

CLAIMS

1. A method of detecting a nucleic acid cleavage contained
in a single cell, comprising the following steps (1) through
5 (5):

(1) a step of arranging one or more cells on a support
having an amino group on the surface thereof;

(2) a step of covering the cell or cells with a
mixture of purified proteolytic enzyme having a weakly
10 alkaline optimum pH value and a weakly acidic solution
containing a gelling agent;

(3) a step of solidifying the weakly acidic solution;

(4) a step of immersing the support carrying thereon
the solidified weakly acidic solution into a weakly alkaline
15 solution containing a condensed phosphoric acid compound, a
surface active agent and a reducing agent; and

(5) a step of separating the nucleic acid derived from
the cell or cells on the support immersed in the weakly
alkaline solution by means of two-dimensional pulse-field gel
20 electrophoresis using a solution for electrophoresis
containing a condensed phosphoric acid compound,
wherein the two-dimensional pulsed field gel electrophoresis
is achieved by using an electrophoresis apparatus having two
or more pairs of electrodes, the two or more pairs of
25 electrodes, being arranged so as to intersect each other at an
angle between 45 degrees and 120 degrees, and by executing the
first pulsed field gel electrophoresis, turning the support by
60 to 160 degrees around the cell arrangement portion thereof,
using the cell arrangement portion as center of revolution,

and executing the second pulsed field gel electrophoresis.

2. A method of manufacturing a cell that is free from nucleic acid cleavage, comprising the following steps (1) through (6):

5 (1) a step of arranging one or more cells found in a test sample on a support having an amino group on the surface thereof;

(2) a step of covering the cell or cells with a mixture of purified proteolytic enzyme having a weakly
10 alkaline optimum pH value and a weakly acidic solution containing a gelling agent;

(3) a step of solidifying the weakly acidic solution;

(4) a step of immersing the support carrying thereon the solidified weakly acidic solution into a weakly alkaline
15 solution containing a condensed phosphoric acid compound, a surface active agent and a reducing agent;

(5) a step of separating the nucleic acid derived from the cell or cells on the support immersed in the weakly alkaline solution by means of two-dimensional pulse-field gel
20 electrophoresis using a solution for electrophoresis containing a condensed phosphoric acid compound, wherein the two-dimensional pulsed field gel electrophoresis is achieved by using an electrophoresis apparatus having two or more pairs of electrodes, the two or more pairs of
25 electrodes, being arranged so as to intersect each other at an angle between 45 degrees and 120 degrees, and by executing the first pulsed field gel electrophoresis, turning the support by 60 to 160 degrees around the cell arrangement portion thereof, using the cell arrangement portion as center of revolution,

and executing the second pulsed field gel electrophoresis; and

(6) a step of determining the cell or cells in the test sample as cell or cells that are free from nucleic acid cleavage when the mode of separation of the nucleic acid that has been subjected to electrophoresis is substantially similar to the mode of separation of the nucleic acid derived from a normal cell or cells.

3. The method according to claim 1 or 2, wherein the purified proteolytic enzyme having a weakly alkaline optimum pH value is active for dissociation of the peptide bond formed between arginine and arbitrary amino acid and/or the peptide bond between lysine and arbitrary amino acid.

4. The method according to claim 1 or 2, wherein the purified proteolytic enzyme having a weakly alkaline optimum pH value is a purified trypsin-like enzyme.

5. The method according to claim 1 or 2, wherein the condensed phosphoric acid compound is at least a phosphoric acid compound selected from a group consisting of polyphosphoric acid, hexametaphosphoric acid and salts thereof.

6. The method according to claim 1 or 2, wherein the cell or cells are sperm cell or sperm cells.

7. A kit for using the method according to claim 1 or 2, comprising the following components (1) through (6):

(1) a support having an amino group on the surface thereof;

(2) purified proteolytic enzyme having a weakly alkaline optimum pH value;

(3) a weakly acidic solution containing a gelling

agent;

(4) a weakly alkaline solution containing a condensed phosphoric acid compound, a surface active agent and a reducing agent;

5 (5) a solution for electrophoresis containing a condensed phosphoric acid compound; and

(6) an electrophoresis apparatus having two or more pairs of electrodes, the two or more pairs of electrodes being arranged so as to intersect each other at an angle between 45
10 degrees and 120 degrees.

CLAIMS

1. A method of detecting a nucleic acid cleavage contained in a single cell, comprising the following steps (1) through (5):

5 (1) a step of arranging one or more cells on a support having an amino group on the surface thereof;

(2) a step of covering the cell or cells with a mixture of purified proteolytic enzyme having a weakly alkaline optimum pH value and a weakly acidic solution containing a gelling agent;

10 (3) a step of solidifying the weakly acidic solution;

(4) a step of immersing the support carrying thereon the solidified weakly acidic solution into a weakly alkaline solution containing a condensed phosphoric acid compound, a surface active agent and a reducing agent; and

15 (5) a step of separating the nucleic acid derived from the cell or cells on the support immersed in the weakly alkaline solution by means of two-dimensional pulse-field gel electrophoresis using a solution for electrophoresis containing a condensed phosphoric acid compound.

20 2. A method of manufacturing a cell that is free from nucleic acid cleavage, comprising the following steps (1) through (6):

(1) a step of arranging one or more cells found in a test sample on a support having an amino group on the surface thereof;

25 (2) a step of covering the cell or cells with a mixture of purified proteolytic enzyme having a weakly alkaline optimum pH value and a weakly acidic solution containing a gelling agent;

(3) a step of solidifying the weakly acidic solution;

(4) a step of immersing the support carrying thereon the

~~solidified weakly acidic solution into a weakly alkaline solution~~

containing a condensed phosphoric acid compound, a surface active agent and a reducing agent;

(5) a step of separating the nucleic acid derived from the cell or cells on the support immersed in the weakly alkaline solution
5 by means of two-dimensional pulse-field gel electrophoresis using a solution for electrophoresis containing a condensed phosphoric acid compound; and

(6) a step of determining the cell or cells in the test sample as cell or cells that are free from nucleic acid cleavage
10 when the mode of separation of the nucleic acid that has been subjected to electrophoresis is substantially similar to the mode of separation of the nucleic acid derived from a normal cell or cells.

3. The method according to claim 1 or 2, wherein the purified
15 proteolytic enzyme having a weakly alkaline optimum pH value is active for dissociation of the peptide bond formed between arginine and arbitrary amino acid and/or the peptide bond between lysine and arbitrary amino acid.

4. The method according to claim 1 or 2, wherein the purified
20 proteolytic enzyme having a weakly alkaline optimum pH value is a purified trypsin-like enzyme.

5. The method according to claim 1 or 2, wherein the condensed phosphoric acid compound is at least a phosphoric acid compound selected from a group consisting of polyphosphoric acid,
25 hexametaphosphoric acid and salts thereof.

6. The method according to claim 1 or 2, wherein the two-dimensional pulsed field gel electrophoresis is achieved by an electrophoresis apparatus having two or more pairs of electrodes,
~~the two or more pairs of electrodes, being arranged so as to~~

intersect each other at an angle between 45 degrees and 120 degrees.

7. The method according to claim 1 or 2, wherein the two-dimensional pulsed field gel electrophoresis is achieved by executing the first pulsed field gel electrophoresis, turning the support by 60 to 160 degrees around the cell arrangement portion thereof, using the cell arrangement portion as center of revolution, and executing the second pulsed field gel electrophoresis.

8. The method according to claim 1 or 2, wherein the cell or cells are sperm cell or sperm cells.

9. A kit for detecting nucleic acid cleavage or for manufacturing a cell with cleavage-free nucleic acid, comprising the following components (1) through (6):

(1) a support having an amino group on the surface thereof;

(2) purified proteolytic enzyme having a weakly alkaline optimum pH value;

(3) a weakly acidic solution containing a gelling agent;

(4) a weakly alkaline solution containing a condensed phosphoric acid compound, a surface active agent and a reducing agent;

(5) a solution for electrophoresis containing a condensed phosphoric acid compound; and

(6) an electrophoresis apparatus having two or more pairs of electrodes, the two or more pairs of electrodes being arranged so as to intersect each other at an angle between 45 degrees and

120 degrees.