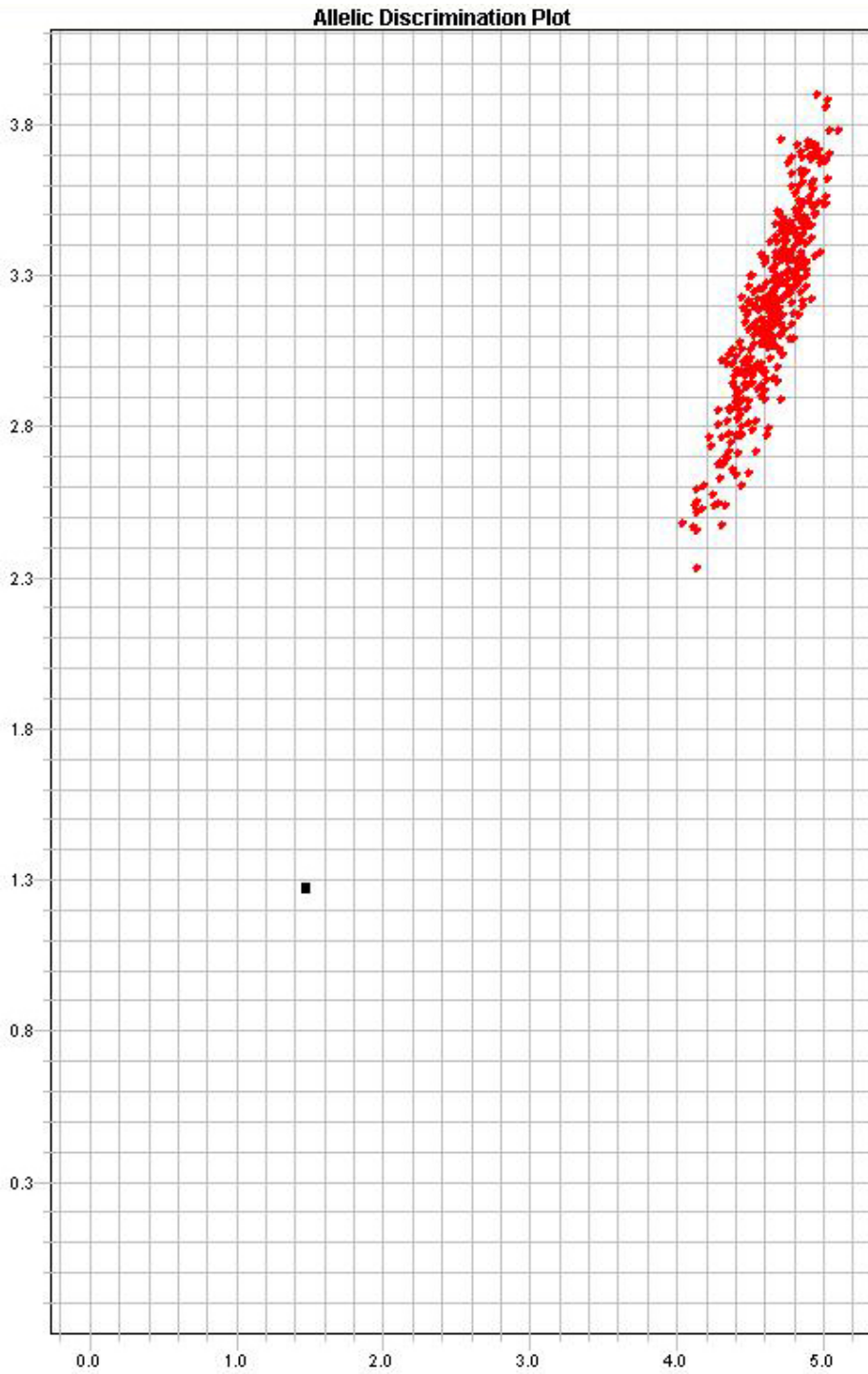
SNP: rs118204011

Plate Name: USA_11

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



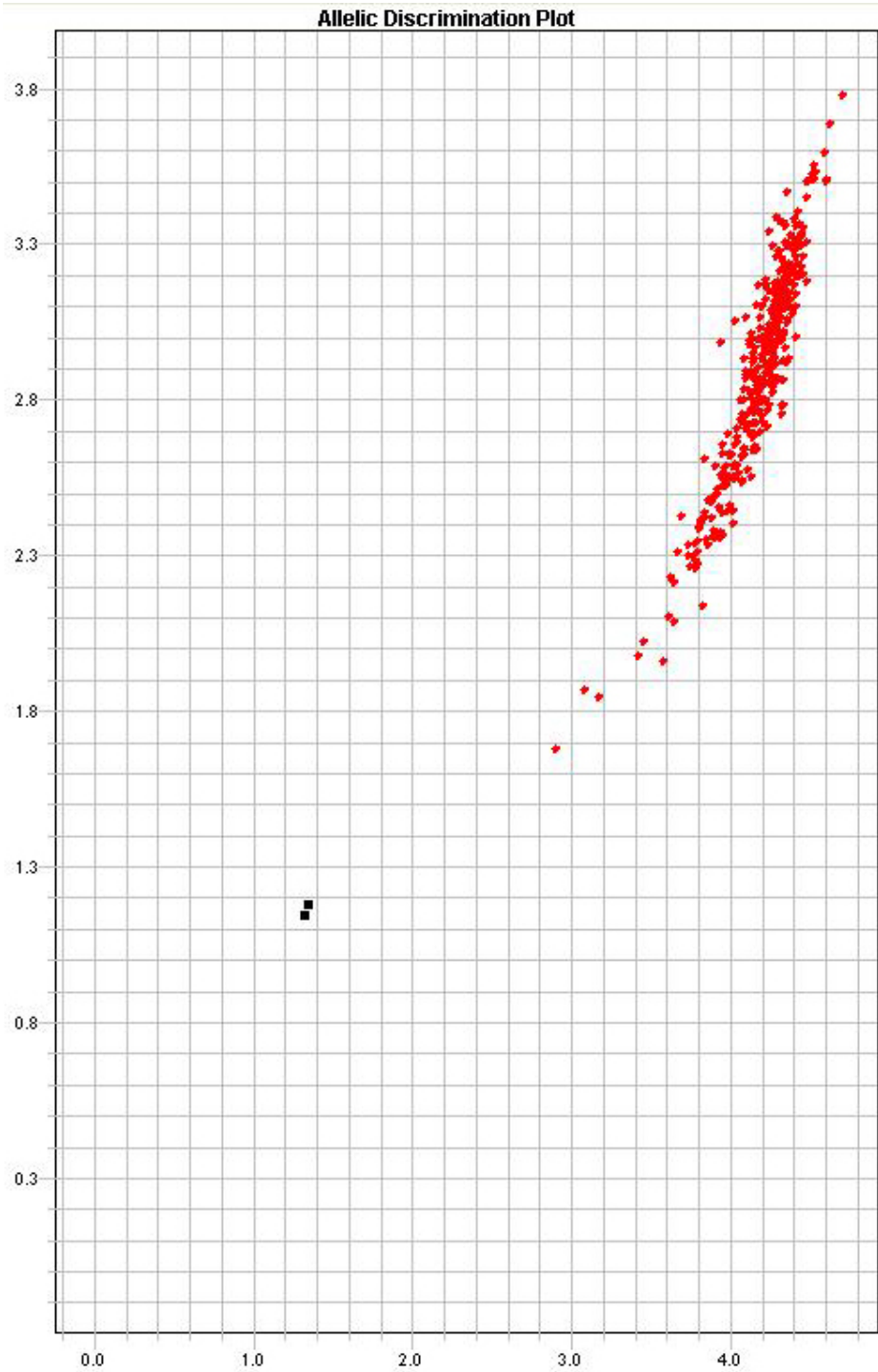
SNP: rs118204011

Plate Name: USA_12

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



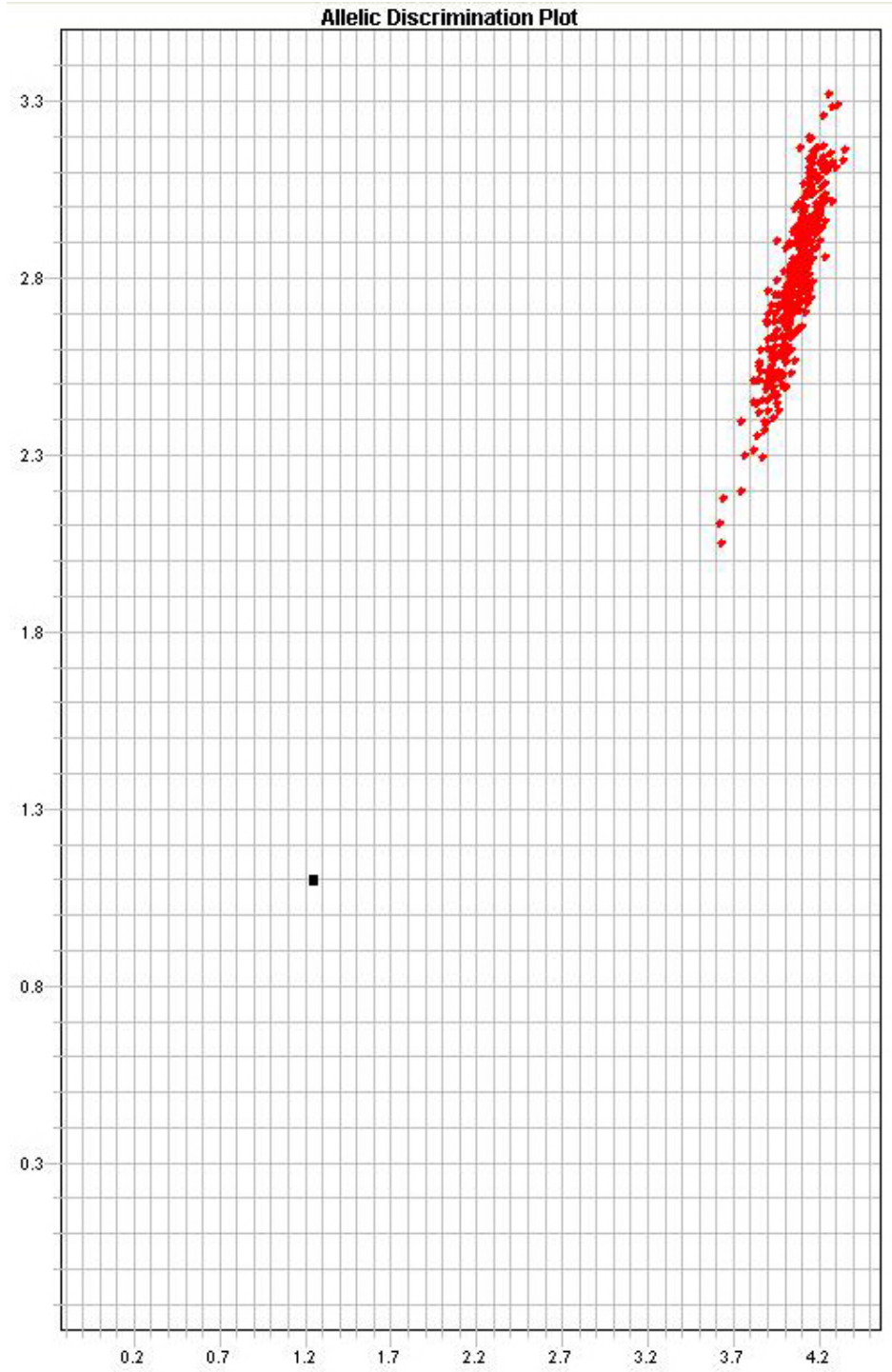
SNP: rs118204011

Plate Name: USA_13

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



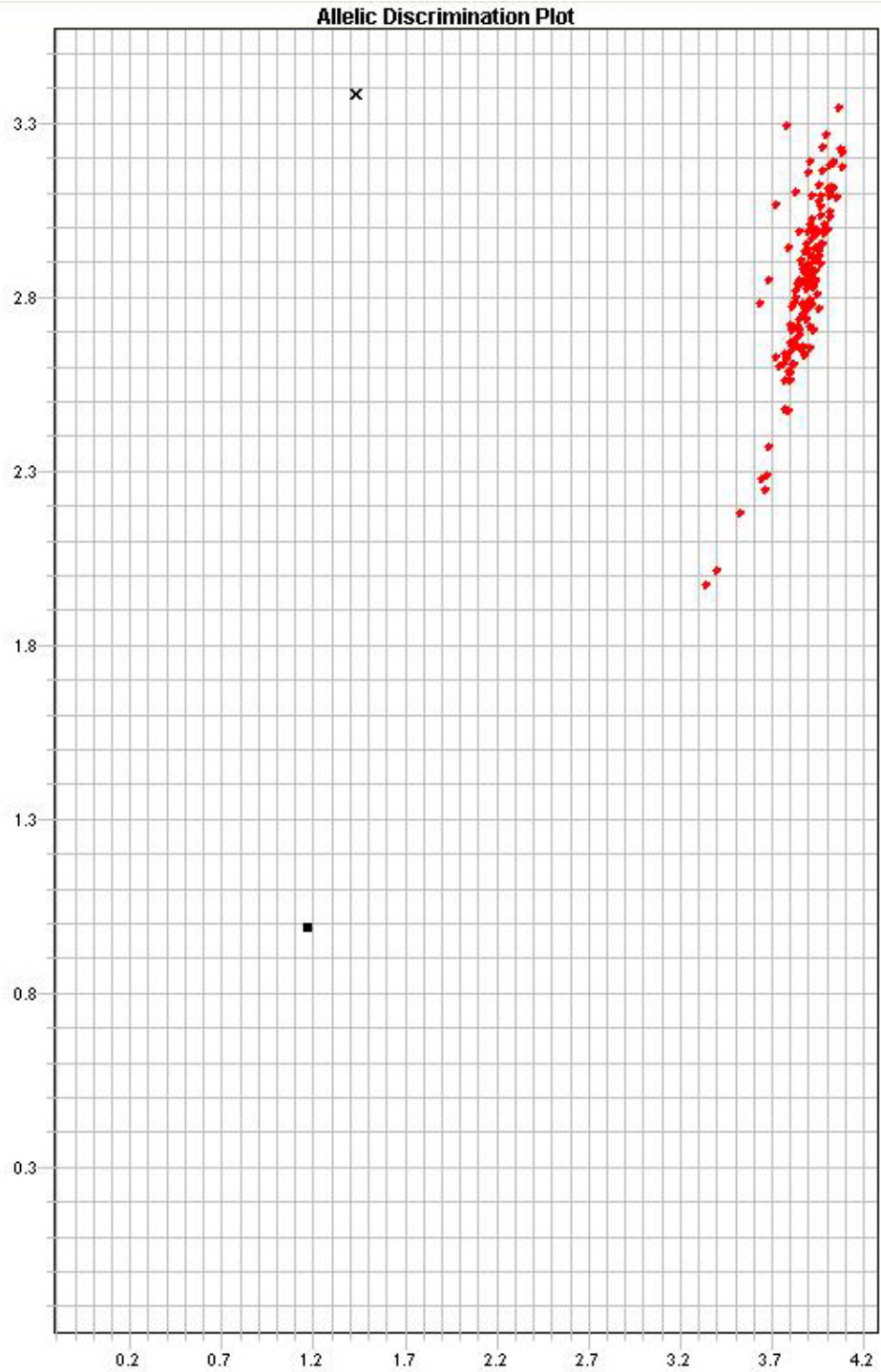
SNP: rs118204011

Plate Name: USA_14

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



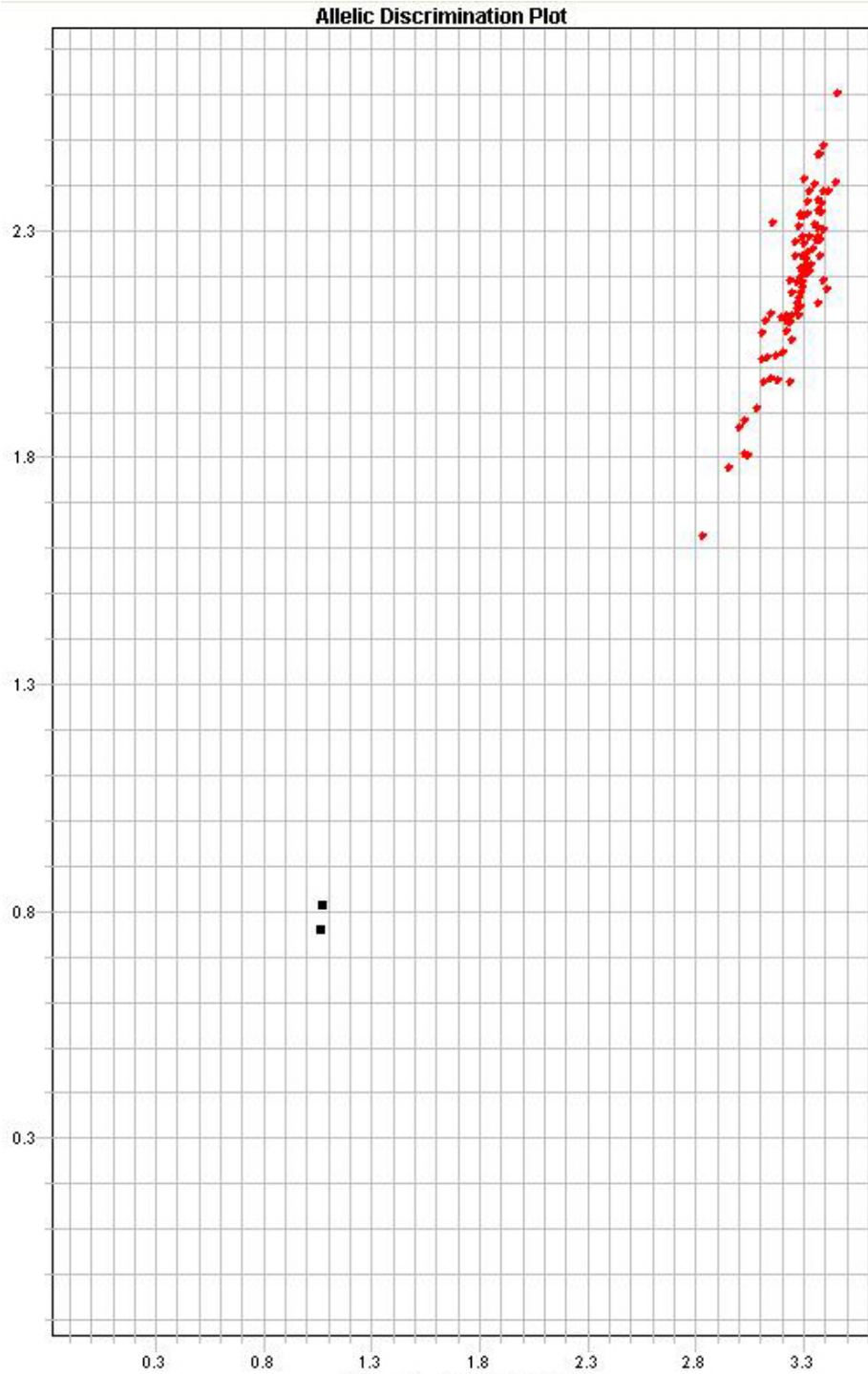
SNP: rs118204011

Plate Name: USA_reruns1

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0



SNP: rs118204011

Plate Name: USA_reruns2

Homozygote Controls on plate: 0

Heterozygote Controls on plate: 0

New Heterozygote's on plate: 0

