

We claim:

1. A method for producing a nucleic acid probe, comprising:

generating at least a first binding region and a second binding region, wherein generating the first

binding region and the second binding region comprises:

(a) separating the genomic target nucleic acid sequence into a plurality of segments;

(b) comparing each segment with a genome comprising the genomic target nucleic acid molecule; and

(c) selecting at least two segments which are uniquely specific to the genomic target nucleic acid molecule, wherein the uniquely specific segments are represented only once in ~~the~~ a haploid genome of an organism comprising the genomic target nucleic acid molecule, which segments are the at least first binding region and second binding region, and wherein the first binding region and second binding region comprise ~~about~~ 20% or less of a genomic target nucleic acid molecule; and

joining the at least first binding region and second binding region in a pre-determined order and orientation, thereby producing the nucleic acid probe,

wherein the pre-determined order and orientation of the at least first binding region and second binding region is generated by:

(a) ordering the at least first binding region and second binding region to produce at least one candidate nucleic acid probe;

(b) separating the candidate nucleic acid probe into a plurality of segments;

(c) comparing each segment of the candidate nucleic acid probe with the genome comprising the genomic target nucleic acid molecule; and

(d) selecting at least one order and orientation of the selected segments that is uniquely specific to the genomic target nucleic acid molecule, which is pre-determined order and orientation.

2. The method of claim 1, wherein generating the first binding region and the second binding region further comprises:

(d) synthesizing the at least two selected uniquely specific segments;

(e) hybridizing the synthesized segments with genomic DNA comprising the genomic target nucleic acid molecule;

(f) detecting presence or absence of hybridization of the genomic DNA with the synthesized segments; and

(g) selecting one or more segments which have an absence of hybridization with the genomic DNA for inclusion in the probe.

3. A method for producing a nucleic acid probe, comprising:

generating at least a first binding region and a second binding region, wherein generating the first binding region and the second binding region comprises:

(a) separating a genomic target nucleic acid sequence into a plurality of nucleic acid segments;

(b) synthesizing the plurality of nucleic acid segments;

(c) attaching the synthesized plurality of nucleic acid segments on an array;

(d) ~~hybridizing the synthesized plurality of nucleic acid segments array~~ with total genomic DNA and blocking DNA; and

(~~e~~) selecting at least two segments which are uniquely specific to the genomic target nucleic acid molecule, wherein the uniquely specific segments are represented only once in a genome of the genomic target nucleic acid molecule, which segments are the at least first binding region and second binding region, and wherein the first binding region and second binding region comprise ~~about~~ 20% or less of a genomic target nucleic acid molecule; and

joining the at least first binding region and second binding region in a pre-determined order and orientation, thereby producing the nucleic acid probe;

wherein the pre-determined order and orientation of the at least first binding region and second binding region is generated by:

(a) ordering the at least first binding region and second binding region to produce at least one candidate nucleic acid probe;

(b) separating the candidate nucleic acid probe into a plurality of segments;

(c) comparing each segment of the candidate nucleic acid probe with the genome comprising the genomic target nucleic acid molecule; and

(d) selecting at least one order and orientation of the selected segments that is uniquely specific to the genomic target nucleic acid molecule, which is pre-determined order and orientation.

4. The method of any one of claims 1 to 3, further comprising removing repetitive DNA sequences from the genomic target nucleic acid molecule to produce a substantially repeat-free genomic target nucleic acid sequence after selecting the at least two segments which are uniquely specific to the genomic target nucleic acid molecule.

5. The method of any one of claims 1 to 4, wherein separating the genomic target nucleic acid sequence into a plurality of nucleic acid segments is performed *in silico*.

6. The method of any one of claims 1 to 5, wherein the plurality of nucleic acid segments are ~~about~~ 20-500 nucleotides in length.

7. The method of any one of claims 1 to 6, wherein the plurality of nucleic acid segments overlap by at least 10 nucleotides.

8. The method of any one of claims 1 to 7, further comprising:

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determining a G/C nucleotide content of the plurality of segments; and

selecting at least two segments having G/C nucleotide content between about 30% and 70% after
selecting the at least two segments which are uniquely specific to the genomic target nucleic acid
molecule.

9. ~~The method of any one of claims 1 to 8, wherein the pre-determined order and orientation of the
at least first binding region and second binding region is generated by:~~

~~(a) ordering the at least first binding region and second binding region to produce at least one
candidate nucleic acid probe;~~

~~(b) separating the candidate nucleic acid probe into a plurality of segments;~~

~~(c) comparing each segment of the candidate nucleic acid probe with the genome comprising the
genomic target nucleic acid molecule; and~~

~~(d) selecting at least one order and orientation of the selected segments that is uniquely specific to
the genomic target nucleic acid molecule, which is pre-determined order and orientation.~~

10. ~~The method of claim 9, wherein the ordering of step (a) is the order and orientation of the at least
first binding region and second binding region of the genomic target nucleic acid.~~

11. The method of claim 1, wherein comparing each segment with the genome comprising the
genomic target nucleic acid molecule, selecting at least two segments which are uniquely specific to the
genomic target nucleic acid molecule, or both is performed *in silico*.

12. The method of any one of claims 1 to 11, wherein the uniquely specific nucleic acid sequences
comprise about 5% or less of the genomic target nucleic acid molecule.

13. The method of any one of claims 1 to 12, wherein joining the at least first binding region and
second binding region comprises oligonucleotide synthesis, an

enzymatic ligation reaction, a chemical ligation reaction, nucleic acid amplification, or a combination of two or more thereof.

5 ~~14~~12. The method of claim ~~13~~11, wherein the enzymatic ligation reaction comprises a ligase or a recombinase.

~~15. The method of any one of claims 1 to 14, wherein the nucleic acid probe hybridizes specifically to the genomic target nucleic acid molecule in the absence of a DNA blocking reagent.~~

10 ~~16~~13. The method of any one of claims 1 to ~~15~~12, further comprising labeling the nucleic acid probe.

~~17~~14. The method of claim ~~16~~13, wherein ~~labeling the nucleic acid probe uses~~ is labeled by nick translation.

15 ~~18~~15. The method of any one of claims 1 to ~~17~~14, wherein the genomic target nucleic acid molecule is from a eukaryotic genome.

~~19~~16. The method of claim ~~18~~15, wherein the eukaryotic genome is a human genome.

20 ~~20~~17. The method of any one of claims 1 to ~~19~~16, wherein the at least first binding region and second binding region are complementary to non-contiguous portions of the genomic target nucleic acid molecule.

25 ~~21~~18. The method of any one of claims 1 to ~~20~~17, wherein the nucleic acid probe comprises at least five binding regions.

~~22~~19. The method of claim ~~21~~18, wherein the nucleic acid probe comprises at least fifty binding regions.

2320. The method of any one of claims 1 to 2219, wherein the at least first binding region and second binding region are at least 50 nucleotides in length.

2421. The method of any one of claims 1 to 2320, ~~wherein further comprising including~~ the at least first binding region and second binding region ~~are included~~ in a vector.

2522. The method of claim 2421, wherein the vector is a plasmid.

2623. The method of claim 2 or claim 3, wherein hybridizing the synthesized nucleic acid segments comprises hybridizing an array of the synthesized nucleic acid segments with total genomic DNA and blocking DNA.

2724. The method of claim 2623, wherein the array further comprises at least one positive control, at least one negative control, or a combination thereof.

2825. The method of claim 2724, wherein the at least one positive control comprises a nucleic acid molecule comprising a sequence with a known copy number in the genome of the organism comprising the genomic target nucleic acid sequence, the negative control comprises a nucleic acid molecule comprising a randomized sequence or a genomic sequence from an organism unrelated to the organism comprising the genomic target nucleic acid sequence, or a combination thereof.

2926. The method of claim 2 or claim 3 or any one of claims 2623-2825, wherein selecting at least two segments which are uniquely specific comprises deriving a linear regression of hybridization scores of total genomic DNA and blocking DNA and selecting sequences falling within a predetermined cutoff.

3027. The method of claim 2926, wherein the predetermined cutoff comprises one or more of the linear regression of the positive control sequences decreased by one standard deviation, mean of the total genomic DNA score of the negative control sequences, or a selected distance from the origin of the mean of all sequences.

~~3128~~. An isolated nucleic acid probe generated using the method of any one of claims 1 to ~~3027~~, wherein the genomic target nucleic acid is from a eukaryotic genome.

5 ~~3229~~. A kit comprising one or more nucleic acid probes generated using the method of any one of claims 1 to ~~2421~~.