

CERTIFICATION OF ANIMAL-DERIVED PRODUCT(S) BASED ON DNA-METHYLATION

FIELD OF THE INVENTION

- 5 The present invention relates to a method of determining certification of an unknown animal derived product sample based on specific panels of genes that provide a source for the generation of DNA methylation profiles which are specific for a distinct certification. In particular, DNA methylation profiling may be used to determine the distinct certification of animal-derived products. The distinct certification may include halal, kosher and the like.

10

BACKGROUND OF THE INVENTION

- Sustainable food production is presently considered among the globally most important societal needs. As the value chains of the agriculture and aquaculture industries are highly complex,
- 15 certificates have been established to reinforce consumer relationships and trust. However, certificates are based on audits at specific farms and can be easily tampered by moving livestock from non-certified farms to certified farms. Furthermore, surveillance of sustainable farming practices is imperfect as it is largely limited to audits. This leaves room for fraudulent activities in the industry, so there is an urgent need for a tamper-proof analysis and certification of meat.
- 20 Further, there are people in the world that abide by dietary restrictions that relate specifically to a tradition or a religion that they follow. For example, some Jewish people eat only foods that have been deemed Kosher in accordance with Jewish law. Similarly, some people from the Muslim religion may desire to eat only foods that have been deemed Halal in accordance with Islamic law. Other people may have other dietary restrictions that relate to other traditions, religions, ethical
- 25 codes, or the like. Both Kosher certification (i.e., designation of a food item as Kosher) and Halal certification (i.e., designation of a food item as Halal) of food, including meat involve the conformance with specific laws that relate to the slaughter of animals. For example, Judaic ritual slaughter (Shechita) and Islamic ritual slaughter (Zabihah) require an animal to be cut across the neck with a non-serrated blade in a single clean attempt to sever main blood vessels of the animal,
- 30 and to drain the blood of the animal. Health, safety and also animal welfare considerations demand that the origins of animal products, and in particular meat products, should be traceable, so that quality assurance audits, and monitoring procedures can be effectively and reliably carried out. There is, however, no present means of accurately and reliably tracing the origins of meat. In particular, outside of trusting the labels found on the meat packets and shops, there is no reliable,
- 35 effective and easy to use method for distinguishing halal meat from non-halal meat and kosher meat from non-kosher meat. That is to say, although meat may be certified as Kosher (i.e., allowed under the Jewish tradition) or Halal (i.e., allowed under the Islamic tradition), buyers may be unable to determine whether the meat was properly slaughtered based on visual inspection. Moreover, when consuming food at a restaurant, customers may be unable to view an inspection label affixed
- 40 to packaging of meat prior to preparation of the meat. Furthermore, a customer may be unable to determine a type of animal from which the meat is derived based on visual inspection. This may result in a person who abides by a dietary restriction being hesitant to consume animal products.

Physical conditions and/or chemical conditions of an animal may be altered based on the way the animal has been slaughtered. For example, it is well known that cortisol levels of animals for which Kosher slaughter or Halal slaughter may be performed may be different from cortisol levels of animals that undergo another means of slaughter. Also, different types of meats obtained from different animals may be associated with physical differences and/or chemical differences. For example, proteins from pork meat of a pig may differ from proteins from beef meat of a cow. Differences may also exist in relation to different breeds of a particular type of animal, different cuts of meat of a particular animal, or the like. This may limit an effectiveness of comparing an unknown sample of meat to a set of known reference samples of meat.

Recent studies have shown that DNA methylation patterns of animals contain important information about their rearing conditions. For example, a comparison of genome-wide patterns of methylation and variation at the DNA level revealed that a highly significant proportion of epigenetic variation could be associated with fitness differences and rearing conditions such as captivity in salmon (Le Luyer J et al. 2017 PNAS vol 114, no 49).

Koop et al. and Rhein et al. also separately confirmed that methylation patterns are maintained postmortem and are consistent regardless of the level of decomposition of the sample. One of the only aspects that still affects the methylation pattern of the sample would be the DNA integrity and amount of DNA that is found in the sample (Koop et al., 2021 International Journal of Legal Medicine 135:167–173 and Rhein et al., 2015 Frontiers in Genetics 6: 182).

In view of the above, there is an urgent need to provide means for identifying and quality controlling the certification of organisms, in particular food and more particularly animal material derived to satisfy the dietary restrictions relating to a tradition, a religion, an ethical code, or the like followed strictly by people.

DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing a method using DNA methylation patterns to distinguish one type of animal derived product from another type of animal derived product and to be able to determine the means of killing or slaughtering the animal from which the animal derived product derives from and thereby certify the product accurately and reliably. The present invention is based on the finding that the means of slaughtering an animal, results in stress in the internal environment of the animal and this can permanently change the genome of the animal through epigenetics. In particular, the capability to adapt to the environment (i.e. means of slaughtering) and maintain the adapted biological pattern depends on epigenetic mechanisms, including DNA methylation. In particular, the present invention is based on the finding that the method of slaughtering an animal for food consumption may also result in changes in epigenetic mechanisms of the animal, including DNA methylation patterns and these patterns may be passed down to the different products that may derive from the animal.

The inventors have unexpectedly found that this property can be utilized to identify "epigenetic fingerprints" on the genome that are specific to a method of slaughter of not just one animal but possibly all the animals that undergo the same method of slaughter. For example, these 'epigenetic fingerprints' may be specific not only for the animal that is slaughtered in a particular way but also

5 for any product that stems from the very animal (an individual). Based on these findings, the present invention provides means to identify the specific method of slaughter of a particular animal, for example rearing animals also known as livestock and poultry from which an animal-derived product comes from. In particular, the method according to any aspect of the present invention may be used to determine if an animal-derived product is derived from an animal that has been

10 slaughtered in a particular way or not. More in particular, the method according to any aspect of the present invention may be used to determine if an animal-derived product is derived from an animal that has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or if the animal has been bled to death. In this way, the method according to any aspect of the present invention may then be used

15 to accurately and reliably determine the method of slaughter of an animal and then provide and/or confirm the certification of any sample that originates from the animal. Further, the method according to any aspect of the present invention may also be used to identify if any animal-derived product is accurately and reliably certified, particularly where the certification is based on the method of slaughter of the animal.

20

According to one aspect of the present invention, there is provided a method of certifying a test animal-derived product sample, the method comprising the steps of:

- (a) determining a test methylation profile of one or more pre-selected methylation sites within the genomic material obtained from the test animal derived product sample; and
- 25 (b) comparing the test methylation profile obtained from (a) with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal
- 30 is not bled to death

wherein a difference in the test methylation profile of (a) compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived

35 product is certified so; and

wherein the pre-selected methylation sites are CpG sites selected from genes or regions of genomic DNA from the control and test animal that shows the highest degree of methylation variation during the training of the method.

40 According to another aspect of the present invention, there is provided a method of certifying a test animal-derived product sample, the method comprising the steps of:

- (a) determining the methylation status of at least one CpG site within the genomic material obtained from the animal-derived product sample,
- (b) comparing the methylation status of the CpG site from (a) with that of a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death,

wherein a difference in the test methylation status of (a) in the test animal compared to the CpG site in the control animal is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product is certified so.

According to yet another aspect of the present invention, there is provided a method of certifying a test animal-derived product sample, the method comprising the steps of:

- (a) determining the test methylation profile of one or more pre-selected methylation sites within the genomic material obtained from the test animal derived product sample; and
- (b) comparing the test methylation profile determined in (a) with a panel of predetermined reference methylation profiles of the same biological taxon of the test animal from which the product sample derives, wherein each of the predetermined reference methylation profiles is specific for a distinctly certified reference animal that
- has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus; and/or
 - bled to death;

wherein if the test methylation profile is significantly similar to one of the predetermined reference methylation profiles, the test animal derived product sample has the same distinct certification as that of the predetermined reference methylation profile and is derived from a test animal that has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or bled to death and the test animal-derived product is certified so.

As used herein, the term 'animal-derived product' refers to products that originate from animals. In particular, the term 'test animal-derived product' refers to the sample or subject in question that is to undergo the method according to any aspect of the present invention. These products from animals may include meat and meat products, also including fat, flesh, blood, and lesser-known products, such as isinglass and rennet, poultry products (meat and eggs), dairy products (milk and cheese), and non-food products such as fiber (wool, mohair, cashmere, leather and the like).

Animal-derived products may also include products that can be made using animal products (e.g. fat) such as soap, creams and such. In one example, the animal-derived product is meat, eggs,

blood, brain, sperm, milk and any other tissue or sample that provides genomic DNA. In particular, the animal-derived product is meat.

The term 'certification of quality' refers to a certificate or a confirmation given by designated
5 certification agencies that endorse the quality of a particular animal derived product, including food for use and/or consumption by human beings. The term 'certification of quality' is used interchangeably with the term 'certification'. These certifications are usually found on the packaging of the animal-derived product including food to be consumed and are printed by the manufactures of the products. Examples of certifications of distinct food quality may include 'Haltungsform',
10 'Tierwohl', 'Ohne Gentechnik', 'halal', 'kosher', and other safe labels that confirm that a product sold has been prepared in accordance with specific religious or safety regulations. Specifically, the term 'certification of food quality' refers to a certificate or a confirmation given by designated certification agencies that endorse the quality, source or means of slaughter of a particular food for consumption by human beings. According to any aspect of the present invention, the certified
15 quality may be a distinct certified food quality, or distinct certification and this may be kosher, non-kosher, halal or non-halal.

In one example, the distinct certified food quality or certification of the sample X according to any aspect of the present invention may be kosher, non-kosher, halal or non-halal. More in particular,
20 kosher or halal refers to the sample X originating from an animal that was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, the trachea and/or the oesophagus. Even more in particular, the animal is drained of blood.

The term 'kosher' used in combination with food according to any aspect of the present invention
25 refers to food that conforms to Jewish dietary regulations of *kashrut* (dietary law) or food that may be consumed according to *halakha* (law). Kosher used in relation to meat relates particularly to a manner in which animals are prepared for consumption. According to Jewish tradition, meat may be considered kosher when the meat comes from animals that have been slaughtered according to Jewish law where the animal is killed by a single cut across the throat to a precise depth, severing
30 both carotid arteries, both jugular veins, both vagus nerves, the trachea and the oesophagus, no higher than the epiglottis and no lower than where cilia begin inside the trachea, causing the animal to bleed to death. Such slaughter is to be carried out using a large, razor-sharp knife, which is checked before each slaughter to ensure that it has no irregularities (such as nicks and dents). The slaughter is usually also carried out by a shochet or a rabbi. Kosher meat usually refers to most
35 meats excluding pig. In particular, kosher meat may be selected from beef, chicken, lamb, mutton, goat meat and mixtures thereof. Kosher meat does not include shellfish, which under Jewish tradition is not permitted for consumption. Although Jewish traditions permit consumption of vertebrate fish, since there is no special method of slaughtering vertebrate fish, all vertebrate fish may be considered kosher. Any food or meat that does not fall within the definition of 'kosher' will
40 then be considered as 'non-kosher'.

The term 'halal' used in combination with food according to any aspect of the present invention refers to food that conforms to Islamic dietary laws and especially meat processed and prepared in accordance with those requirements. Similar to the way kosher meat is prepared, in Islamic tradition, animals are slaughtered according to *Dhabīḥah* where the animal is slaughtered using a cut across the neck with a non-serrated sharp blade in a single clean attempt to make an incision that cuts the front of the throat, oesophagus and jugular veins but not the spinal cord. In addition to the direction, permitted animals should be slaughtered upon utterance of the Islamic prayer Bismillah. The animal must also be drained of blood after the slaughter. The slaughter must be performed by an adult Muslim. Halal meat usually refers to most meats excluding pig. In particular, halal meat may be selected from beef, chicken, lamb, mutton, goat meat and mixtures thereof. Although Islamic traditions permit consumption of shellfish and vertebrate fish, since there is no special method of preparing shellfish, all shellfish and vertebrate fish may be considered halal. Any food or meat that does not fall within the definition of 'halal' will then be considered as 'non-halal'. The definition of halal is further provided in <https://www.smiic.org/en/project/24> (Organisation of Islamic Cooperation (OIC)/Standards and Metrology Institute for the Islamic Countries (SMIIC), OIC/SMIIC 1:2019 General Requirements for Halal Food. accessed on 08 June 2022)

The term "test" used in conjunction with the term subject and/ or animal in the present disclosure refers to an entity that is subjected to the method according to any aspect of the present invention and is the basis for an analysis application of the present invention. An "(individual) test subject", an "(individual) group of test subjects" or a "test profile" or an 'test animal derived product' is therefore a (individual) subject or group of subjects being tested according to the invention or a profile being obtained or generated in this context. Conversely, the term "reference" shall denote, mostly predetermined, entities which are used for a comparison with the test entity. For example, the term 'reference animal' refers to an animal used for comparison or as a control in reference to the 'test animal'. Similarly, the term 'sample' and/or 'test animal-derived product sample' used in accordance with any aspect of the present invention refers to an entity that may be subject to the method of the present invention. In particular, a sample may be any (test) animal-derived product that may be subject to the method of the present invention to determine the distinct certification by first determining the DNA methylation profile and then comparing this test methylation profile with a control. Blockchain may also be used to make the information easily available for the consumer.

The method according to any aspect of the present invention may be used to identify an unknown sample (i.e. animal-derived product sample) based on DNA methylation patterns. These DNA methylation patterns may then be compared with reference DNA methylation patterns to trace the animal-derived product sample back to the slaughterhouse or farmhouse from which the sample originates and then determine whether the unknown sample corresponds to an animal which has been slaughtered according to Kosher, Halal, non-Kosher or non-Halal practices. In this way, a buyer or a consumer of meat can verify that meat being sold as or marketed as being Kosher or Halal is genuine.

As used herein, the term "comprising" is to be construed as encompassing both "including" and "consisting of", both meanings being specifically intended, and hence individually disclosed aspects of the present invention. Where used herein, "and/or" is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. In the context of the present invention, the terms "about" and "approximately" denote an interval of accuracy that the person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates deviation from the indicated numerical value by $\pm 20\%$, $\pm 15\%$, $\pm 10\%$, and for example $\pm 5\%$. As will be appreciated by the person of ordinary skill, the specific deviation for a numerical value for a given technical effect will depend on the nature of the technical effect. For example, a natural or biological technical effect may generally have a larger such deviation than one for a man-made or engineering technical effect. Where an indefinite or definite article is used when referring to a singular noun, e.g. "a", "an" or "the", this includes a plural of that noun unless something else is specifically stated.

In context of the present invention, the terms "methylation profile", "methylation pattern", "methylation state" or "methylation status," are used herein to describe the state, situation or condition of methylation of a genomic sequence, and such terms refer to the characteristics of a DNA segment at a particular genomic locus in relation to methylation. Such characteristics include, but are not limited to, whether any of the cytosine (C) residues within this DNA sequence are methylated, location of methylated C residue(s), percentage of methylated C at any particular stretch of residues, and allelic differences in methylation due to, e.g., difference in the origin of the alleles.

The term "methylation status" refers to the status of a specific methylation site (i.e. methylated vs. non-methylated) which means a residue or methylation site is methylated or not methylated. Then, based on the methylation status of one or more methylation sites, a methylation profile may be determined. Accordingly, the term "methylation profile" or also "methylation pattern" refers to the relative or absolute concentration of methylated C residues or unmethylated C residues at any particular stretch of residues in the genomic material of a biological sample. For example, if cytosine (C) residue(s) not typically methylated within a DNA sequence are methylated, it may be referred to as "hypermethylated"; whereas if cytosine (C) residue(s) typically methylated within a DNA sequence are not methylated, it may be referred to as "hypomethylated". Likewise, if the cytosine (C) residue(s) within a DNA sequence (e.g., the DNA from a sample nucleic acid from a test subject) are methylated as compared to another sequence from a different region or from a different individual (e.g., relative to normal nucleic acid or to the standard nucleic acid of the reference sequence), that sequence is considered hypermethylated compared to the other sequence. Alternatively, if the cytosine (C) residue(s) within a DNA sequence are not methylated as compared to another sequence from a different region or from a different individual, that sequence is considered hypomethylated compared to the other sequence. These sequences are said to be "differentially methylated". Measurement of the levels of differential methylation may be

done by a variety of ways known to those skilled in the art. One method is to measure the methylation level of individual interrogated CpG sites determined by the bisulfite sequencing method, as a non-limiting example.

- 5 The term “hypermethylation” refers to the average methylation state corresponding to an increased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample. In particular, control refers to an animal derived product not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or not having been bled to death.

- 10 The term “hypomethylation” refers to the average methylation state corresponding to a decreased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample. In particular, control refers to an animal derived product not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or not having been bled to death.

- 20 As used herein, a “methylated nucleotide” or a “methylated nucleotide base” refers to the presence of a methyl moiety on a nucleotide base, where the methyl moiety is usually not present in a recognized typical nucleotide base. For example, cytosine in its usual form does not contain a methyl moiety on its pyrimidine ring, but 5-methylcytosine contains a methyl moiety at position 5 of its pyrimidine ring. Therefore, cytosine in its usual form may not be considered a methylated nucleotide and 5-methylcytosine may be considered a methylated nucleotide. In another example, thymine may contain a methyl moiety at position 5 of its pyrimidine ring, however, for purposes herein, thymine may not be considered a methylated nucleotide when present in DNA. Typical nucleotide bases for DNA are thymine, adenine, cytosine and guanine. Typical bases for RNA are uracil, adenine, cytosine and guanine. Correspondingly a “methylation site” is the location in the target gene nucleic acid region where methylation has the possibility of occurring. For example, a location containing CpG is a methylation site wherein the cytosine may or may not be methylated. In particular, the term “methylated nucleotide” refers to nucleotides that carry a methyl group attached to a position of a nucleotide that is accessible for methylation. These methylated nucleotides are usually found in nature and to date, methylated cytosine that occurs mostly in the context of the dinucleotide CpG, but also in the context of CpNpG- and CpNpN-sequences may be considered the most common. In principle, other naturally occurring nucleotides may also be methylated but they will not be taken into consideration with regard to any aspect of the present invention.

- 40 As used herein, a “CpG site” or “methylation site” is a nucleotide within a nucleic acid (DNA or RNA) that is susceptible to methylation either by natural occurring events *in vivo* or by an event instituted to chemically methylate the nucleotide *in vitro*.

As used herein, a "methylated nucleic acid molecule" refers to a nucleic acid molecule that contains one or more nucleotides that is/are methylated.

- 5 The term 'epigenetic change' as used herein refers to a chemical (e.g., methylation) change or protein (e.g., histones) change that takes place to a gene body or a promoter thereof. Through epigenetic changes, environmental factors like diet, stress and prenatal nutrition can make an imprint on genes passed from one generation to the next.
- 10 The term "bisulfite" as used herein encompasses any suitable type of bisulfite, such as sodium bisulfite, or another chemical agent that is capable of chemically converting a cytosine (C) to a uracil (U) without chemically modifying a methylated cytosine and therefore can be used to differentially modify a DNA sequence based on the methylation status of the DNA, e.g., U.S. Pat. Pub. US 2010/0112595 (Menchen et al.). As used herein, a reagent that "differentially modifies"
- 15 methylated or non-methylated DNA encompasses any reagent that modifies methylated and/or unmethylated DNA in a process through which distinguishable products result from methylated and non-methylated DNA, thereby allowing the identification of the DNA methylation status. Such processes may include, but are not limited to, chemical reactions (such as a C to U conversion by bisulfite) and enzymatic treatment (such as cleavage by a methylation-dependent endonuclease).
- 20 Thus, an enzyme that preferentially cleaves or digests methylated DNA is one capable of cleaving or digesting a DNA molecule at a much higher efficiency when the DNA is methylated, whereas an enzyme that preferentially cleaves or digests unmethylated DNA exhibits a significantly higher efficiency when the DNA is not methylated.
- 25 In context of the present invention also any "non-bisulfite-based method" and "non-bisulfite-based quantitative method" are comprised to test for a methylation status at any given methylation site to be tested. Such terms refer to any method for quantifying methylated or non-methylated nucleic acid that does not require the use of bisulfite. The terms also refer to methods for preparing a nucleic acid to be quantified that do not require bisulfite treatment. Examples of non-bisulfite-based
- 30 methods include, but are not limited to, methods for digesting nucleic acid using one or more methylation sensitive enzymes and methods for separating nucleic acid using agents that bind nucleic acid based on methylation status. The terms "methyl-sensitive enzymes" and "methylation sensitive restriction enzymes" are DNA restriction endonucleases that are dependent on the methylation state of their DNA recognition site for activity. For example, there are methyl-sensitive
- 35 enzymes that cleave or digest at their DNA recognition sequence only if it is not methylated. Thus, an unmethylated DNA sample will be cut into smaller fragments than a methylated DNA sample. Similarly, a hypermethylated DNA sample will not be cleaved. In contrast, there are methyl-sensitive enzymes that cleave at their DNA recognition sequence only if it is methylated. As used herein, the terms "cleave", "cut" and "digest" are used interchangeably.

A “biological sample” in context of the invention may comprise any biological material obtained from the subject or group of subjects that contains genomic material, and may be liquid, solid or both, may be tissue or bone, or a body fluid such as blood, lymph, etc. In particular, the biological sample useful for the present invention may comprise biological cells or fragments thereof.

- 5 As used herein, the term “pre-selected methylation sites” refers to methylation sites that were selected from genes or regions that showed the highest degree of methylation variation during the training of the method and fulfils certain quality criteria such as a minimum sequencing coverage of $\geq 5x$ were considered and for ≥ 5 qualified CpG sites. Additionally, genes that have an average methylation level < 0.1 or an average methylation level > 0.9 can be excluded due to their limited
- 10 dynamic range. “Reference methylation profiles” may be defined on the basis of multiple training samples using multivariate statistical methods, such as such as Principal Component analysis or Multi-Dimensional Scaling. In particular, the CpG site according to any aspect of the present invention, particularly in the pre-selected methylation sites is selected from the Table A below:

Table A Relevant CpG sites that are differentially methylated

No	chrom	position	No	chrom	position	No	chrom	position	No	chrom	position	No	chrom	position	No	chrom	position	No	chrom	position	No	chrom	position
1	2	34511190	50	20	5577995	99	10	3477178	148	21	5073396	197	7	9938317	295	12	1581083	344	8	16589375	393	18	7373876
2	2	5752893	51	27	3637822	100	1	165873023	149	20	533436	198	17	1085495	296	6	10819506	345	9	5089285	394	3	67720493
3	2	20693997	52	19	2411046	101	2	16103001	150	11	10452379	199	7	29790478	297	19	176255	346	9	8479123	395	2	129925091
4	2	17123376	53	17	6189674	102	5	27885744	151	4	49890681	200	8	125171980	298	11	10950629	347	3	8952808	396	4	2123054
5	2	115673685	54	3	105678589	103	2	4575039	152	5	52943641	201	28	2443299	299	9	9528198	348	7	11248389	347	20	10287258
6	2	24913614	55	1	113283028	104	33	431970	153	2	46089114	202	2	16048197	251	7	16281087	300	2	121383106	349	13	13028004
7	4	58554384	56	11	1459001	105	14	6596177	154	23	1870014	203	19	4088736	252	2	1390782	301	2	85904873	350	5	48066943
8	18	8977183	57	7	35189129	106	7	301591	155	2	41841626	204	26	1519097	302	2	129933052	351	8	28086175	400	26	3571402
9	4	45296016	58	4	32571055	107	7	72829308	156	24	4885733	205	6	11221033	254	3	11047446	303	3	39148221	352	2	26792587
10	7	18690074	59	28	3800027	108	5	43891403	157	4	6740626	206	9	10619341	256	3	108708555	305	1	35484379	354	5	39173222
11	2	123206194	60	11	18808917	109	10	18678519	158	3	76507235	207	9	7925956	257	4	5202992	306	17	2360133	355	4	86456090
12	1	49770596	61	1	168855019	110	3	38237516	159	5	58997382	208	9	7925956	258	6	1549721	258	3	19170252	307	8	38166099
13	5	29057117	62	3	76755036	111	19	5832481	160	17	8203614	209	6	1549721	258	3	19170252	307	8	1401347	356	5	3421530
14	21	2688710	63	1	34377737	112	1	50837347	161	27	3464008	210	5	23458500	259	17	10895665	308	2	66497871	357	3	4606658
15	1	47926496	64	3	76739615	113	2	33678917	162	11	841570	211	28	1477408	260	7	6603676	309	15	5027241	358	20	5519899
16	13	11458665	65	1	56825511	114	4	89259160	163	4	43608241	212	19	50552	261	3	105215220	310	12	7055474	359	11	6629490
17	5	19158349	66	1	108957860	115	19	5767147	164	7	22569247	213	26	3246923	262	26	1651982	311	2	3856589	360	18	9435327
18	11	1817296	67	25	1876704	116	4	40113483	165	2	78548594	214	8	6089739	263	10	10824700	312	1	193821130	361	3	102971530
19	10	12400953	68	1	130278359	117	5	14162871	166	5	28115369	215	13	3700593	264	4	39012274	313	13	18308448	362	19	4492763
20	19	8867093	69	17	7904953	118	10	10895845	167	1	84532408	216	13	16065472	265	14	7327840	314	1	68455273	363	23	4152128
21	4	39537311	70	3	4193791	119	20	10007818	168	6	12121146	217	4	5353037	266	2	42356641	315	2	114090502	364	2	63907320
22	13	6729058	71	3	96461436	120	12	9675444	218	1	119440733	219	5	52943689	267	5	53918500	316	4	60369319	365	15	9763222
23	10	11406434	72	2	5096922	121	3	58874863	170	6	32236382	219	27	1541894	268	4	57532309	317	27	4720035	366	15	9763222
24	4	21041627	73	12	15606143	122	20	10511507	171	10	12734127	220	26	4856660	269	6	11915935	318	10	967899	367	5	NT_460419v1
25	26	5003532	74	2	20877142	123	2	81360739	172	2	11287708	221	12	2372012	270	5	28318496	319	4	1985540	368	19	199882
26	8	16729492	75	12	710959	124	15	8003087	173	8	16370376	222	1	110237976	271	7	11358921	320	1	175460817	370	3	32362994
27	4	39536841	76	2	27177202	125	6	17322989	174	19	1401235	223	1	84409563	272	7	11358921	321	1	175460817	370	3	32362994
28	7	24170000	77	6	28147880	126	3	104905440	175	1	144796538	224	1	38877658	273	1	15441151	322	3	33946497	371	NT_465658v1	
29	2	23153225	78	25	1107409	127	19	602617	176	2	102567607	225	5	11621859	274	6	10819414	323	12	2259754	372	23	2381593
30	1	130973501	79	2	115921454	128	9	15414814	177	8	7548136	226	25	976468	275	1	39877753	324	3	12583058	373	19	7203329
31	19	8481362	80	15	7347328	129	8	16295920	178	14	5000387	227	4	8695640	276	8	2118531	325	24	804341	374	3	65981131
32	1	14382900	81	2	4980847	130	3	32261769	179	1	56606169	228	1	80519994	277	17	2935626	326	2	27444073	375	5	13107975
33	10	6806714	82	10	15213509	131	2	62413006	180	9	9539267	229	19	185280	278	17	7463984	327	3	104543849	376	2	57948024
34	10	12500816	83	2	127761601	132	2	118636996	181	18	3700175	230	3	48423158	279	20	5394896	328	1	53092288	377	1	56521314
35	2	7668187	84	2	15482005	133	21	927717	182	1	61708721	231	1	39858634	280	17	4082722	329	1	14903055	378	2	34328034
36	1	48250341	85	24	3786074	134	25	1946574	183	8	29363057	232	8	16032167	281	15	7571659	330	1	122962624	379	4	34288785
37	26	1544666	86	12	4380982	135	3	22702842	184	6	11517878	233	1	91422737	282	1	144796614	331	3	76739886	380	3	30399012
38	3	26860812	87	21	2332328	136	5	12488676	185	4	88992493	234	18	3777721	283	4	165873085	332	8	11915537	381	18	3700112
39	3	40601306	88	2	11287812	137	1	169374378	186	4	10802755	235	12	7012484	284	4	66670233	333	8	16296168	382	13	12290255
40	9	7642489	89	1	36508947	138	1	35104436	187	3	12755149	236	1	77229485	285	16	168962	334	9	7724217	383	7	35847537
41	7	5077349	90	7	301604	139	28	153426	188	2	21041245	237	3	49813062	286	17	7673395	335	9	4626749	384	20	8156579
42	11	18808803	91	20	9983514	140	15	7003924	189	14	957744	238	3	27270228	287	17	5741333	336	4	60368609	385	19	580436
43	26	2590632	92	5	10355756	141	14	2268610	190	7	27177213	239	5	1727214	289	15	6891797	337	2	37529420	386	19	9279301
44	9	22225808	93	9	10672938	142	3	105678613	191	7	28179710	240	5	5177214	290	18	2724676	338	13	3283054	387	5	9279301
45	2	78548181	94	20	4533305	143	1	144628599	192	4	11719574	241	3	36638958	291	14	6696623	339	1	194236534	388	23	375697
46	12	6363727	95	21	2332272	144	9	14072787	193	5	24369896	242	7	15096160	291	12	10148958	340	18	10185868	389	24	375697
47	3	107890733	96	5	53496100	145	19	5363084	194	19	5363084	243	2	121482517	292	12	10148958	341	18	1708416	390	1	75199586
48	3	27258633	97	9	22079838	146	17	6765666	195	24	4459949	244	7	17289720	293	6	12121122	342	8	16374372	391	17	8205987
49	9	5216234	98	2	49110506	147	5	48084582	196	6	32533539	245	NT_464303v1	5736	294	1	49441925	343	8	14713737	392	3	26344701

[illegible]

The term "pre-determined reference profile" used in the context of the present invention refers to a typical or standard methylation profile of the genomic material of a type of reference animal-derived product that is confirmed to be correctly labelled or certified. In one example, the pre-determined reference profile may be used in the context of a control animal, where the control animal has been correctly certified (i.e. where the control animal has neither been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus nor of having been bled to death). The control animal may thus have been slaughtered using non-halal and non-kosher means of slaughtering that may be termed the normal means of slaughtering. A panel of pre-determined reference profiles for control animals may include profiles from different samples that have been obtained from different parts of control animals (not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus nor of having been bled to death). For example, the panel of pre-determined reference profiles may include at least one profile for egg, at least one profile for meat (muscle, tissue, organs etc.), at least one profile for milk and the like. Each of these samples may have its own unique pre-determined methylation reference profile that also forms a part of the panel of pre-determined reference profiles

In another example, the pre-determined reference profile may be used in the context of reference animals that may have been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death. A panel of pre-determined reference profiles may be prepared for different samples that are from animals that have been confirmed to be slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death. Again here, there may be a panel of pre-determined reference profiles for each product that is derived from the animal that has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death. For example, the panel of pre-determined reference profiles may include at least one profile for egg, at least one profile for meat (muscle, tissue, organs etc.), at least one profile for milk and the like from animals that have been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death. Each of these samples may have its own unique pre-determined methylation reference profile that also forms a part of the panel of pre-determined reference profiles

For example, the control may a type of meat of the same species or the same animal taxon as the animal-derived product sample or unknown sample which has a known certification. Alternatively, the pre-determined reference profile may be obtained from different types of meat with a known certification. In one example, the control may be chicken meat that is certified 'halal'. The halal chicken meat may be a mix of different parts of the chicken (i.e. breast, thigh, kidney, liver etc.). In another example, the halal chicken meat may be from one part of a chicken. From the halal chicken meat, a methylation profile may be obtained to be the pre-determined reference profile (the

control) that may be used to determine if the test sample (meat) is halal or non-halal by determining if the methylation profile of the test sample is significantly similar to the pre-determined reference profile. The methylation profile of different types of halal meat from one species of animal may be identical. The methylation profile of different types of halal meat from different species of animals may also be identical. In one example, the control may be chicken meat that is certified 'kosher'. The kosher chicken meat may be a mix of different parts of the chicken (i.e. breast, thigh, kidney, liver etc.). In another example, the kosher chicken meat may be from one part of a chicken. From the kosher chicken meat, a methylation profile may be obtained to be the pre-determined reference profile (the control) that may be used to determine if the test sample (meat) is kosher or non-kosher by determining if the methylation profile of the test sample is significantly similar to the pre-determined reference profile. The methylation profile of different types of kosher meat from one species of animal may be identical. The methylation profile of different types of kosher meat from different species of animals may also be identical.

There may be a compilation of several pre-determined reference profiles and comparing the methylation profile of the test sample with the pre-determined reference profiles in the compilation may enable identifying the specific pre-determined reference profile that is (significantly) similar to the methylation profile of the test sample and then the test sample may be confirmed to be slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death or not. In one example, the pre-determined reference profiles may include methylation profiles of different parts of meat (i.e. breast, thigh, kidney, liver, shoulder, ribs, intestines, etc.) from an animal (chicken, goat, cow, lamb, sheep etc.) that has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or having been bled to death.

In particular, the panel of predetermined reference methylation profiles according to any aspect of the present invention is distinct for different test animal-derived products. That is to say, each predetermined reference methylation profile is distinct for a single animal-derived product. The panel of predetermined reference methylation profiles may thus include many different predetermined reference methylation profiles from different parts of an animal or several animals of the same biological taxon as the test animal. There will also be different panels of predetermined reference methylation profiles for different animal taxon, and the relevant panel of predetermined reference methylation profiles unique for an animal taxon will depend on the animal taxon of the test animal.

The term "significantly similar" in context of the present disclosure, and in particular in context with the comparison of methylation profiles (such as the comparison between test profiles (from test subject(s) (i.e. sample X) and reference profiles) shall mean a similarity observed by statistical means (i.e. by using bioinformatics) and/or also by observation using the eye. A significant similarity is observed for example if a test profile overlaps with a reference profile that is defined by

multiple training samples through multivariate statistical methods, such as Principal Component analysis or Multi-Dimensional Scaling. In particular, a test profile is significantly similar to the pre-determined reference profile if more than 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99% % of the methylation pattern/ profile overlaps with that of the reference profile. A similarity of a test
5 profile to more than one, such as two, three or even all reference profile reduces the significance of the similarity. Of course, the similarity of the test methylation profile to the reference methylation profile takes into consideration the experimental error that occurs in all methods.

The animal-derived product sample may be a single type of meat, different types of meat, a single
10 part of a type of meat, different parts of a single type of meat or different parts of different types of meat. The sample may be from any biological entity having a DNA genome and DNA genome methylation. In particular, the methylation site is a CpG site. The biological entity may be any animal excluding a pig. In particular, the animal may be selected from the group consisting of chicken, lamb, camel, cow, goat, sheep, horse, donkey, turkey, duck, goose, quail, rabbit and mule.
15 More in particular, the animal may be selected from the group consisting of cow, sheep, goat, camel, chicken, goose, duck and turkey. The term 'meat' herein may thus be understood to include chicken, lamb, beef, mutton, goat, camel, chicken, goose, duck, turkey and mixtures thereof.

The one or more pre-selected methylation sites in (a) are methylation sites associated with tissue
20 specific gene expression, preferably wherein the pre-selected methylation sites are associated with gene expression of one distinct tissue.

The tissue may be selected from

- (i) metabolic tissue such as gut tissue, said gut tissue preferably being ileum or jejunum,
- 25 (ii) muscular tissue,
- (iii) skin tissue, and
- (iv) organ tissue, said organ tissue preferably being hepatic and / or pancreatic tissue.

30 According to yet another aspect of the present invention, there is provided a method of verifying certification of a test animal-derived product sample that has been certified to derive from a test animal that was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or that was bled to death, the method comprising the steps of:

- 35 (a) determining a test methylation profile of one or more pre-selected methylation sites within the genomic material obtained from the test animal derived product sample; and
- (b) comparing the test methylation profile obtained from (a) with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not
40 slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death

- wherein a difference in the test methylation profile of (a) compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product being correctly certified, and
- wherein a similarity in the test methylation profile of (a) compared to the reference methylation profile of the control animal, is indicative of the test animal not having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product being wrongly certified, and
- wherein the pre-selected methylation sites are CpG sites selected from genes or regions of genomic DNA from the control and test animal that shows the highest degree of methylation variation during the training of the method.
- According to a further aspect of the present invention, there is provided a method for confirming or rejecting an assumed certification of an animal derived product sample, the method comprising
- comparison of a test methylation profile obtained from genomic material of the animal derived product sample with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death,
- wherein a difference in the test methylation profile compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death
- wherein the assumed certification is that the test animal derived product sample is kosher or halal where the test animal is slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the test animal is bled to death.
- According to another aspect of the present invention, there is provided a method of determining if a test animal-derived product sample, is obtained from a test animal that has been bled to death or slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus the method comprising:
- comparison of a test methylation profile obtained from genomic material of the test animal derived product sample with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death.

In particular, the certification is kosher or halal where the animal is slaughtered by a cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, the trachea and/or the oesophagus. Additionally, or alternatively, the certification kosher or halal where the animal is drained of blood.

5

According to yet a further aspect of the present invention, there is provided a certification of a test animal derived product sample certifying that the test animal was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, the trachea and/or the oesophagus,

10 wherein the method for determining that the test animal was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, the trachea and/or the oesophagus comprises the steps of:

- (a) determining a test methylation profile of one or more pre-selected methylation sites within the genomic material contained in the test animal-derived product sample; and
- 15 (b) comparing the test methylation profile determined in (a) with a panel of predetermined reference methylation profiles of the same biological taxon of the test animal from which the product sample derives, wherein each of the predetermined reference methylation profiles is from a different reference animal and/or different supplier,

wherein if the test methylation profile of (a) is significantly similar to one of the predetermined
20 reference methylation profiles, the test animal was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, the trachea and/or the oesophagus.

Additionally, or alternatively, the test animal is drained of blood.

25

According to yet another aspect of the present invention, there is provided a use of DNA methylation profiling for determining for certifying of an animal-derived product sample. In particular, the certification of the animal-derived product sample is kosher or halal where the test animal is slaughtered by a single cut across the throat severing both carotid arteries, both jugular
30 veins, both vagus nerves, trachea and/or oesophagus and/or the test animal is bled to death.

BRIEF DESCRIPTIONS OF FIGURES

Figure 1 is the results of Principle Component Analysis (PCA) of 6706643 CpG sites with min
35 coverage 10 in all halal and non-halal samples.

Figure 2 is the results of Principle Component Analysis (PCA) of 76339 Differentially methylated positions (DMPs) identified.

EXAMPLES

40

The foregoing describes preferred embodiments, which, as will be understood by those skilled in the art, may be subject to variations or modifications in design, construction or

operation without departing from the scope of the claims. These variations, for instance, are intended to be covered by the scope of the claims.

Example 1

Wet-Lab methodology

- 5
- Halal and non halal chicken breast meat were purchased from five different vendors. The vendors for halal meat include Pasar, ZAC Butchery, Hego premium, RedMart and Keesong, whereas non halal meat was purchased from Pasar, Tegel, Farm fresh, Ryan and AW's in Singapore. Genomic DNA was purified from the breast tissue samples using DNeasy Blood & Tissue Kit (Qiagen) and is quantified using PicroGreen or NanoDrop™ 2000.
- 10
- The genomic DNA (500ng) from breast tissue samples was used to prepare libraries for Whole Genome Bisulfite Sequencing (WGBS). The sequencing of the libraries was performed by a third party on a NovaSeq platform which generated 125GB data per sample with 20X coverage.

Halal	Non-Halal
Brand	Brand
Pasar	Pasar
ZAC Butchery	Tegel
Hego premium	Farm fresh
RedMart	Ryan
Keesong	AW's

- 15
- Computational methodology

Processing:

Sequenced reads were trimmed and mapped with BSMAP1 version 2.5 using the assembly version 5.0 of the chicken (*Gallus gallus*) genome as reference sequence. After deduplication using picard2 (<http://broadinstitute.github.io/picard/>), the methylation ratios were determined using a Python script (methratio.py) distributed with the BSMAP package. BSMAP is further explained in Xi, Y. et al., BMC Bioinform. 10, 232 (2009). For all further analysis, only CpGs covered by at least ten reads were considered, which resulted in 6706643 CpG sites.
- 20

Differential methylation analysis

- 25
- Differential methylation analysis was performed using MethylKit3 (version 1.12.0) between the halal and non-halal group. MethylKit uses logistic regression to calculate p-values and sliding linear model method4 to adjust the p-values to q-values. The use of the linear model method is further described here, Wang H-Q, et al., Bioinformatics. 2011; 27:225–231. CpG sites with an FDR below 0.05 and a methylation change larger than 25% between the groups were considered as
- 30
- significantly differentially methylated Positions (DMPs), resulting in 76339 CpG sites. 8000 of the exact positions of CpG sites are provided below in Table 1.

- MethylKit generates a 'prcomp' object, which can be used to extract and plot the principal components. The use of MethylKit is disclosed in Akalin A, et al. Genome Biol. 2012;13(10): R87. Principle Component Analysis (PCA) is a dimensionality-reduction method which can transform large data sets to a few principal components. The first few principal components generally retain
- 5 most of the variation present in the dataset and are useful for emphasizing the grouping structure in the data
- Principle Component Analysis (PCA) of CpG sites with min coverage 10 in all samples: 6706643 as shown in Figure 1.
- Principle Component Analysis (PCA) of 76339 DMPs identified is shown in Figure 2.
- 10 Plotting of the first two principal components of the CpGs before and after differential methylation analysis reveal meaningful clustering of the samples. Differentially methylated Positions (DMPs) were able to effectively cluster of the halal and non-halal groups in a meaningful way.
- 15 Table 1. 8000 CpG sites of the 76339 significantly differentially methylated Positions (DMPs) are provided.

[illegible]

[illegible]

PCT/EP2023/068584

[illegible]

[illegible]

[illegible]

[illegible]

PCT/EP2023/068584

Chrom. 5	Chrom. 5	Chrom. 5	Chrom. 6	Chrom. 6	Chrom. 6	Chrom. 6	Chrom. 6	Chrom. 7	Chrom. 7	Chrom. 7	Chrom. 7	Chrom. 7
52654526	53350298	54595828	692734	5037117	31470098	16090586	28881426	301604	11615616	15108657	17966477	27343972
52659548	53363620	54598721	1066199	5061613	31919184	16101389	28881458	320403	11661181	15103615	18526987	27364470
52663733	53365711	54781709	1066321	5091778	32222841	16107870	28888268	805868	12757217	15135188	20724566	27365842
52666269	53366711	54861907	1295055	5227438	32421797	16147759	29010153	814038	12866588	15137472	21629066	27365964
52687414	53367838	54893529	1317745	5242145	32533531	16150161	29062161	826098	13023345	15143187	21649639	27370083
52739657	53370753	55116043	1549720	5324181	32533539	16151011	29064754	855737	13025693	15143894	22062321	27375270
52746737	53370882	55126031	1688316	5381946	32553836	16181782	29074340	1327352	13210100	15160572	22576585	27375530
52767738	53372280	55127763	1751703	5481759	33316952	16189407	29093179	1810606	13273118	15166127	25583207	27375560
52769858	53373358	55148658	1766425	5483099	33337923	16255777		1917474	13344769	15166295	25975729	27412304
52812262	53373821	55150342	1798861	5603835	33342133	17160506		1946070	13385528	15167737	26039205	27476404
52818991	53373981	55183258	1829961	5710720	33651448	17248563		2048597	13387036	15169012	26249224	27489012
52820946	53373958	55612488	1831413	5736217	33668577	17912254		2242338	13399507	15174864	26422355	27502405
52826960	53376325	55752915	1832444	5737206	33721908	20126863		2249065	13412225	15178156	26629955	27528418
52843844	53376633	55798281	1833736	5796834	33782061	20383682		2509514	13439227	15281331	26703705	27568155
52843862	53377206	56074892	1838966	5909340	33807947	21112445		3339014	13445771	15284520	26850748	27574240
52846856	53379760	56284306	1839531	5940725	33864364	21419070		3433287	13456350	15290117	26852789	27575855
52851996	53381231	56386561	1842351	5983112	33865901	22207618		3449492	13487751	15291269	26878501	27689645
52941718	53390948	56456436	1843581	6174972	34333441	23459027		3746492	13870607	15292396	26878892	27694641
53024807	53399924	57274219	1843645	6204887	34645963	23749096		3748218	13935768	15296100	26898808	27739292
53031528	53412026	57608365	2282080	6243492	34649194	23755220		3805367	13942865	15297427	26978699	27746745
53114393	53420075	57764789	2496618	6246540	34650330	23764933		4050803	13996490	15297686	26992355	27753430
53127784	53420319	58003624	2535628	6251296	34689348	23833335		4149279	14024835	15409629	26992412	27759841
53136411	53429321	58040379	2573835	6402282	34692964	23962968		4789081	14033564	15436364	26994142	27764841
53136460	53430360	58238117	2581214	6429832	34995063	23967945		4840777	14117072	15450801	27002696	27765340
53164992	53433458	58257556	2581713	6558093	8361994	23975115		5004635	14134663	15454819	27014135	27768432
53169152	53438323	58263133	2582533	6669093	9123356	23992089		5007217	14146913	15564620	27020531	27835693
53169162	53441645	58344094	2618198	6685056	9224908	24008084		5045399	14173371	15610517	27021504	27849579
53175644	53442108	58563317	2624866	6789427	9884913	24030922		5077349	14201994	15636705	27033840	27852031
53198315	53446675	58563592	2644275	7450801	9901669	24249966		5084808	14219853	15642588	27034127	27905229
53227075	53448879	58595400	2768921	7826925	10262615	24250281		5089381	14234292	15642903	27034234	27985925
53245954	53454674	58606679	3063502	7896752	10268059	24274814		5252132	14240796	15651826	27039439	27986934
53248048	53461819	58632869	3187511	7900195	10580036	24288904		5275128	14247995	15702282	27075704	28020458
53248838	53461822	58688607	3396271	8107243	10858989	24739741		5363679	14271330	16099788	27090685	28030569
53250287	53463537	58691364	3396276	8355283	11075787	24823394		5449080	14321603	16501998	27139249	28137794
53245954	53466724	58704760	3396299	8359024	11760770	25795308		5500743	14323858	16503815	27163747	28144761
53253659	53467872	58707791	3419176	29142506	11821707	26065894		9014407	14330634	16810810	27163759	28434849
53254508	53469344	58725598	3439338	29170831	11830253	26092592		9085792	14330767	16924331	27164035	28631917
53259108	53470944	58744255	3462007	29183045	11862119	26092603		9591840	14330805	16959947	27166549	28633531
53263774	53476348	58890151	3662611	29203352	11936642	26092617		9650990	14337208	16960117	27178113	28648562
53263787	53496346	58890658	3683701	29621056	11982430	26229638		9665743	14337819	16960276	27186177	28655230
53264782	53498902	58891974	4451463	30723388	12074751	26688926		9692745	14539304	16960401	27221765	28680207
53264824	53500408	58956549	4454145	30915629	12097291	26812092		9694189	14719071	16963960	27231920	28721912
53297330	53504404	58971642	4687447	30963710	12120023	26876649		9695252	1483287	17119746	27232125	28830284
53299189	53504847	59352204	4692777	31052460	12120483	27879287		9695380	14834287	17204584	27236531	28838337
53307827	53505057	59360896	4762220	31069085	12120525	27883220		9697869	14845995	17277388	27236655	28849611
53308188	53566111		4870197	31211983	12121011	27892026		10325731	14848549	17418071	27255358	28873399
53309222	53568275		4876925	31302563	12251007	28702992		10382166	14951905	17430463	27297867	28991186
53311801	53967309		4975292	31326918	14303206	28773967		10452451	14954513	17433914	27299956	29035494
53316042	54373351		5003289	31350235	14317094	28791280		11036687	14972459	17567314	27304944	29042565
53316205	54591129		5013941	31379349	15248209	28833556		11358921	15089740	17879716	27307241	29053147
53347473	54594211		5035438	31379735	16089401	28838346		11578601	15095800	17900863	27310870	29057299

[illegible]

Chrom. 9	Chrom. 9	Chrom. 10	Chrom. 10	Chrom. 10	Chrom. 11	Chrom. 11	Chrom. 12	Chrom. 12	Chrom. 12	Chrom. 12	Chrom. 12	Chrom. 12
9494132	16710836	1495914	6682737	9967973	14741617	11351199	11218443	18143571	51732	1543062	4868813	8757625
9498792	16731182	801341	6713636	9984099	14745710	891300	11240157	18175001	176426	1621397	4880409	9217309
95111309	16811311	866499	6723870	10038430	14746384	1581770	11280638	18189043	276209	1636027	4889641	9217691
9515502	16819626	968395	6791880	10040283	14754379	1600067	11283151	18459577	397384	1656909	4957095	9399852
9591055	17361368	1350919	6808888	10161735	14758510	1683918	11283389	18503235	398610	1665995	4976981	9428945
9593950	17659538	1462850	6823704	10315219	14759237	1686571	11293534	18767161	450553	1687066	4977763	9436091
9599799	17847921	1609501	6846208	10678185	14763048	1728743	11297518	18768975	521880	1687135	4979539	9437805
9644753	17851246	1683660	6853382	10763460	14786627	1863208	11299037	18768986	542510	1691864	4979706	9567478
9653489	17851648	1719622	6918143	11145709	14788655	1906700	11318104	18772517	542557	1699838	5302382	9697070
9864856	18179118	1914635	6925317	11180297	14792354	1911888	11322256	18795180	563307	1700768	5399238	10122859
9880379	18179225	2090260	6932411	11183679	14811735	2056187	11352184	18795189	661095	1703169	5449655	10394296
9881305	19237779	2119292	6937763	11233813	14812739	2063197	11367752	18864943	661440	1706337	5472277	10423289
9905138	19735122	2186376	6955809	11362740	14818072	2081284	11582590	19178877	661517	1736433	5497341	10512222
10475669	19745125	2240597	6957849	11391812	14821963	2138828	11619742		661665	1788357	5529097	10609404
10625990	20011248	2277641	6962213	11425184	14838025	2155195	11621886		662661	2244294	5619289	10858472
10645249	21055992	2277755	7421331	11506271	14891750	2224652	11622879		690015	2446121	5625183	10880311
10672930	21421796	2302370	7421385	11818149	14892276	2225923	11628550		695529	2457549	5636033	11730907
10672938	21494626	2333605	7508499	12185748	14896510	2228119	11629227		697750	2458438	5795446	11783849
10754378	21774684	2333609	7605752	12185793	14932274	3969220	11641156		698223	2594356	5826085	11787324
10807294	22009352	2604032	7669584	12189467	14972614	4291412	11644450		708358	2607630	5873446	11824369
10823684	22010245	2772386	7680492	12214463	15023789	4463364	11647778		720138	2687627	5873556	11856004
10861281	22056779	2802715	7684775	12323249	15141800	4494342	11650702		733457	2747914	5903803	11905675
10868266	22056779	2803847	7691610	12321116	15144334	4622583	11650769		736291	2762515	5908603	11924333
11987274	22074536	2823201	7705076	12409569	15220584	4655945	11653804		737246	3335463	6210848	11971411
12058144	22387551	2824311	7708600	12536882	15392718	4715967	11663170		740337	3372783	6212759	12011879
12901654	22555515	2844753	7767102	12549842	15394359	4833405	11689525		742588	3382627	6279058	12011888
13002733	22685443	2848203	7945551	12595775	15527611	4837700	11693345		748792	3479415	6304121	12021455
13163498	22693262	2849767	7958502	12598176	15541212	5151149	11694209		752453	3575255	6305062	12021659
13173429	22698457	2851840	7972061	12598853	1609829	5940706	11702974		754563	3656884	6306552	12024511
13347989	22703007	2857010	8501730	12601339	16616818	7647894	11707148		763364	3689679	6334170	12027892
13425070	22730366	2864180	8712363	12684821	16842942	7761730	11716714		769021	3700933	6337183	12477335
13427496	22896679	2877295	8743690	12810954	16856719	7801704	11968452		769965	3711136	6342556	12485199
13608199	22962150	2917390	8744138	13213072	16937814	7896980	12106514		799588	3712025	6352811	12491940
13757110	23298083	2917746	8744316	13220134	16942287	7908053	14618722		1027253	3719080	6367810	12511016
14156750	2991274	8770467	8770467	13282947	17904460	8115329	14704540		1035358	3726040	6369556	12521397
14185203	3221516	8898004	13319446	13319446	18492514	8115768	14832419		1042407	3726438	6370024	12545271
14194773	3329587	8956013	8956013	13354511	18674710	8116236	14973901		1042944	3727589	6379514	12546969
14289974	3655013	9079134	9079134	13387814	18678799	8409843	15067082		1165509	3822633	6385026	13485946
14463910	3817963	9088412	9088412	13447134	19061304	8538463	15275895		1174170	3928204	6387502	13509694
14628095	4401887	9100952	9100952	13472869	19454535	8962219	15828549		1175485	4010287	6393292	13538909
15242003	4527548	9106742	9106742	13761009	19457157	9139740	15829930		1212203	4036336	6520842	14177283
15250109	5240401	9106763	14006299	14006299	19457917	9384878	15956238		1272418	4116420	6521009	14225420
15287949	6254070	9130046	14208307	19458128	9397891	16114058	16114058		1329079	4247390	6943359	14427210
15293390	6285147	9130175	14219109	19999395	9652177	16205941	16205941		1329520	4260509	6944328	14554492
15341820	6296424	9135222	14266594	10092795	16591272	16591272	16591272		1360707	4460565	6969420	14789914
15446995	6358155	9175618	14297099	10320291	16643137	16643137	16643137		1371634	4552300	7012484	15260838
15557669	6373115	9199783	14523767	10361018	16825614	16825614	16825614		1374116	4562948	7025099	15388234
15582428	6405418	9285137	14570377	10381934	16919794	16919794	16919794		1377862	4686899	7086951	15542923
15660852	6412805	9701297	14570429	10402877	16972201	16972201	16972201		1432310	4686340	7722653	16026339
15774230	9723630	14667889	14667889	10487159	18141309	18141309	18141309		1490269	4686578	7824724	16132562
16700369	6611317	9728149	14740902	10960086	18141324	18141324	18141324		1509999	4824581	8482351	16419180

Chrom. 12	Chrom. 13	Chrom. 13	Chrom. 13	Chrom. 13	Chrom. 14	Chrom. 14	Chrom. 14	Chrom. 14	Chrom. 14	Chrom. 15	Chrom. 15	Chrom. 15	Chrom. 17	Chrom. 17	Chrom. 17
16425162	754274	11026377	17454605	3790879	7209342	12888750	2194376	9525027	863806	6340367	8655022				
893530	11028313	17482809	1184277	3806797	7210856	12925013	2232471	9600201	906851	6559597	8655179				
16425264	11027110	17494234	1477415	3942165	7278970	12940572	2666055	9643276	1098618	6573224	8658931				
16730119	11075965	17495930	1540679	3966352	7310590	12944800	3173586	9648925	1439118	6588015	8660545				
16787346	11705116	17519569	3974602	7336448	12944881	12944881	4173258	9662551	1442589	6588677	8690000				
17292280	2443603	17709529	17519569	1543541	7344432	12948156	4627060	9707692	1451834	6611979	8694078				
17293207	2521595	11792287	2081096	4592247	7344432	12948156	4627060	9707692	1451834	6611979	8694078				
17335011	2832242	11822391	17873691	4592785	7398568	13062440	4633267	9742644	1569753	6700041	8771538				
17347985	2870925	11934618	17925090	2265777	7493466	13531394	4710758	9742743	1592010	6722116	8722184				
17363790	3285202	11990165	18052586	2279076	7524051	13566120	4850805	9749487	1661517	6774639	8787695				
17685277	3745707	12021075	2304780	4633141	7880754	13574609	4978411	9750184	1725150	6798651	9105384				
17685380	3745722	12045999	2324654	4648452	7893704	13980135	5072570	9760273	1725150	6798651	9105384				
17784817	4611051	12057725	2330596	4682019	8059214	13980135	5072570	9760273	1725150	6798651	9105384				
17917172	4655648	12103441	2331106	4714756	8069226	13980135	5072570	9760273	1725150	6798651	9105384				
18165884	5408399	12103706	2331114	4751178	8069226	13980135	5072570	9760273	1725150	6798651	9105384				
18555850	5439104	12104389	2334011	4769384	8071662	13980135	5072570	9760273	1725150	6798651	9105384				
18565669	5444321	12111103	2436344	4807786	8071665	13980135	5072570	9760273	1725150	6798651	9105384				
18568400	5445388	12116512	2445621	4913952	8099475	13980135	5072570	9760273	1725150	6798651	9105384				
18568854	5493049	12138572	2504379	4914002	8115429	13980135	5072570	9760273	1725150	6798651	9105384				
18596275	5695438	12161905	2534559	4959032	8126869	13980135	5072570	9760273	1725150	6798651	9105384				
18596396	5700866	12161905	2863000	4959128	8127796	13980135	5072570	9760273	1725150	6798651	9105384				
18691310	5965190	12228537	2877893	4961156	8128165	13980135	5072570	9760273	1725150	6798651	9105384				
18694275	6050483	12238754	2888580	5000387	8133352	13980135	5072570	9760273	1725150	6798651	9105384				
18709215	6137657	12240815	2900733	5001220	8133540	13980135	5072570	9760273	1725150	6798651	9105384				
18709519	6137673	12257109	2919741	5011687	8142769	13980135	5072570	9760273	1725150	6798651	9105384				
19186431	6199135	12275710	2928803	5019119	8148118	13980135	5072570	9760273	1725150	6798651	9105384				
	6266908	12298652	2931291	5056515	8162012	13980135	5072570	9760273	1725150	6798651	9105384				
	6271195	12759552	2932764	5234564	8210184	13980135	5072570	9760273	1725150	6798651	9105384				
	6290088	13374481	2954320	5283059	8218710	13980135	5072570	9760273	1725150	6798651	9105384				
	6296571	13475246	2958241	5331820	8940474	13980135	5072570	9760273	1725150	6798651	9105384				
	6306927	13575463	2958371	5361858	9595312	13980135	5072570	9760273	1725150	6798651	9105384				
	6347591	14066325	3026314	5367483	9952697	13980135	5072570	9760273	1725150	6798651	9105384				
	6347626	14510508	3092581	5369311	10005309	13980135	5072570	9760273	1725150	6798651	9105384				
	6486823	14510801	3097743	5413765	10106277	13980135	5072570	9760273	1725150	6798651	9105384				
	6565794	14890912	3108633	5479705	10335114	13980135	5072570	9760273	1725150	6798651	9105384				
	6706060	14989832	3192556	5492240	10399461	13980135	5072570	9760273	1725150	6798651	9105384				
	6727085	15861035	3385119	5508343	10413814	13980135	5072570	9760273	1725150	6798651	9105384				
	6729058	16008059	3404797	5511388	10476927	13980135	5072570	9760273	1725150	6798651	9105384				
	6739588	16065472	3428785	5518179	10508240	13980135	5072570	9760273	1725150	6798651	9105384				
	6888646	16251932	3442312	5547996	10517012	13980135	5072570	9760273	1725150	6798651	9105384				
	7170932	16399761	3451277	5555946	10552049	13980135	5072570	9760273	1725150	6798651	9105384				
	7331658	16627002	3455949	5841809	10653992	13980135	5072570	9760273	1725150	6798651	9105384				
	7827138	17092078	3615271	5983642	10668907	13980135	5072570	9760273	1725150	6798651	9105384				
	8401337	17133072	3625853	6370002	10992692	13980135	5072570	9760273	1725150	6798651	9105384				
	8567731	17183261	3639827	6595306	11960776	13980135	5072570	9760273	1725150	6798651	9105384				
	8938041	17330579	3665270	6596177	12117345	13980135	5072570	9760273	1725150	6798651	9105384				
	9012796	17373832	3666147	6600169	12253079	13980135	5072570	9760273	1725150	6798651	9105384				
	9023548	17428611	3720087	6740436	12413874	13980135	5072570	9760273	1725150	6798651	9105384				
	9544248	17428374	3722871	6753103	12433516	13980135	5072570	9760273	1725150	6798651	9105384				
	9888714	17434757	3726177	6785274	12721749	13980135	5072570	9760273	1725150	6798651	9105384				
	10526785	17445579	3727676	6947447	12852505	13980135	5072570	9760273	1725150	6798651	9105384				
	10660063	17453099	3789566	7208751	12853507	13980135	5072570	9760273	1725150	6798651	9105384				

Chrom. 18	Chrom. 18	Chrom. 19	Chrom. 19	Chrom. 20	Chrom. 20	Chrom. 21	Chrom. 22	Chrom. 23	Chrom. 24	Chrom. 25		
902645	4749214	6735711	4724668	7583548	625393	6460722	13087908	433173	1663230	303275	126822	978009
977710	4808924	6760809	5451419	7604030	813936	6484087	13207717	912762	2100481	541486	149303	1102452
1093065	4841700	6818457	5465889	7633191	837059	7157261	13214456	1029141	2547055	573262	200583	1103192
1124752	4903508	6992754	5500154	7669414	911318	7305471	13215531	1390021	2552848	573539	284769	1109695
1165817	4913686	717630	5726543	7677916	927790	8111695	13216111	1554352	2729474	584175	1405250	1150999
1208234	5039644	271482	5747298	7757436	971372	8191456	13334571	1641909	2798914	609173	1421182	1320004
1209138	5517339	580436	5747488	8014377	974467	8408068	13344724	1653319	3700626	616284	1912123	1328120
1231936	5522749	747638	5830696	8071886	974560	8412195	13364176	1653357	3703666	648063	1986908	1528888
1256978	5602842	838192	5833728	8259175	978996	8443839	13483320	1831102	3704248	660965	1988653	1669503
1522336	5602848	876247	5833753	8300311	989001	8489417	1393385	2333072	3716675	663857	2059417	1704211
1529107	6141471	1003675	5841962	8416214	993560	8582641		2489788		664421	2059419	1704246
1543545	6189234	1408165	5891453	8434392	1000718	8789679		2559790		738474	2097033	1778485
1550632	6252403	1502032	5894315	9303967	1004332	8931031		2610337		772230	2123113	1856815
1590860	6633350	1505279	5965904		1073683	8938913		2618503		775923	2145047	2053338
1600890	6636041	1539817	5989183		1395259	8944640		2624195		792876	2330171	2379274
1639026	7358034	1547313	5989235		1404631	9254545		2688709		804158	2330498	2492072
1706460	7527461	1569669	6010174		1412280	9271756		2832321		808523	2652186	2534366
1798888	7540992	1580462	6020007		1419154	9286346		2974147		829432	2659757	
1859042	7547309	1751180	6022617		1460398	9294047		2994600		853467	2688398	
2179391	7574920	1796257	6025436		1530162	9325396		3001665		873415	2872478	
2353528	8520358	1796923	6027194		1532476	9805264		3005099		874662	2873532	
2870516	8892020	1809408	6047730		1538650	9849284		3024714		882886	2875411	
2885180	9491434	1876738	6054505		1561203	10114240		3516402		1002478	2922961	
2895564	9521363	1985088	6095684		1626986	10285739		3528476		1045090	2924593	
2914143	9521864	2259820	6095884		1759206	10508386		3536570		1156676	2992122	
2950413	9884590	2356804	6096481		1759384	10763997		3558629		1531846	3211402	
2951088	9987506	2434748	6100849		1801935	10764208		3684754		1577757	3211739	
3182918	10010477	2619904	6101015		2305416	10930631		3686478		1614192	3217940	
3274870	10267891	2641336	6109777		2377369	10959899		3723987		1615332	3225884	
3285062		2845568	6118616		2426602	10991662		4054918		1637697	3231008	
3286431		3550591	6161955		2477792	11075418		4836900		1644570	3494353	
3289038		3585622	6371540		2568813	11505812		4939003		1657014	3496578	
3306804		3651040	6527535		2569043	11676390		4948026		1659546	3572763	
3307774		3651331	6531519		2668097	11814885		4976109		1929541	3636773	
3406039		3652052	6549159		2677844	11824650		4997453		1948145	3666284	
3422806		3678657	6597719		2836739	11830800		5022715		2008332	3758125	
3550356		3678661	6602651		2900096	12066976		5128628		2410540	3863025	
3575439		3687105	6673957		2936787	12075797		5291715		2686617	4332717	
3796117		3759136	6702522		2982291	12186799		5314232		2704778	4459949	
4057707		3759162	7189659		2982302	12269210		5323538		2769886	4769482	
4089443		3766184	7194757		2995437	12670983		5678012		2950084	5102087	
4104363		3779485	7194939		3130379	12671317		5759162		3111000	5890836	
4133004		3779599	7195075		3181759	12687999		5762368		3272938	6037678	
4133737		3782009	7200145		3199954	12716334		5762381		3368358		
4134351		3814901	7203329		3201300	12761427		5764164		3772854		
4134920		3939895	7224289		3787277	12837139		6571837		4450830		
4135566		3962214	7417096		4057226	12861404		6571938		4450876		
4138386		4000300	7430290		4922176	12892794		6576343		4470184		
4273700		4063257	7435989		5358625	12905285		6580599		4567627		
4296051		4372316	7532028		6017404	12923319				4884561		
4583312		4638239	7544763		6146979	12946708		4916097				

Chrom. 26	Chrom. 27	Chrom. 28	Chrom. 33	Unlocalised Scaffolds
382874	1432737	151802	414716	chr1_NT_456097v1_random: 43874
504218	1435380	151819	599116	chr1_NT_456113v1_random: 3429
506657	1436167	1062404	1461466	chr1_NT_456143v1_random: 7614
509283	1445658	1957082	1485829	chr1_NT_456856v1_random: 5170
528461	1454549	2443298		chr1_NT_457819v1_random: 308
543884	1457215	2641875		chr13_NT_462202v1_random: 5933
550031	1490347	2675966		chr14_NT_462351v1_random: 19241
568259	1490619	2791367		chr14_NT_462351v1_random: 19095
588357	1564845	2835337		chr3_NT_459020v1_random: 12054
602694	1565230	2912314		chr3_NT_459020v1_random: 118744
699097	1570143	2935182		chr3_NT_459020v1_random: 64013
713348	1571190	3218402		chr3_NT_459020v1_random: 106471
715959	1582033	3270473		chr3_NT_459020v1_random: 64289
775775	1591385	3491291		chr3_NT_459020v1_random: 13514
808129	1594214	3501746		chr4_NT_459801v1_random: 31636
808162	1596504	3548523		chr4_NT_459801v1_random: 166828
828736	1598914	3593309		chr5_NT_460430v1_random: 18341
1121275	1601776	3623347		chr5_NT_460548v1_random: 7262
1941887	1603418	3635463		chr6_NT_460840v1_random: 23366
2225324	1609162	3878808		chr22_NT_463002v1_random: 9829
2282561	1613400	3907769		chr33_NT_463452v1_random: 15025
2591052	1619962	3985591		chr33_NT_463454v1_random: 57531
2621536	1619971	3985871		chr33_NT_463456v1_random: 36158
2773637	1619989			chrLGE64: 868044
2820275	1620516			chrUn_NT_464128v1: 67946
2874261	1625362			chrUn_NT_464135v1: 21542
2876457	1628371			chrUn_NT_464148v1: 5652
2876551	1639263			chrUn_NT_464215v1: 7282
2880150	1909749			chrUn_NT_464327v1: 17129
2888638	1949283			chrUn_NT_464492v1: 18892
2890690	2053097			chrUn_NT_465142v1: 16062
2999174	2053967			chrUn_NT_465200v1: 5520
3010775	2158431			chrUn_NT_466029v1: 6006
3013540	2752990			chrUn_NT_466029v1: 3712
3045860	2927799			chrUn_NT_466086v1: 822
3073425	2966444			chrUn_NT_466086v1: 8623
3103563	3052200			chrUn_NT_467376v1: 1658
3115215	3195680			chrUn_NT_467869v1: 10766
3115232	3458427			chrUn_NT_469469v1: 2442
3129055	3458886			chrUn_NT_469469v1: 2838
4772959	3475696			chrUn_NT_471611v1: 6225
4876868	3551689			chrUn_NT_473432v1: 5412
	3632525			chrUn_NT_473478v1: 911
	3637822			chrUn_NT_473624v1: 3973
	3875240			chrUn_NT_473988v1: 822
	3988099			chrUn_NT_479485v1: 871
	4038299			chrUn_NT_479485v1: 432
	4139773			

CLAIMS

1. A method of certifying a test animal-derived product sample, the method comprising the steps of:
- 5 (a) determining a test methylation profile of one or more pre-selected methylation sites within the genomic material obtained from the test animal derived product sample; and
- (b) comparing the test methylation profile obtained from (a) with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not
- 10 slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death
- wherein a difference in the test methylation profile of (a) compared to the reference methylation profile from the control animal, is indicative of the test animal having been
- 15 slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product is certified so; and
- wherein the pre-selected methylation sites are CpG sites selected from genes or regions of genomic DNA from the control and test animal that shows the highest degree of
- 20 methylation variation during the training of the method.
2. A method of certifying a test animal-derived product sample, the method comprising the steps of:
- (a) determining the test methylation profile of one or more pre-selected methylation sites
- 25 within the genomic material obtained from the test animal derived product sample; and
- (b) comparing the test methylation profile determined in (a) with a panel of predetermined reference methylation profiles of the same biological taxon of the test animal from which the product sample derives, wherein each of the predetermined reference methylation profiles is specific for a distinctly certified reference animal that
- 30 - has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus; and/or
- bled to death;
- 35 wherein if the test methylation profile is significantly similar to one of the predetermined reference methylation profiles, the test animal derived product sample has the same distinct certification as that of the predetermined reference methylation profile and is derived from a test animal that has been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or
- 40 oesophagus and/or bled to death and the test animal-derived product is certified so; and

wherein the pre-selected methylation sites are CpG sites selected from genes or regions of genomic DNA from the control and test animal that shows the highest degree of methylation variation during the training of the method.

- 5 3. A method of certifying a test animal-derived product sample, the method comprising the steps of:
- (a) determining the methylation status of at least one CpG site within the genomic material obtained from the animal-derived product sample,
- 10 (b) comparing the methylation status of the CpG site from (a) with that of a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death,
- wherein a difference in the test methylation status of (a) in the test animal compared to the
- 15 CpG site in the control animal is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product is certified so.
- 20 4. The method according to any one of the preceding claims, wherein test animal derived product sample is distinctly certified kosher or halal where the test animal is slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the test animal is bled to death.
- 25 5. A method of verifying certification of a test animal-derived product sample that has been certified to derive from a test animal that was slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or that was bled to death, the method comprising the steps of:
- (a) determining a test methylation profile of one or more pre-selected methylation sites
- 30 within the genomic material obtained from the test animal derived product sample; and
- (b) comparing the test methylation profile obtained from (a) with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not
- slaughtered by a single cut across the throat severing both carotid arteries, both
- 35 jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death
- wherein a difference in the test methylation profile of (a) compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular

- veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product being correctly certified, and wherein a similarity in the test methylation profile of (a) compared to the reference methylation profile of the control animal, is indicative of the test animal not having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death and the test animal-derived product being wrongly certified; and wherein the pre-selected methylation sites are CpG sites selected from genes or regions of genomic DNA from the control and test animal that shows the highest degree of methylation variation during the training of the method.
6. A method according to any one of the preceding claims, wherein the CpG site is selected from list provided in Table A.
7. A method for confirming or rejecting an assumed certification of an animal derived product sample, the method comprising
- comparison of a test methylation profile obtained from genomic material of the animal derived product sample with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death, wherein a difference in the test methylation profile compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death wherein the assumed certification is that the test animal derived product sample is kosher or halal where the test animal is slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the test animal is bled to death.
8. A method of determining if a test animal-derived product sample, is obtained from a test animal that has been bled to death or slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus the method comprising:
- comparison of a test methylation profile obtained from genomic material of the test animal derived product sample with a reference methylation profile obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut

across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death.

9. The method according to claim 8, wherein the method comprises the steps of:
- 5 a) determining the test methylation profile of one or more pre-selected methylation sites within the genomic material obtained from the test animal derived product sample; and
- b) comparing the test methylation profile determined in (a) with a panel of predetermined reference methylation profiles of the same biological taxon of the test animal from which the product sample derives, wherein with a reference methylation profile
- 10 obtained from a control animal of the same biological taxon of the test animal from which the product sample is derived from, where the control animal is not slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the control animal is not bled to death,
- 15 wherein a difference in the test methylation profile of (a) compared to the reference methylation profile from the control animal, is indicative of the test animal having been slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or of the test animal having been bled to death.
- 20
10. The method according to any one of the preceding claims, wherein the animal is selected from the group consisting of cow, sheep, goat, camel, chicken, goose, duck and turkey.
11. The method according to any one of the preceding claims, wherein the test animal-derived product sample is meat, eggs, milk and any other tissue or sample that provides genomic
- 25 DNA.
12. The method according to any one of the preceding claims, wherein the test animal-derived product sample is meat.
13. The method according to any one of the preceding claims, wherein the difference is hypomethylation or hypermethylation.
- 30
14. Use of DNA methylation profiling for certifying of an animal-derived product sample, wherein the certification of the animal-derived product sample is kosher or halal where the
- 35 test animal is slaughtered by a single cut across the throat severing both carotid arteries, both jugular veins, both vagus nerves, trachea and/or oesophagus and/or the test animal is bled to death.