

unique molecular pathology. The human genome project laid the groundwork for genome-wide analysis of cancers.

For example, the introduction of next-generation sequencing technologies (2004-present) has accelerated the discovery pace of causal genomic factors that
5 underlie the diagnosis of NSCLC, making it clear that NSCLC is really a family of related diseases, each of which may be responsive to a different targeted therapy.

The art lacks reliable and robust molecular analysis methods for the analysis of genetic diseases. Traditionally, molecular diagnostics have consisted of antibody-based tests (immunohistochemistry), in-situ hybridization with DNA probes (fluorescence in
10 situ hybridization), and hybridization or PCR-based tests that query specific nucleotide sequences. Until recently, DNA sequencing as a molecular diagnostic tool has been generally limited to the coding exons of one or two genes. While DNA sequencing has been used in the diagnosis and treatment of solid cancers, one of the most significant drawbacks of these methods is that they require direct access to tumor tissues. Such
15 material is often difficult to obtain from the initial biopsy used to diagnose the disease and virtually impossible to obtain in multiple repetitions over time. Similarly, biopsies are not possible in patients with inaccessible tumors and not practical in individuals suffering from metastatic disease.

Thus, the vast potential of molecular diagnostics for genetic diseases; fetal
20 testing; paternity testing; predicting response to drug treatment; diagnosing or monitoring a medical condition; mendelian disorders; genetic mosaicism; pathogen screening; microbiome profiling; and organ transplant monitoring; has yet to be realized. To date, existing molecular diagnostics approaches lack efficient solutions to clone and amplify individual DNA molecules, as well as solutions to efficiently target
25 sequencing to specific genomic loci, with sensitivity sufficient to discriminate true positive test results from false positive signals that arise during sample processing.

BRIEF SUMMARY

[In one aspect, provided herein is a method for targeted genetic analysis of circulating cell-free DNA \(cfDNA\) comprising:](#)

(a) hybridizing one or more capture probe modules to a target genetic locus in a tagged circulating cfDNA library to form one or more capture probe module-circulating cfDNA clone complexes,

5 wherein the one or more capture probe modules hybridizes to a specific DNA target region wherein the capture probe modules comprise capture probes that are substantially smaller than 60 nucleotides, and wherein the tagged circulating cfDNA library comprises circulating cfDNA ligated at each end to an adaptor module;

(b) isolating the one or more isolated capture probe module-circulating cfDNA complexes from (a):

10 (c) enzymatically processing the one or more isolated capture probe module-circulating cfDNA complexes from (b), wherein the enzymatic processing comprises performing 5'-3' DNA polymerase extension of the capture probe using the isolated circulating cfDNA clone in the complex as a template;

15 (d) performing PCR on the enzymatically processed complex of (c) to generate amplified hybrid nucleic acid molecules, wherein the amplified hybrid nucleic acid molecules comprise a target sequence in the target genetic locus capable of hybridizing to the capture probe and the complement of the capture probe module tail sequence; and

20 (e) performing a quantitative genetic analysis of on the amplified hybrid nucleic acid molecules.

The invention relates generally to compositions and methods for improved compositions and methods for the genetic analysis of cfDNA.