

## CLAIMS

1. A method of determining a risk of gastrointestinal cancer in a human subject, the method comprising:

5 determining in a biological sample of the subject, whether mutation is present in at least one of the CTCF-binding sites (CBS) overlapping regions set forth in the table 1 below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 6          | 50570094   | 50570120  |
| 8          | 71000992   | 71001012  |
| 1          | 209422184  | 209422222 |
| 2          | 49173770   | 49173816  |
| 4          | 182064578  | 182064613 |
| X          | 104435106  | 104435140 |
| 14         | 70285576   | 70285601  |
| 6          | 73122084   | 73122123  |
| 8          | 70576141   | 70576184  |
| 13         | 36552821   | 36552860  |
| 3          | 164903700  | 164903728 |

or portions or flanking sequences thereof,

and/or at least one of the non-CBS regions set forth in the table 2 below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 7          | 68391104   | 68391132  |
| 7          | 136495924  | 136495948 |
| 2          | 57627616   | 57627640  |
| 16         | 8381278    | 8381302   |
| 5          | 23824204   | 23824224  |
| 7          | 67614923   | 67614943  |
| 8          | 65161396   | 65161420  |
| 7          | 4937707    | 4937736   |

|    |           |           |
|----|-----------|-----------|
| 12 | 126996666 | 126996686 |
| 4  | 5415060   | 5415082   |
| X  | 137405623 | 137405655 |
| 3  | 171164993 | 171165017 |
| 4  | 144748744 | 144748764 |
| 9  | 25481736  | 25481758  |
| 2  | 77150455  | 77150477  |
| 3  | 104801455 | 104801477 |
| X  | 125548690 | 125548710 |
| 14 | 83046706  | 83046744  |

or portions or flanking sequences thereof,

wherein presence of mutation in at least one of the CBS overlapping regions, or portions or flanking sequences thereof, and/or at least one of the non-CBS regions, or portions or flanking sequences thereof, is indicative of a risk of gastrointestinal cancer in the subject.

2. The method of claim 1, the method comprising:

determining in the biological sample of the subject, whether mutation is present in at least one of the CTCF-binding sites (CBS) overlapping regions set forth in the table below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 6          | 50570094   | 50570120  |
| 8          | 71000992   | 71001012  |
| 1          | 209422184  | 209422222 |
| 2          | 49173770   | 49173816  |
| 4          | 182064578  | 182064613 |
| X          | 104435106  | 104435140 |
| 14         | 70285576   | 70285601  |
| 6          | 73122084   | 73122123  |
| 8          | 70576141   | 70576184  |
| 13         | 36552821   | 36552860  |

|   |           |           |
|---|-----------|-----------|
| 3 | 164903700 | 164903728 |
|---|-----------|-----------|

or portions or flanking sequences thereof.

3. The method of claim 2, the method further comprising determining in the biological sample of the subject, whether mutation is present in at least one of the non-CBS regions set forth in the table below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 7          | 68391104   | 68391132  |
| 7          | 136495924  | 136495948 |
| 2          | 57627616   | 57627640  |
| 16         | 8381278    | 8381302   |
| 5          | 23824204   | 23824224  |
| 7          | 67614923   | 67614943  |
| 8          | 65161396   | 65161420  |
| 7          | 4937707    | 4937736   |
| 12         | 126996666  | 126996686 |
| 4          | 5415060    | 5415082   |
| X          | 137405623  | 137405655 |
| 3          | 171164993  | 171165017 |
| 4          | 144748744  | 144748764 |
| 9          | 25481736   | 25481758  |
| 2          | 77150455   | 77150477  |
| 3          | 104801455  | 104801477 |
| X          | 125548690  | 125548710 |
| 14         | 83046706   | 83046744  |

or portions or flanking sequences thereof.

4. The method of any of claims 1-3, further comprising determining in the biological sample of the subject, whether mutation is present in at least one of the genes selected from the group consisting of: *KRAS*, *TP53*, *RHOA*, *ARID1A*, *PIK3CA*, *ERBB3* and *PTEN*, wherein presence of

mutation in at least one of the CBS overlapping regions, or portions or flanking sequences thereof, at least one of the non-CBS regions, or portions or flanking sequences thereof, and/or at least one of the genes is indicative of a risk of gastrointestinal cancer in the subject.

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5. The method of any of claims 1-4, wherein determining whether mutation is present in the CBS overlapping regions, or portions or flanking sequences thereof, and/or the non-CBS regions, or portions or flanking sequences thereof, does not comprise determining whether mutation is present in a region spanning more than 50 nucleotides.

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6. The method of any of claims 1-5, wherein determining whether mutation is present in at least one of the CBS overlapping regions, or portions or flanking sequences thereof, comprises contacting the biological sample with a primer having at least about 85% sequence identity to a sequence selected from the group consisting of: SEQ ID NO. 35, SEQ ID NO. 36, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 45, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52, SEQ ID NO. 57, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 60, SEQ ID NO. 65, SEQ ID NO. 66, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. 83, SEQ ID NO. 84 and combinations thereof and/or wherein determining whether mutation is present in at least one of the non-CBS regions, or portions or flanking sequences thereof, comprises contacting the biological sample with a primer having at least about 85% sequence identity to a sequence selected from the group consisting of: SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 56, SEQ ID NO. 61, SEQ ID NO. 62, SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 71, SEQ ID NO. 72, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 85, SEQ ID NO. 86, SEQ ID

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NO. 87, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91, SEQ ID NO. 92, and combinations thereof.

- 5 7. The method of any of claims 1-6, wherein the method comprises determining whether mutation is present in at least two of the CBS overlapping regions, or portions or flanking sequences thereof, set forth in the table 1, optionally wherein the method comprises determining whether mutation is present in all of the CBS overlapping regions, or portions or flanking sequences thereof, set forth in the table  
10 1, further optionally wherein the method comprises determining whether mutation is present in all of the CBS overlapping regions, or portions or flanking sequences thereof, and all of the non-CBS regions, or portions or flanking sequences thereof, set forth in the tables 1 and 2.
- 15 8. The method of any of claims 1-7, wherein the method has a detection sensitivity of no less than about 50%.
- 20 9. The method of any of claims 1-8, wherein if the biological sample shows a presence of mutation in at least one of the CBS overlapping regions, or portions or flanking sequences thereof, and/or at least one of the non-CBS regions, or portions or flanking sequences thereof, and optionally a mutation is present in at least one of the genes selected from the group consisting of: *KRAS*, *TP53*, *RHOA*, *ARID1A*, *PIK3CA*, *ERBB3*  
25 *and PTEN*, the subject is to be administered a therapeutic agent for treating gastrointestinal cancer.
- 30 10. The method of claim 9, wherein the therapeutic agent is selected from the group consisting of chemotherapy, radiation therapy, immunotherapy and combinations thereof.
11. The method of any of claims 1-10, wherein the biological sample is a fluid biological sample.

12. The method of claim 11, wherein the fluid biological sample is selected from the group consisting of: blood, plasma, serum and combinations thereof.

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13. The method of any of claims 1-12, wherein the gastrointestinal cancer is selected from the group consisting of gastric cancer, colorectal cancer, colon cancer and rectal cancer.

- 10 14. A kit for detecting gastrointestinal cancer in a human subject, the kit comprising:

an agent for detecting mutation in at least one of the CBS overlapping regions set forth in the table 1 below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 6          | 50570094   | 50570120  |
| 8          | 71000992   | 71001012  |
| 1          | 209422184  | 209422222 |
| 2          | 49173770   | 49173816  |
| 4          | 182064578  | 182064613 |
| X          | 104435106  | 104435140 |
| 14         | 70285576   | 70285601  |
| 6          | 73122084   | 73122123  |
| 8          | 70576141   | 70576184  |
| 13         | 36552821   | 36552860  |
| 3          | 164903700  | 164903728 |

or portions or flanking sequences thereof,

15

and/or in at least one of the non-CBS regions set forth in the table 2 below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 7          | 68391104   | 68391132  |
| 7          | 136495924  | 136495948 |
| 2          | 57627616   | 57627640  |

|    |           |           |
|----|-----------|-----------|
| 16 | 8381278   | 8381302   |
| 5  | 23824204  | 23824224  |
| 7  | 67614923  | 67614943  |
| 8  | 65161396  | 65161420  |
| 7  | 4937707   | 4937736   |
| 12 | 126996666 | 126996686 |
| 4  | 5415060   | 5415082   |
| X  | 137405623 | 137405655 |
| 3  | 171164993 | 171165017 |
| 4  | 144748744 | 144748764 |
| 9  | 25481736  | 25481758  |
| 2  | 77150455  | 77150477  |
| 3  | 104801455 | 104801477 |
| X  | 125548690 | 125548710 |
| 14 | 83046706  | 83046744  |

or portions or flanking sequences thereof.

15. The kit of claim 14, the kit comprising:

an agent for detecting mutation in at least one of the CBS

- 5 overlapping regions set forth in the table below:

| <b>Chromosome</b> | <b>Start site</b> | <b>End site</b> |
|-------------------|-------------------|-----------------|
| 6                 | 50570094          | 50570120        |
| 8                 | 71000992          | 71001012        |
| 1                 | 209422184         | 209422222       |
| 2                 | 49173770          | 49173816        |
| 4                 | 182064578         | 182064613       |
| X                 | 104435106         | 104435140       |
| 14                | 70285576          | 70285601        |
| 6                 | 73122084          | 73122123        |
| 8                 | 70576141          | 70576184        |
| 13                | 36552821          | 36552860        |

|   |           |           |
|---|-----------|-----------|
| 3 | 164903700 | 164903728 |
|---|-----------|-----------|

or portions or flanking sequences thereof.

16. The kit of claim 15, further comprising an agent for detecting mutation in at least one of the non-CBS regions set forth in the table below:

| Chromosome | Start site | End site  |
|------------|------------|-----------|
| 7          | 68391104   | 68391132  |
| 7          | 136495924  | 136495948 |
| 2          | 57627616   | 57627640  |
| 16         | 8381278    | 8381302   |
| 5          | 23824204   | 23824224  |
| 7          | 67614923   | 67614943  |
| 8          | 65161396   | 65161420  |
| 7          | 4937707    | 4937736   |
| 12         | 126996666  | 126996686 |
| 4          | 5415060    | 5415082   |
| X          | 137405623  | 137405655 |
| 3          | 171164993  | 171165017 |
| 4          | 144748744  | 144748764 |
| 9          | 25481736   | 25481758  |
| 2          | 77150455   | 77150477  |
| 3          | 104801455  | 104801477 |
| X          | 125548690  | 125548710 |
| 14         | 83046706   | 83046744  |

or portions or flanking sequences thereof.

17. The kit of any of claims 14-16, further comprising an agent for detecting mutation in at least one of the genes selected from the group consisting of: *KRAS*, *TP53*, *RHOA*, *ARID1A*, *PIK3CA*, *ERBB3*, *PTEN*.

18. The kit of any of claims 14-17, wherein the agent for detecting mutation in at least one of the CBS overlapping regions, or portions or flanking sequences thereof, comprises a primer having at least about 85% sequence identity to a sequence selected from the group consisting of:  
5 SEQ ID NO. 35, SEQ ID NO. 36, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 45, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52, SEQ ID NO. 57, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 60, SEQ ID NO. 65, SEQ ID NO. 66, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. SEQ ID  
10 NO. 83 and SEQ ID NO. 84 and/or wherein the agent for detecting mutation in at least one of the non-CBS regions, or portions or flanking sequences thereof, comprises a primer having at least about 85% sequence identity to a sequence selected from the group consisting of:  
15 SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 56, SEQ ID NO. 61, SEQ ID NO. 62, SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 71, SEQ ID NO. 72, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 85, SEQ ID NO. 86,  
20 SEQ ID NO. 87, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91 and SEQ ID NO. 92.
19. The kit of any of claims 14-18, the kit comprising agents for detecting mutation in at least two of the CBS overlapping regions, or portions or  
25 flanking sequences thereof, set forth in the table 1, optionally wherein the kit comprises agents for detecting mutation in all of the CBS overlapping regions, or portions or flanking sequences thereof, set forth in the table 1, further optionally wherein the kit comprises agents for detecting mutation in all of the CBS overlapping regions, or portions  
30 or flanking sequences thereof, and all of the non-CBS regions, or portions or flanking sequences thereof, set forth in the tables 1 and 2.

20. The method or the kit of any of claims 1-19, wherein the human subject is an Asian subject.