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Filaggrin genotyping using Taqman SNP genotyping assays

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Protocol status: Working
We use this protocol and it's working

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Abstract

The *FLG* gene encodes the filaggrin protein which is essential for epidermal barrier formation and hydration. Mutations in the filaggrin gene are associated with a broad range of skin and allergic diseases. This protocol is used to genotype four mutations (R501X, 2282del4, R2447X and S3247X) within the FLG gene.

Materials

Applied Biosystems QuantStudio5

Taqman Genotyping Master Mix (Applied Biosystems Cat. no. 4371355)
MicroAmp Optical 384-Well Reaction Plate, 1,000 plates (Applied Biosystems Cat. no. 4343370)
MicroAmp Optical Adhesive Film, 100 films (Applied Biosystems Cat. no.4311971)
UltraPure Nuclease-free water, 500ml (Invitrogen Cat. no. 10977035)

FLG mutation-specific primers and probes:
Sequence Detection Primer (Applied Biosystems Cat. no. 4304970)
Taqman TAMRA Probe (Applied Biosystems Cat. no. 450025)
Taqman MGB Probe (Applied Biosystems Cat. no. 4316034)

	A	B	C
	Mutation	Primer pairs	Probes
	R501X	Forward: 5’ CAC TGG AGG AAG ACA AGG ATC G 3’ Reverse: 5’ CCC TCT TGG GAC GCT GAA 3’	Wild type 5’ 6-FAM-CAC GAG ACA GCT C-MGBNFQ 3’ Mutant 5’ VIC-CAT GAG ACA GCT CC-MGBNFQ 3’ FAM=C; VIC=T
	S3247X	Forward: 5’ CCA GAA ACC ATC GTG GAT CTG 3’ Reverse: 5’ TGC CTG ATT GTC TGG AGC G 3’	Wildtype: 5’ 6-FAM-CAG TCA AGG CAC GG-MGBNFQ 3’ Mutant: 5’ VIC-AGC AGT AAA GGC ACG-MGBNFQ 3’ FAM=C; VIC=A
	2282del4	Forward-1: 5’ TCC CGC CAC CAG CTC C 3’ Forward-2: 5’ CCA CTG ACA GTG AGG GAC ATT CA 3’ Reverse: 5’ GGT GGC TCT GCT GAT GGT GA 3’	Wild type: 5’ 6-FAM-CAC AGT CAG TGT CAG GCC ATG GAC A-TAMRA 3’ Mutant: 5’ VIC-AGA CAC ACA GTG TCA GGC CAT GGA CA-TAMRA 3’ FAM=T; VIC=A
	R2447X	Forward-1: 5’ ACG TGG CCG GTC AGC A Forward-2: 5’ AGC ACT GGA GGA AGA CAA GGA T 3’ Reverse: 5’ CCT GAC CCT CTT GGG ACG T 3’	Wild type 5’ 6-FAM-CAC GAG ACA GCT C-MGBNFQ 3’ Mutant 5’ VIC-CAT GAG ACA GCT CC-MGBNFQ 3’ FAM=C; VIC=T

Primers and probes for FLG mutation screening by Taqman allelic discrimination

Prepare primers and probes

1 Primers are sent in a concentration of 20pmol/µL and volume 500µL. To prepare primers mix 500µL of primers at 20pmol/µL with 100µL of nuclease-free water.

2 To prepare probes mix an equal volume of probe with nuclease-free water (based on a probe concentration of 6,000 pmol).

Prepare reaction mix

3 The tables below are for one sample based on 10µL reaction volume (2µL of DNA and 8µL of reaction mix). Multiply up reaction mix by number of samples plus extra for dead-volume. Ensure a sensible amount for accurate pipetting.
(This means that for R501X and S3247X primers will be at 300nM & probes at 100nM; and for 2282del4 and R2447X - Forward and Reverse primers will be at 500nM, Inner Forward Primer 2 at 100nM & probes at 100nM).

4 For R501X and S3247X prepare a mastermix based on per sample volumes in the table.
Prepare a separate mastermix for R501X and for S3247X:

	A	B
	Reagent	Volume (µL)
	Primer Forward	0.03
	Primer Reverse	0.03
	Probe 1	0.02
	Probe 2	0.02
	Taqman Genotyping Master Mix (2x)	5
	Nuclease-free water	2.90

5 For 2282del4 and R2447X prepare a mastermix based on per sample volumes in the table.

	A	B
	Reagent	Volume (µL)
	Primer Forward	0.05
	Primer Reverse	0.05
	Probe 1	0.02
	Probe 2	0.02
	Inner Forward Primer 2	0.01
	Taqman Genotyping Master Mix (2x)	5
	Nuclease-free water	2.85

Prepare reaction plate

6 Add 2µL of DNA at a concentration of 10ng/µL, for each sample to be genotyped, to a 384-well plate. Ensure at least 16 wells of the 384 have no DNA added for blank controls (these wells should still have reaction mix added).

PCR amplification

7 Create an experiment on QuantStudio5 or load an existing experiment template (EDT file) using QuantStudio Design & Analysis Software. Ensure assay file is uploaded into library. See QuantStudio user manual for details.

8 Load 384-well plate onto heating block ensuring plate is in correct orientation with well A1 positioned top-left.

9 Cycle with the following settings: 12m 15s

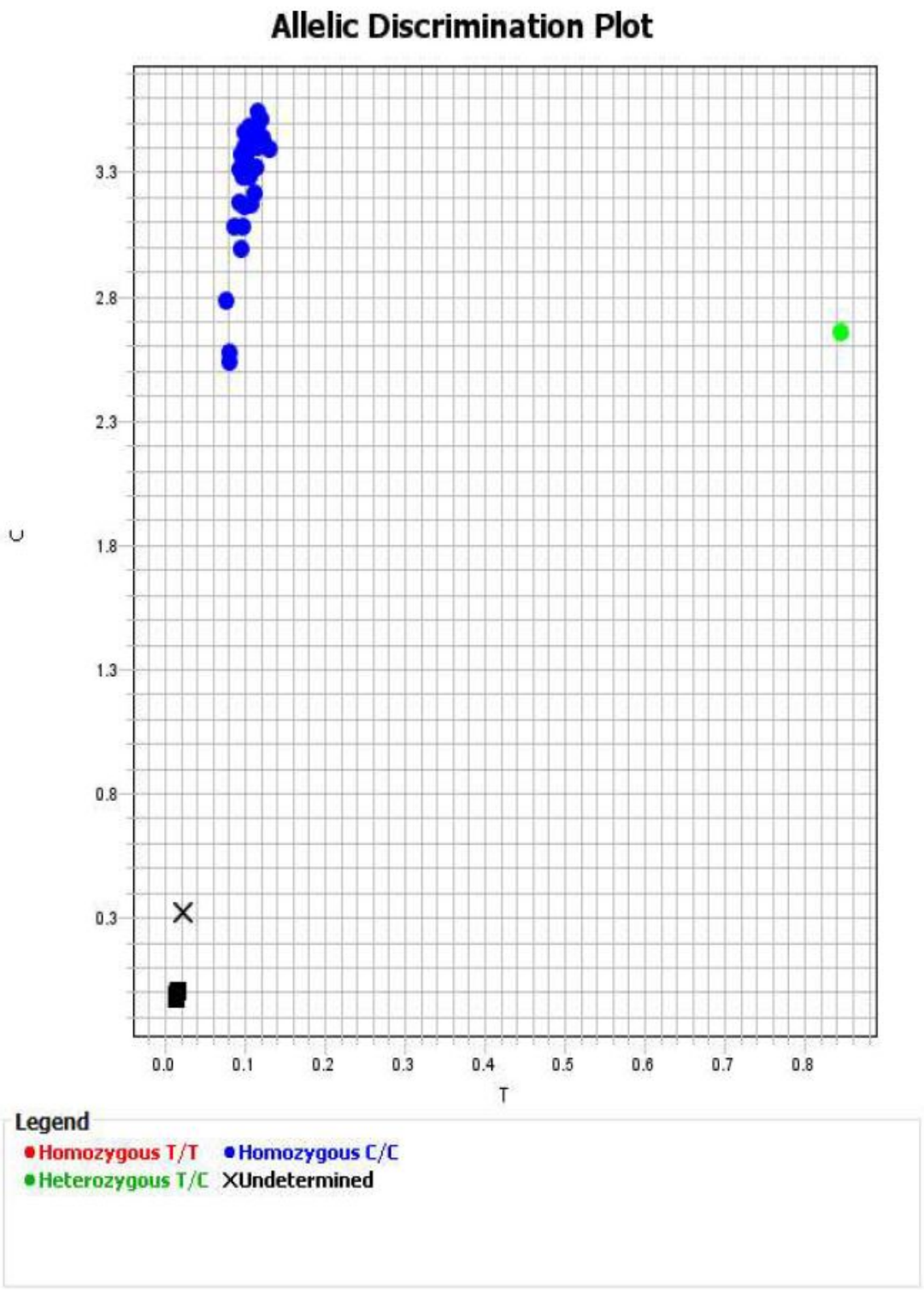
60 °C for 00:00:30
95 °C for 00:10:00
Then 40 cycles of
Denaturation: 95 °C for 00:00:15
Annealing/extension: 60 °C for 00:01:00
Then 60 °C for 00:00:30

Data analysis

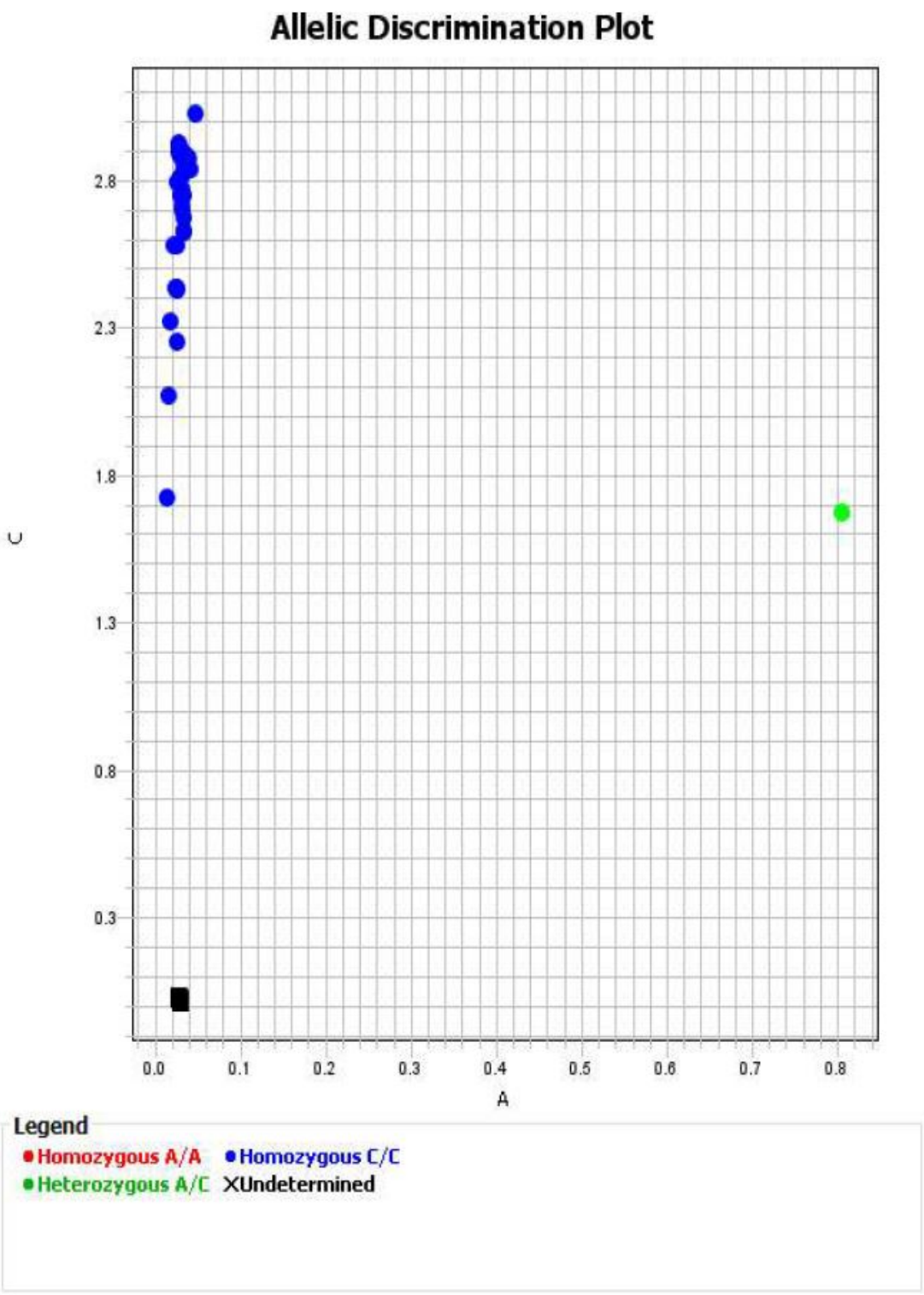
10 Within QuantStudio Design & Analysis Software review data to ensure clear clusters are visible and that blanks are not called.

11 For each genotype, export data to excel and save a copy of each allelic discrimination plot as a pdf file.

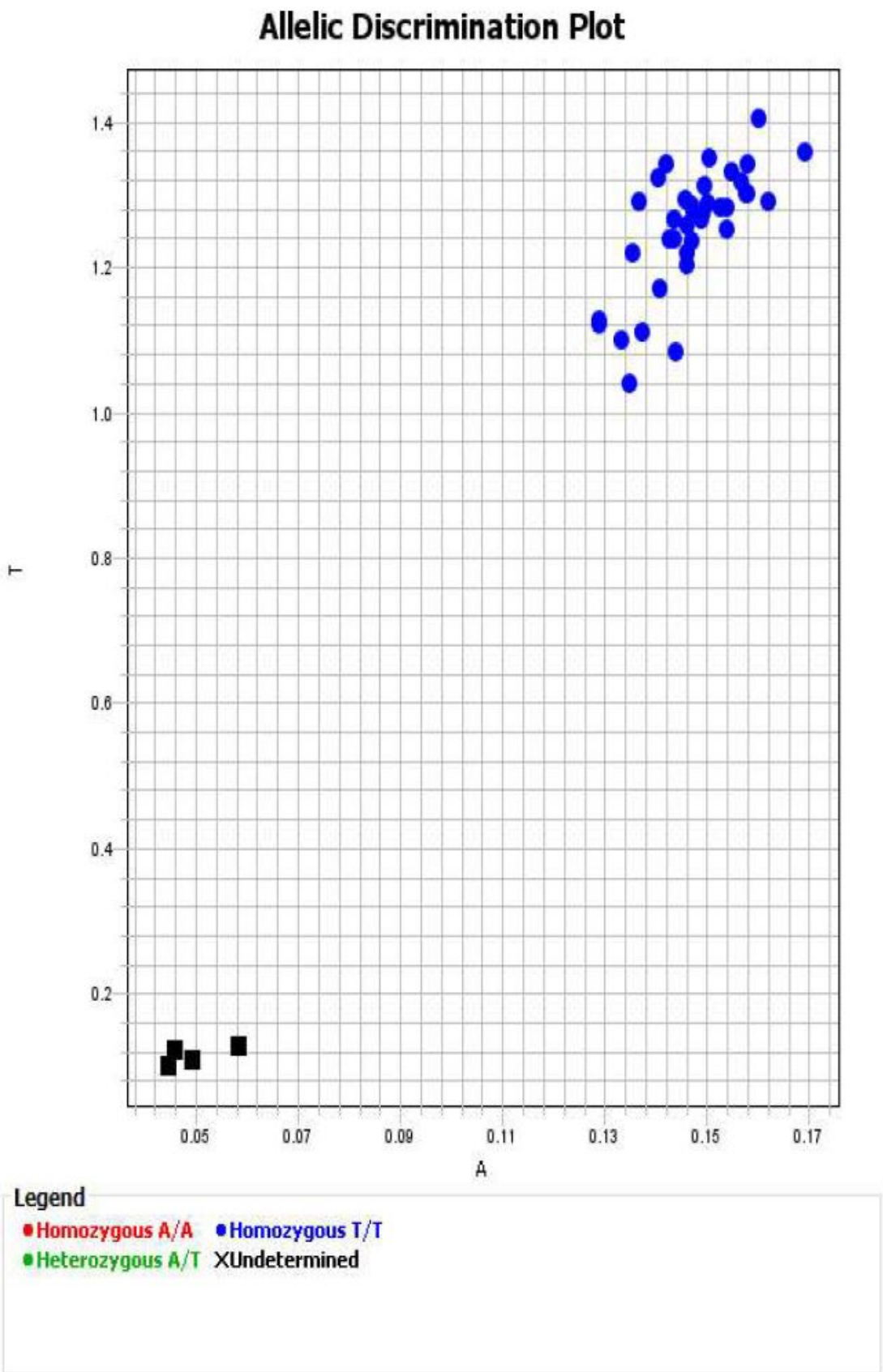
12 The FLG genotyping is looking for rare alleles so does not form the usual three clusters. The following clusters are examples to help determine final genotypes.



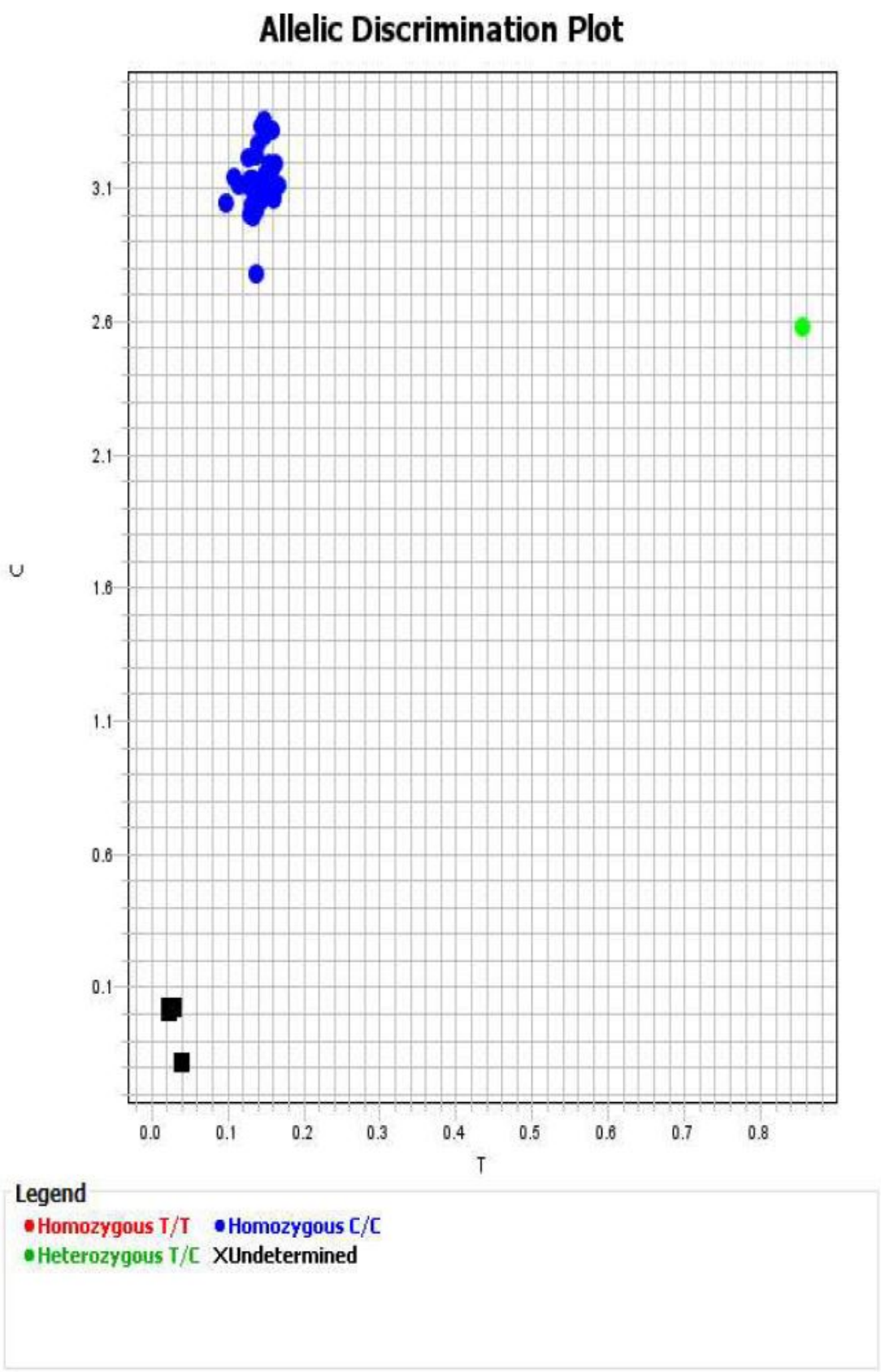
Cluster plot for R501X



Cluster plot for S3247X



Cluster plot for 2282del4



Cluster plot for R2447X

Data Report

13 Prepare the data summary report. Include information on scanner used, SOP, analysis software version and assays used. Detail call rates and any false call rates (blanks that gave a genotype). Return summary report with raw data (eds files), exported data (excel files) and allelic cluster plots (pdf).

Protocol references

Sandilands, A., Terron-Kwiatkowski, A., Hull, P. et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 39, 650–654 (2007). https://doi.org/10.1038/ng2020.

Genotyping Analysis Module User Guide for use with QuantStudio Design and Analysis Software v2. Publication Number: MAN0018749. Revision: D.0
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018749_GenotypingAnalysisModule_QSDASW_v2_UG.pdf
(Accessed 8 Apr 2024)