

function of the gene in which they reside. The implication is that while normal diploid humans possess two functional copies of most genes, there are many instances in all humans where only one (or zero) functional gene copies are present. Similarly, instances where genes are overrepresented by gene duplication/amplification events are also encountered with significant frequency.

One of the key features in biological networks is functional redundancy. Normal, healthy individuals can tolerate the average load of genetic lesions because they possess, on average, two copies of every gene such that loss of one copy is inconsequential. Moreover, sets of genes often perform similar functions such that minor perturbations in specific gene functions are generally compensated for within a larger network of functional elements. While functional compensation in biological systems is a general theme, there are many instances in which specific gene loss can trigger acute disruptive events. By way of example, cancers appear to be the consequence of genetic disease in which the compound effect of multiple individual lesions is uncontrolled cell proliferation. Similarly, prescribed medicines are often specific chemical entities that are transported, metabolized and/or eliminated by very specific genes. Perturbations in these genes, while generally inconsequential under normal circumstances, can manifest as adverse events (*e.g.*, side effects) during chemical therapy.

The central aim of “personalized medicine”, increasingly referred to as “precision medicine,” is to merge genetic information that is specific to the patient with treatment options that are compatible with the individual’s genetic profile. However, the vast potential of personalized medicine has yet to be realized. To realize this goal, there must be clinically acceptable, robust genetic diagnostic tests that can reliably determine the genetic status of relevant genes.

SUMMARY

Particular embodiments contemplated herein provide a method for generating a tagged DNA library comprising treating fragmented DNA with end-repair enzymes to generate fragmented end-repaired DNA; and ligating a random nucleic acid tag

sequence, and optionally a sample code sequence and/or a PCR primer sequence to the fragmented end-repaired DNA to generate the tagged DNA library.

In particular embodiments, the random nucleic acid tag sequence is from about 2 to about 100 nucleotides. In some embodiments, the present disclosure provides that
5 the random nucleic acid tag sequence is from about 2 to about 8 nucleotides.

In certain embodiments, the fragmented end-repaired DNA contains blunt ends. In some embodiments, the blunt ends are further modified to contain a single base pair overhang.

In certain embodiments, the ligating comprises ligating a multifunctional
10 adaptor module to the fragmented end-repaired DNA to generate the tagged DNA library, wherein the multifunctional adaptor molecule comprises: i) a first region comprising a random nucleic acid tag sequence; ii) a second region comprising a sample code sequence; and iii) a third region comprising a PCR primer sequence.

In additional embodiments, the method further comprises hybridizing a tagged
15 DNA library with at least one multifunctional capture probe module to form a complex, wherein the multifunctional capture probe module hybridizes to a specific target region in the DNA library.

In further embodiments, the method further comprises isolating the tagged DNA library-multifunctional capture probe module complex.

20 In some embodiments, the method further comprises 3'-5' exonuclease enzymatic processing of the isolated tagged DNA library-multifunctional capture probe module complex to remove the single stranded 3' ends. In some embodiments, the enzyme for use in the 3'-5' exonuclease enzymatic processing is T4 polymerase.

In particular embodiments, the method further comprises 5'-3' DNA polymerase
25 extension of the isolated tagged DNA library-multifunctional capture probe module complex from the 3' end of the multifunctional capture probe utilizing the isolated tagged DNA library fragments as template.

In certain embodiments, the method further comprises joining of the multifunctional capture probe and isolated tagged DNA library fragments through the
30 concerted action of a 5' FLAP endonuclease, DNA polymerization and nick closure by a DNA ligase.

In additional embodiments, a hybrid tagged DNA library comprises hybrid tagged DNA sequences for use in targeted genetic analysis, wherein each hybrid tagged DNA sequence comprises: i) fragmented end-repaired DNA; ii) a random nucleotide tag sequence; iii) a sample code sequence; iv) a PCR primer sequence; and v) a multifunctional capture probe module tail sequence.

In further embodiments, a multifunctional adaptor module comprises: i) a first region comprising a random nucleotide tag sequence; ii) a second region comprising a sample code sequence; and iii) a third region comprising a PCR primer sequence.

In particular embodiments, a multifunctional capture probe module comprises: i) a first region capable of hybridizing to a partner oligonucleotide; ii) a second region capable of hybridizing to a specific target region; and iii) a third region comprising a tail sequence. In some embodiments, the first region of the capture probe module is bound to a partner oligonucleotide. In some embodiments, the partner oligonucleotide is chemically modified.

In one embodiment, compositions comprise a tagged DNA library, a multifunctional adaptor module and a multifunctional capture probe module.

In particular embodiments, a composition comprises a hybrid tagged genomic library according to the methods of the present disclosure.

In certain embodiments, a composition comprises a reaction mixture for performing the methods contemplated herein.

In particular embodiments, a reaction mixture capable of generating a tagged DNA library comprises: a) fragmented DNA and b) DNA end-repair enzymes to generate fragmented end-repaired DNA.

In certain embodiments, a reaction mixture further comprises a multifunctional adaptor module.

In additional embodiments, a reaction mixture further comprises a multifunctional capture probe module.

In some embodiments, a reaction mixture further comprises an enzyme with 3'-5' exonuclease activity and PCR amplification activity.

In one embodiment, the reaction mixture comprises a FLAP endonuclease, a DNA polymerase, and DNA ligase.

In further particular embodiments, the enzyme for use in the 3'-5' exonuclease enzymatic processing is T4 DNA polymerase.

5 In some particular embodiments, the methods contemplated herein comprise performing PCR on the 3'-5' exonuclease enzymatically processed complex from the preceding claims, wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence.

10 In various embodiments, a method for targeted genetic analysis is provided comprising: (a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library; (b) isolating the tagged genomic library-multifunctional capture probe module complex from a); (c)
15 performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends; (d) performing PCR on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic
20 acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; and (e) performing targeted genetic analysis on the hybrid nucleic acid molecule from d).

25 In one aspect, there is provided a method for targeted genetic analysis comprising:

(a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library, and wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor
30 molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;
(b) isolating the tagged genomic library-multifunctional capture probe module complex from a);
(c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with
35 3'-5' exonuclease activity to remove the single stranded 3' ends;
(d) performing PCR on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises
40 the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; and
(e) performing targeted genetic analysis on the hybrid nucleic acid molecule from d).

45 In particular embodiments, steps a) through d) are repeated at least about twice and the targeted genetic analysis of e) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

In further embodiments, at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

capture probe module complex from a); (c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends; (d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; (e) performing PCR amplification of the hybrid nucleic acid molecule in d); and (f) performing targeted genetic analysis on the hybrid nucleic acid molecule from e).

In another aspect, there is provided a method for determining copy number of a specific genomic target region comprising:

- (a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library, and wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;
- (b) isolating the tagged genomic library-multifunctional capture probe module complex from a);
- (c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;
- (d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence;
- (e) performing PCR amplification of the hybrid nucleic acid molecule in d); and
- (f) performing targeted genetic analysis on the hybrid nucleic acid molecule from e).

In particular embodiments, steps a) through e) are repeated at least about twice and the targeted genetic analysis of f) comprises performing a sequence alignment of the hybrid nucleic acid molecule sequences from the at least two e) steps.

5 In certain embodiments, at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

In additional embodiments, at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

10 In various embodiments, a method for targeted genetic analysis is provided comprising: (a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library; (b) isolating the tagged genomic library-multifunctional capture probe hybrid
15 module complex from a); (c) performing 5' to 3' DNA polymerase extension of the multifunctional capture probe on the complex from b) to replicate a region of the captured, tagged genomic target region that is 3' of the multifunctional capture probe in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the multifunctional capture probe hybrid module and the
20 complement of a region of the tagged genomic target region that is located in the 3'

direction from the location where the multifunctional capture probe hybrid module hybridizes to the genomic target region; and (d) performing targeted genetic analysis on the hybrid nucleic acid molecule from c).

5 In another aspect, there is provided a method for targeted genetic analysis comprising:

(a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library, and
10 wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;

(b) isolating the tagged genomic library-multifunctional capture probe hybrid module complex from a);

15 (c) performing 5' to 3' DNA polymerase extension of the multifunctional capture probe on the complex from b) to replicate a region of the captured, tagged genomic target region that is 3' of the multifunctional capture probe in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the multifunctional capture probe hybrid module and the complement of a region of the
20 tagged genomic target region that is located in the 3' direction from the location where the multifunctional capture probe hybrid module hybridizes to the genomic target region; and

(d) performing targeted genetic analysis on the hybrid nucleic acid molecule from c).

25 In further embodiments, steps a) through c) are repeated at least about twice and the targeted genetic analysis of d) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

 In some embodiments, at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

30 In particular embodiments, at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

In various embodiments, a method for determining copy number of a specific genomic target region is provided comprising: (a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library; (b) isolating the tagged genomic library-multifunctional capture probe hybrid module complex from a); (c) performing 5' to 3' DNA polymerase extension of the multifunctional capture probe on the complex from b) to replicate a region of the captured tagged genomic target region that is 3' of the multifunctional capture probe in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the multifunctional capture probe hybrid module and the complement of a region of the tagged genomic target region that is located in the 3' direction from the location where the multifunctional capture probe hybrid module hybridizes to the genomic target region; and (d) performing PCR amplification of the hybrid nucleic acid molecule in c); and (e) quantitating the PCR reaction in d), wherein the quantitation allows for a determination of copy number of the specific genomic target region.

In particular embodiments, the methods contemplated herein comprise obtaining the sequences of the hybrid nucleic acid molecules from step d).

CLAIMS:

1. A method for targeted genetic analysis comprising:

- (a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library, and wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;
- (b) isolating the tagged genomic library-multifunctional capture probe module complex from a);
- (c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;
- (d) performing PCR on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; and
- (e) performing targeted genetic analysis on the hybrid nucleic acid molecule from d).

2. The method of claim 1, wherein steps a) through d) are repeated at least about twice and the targeted genetic analysis of e) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

3. The method of claim 2, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

4. The method of claim 3, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

5. A method for determining copy number of a specific genomic target region comprising:

- (a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library, and wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;

- (b) isolating the tagged genomic library-multifunctional capture probe module complex from a);
 - (c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;
 - (d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence;
 - (e) performing PCR amplification of the hybrid nucleic acid molecule in d); and
 - (f) performing targeted genetic analysis on the hybrid nucleic acid molecule from e).
6. The method of claim 5, wherein steps a) through e) are repeated at least about twice and the targeted genetic analysis of f) comprises performing a sequence alignment of the hybrid nucleic acid molecule sequences from the at least two e) steps.
7. The method of claim 6, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.
8. The method of claim 7, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.
9. A method for targeted genetic analysis comprising:
- (a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library, and wherein the tagged genomic library comprises genomic DNA ligated to a multifunctional adaptor molecule comprising one or more nucleic acid tags, wherein the one or more nucleic acid tags identify a genomic DNA clone;
 - (b) isolating the tagged genomic library-multifunctional capture probe hybrid module complex from a);
 - (c) performing 5' to 3' DNA polymerase extension of the multifunctional capture probe on the complex from b) to replicate a region of the captured, tagged genomic target region that is 3' of the multifunctional capture probe in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the multifunctional capture probe hybrid module and the complement of a region of the tagged genomic target region that is located in the 3' direction from the location where the multifunctional capture probe hybrid module hybridizes to the genomic target region; and

(d) performing targeted genetic analysis on the hybrid nucleic acid molecule from c).

10. The method of claim 9, wherein steps a) through c) are repeated at least about twice and the targeted genetic analysis of d) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

11. The method of claim 10, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

12. The method of claim 11, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

13. The method of any of the preceding claims wherein the targeted genetic analysis is sequence analysis.

14. The method of claim 1, wherein the one or more nucleic acid tags is randomly selected from a pool of known nucleic acid tags.

15. The method of claim 14, wherein the multifunctional adaptor molecule further comprises a sample code tag sequence.

16. The method of claim 5, wherein the one or more nucleic acid tags is randomly selected from a pool of known nucleic acid tags.

17. The method of claim 16, wherein the multifunctional adaptor molecule further comprises a sample code tag sequence.

18. The method of claim 9, wherein the one or more nucleic acid tags is randomly selected from a pool of known nucleic acid tags.

19. The method of claim 18, wherein the multifunctional adaptor molecule further comprises a sample code tag sequence.