DEFRA

STANDARD OPERATING PROCEDURE (SOP) 1

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STANDARD OPERATING PROCEDURE FOR THE GENETIC IDENTIFICATION OF TRADITIONAL BREEDS OF CATTLE AND PIG USING DNA SNP MARKERS

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CONTENTS

1.   HISTORY / BACKGROUND

The production and sale of meat from traditional cattle and pig breeds in the United Kingdom represents a niche premium market that relies on the strength of the traditional breed brand to achieve higher market value. There is a financial incentive to unscrupulous meat producers and retailers to substitute meat from other cheaper breeds, thereby defrauding the consumer and competing unfairly against genuine traditional meat producers. There is a need for the UK authorities to be able to authenticate the breed of origin of meat labelled by breed, in order to deter mislabelling and potentially prosecute deliberate fraudsters.

Animals, their parts and derivatives can be identified using molecular genetic markers termed Single Nucleotide Polymorphisms (SNPs). The Standard Operating Procedure (SOP) described here is the result of an FSA project (no. Q01130) to develop a set of SNP genotyping assays capable of identifying cattle and pig meats sold by breed.

2.   PURPOSE

The purpose of this SOP is to provide a cattle and pig breed identification method to laboratory investigators that have the capacity to perform SNP genotyping assays.

3.   SCOPE

This method is suitable for the qualitative identification of DNA (deoxyribonucleic acid) belonging to any of eleven cattle breeds, thirteen pig breeds and wild boar, as listed in Appendix 1. The assay is designed to work with raw, boiled, fried, or baked meat as well as skin samples. It is not suitable for the identification of complex meat products containing DNA from multiple breeds, or breeds not listed in Appendix I.

4.   DEFINITIONS AND ABBREVIATIONS

DNA	Deoxyribonucleic acid
FSA	UK Food Standards Agency
PCR	Polymerase Chain Reaction
SOP	Standard Operating Procedure
UV	Ultraviolet

5.   PRINCIPLE OF THE METHOD

From a biological perspective, breed identification is equivalent to population assignment. Populations can be defined as groups of individuals that share greater genetic similarity with each other than they do with members of other populations. DNA markers can be used to differentiate populations and assign unknown samples to their correct population of origin. By targeting these markers during genetic analysis, it is possible to identify the breed of an unknown meat sample. The method presented here consists of three principal stages: i)

DNA extraction, ii) SNP genotyping, iii) data analysis and breed assignment. Briefly, DNA is extracted from unknown meat samples using standard laboratory protocols. A 'SNP-chip' (genotyping array) is then employed to generate genotypes for 72 SNP markers. The resulting genotypes for the unknown sample are combined to form a profile and this is submitted for analysis against a reference data set of multiple breed genotypes. The analysis assigns the unknown sample to the most likely breed and generates a probability of belonging to that breed relative to the claimed breed.

It is assumed that the person performing this SOP is familiar with standard genetic analysis techniques and molecular laboratory practice.

6.   MATERIALS AND EQUIPMENT
N.B. Batch numbers of kits used must be recorded

6.1   Water

General use: Distilled or de-ionised water
PCR procedures: Sterile, DNase-, RNase- and Protease-free water
                        e.g. Fisher Scientific DNA free water, product code:BPE2470-1

6.2   Solutions, standards and reference materials

1. Reference DNA:
    Positive control reference DNA for pig breeds (one of each)
    A positive control of known genotype (96-SNP profile) = QC control

6.3   Commercial kits

DNA Extraction

The method has been validated using the 'DNeasy Blood & Tissue Kit' supplied by Qiagen (Product code 69504). DNA extraction kits from other suppliers <u>must</u> be shown to be appropriate before use.

Illumina genotyping

Custom Bead-Express bovine-porcine genotyping SNP chips. This product is custom made by Illumina with a minimum order size of 480 samples. The order template, including sequences for each SNP on the assay, is in the accompanying file, 'Illumina Vercode template order.csv'

6.4   Plasticware

<u>It is essential that all plasticware is sterile before use</u>

Item		Detail	Supplier	Product code
1	1.5ml tubes	flat cap style	Starlab	S1605
2	200 µl PCR tubes	single, strip or 96-well	Starlab	B1402
3	Pipette tips (filtered)	10, 20 and 200 µl	Starlab	S1120

6.5   Equipment

The following items of equipment are required to undertake the analysis. With the exception of item 5 (Illumina BeadExpress), alternative suppliers/models are available for each item. These <u>must</u> be shown to be appropriate before use.

Optional items are referenced in the procedures and should be included in the SOP whenever possible.

Item		Detail	Example supplier	Product code
1	Precision pipettes	delivery of 1-200 µl	Starlab	G8900
2	Benchtop vortex		Labnet	VX-100
3	Micro centrifuge	to hold 1.5 ml tubes	Eppendorf	5452
4	Thermal mixer	to hold 1.5 ml tubes	Eppendorf	5355
5	Genotyping platform	BeadExpress	Illumina	
6	DNA Quantifier	Accurate to +/- 1 ng	Nanodrop	ND1000

Optional

7	Laminar flow hood	PCR Workstation	Gelman	

6.6   Other materials

Disposable plastic gloves, sterile dissection equipment

6.7   Electronic Files / Computer Software

The following electronic files and computer software are required as part of the method:

- a) Microsoft excel (version 2003 or later)
- b) GENECLASS2 population genetic software
- c) Excel file: Data conversion.xlsm (N.B. this requires macros to be enabled)
- d) Excel file: Porcine Analysis.xlsx
- e) Excel file: Bovine Analysis.xlsx
- f) Excel file: Illumina Vercode template order.csv

7.   PROCEDURES

It is <u>essential</u> to wear disposable plastic gloves during all procedures and to use pipette tips that are sterile and fitted with filters.

7.1 Sample Preparation

All samples should be stored frozen at -20°C until processed. Samples can be stored frozen indefinitely. Prior to processing, thaw samples at room temperature.

The external surfaces of samples submitted for analysis may have been affected through preservation treatments or bacterial breakdown. Where possible, obtain subsamples for DNA extraction from the least degraded area of tissue in order to minimize contaminant DNA and DNA degradation. This will typically mean removing outer layers of tissue in contact with the environment before taking a subsample. Use sterile dissection equipment where appropriate.

Test samples must be run in duplicate from sampling to results interpretation. Positive QC controls must be run in duplicate from the DNA extract stage

7.2   DNA Extraction

Materials:
The extraction should be carried out with the Qiagen DNeasy Blood & Tissue Kit, following the manufacturer's protocol.

Procedure:
1.   Cut up approx. 25 mg tissue into small pieces and place into a 1.5 ml tube.
2.   Include an empty 1.5 ml tube as an extraction control. This is treated following the same procedure and carried through to the PCR stage (7.3).
3.   Add 180 µl Buffer ATL (tissue lyser).
4.   Add 20 µl proteinase K and vortex for 15 seconds.
5.   Incubate in a thermal mixer at 56°C for 2 hours.
6.   Vortex for 15 seconds.
7.   Add 200 µl Buffer AL (cell lyser) to the sample and vortex for 15 seconds.
8.   Add 200 µl 100% ethanol and vortex for 15 seconds.
9.   Pipette the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.
10.   Centrifuge at 8000 x g for 1 minute
11.   Discard the eluate and replace the collection tube.
12.   Add 500 µl Buffer AW1 (wash 1).
13.   Centrifuge at 8000 x g for 1 minute
14.   Discard the eluate and replace the collection tube.
15.   Add 500 µl Buffer AW2 (wash 2).
16.   Centrifuge at 13,000 x g for 3 minutes
17.   Discard the eluate and collection tube. Place the spin column in a 1.5 ml tube.
18.   Pipette 100 µl Buffer AE (elution) directly onto the spin column membrane.
19.   Incubate at room temperature for 1 minute
20.   Centrifuge at 8000 rpm for 1 minute to elute DNA.
21.   Discard the spin column, close the tube and store the eluate containing DNA at 4°C for up to one week or in a freezer (-20°C) long term.
22.   DNA extract quantification. Extracted DNA must be quantified to assess the extraction process and enable normalisation of DNA concentration. DNA should be diluted to 50ng / µl using DNA-free water.

The DNA samples should now be prepared for delivery to an Illumina BeadXpress service provider, by aliquoting 20 µl of the 50ng/ul DNA into 200 µl PCR tubes.

Controls:
See section 7.6 for a list of sample controls to include with the test samples sent to the service provider.

7.3   SNP Genotyping

Materials:
Illumina Veracode custom assay (as per file: Illumina Vercode template order.xlsx
Illumina BeadExpress platform and associated equipment

DNA from test samples: 20 µl volume of 50ng/µl

Procedure:
The procedure should follow the standard protocol described in the 'Illumina GoldenGate Genotyping Assay for Veracode Manual Protocol' (Appendix II)

Output requirements:
Sample data should be provided in the form of a final DNA report with the following report criteria:

Zeroed SNPs	included
Excluded samples	retained
Replicate samples	report separately
Data format	matrix
GC score	removed
File type	tab delimited

The final data report should be saved as a standard text file, named 'FinalReportData.txt'.

7.4   Data analysis

ANALYSIS SECTION A

Conversion of Illumina output files

The data received from the Illumina service provider will include a file named 'FinalReportData.txt'. This file contains the DNA profiles for each sample tested. The DNA profiles will consist of a string of 192 SNPs (96 bovine and 96 porcine). Only 96 of these SNPs will be relevant to the analysis, depending on the product under test.

The first stage of the data analysis involves extracting the necessary information from the Illumina file and converting it into a format suitable for downstream analysis. The required format is known as 'GenePop' format.

File conversion should be undertaken as follows:

1.   Open the excel file named 'Data conversion.xlsm'
2.   Save a copy of the file with a sample specific name. Continue to use this file.
3.   On the 'Import data' worksheet place the cursor in cell A1, then select 'Data' from the toolbar, then 'Import Text file'. Select the Illumina output file, 'FinalReportData.txt'
4.   In the dialogue box, select the 'Delimited' box, then click 'Next'
5.   Select 'tab' as the only 'Delimiter'
6.   Select 'none' from the 'text qualifier' drop down, then click 'Next'
7.   Click 'Finish', then Click 'OK' on the next box.
8.   Highlight the first nine rows and delete them.
9.   Highlight all of the data, select 'Copy', Click onto the 'Transpose sheet' of the excel workbook, select 'Paste Special', 'Transpose', 'OK'.

     The data should now appear in horizontal rows, with each row representing a sample (Figure 1). The header row lists the names of the SNPs

10. Remove the 96 SNPs that relate to the species (cow or pig) that is NOT being tested:
     a. If the test sample is beef, run the bovine macro (Press Ctl+b)
     b. If the test sample is pork, run the porcine macro (Press Ctl+p)

11. Porcine test: locate and highlight the column with SNP 'DIAS0000661' (Column C?). Press 'Control + F' on the keyboard to bring up the 'Find/Replace' dialogue box. Find 'CC' - Replace with 'AA'; Find CG – Replace with AG.

12. Highlight the SNP genotype data (i.e. the cells with the two-letter codes). Press 'Control + F' on the keyboard to bring up the 'Find/Replace' dialogue box. Perform the following Find/Replace commands:

Find	Replace
AA	'0101
AC	'0102
AG	'0102
AT	'0102
CC	'0202
GG	'0202
TT	'0202
--	'0000

13. Select each sample name cell, one at a time, and add a comma (,) to the end of each sample name.
14. Select all of the rows containing sample data and press 'Copy'
15. Select either the 'Porcine' or 'Bovine' worksheet, as appropriate, place the cursor in cell A99 and press 'Paste'.
16. Save the excel file.
17. Save the worksheet as a Tab delimited text file:
    a. Select 'Save as'
    b. Select 'Text (tab delimited)' from the file type drop down
    c. Name the file according to the samples within in. Click 'Save'
    d. Click 'OK' to accept only one worksheet to be saved
    e. Click 'Yes' to accept format
18. Close the excel file, select 'No' to saving changes when prompted
19. Open the text file and check that the format agrees with Figure 2
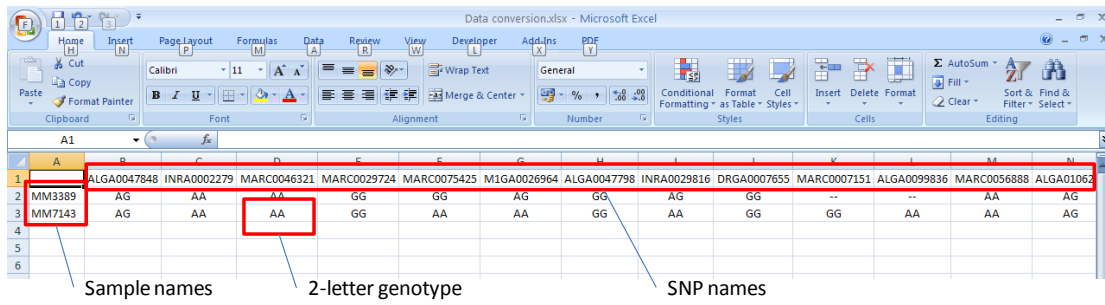
Figure 1   Screenshot of the 'Transpose sheet' from the Data Conversion excel file, showing the data for two samples. The header row lists the SNPs on the assay, the first column list the samples. The DNA results for each sample consist of a two-leter code for each SNP. ('--' signifies that the assay has failed for a sample at that SNP).
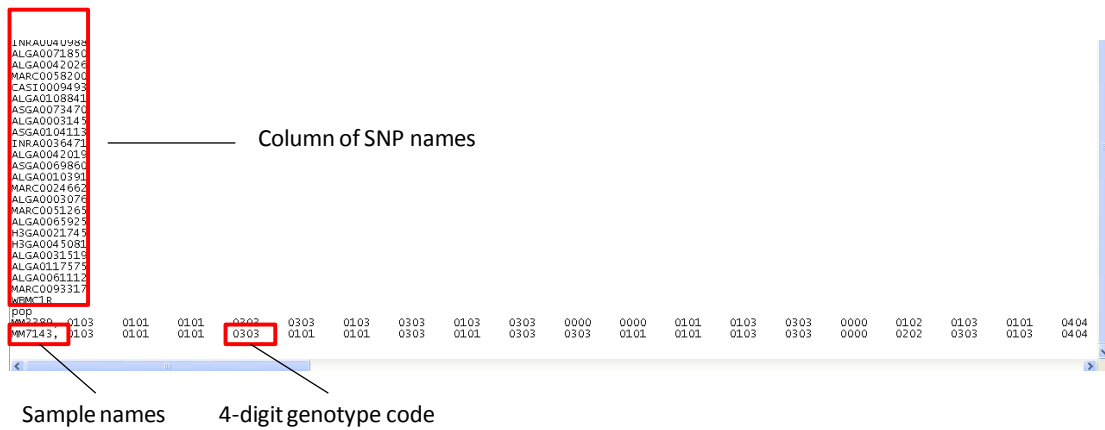

Figure 2   Screenshot of an example text file for two porcine samples, showing the bottom of the SNP list and sample names in the first column and the 4-digit genotype code in rows for each sample.

ANALYSIS SECTION B

GeneClass software

In order to perform the statistical assignment of the test sample to a breed it is necessary to use the population genetic software package, GENECLASS2.

GENECLASS2 is available to download for free from: http://www.ensam.inra.fr/URLB/.

1. Download the set-up file from the webpage (normally the English Windows version)
2. Install the program in an appropriate folder.

The program is used to calculate the likelihoods associated with the test sample originating from each of the breeds in the reference data. Both the reference data and the test sample data need to be inputted into the GENECLASS2 programme.

3. Create two folders, one named 'Geneclass input', the other named 'Geneclass output'.
4. Copy the 'porine_geneclass.txt' and 'bovine_geneclass.txt' files that have been provided into the 'Geneclass input' folder.
5. Copy the test sample text file generated from the ANALYSIS SECTION A into the 'Geneclass input' folder.
6. Copy the 'Porcine Analysis.xlsx' and 'Bovine Analysis' files that have been provided into the Geneclass output folder.

7. Open the GENECLASS2 software by double-clicking on the programme icon. By default, the programme will open on the 'Parameters' tab (top left of screen)
8. Load the appropriate reference data (bovine or porcine) by clicking on the 'Open'button to the right of the 'Reference population' bar. Select either 'porine_geneclass.txt' or 'bovine_geneclass.txt' from the Geneclass input folder you have created (Figure 3).
9. Load the appropriate test sample by clicking on the 'Open'button to the right of the 'Sample to be assigned' bar. Select the test sample text file you saved within the Geneclass input folder you have created (Figure 3).
10. Method. Within the 'Parameters' tab, there are four tabs that relate to the method.
    a. Tab 1) 'Computation goal'.
        i. Ensure that the 'Assign / exclude population as origin of individuals' box is checked.
        ii. Ensure that the 'Assign: Individuals' box is checked
        iii. Ensure 'Assignment threshold' bar is set to 0.05
    b. Tab 2) 'Criteria for Computation'.
        i. Ensure that the 'Bayesian method: Rannala & Mountain' box is checked
    c. Tab 3) 'Probability Computation'
        i. Ensure that no boxes are checked
    d. Tab 4) 'Locus selection'
        i. Ensure that all boxes are checked
11. Press the 'Start' bar at the bottom.

The programme should only take a few seconds to run. On completion, the screen will display the 'Results' tab (Figure 4).   There should be one line of data per sample with your sample file names on the left hand side.

12. Examine the number of SNPs used in the 'Nb of loci' column.

    DECISION:
    If the number of loci used is below 90, the test is assumed to have failed and should be re-run.

13. Examine the breed code listed under the Rank 1 column header.

    DECISION:
    If this breed code matches the breed of origin that is being claimed for the sample, the claim has been verified and the analysis is complete. GO TO 7.5 - Interpretation.

    If this breed code differs from the breed of origin that is being claimed for the sample, subsequent statistical analysis needs to be undertaken.   Record the Rank 1 breed listed for the test sample and continue with this SOP (Part 13)

14. In the 'Results' tab, click on 'Export' and save the file (as a .csv file type) to the Geneclass output folder you have created,using the test sample name as the file name, e.g. 'test_sample.csv'

15. Return to the 'Parameters' tab. The analysis needs to be re-run with a different method, as follows:
    a. Tab 1) 'Computation goal'.
        i. Ensure that the 'Assign / exclude population as origin of individuals' box is checked.
        ii. Ensure that the 'Assign: Individuals' box is checked
        iii. Ensure 'Assignment threshold' bar is set to 0.05
    b. Tab 2) 'Criteria for Computation'.
        i. Ensure that the 'Bayesian method: Rannala & Mountain' box is checked
    c. Tab 3) 'Probability Computation'
        i. Ensure that 'Enable probability calculation' box is checked
        ii. Ensure that 'Paetkau et al 2004' box is checked
        iii. Ensure that 'No. Simulated individuals' = 1000 and 'type I error (alpha)' = 0.05
    d. Tab 4) 'Locus selection'
        i. Ensure that all boxes are checked

16. Press the 'Start' bar at the bottom.

The programme will take a little longer (~15-20 seconds) to run. On completion, the screen will display the 'Results' tab, slightly different to the previous one (Figure 5). There should be a single line of data with your test sample file name on the left hand side.

The first column of data (immediately to the right of the sample name) shows the probability associated with the most likely breed.

    DECISION:
    If this probability is less than 0.05, it indicates that the true breed from which the sample originated is not in the data base and therefore cannot be identified. GO TO 7.5 - Interpretation.

    If this probability is greater than 0.05, then the analysis should proceed, following the SOP (Part 16).

17. Close the GENECLASS2 programme. Proceed to **Analysis Section C**
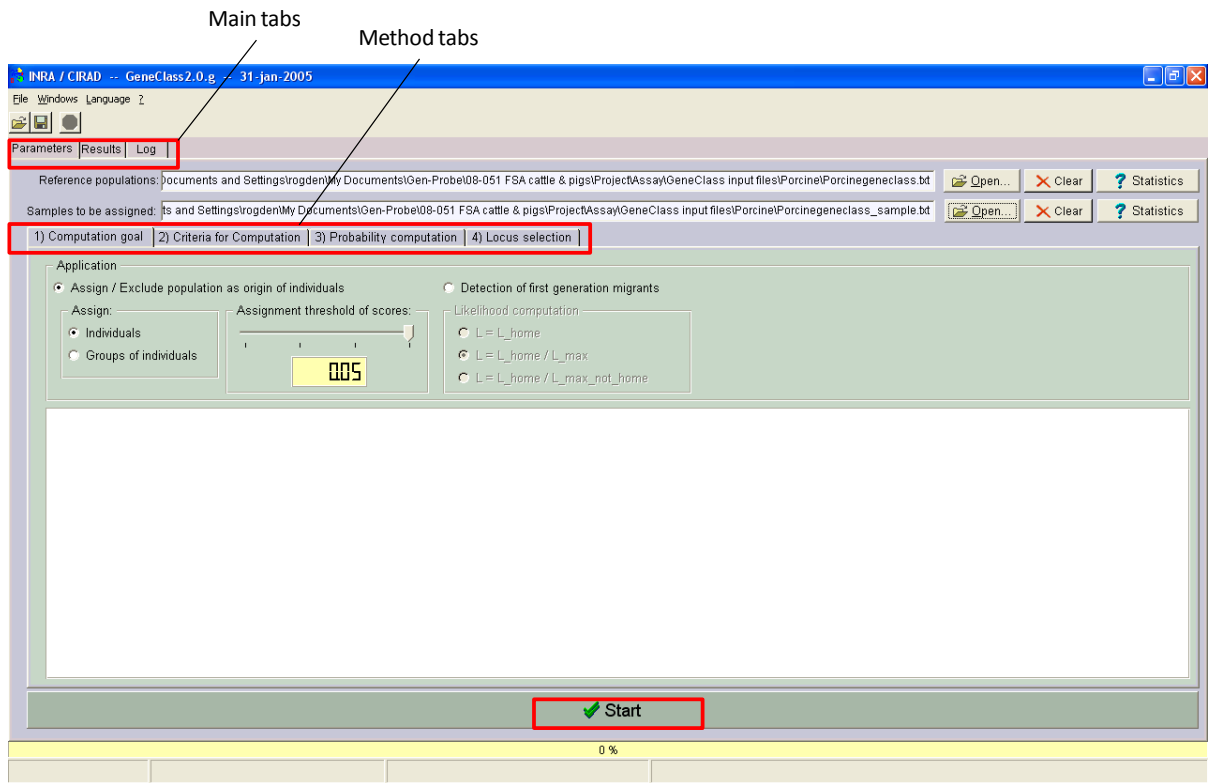
Main tabs

Method tabs

Figure 3   Screenshot of GENECLASS2 software opening page, showing reference and test file dialogue boxes, method tabs and analysis start button.
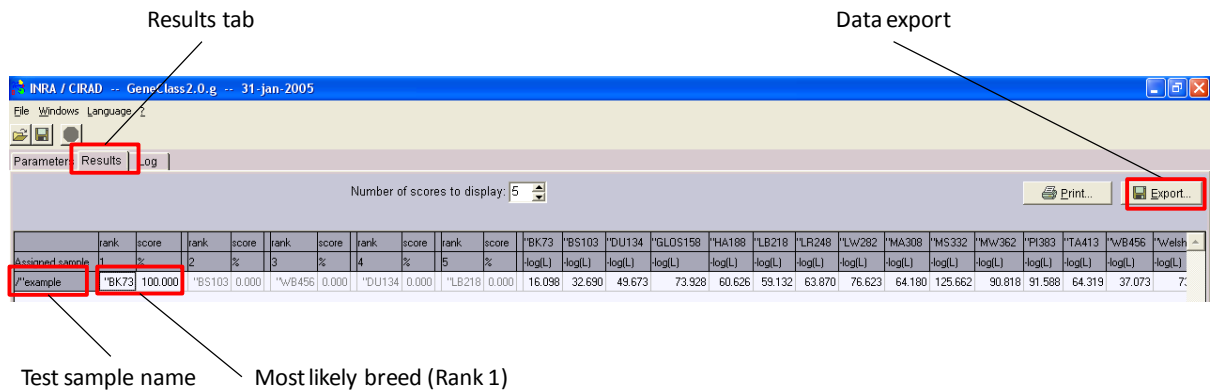
Figure 4   Screenshot of GENECLASS2 results page showing test sample number, mostly likely breed assignment and export function.
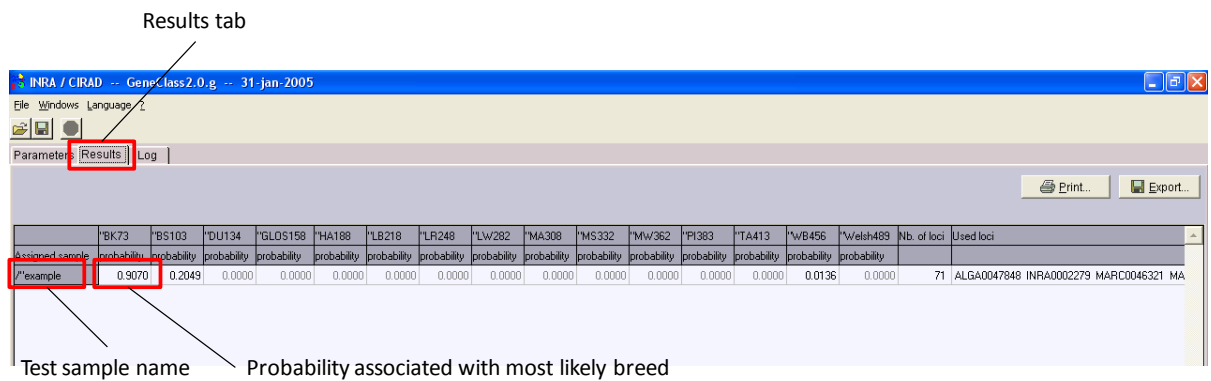

Figure 5   Screenshot of GENECLASS2 results page showing test sample number and the probability associated with the most likely breed (first data column). If this probability is greater than 0.05, then the analysis should proceed. If this probability is less than 0.05, it indicates that the true breed from which the sample originated is not in the data base and cannot be identified.

ANALYSIS SECTION C

Excel Spreadsheet Analysis

1. Open a new excel spreadsheet.
2. From the 'Data' tab, select 'import from text file'
3. Select the sample file from the Geneclass output folder you created (e.g. test_sample.csv)
4. In the dialogue box, select the 'Delimited' box, then click 'Next'
5. Select 'semicolon' as the only 'Delimiter'
6. Select 'none' from the 'text qualifier' drop down, then click 'Next'
7. Click 'Finish', then Click 'OK' on the next box.

The data should now be entered into the excel spreadsheet and look similar to the porcine example in Figure 6.

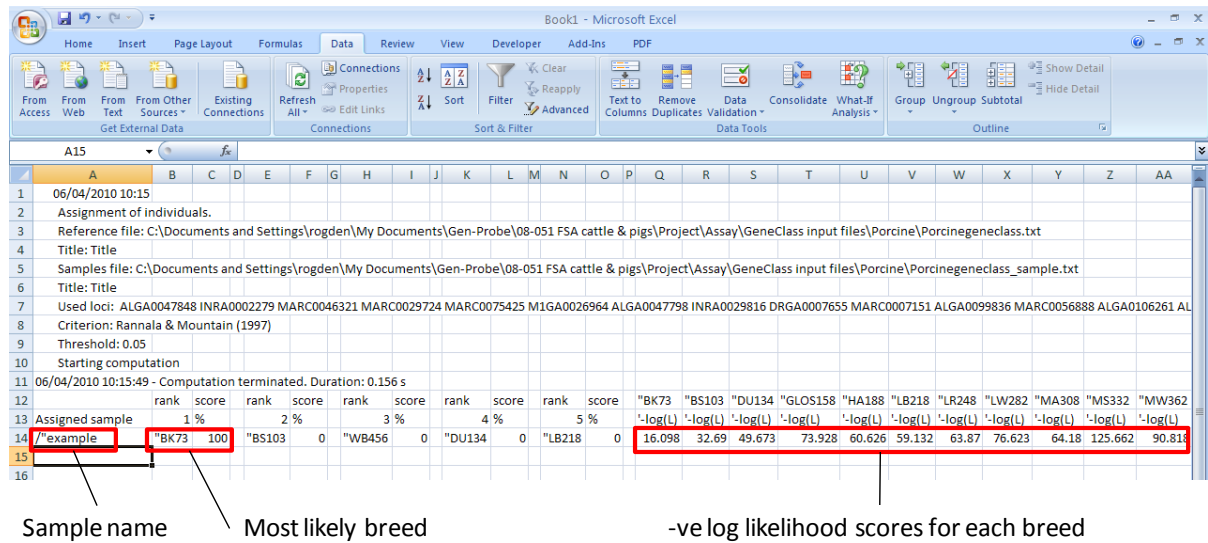Sample name   Most likely breed   -ve log likelihood scores for each breed

Figure 6   Example of successfully imported GENECLASS2 data within excel. The sample name, most likely breed and breed likelihood scores are highlighted.

8. Save the excel file in the Geneclass output folder. Keep the excel file open.
9. Open the excel file named 'Porcine Analysis'.
10. Save a copy of the relevant Analysis Sheet using the test sample name in the file name. Continue working with this copy. Close the original copy.

    This file contains three worksheets. Do NOT make any changes to these worksheets, other than described in the following instructions.

11. From the Geneclass output file created at (8), copy the value in the '-log(L)' column that relates to the Rank 1 breed in Column B (for example, in Figure 6, this would be the '-log(L) value for the BK73 breed (= 16.098 in cell Q14).
12. Paste this value into the corresponding cell in Column D on Sheet 1, Table A, of the Analysis sheet (Figure 7)
13. From the Geneclass output file created at (8), copy the value of the '-log(L)' column from the claimed breed (for example, in Figure 6, if the claimed breed was 'Hampshire' this would be the value of 60.626 in cell U14).
14. Paste this value into the corresponding cell in Column I on Sheet 1, Table A of the Analysis sheet (Figure 7)
15. Check: On the Analysis sheet, a value for the likelihood ratio should now appear in cell C20.
16. The appropriate probability for the test sample originating from the breed identified in GENCLASS2, rather than the breed claimed, is provided in Table B. The relevant probability should be copied from Table B and pasted (Paste special – Paste value) into the 'Result' box in cell G41. For example, the probability for a sample that claimed to be from a Hampshire pig, but which GENECLASS2 analysis identified as a Berkshire pig, should be copied from cell C29 of Table B (Figure 8).
17. Save the Analysis file. STOP. **GO TO 7.5 - Interpretation**

Table A

Breed identified in GeneClass2 (Rank 1)	Code	-Log(L)	Claimed bred of origin		Code	-Log(L)
Berkshire	BK73	16.098	Berkshire		BK73	
British Saddleback	BS103		British Saddleback		BS103	
Duroc	DU134		Duroc		DU134	
Gloucester Old Spot	GLOS158		Gloucester Old Spot		GLOS158	
Hampshire	HA188		Hampshire		HA188	60.626
Large Black	LB218		Large Black		LB218	
Landrace	LR248		Landrace		LR248	
Large White	LW282		Large White		LW282	
Mangalica	MA308		Mangalica		MA308	
Meishan	MS332		Meishan		MS332	
Middle White	MW362		Middle White		MW362	
Pietrain	PI383		Pietrain		PI383	
Tamworth	TA413		Tamworth		TA413	
Wild Boar	WB456		Wild Boar		WB456	
Welsh	Welsh489		Welsh		Welsh489	

Figure 7   Screenshot of Table A from Porcine Analysis sheet. −log(L) values have been entered for the most likely breed (Berkshire, BK73) and for the claimed breed (Hampshire, HA188).

Probability for sample, cell C29

Table B — Probability table

Claimed breed	Breed identified in GeneClass2 (Rank 1)														
		BK73	BS103	DU134	GLOS15	HA188	LB218	LR248	LW282	MA308	MS332	MW362	PI383	TA413	WB456	Welsh
	Berkshire	x	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	British Saddleback	1	x	1	1	1	1	1	1	1	1	1	1	1	1	1
	Duroc	1	1	x	1	1	1	1	1	1	1	1	1	1	1	1
	Gloucester Old Spot	1	1	1	x	1	1	1	1	1	1	1	1	1	1	1
	Hampshire	1	1	1	1	x	1	1	1	1	1	1	1	1	1	1
	Large Black	1	0.9336	1	1	1	x	1	1	1	1	1	1	1	1	1
	Landrace	1	1	1	1	1	1	x	1	1	1	1	1	1	1	0
	Large White	1	1	1	1	1	1	0	x	1	1	1	1	1	1	0
	Mangalica	1	1	1	1	1	1	1	1	x	1	1	1	1	1	1
	Meishan	1	1	1	1	1	1	1	1	1	x	1	1	1	0.9997	1
	Middle White	1	1	1	1	1	1	0.9999	0	1	1	x	1	1	1	1
	Pietrain	1	1	1	1	1	1	0.9929	0.9995	1	1	1	x	1	1	1
	Tamworth	1	1	1	1	1	1	1	1	1	1	1	1	x	1	1
	Wild Boar	1	1	1	1	1	1	1	1	1	1	1	1	1	x	1
	Welsh	1	1	1	1	1	1	0	0	1	1	1	1	1	1	x

Result P(belonging to identified breed, given the claimed breed)   =   1

Probability result, cell G51

Figure 8   Table B showing probabilities associated with breed assignment. For a sample claimed to be from Hampshire pig but identified during analysis as being from Berkshire pig, the relevant probability is found in cell C29 at the intersection of these breeds. The probability value of 1, indicated that it is a cetainty that the sample originated from a Berkshire pig, given the alternative hypothesis of a Hampshire pig.
Note that all other values in Table B are meaningless.

7.5   Interpretation

The data relating to the test sample has now been processed. There are three possible outcomes.

1) **The GENECLASS2 analysis identifies the claimed breed as the most likley source of the sample.**
   In this event, the breed of origin has been verified.

2) **The GENECLASS2 analysis identifies an alternate breed as being most likely, but it's associated probability in GENECLASS2 (Figure 5) is less than 0.05.**
   In this event, the claimed breed can be excluded as the breed of origin, however no other breed can be identified as the true source of the sample.

3) **The GENECLASS2 analysis identifies an alternative breed (P>0.01). The subsequent Excel spreadsheet analysis compared the relative likelihoods of the claimed breed and identified breed and calculated a probability.**
   This probability is sample specific and takes into account the possibility of samples from each breed being mistakenly identified as originating from the other.

   Where the probability value is 1, this means that the distribution of genetic variation observed in the two breeds does not overlap for the DNA result observed in the test sample. It can be concluded that the sample must have come from the identified breed, rather than the claimed breed.

   Where the probability value is less than 1, this means that the distribution of genetic variation observed in the two breeds may overlap for the DNA result observed in the test sample. The probability value indicates the level of certainty with which it can be concluded that the sample came from the identified breed, rather than the claimed breed.

7.6   Quality Assurance

Extraction Control
Included to check for extraction kit contamination. Only negligible DNA should be detected during quantification (<2ng/ul). If significant levels of DNA are detected, sterilize all equipment and repeat DNA extractions.

Negative SNP Assay Control
Included to check for background laboratory contamination. No SNP genotypes should be produced.

Positive SNP Assay Control (QC control)
The profile generated from the duplicated positive controls should match the control breed. If a mismatch is detected, it is likely the SNP genotyping assay has not run correctly.

The profile generated from the positive controls should show identical genotypes to the reference genotype in at least 90 of the 96 SNPs on the assay.

Repeat SNP Assay Control
One sample, or the positive control, should be run twice to ensure that the genotypes produced are identical among sample replicates.

APPENDICES

Appendix I

The list of breeds that can be identified using this SOP:

Welsh/Landrace

Gloucester Old Spot

Berkshire

Hampshire

Large Black

Large White

Middle White

Oxford Sandy & Black

British Saddleback

Mangalica

Tamworth

Pietrain

Duroc

Wild boar

Appendix II

---------- END OF DOCUMENT ----------