normalize a specific unrelated RNA-Y with a genomic DNA-X sequence if that sequence is transcribed.

The present invention seeks to overcome one or more of the difficulties existing in the prior art by preparing nucleic acid samples comprising both DNA and RNA (e.g. TNA), and co-amplifying (i) one gene (DNA-X) and its associated RNA transcription products (RNA-X), along with an unrelated region of DNA (DNA-N) which is not transcribed. The DNA-N can then be used to normalize levels of the DNA-X plus RNA-X. Further, it is possible to use multiple primer sets targeting multiple transcribed genes or regions and their associated transcripts, for example DNA-X plus RNA-X and DNA-Y plus RNA-Y can be normalized to a non-transcribed DNA-N. Optionally, for convenience, the ratio obtained by dividing the data from analysis of multiple transcribed DNA/RNA targets by that from a single non-transcribed DNA-N can be again be divided by the number of primer sets targeting the sets of transcribed DNA/RNA species. The invention will be further clarified by way of the following examples.

## *Summary ~~of the Invention~~*

The present ~~invention~~disclosure may involve co-amplification of three nucleic acid species (e.g. simultaneously) in a sample comprising both DNA and RNA (e.g. of a sample of total nucleic acid (TNA)). These species are (i) gene(s), (ii) transcripts expressed from those gene(s), and (iii) non-expressed (i.e. non-transcribed) DNA sequences. Together the combined measurement of a gene and its corresponding transcripts is often referred to herein as GAT (**G**ene **A**nd **T**ranscript) and the measurement of non-transcribed DNA is often referred to herein as NED (**N**on-**E**xpressed **D**NA). The methods of the present ~~invention~~disclosure involve co-amplifying these species together or separately, and using the ratio of the estimates of GAT and the NED as an indicator of transcriptional capacity, which can in turn be used to assess characteristics such as cell viability, cell death, functional perturbation of cells resulting from an external stimulus or a disease state, and the like.

In one aspect, the present disclosure refers to a method for normalising quantitative data obtained by amplification of nucleic acids from a cell, organism, or virus, the method comprising:

(i) obtaining quantitative data

- from amplification of genomic DNA from a first gene and RNA transcribed from the first gene, and
- from amplification of a sequence of non-transcribed DNA in the cell, organism, or virus; and

(ii) using the quantitative data to derive a normalised value (nV) representative of the relative amounts of:

- said genomic DNA and RNA transcripts of the first gene, to
- said genomic DNA that is not transcribed,

present within the nucleic acid sample prior to said amplification;

wherein said genomic DNA from the first gene and RNA transcribed from the first gene, and said sequence of non-transcribed DNA, are co-amplified in the same reaction.

The present ~~invention~~disclosure relates, at least in part, to ~~embodiment~~examples 1-48 listed below:

~~Embodiment~~Example 1. A method for normalising quantitative data obtained by amplification of nucleic acids from a cell, organism, or virus, the method comprising:

(i) obtaining quantitative data

- from amplification of genomic DNA from a first gene and RNA transcribed from the first gene, and
- from amplification of a sequence of non-transcribed DNA in the cell, organism, or virus; and

(ii) using the quantitative data to derive a normalised value (nV) representative of the relative amounts of:

- said genomic DNA and RNA transcripts of the first gene, to
- said genomic DNA that is not transcribed,

present within the nucleic acid sample prior said amplification.

~~Embodiment~~Example 2. The method of ~~embodiment~~example 1, wherein the quantitative data is amplicon copy number, and the method comprises:

- obtaining a value A (vA) representing total amplicon number generated from the amplification of said genomic DNA and RNA transcripts of the first gene, and a value B (vB) representing total amplicon number generated from the sequence of non-transcribed DNA;
- calculating a normalised value (nV) using the equation:

$$vA/vB = nV$$

or an equivalent form thereof.

~~Embodiment~~Example 3. The method of ~~embodiment~~example 1, wherein the quantitative data is amplicon copy number, and the method comprises:

- obtaining a value X (vX) representing total amplicon number generated from: the amplification of genomic DNA and RNA transcripts from the first gene, and amplification of genomic DNA and RNA transcripts from at least one additional gene;
- obtaining a value B (vB) representing total amplicon number generated from the sequence of non-transcribed DNA,
- calculating a normalised value (nV) using the equation:

$$vX / (vB \times (X+1)) = nV$$

or an equivalent form thereof,

wherein X is the number of said additional gene(s).

~~Embodiment~~Example 4. The method of ~~embodiment~~example 2 or ~~embodiment~~example 3, wherein the amplification is digital polymerase chain reaction (dPCR).

~~Embodiment~~Example 5. The method of ~~embodiment~~example 1, wherein the quantitative data is threshold value (Ct), and the method comprises:

- obtaining a cycle threshold value CtA from the amplification of said genomic DNA and RNA transcripts of the first gene,
- obtaining a cycle threshold value CtB from the amplification of the sequence of non-transcribed DNA; and
- calculating a normalised value (nV) using the equation:

$$2^{CtB - CtA} = nV$$

or an equivalent form thereof.

~~Embodiment~~Example 6. The method of ~~embodiment~~example 1, wherein the quantitative data is threshold value (Ct), and the method comprises:

- obtaining a cycle threshold value CtX from: the amplification of said genomic DNA and RNA transcripts of the first gene, and from amplification of genomic DNA and RNA transcripts from at least one additional gene;
- obtaining a cycle threshold value CtB from the amplification of the sequence of non-transcribed DNA; and
- calculating a normalised value (nV) using the equation:

$2^{CtB - CtX} / (X + 1) = nV$

or an equivalent form thereof,

wherein X is the number of said additional gene(s).

~~Embodiment~~Example 7. The method of ~~embodiment~~example 5 or ~~embodiment~~example 6, wherein the amplification is quantitative polymerase chain reaction (qPCR).

~~Embodiment~~Example 8. The method of any one of ~~embodiment~~examples 1 to 7, wherein the sequence of non-transcribed DNA is genomic DNA.

~~Embodiment~~Example 9. The method of any one of ~~embodiment~~examples 1 to 8, further comprising conducting said amplification of the nucleic acids from the cell, organism, or virus.

~~Embodiment~~Example 10. The method of any one of ~~embodiment~~examples 1 to 9, wherein the nucleic acids from the cell, organism, or virus are an extract of total nucleic acids.

~~Embodiment~~Example 11. The method of any one of ~~embodiment~~examples 1 to 10, wherein any said amplification is conducted using: polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), helicase dependant amplification (HDA), strand invasion based amplification (SIBA), transcript-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), or any combination thereof.

~~Embodiment~~Example 12. The method of any one of ~~embodiment~~examples 1 to 11, further comprising using the normalised value (nV) to assess the level of transcriptional activity in the cell, organism, or virus.

~~Embodiment~~Example 13. The method of any one of ~~embodiment~~examples 1 to 12, further comprising:

- obtaining a transcription-negative normalised value (nV-) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known not to have transcriptional activity; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the transcription-negative normalised value (nV-), to thereby assess the level of transcriptional activity in the cell, organism, or virus.

~~Embodiment~~Example 14. The method of ~~embodiment~~example 13, wherein the transcription-negative normalised value (nV-) is a mean value generated from said series of said normalised values (nV).

~~Embodiment~~Example 15. The method of ~~embodiment~~example 13 or ~~embodiment~~example 14, wherein:

- the transcription-negative normalised value (nV-) is used as a base value for assessing a presence or an absence of transcriptional activity in the cell, organism or virus; and
- an absence of transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or below the transcription-negative normalised value (nV-); or
- transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is above the transcription-negative normalised value (nV-).

~~Embodiment~~Example 16. The method of any one of ~~embodiment~~examples 13 to 15, wherein said transcription-negative normalised value (nV-):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known not to have transcriptional activity; and/or
- is provided with a confidence interval that said transcription-negative normalised value (nV-) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

~~Embodiment~~Example 17. The method of ~~embodiment~~example 16, wherein the confidence interval is more than 90%, or more than 95%.

~~Embodiment~~Example 18. The method of any one of ~~embodiment~~examples 13 to 17, further comprising:

- obtaining a transcription-positive normalised value (nV+) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to have transcriptional activity; and
- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the transcription-positive normalised value (nV+), to thereby assess the level of transcriptional activity in the cell, organism, or virus.

~~Embodiment~~Example 19. The method of ~~embodiment~~example 18, wherein the transcription-positive normalised value (nV+) is a mean value generated from said series of said normalised values (nV).

~~Embodiment~~Example 20. The method of ~~embodiment~~example 18 or ~~embodiment~~example 19, wherein:

- the transcription-positive normalised value (nV+) is used as a base value for transcriptional activity in the cell, organism or virus; and
- a lack or absence of transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is below the transcription-positive normalised value (nV+); or
- transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or above the transcription-positive normalised value (nV+).

~~Embodiment~~Example 21. The method of any one of ~~embodiment~~examples 18 to 20, wherein said transcription-positive normalised value (nV+):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to have transcriptional activity; and/or
- is provided with a confidence interval that said transcription-positive normalised value (nV+) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

~~Embodiment~~Example 22. The method of ~~embodiment~~example 21, wherein the confidence interval is more than 90%, or more than 95%.

~~Embodiment~~Example 23. The method of ~~embodiment~~example 13, further comprising:

- obtaining a transcription-negative normalised value (nV-) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known not to have transcriptional activity;
- obtaining a transcription-positive normalised value (nV+) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to have transcriptional activity; and
- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to:

(i) the transcription-negative normalised value (nV-) and to the transcription-positive normalised value (nV+), or

(ii) to a combined transcription normalised value ($nV^{\pm}$) intermediate to the transcription-negative normalised value (nV-) and the transcription-positive normalised value (nV+),

to thereby assess the level of transcriptional activity in the cell, organism, or virus.

~~Embodiment~~Example 24. The method of ~~embodiment~~example 23, wherein the combined transcription normalised value ($nV^{\pm}$) is calculated using the equation:

$$(nV+) + (nV-) / 2 = (nV^{\pm})$$

or an equivalent form thereof.

~~Embodiment~~Example 25. The method of ~~embodiment~~example 24, wherein said combined transcription normalised value ($nV^{\pm}$):

- incorporates statistical variation in said series of transcription-negative normalised value (nV-) and/or said transcription-positive normalised value (nV+); and/or
- is provided with a confidence interval that said combined transcription normalised value ($nV^{\pm}$) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

~~Embodiment~~Example 26. The method of ~~embodiment~~example 25, wherein the confidence interval is more than 90%, or more than 95%.

~~Embodiment~~Example 27. The method of any one of ~~embodiment~~examples 12 to 26, wherein the level of transcriptional activity in the cell, organism, or virus is assessed for the purpose of determining any one or more of:

- viability of the test cell or the test organism;
- whether the test cell, organism or virus is alive or dead;
- transcriptional perturbation within the test cell, organism, or virus.

~~Embodiment~~Example 28. The method of any one of ~~embodiment~~examples 1 to 11, further comprising using the normalised value (nV) to assess the level of drug resistance or drug sensitivity in the cell, organism, or virus, wherein:

- said cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids, and
- said normalised value (nV) is compared to a control normalised value (cnV) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to be:
  - (i) resistant to the drug; or
  - (ii) sensitive to the drug,

to thereby assess the level of drug resistance or drug sensitivity in the cell, organism, or virus.

~~Embodiment~~Example 29. The method of any one of ~~embodiment~~examples 1 to 11 or 28, further comprising:

- obtaining a drug-sensitive normalised value (dsV) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to be sensitive to the drug; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the drug-sensitive normalised value (dsV), to thereby assess the level of drug resistance or drug sensitivity or in the cell, organism, or virus, wherein the cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids.

~~Embodiment~~Example 30. The method of ~~embodiment~~example 29, wherein the drug-sensitive normalised value (dsV) is a mean value generated from said series of said normalised values (nV).

~~Embodiment~~Example 31. The method of ~~embodiment~~example 29 or ~~embodiment~~example 30, wherein:

- the drug-sensitive normalised value (dsV) is used as a base value for assessing a presence or an absence of resistance or sensitivity to the drug in the cell, organism or virus; and

- resistance to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is above the drug-sensitive normalised value (dsV); or

- sensitivity to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or below the drug-sensitive normalised value (dsV).

~~Embodiment~~Example 32. The method of any one of ~~embodiment~~examples 29 to 31, wherein said drug-sensitive normalised value (dsV):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to be sensitive to the drug; and/or

- is provided with a confidence interval that said drug-sensitive normalised value (dsV) is predictive of a presence or an absence of:

  (i) resistance to the drug in the cell, organism or virus; or

  (ii) sensitivity to the drug in the cell, organism or virus.

~~Embodiment~~Example 33. The method of ~~embodiment~~example 32, wherein the confidence interval is more than 90%, or more than 95%.

~~Embodiment~~Example 34. The method of any one of ~~embodiment~~examples 1 to 11 or 28, further comprising:

- obtaining a drug-resistant normalised value (drV) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to resistant to the drug; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the drug-resistant normalised value (drV), to thereby assess the level of drug resistance or drug sensitivity or in the cell, organism, or virus, wherein the cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids.

~~Embodiment~~Example 35. The method of ~~embodiment~~example 34, wherein the drug-resistant normalised value (drV) is a mean value generated from said series of said normalised values (nV).

~~Embodiment~~Example 36. The method of ~~embodiment~~example 34 or ~~embodiment~~example 35, wherein:

- the drug-resistant normalised value (drV) is used as a base value for assessing a presence or an absence of resistance or sensitivity to the drug in the cell, organism or virus; and

- resistance to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or above the drug-resistant normalised value (drV); or

- sensitivity to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is below the drug-resistant normalised value (drV).

~~Embodiment~~Example 37. The method of any one of ~~embodiment~~examples 34 to 36, wherein said drug-resistant normalised value (drV):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to be resistant to the drug; and/or

- is provided with a confidence interval that said drug-resistant normalised value (drV) is predictive of a presence or an absence of:

(i) resistance to the drug in the cell, organism or virus; or

(ii) sensitivity to the drug in the cell, organism or virus.

~~Embodiment~~Example 38. The method of ~~embodiment~~example 37, wherein the confidence interval is more than 90%, or more than 95%.

~~Embodiment~~Example 39. The method of one of ~~embodiment~~examples 1 to 11, further comprising using the normalised value (nV) to assess the level of drug resistance or drug sensitivity in the cell, organism, or virus, wherein:

- a first population of said cell, organism, or virus which has been treated with a drug prior to said amplification of nucleic acids, is used to generate a first said normalized value (nV),
- a second population of said cell, organism, or virus which has not been treated with a drug prior to said amplification of nucleic acids, is used to generate a second said normalized value (nV),
- said first normalized value (nV) and said second normalized value (nV) are compared to assess the level of transcriptional activity in the cell, organism, or virus with or without drug treatment, and thereby assess the level of drug resistance or drug sensitivity in the cell, organism, or virus.

~~Embodiment~~Example 40. The method of ~~embodiment~~example 39, wherein said drug sensitivity is indicated when said first normalized value (nV) is lower than said second normalized value (nV).

~~Embodiment~~Example 41. The method of any one of ~~embodiment~~examples 27 to 40, wherein the drug is an antimicrobial.

~~Embodiment~~Example 42. The method of any one of ~~embodiment~~examples 27 to 41, wherein the drug is an antimicrobial of a class selected from: Aminoglycosides, ansamycins, carbacephem, carbapenems, cephalosporins, glycopeptides, macrolidespenicillins, monobactams, polypeptides, quinolones, sulphonamides, tetracyclines.

~~Embodiment~~Example 43. The method of any one of ~~embodiment~~examples 27 to 42, wherein the drug is Ciprofloxacin, Azithromycin, Rifampicin, or Doxycycline.

~~Embodiment~~Example 44. The method of any one of ~~embodiment~~examples 27 to 43, wherein the first gene is a gene from a Chlamydia species (e.g. Chlamydia trachomatis), a Gonorrhea species, or a mycoplasma species (e.g. Mycoplasma genitralium).

~~Embodiment~~Example 45. The method of any one of ~~embodiment~~examples 1 to 44, wherein said genomic DNA from the first gene and RNA transcribed from the first gene, and said sequence of non-transcribed DNA are co-amplified in the same reaction.

~~Embodiment~~Example 46. The method of ~~embodiment~~example 45, wherein said reaction comprises using reverse transcriptase.

~~Embodiment~~Example 47. The method of any one of ~~embodiment~~examples 1 to 46, wherein the cell is a mammalian cell, a human cell, an animal cell, a plant cell, a bacterial cell, a host cell infected by viruses, or a host cell of infected by bacteria.

~~Embodiment~~Example 48. The method of any one of ~~embodiment~~examples 1 to 47, wherein the organism is a mammal, a human, a plant, a bacterium, a virus, a fungus, an alga, an archaeon or a protozoan.

The present ~~invention~~disclosure also relates, at least in part, to ~~embodiment~~examples 1-23 listed below:

~~Embodiment~~Example 1. A method for assessing the transcriptional activity of a cell or an organism, the method comprising:

- performing a nucleic acid amplification reaction on total nucleic acid from the cell or organism, wherein the nucleic acid amplification comprises:

contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a first gene and RNA transcribed from the first gene,

contacting the total nucleic acid sample with one or multiple primers capable of amplifying a region of non-coding genomic DNA that is not transcribed in the cell or organism;

- analysing kinetics of the nucleic acid amplification reaction to derive a ratio of:

(i) said genomic DNA and RNA transcripts of the first gene, to

(ii) said region of non-coding DNA,

wherein the ratio is representative of the relative amount of (i) and (ii) present within the total nucleic acid sample prior to performing the nucleic acid amplification reaction; and

- analysing the ratio to assess the transcriptional activity of the cell or organism, wherein:

a ratio of 1 indicates an absence of transcriptional activity in the cell or organism, and

a ratio of more than 1 indicates that transcriptional activity exists or may exist in the cell or organism.

~~Embodiment~~Example 2. The method of ~~embodiment~~example 1, wherein a ratio of more than: 1.5, 1.7, 1.9, 2, 2.2, 2.4, 2.5, 2.7, 2.9 or 3, indicates transcriptional activity in the cell or organism.

~~Embodiment~~Example 3. The method of ~~embodiment~~example 1, wherein a ratio of less than: 1.5, 1.4, 1.3, 1.2 or 1.1 indicates an absence of transcriptional activity in the cell or organism.

~~Embodiment~~Example 4. A method for assessing the viability of a cell or an organism, the method comprising:

- performing a nucleic acid amplification reaction on total nucleic acid the cell or organism, wherein the nucleic acid amplification comprises:

contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a first gene and RNA transcribed from the first gene,

contacting the total nucleic acid sample with one or multiple primers capable of amplifying a region of non-coding genomic DNA that is not transcribed in the cell or organism;

- analysing kinetics of the nucleic acid amplification reaction to derive a ratio of:

(i) said genomic DNA and RNA transcripts of the first gene, to

(ii) said region of non-coding DNA,

wherein the ratio is representative of the relative amount of (i) and (ii) present within the total nucleic acid sample prior to performing the nucleic acid amplification reaction; and

- analysing the ratio to assess the transcriptional activity of the cell or organism, wherein:

a ratio of 1 indicates the cell or organism is not viable, and

a ratio of more than 1 indicates that the cell or organism is or may be viable.

~~Embodiment~~Example 5. The method of ~~embodiment~~example 4, wherein a ratio of more than: 1.5, 1.7, 1.9, 2, 2.2, 2.4, 2.5, 2.7, 2.9 or 3, indicates that the cell or organism is viable.

~~Embodiment~~Example 6. The method of ~~embodiment~~example 4, wherein a ratio of less than: 1.5, 1.4, 1.3, 1.2 or 1.1 indicates that the cell or organism is not viable.

~~Embodiment~~Example 7. A method for determining whether a cell or an organism is dead, the method comprising:

- performing a nucleic acid amplification reaction on total nucleic acid from the cell or organism, wherein the nucleic acid amplification comprises:

contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a first gene and RNA transcribed from the first gene,

contacting the total nucleic acid sample with one or multiple primers capable of amplifying a region of non-coding genomic DNA that is not transcribed in the cell or organism;

- analysing kinetics of the nucleic acid amplification reaction to derive a ratio of:

(i) said genomic DNA and RNA transcripts of the first gene, to

(ii) said region of non-coding DNA,

wherein the ratio is representative of the relative amount of (i) and (ii) present within the total nucleic acid sample prior to performing the nucleic acid amplification reaction; and

- analysing the ratio to assess the transcriptional activity of the cell or organism, wherein:

a ratio of 1 indicates that the cell or organism is dead, and

a ratio of more than 1 indicates that the cell or organism is alive or may be alive.

~~Embodiment~~Example 8. The method of ~~embodiment~~example 7, wherein a ratio of more than: 1.5, 1.7, 1.9, 2, 2.2, 2.4, 2.5, 2.7, 2.9 or 3, indicates that the cell or organism is alive.

~~Embodiment~~Example 9. The method of ~~embodiment~~example 7, wherein a ratio of less than: 1.5, 1.4, 1.3, 1.2 or 1.1 indicates that the cell or organism is not alive.

~~Embodiment~~Example 10. A method for detecting transcriptional perturbation within a cell, the method comprising:

- performing a nucleic acid amplification reaction on total nucleic acid from the cell or organism, wherein the nucleic acid amplification comprises:

contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a first gene and RNA transcribed from the first gene,

contacting the total nucleic acid sample with one or multiple primers capable of amplifying a region of non-coding genomic DNA that is not transcribed in the cell or organism;

- analysing kinetics of the nucleic acid amplification reaction to derive a ratio of:

(i) said genomic DNA and RNA transcripts of the first gene, to

(ii) said region of non-coding DNA,

wherein the ratio is representative of the relative amount of (i) and (ii) present within the total nucleic acid sample prior to performing the nucleic acid amplification reaction; and

- analysing the ratio to assess the transcriptional activity of the cell or organism, wherein:

a ratio of 1 indicates complete transcriptional perturbation in the cell or organism, and

a ratio of more than 1 indicates a lack of transcriptional perturbation in the cell or organism.

~~Embodiment~~Example 11. The method of ~~embodiment~~example 10, wherein a ratio of more than: 1.5, 1.7, 1.9, 2, 2.2, 2.4, 2.5, 2.7, 2.9 or 3, indicates a lack of transcriptional perturbation in the cell or organism.

~~Embodiment~~Example 12. The method of ~~embodiment~~example 10, wherein a ratio of less than: 1.5, 1.4, 1.3, 1.2 or 1.1 indicates partial or complete transcriptional perturbation in the cell or organism.

~~Embodiment~~Example 13. The method of any one of ~~embodiment~~examples 1 to 12, comprising generating and a cycle threshold (Ct) value for:

(i) said region of non-coding DNA, and

(ii) said genomic DNA and RNA transcripts of the first gene;

during said nucleic acid amplification reaction and deriving the ratio by comparing said values.

~~Embodiment~~Example 14. The method of any one of ~~embodiment~~examples 1 to 13, wherein the method comprises:

- generating a cycle threshold value ctA for said genomic DNA and RNA transcripts of the first gene, and, generating a cycle threshold value CtB for said region of non-coding DNA, following commencement of the nucleic acid amplification reaction;
- calculating a fold change between the CtB and ctA values using the equation:

$2^{CtB - CtA} = 2^{\Delta Ct}$; and

- generating the ratio using the equation:

$2^{\Delta Ct}/TR$

wherein TR is a ratio of copies of the genomic DNA of the first gene to copies of the region of non-coding genomic DNA in the total nucleic acid sample of the cell.

~~Embodiment~~Example 15. The method of any one of ~~embodiment~~examples 1 to 14, wherein:

- performing the nucleic acid amplification reaction further comprises contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a second gene and RNA transcribed from the second gene, and
- the kinetics of the nucleic acid amplification reaction are analysed to derive a ratio representative of the relative amount of:

(i) said region of non-coding DNA, to

(ii) said genomic DNA and RNA transcripts of the first and second genes present within the total nucleic acid sample prior to performing the nucleic acid amplification reaction.

~~Embodiment~~Example 16. The method of ~~embodiment~~example 15, wherein the method comprises:

- generating a cycle threshold value ctA for said genomic DNA and RNA transcripts of the first and second genes, and, generating a cycle threshold value CtB for said region of non-coding DNA, following commencement of the nucleic acid amplification reaction;
- calculating a fold change between the CtB and ctA values using the equation:

$2^{CtB - CtA} = 2^{\Delta Ct}$

- generating the ratio using the equation

$2^{\Delta Ct}/TR$

wherein TR is a ratio of copies of the genomic DNA of the first and second genes to copies of the region of non-coding genomic DNA in the total nucleic acid sample of the cell.

~~Embodiment~~Example 17. The method of any one of ~~embodiment~~examples 1 to 16, wherein said analysing the ratio comprises comparing the ratio to a threshold ratio derived from performing the method on a series of negative or positive control cells or organisms and deriving a mean threshold ratio value indicative of a phenotype.

~~Embodiment~~Example 18. A method for detecting drug resistance or drug sensitivity in a cell or organism, the method comprising:

- performing a first nucleic acid amplification reaction on total nucleic acids obtained from a first sample of the cell or organism that has not been treated with the drug; and
- performing a second nucleic acid amplification reaction on total nucleic acids obtained from a second sample of the cell or organism that has been treated with the drug;

wherein each said nucleic acid amplification reaction is performed separately and comprises:

contacting the total nucleic acid sample with one or multiple primers capable of amplifying genomic DNA from a first gene and RNA transcribed from the first gene,

contacting the total nucleic acid sample with one or multiple primers capable of amplifying a region of non-coding genomic DNA that is not transcribed in the cell or organism;

- analysing kinetics of each said nucleic acid amplification reaction to derive first and second ratios of:

(i) said genomic DNA and RNA transcripts of the first gene, to

(ii) said region of non-coding DNA,

wherein the ratio is representative of the relative amount of (i) and (ii) present in the total nucleic acid sample prior to performing the nucleic acid amplification reaction; and

- comparing the ratios to assess whether the cell or organism has resistance to the drug, wherein:

complete drug resistance in the cell or organism is indicated when the first and second ratios are equal, and

sensitivity of the cell or organism to the drug is indicated when the second ratio is lower than the first ratio.

~~Embodiment~~Example 19. The method of ~~embodiment~~example 18, wherein the cell or organism is deemed to have resistance to the drug when the value of second ratio is no more than: 1%, 2%, 5%, 7.5%, 10%, 12%, 15% or 20%; lower than the value of first ratio.

~~Embodiment~~Example 20. The method of ~~embodiment~~example 18, wherein the cell or organism is deemed to be sensitive to the drug when the value of second ratio is at least: 10%, 20%, 30%, 40% or 50%; than the first ratio.

~~Embodiment~~Example 21. The method of any one of ~~embodiment~~examples 1 to 20, wherein the amplification method is selected from the group consisting of: polymerase chain reaction (PCR), strand displacement amplification (SDA), helicase dependent amplification (HDA), Recombinase Polymerase Amplification (RPA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), transcription-mediated

amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), reverse transcription polymerase chain reaction (RT-PCR), and any combination thereof.

~~Embodiment~~Example 22. The method of any one of ~~embodiment~~examples 1 to 21, wherein the cell is a mammalian cell, a human cell, a plant cell, a bacterial cell, a host cell infected by viruses, or a host cell of infected by bacteria.

~~Embodiment~~Example 23. The method of any one of ~~embodiment~~examples 1 to 21, wherein the organism is a bacterium, a virus, a fungi, an algae, an archaeon or a protozoan.

The present ~~invention~~disclosure is generally compatible with current procedures used in research or diagnostic laboratories and can use the same sample types, extraction methods and amplification methods. The nucleic acids (e.g. TNA) to be analysed may be a derived from any suitable source such as, for example, prokaryotic or eukaryotic cells, or from a virus. The present ~~invention~~disclosure can determine the presence or absence of active transcription, an increase or decrease in RNA expression levels or unchanged RNA expression levels.

## *Brief Description of the Drawings*

Embodiments of the present invention will now be described, by way of non-limiting example only, with reference to the accompanying Figures 1-12 as set out below.

**Figure 1: Schematic showing steps in VITA PCR.** A Reverse Transcriptase PCR (RT-PCR) is performed using total nucleic acid as template. This VITA PCR assay has at least one set of primers which is capable of co-amplifying the DNA of a specific gene (DNA-X) together with the RNA transcribed from this gene (RNA-X). It also contains a set of primers targeting a region of DNA which is not transcribed into RNA (DNA-N). During the reverse transcriptase step the RNA-X is copied into cDNA-X and then during PCR thermocycling DNA-X, cDNA-X and DNA-N are all amplified. The signal from the GAT (Gene And Transcript), which measures the sum of amplicons originating from DNA-X plus RNA-X can be read through a single Fluorophore Channel A, whilst the signal from the NED (Non-Expressed DNA), which is a measure of amplicons from DNA-N, can be read through a second Fluorophore Channel B. The difference in Cycle threshold (Ct) values between GAT and NED in a specimen allows an estimate of the fold change (FC) in a specimen observed for these targets, wherein $FC = 2^{Ct\ NED - Ct\ GAT} = 2^{\Delta Ct}$. The VITA Index can be calculated by dividing the fold change observed in the sample by the theoretical ratio (TR) anticipated in the absence of active transcription, assuming the gene and NED locus are present in equal copy numbers. If one transcript & gene are used to measure the GAT and one DNA sequence is used to measure NED, then the TR = (1 x