

CLAIMS

1. A molecular detection assay comprising:
treating a biological sample directly with a bisulphite agent under conditions that allow cell disruption and nucleic acid treatment;
5 removing the bisulphite agent from the treated sample; and
detecting a target nucleic acid in the treated sample.
2. An assay according to Claim 1, in which the biological sample is selected from faecal, nasal, blood, plasma, serum, buccal cells, pus, wound, concentrated filtrates, cerebrospinal fluid, semen, liquid based cytology (LBC), tissue, FFPE, laser captured cells, cultured cells,
10 pelleted cells, bacterial cultures, bacterial colonies, viral suspension, aspirate, bronchial lavage, sputum sample, environmental sample, environmental concentrate, foodstuff, raw ingredient, water sample or water concentrate.
3. An assay according to Claim 1 or 2 for detecting infectious disease, genetic disease or genetic trait in an animal.
- 15 4. An assay according to Claim 3 in which the infectious disease is caused by a microorganism.
5. An assay according to Claim 4 in which the microorganism is bacterium, virus, viroid, yeast, fungi, prion, parasites, or amoeba.
6. An assay according to Claim 2 in which the genetic disease is cancer, a disease
20 associated with copy number variation, inherited disorder, environmental induced disease, disease caused by exposure to carcinogens, disease characterised by expansion or reduction in a nucleotide repeat length.
7. An assay according to any of Claims 1 to 6 in which the bisulphite agent is sodium bisulphite or sodium metabisulphite.
- 25 8. An assay according to Claim 7 in which the bisulphite agent is used at a concentration of 2.5M to 3.5M having a pH range of 4.5 to 5.5.
9. An assay according to Claim 8 in which the bisulphite agent is used at a concentration of 3M at a pH of 5.0.

10. An assay according to any of Claims 1 to 9 in which the treating step involves heating between 75oC and 95oC for 1 to 30 minutes.
11. An assay according to Claim 10 in which the sample is heated for 10 to 20 minutes at a temperature of between 80oC and 95oC.
- 5 12. An assay according to any of Claims 1 to 11 in which the bisulphite is removed by column based purification, bead based purification or precipitation.
13. An assay according to any of Claims 1 to 12 in which after removal of the bisulphite, the treated sample is resuspended in an elution buffer having a pH of at least 10.
14. An assay according to Claim 13 in which the elution buffer has a pH of 11.5 to 12.5.
- 10 15. An assay according to Claim 14 in which the elution buffer has pH of at least 12.
16. An assay according to any of Claims 1 to 15 further including providing nucleic acid primers or probes to the treated sample.
17. An assay according to Claim 16 in which the target nucleic acid molecule is detected by amplification.
- 15 18. An assay according to claim 17 in which the amplification is PCR, qPCR, reverse transcription PCR, digital PCR, isothermal amplification or signal amplification.
19. An assay according to any of Claims 1 to 18 where the target nucleic acid is detected by sequencing the treated material.

CLAIMS

1. A molecular detection assay comprising:

treating a biological sample directly with a bisulphite agent under conditions that allow cell disruption and nucleic acid treatment;

5 removing the bisulphite agent from the treated sample; and

detecting a target nucleic acid in the treated sample,

in which the assay is carried out without pre-treatment of the biological sample prior to treating with the bisulphite agent.
2. An assay according to Claim 1, in which the biological sample is selected from faecal,

10 nasal, blood, plasma, serum, buccal cells, pus, wound, concentrated filtrates, cerebrospinal fluid, semen, liquid based cytology (LBC), tissue, FFPE, laser captured cells, cultured cells, pelleted cells, bacterial cultures, bacterial colonies, viral suspension, aspirate, bronchial lavage, sputum sample, environmental sample, environmental concentrate, foodstuff, raw ingredient, water sample or water concentrate.
- 15 3. An assay according to Claim 1 or 2 for detecting infectious disease, genetic disease or genetic trait in an animal.
4. An assay according to Claim 3 in which the infectious disease is caused by a microorganism.
5. An assay according to Claim 4 in which the microorganism is bacterium, virus, viroid,

20 yeast, fungi, prion, parasites, or amoeba.
6. An assay according to Claim 2 in which the genetic disease is cancer, a disease associated with copy number variation, inherited disorder, environmental induced disease, disease caused by exposure to carcinogens, disease characterised by expansion or reduction in a nucleotide repeat length.
- 25 7. An assay according to any of Claims 1 to 6 in which the bisulphite agent is sodium bisulphite or sodium metabisulphite.
8. An assay according to Claim 7 in which the bisulphite agent is used at a concentration of 2.5M to 3.5M having a pH range of 4.5 to 5.5.

9. An assay according to Claim 8 in which the bisulphite agent is used at a concentration of 3M at a pH of 5.0.
10. An assay according to any of Claims 1 to 9 in which the treating step involves heating between 75°C and 95°C for 1 to 30 minutes.
- 5 11. An assay according to Claim 10 in which the sample is heated for 10 to 20 minutes at a temperature of between 80°C and 95°C.
12. An assay according to any of Claims 1 to 11 in which the bisulphite is removed by column based purification, bead based purification or precipitation.
13. An assay according to any of Claims 1 to 12 in which after removal of the bisulphite, the
10 treated sample is resuspended in an elution buffer having a pH of at least 10.
14. An assay according to Claim 13 in which the elution buffer has a pH of 11.5 to 12.5.
15. An assay according to Claim 14 in which the elution buffer has pH of at least 12.
16. An assay according to any of Claims 1 to 15 further including providing nucleic acid primers or probes to the treated sample.
- 15 17. An assay according to Claim 16 in which the target nucleic acid molecule is detected by amplification.
18. An assay according to Claim 17 in which the amplification is PCR, qPCR, reverse transcription PCR, digital PCR, isothermal amplification or signal amplification.
19. An assay according to any of Claims 1 to 18 where the target nucleic acid is detected by
20 sequencing the treated material.