SUMMARY

[0006] In one aspect, the present disclosure provides a method of determining the complexity of a nucleic acid template by:

i) providing a nucleic acid template;

ii) providing a plurality of primer pairs, comprising a first primer and a second primer, wherein the first primer comprises a sequence complementary to a portion of the nucleic acid template, and the second primer comprises a sequence complementary to a portion of the complement of the nucleic acid template;

iii) attaching a codeword to the 5' end of the first primer, the 5' end of the second primer, or both, to form a codeword-primer molecule, or to the nucleic acid template to form a codeword-template molecule;

iv) performing an amplification reaction with the paired codeword-primer molecules and the nucleic acid template or with the primer pairs and the codeword-template molecule for a defined number of cycles to obtain an amplification reaction product;

v) obtaining the sequence of the amplification reaction product at the end of each cycle, at the end of the defined number of cycles, or at an intermediate number of cycles;

vi) determining the abundance of each codeword present in the amplification reaction product at the end of each cycle, at the end of the defined number of cycles, or at an intermediate number of cycles;

vii) determining the observed codeword entropy of each cycle; and

viii) comparing the observed codeword entropy to an estimated codeword entropy,

to determine the complexity of the nucleic acid template.

[0007] In an alternative aspect, the present disclosure provides a method of identifying a true sequence variant by:

i) providing a nucleic acid template;

ii) providing a plurality of primer pairs, comprising a first primer and a second primer, wherein the first primer comprises a sequence complementary to a portion of the nucleic acid template, and the second primer comprises a sequence complementary to a portion of the complement of the nucleic acid template;

iii) attaching a codeword to the 5' end of the first primer, the 5' end of the second primer, or both, to form a codeword-primer molecule, or to the nucleic acid template to form a codeword-template molecule;

iv) performing an amplification reaction with the paired codeword-primer molecules and the nucleic acid template or with the primer pairs and the codeword-template molecule for a defined number of cycles to obtain an amplification reaction product;

v) obtaining the sequence of the amplification reaction product at the end of each cycle, at the end of the defined number of cycles, or at an intermediate number of cycles;

vi) determining the abundance of each codeword present in the amplification reaction product at the end of each cycle, at the end of the defined number of cycles, or at an intermediate number of cycles;

vii) determining the observed codeword entropy of each cycle; and

viii) performing a supervised classification method based on the results of steps vi) and vii),

to identify the true sequence variant.

[0008] The true sequence variant may be a low prevalence sequence variant.

[0009] The nucleic acid template may be a DNA template.

[0024] The method may be performed using a single target sequence or using two or more target sequences.

[0025] The method may be performed a single time or may be performed two or more times.

[0026] The method may include determination of codeword performance as function of subsequence and location.

[0027] In some aspects, the present disclosure provides primers comprising one or more of the sequences set forth in SEQ ID NOs: 1-146.

[0028] In some aspects, the present disclosure provides a set of primer pairs, comprising a first primer and a second primer, where the first primer comprises a sequence set forth in any one of SEQ ID NOs: 1-73 and the second primer comprises a sequence set forth in any one of SEQ ID NOs: 74-146.

[0029] In some aspects, the present disclosure provides kits comprising the primer or primer pairs as described herein, further comprising suitable reagents for storage, transport, delivery or use of the primers or primer pairs, optionally with instructions for use.

[0030] This summary does not necessarily describe all features of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0032] **FIGURE 1** is a flow chart showing patient sample workflow;

[0033] **FIGURE 2** is a flow chart showing sequence analysis workflow;

[0034] **FIGURE 3** is a matrix showing codeword performance as a function of subsequence composition and location;

[0035] **FIGURE 4** is an algorithm to determine parameters with high influence in codeword performance;