

NUCLEIC ACID PROBES

FIELD

The present invention relates to fluorescence in situ hybridization (FISH). In particular, the invention relates to a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte for fluorescence in situ hybridization.

BACKGROUND

As an attractive approach to spatial transcriptomics, multiplexed fluorescent *in situ* hybridization (FISH) allows combinatorial imaging of the transcriptome, and promises to reveal the state-to-function relationships of single cells in native tissues. A key challenge to making multiplexed FISH more broadly applicable to all tissue types is the difficulty in accurately detecting individual RNA molecules in complex tissue environments, which often suffer from low signals and tissue-dependent background. To address this limitation, much effort has been focused on signal amplification to generate brighter RNA spots. However, such approaches can only improve the signal relative to the tissue auto-fluorescence. In addition, since all probes are equally amplified, these amplification methods do not help to distinguish between real RNA spots (true positives) from non-specifically bound probes (false positives).

Off-target binding of FISH probes generates background fluorescence and spurious signals. These problems are exacerbated in multiplexed FISH because of the use of highly diverse (usually consisting of thousands of sequences) and concentrated probe solutions. One approach to solve these problems is to use customized tissue clearing approaches to remove cellular proteins and lipids, thereby reducing non-specific probe binding. However, clearing does not remove the non-specific binding of probes to non-target RNAs inside the cells and tissues. In addition, tissue clearing creates another source of technical variability from sample to sample, and it entails lengthy protocols that may require customization for each tissue type.

Accordingly, it is generally desirable to overcome or ameliorate one or more of the above mentioned difficulties.

SUMMARY

In one aspect, there is provided a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte, comprising:

- i. a first nucleic acid probe comprising:
 - a) a first probe binding arm that is complementary to a first probe target region of a bridge probe; and
 - b) a first polynucleotide analyte binding arm that is complementary to a first analyte target region of the polynucleotide analyte, and
- ii. a second nucleic acid probe comprising:
 - a) a second probe binding arm that is complementary to a second probe target region of the bridge probe; wherein the first probe target region is located downstream of the second probe target region on the bridge probe, and
 - b) a second polynucleotide analyte binding arm that is complementary to a second analyte target region of the polynucleotide analyte, wherein the second analyte target region is located downstream of the first analyte target region on the polynucleotide analyte,

wherein binding of the first polynucleotide analyte binding arm to the first analyte target region and binding of the second polynucleotide analyte binding arm to the second analyte target region permit binding of the first probe binding arm to the first probe target region and binding of the second probe binding arm to the second probe target region, thereby detecting the polynucleotide analyte.

In one aspect, there is provided a probe system as defined herein.

In one embodiment, there is provided a probe system comprising:

- i. a first nucleic acid probe that comprises:
 - a) a first probe binding arm that is complementary to a first probe target region of a bridge probe, and
 - b) a first polynucleotide analyte binding arm that is complementary to a first analyte target region of a polynucleotide analyte; and
- ii. a second nucleic acid probe that comprises:

- a) a second probe binding arm that is complementary to a second probe target region of the bridge probe, wherein the first probe target region is located downstream of the second probe target region on the bridge probe, and
- b) a second polynucleotide analyte binding arm that is complementary to a second analyte target region of the polynucleotide analyte, wherein the second analyte target region is located downstream of the first analyte target region on the polynucleotide analyte;

wherein binding of the first polynucleotide analyte binding arm to the first analyte target region and binding of the second polynucleotide analyte binding arm to the second analyte target region permit binding of the first probe binding arm to the first probe target region and binding of the second probe binding arm to the second probe target region, thereby detecting the polynucleotide analyte.

In one embodiment, the probe binding arm in the first and/or second nucleic acid probe comprises an identification portion for binding to a unique bridge probe. The identification portion may allow a pair (or multiple pairs) of nucleic acid probes to be recognized by a unique bridge probe. Multiple pairs of nucleic acid probes may comprise the same identification portion for binding to the same unique bridge probe, this may allow each pair of nucleic acid probes (or a set of nucleic acid probe pairs) to be distinguishable from one another in a library comprising a plurality of nucleic acid probe pairs.

In one aspect, there is provided a method of detecting a polynucleotide analyte in a sample, the method comprising:

- (a) contacting the sample with a pair of non-naturally occurring nucleic acid probes or a probe system as defined herein; and
- (b) detecting the polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.

In one aspect, there is provided a library for detecting two or more polynucleotide analytes in a sample; the library comprising two or more pairs of non-naturally occurring nucleic acid probes or a plurality of probe systems as defined herein,

wherein each pair of nucleic acid probes is specific to each polynucleotide analyte; and wherein each pair of nucleic acid probes is configured to hybridize to a unique bridge probe in the presence of the polynucleotide analyte.

In one aspect, there is provided a method of detecting two or more polynucleotide analytes in a sample, the method comprising:

- a) contacting a sample with a library as defined herein, and
- b) detecting each polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.

The method may comprise providing a unique bridge probe that is configured to bind to a specific pair (or multiple pairs) of nucleic acid probes prior to step b). A plurality of unique bridge probes may be provided either concurrently, sequentially or combinatorically to enable detection of a plurality of polynucleotide analytes.

In one aspect, there is provided a method of detecting or visualising the expression of one or more polynucleotide analytes in a sample, the method comprising

- a) contacting a sample with a library as defined herein, and
- b) detecting or visualising each polynucleotide analyte based on hybridisation to a unique bridge probe.

In one aspect, there is provided a kit comprising a pair of non-naturally occurring nucleic acid probes as defined herein or a plurality of probe systems or a library as defined herein.

In one embodiment, the kit further comprises one or more bridge probes.

BRIEF DESCRIPTION OF THE FIGURES

Certain embodiments are illustrated by the following figures. It is to be understood that the following description is for the purpose of describing particular embodiments only and is not intended to be limiting with respect to the description.

Figure 1: Optimization of the bridge sequence length (a) Split probes were designed to target a polymorphic repeat region (SEQ ID NO: 591) of the MUC5AC transcripts in A549 cell lines. RNA FISH images of split bridge sequence length (x) 7-12 nucleotides (nt) in (b) unpaired and (c) paired split probes (orange and light blue sequences). Shorter (7-9 nucleotides) bridge lengths were able to suppress the binding of unpaired probes. However, using bridge lengths

that were too short (7 + 7 nucleotides) resulted in poor binding even in paired probes. 9+9 nucleotides appeared to be the most optimal length.

Figure 2: Optimization of split-FISH workflow. Split-FISH image (a) with, and (b) without amplification primers removed from the probes via restriction digestion. (c) Same as b, but at 10x contrast. (d) Normalized RNA brightness after hybridization of bridge probe for split-FISH (blue) versus conventional readout probe (red) for 1, 5, 10, 30, and 60 minutes. Additional round of dye labelled readout probe hybridization (10 minutes) is needed for split-FISH.

Figure 3: Optimization of the split probe construct. (a-f) Six different constructs - circular, cruciform, double 'C', and double 'Z', conventional, and unpaired were tested (SEQ ID NOs; 344-353). The targeted RNA (SEQ ID NO: 591) and probe sequences are shown. (g-k) Example RNA FISH images of the tested constructs with DAPI nucleus (blue) staining. It was found that the circular construct (g) resulted in the best RNA signals, which achieved similar brightness to the conventional scheme (k). (l) In contrast, unpaired probe showed no signal (negative control). (m) Box plots of the brightness of single RNA molecules ($n = 1,000$ randomly selected RNAs from 5 FOVs) for each of the probe constructs. Center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.

Figure 4: Two channels co-localization control for the split probe construct. (a) 75 unique probes (Cy3) against non-repeat regions on *MUC5AC* transcripts were simultaneously hybridized with split probe constructs (Cy5) - circular, cruciform, double 'C', double 'Z', and conventional. (b-e) Sample RNA FISH images from Cy3 and Cy5 channels for the circular and double 'Z' constructs, with DAPI staining (blue) for cell nucleus. Double 'Z' Cy5 is displayed at 4x enhanced contrast compared to 'circular'. This experiment was repeated twice with similar results. (f) Box plots of the fraction of Cy5 spots that co-localized with Cy3 spots ($n = 10$ FOVs) for each of the probe constructs. Center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.

Figure 5: Split-probes eliminate false positive signals associated with known off targets (a) Using conventional read-out, the false positive signals were also observed in the nucleus (blue). (b) Removal of the 'rogue' probe (red) eliminated the false positive signals in the nucleus. (c) Using split readouts, no false positive signals were observed in the nucleus despite the presence of the 'rogue' sequence. (d) Readout probes are unable to bind to the unpaired

'rogue' sequence.

Figure 6. Split probe-based multiplexed FISH (split-FISH) in mammalian cell line and tissues. (a) Scheme of multiplexed split-FISH protocol. Encoding probes are hybridized first. At each round of imaging, bridge probes are introduced and allowed to hybridize, followed by dye-labelled readout probes. After imaging, both bridge and readout probes are washed out in preparation for the next round. (b) Decoded transcript locations for the region in Fig. 8d from split-FISH in AML12 cells. Maximum intensity projections across all rounds of hybridization are shown with decoded transcript locations overlaid. Each dot denotes a single transcript. Colors represent different genes. Length of the scale bar is 10µm. Scatter plot of total counts per gene vs bulk RNA-sequencing FPKM values for AML12, with Log Pearson correlation in red. Scatter plot of counts per cell between split-FISH and conventional, for the 10 genes common to both schemes. The $y = x$ line is shown in red. (c) Scatter plot of total counts per gene vs bulk RNA-sequencing FPKM values for brain, kidney, ovary, and liver tissues. Log Pearson correlation values in red. (d) Comparison of 'blank' counts per cell between conventional multiplexed FISH and split-FISH for mouse brain and liver tissues. Eight and seven 'blank' barcodes were tested for split-FISH (317 genes) and conventional (133 genes) schemes respectively. Centre line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; all data points shown in blue.

Figure 7: Optimized split-FISH allows repeated cycles of hybridization and wash (a) Alternating hybridization and wash of the FLNA transcripts in the same A549 cells for 20 cycles. (b) Box plots of number of spots detected per cell ($n = 38$ cells) over the 20 hybridization and wash cycles. Center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range. (c) Box plots of RNA brightness ($n = 1,000$ randomly selected RNAs from 4 FOVs) over the 20 hybridizations. Center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.

Figure 8. Comparison of conventional and split probe approaches to multiplexed FISH. (a) Schematic comparison of the two approaches. Cellular RNA in black, encoding probes in red, dye-labelled readout probes in orange. Bridge probes (split scheme only) are in green, which bind only when two matching encoding probes are coincident within close proximity. (b to e) Unprocessed images from a single imaging round of multiplexed FISH, from AML12 cells and mouse brain slices using conventional (b and c) and split probe (d and e) schemes.

Images in b, c, d and e are scaled to the same camera intensity range (30k, orange dashed box on histogram). Inset shows full field of view, of which the main image shows a zoomed-in region (red box). The length of the scale bars are 10 μ m. Histograms show distribution of raw pixel intensity from the entire field of view. X-axis of histograms are scaled to the maximum camera sensor output of 65535. Red lines show median.

Figure 9: Tissue auto-fluorescence was negligible compared to real RNA signals (a) Representative image from split-FISH with DAPI stain (blue). (b) Post-wash images, showing no detectable RNA spots. (c) Same image as b, but at 10x contrast, to highlight tissue auto-fluorescence and un-washed single fluorescent dye molecules.

Figure 10. Distinct transcriptomic localization patterns in four types of un-cleared mouse tissue revealed by split-FISH. Decoded transcript locations of selected genes overlaid on stitched image from one round of imaging. The length of the scale bars are 100 μ m. (a) Brain tissue showing differential localization of transcripts in neuronal processes (Map4) and regions containing cell bodies (e.g. Itpr1). (b) Zonation patterns of 5 genes (Ppl, Sptbn2, Irs1, Notch3, and Osbpl8) in a kidney section. (c) Compartmentalized localization of Plxnc1, Dsp, and Slc12a7 transcripts within ovarian follicles, localization of Myh11 transcripts surrounding follicles and Rnf213 transcripts near the outer surface of the ovary (d) Localization of genes around portal veins of the liver section.

Figure 11: Correlations between total counts and bulk RNA-sequencing FPKM values for conventional multiplexed FISH. (a) AML-12 (b) Liver (c) Brain.

Figure 12: Additional images from 5 bits of the AML-12 dataset shown in Figure 1. In the bottom right images, detected genes in the same region are annotated by gene name, with different colors for each gene. (a) Conventional (b) Split-FISH.

Figure 13: Additional images from 5 bits of the mouse brain dataset shown in Figure 1. In the bottom right images, detected genes in the same region are annotated by gene name, with different colors for each gene. (a) Conventional (b) Split-FISH.

Figure 14: Additional images from 5 bits of the mouse liver dataset. In the bottom right images, detected genes in the same region are annotated by gene name, with different colors for each gene. (a) Conventional (b) Split-FISH.

DETAILED DESCRIPTION

The specification discloses a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte.

Provided herein is a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte, comprising

- i. a first nucleic acid probe comprising:
 - a) a first probe binding arm that is complementary to a first probe target region of a bridge probe; and
 - b) a first polynucleotide analyte binding arm that is complementary to a first analyte target region of a polynucleotide analyte, and
- ii. a second nucleic acid probe comprising:
 - a) a second probe binding arm that is complementary to a second probe target region of the bridge probe, wherein the first probe target region is located downstream of the second probe target region on the bridge probe, and
 - b) a second polynucleotide analyte binding arm that is complementary to a second analyte target region of the polynucleotide analyte wherein the second analyte target region is located downstream of the first analyte target region on the polynucleotide analyte,

wherein binding of the first polynucleotide analyte binding arm to the first analyte target region and binding of the second polynucleotide analyte binding arm to the second analyte target region permit binding of the first probe binding arm to the first probe target region and binding of the second probe binding arm to the second probe target region, thereby detecting the polynucleotide analyte.

In one aspect, there is provided a probe system comprising:

- i. a first nucleic acid probe that comprises:
 - a) a first probe binding arm that is complementary to a first probe target region of a bridge probe, and

- b) a first polynucleotide analyte binding arm that is complementary to a first analyte target region of a polynucleotide analyte; and
- ii. a second nucleic acid probe that comprises:
 - a) a second probe binding arm that is complementary to a second probe target region of the bridge probe, wherein the first probe target region is located downstream of the second probe target region on the bridge probe, and
 - b) a second polynucleotide analyte binding arm that is complementary to a second analyte target region of the polynucleotide analyte, wherein the second analyte target region is located downstream of the first analyte target region on the polynucleotide analyte;

wherein binding of the first polynucleotide analyte binding arm to the first analyte target region and binding of the second polynucleotide analyte binding arm to the second analyte target region permit binding of the first probe binding arm to the first probe target region and binding of the second probe binding arm to the second probe target region, thereby detecting the polynucleotide analyte.

Without being bound by theory, the inventors have found a way to decrease non-specific background when detecting polynucleotide analytes in a cell or tissue (such as using Fluorescence in-situ hybridization). This can be done by using a set of split probes whereby a fluorescence signal is generated only when two independent hybridization events are co-localized (termed as split-FISH). In the split-FISH scheme (Figs. 6a and 8a), a bridge sequence is shared between a pair of adjoining encoding probes. The bridge probe can be designed to be unable to hybridize with sufficient affinity to any single encoding probe. Only when a pair of encoding probes is hybridized at adjacent locations on the polynucleotide analyte (such as a target RNA) will there be sufficient complementary base pairing in close proximity to enable the bridge probe to bind efficiently. A fluorescently labeled readout probe may then hybridize to the bridge probes to generate on-target signals. By improving the probe design at the single-molecule level and designing custom-barcoded bridge sequences, split-FISH can be used for accurate transcriptomic profiling even in uncleared tissues.

The probe system may further comprise the bridge probe.

The pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte may also be referred to a pair of non-naturally occurring nucleic acid split probes.

The pair of non-naturally occurring nucleic acid probes may also be referred to as “encoding probes”.

The pair of nucleic acid probes may be a pair of single-stranded nucleic acid probes.

The “bridge probe” may hybridize to the nucleic acid probes when the first and second nucleic acid probes hybridizes with the polynucleotide analyte. The “bridge probe” may therefore detect the binding of the first and second nucleic acid probes to the polynucleotide analyte.

Each pair of nucleic acid probes may be configured to hybridize to a unique bridge probe. In one embodiment, the probe binding arm in the first and/or second nucleic acid probes comprises an identification portion for binding to a unique bridge probe. The identification portion may allow a pair (or multiple pairs) of nucleic acid probes to be recognized by a unique bridge probe. This may allow each pair of nucleic acid probes (or a set of nucleic acid probe pairs) to be distinguishable from one another in a library comprising a plurality of nucleic acid probe pairs.

Also provided herein is the use of a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte. Also provided herein is a pair of non-naturally occurring nucleic acid probes when used to detect a polynucleotide analyte

In one embodiment, the probe binding arm in the first and/or second nucleic acid probes consists of 9 or 10 nucleotides. In one embodiment, the probe binding arm in the first and/or second nucleic acid probes consists of 9 nucleotides. It was found that the length of the split bridge may affect non-specific background signal and a length of about 9 nucleotides was surprisingly able to produce a level of non-specific background signal that is virtually undetectable. For example, the first nucleic acid probe may comprise a first probe binding arm at the 3' terminus that is complementary to and selectively hybridizes to a first probe target region of a bridge probe, wherein the first probe binding arm is ATTTAACCG (SEQ ID NO: 592) (see Table 9). The second nucleic acid probe may comprise a second probe binding arm at the 5' terminus that is complementary to and selectively hybridizes with a second probe target region of the bridge probe, wherein the second probe binding arm is CCCATTACC (SEQ ID NO: 593). The bridge probe may have a sequence of GGTAATGGGCGGTAAAT (SEQ ID

NO: 594). The bridge probe may further comprise one or two readout sequences (e.g. ATTGTAAGCGTGAGAAA (SEQ ID NO: 595)) that allows the bridge probe to be detected or recognised by a readout probe.

In one embodiment, the polynucleotide analyte binding arm in the first or second nucleic acid probes consists of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides. In one embodiment, the polynucleotide analyte binding arm in the first or second nucleic acid probes consists of 25 nucleotides.

In one embodiment, a linker is positioned between the probe binding arm and the polynucleotide analyte binding arm. The linker may be a short linker that is about 1 to 10 nucleotides. The linker may be a short linker of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleobases. In one embodiment, the linker is about 1 to 10, 1 to 9, 1 to 8; 1 to 7; 1 to 6; 1 to 5, 1 to 4, 1 to 3, 1 to 2 nucleobases. In one embodiment, the linker is about 1 to 5 nucleobases. In one embodiment, the linker is 1, 2, 3, 4 or 5 nucleobases. In one embodiment, the linker is 2 or 3 nucleobases. In one example, the linker is TAT (see Table 8a under Paired (circular) split probe sequences).

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides.

As used herein, the term "nucleic acid", and equivalent terms such as "polynucleotide", refer to a polymeric form of nucleotides of any length, such as ribonucleotides, deoxyribonucleotides or peptide nucleic acids (PNAs), that comprise purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The nucleic acid may be double stranded or single stranded. References to single stranded nucleic acids include references to the sense or antisense strands. The backbone of the polynucleotide can comprise sugars and phosphate groups, as may typically be found in RNA or DNA, or modified or substituted sugar or phosphate groups. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. The terms nucleoside, nucleotide, deoxynucleoside and deoxynucleotide generally include complements, fragments

and variants of the nucleoside, nucleotide, deoxynucleoside and deoxynucleotide, or analogs thereof.

In one embodiment, the first analyte target region is immediately adjacent to the second analyte target region. In another embodiment, the first analyte target region is spaced from the second analyte target region by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleobases.

In one embodiment, the first probe target region is immediately adjacent to the second probe target region. In another embodiment, the first probe target region is spaced from the second probe target region by no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 nucleobases.

An "oligonucleotide" as used herein is a single stranded molecule which may be used in hybridization or amplification technologies. In general, an oligonucleotide may be any integer from about 15 to about 100 nucleotides in length, but may also be of greater length.

The term "probe" refers to any molecule which is capable of selectively binding to a specifically intended target molecule, for example, a nucleotide transcript. Probes can be either synthesized by one skilled in the art, or derived from appropriate biological preparations.

The nucleic acid probes (or nucleic acid split probes) of the present invention may be useful for detecting the presence or absence of one or more polynucleotide analytes in one or more samples known to contain or suspected of containing the polynucleotide analytes. The nucleic acid probes can also be used to quantify the amount of polynucleotide analytes within the sample. The nucleic acid probes are useful for detecting unamplified polynucleotide target in a sample such as for example RNA, MRNA, rRNA, plasmid DNA, viral DNA, bacterial DNA, and chromosomal DNA. Additionally, the nucleic acid probes may be useful in conjunction with the amplification of a polynucleotide target by well-known methods such as PCR, ligase chain reaction, Q-B replicase, strand-displacement amplification (SDA), rolling-circle amplification (RCA), nucleic acid sequence-based amplification (NASBA), and the like.

In one embodiment, the bridge probe is coupled or conjugated to a label (such as a fluorescent label). Such a bridge probe may be referred to as a readout probe.

In one embodiment, the bridge probe is detected via hybridization to a secondary detection probe (or readout probe) that is conjugated to a label (such as a fluorescent label). The bridge probe may comprise a specific (or unique) tag or barcode sequences that enable it to be recognised via hybridisation to a secondary detection probe (or readout probe).

Examples of fluorescent labels include, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, phycocrytherin, phycocyanin, spectrum orange, spectrum green, and/or derivatives of any one or more of the above. Multiple probes used in the assay may be labeled with more than one distinguishable fluorescent or pigment color. These color differences provide a means to identify, for example, the hybridization positions of specific probes. Moreover, probes that are not separated spatially can be identified by a different color light or pigment resulting from mixing two other colors (e.g., light red+green=yellow) pigment (e.g., blue+yellow=green) or by using a filter set that passes only one color at a time. Probes can be labeled directly or indirectly with the fluorophore, utilizing conventional methodology. Additional probes and colors may be added to refine and extend this general procedure to include more genetic abnormalities or serve as internal controls.

In one embodiment, the secondary detection probe (or readout probe) hybridizes to a terminal region of the bridge probe.

In one embodiment, two secondary detection probes hybridize to both terminal regions of the bridge probe.

In one embodiment, the secondary detection probe or probes (or readout probes) hybridize to a central region of the bridge probe.

In one embodiment, the bridge probe has the same sequence as the polynucleotide analyte.

In one embodiment, the readout probe has the same sequence as the polynucleotide analyte.

In one embodiment, there is provided a pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte, the pair of nucleic acid probes comprising two anti-parallel nucleic acid strands, wherein:

- i. a first nucleic acid strand comprises:
 - a) a readout binding arm at the 3' terminus that is complementary to and selectively hybridizes to a first region of a readout probe; and
 - b) a polynucleotide analyte binding arm at the 5' terminus that is complementary to and selectively hybridizes with a first region of the polynucleotide analyte, and
- ii. a second nucleic acid strand comprises:
 - a) a readout binding arm at the 5' terminus that is complementary to and selectively hybridizes with a second region of a readout probe; and
 - b) a polynucleotide analyte binding arm at the 3' terminus that is complementary to and selectively hybridizes with a second region of the polynucleotide analyte positioned at the 3' end of the first region;

wherein hybridization of the first and second nucleic acid strands with the polynucleotide analyte enables hybridization to the readout probe and detection of the polynucleotide analyte.

The term "complementary" refers to the base pairing between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single stranded nucleic acid to be sequenced or amplified. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single stranded RNA or DNA molecules are said to be complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the nucleotides of the other strand, usually at least about 90% to 95%, and more preferably from about 98 to 100% of the nucleotides of the other strand. Alternatively, complementarity exists when an RNA or DNA strand will hybridize under selective hybridization conditions to its complement. Typically, selective hybridization will occur when there is at least about 65% complementarity over a stretch of at least 14 to 25 nucleotides, preferably at least about 75%, and more preferably at least about 90% complementarity.

As used herein, the term "hybridization" or "hybridizes" refers to the process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide. The term "hybridization" may also refer to triple-stranded hybridization. The resulting (usually) double-stranded polynucleotide is a "hybrid". The proportion of the

population of polynucleotides that forms stable hybrids is referred to herein as the "degree of hybridization."

Hybridization conditions will typically include salt concentrations of less than about 1M, more usually less than about 500 mM and less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, more typically greater than about 30°C, and preferably in excess of about 37°C. Hybridizations are usually performed under stringent conditions, i.e. conditions under which a probe will hybridize to its target. Stringent conditions are sequence-dependent and are different under different circumstances. Longer fragments may require higher hybridization temperatures for specific hybridization. As other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid composition) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Typically, stringent conditions include salt concentration of at least 0.01 M to no more than 1 M Na ion concentration (or other salts) at a pH 7.0 to 8.3 and a temperature of at least 25°C. For example, conditions of 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30°C are suitable for allele-specific probe hybridizations.

A "label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or non-covalently joined to a polynucleotide.

The term "labelled", with regard to, for example, a probe, is intended to encompass direct labelling of the probe by coupling (i.e., physically linking) a detectable substance to the probe, as well as indirect labelling of the probe by reactivity with another reagent that is directly labelled. Examples of indirect labelling include detection of a bridge probe (bound to a nucleic acid pair in the presence of a polynucleotide analyte) using a fluorescently labelled secondary probe (or readout probe).

The term "polynucleotide analyte" may be any polynucleotide that may be detected or analyzed

by a pair of nucleic acid probes or probe system as defined herein. The analyte may be naturally-occurring or synthetic. A polynucleotide analyte may be present in a sample obtained using any methods known in the art. In some cases, a sample may be processed before analyzing it for a polynucleotide analyte. The polynucleotide may include DNA, RNA, peptide nucleic acids, and any hybrid thereof, where the polynucleotide contains any combination of deoxyribo- and/or ribo-nucleotides. Polynucleotides may be single stranded or double stranded, or contain portions of both double stranded or single stranded sequence. Polynucleotides may contain any combination of nucleotides or bases, including, for example, uracil, adenine, thymine, cytosine, guanine, inosine, xanthine, hypoxanthine, isocytosine, isoguanine and any nucleotide derivative thereof. As used herein, the term "nucleotide" may include nucleotides and nucleosides, as well as nucleoside and nucleotide analogs, and modified nucleotides, including both synthetic and naturally occurring species. Polynucleotides may be any suitable polynucleotide, including but not limited to cDNA, mitochondrial DNA (mtDNA), messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), nuclear RNA (nRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), small Cajal body-specific RNA (scaRNA), microRNA (miRNA), double stranded (dsRNA), ribozyme, riboswitch or viral RNA. Polynucleotides may be contained within any suitable vector, such as a plasmid, cosmid, fragment, chromosome, or genome. The polynucleotide analyte can be a nucleic acid endogenous to the cell. As another example, the polynucleotide analyte can be a nucleic acid introduced to or expressed in the cell by infection of the cell with a pathogen, for example, a viral or bacterial genomic RNA or DNA, a plasmid, a viral or bacterial mRNA, or the like.

Genomic DNA may be obtained from naturally occurring or genetically modified organisms or from artificially or synthetically created genomes. Polynucleotide analytes comprising genomic DNA may be obtained from any source and using any methods known in the art. For example, genomic DNA may be isolated with or without amplification. Amplification may include PCR amplification, rolling circle amplification and other amplification methods. Genomic DNA may also be obtained by cloning or recombinant methods, such as those involving plasmids and artificial chromosomes or other conventional methods (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, cited supra.) Polynucleotide analytes may be isolated using other methods known in the art, for example as disclosed in *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) or *Molecular Cloning: A Laboratory Manual*. If the isolated polynucleotide analyte is an mRNA, it may be reverse transcribed into

cDNA using conventional techniques, as described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*., cited supra.

The term "gene" is used broadly to refer to any nucleic acid associated with a biological function. Genes typically include coding sequences and/or the regulatory sequences required for expression of such coding sequences. The term gene can apply to a specific genomic sequence, as well as to a cDNA or an mRNA encoded by that genomic sequence. Genes also include non-expressed nucleic acid segments that, for example, form recognition sequences for other proteins. Non-expressed regulatory sequences include promoters and enhancers, to which regulatory proteins such as transcription factors bind, resulting in transcription of adjacent or nearby sequences.

As used herein, the term "sample" includes tissues, cells, body fluids and isolates thereof etc., isolated from a subject, as well as tissues, cells and fluids etc. present within a subject (i.e. the sample is in vivo). Examples of samples include: whole blood, blood fluids (e.g. serum and plasm), lymph and cystic fluids, sputum, stool, tears, mucus, hair, skin, ascitic fluid, cystic fluid, urine, nipple exudates, nipple aspirates, sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, archival samples, explants and primary and/or transformed cell cultures derived from patient tissues etc.

The sample (such as a tissue or cell sample) may be fixed and permeabilized before hybridization with a pair of nucleic acid probe as defined herein, to retain the polynucleotide analytes in the cell and to permit the nucleic acid probes, bridge probes, etc. to enter the sample. The sample is optionally washed to remove materials not captured to one of the polynucleotide analytes. The sample can be washed after any of the various steps, for example, after hybridization of the nucleic acid probes to the polynucleotide analytes to remove unbound nucleic acid probes or after hybridization with the nucleic acid probes and bridge probes, before removing unbound nucleic acid probe and bridge probes.

The terms "restriction enzyme" and "restriction endonuclease" as used herein means an endonuclease enzyme that recognises and cleaves a specific sequence of DNA (recognition sequence).

In one aspect, there is provided a method of detecting a polynucleotide analyte in a sample, the method comprising:

- (a) contacting the sample with a pair of non-naturally occurring nucleic acid probes or a probe system as defined herein; and
- (b) detecting the polynucleotide analyte based on hybridization to a bridge probe in the presence of the polynucleotide analyte.

In one embodiment, there is provided a method of determining the level of a polynucleotide analyte in a sample, the method comprising:

- (a) contacting the sample with a pair of non-naturally occurring nucleic acid probes or a probe system as defined herein; and
- (b) detecting the polynucleotide analyte based on hybridization to a bridge probe in the presence of the polynucleotide analyte.

The various hybridization steps can be performed simultaneously or sequentially, in essentially any convenient order. In one embodiment, a hybridization step with the multiple pairs (or library) of nucleic acid probes is accomplished for all of the polynucleotide analytes at the same time. For example, all the nucleic acid probes can be added to the sample at once and permitted to hybridize to their corresponding targets, the sample can then be washed. Corresponding bridge probes can be hybridized to the nucleic acid probes and sample can be washed again prior to detection of the bridge probes. It will be evident that double-stranded polynucleotide analyte(s) are preferably denatured, e.g., by heat, prior to hybridization of the corresponding pair(s) of nucleic acid probes to the polynucleotide analyte.

The method may comprise the step of hybridizing a bridge probe to the pair of non-naturally occurring nucleic acid probes that are bound to the polynucleotide analyte that is present. Any unbound bridge probe may be removed or washed off.

The bridge probe may be coupled or conjugated to a label (such as a fluorescent label) that enables detection of the bridge probe and thus enables detection of the polynucleotide analyte. Such a bridge probe may also be referred to as a “readout probe”.

Alternatively, a secondary detection probe (i.e. a readout probe) may be hybridized to the bridge probe and allows the bridge probe (and the polynucleotide analyte) to be detected.

The bridge probe may comprise a specific tag or barcode sequence (such as a 6 nucleotide sequence). This may enable the bridge probe to be recognised by the secondary detection probe (or readout probe).

The method may allow the detection of the presence or levels of the polynucleotide analyte based on the signal that is detected.

The method may involve detecting one or more polynucleotide analytes. The polynucleotide analytes may be detected concurrently or sequentially.

In the case where the polynucleotide analytes are detected sequentially, this may involve multiple rounds of hybridization for each polynucleotide analyte with a specific pair of nucleic acid probes, and subsequent detection with bridge and/or readout probes. There may also be a step of washing or removal of signal (by, for example, bleaching) in between detection of each polynucleotide analyte.

In one aspect, there is provided a library for detecting two or more polynucleotide analytes in a sample; the library comprising two or more pairs of non-naturally occurring nucleic acid probes or a plurality of probe systems as defined herein, wherein each pair of nucleic acid probes is specific to each polynucleotide analyte; and wherein each pair of nucleic acid probes is configured to hybridize to a unique bridge probe in the presence of the polynucleotide analyte.

The term “unique bridge probe” may refer to the ability of a bridge probe to recognise a specific pair of nucleic acid probes. Each pair of nucleic acid probes in a library may comprise an “identification portion” (or barcode) in the probe binding arm of either the first or second nucleic acid probe (or both) for binding to a unique bridge probe. In one embodiment, the identification portion consists of 6 nucleotides (e.g. actcta). The bridge probe may have a corresponding barcode sequence that recognises the identification portion in the pair of nucleic acid probes.

More than one pair of nucleic acid probes (e.g. a set of nucleic acid probes) may comprise the same identification portion (or barcode) that allows them to bind to a unique bridge probe. A

library of nucleic acid probe pairs may be grouped according to nucleic acid probe pairs that share the same identification portion (or barcode). This may allow for the combinatorial detection of polynucleotide analytes based on addition of a corresponding unique bridge probe that recognises nucleic acid probe pairs that share the same identification portion.

A library of identification portions (or barcodes) may be used in certain embodiments, e.g., containing at least 10, at least 10^2 , at least 10^3 , at least 10^4 , at least 10^5 , at least 10^6 , at least 10^7 , at least 10^8 , etc. unique sequences. The unique sequences may be all individually determined (e.g., randomly), although in some cases, the identification portion may be defined as a plurality of variable portions (or "bits"), e.g., in sequence. For example, an identification portion may include at least 2, at least 3, at least 5, at least 6, at least 7, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, or at least 50 variable portions. Each of the variable portions may include at least 2, at least 3, at least 4, at least 5, at least 7, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, or more possibilities. In one embodiment, the identification portion consists of 6 variable portions.

Thus, for example, an identification portion defined with 22 variable regions and 2 unique possibilities per variable region would define a library of identification portions with $2^{22} = 4,194,304$ members. As another non-limiting example, an identification portion may be defined with 10 variable regions and 7 unique possibilities per variable region to define a library of identification portions with 7^{10} members. It should be understood that a variable portion may include any suitable number of nucleotides, and different variable portions within an identification portion may independently have the same or different numbers of nucleotides. Different variable regions also may have the same or different numbers of unique possibilities. For example, a variable portion may be defined having a length of at least 2, at least 3, at least 4, at least 5, at least 7, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, or more nucleotides, and/or a maximum length of no more than 50, no more than 40, no more than 30, no more than 25, no more than 20, no more than 15, no more than 10, no more than 7, no more than 5, no more than 4, no more than 3, or no more than 2 nucleotides. Combinations of these are also possible, e.g., a variable portion may have a length of between 5 and 50 nt, or between 15 and 25 nt, etc. A non-limiting example of a library is illustrated with identification sequences 1-1, 1-0, 2-1, 2-0, etc. through 22-1 and 22-0, which may be concatenated together (e.g., identification sequence 1— identification sequence 2— identification sequence 3— ...— identification 22) to produce an bridge sequence (in this non-

limiting example, each sequence position 1, 2, ... 22 may have one of two possibilities, identified with -0 and -1, e.g., sequence position 1 can be either identification sequence 1-1 or 1-0, sequence position 2 can be either identification sequence 2-1 or 2-0, etc.). Similarly, according to certain embodiments, information could also be included in the absence of such sequences. For example, the same information included in the presence of one sequence (e.g. sequence 1-0), could also be determined from the absence of another sequence (e.g., sequence 1-1) Each identification sequence position may be thought of as a "bit" (e.g., 1 or 0 in this example), although it should be understood that the number of possibilities for each "bit" is not necessarily limited to only 2, unlike in a computer. In other embodiments, there may be 3 possibilities (i.e., a "trit"), 4 possibilities (i.e., a "quad-bit"), 5 possibilities, etc., instead of only 2 possibilities as in some embodiments.

The method for generating a library may comprise (a) associating barcode sequences with a plurality of oligonucleotide sequences and a plurality of codewords, wherein the codewords comprise a number of positions that is less than the number of targets, and b) grouping the pairs of nucleic acid probes based on a plurality of codewords, wherein each of the bridge probe corresponds to a specific value of a unique position within the codewords. The method may comprise exposing a sample to one of the bridge probes; imaging the sample; and repeating the exposing and imaging steps one or more times, before repeating with a different bridge probe. This process may be repeated for at least 10, 15, 20, 50, 80, 100, 500 repetitions.

In one aspect, there is provided a method of detecting two or more polynucleotide analytes in a sample, the method comprising:

- a) contacting a sample with a library or a probe system as defined herein, and
- b) detecting each polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.

In one embodiment, there is provided a method for combinatorial detection of two or more polynucleotide analytes in a sample, the method comprising:

- a) contacting a sample with a library or a probe system as defined herein, and
- b) detecting the two or more polynucleotide analytes based on hybridization to a unique bridge probe in the presence of the two or more polynucleotide analyte.

In one embodiment, there is provided a method of determining the levels of two or more polynucleotide analytes in a sample, the method comprising:

- a) contacting a sample with a library or a probe system as defined herein, and
- b) detecting each polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.

In one embodiment, two or more nucleic acid probe pairs may be configured to bind to the same unique bridge probe to allow the two or more polynucleotide analytes to be detected combinatorically.

The terms “detecting”, “determining”, “measuring”, “evaluating”, “assessing” and “assaying” are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assessing may be relative or absolute. The method as defined herein may comprise measuring or visualising the levels of two or more polynucleotide analytes in a sample.

In one embodiment, the method comprises contacting the sample with a unique (or bar-coded) bridge probe for each polynucleotide analyte.

In one embodiment, the multiple polynucleotide analytes are detected concurrently based on hybridization to a unique bridge probe for each polynucleotide analyte.

In one embodiment, the multiple polynucleotide analytes are detected sequentially based on multiple rounds of hybridization to a unique bridge probe for each polynucleotide analyte.

In one embodiment, the method comprises detecting the unique bridge probe via hybridization to a readout probe that is conjugated to a label.

In one embodiment, the method comprises contacting the sample with a unique readout probe for each polynucleotide analyte.

The method may comprise removing any bound or unbound bridge and/or readout probe (such as by washing) in between detection of each polynucleotide analyte.

The method may comprise removing any signal from any bound or unbound readout probe in between detection of each polynucleotide analyte. This may be done by, for example, bleaching or quenching a signal.

In one aspect, there is provided a kit comprising a pair of non-naturally occurring nucleic acid probes as defined herein or a library as defined herein. The kit may further comprise bridge probes for detecting nucleic acid probes that are bound to polynucleotide analytes. The bridge probes may be labelled to enable detection or measurement of the analyte. Alternatively, the kit may further comprise readout probes that bind to the bridge probes. The kit optionally also includes instructions for detecting one or more polynucleotide analytes in a sample, one or more buffered solutions (e.g., diluent, hybridization buffer, and/or wash buffer), reference cell(s) comprising one or more of the polynucleotide analytes.

In one embodiment, there is provided a method of performing an array-based assay. Provided herein is also an array-based assay. The term “array” encompasses the term “microarray” and refers to an ordered array presented for binding to nucleic acids and the like. An “array,” includes any two-dimensional or substantially two-dimensional (as well as a three-dimensional) arrangement of spatially addressable regions bearing nucleic acids, particularly oligonucleotides or synthetic mimetics thereof, and the like.

Provided herein is a method of performing a multiplex fluorescence *in situ* hybridisation (FISH) assay.

Provided herein is a composition, the composition comprising a pair of non-naturally occurring nucleic acid probes as defined herein.

Essentially any type of cell that can be differentiated based on its nucleic acid content (presence, absence, expression level or copy number of one or more nucleic acids) can be detected and identified using the nucleic acid probes as defined herein to detect a suitable selection of polynucleotide analytes. The cell can, for example, be a circulating tumor cell, a virally infected cell, a fetal cell in maternal blood, a bacterial cell or other microorganism in a biological sample (e.g., blood or other body fluid), an endothelial cell, precursor endothelial cell, or myocardial cell in blood, a stem cell, or a T-cell. Rare cell types can be enriched prior

to performing the methods, if necessary, by methods known in the art (e.g., lysis of red blood cells, isolation of peripheral blood mononuclear cells, further enrichment of rare target cells through magnetic-activated cell separation (MACS), etc.). The methods are optionally combined with other techniques, such as DAPI staining for nuclear DNA. It will be evident that a variety of different types of nucleic acid markers are optionally detected simultaneously by the methods and used to identify the cell. For example, a cell can be identified based on the presence or relative expression level of one nucleic acid target in the cell and the absence of another nucleic acid target from the cell; e.g., a circulating tumor cell can be identified by the presence or level of one or more markers found in the tumor cell and not found (or found at different levels) in blood cells, and its identity can be confirmed by the absence of one or more markers present in blood cells and not circulating tumor cells. The principle may be extended to using any other type of markers such as protein based markers in single cells.

Provided herein are methods of diagnosis of a disease. The disease may be cancer, or viral or bacterial infection or a genetic disorder due to the presence of a defective gene. The method may comprise detecting the presence or absence of one or more polynucleotide analytes in a sample obtained from a subject. Provided herein are also methods of treating the disease following detection of the disease.

By “subject” or “patient” is meant any single subject for which therapy is desired, including humans, cattle, horses, pigs, goats, sheep, dogs, cats, guinea pigs, rabbits, chickens, insects and so on. Also intended to be included as a subject are any subjects involved in clinical research trials not showing any clinical sign of disease, or subjects involved in epidemiological studies, or subjects used as controls.

One or more polynucleotide analytes associated with cancer can be detected using the nucleic acid probes as defined herein, e.g., those that encode over expressed or mutated polypeptide growth factors (e.g., sis), overexpressed or mutated growth factor receptors (e.g., erb-B1), over expressed or mutated signal transduction proteins such as G-proteins (e.g., Ras), or non-receptor tyrosine kinases (e.g., abl), or over expressed or mutated regulatory proteins (e.g., myc, myb, jun, fos, etc.) and/or the like. In general, cancer can often be linked to signal transduction molecules and corresponding oncogene products, e.g., nucleic acids encoding Mos, Ras, Raf, and Met; and transcriptional activators and suppressors, e.g., p53, Tat, Fos, Myc, Jun, Myb, Rel, and/or nuclear receptors. p53. For detection of circulating tumor cells

(CTC), a variety of suitable polynucleotide analytes are known. For example, a multiplex panel of markers for CTC detection could include one or more of the following markers: epithelial cell-specific (e.g. CK19, Muc1, EpCAM), blood cell-specific as negative selection (e.g. CD45), tumor origin-specific (e.g. PSA, PSMA, HPN for prostate cancer and mam, mamB, her-2 for breast cancer), proliferating potential-specific (e.g. Ki-67, CEA, CA15-3), apoptosis markers (e.g. BCL-2, BCL-XL), and other markers for metastatic, genetic and epigenetic changes.

Similarly, one or more polynucleotide analytes from pathogenic or infectious organisms can be detected by the nucleic acid probes as defined herein, e.g., for infectious fungi, e.g., *Aspergillus*, or *Candida* species; bacteria, particularly *E. coli*, which serves a model for pathogenic bacteria (and, of course certain strains of which are pathogenic), as well as medically important bacteria such as *Staphylococci* (e.g., *aureus*), or *Streptococci* (e.g., *pneumoniae*); protozoa such as sporozoa (e.g., *Plasmodia*), *rhizopods* (e.g., *Entamoeba*) and flagellates (*Trypanosoma*, *Leishmania*, *Trichomonas*, *Giardia*, etc.); viruses such as (+) RNA viruses (examples include Poxviruses e.g., *vaccinia*; Picornaviruses, e.g., *polio*; Togaviruses, e.g., *rubella*; Flaviviruses, e.g., HCV; and Coronaviruses), (-) RNA viruses (e.g., Rhabdoviruses, e.g., VSV; Paramyxoviruses, e.g., RSV; Orthomyxoviruses, e.g., influenza; Bunyaviruses; and Arenaviruses), dsDNA viruses (e.g. Reoviruses), RNA to DNA viruses, i.e., Retroviruses, e.g., HIV and HTLV, and certain DNA to RNA viruses such as Hepatitis B.

Gene amplification or deletion events can be detected at a chromosomal level using the nucleic acid probes as described herein, as can altered or abnormal expression levels. Some polynucleotide analytes include oncogenes or tumor suppressor genes subject to such amplification or deletion. Exemplary nucleic acid targets include, integrin (e.g., deletion), receptor tyrosine kinases (RTKs; e.g., amplification, point mutation, translocation, or increased expression), NF1 (e.g., deletion or point mutation), Akt (e.g., amplification, point mutation, or increased expression), PTEN (e.g., deletion or point mutation), MDM2 (e.g., amplification), SOX (e.g., amplification), RAR (e.g., amplification), CDK2 (e.g., amplification or increased expression), Cyclin D (e.g., amplification or translocation), Cyclin E (e.g., amplification), Aurora A (e.g., amplification or increased expression), P53 (e.g., deletion or point mutation), NBS1 (e.g., deletion or point mutation), Gli (e.g., amplification or translocation), Myc (e.g., amplification or point mutation), HPV-E7 (e.g., viral infection), and HPV-E6 (e.g., viral infection).

If a polynucleotide analyte is used as a reference, suitable reference nucleic acids have similarly been described in the art or can be determined. For example, a variety of genes whose copy number is stably maintained in various tumor cells is known in the art. Housekeeping genes whose transcripts can serve as references in gene expression analyses include, for example, 18S rRNA, 28S rRNA, GAPD, ACTB, and PPIB.

Provided herein is a method of detecting or visualising the expression of one or more polynucleotide analytes in a sample, the method comprising a) contacting a sample with a library as defined herein, and b) detecting or visualising the expression of each polynucleotide analyte based on hybridisation to a unique bridge probe in the presence of the one or more polynucleotide analytes.

The method may comprise detecting the presence or level of mRNA in a sample.

The sample may be a cell or tissue sample.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or method step or group of elements or integers or method steps but not the exclusion of any other element or integer or method steps or group of elements or integers or method steps.

As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a method" includes a single method, as well as two or more methods; reference to "an agent" includes a single agent, as well as two or more agents; reference to "the disclosure" includes a single and multiple aspects taught by the disclosure; and so forth. Aspects taught and enabled herein are encompassed by the term "invention". Any variants and derivatives contemplated herein are encompassed by "forms" of the invention.

EXAMPLES

Materials and Methods

SPLIT-FISH library design. Targeting regions (pairs of 25-nt sequences with 2-nt spacing in between the pair) were identified using a previously published algorithm. First, reference transcript sequences were downloaded from the GENCODE website (human v24 and mouse m4 respectively). A specificity table was calculated using 15-nt seed and 0.2 specificity cut-off was used. Quartet repeats ('AAAA', 'TTTT', 'GGGG', and 'CCCC'), KpnI restriction sites ('GGTACC' (SEQ ID NO: 1) and 'CCATGG' (SEQ ID NO: 2)), and EcoRI restriction sites ('GAATTC' (SEQ ID NO: 3) and 'CTTAAG' (SEQ ID NO: 4)) were excluded from the possible target regions. Then, the right targeting region pairs were concatenated with the right bridge sequence (e.g. 'CactctaCC TAT' (SEQ ID NO: 5), lowercase indicates variable bases that form the 6-nt barcode, TAT is a linker between the bridge sequence and the targeting region). The left targeting region pairs were concatenated with the left bridge sequence 'TAT ATTTAACCG' (SEQ ID NO: 6). Finally, KpnI and EcoRI restriction sites, as well as the forward and reverse PCR primers were introduced at both ends of each side of the probes. Removal of the PCR primers via restriction digestion is required for efficient subsequent hybridization of the bridge sequence. The list of encoding probes can be found in Table 1. The bridge sequences were flanked by readout sequences at both ends. The list of bridge sequence can be found in Table 3. The readout sequences used were '5Cy5/TTACTCACGCACCCATCA' (SEQ ID NO: 7) and '5Alex750N/TTTCTCACGCTTTACAAT' (SEQ ID NO: 8). To construct the 317-genes combinatorial library, a '26 choose 2' coding scheme was used. Eight of the 325 possible code-words were blanks, which are not assigned to any gene (no encoding probes), to act as negative controls that estimate the levels of the false-positive background. For each gene, 72 pairs of target regions were split into two pools. Each pool was assigned a 6-nt barcode according to the gene's 'on' bits. The gene codebook assignment for the 317-genes library can be found in Table 2. The conventional multiplexed FISH probe and library were designed as previously described. The conventional encoding probe library and readout sequences can be found in Table 4 and 5 respectively. The conventional codebook can be found in Table 6.

Probe amplification and preparation. Probe library (Twist Bioscience) was made using a slightly modified version of a previously published protocol. Briefly, the oligopool was first amplified by limited cycle PCR using Phusion Hot Start Flex 2X master mix (NEB, Cat: M0536L) with an annealing temperature of 66°C, followed by an overnight *in vitro* transcription using a high yield *in vitro* transcription kit (NEB, Cat: E2050S). T7 promoter sequence was introduced on the reverse primer during the PCR. Next, reverse transcription

from the RNA template (ThermoFisher Cat: EP0753) was performed. The RNA was then cleaved off using alkaline hydrolysis, leaving behind ssDNA which was then purified via spin column purification (Zymo Cat: C1016-50), and eluted in nuclease free water (Ambion, Cat: AM9930). Cut primers, complementary to the EcoRI and KpnI restriction sites were then annealed to the ssDNA probes before performing a double restriction digest for 16 hours at 37°C using high fidelity enzymes (NEB Cat: R3101M, R3142M) to cleave off the forward and reverse primers. Finally, the ssDNA probes were purified using a spin column (Zymo, Cat: C1016-50) or magnetic beads (Beckman Coulter, Cat: A63882) and eluted in nuclease-free water. Probes were dried and stored at -20°C. The primers used for PCR are f'AACGAACGGAGGGTCATTGG' (SEQ ID NO: 9) and

'TAATACGACTCACTATAGGGAGGCTCTACTCGCATTAGGG' (SEQ ID NO: 10); the primers used for restriction digestion are 'TACTCGCATTAGGGGAATTCNN' (SEQ ID NO: 11) and 'NNGTACCCCAATGACCCTCCGT' (SEQ ID NO: 12).

Cell culture sample preparation. Human foreskin fibroblasts (ATCC® CRL-2097™), human A549 (ATCC® CCL-185™), and AML12 (ATCC® CRL-2254™) cells were cultured in Dulbecco's High Glucose Modified Eagles Medium (Hyclone™ Cat: SH30022.01), supplemented with 10% fetal bovine serum (Thermofisher, Cat: 26140079). A549 cells were cultured in DMEM/F12 1:1, supplemented with 10% fetal bovine serum. Cells were grown in 6-well plates on 22 mm x 22 mm No.1 coverslips (Marienfeld-Superior Cat: 0101050) for the XLOC_010514 and MUC5AC experiments, or 40 mm diameter No.1 coverslips (Warner Instruments Cat: 64-1500) for the FLNA experiments. Cells were grown to ~80% confluency before fixation in 4% vol/vol paraformaldehyde (Electron Microscopy Sciences Cat: 15714) in 1x PBS for 15 minutes at room temperature. Following fixation, the samples were quenched in 0.1 M Glycine (1st BASE) for 1 minute at room temperature. The cells were then permeabilized in 70% ethanol overnight at 4°C.

Tissue sample preparation and coverslip functionalization. All animal care and experiments were carried out in accordance with Agency for Science, Technology and Research (A*STAR) Institutional Animal Care and Use Committee (IACUC) guidelines. Coverslip functionalization and tissue processing were based on a slightly modified version of a previously published protocol³. Briefly, coverslips (Warner Instruments Cat: 64-1500) were cleaned with 1 M KOH in an ultrasonic water bath for 20 minutes, rinsed thrice with MilliQ water followed by 100% methanol. Then, the coverslips were immersed in an amino-silane

solution (3% vol/vol (3-Aminopropyl)triethoxysilane [MERCK Cat: 440140] 5% vol/vol acetic acid [Sigma Cat: 537020] in methanol) for 2 minutes at room temperature before rinsing thrice with MilliQ water and air dried. Functionalized coverslips can then be used immediately or stored in a dry, desiccated environment at room temperature for several weeks. Histology work was performed by the Advanced Molecular Pathology Laboratory, IMCB, A*STAR, Singapore. Briefly, C57BL/6NTac mice aged 8 weeks (InVivos) were euthanized with ketamine, the kidney, liver, brain, and ovary were quickly harvested, cut to smaller pieces, and frozen immediately in Optimal Cutting Temperature compound (Tissue-Tek O.C.T.; VWR, 25608-930), and stored at -80°C. 7 µm sections of fresh frozen tissues were cut using a cryotome onto functionalized coverslips. Sections were air-dried for 5 minutes at room temperature prior to fixation in 4% vol/vol paraformaldehyde in 1x PBS for 15 minutes. Following fixation, samples were rinsed once with 1x PBS and either permeabilized in 70% ethanol overnight at 4°C or stored at -80°C.

XLOC_010514, MUC5AC, and FLNA experiments. After permeabilization, the cultured cells were equilibrated to room temperature before rehydration in 2x saline-sodium citrate (SSC, Axil Scientific Cat: BUF-3050-20X1L) for 5 minutes. Samples were incubated in a 10% formamide wash buffer, containing 10% deionized formamide (Ambion™ Cat: AM9342, AM9344) and 2x SSC, for 30 minutes at room temperature. The split probes were diluted in a 10% hybridization buffer to a final concentration of 20 nM per probe. The 10% hybridization buffer composed of 10% deionized formamide (vol/vol) and 10% dextran sulfate (Sigma Cat: D8906) (wt/vol) in 2x SSC. The encoding probes were stained overnight at 37°C in a humidified chamber. Following hybridization of the encoding probes, the samples were washed in a 10% formamide wash buffer twice, incubating for 15 minutes at 37°C per wash. The samples were then removed from the 10% formamide wash buffer and stained with either the bridge probe or the conventional readout probe. The probes were diluted to a concentration of 10 nM in 10% hybridization buffer and stained for 20 minutes at room temperature. The cells were then washed once with 10% formamide wash buffer and then twice with 2x SSC at room temperature. DAPI (Sigma Cat: D9564) was stained at a concentration of 1 µg/mL in 2x SSC for 10 minutes at room temperature. The samples were then washed twice with 2x SSC and either imaged immediately or stored for no longer than 12 hours at 4°C in 2x SSC before imaging. The list of XLOC_010514, MUC5AC, and FLNA sequences can be found in Table 7, 8, and 9 respectively.

Multiplexed FISH experiments in tissue. Tissue samples were stained as described above, using 20% formamide concentration in the hybridization and wash buffers instead of 10%. For tissue samples, pre-hybridization was also extended to 3 hours at 37°C in 20% formamide wash buffer. The samples were stained overnight or longer at a final probe concentration of 500 μ M (2 to 3 fold higher concentration than used in the conventional experiment) in 20% hybridization buffer. After two 20% formamide washes, the samples were washed twice with 2x SSC and either imaged immediately or stored in 2x SSC for no longer than one week at 4°C prior to imaging.

Split-FISH imaging cycle. Samples were then mounted into a flow chamber (Biopetechs Cat: FCS2), which was secured to the microscope stage. Hybridization of the bridge and readout probes in the flow chamber was done sequentially by buffer exchange controlled by a custom-built, computer-controlled fluidics system. The system consisted of three daisy-chained eight-way valves for buffer selection and a peristaltic pump providing the driving force for fluid flow, as previously described. The bridge probe solution contained 5 nM of each bridge sequence in a 10% hybridization buffer. The sample was incubated in the solution for 10 minutes at room temperature. Next, 5 nM of fluorescently labeled readout probe in 10% hybridization buffer was flowed into the chamber and incubated for another 10 minutes at room temperature. Following hybridization, the sample was washed with 10% formamide wash buffer to remove unbound probes. Imaging buffer was then flowed into the chamber before images were acquired. The imaging buffer consisted of 2x SSC, 50 mM Tris-HCl pH 8, 10% glucose, 2 mM Trolox (Sigma, Cat: 238813), 0.5 mg/ml glucose oxidase (Sigma, Cat: G2133) and 40 μ g/ml catalase (Sigma, Cat: C30). To remove the fluorescent signals, the samples were washed with 40% formamide wash buffer. This hybridization and wash cycle was repeated until all the bits were imaged. With two-color imaging, 26 bits were completed in 13 cycles. 133-genes (Modified Hamming Distance 4) multiplexed FISH imaging using the conventional probes was performed as previously described. The conventional probe library correlated well with bulk RNA-seq (Fig. 11).

Imaging Setup 1. The XLOC_010514 and MUC5AC experiments were performed using a custom-built microscope that was constructed around a Nikon Ti-E body, MS-200 ASI X-Y stage, CFI Plan Apo Lambda 100 $\times$ 1.45 N.A. oil-immersion objective, and Andor iXon Ultra 888 EMCCD camera. DAPI was excited by 405 nm (LuxX, 405-20), and Cy5 was excited by

638 nm (LuxX, 638-100) solid-state lasers (Omicron). Z-stacks, of 400 nm apart, were obtained for each laser excitation for five different Z positions. The exposure time was 1 second.

Imaging Setup 2. The FLNA and multiplexed FISH experiments were performed using a second custom-built microscope that was constructed around a Nikon Ti2-E body, Marzhauser SCANplus IM 130 x 85 motorized X-Y stage, a Nikon CFI Plan Apo Lambda 60x 1.4 N.A. oil-immersion objective, and an Andor Sona 4.2B-11 sCMOS camera. Focus was maintained using the Nikon Perfect Focus system and only one Z position was imaged per field of view per cycle. The DAPI channel was excited by a Coherent Obis 405 100 mW laser. The following two fiber lasers from MPB Communications: 2RU-VFL-P-1000-647-B1R (1000 mW), 2RU-VFL-P-500-750-B1R (500 mW) were used as illumination for Cy5 (647 nm) and Alexa750 (750 nm) respectively. All laser channels were combined and launched into a Newport F-SM8-C-2FCA fiber. The resulting beam was collimated and flattened using an AdlOptica 6_6 series Pi-shaper, then expanded before being sent into a 300 mm lens near the back-port of the Ti-2 to illuminate an approximately 230 μm x 230 μm field of view. Custom multi-wavelength filters ZET488/532/592/647/750m (Chroma) and ZT488/532/592/647/750rpc-UF2 (Chroma) were used. A Finger Lakes Instrumentation HS-632 High Speed Filter Wheel, containing FF01-433/24-32, FF02-684/24-32 and FF01-776/LP-32 emission filters (Semrock), was attached to the output port between the microscope and the camera, allowing different emission filters to be used when imaging respective channels. The exposure time was 500 ms.

Image analysis. The multiplexed FISH images were processed by a custom Python pipeline, following a previously published approach but with modified pre-processing, gene callout filtering, and mosaic-stitching procedures. Briefly, the images from each hybridization cycle were first corrected for field and chromatic distortion. Images were then registered for translation relative to a selected frame in the Cy5 channel by phase correlation using a subpixel registration algorithm provided in the Scikit-image package. For each dataset, a global bit-wise normalization was performed by pooling all pixels above the 99.9th percentile of intensity in each field of view, then taking the 50th percentile of the pooled pixel intensities as a normalization value for the bit. Images were filtered in the frequency domain using a second order 2D band-pass Butterworth filter to remove cell background (low frequency cutoff) and camera noise (high frequency cutoff). The n-dimensional vector (where n is the number of bits) for each aligned pixel is then normalized to the unit length by dividing by its magnitude (L2 norm). The same normalization was done for each code-word in the set of genes. The Euclidean

distance from the pixel vector to each gene's code-word was then calculated. All pixels were filtered for maximum Euclidean distance (distance threshold) to a gene's code-word, using a threshold of 0.52 for conventional and 0.33 for split-FISH. The L2 norm of each pixel vector was used as a second filter (magnitude threshold) to remove called pixels with too low intensities. The called and filtered pixels were then grouped into connected regions (4-connected neighbourhood) for each gene. Regions with only 1 pixel were subject to a second more stringent intensity threshold. Sets of parameters which yielded both good correlation to bulk FPKM counts and high gene counts were chosen. The number of regions for each gene across all fields of view was then summed, and total counts for each gene compared to the respective FPKM values by calculating the Pearson correlation. The FPKM values from bulk RNA sequencing of mouse tissues were downloaded from the ENCODE portal (<https://www.encodeproject.org/>) with the following identifiers: ENCSR000BZC (ovary), ENCFF478QMU (kidney replicate 1), ENCFF638NYA (kidney replicate 2), ENCFF844MJF (liver replicate 1), ENCFF271DWG (liver replicate 2), ENCFF653BKJ (frontal cortex replicate 1), and ENCFF703SOK (frontal cortex replicate 2). The FPKM values of AML12 cell line was obtained by performing bulk RNA sequencing in-house. Briefly, RNA was extracted using Isolate II RNA Mini Kit (Bioline), sequencing was performed at the GIS next generation sequencing platform, A*STAR, Singapore, and the sequences were analyzed using Salmon. The list of FPKM values (or their mean if the tissue has sequencing replicates) used for the Pearson correlation analysis is listed in Table 10. Cells were manually counted using the DAPI and RNA images. For the split-FISH library, 789, 4043, 7484, 13405, and 26001 cells were imaged for the AML-12, brain, liver, ovary and kidney experiments respectively. For the conventional library, 1382, 2581 and 2729 cells were imaged for the AML-12, brain and liver experiments respectively. Brightness and spot counting analysis for the MUC5AC and FLNA experiments (for Figure 3 and 7) were done using a multi-Gaussian-fitting algorithm, as previously described. For mosaic stitching in tissue samples, adjacent field of view (FOV) alignments were estimated using the phase correlation algorithm from Scikit-Image modified to output a value for the phase correlation peak magnitude, which is an indication of registration accuracy. A graph with FOVs as vertices and edges weighted by the negative of the phase correlation peak value was generated. The full mosaic was then stitched by calculating the minimum spanning tree (SciPy) and shifting each field of view accordingly. Overlapping regions were blended using maximum intensity projection.

EXAMPLE 1

First, the split probe sequence was optimized using single-molecule FISH on MUC5AC transcripts in A549 cells (Fig. 1). It was reasoned that the length of the complementary sequences between the bridge probe and either of the encoding probes has to be shorter in length than in conventional multiplexed FISH to prevent any single and unpaired off-target encoding probe from binding to the readout probe. Thus, the length of the split bridge sequence was titrated and it was discovered that nine or fewer nucleotides is required to produce a level of non-specific background signal that is virtually undetectable (Fig. 1). Several pairing schemes were further screened, including circular, cruciform, double 'C', and double 'Z' (Fig. 3), and it was found that the circular construct produces the brightest on-target signal. It had a mean brightness that was ~4.7 fold higher than the double 'Z' construct. Importantly, the circular construct scheme produced a signal intensity that is comparable to the conventional readout scheme, indicating that RNA brightness was not compromised as a result of eliminating non-specific probe binding. To further test the optimized split probe construct, single-molecule FISH was performed on the long non-coding RNA XLOC_010514, for which one of the probes is known to non-specifically bind to off-targets within the cell nuclei, which was shown in a previous study (Fig. 5). The split probe approach successfully quells the signals arising from the non-specific binding, suggesting that there is no need to remove or even know the non-specific 'rogue' sequence *a priori*.

Next, the inventors focused on optimizing the split-FISH workflow (Fig. 6a). It was found that the primers used for oligo library amplification impeded the circularization of the adjoining probe pairs, so restriction sites adjacent to primer sequences were incorporated, allowing the primers to be cleaved off by restriction digestion (Fig. 2). It was also observed that different bridge probe sequences yielded varying RNA spot brightness. Thus, several sequences were screened, and those that yielded the highest brightness within 10 minutes of hybridization time were selected. With the optimized design, the inventors were able to perform multiple iterations of hybridization and washing (at least 20 rounds) without any observable loss of FISH signal or RNA counts (Fig. 7).

The performance of split-FISH was then compared against conventional multiplexed FISH in mouse cell cultures and mouse tissue slices. To demonstrate the combinatorial labelling of RNAs, 317 genes were randomly selected as targets, and 26 barcoded bridge sequences were designed. An 'N Choose 2' barcoding scheme (Table 2) was designed by assigning each of the

two required barcodes to half of the available encoding probes (Table 1). Compared with samples stained with the conventional probe library, samples stained with the split probe library showed decreases in non-specific background (estimated as the median value of all the raw images) that was about 16% in cultured mouse hepatocytes (AML12, Fig. 8b, c) and about 44% in brain tissue slices (Fig. 8d, e). The number of detected RNAs in AML12 correlated well with bulk RNA-seq (log Pearson correlation of 0.7) and conventional multiplexed FISH (10 common genes, log Pearson correlation of 0.97) (Fig. 5a). The average false positive rate (estimated using number of blank code-words detected per cell) in AML12 (0.13 ± 0.015 per cell, S.E.M. $n = 8$ replicates) was comparable to that previously reported while using conventional multiplexed FISH in a cleared U-2OS cell-line sample (0.08 ± 0.03 per cell).

To demonstrate that split-FISH works robustly without any tissue-specific clearing, the same probe set for the 317 genes was used and split-FISH imaging of three additional mouse tissues—kidney, liver, and ovary was performed. The transcript counts from all the tissues also correlated strongly with bulk RNA-seq results, with log Pearson correlation values between 0.54 and 0.75 (Fig. 5b). Images taken after washing also confirmed that off-target binding is the main contributor to background signal, and tissue auto-fluorescence in our detection channels was insignificant in comparison (Fig. 9). The average false positive rates of split-FISH in brain, kidney, liver, and ovary were 0.012 ± 0.002 , 0.0042 ± 0.0004 , 0.008 ± 0.003 , and 0.03 ± 0.009 per cell respectively (S.E.M., $n = 8$ replicates). In fact, the false positive rates were lower by ~44 fold (in brain tissue), and ~19 fold (in liver tissue) compared to the conventional multiplexed FISH (Welch's t-test, p-values of 0.020 and 0.014 respectively, Fig. 5a), despite employing a barcoding scheme with lower Hamming distance. This confirmed that non-specific probe binding was contributing to false positive signals.

For each tissue type that was imaged, diverse localization patterns of the single-cell transcriptome was observed. For example, *Map4* transcripts were found to be highly enriched in the neuronal processes in the frontal cortex (Fig. 10a), and *Ahnak* was found predominantly lining the portal veins in the liver (Fig. 10d). Distinct zonation patterns of certain transcripts (e.g., *Osbpl8*, *Ppl*, and *Notch 3*) in the kidney tissue (Fig. 10b) suggest a spatial division of labor previously observed in liver via single molecule FISH. Some transcripts, such as *Slc12a7*, *Plxnc1*, and *Dsp*, were highly compartmentalized in the mouse ovary, possibly corresponding to different maturation stages of the follicles (Fig. 10c). In the mouse liver tissue, the transcripts

of Son and Abcc2 were found to be highly localized to the nucleus in the cells, highlighting the power of multiplexed FISH to distinguish subcellular features in tissue samples.

In conclusion, the inventors showed accurate multiplexed FISH of 317 genes in diverse mouse tissues without requiring tissue clearing, demonstrating the prowess of split-FISH not only in simplifying tissue preparation protocols for multiplexed FISH, but also in broadening the range of accessible tissue types.

Table 1: 317-genes split library template sequences. Template sequences include the forward and reverse primer sequences necessary for library amplification. The template sequences for 1 target gene is shown below.

Target Gene	Transcript ID	Template Sequence
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GGGTTACTGCCGGCTGCAGCAGCCA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 13)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CGGAGGCGGAGGCCGAGGAGGCAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 14)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AGCAGCTCCACGGGCTTGACCGCGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 15)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCGCATGATGCGCTCGCGCTCGGCG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 16)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GGCTCTTTCTACCTGTCCGAGAAC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 17)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTGTTCTTGGCGAAGCTCGAGGTG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 18)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AGTGGCTCAGGTCTCCGAGGCTGAA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 19)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT TGGTGCCGGCCAGCATGAGGGCGG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 20)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CGCTGCTTCTCTCGCTCCTCGCGCT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 21)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTCCGCCCGCAGCAGTCCCTGACGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 22)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCGCCGCCGCTGGGCGCGCTCCG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 23)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCTGCTGCCGACTCGGCCTTAAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 24)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CGCGTCCCGCAGCTCCCGGGCGCG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 25)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCCGTGTTCTCCGCCCGGCCAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 26)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GTCTTCGTCTCTCTACCTTCTCC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 27)

Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CCGGGCCGTGGAGCGAGCGCTCTCC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 28)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AGAGAGGCCACACCTGGCCATATCC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 29)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AATACCATGTCTGCCTTCTCAGTGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 30)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTACAGGGACCTTCTCTCCAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 31)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AAGATAGAGAGGTAACTCTGGGAGA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 32)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GGGAACAGGATAATTTGTTACGTG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 33)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTGGAGGTTGGAGGGCTCCATTAA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 34)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GAAGGTAGCTCTACTTCGTAAGGCA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 35)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCAGCCAAGGATCCTAAATTGTCTT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 36)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTTCAGGTATAAGTGAGAGGGTACC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 37)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GCCTCCCACTCCAGGTCAAGAAAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 38)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CCACACACCTTGGATGCTAAATG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 39)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GACTTTCCACCTCCCTGGTGAAGA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 40)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CATTATCTACACGTGACACCTAAA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 41)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTGTCTTAGAGACAACACCAGTTAT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 42)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT AATGCTCATGCTTTGAAGTAAGTAC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 43)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT GTCGGTGAGGACTAAGAAAGGTGAC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 44)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CCGTGTACGGCACTGCACCTTCAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 45)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT ACGTACATGTGACTCTGGATCTGA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 46)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT ATATGGTCTGGAACCTGAAGGTACC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 47)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTCTACC TAT CTATCTGGTATGGGCTGACTCAATA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 48)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTCTCTCCATGGGAGACTACGAACCT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 49)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCTGCTGGGCTCCCTGGGCATCGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 50)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGGGCGCAGGCCTCCAGAGAGCGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 51)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCACTGCTGCAGTTTGGCCAGGCGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT

		(SEQ ID NO: 52)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGGGCGGAGGCCGGCTCCTGGCGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 53)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAGCGAAGCTCCCGAAGGCTCTCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 54)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TGCGGGCCGTGAGCGGAACCAGAGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 55)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ATCTTGCGGTGCGCTCGGGTACCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 56)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGCCGCTGTTGCGCGCGCACGCGCT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 57)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGCCAGCTCCCGCCGCGCTCCTCG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 58)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGACCAGCTCCGCGCGCTCCCGCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 59)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCGCCTCGTGGCGCGCCGCTCGGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 60)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTTCCTGCAGCTGCTCGTAGTTCTC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 61)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CCTCCAAGTGCCTGATGCTCCCG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 62)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTTGGCACGCTGGGCCCTCTCCTTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 63)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGCGCTTCGCGGTCTCGAGAGAGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 64)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAGCACGGTCTGTCCAGGGCGGCTG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 65)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CATTAAATGTTTCATTAGTCTGGAGA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 66)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CTTTCTATTCCAACCCAAAGACACC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 67)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTCCCTCCGCTGCTGCTACTTAAAT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 68)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAATTCACAGTCTAGCGATAGCTTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 69)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CTAAGCAGCCTCACCGATCCAGCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 70)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGCTTCTGTCTGCAGGAGCTGGCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 71)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GAACGCTGAACTAAGTCACAGAGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 72)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GAATTTAAAGAGGGAAGACTCCTTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 73)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ACAAGGAGACCAACAATAACACT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 74)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CATTTGTCTGTGATGCACTGCCCTG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 75)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC AAGCTGACGGGTGGATCTGAGTGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 76)

Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAGCTTTCTTCTACACAAGACTCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 77)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAGCTCACTGACAAGGAAATTAAGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 78)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TCAAGGGTAGAACTTTCCATGGAGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 79)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ACCTGAAGTCGCTCAGTAAGGTGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 80)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CCAAGGTGACAGAAACAGGAAATAT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 81)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GAGCTTCTCAAGACTGTTCTTCTG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 82)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTCTTACTGTCACTAGGTCTGAATT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 83)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ATTATCCATCTACTTCAGCATCCTC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 84)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT TCTTCTGGCACC GGTCACCATAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 85)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT AAGAGATCGAGGTGCAGCAACGGAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 86)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CGCCTCGTAGGCCTCGTACAGGCCG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 87)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GGCGGGCGAGGCCGGGAGGCTGCTG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 88)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CGGACTCGGAGCTCAGGCAACGGTC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 89)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GGGCCGAAGGGCGCCAGTGGTT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 90)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GCTCGGCGCGCACTTGGCGGACGAT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 91)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT TGCTCCCACTGGCCGTGGGCCGCGG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 92)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GCGCTCGCACTGCTGCTGCCGCTC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 93)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CTGCTCCTGCTGCAGCTTGCACAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 94)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GGCTCAGCTCGCGCTTCTCGCGCTG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 95)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT GCGCGCGGCCAAGCTGGCCTCCAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 96)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CTTTGCGCTCGGCCGCTTCTTCGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 97)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CGCGCCGGGCTTCTGCTGCACAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 98)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT CTCACTGCGCTCCAGCTTGCGCCA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 99)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT ATTTGCGGTCCAGGCTGGCATGTAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 100)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CACTTACCC TAT TCATAGTGTGGCAACTGGACTGGAG GAATTC CCCTAATGCGAGTAGAGCCT

		(SEQ ID NO: 101)	
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TGGATGTGGGTAGATTGTGCCATGT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 102)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TCTTGAACACAATTGTTAGAAAGAT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 103)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC AGACGAGCTGTGCTTCTTGGGAAA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 104)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TTATTATCTGTAGATGATTGTCTAA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 105)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC CATCAGACACTGCCTCCACTTACGT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 106)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TCCAGACTGCATTCAAGGCTGGAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 107)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC AACATAGCACAGCTCCACACAGGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 108)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC AGTGTGCTTTGGATCCAAGAGACC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 109)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TAATGCCAGCAATCCATGTACCAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 110)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC AACTGAACCTTCTGACCAGGGAGCG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 111)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC AGACACACTAACACGTCTAATGCAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 112)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC GAACCCACCCACTCAAGAAACAAGC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 113)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TCAGAAGCAACCCAGGGACCACAAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 114)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TCGCTCGCGTCTTCCCAGTGCTTG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 115)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC CCGTCAATTCGTTTGCACAACTA GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 116)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC CCAAATCCTGATGGTACATTACACT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 117)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC TTACATAGTGGTTCTCTCAGTGTCT GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 118)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC GGTTAGGTATAGAGATGTGAGCCAC GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 119)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTAC CACTTACCC GAAAGAAACATGAACATTTCTTTAG GAATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 120)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GAACCTCGTTCTTTGTTGGAGTCCC ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 121)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TGCTCCCGACGTCCACCTTCTGCC ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 122)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGCCACGCGCATGGAGCGGCCGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 123)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCTGCTGCAGCTGCTGCTGCTGCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 124)	TAT
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TCCGCGGGAGAGCGAGTCCAGCGA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 125)	TAT

Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTGCAGACGACGCCGAGCCCGCGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 126)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCTCCACATGCTGGGCTGTCTGCGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 127)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGCTGCTCCAGCAACAGGCGCTCTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 128)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGCGGCCCTCGCGCTCCTCCCGGCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 129)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTCGTCGCCGCGCGCGCTTCCACC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 130)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GTTGGCCCTCTTGCGCTCTCGGGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 131)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC AGCTCCGACGGCCCTCCCGCTCCTC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 132)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCCGACCCCTGGAGCTCCTCTCTCCG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 133)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CTTGCGCCACCTGCGCCGCTGCTG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 134)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGCCTGCAGCAGCTCGCGCGCGCGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 135)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGGCCTCTCGACCATGCGGTCAA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 136)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GCCTGGAGTTTGGTCAAGGTGACTA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 137)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTTCCAAATGGAGTAAGTCTGTAAA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 138)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CCAAGGCAGCTGTGGCAGAGAGCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 139)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTCAAGTCCTGTGCAACTTGATTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 140)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC AGAAAGCCAAGGGAAGTCAACTGAAA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 141)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TCACCTTCCATGGAAGCCTCATGGG TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 142)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TAAGCAAGAGTGGCCTGGGCAGGCA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 143)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTCTAGCCCTTCAGCCACCACAGGC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 144)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GAGGTACCAGAAGAGTTGATGGCCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 145)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ACAAACTAGGCTAACAGCTGTCTC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 146)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTGGTATTGCAAGTACCAAGTACCC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 147)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CGTCTCTACCCTGATTCTTTCTTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 148)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAGTACATAGCTCCTTTCCTGGTAC TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 149)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC CAAAGGACCATAATCACATTAATTT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT

		(SEQ ID NO: 150)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TCAGAACACCCGATTGCCTCCTCAA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 151)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC GGTACGGAACCATGATCTCAGTTCT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 152)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC ACAGTACGGCGCAGGAGCCATGGAG TAT ATTAAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 153)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TTTGTCAACATTCTGGCCTGGGCCT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 154)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TGTCCAACCTTCAGTAGAGTATCCTA TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 155)
Ccdc177	ENSMUST00000073251.6	AACGAACGGAGGGTCATTGG GGTACC TATACAGGTATTCTCCTTGCAATT TAT ATTTAACCG AATTC CCCTAATGCGAGTAGAGCCT (SEQ ID NO: 156)

Table 2 Codebook for each gene in the 317-genes split probe library. The binary code-word assigned to each gene in the 317-genes split probe library.

Gene	Code-word
Ccdc177	010010000000000000000000000000
RP23-383N15.1	0000000000000000000001010000
RP24-338G10.1	0000000100000000000001000000
Myh11	0000000010000100000000000000
Xlrp1	00000000000000000000000000011
Col6a5	000000000000000010000000100
Abca9	0000001000000000000100000000
Pkdrej	0000100000000000100000000000
Col6a6	0000001000100000000000000000
Igsf10	0101000000000000000000000000
Tmc3	0000000000100100000000000000
Wdfy4	000000000000000000000100100
Scn10a	0000010010000000000000000000
Fat4	000000000000000000000010001
Hmcn2	0000000001000010000000000000
Zfp831	0010000000000000100000000000
Map3k9	000010000000000000000010000
Scn7a	0000000000100000100000000000
Nat8l	0001010000000000000000000000
Sdk1	0001000000000000000000000010
Dnah8	0000000000001010000000000000
Dnah11	0010000000001000000000000000
Pappa	0010000000000010000000000000
Spag17	0000000010000000000100000000
Trabd2b	0000000000000001000010000000
Prtg	0001000000100000000000000000

Grik3	00000000001010000000000000
Gpr179	0000000000100010000000000000
Edaradd	000000000010000000000001000
Akap6	000000000001000000000000100
Adamts17	0000000000000110000000000000
Map1a	0000001010000000000000000000
Bend4	0001100000000000000000000000
Reps2	000000000000000100000010000
Armxc4	0010000000000100000000000000
Plcb2	0000000000110000000000000000
Adam19	0000001000000000100000000000
Galnt15	0010100000000000000000000000
Pygo1	0000000000000000000001000010
Trxb	1001000000000000000000000000
Stab2	1000000000000010000000000000
Ptprz1	0010000000000000000000000100
Kif5a	0000000000000010000001000000
Dnah1	0000110000000000000000000000
Chst2	0000001000000100000000000000
Cacna1c	0010000000000000000000000010
Col4a4	0000000001000000000000000010
Scn4a	000000000000000001000001000
Sdk2	0000000000000011000000000000
Pcdhb19	0000000000000000000011000000
Myom2	0100000001000000000000000000
Fgd5	00000000001000000000010000
Kif1a	0100010000000000000000000000
Caskin1	0000010000000000100000000000
Csmd1	0000000000000000100010000000
Acacb	0100000000000000100000000000
Ksr2	0000100000000100000000000000
Mgam	0000000000000000001100000000
Plxnc1	1000000000000001000000000000
Pcdhb22	0000000000000100000010000000
Impg2	0000000000000010000000000001
Sptbn2	0100000000000000000000010000
Rnf150	0000000100000100000000000000
Myh7b	0001000000000000010000000000
Adamts2	1000000000000000000000000100
Ppfia2	0000010000000000000001000000
Camk4	1000010000000000000000000000
Rims3	0000000000000010000000000010
Figf	0100000000010000000000000000
Gpr116	1000100000000000000000000000
Prune2	1000000000000000000000000010

Erc2	000000010000000000000001000
Abca1	000000000000010001000000000
Notch3	000000000000000000100010000
Dock10	000000000001000000000000001
Ntrk2	100000000100000000000000000
Sym	000000000000000000000100001
Pkd1l3	0000001000000000000000010000
Mrc2	000000000000000001010000000
Foxo1	0000100000000000000001000000
Il17rd	1000000000000000000000010000
Myom3	000000010001000000000000000
Rassf4	000000010000000010000000000
Ddr2	0100000000000000000100000000
Trpm6	000000010000000000000000010
Spon1	0000001000000000000000000100
Celsr1	0000010100000000000000000000
Aldh1l2	0000000000000100000000100000
Parp14	00010000000000000000010000000
Daam2	0000000000000000001000000100
Ago4	0000000010000000000001000000
Ctlf	0000000000000000000000010100
Setd1b	0000001000000010000000000000
Pla2r1	00000000000100000000000000001
Gpr161	0000010000000000000000000100
Dock6	0010010000000000000000000000
Gm20342	0000000000000000000100001000
Trpm2	0000000000000000000100100000
Lgr6	0000000000010000000000000010
Fry	0000000001001000000000000000
Flrt2	00000000000000000100000000001
Ptpm	01000000000000000000000000010
Srgap3	0001000000000000000000010000
Dock8	0000000000001000100000000000
Shroom3	0001000000001000000000000000
Sned1	0000000000000000000000001100
Gm29666	0000000000001000000000010000
Nhs12	00000000000000000000000001010
Arhgap31	0000000100000000001000000000
Zfp334	0001000000000000000001000000
Cdyl2	0000000000001000000100000000
Veph1	10000000000000000010000000000
Slc38a1	00000000000000000001000100000
Hspg2	000000001000000000000100000
Itga4	1000000000000000000001000000
Amer1	000000000000000000000100010

Thsd7a	0000001000010000000000000000
Plxnd1	0000000000000100000000000100
Crb2	0000100000000001000000000000
Dock4	1000000100000000000000000000
Tbc1d32	0100000000000000010000000000
Onecut2	00000000000000000010100000
Polr2a	0011000000000000000000000000
Zfyve26	0001000000000100000000000000
Irgq	0100000000000000000000000100
Cacna1a	0100000000010000000000000000
Alms1	0100000000000100000000000000
Arhgef10	0100000000000001000000000000
Zbtb1	00000000010000000000010000
Dip2c	00000010000000000100000000
Rttm	000000100000000000001000000
Prdm15	000000000000100000000001000
Kmt2c	0000000000100010000000000000
Lsm11	000000000000000100000000100
Kif13a	000000001000001000000000000
Usp36	00000001000000000001000000
Rnf213	000000000000000000000000110
Spg11	00000000000010000010000000
Dcaf5	1100000000000000000000000000
Cmah	0000000000000000000000011000
Trim56	000010000000000000010000000
Thada	000000000001010000000000000
Col7a1	000000000010000100000000000
Fancm	001000000000000000001000000
Rprd2	000010000000010000000000000
Rnf217	000000001100000000000000000
Med12	00000000000100000000100000
Sestd1	00000000000010000000000001
Flnc	000010000000000000000100000
Itpr1	000010000000000000000000010
AU022252	000001000010000000000000000
Sh3pxd2b	000100000000100000000000000
Rfx7	000000000000010100000000000
Wdr7	000001000000000000000001000
Brca2	01000000000000000000100000
Wdr81	00000000000000000000101000
Trnc18	00000000000000010010000000
Ep300	100000000000000000000001000
Piezo1	000000000000100100000000000
Atxn1l	00100000000000000000010000
Plcxd2	000000001001000000000000000

Atg2b	00000000010000000010000000
Nf1	000000000000000001001000000
Wdr90	000000100000000100000000000
Rnf169	100000000000100000000000000
Myo9a	0000100000000000000000000001
Ly75	000001000001000000000000000
Sox11	001000010000000000000000000
Klf21a	000000000000000100000000010
Btrc	100000001000000000000000000
Dock9	100000000000000001000000000
Ubn1	000000000000000010000010000
Pask	000000000100000001000000000
Svil	000000001010000000000000000
Rapgef1	00000000000000000010000010
Nedd4l	000000000000000001000000001
Gas2l3	00000000000000000010010000
2410089E03Rik	000000100000000000000100000
Sec16a	000100000000000000000001000
Dpy19l4	00000000010000000001000000
Ep400	000000010100000000000000000
Nin	000000000000000000100000100
Nipbl	000000000000000010000001000
Utp14b	000000000000001000100000000
Slc12a7	0000000000000000100000001000
Akap11	000000000000000010000000010
Smg7	000100001000000000000000000
Brip1	000000000001100000000000000
Ythdc2	000000000000000000100000001
Rc3h1	000100000000000000100000000
Utrn	00000000001000000001000000
Rsf1	000000000000000000001001000
Zfyve16	000100000000000000000000100
4932438A13Rik	000000000000000001000000010
Cdc42bpb	000000000010000000010000000
Trove2	000000000000000101000000000
Abcc2	000000100000000010000000000
Gtf3c4	1000000000000000000000000001
1700020114Rik	000000000000000000010000100
Luzp1	100000000001000000000000000
Abca3	100000100000000000000000000
Dicer1	001000100000000000000000000
Larp1	000000001000100000000000000
Map4	000000100100000000000000000
Ehbp1l1	000000000000000000101000000
Uhrf1bp1l	00000000000100000000001000

Aspm	00000000011000000000000000
Osbpl8	00000000000100000001000000
Dsp	01000001000000000000000000
Mysm1	00000100010000000000000000
Arhgap35	00000000100000000000001000
Plxna1	00000000100000000000000100
Ppl	00000000000000010000100000
Elf5b	00000001000000001000000000
Atr	00100000010000000000000000
Psd3	00001000001000000000000000
Tmem2	00000000100000000100000000
Cenpe	00000010000000000000000001
Als2cl	00000100000000000100000000
Kntc1	00000000001000000000100000
Zfp26	00000000010000000000001000
Tspan7	10100000000000000000000000
Nvl	00010000000000000000100000
Cacna2d1	00000001000000000000000100
Arhgef12	00000000000000000001000100
Slc4a7	00000000000000000100000001
Sart1	00000011000000000000000000
Brwd1	00000000100000000000000001
Trim33	00100000000000000000100000
Letm1	00000100000000000010000000
Arfgef1	10000000000000000000100000
Nbeal2	00001010000000000000000000
Golga4	10000000000000000010000000
Zbtb41	00010001000000000000000000
Cgnl1	00001000000000001000000000
Sbf1	00000000010000010000000000
Prrc2c	00100000000000010000000000
Myo6	00001000000000000000000100
Spag9	01000000000000000000000100
Edem1	0000000000000000000110000
Heatr1	00000000100000010000000000
Arhgef5	00000000010000001000000000
Dync1h1	01000000000000000001000000
Ogt	00000000000010000000000010
Macf1	00000001001000000000000000
Utp20	00000000000001000000000100
Smchd1	00000000001000000000000100
Pdzd8	00000001000000100000000000
Polr1a	00000100000000000000000010
Arhgef28	00000000100000001000000000
Mki67	00000000000000000001000001

Myo5c	00100000000000000010000000
Tjp1	001000000000000000000000001
Son	000100000000000000000000001
Cnot1	01000000000010000000000000
Mlec	00000100000000100000000000
Mlt4	00000100000000000000000100
Prpf8	00000001000000000000000001
Abhd2	00000000000100000000000010
Myo18a	00000000000010000000010000
Sptlc2	00000000000100100000000000
Ppp2ca	00000000000100001000000000
Aars	01000000100000000000000000
Pten	00000100000000000000010000
Pik3c2a	00010000000000001000000000
Slk	01100000000000000000000000
Cand1	00100000000000001000000000
Trpo1	10000000000000000010000000
Myof	00001001000000000000000000
Flna	00000100000000000000000001
Kprb1	00000100000000010000000000
Ahrak	00000000000001000000000100
Blank1	00000000000000000010001000
Blank2	00000000000001000000010000
Blank3	01000000000000000000000001
Blank4	00000010000000000000000010
Blank5	00000000000001000001000000
Blank6	00000001000000000000010000
Blank7	00000000010000000000000001
Blank8	00010010000000000000000000

Table 3 Bridge sequence for each bit in the 317-genes split probe library. Each bridge sequence consists of three blocks (separated by spaces): a split probe binding block in the centre, flanked by two readout binding blocks. In the split probe binding block, the barcode sequences are in lowercase. Bridge sequences used in AML-12, kidney, frontal cortex, and liver experiments are shown. B1 to B13 were read out by Alexa750, and B14 to B26 were read out by Cy5. For ovary experiments, B1, B3, B8 to B13, B15, and B17 to B20 were read out by Cy5 and B2, B4 to B7, B14, B16, and B21 to B26 were read out by Alexa750.

Bit	Bridge sequence
B1	ATTGTAAAGCGTGAGAAA GGgatagtgGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 157)
B2	ATTGTAAAGCGTGAGAAA GGtagagtgGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 158)
B3	ATTGTAAAGCGTGAGAAA GGattgaggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 159)
B4	ATTGTAAAGCGTGAGAAA GGgtgtgggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 160)

B5	ATTGTAAAGCGTGAGAAA GGgtaagtGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 161)
B6	ATTGTAAAGCGTGAGAAA GGtttgGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 162)
B7	ATTGTAAAGCGTGAGAAA GGaaggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 163)
B8	ATTGTAAAGCGTGAGAAA GGaatggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 164)
B9	ATTGTAAAGCGTGAGAAA GGaagaaggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 165)
B10	ATTGTAAAGCGTGAGAAA GGagagGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 166)
B11	ATTGTAAAGCGTGAGAAA GGtggtGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 167)
B12	ATTGTAAAGCGTGAGAAA GGgagGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 168)
B13	ATTGTAAAGCGTGAGAAA GGattggGCGGTTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 169)
B14	TGATGGGTGCGTGAGTAA GGtatgGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 170)
B15	TGATGGGTGCGTGAGTAA GGgaagtGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 171)
B16	TGATGGGTGCGTGAGTAA GGagatGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 172)
B17	TGATGGGTGCGTGAGTAA GGaggaagGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 173)
B18	TGATGGGTGCGTGAGTAA GGgagaggGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 174)
B19	TGATGGGTGCGTGAGTAA GGtaagtGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 175)
B20	TGATGGGTGCGTGAGTAA GGgtttGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 176)
B21	TGATGGGTGCGTGAGTAA GGtttgGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 177)
B22	TGATGGGTGCGTGAGTAA GGagtGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 178)
B23	TGATGGGTGCGTGAGTAA GGtagGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 179)
B24	TGATGGGTGCGTGAGTAA GGttgtGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 180)
B25	TGATGGGTGCGTGAGTAA GGtggaGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 181)
B26	TGATGGGTGCGTGAGTAA GGtgagGCGGTTAAAT TGATGGGTGCGTGAGTAA (SEQ ID NO: 182)

Table 4: 133-genes conventional library template sequences. Template sequences include the forward and reverse primer sequences necessary for library amplification. The primers used for PCR are ‘TGGTTCAATCGTATGCCCCGT’ (SEQ ID NO: 183) and ‘TAATACGACTCACTATAGGGGTCACCTTAGCCAACGCCGAT’ (SEQ ID NO: 184).

Target Gene	Transcript ID	Template Sequence
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT GTCTGATTCAAATAAGTCAAACACACCTT TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 185)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT ACCTGTGATTGTAATAGATACTATTTCAA TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 186)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT GGAGCTTTCCTGTAACCTTAATATACACTG TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 187)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AAGGTTCAAGACAGTCTTATCTTTCTTT TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 188)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT GGACTAGGAGATATAGTTATATATATTT TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 189)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AGCTGATCCTTCTTCTAAGCAGACACTA TTAGTCGTGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC

		(SEQ ID NO: 190)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT GAGCGTATGCACGCATGTGCAAATGCACAT TTAGTCGTGCGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 191)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT CAACTGGATCTCTACAACATCCATGCATTT TTAGTCGTGCGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 192)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AATGAAATGATTGAGCCATCTAACTTTAAA TTAGTCGTGCGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 193)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT ACATTCGCCTTCTCTCTCTCCCTCTTCT TTAGTCGTGCGCAACGCCAGGCAACTCATGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 194)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AAACCCAAACCCAAACAACAACAACA GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 195)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TGTGATCTCTTTATCATGTGCCTTACGAAT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 196)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AGACAGCTTTAGTCTGGCCAGCCTCTAAA GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 197)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT AATACAAGTGTAGGAAAGGGTGTGTCTAA GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 198)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TATTGTTATTTGGTAGGTGTTTCGATGTTT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 199)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT ATGAATGACCAAATATTACTTTTCAGGTTTC GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 200)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TGCGCATCTCCCAAGTGCACAAACCATAA GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 201)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TGTGTTTGGCAAACCTGAGTAGAGTCTCATT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 202)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT ACAATCTTGCTATATACATAGAGAACATTT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 203)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TTCCTTGCACTCTAGCAATTCAAGGGTTCT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 204)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT CATCACCTTGGCCTTTAAAGTGTGATAAA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 205)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TAAATGTAAGATAATGATTAGTTAGTTAC CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 206)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT CTTCCCTTTCTTTGTGAGTAGATAAGCCCA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 207)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTTGT TAAGGGAACCATTCATAAACCCACCTCTACA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 208)

Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT AGTTGGAGAGAAATTAGGTATTGGATAAAGG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 209)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT CAAACAAGGGCAAGACTACATCTGCTACAG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 210)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT ATTCAATTGGGCAGTGACATTTAGAATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 211)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT ATTATTATGATTAATTAGTACAATTAATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 212)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT AAATCAACAGGAAACCAACAATCAACAAA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 213)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT CCCATGATCGTCCGATCTGGTCGGATTGT ACAATTTACCTCCATAGCAAGTCTACAAA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 214)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC GACTGAGTCCTTAGCTTGCTACTAATTAGT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 215)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC AAACGAAATGAGACTAGGTGATAGATCAAC GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 216)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC ATTCTTCTCTACTATCTTTATAGTTCTTT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 217)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC ACTTCAGATTGGGTGAAGTATTGCCAATTT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 218)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC AATGTAATTACTAAAGAAAGATATACCATT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 219)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC CCCAGTTGAATGCATCCAACACATTTCCAG GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 220)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC CAGCTAGACAAGATGATTAGACTCTGTAAG GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 221)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC ATACTATAGGATTAAGCACTAGCATTGAAA GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 222)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC TCAAGTACTTTCTTAAAGAAACAATAGCAC GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 223)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC TCTTAGGCTCCAGGTTTCGTTTGTTGTTT GTAAGCGCAACGGTGGACCGGAGACGACGG ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 224)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC AGCAAAGGATCCTGCGGTGATGTGGCATT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 225)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC AGTGTTTAGCAGTAGGTACAATGTAAATAT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 226)

Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC TAAATAATTGAGTTGGAAGGCTGCTGATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 227)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC CTGGCCGAAGGAGAGCTAAAGGTGTCTTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 228)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC TTTACACAAGTTTCACTTTGGGCAAGAGAAA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 229)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC GAGAACCTCAGGCTTGAGAAGGAAGAATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 230)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC CATGTACACAGACATGCCACATTTTCATT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 231)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC ACTTCCTTCTGAGTCTTGGGCATGTCACTG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 232)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC CAAATGTCTCCTGCCGTGTGACATGCTGGG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 233)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT TTAGTCGTGCGAACGCCAGGCAACTCATGC ACTTGTGAGTGCACATAGTTGAAAGGTGG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 234)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG TAAATGTCCCTTTCTATCAATCTTGAGTCA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 235)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG TAATTAATTACCTTTAATGAGAGTGGATA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 236)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG AGATGTTAGAGCAATGTACATTTCAATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 237)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG ACAGATCTGGAGAAGGCCACCTATGTGTTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 238)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG GGAGCTACATTGTTAGTGGTTATTTACAG CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 239)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG CACAGCTTCACCAATAAGAACTTGATATT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 240)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG CTTTATCTTCAAGAAGTCACAGAGGCAGAT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 241)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG TAGATTATAATGAGAGATGTATTACATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 242)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG AAGGATTGAACAGATATGGCTTGTTATTT CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 243)
Igf1	ENSMUST00000095360.6	TGGTTCAATCGTATGCCCGT GTAAGCGCAACGGTGGACCGGAGACGACGG AGCCATAGCCTGTGGGCTTGTTGAAGTAAA CGGCCGAATTACATCCGTCGTAATCGAGGC ATCGGCGTTGGCTAAGTGAC (SEQ ID NO: 244)

* Only the template sequence for the first gene is shown in this PDF as the table is too large. The full sequence table can be downloaded as excel file.

Table 5: Readout probe sequences for each of the 16 bits used in the 133-genes conventional library.

Bit	Readout Sequence
B1	CGCAACGCTTGGGACGGTTCCAATCGGATC/3Cy5Sp/ (SEQ ID NO: 245)
B2	CGAATGCTCTGGCCTCGAACGAACGATAGC/3Cy5Sp/ (SEQ ID NO: 246)
B3	ACAAATCCGACCAGATCGGACGATCATGGG/3Cy5Sp/ (SEQ ID NO: 247)
B4	CAAGTATGCAGCGCGATTGACCGTCTCGTT/3Cy5Sp/ (SEQ ID NO: 248)
B5	GCGGGAAGCACGTGGATTAGGGCATCGACC/3Cy5Sp/ (SEQ ID NO: 249)
B6	AAGTCGTACGCCGATGCGCAGCAATCACT/3Cy5Sp/ (SEQ ID NO: 250)
B7	CGAAACATCGGCCACGGTCCCGTTGAACTT/3Cy5Sp/ (SEQ ID NO: 251)
B8	ACGAATCCACCGTCCAGCGCGTCAACAGA/3Cy5Sp/ (SEQ ID NO: 252)
B9	/5IRD800CWN/CGCGAAATCCCCGTAACGAGCGTCCCTTGC (SEQ ID NO: 253)
B10	/5IRD800CWN/GCATGAGTTGCCTGGCGTTGCGACGACTAA (SEQ ID NO: 254)
B11	/5IRD800CWN/CCGTCTCTCCGGTCCACCGTTGCGCTTAC (SEQ ID NO: 255)
B12	/5IRD800CWN/GGCCAATGGCCCAGGTCCGTCACGCAATTT (SEQ ID NO: 256)
B13	/5IRD800CWN/TTGATCGAATCGGAGCGTAGCGGAATCTGC (SEQ ID NO: 257)
B14	/5IRD800CWN/CGCGCGGATCCGCTTGTCGGAACGATAC (SEQ ID NO: 258)
B15	/5IRD800CWN/GCCTCGATTACGACGGATGTAATTCGGCCG (SEQ ID NO: 259)
B16	/5IRD800CWN/GCCCGTATTCGCGTTGCGAGTAGGGCAAT (SEQ ID NO: 260)

Table 6: Codebook for each gene in the 133-genes conventional library. The binary code-word assigned to each gene in the 133-genes conventional library.

Gene	Code-word
Igf1	0 0 1 0 0 0 0 0 0 1 1 0 0 0 1 0
Cps1	1 0 0 0 0 0 0 1 1 0 0 0 1 0 0 0
Glul	0 0 0 0 1 1 0 1 0 0 0 1 0 0 0 0
Glud1	0 0 0 0 0 0 0 1 0 0 0 0 1 1 1 0
Pigr	0 1 0 0 0 1 0 0 0 0 1 0 0 0 1
Pck1	1 0 0 1 0 0 0 0 0 0 1 1 0 0 0 0
Uox	0 1 0 0 0 0 0 0 1 1 0 0 0 1 0 0
Hnf4a	0 0 0 0 1 1 0 0 1 0 0 0 0 0 0 1
Abcc3	0 1 0 1 0 0 0 0 0 1 0 0 0 0 0 1
G6pc	0 0 1 0 1 0 1 1 0 0 0 0 0 0 0 0
Paqr9	0 0 0 0 0 1 1 0 0 1 0 0 0 0 0 1
Cpt1a	0 1 0 0 1 0 0 1 0 1 0 0 0 0 0 0
Acly	1 0 0 0 0 0 1 0 0 1 1 0 0 0 0 0
Ganab	0 0 0 1 0 0 0 0 1 1 1 0 0 0 0 0

Gpam	0010100001000100
Plxnb2	0010010100000010
Dpyd	0111000000010000
Clptm1	0001001001010000
Ddx17	0101011000000000
Scap	0010000000000111
Uba1	0001101000100000
Gns	0101100000000010
Lrrc3	0100001110000000
Fads6	0100000010100001
Hs3st3b1	1010001000000010
Zcchc24	0100001000100010
Cdc42bpb	1000100000001100
Zdhhc5	1010110000000000
Pdxk	0010010010100000
Vwa8	1000001000000101
Flii	0010000001010001
Ube2z	0000001100010010
Slc38a2	0000001101000100
Stat2	0100000100110000
Bcl9l	0000100001110000
Tomm20	1011000000000100
Il4ra	0000111000000010
Nol6	0100000010010010
Ide	1100001000001000
Ddx3x	0000000110100010
Rnf144b	1100010010000000
Mybbp1a	1100000000010100
Hnf1b	1000100010100000
Flnb	0101000000100100
Golga2	0000000001100101
Cdh5	0011100000001000
Tbx3	0000100000100110
Paxip1	0010001000101000
Dennd5a	1000000011010000
Rhbdd2	0000010100000101
Mob3b	0010010000001100
Plekha2	1000000000101010
Ube3a	0001110000000100
Dgkq	1000011100000000
Tecpr1	0001001000000110
Iffo2	1001001010000000
Zhx2	0000101011000000
Gpd2	0110010000000001
Lrat	0001000101000010

Lrtm1	1001010000001000
Tbl1xr1	0100000000001101
Pecam1	0000100010001010
Cog3	0000001010110000
Atp11b	0010011000010000
Add3	0110100010000000
Mcam	0101000010001000
1700017B05Rik	0100101000000100
Megf8	1010000100010000
Tppp	1000000010000110
Vps13c	1100100000000001
Exoc2	0010000010011000
Taok1	1000010001000100
Enpp4	0000011000100100
Des	0000001001001010
Ranbp2	0001100100000001
Golga4	1000100100000010
Sptlc2	0000000110010001
Sox9	0010001010000100
Capn5	0001100010010000
Rasa1	0000001010000011
Vcpip1	1010000001001000
Tln2	1001100001000000
Gpc1	0000001100100001
Adcy7	0100010100001000
Zxdb	0000010011000010
Cdk19	0001000010000101
Chic1	0100010001010000
C3ar1	0010000000110100
Ccdc88b	0011010001000000
Aatk	0001010000010001
Tmed8	1000000101000001
Zfp174	1010000010000001
Fastkd2	0000010101100000
Zfp870	0001000000101001
Dsg1c	0000100001000011
Wdr19	0000110001001000
Mphosph9	0010100000010010
Abca5	0000000010101100
Atp8b5	0001000100010100
Ankrd26	0010000100001001
Tbc1d9	0110000000001010
Zfp560	0010100000100001
Ggt1	0100010000000110
AW554918	0100000001101000

Itga4	0011000100100000
Ammeecr1	0000010010010100
Hecw2	0000000000110011
Fam102b	0000100000010101
Nova2	0000000001010110
Krt7	1000101000010000
Prrg3	0000010000001011
Klf7	0001000000011010
Setbp1	1000000100100100
Pcdh17	0011000010000010
Brip1	0001001100001000
Armcx4	0000011010001000
Scube1	0000100110000100
Alms1	0000000011001001
Dync2h1	1001000000000011
Hcn3	1101000100000000
Sdk1	0110000100000100
Slit3	0000000101011000
Plag1	1000010000010010
Sema3g	0000001000011100
Ncs1	0001000001001100
Egflam	0100110000100000
Lama3	1000000000011001
Igdcc4	0000010000111000
Sgpp2	0000100100101000
Lrrc16b	0001010000100010
Col4a4	1000010000100001
Cnnm1	0100000100000011
Cftr	0110001001000000
Blank1	0001010110000000
Blank2	0010000111000000
Blank3	1110000000100000
Blank4	0100100000011000
Blank5	0011001000000001
Blank6	1100000001000010
Blank7	0000101000001001

Table 7: Probe sequences for the conventional, split probe pairs, and readout probe used in the XLOC_010514 experiment (Figure 4). The known off-target sequence is shown in bold.

XLOC conventional probe sequences

XLOC_1	CATTCCCAGGGACATT CATT TATCCACCGAACCCTTAC (SEQ ID NO: 261)
XLOC_2	GATGACTTCTCTATCCCACTTATCCACCGAACCCTTAC (SEQ ID NO: 262)

XLOC_3	GAAGTGCCTCGTCATTCTGGTATCCACCGAACCCCTTAC (SEQ ID NO: 263)
XLOC_4	GCTCCAGTAACTAGCTGACATATCCACCGAACCCCTTAC (SEQ ID NO: 264)
XLOC_5	AAGCCCAGGGGTACTCCTTATATCCACCGAACCCCTTAC (SEQ ID NO: 265)
XLOC_6	GGAGCCTTGTAATACTGATTATCCACCGAACCCCTTAC (SEQ ID NO: 266)
XLOC_7	GCTGGAACCCCTTAGGAAATATATCCACCGAACCCCTTAC (SEQ ID NO: 267)
XLOC_8	GAGAATGGCAAGAACTCCATTATCCACCGAACCCCTTAC (SEQ ID NO: 268)
XLOC_9	ATGAACAGGCAGAGGCTAGATATCCACCGAACCCCTTAC (SEQ ID NO: 269)
XLOC_10	TACTGATCTGAAGCAGCAGGTATCCACCGAACCCCTTAC (SEQ ID NO: 270)
XLOC_11	CAGACTAAAAGGAGGCCAGGTATCCACCGAACCCCTTAC (SEQ ID NO: 271)
XLOC_12	AGTGAATTGAGAATATGAGGTATCCACCGAACCCCTTAC (SEQ ID NO: 272)
XLOC_13	AGGATAGTAACTCAGTAGAATATCCACCGAACCCCTTAC (SEQ ID NO: 273)
XLOC_14	TGATAGGAGGCATTGTCATTATCCACCGAACCCCTTAC (SEQ ID NO: 274)
XLOC_15	TTGTATCAAACCAAGTCTCTTATCCACCGAACCCCTTAC (SEQ ID NO: 275)
XLOC_16	ACGAGCAGTTGTATGTGAATTATCCACCGAACCCCTTAC (SEQ ID NO: 276)
XLOC_17	TTCCCCAGTTATAGAAATCATATCCACCGAACCCCTTAC (SEQ ID NO: 277)
XLOC_18	TATCTGGGGTAATGGAGACATATCCACCGAACCCCTTAC (SEQ ID NO: 278)
XLOC_19	CGAATTCCTGCACTGGTAATATCCACCGAACCCCTTAC (SEQ ID NO: 279)
XLOC_20	TCAGGTTGTATGATATAGTGTATCCACCGAACCCCTTAC (SEQ ID NO: 280)
XLOC_21	ACTGTCTACTTCACCATGATATCCACCGAACCCCTTAC (SEQ ID NO: 281)
XLOC_22	TAAACATCCATTGCCCTGGTATCCACCGAACCCCTTAC (SEQ ID NO: 282)
XLOC_23	ACAGACCGGTTAACCATCCATATCCACCGAACCCCTTAC (SEQ ID NO: 283)

XLOC split probe sequences

<i>Name</i>	<i>Sequence</i>
XLOC_SplitLeft_1	AACCCTTAC TAT AAATTAACATGCTCGGCCAG (SEQ ID NO: 284)
XLOC_SplitLeft_2	AACCCTTAC TAT AATTCTCCTTGAAGATCATT (SEQ ID NO: 285)
XLOC_SplitLeft_3	AACCCTTAC TAT TCAGACACAGGTGCAGATAG (SEQ ID NO: 286)
XLOC_SplitLeft_4	AACCCTTAC TAT TCCAGTAACTAGCTGACATG (SEQ ID NO: 287)
XLOC_SplitLeft_5	AACCCTTAC TAT CTGATGAAGTGATTAAGGAG (SEQ ID NO: 288)
XLOC_SplitLeft_6	AACCCTTAC TAT AATTACACTGCCTGGCACAT (SEQ ID NO: 289)
XLOC_SplitLeft_7	AACCCTTAC TAT AGTGCCTCAGACACTGCCTG (SEQ ID NO: 290)
XLOC_SplitLeft_8	AACCCTTAC TAT TATCCATGGGCTGCTGGAAC (SEQ ID NO: 291)
XLOC_SplitLeft_9	AACCCTTAC TAT TCTGAGAATGGCAAGAACTC (SEQ ID NO: 292)
XLOC_SplitLeft_10	AACCCTTAC TAT GCAGAGGCTAGAGATGCTCA (SEQ ID NO: 293)
XLOC_SplitLeft_11	AACCCTTAC TAT AGCAGGTCCACGCCAGGTG (SEQ ID NO: 294)
XLOC_SplitLeft_12	AACCCTTAC TAT CCATCCAACCTGAGTTCATTG (SEQ ID NO: 295)
XLOC_SplitLeft_13	AACCCTTAC TAT GTAGAAAGAGTGAATTGAGA (SEQ ID NO: 296)
XLOC_SplitLeft_14	AACCCTTAC TAT TAGATGATAGGAGGCATTGT (SEQ ID NO: 297)
XLOC_SplitLeft_15	AACCCTTAC TAT CGAGCAGTTGTATGTGAATG (SEQ ID NO: 298)
XLOC_SplitLeft_16	AACCCTTAC TAT ACATCCTCCATTGCACGCA (SEQ ID NO: 299)
XLOC_SplitLeft_17	AACCCTTAC TAT TCGAACGAATTCCTGCACT (SEQ ID NO: 300)
XLOC_SplitLeft_18	AACCCTTAC TAT TTTGAGGTTGTATGATAG (SEQ ID NO: 301)
XLOC_SplitLeft_19	AACCCTTAC TAT TCCACCTCATGGCCACAGAC (SEQ ID NO: 302)
XLOC_SplitLeft_20	AACCCTTAC TAT TAACTTGATTGTGATGATGG (SEQ ID NO: 303)

XLOC_SplitLeft_21	AACCCTTAC TAT TTTAGGAGGGAGGAATTACC (SEQ ID NO: 304)
XLOC_SplitLeft_22	AACCCTTAC TAT ACAAACAAATCTATAGTGAT (SEQ ID NO: 305)
XLOC_SplitLeft_23	AACCCTTAC TAT CCGTGGTGGTTTCGGGGCAG (SEQ ID NO: 306)
XLOC_SplitRight_1	GCGCGGTGGCTCACACCTGT TAT TATCCACCG (SEQ ID NO: 307)
XLOC_SplitRight_2	CCCAGGGACATTCATTGCT TAT TATCCACCG (SEQ ID NO: 308)
XLOC_SplitRight_3	ATGACTTCTCTATCCACTT TAT TATCCACCG (SEQ ID NO: 309)
XLOC_SplitRight_4	GAAGTGCCTCGTCATTCTGG TAT TATCCACCG (SEQ ID NO: 310)
XLOC_SplitRight_5	CCTTGTAATACTGATTAAA TAT TATCCACCG (SEQ ID NO: 311)
XLOC_SplitRight_6	AGTAGGTGCTCAATGAATTG TAT TATCCACCG (SEQ ID NO: 312)
XLOC_SplitRight_7	GCACACAGCAGGTGCTCAAT TAT TATCCACCG (SEQ ID NO: 313)
XLOC_SplitRight_8	CCTTAGGAAATAATAGCTAG TAT TATCCACCG (SEQ ID NO: 314)
XLOC_SplitRight_9	CATTAACCTCTCTTCCAAA TAT TATCCACCG (SEQ ID NO: 315)
XLOC_SplitRight_10	TTCCTAAAGGGATCAAATAA TAT TATCCACCG (SEQ ID NO: 316)
XLOC_SplitRight_11	CAGATTGTCCAGATGAACA TAT TATCCACCG (SEQ ID NO: 317)
XLOC_SplitRight_12	TGATACCTACTGATCTGAAG TAT TATCCACCG (SEQ ID NO: 318)
XLOC_SplitRight_13	ATATGAGGAATGAGAGTGGG TAT TATCCACCG (SEQ ID NO: 319)
XLOC_SplitRight_14	CATTACTAGGATAGTAATC TAT TATCCACCG (SEQ ID NO: 320)
XLOC_SplitRight_15	CATTGTATCAAACCAAGTCT TAT TATCCACCG (SEQ ID NO: 321)
XLOC_SplitRight_16	TATTTGCTTTCTGTATTCCC TAT TATCCACCG (SEQ ID NO: 322)
XLOC_SplitRight_17	GGTAAGTAGGTGAAGAATTT TAT TATCCACCG (SEQ ID NO: 323)
XLOC_SplitRight_18	TGCCTGACAACTCTCCATTT TAT TATCCACCG (SEQ ID NO: 324)
XLOC_SplitRight_19	CGGTTAACCATCCATATAAA TAT TATCCACCG (SEQ ID NO: 325)
XLOC_SplitRight_20	CTTCACAGGTGTACACAGCT TAT TATCCACCG (SEQ ID NO: 326)
XLOC_SplitRight_21	AAAGGGCATGAGAACAATTT TAT TATCCACCG (SEQ ID NO: 327)
XLOC_SplitRight_22	AGAAAGTTGGTCAGCGGTTT TAT TATCCACCG (SEQ ID NO: 328)
XLOC_SplitRight_23	AAGCCCAGGGGTACTCCTTA TAT TATCCACCG (SEQ ID NO: 329)

XLOC readout sequence

Name	Readout Sequence
XLOC_Cy5_Readout	/5Cy5/GTAAGGGTT CGGTGGATA (SEQ ID NO: 330)

Table 8: Probe sequences used in the MUC5AC experiment (Figures 1, 2 and 3). Sheet 8a: Sequences of the unpaired, paired (circular), and readout probes used in Figure 1. Sheet 8b: Sequences of the MUC5AC split-probe constructs and readout probe used for Figure 3. Sheet 8c: Sequences of the MUC5AC split-probe, conventional probe, bridge probe, and readout probes used for the kinetic experiment in Figure 2. Lowercase letters denotes the target gene (MUC5AC) binding sequence. Uppercase letters denotes the 3 nucleotide linker and readout binding sequence.

Table 8a
Unpaired split probe sequences

Name	Sequence
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12nt_Split_probe_2	acagggtgggagtagttccag TAT TATCCACCGAAC (SEQ ID NO: 331)
11nt_Split_probe_2	acagggtgggagtagttccag TAT TATCCACCGAA (SEQ ID NO: 332)
10nt_Split_probe_2	acagggtgggagtagttccag TAT TATCCACCGA (SEQ ID NO: 333)
9nt_Split_probe_2	acagggtgggagtagttccag TAT TATCCACCG (SEQ ID NO: 334)
8nt_Split_probe_2	acagggtgggagtagttccag TAT ATCCACCG (SEQ ID NO: 335)
7nt_Split_probe_2	acagggtgggagtagttccag TAT TCCACCG (SEQ ID NO: 336)

Paired (circular) split probe sequences

Name	Sequence
9nt_Split_probe_1	AACCCTTAC TAT agagggtgtgctggtggtgga (SEQ ID NO: 337)
9nt_Split_probe_2	acagggtgggagtagttccag TAT TATCCACCG (SEQ ID NO: 338)
8nt_Split_probe_1	AACCCTTA TAT agagggtgtgctggtggtgga (SEQ ID NO: 339)
8nt_Split_probe_2	acagggtgggagtagttccag TAT ATCCACCG (SEQ ID NO: 340)
7nt_Split_probe_1	AACCCTT TAT agagggtgtgctggtggtgga (SEQ ID NO: 341)
7nt_Split_probe_2	acagggtgggagtagttccag TAT TCCACCG (SEQ ID NO: 342)

Readout sequence

Name	Readout sequence
Readout	/5Cy5/GTAAGGGTT CGGTGGATA (SEQ ID NO: 343)

Table 8b
MUC5AC split probe construct sequences

Name	Sequence
Circular_probe_1	AACCCTTAC TAT agagggtgtgctggtggtgga (SEQ ID NO: 344)
Circular_probe_2	acagggtgggagtagttccag TAT TATCCACCG (SEQ ID NO: 345)
Cruciform_probe_1	agagggtgtgctggtggtgga TAT AACCCTTAC (SEQ ID NO: 346)
Cruciform_probe_2	TATCCACCG TAT acagggtgggagtagttccag (SEQ ID NO: 347)
Double_'C'_probe_1	agagggtgtgctggtggtgga TAT AACCCTTAC (SEQ ID NO: 348)
Double_'C'_probe_2	acagggtgggagtagttccag TAT TATCCACCG (SEQ ID NO: 349)
Double_'Z'_probe_1	agagggtgtgctggtggtgga TAT TATCCACCG (SEQ ID NO: 350)
Double_'Z'_probe_2	acagggtgggagtagttccag TAT AACCCTTAC (SEQ ID NO: 351)
Conventional_probe	TATCCACCGAACCTTAC agagggtgtgctggtggtggaacagggtgggagtagttccag (SEQ ID NO: 352)
Negative_control	AACCCTTAC TAT agagggtgtgctggtggtgga (SEQ ID NO: 353)

MUC5AC split probe bridge sequence

Name	Readout Sequence
Cy5_bridge	/5Cy5/GTAAGGGTT CGGTGGATA (SEQ ID NO: 354)

Table 8c**MUC5AC colocalization Cy3 probe sequences**

Name	Sequence
MUC5AC_Cy3_1	tgctcctcagcagcctctga TAT ATTTAACCGCACTATCCC (SEQ ID NO: 355)

MUC5AC_Cy3_2	actcattgtgtggacggcgg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 356)
MUC5AC_Cy3_3	tcttgccatggcctgtatg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 357)
MUC5AC_Cy3_4	gtagctgattcgaggagc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 358)
MUC5AC_Cy3_5	ataggagagaggcgagggtg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 359)
MUC5AC_Cy3_6	agatgggaagacagtcgccc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 360)
MUC5AC_Cy3_7	ttggaggctcgtaccacagg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 361)
MUC5AC_Cy3_8	ggtctttagtggaagctgc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 362)
MUC5AC_Cy3_9	gaacacgtagtgcagaggc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 363)
MUC5AC_Cy3_10	aaatcctcgtaggcgccacc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 364)
MUC5AC_Cy3_11	atgaggaccctgctcagcgt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 365)
MUC5AC_Cy3_12	gatgaccacgccatccact TAT ATTTAACCGCACTATCCC (SEQ ID NO: 366)
MUC5AC_Cy3_13	ttgaccaggacggagccctt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 367)
MUC5AC_Cy3_14	agactggctgaaggcgagca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 368)
MUC5AC_Cy3_15	tgctctgctgaatgaggacc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 369)
MUC5AC_Cy3_16	gctccaccttggtgtagct TAT ATTTAACCGCACTATCCC (SEQ ID NO: 370)
MUC5AC_Cy3_17	acatgaggacaaggcccagc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 371)
MUC5AC_Cy3_18	agcaggctgtcatcgtgtt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 372)
MUC5AC_Cy3_19	gtatttggtgtccagctcca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 373)
MUC5AC_Cy3_20	acgggcatccgtgaagtc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 374)
MUC5AC_Cy3_21	gcttggtgttgaggagg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 375)
MUC5AC_Cy3_22	ttccgaattccatgggtgt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 376)
MUC5AC_Cy3_23	acagggtcctgacactggtc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 377)
MUC5AC_Cy3_24	agccagtgagcagttctc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 378)
MUC5AC_Cy3_25	aggagctcctcacagatgcc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 379)
MUC5AC_Cy3_26	acgcagccagagaacagctg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 380)
MUC5AC_Cy3_27	tgcaagcctccaggtagctg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 381)
MUC5AC_Cy3_28	tcacagaagcagaggtcttg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 382)
MUC5AC_Cy3_29	tactcggcaagggtgtgca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 383)
MUC5AC_Cy3_30	tggtactgcatgttggtgg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 384)
MUC5AC_Cy3_31	ttggagcaggtgtctgcgca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 385)
MUC5AC_Cy3_32	acacagtggctcctcacaggc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 386)
MUC5AC_Cy3_33	atgtcgtcaagcacgctccc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 387)
MUC5AC_Cy3_34	ttgacacagggaacacagcg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 388)
MUC5AC_Cy3_35	ccgttgtagacgcaggcaca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 389)
MUC5AC_Cy3_36	agtctgtgagtaggtggcc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 390)
MUC5AC_Cy3_37	atggaacctcctggcagctc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 391)
MUC5AC_Cy3_38	aagcacagagcaggtaccgg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 392)
MUC5AC_Cy3_39	acgttgagaagtggcacct TAT ATTTAACCGCACTATCCC (SEQ ID NO: 393)
MUC5AC_Cy3_40	accgtgtattgctcccgtc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 394)
MUC5AC_Cy3_41	cacatagctgcagtcgctg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 395)
MUC5AC_Cy3_42	tgctgtcacagggttggtc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 396)
MUC5AC_Cy3_43	tcagccagtacagtgaaggc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 397)
MUC5AC_Cy3_44	ttcaggcaggtctcgtctc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 398)
MUC5AC_Cy3_45	atccaggctcagtgctacgc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 399)

MUC5AC_Cy3_46	tgatcaccaccacgtctgc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 400)
MUC5AC_Cy3_47	ctggttcaggaaactccc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 401)
MUC5AC_Cy3_48	agatgggcagctgggtgtag TAT ATTTAACCGCACTATCCC (SEQ ID NO: 402)
MUC5AC_Cy3_49	aagatgggtgacgttggctgc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 403)
MUC5AC_Cy3_50	gatgaagaaggttgagggtc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 404)
MUC5AC_Cy3_51	agctgcagggtcagctgcag TAT ATTTAACCGCACTATCCC (SEQ ID NO: 405)
MUC5AC_Cy3_52	gaacagctgcatggtgggca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 406)
MUC5AC_Cy3_53	ttccacagagaccaggt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 407)
MUC5AC_Cy3_54	atcggtcgtggtgctgga TAT ATTTAACCGCACTATCCC (SEQ ID NO: 408)
MUC5AC_Cy3_55	ttgaagaaggccgacagcgt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 409)
MUC5AC_Cy3_56	aagctgttctgattgtggg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 410)
MUC5AC_Cy3_57	tctccagctcagagagcag TAT ATTTAACCGCACTATCCC (SEQ ID NO: 411)
MUC5AC_Cy3_58	cagtgcctgagcactctc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 412)
MUC5AC_Cy3_59	agtaggttcccgcttcacg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 413)
MUC5AC_Cy3_60	gtgtcaaacatgcagttcga TAT ATTTAACCGCACTATCCC (SEQ ID NO: 414)
MUC5AC_Cy3_61	tcctgcctccgtcacagt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 415)
MUC5AC_Cy3_62	ataggcttcgtgcagacgcc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 416)
MUC5AC_Cy3_63	tggtacgtcattgactggg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 417)
MUC5AC_Cy3_64	ctggcaggtgctgacatggt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 418)
MUC5AC_Cy3_65	aacactgcaggtgatgtccc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 419)
MUC5AC_Cy3_66	acagatgcagccatccacgg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 420)
MUC5AC_Cy3_67	tcgtccaggaaaggtgccctt TAT ATTTAACCGCACTATCCC (SEQ ID NO: 421)
MUC5AC_Cy3_68	tgctggcctgcacacactg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 422)
MUC5AC_Cy3_69	cctctgtgtgtagcagggaca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 423)
MUC5AC_Cy3_70	tggtcaggtgcagatagccc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 424)
MUC5AC_Cy3_71	cgatgcagctcagctccca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 425)
MUC5AC_Cy3_72	aacacatgggagcagcaca TAT ATTTAACCGCACTATCCC (SEQ ID NO: 426)
MUC5AC_Cy3_73	cgtggcatttcggcagtcga TAT ATTTAACCGCACTATCCC (SEQ ID NO: 427)
MUC5AC_Cy3_74	agctcttctgacagccagcc TAT ATTTAACCGCACTATCCC (SEQ ID NO: 428)
MUC5AC_Cy3_75	caggatcatgtccagtggtg TAT ATTTAACCGCACTATCCC (SEQ ID NO: 429)

Colocalization readout sequence

Name	Readout sequence
Readout	/5Cy3/GGGATAGTGCGGTTAAAT (SEQ ID NO: 430)

Table 8d
Kinetic experiment probe sequences

Name	Sequence
MUC5AC_Left_1	AACCTTAC TAT agaggtgtgctggtgtggga (SEQ ID NO: 431)
MUC5AC_Right_1	acagggctgggagtagttccag TAT TATCCACCG (SEQ ID NO: 432)
MUC5AC_Conventional	acagggctgggagtagttccag TATCCACCGAACCTTAC (SEQ ID NO: 433)

Kinetic experiment bridge sequence

Name	Sequence
Bridge_1	TGATGGGTGCGTGAGTAAGTAAGGGTTCGGTGGATATGATGGGTGCGTGAGTAA (SEQ ID NO: 434)

Kinetic experiment readout sequence

Name	Sequence
Bridge Readout	/5Cy5/TTACTCAGC CACCCATCA (SEQ ID NO: 435)
Conventional Readout	/5Cy5/GTAAGGGTT CGGTGGATA (SEQ ID NO: 436)

Table 9 Probe sequences used in the FLNA experiment (Figure 2). The template sequence includes the forward and reverse primer sequences for amplifying the template sequence. The primers used for PCR amplification are 'TACCATCTCGTGTTCGTACC' (SEQ ID NO: 437) and 'TAATACGACTCACTATAGTTCGTTCCGCTACTCACCAC' (SEQ ID NO: 438).

FLNA split probe sequences

Name	Sequence
FLNA_1	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CAGGGAGCAGAGGTTGCGCTGCTGT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 439)
FLNA_2	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGAGCCGCGCCTGCTGCGCTCTGGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 440)
FLNA_3	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGTCCTTCTCGGTGGCCGGCATCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 441)
FLNA_4	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CACCAGCGCGTGAAAGTGTTCTGCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 442)
FLNA_5	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGTCCGTCTGCAGGTTGGCGATGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 443)
FLNA_6	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ATCTTCTTCTGCGCTGAGCACCTCCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 444)
FLNA_7	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ACGTTCTCAAGCTGCATTGGCGGA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 445)
FLNA_8	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCGATGGACACCAGTTTGATGCTCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 446)
FLNA_9	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GAGTAGTGACAGGATCAGGGTCCAGA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 447)
FLNA_10	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GTCTGCTTCTTGGCCTCCTCATCCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 448)
FLNA_11	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ATGGGAGCTGCGGCAGCTTGTCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 449)
FLNA_12	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCCACCAGGGCGCCAGGGCCCGGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 450)
FLNA_13	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGTAACGGGCTTGCTGGCGTCCCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 451)
FLNA_14	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGCCCGTAGGCACGGGCTTCTTCG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 452)
FLNA_15	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGACGTACCAGACGAGAAGGTGCG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 453)
FLNA_16	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGCTCTTGGCGATGTGCTGCCAGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 454)
FLNA_17	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GCCGTAAGATCTCAAAGTAGGTGG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 455)
FLNA_18	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGGATGGGCACGCCGGCAAACGTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 456)
FLNA_19	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CCGCCCGGCAGGCACTCGGGTTACA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 457)
FLNA_20	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CACCTTGAAGTCAGCTGTCTCCTTC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 458)
FLNA_21	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CTCGGTGCCACCTTCACTTCAAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 459)
FLNA_22	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CACCACAAAGTCTGCTGACTTGCCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 460)

FLNA_23	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TTAGCCTGCGATGGCCCTTCACCG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 461)
FLNA_24	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGCGGCCAGTAGCGCACATCACAGG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 462)
FLNA_25	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGCGGATGTCTTCGTGTTGCACA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 463)
FLNA_26	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TTCTCCAATCCAGGCCACGTGCCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 464)
FLNA_27	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CCGTGCTTGGCATCCACTGTGAACCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 465)
FLNA_28	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CAACGCCTCCACAGGGCAGCCTTCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 466)
FLNA_29	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CGGCTTCTGGGCAGTAGGAGCAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 467)
FLNA_30	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GCCAGCCTCGGCGCAGTCCACAGTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 468)
FLNA_31	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGTGTCATTGTCATTGCGGATGAT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 469)
FLNA_32	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GTGGGCGTGGCCTGGTCAGCAAAGA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 470)
FLNA_33	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CGGCCCTTACCTTACTGGCGTCATG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 471)
FLNA_34	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGAAGTGGGTGGGCTTGCCAAAGCTC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 472)
FLNA_35	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GTGTAGGTGTTGTCATGGTGGTCTGA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 473)
FLNA_36	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CCATAAGTGACATTGACGCCTACTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 474)
FLNA_37	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCCAGGCTTGAGATACTGCCACTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 475)
FLNA_38	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCTTTGCCAACGTCCACCTTCTCTC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 476)
FLNA_39	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GATGCCACTTTGCCTTGACCACCAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 477)
FLNA_40	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ACGGGCAGCCGTCATAGGTCACCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 478)
FLNA_41	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGCCAGGCCACCTGTGCCGGCGCC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 479)
FLNA_42	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GCACCTTGACCTTGATGCGTCAAAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 480)
FLNA_43	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGCTCCGCGCTGCCCGCGCTCGAGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 481)
FLNA_44	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGGTGTGCGTGCCATCACCGTGGTC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 482)
FLNA_45	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGGAAGTTGGGCACGGGCTGGCCGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 483)
FLNA_46	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CCATAGCACTGGACACCGGAAGTGT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 484)
FLNA_47	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ACACTGAACCTCAGTGGTGGCTCAC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 485)
FLNA_48	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GCCACACGGCCTTGACGTGCGGCC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 486)
FLNA_49	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ACATGCCATCGCCACGGTCTCTGAAC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 487)
FLNA_50	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGGTACAGTCCACGGAGTGACAGTCC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 488)
FLNA_51	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CCGAGCAGCTGCCGTCTTGTATC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 489)
FLNA_52	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CACCATAGGTGACGTTGAGGCTGTA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 490)
FLNA_53	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CATCTGTACATCATGCACAGGGAC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 491)
FLNA_54	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGGCACGAACCATGCCTGGGCTCAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 492)
FLNA_55	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GTCCACTGGCTCCACCAGGCCTTTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 493)
FLNA_56	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TTCTCGGCTGGGCACATAATTGACG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 494)
FLNA_57	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGGCCTTACCTTGCTGGCATCATG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 495)
FLNA_58	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TGGTGAACCTCACGGGCAGGCTGGC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 496)

FLNA_59	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TTCTTCGGCTTGCCTTCGGGATCCG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 497)
FLNA_60	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCTGGCACGTAGGCCACTGTATACG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 498)
FLNA_61	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT ACTTTGCCTTTGCCTGCCGCCTTAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 499)
FLNA_62	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCCACCACGTCCACATCCACCTCTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 500)
FLNA_63	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGCACGTGCTGCCACCAAAGCGCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 501)
FLNA_64	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TTGTCAGTGATGGTGGGCTGCGCCA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 502)
FLNA_65	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT TCGTGCAGGCCAGCCTCGCTGGGTG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 503)
FLNA_66	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CATAGGCAGTGACATGGCCACAGTT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 504)
FLNA_67	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CTGCATCCTTGGTGTGACGGTGAA GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 505)
FLNA_68	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGTGCAGCTGATTTCTGCTTTGGAC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 506)
FLNA_69	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGGCTGCCTGGGACGTGCTGTTCAT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 507)
FLNA_70	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGACCACAGTGGCCGTGACAGGCT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 508)
FLNA_71	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CGTGGCCATTACGCAGCCGCTTCAG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 509)
FLNA_72	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GTGTGGCCTTCGTGAAGGCCCTGAC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 510)
FLNA_73	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT AGCCACCACATAGCCTGCATCGCGGG GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 511)
FLNA_74	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT CGTGCTGGTCGGCAAACCTGATGTT GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 512)
FLNA_75	TACCATCTCGTGTTCGTACC GGTAC CCCATTACC TAT GGGTGATGCTCTCTTTCACCCGGCC GAATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 513)
FLNA_76	TACCATCTCGTGTTCGTACC GGTACC CTGAGAGCGACCGGTGACCGATGAC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 514)
FLNA_77	TACCATCTCGTGTTCGTACC GGTACC GCCCGAGAGTGGGAGCTACTCATTT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 515)
FLNA_78	TACCATCTCGTGTTCGTACC GGTACC GCGTCCCGCGTGTGACGCGCCGCGC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 516)
FLNA_79	TACCATCTCGTGTTCGTACC GGTACC ATCTTCTTCCACGGCGCGTCTCCG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 517)
FLNA_80	TACCATCTCGTGTTCGTACC GGTACC TTGCTCAGCACTTCAGGTGCTCGT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 518)
FLNA_81	TACCATCTCGTGTTCGTACC GGTACC AGCGCGATAAGCCGACGCCGCTGCGC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 519)
FLNA_82	TACCATCTCGTGTTCGTACC GGTACC GTGGGCCGCTGTTGTGCTTGCGGT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 520)
FLNA_83	TACCATCTCGTGTTCGTACC GGTACC CCGTCCAGGAACCTCGAGCGCCACCG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 521)
FLNA_84	TACCATCTCGTGTTCGTACC GGTACC AGGCCAGGATCAGCTTCAGGTTCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 522)
FLNA_85	TACCATCTCGTGTTCGTACC GGTACC TCCTCGTCCCACATGGGCATGGAGA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 523)
FLNA_86	TACCATCTCGTGTTCGTACC GGTACC ATCCAGCCCAGGAGCCTCTGCTTGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 524)
FLNA_87	TACCATCTCGTGTTCGTACC GGTACC CTCTGCCAGTCCCGGCTGAAGTTGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 525)
FLNA_88	TACCATCTCGTGTTCGTACC GGTACC GTCCCAGTCAGGACACAGGCCCGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 526)
FLNA_89	TACCATCTCGTGTTCGTACC GGTACC TTCAGTTTGGCCGCAAGGGAGCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 527)
FLNA_90	TACCATCTCGTGTTCGTACC GGTACC TCTTGTGCTTATTGGCGGTCACTTT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 528)
FLNA_91	TACCATCTCGTGTTCGTACC GGTACC AGAGCACAGTAACCTTATGAGTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 529)
FLNA_92	TACCATCTCGTGTTCGTACC GGTACC TTGTTGGCGATGTTGCCACTGGGCT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 530)
FLNA_93	TACCATCTCGTGTTCGTACC GGTACC GTGCACGGTGTGGACGCCCTCCATG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 531)
FLNA_94	TACCATCTCGTGTTCGTACC GGTACC CTGGCCAACAGTGACAGTGTAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 532)

FLNA_95	TACCATCTCGTGTTCGTACC GGTACC CCGCACACCCTTGGGCTGGAGGCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 533)
FLNA_96	TACCATCTCGTGTTCGTACC GGTACC ACTGCCCGCATGTTCTGACCACCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 534)
FLNA_97	TACCATCTCGTGTTCGTACC GGTACC GACGCCGCCCTCCAGCCCAGGGCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 535)
FLNA_98	TACCATCTCGTGTTCGTACC GGTACC AAGCCCAGCGTCCCCACGTCGTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 536)
FLNA_99	TACCATCTCGTGTTCGTACC GGTACC CCGTCGCCCTTGTCGTACATTCTGA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 537)
FLNA_100	TACCATCTCGTGTTCGTACC GGTACC ACGTGAACGGCATACTCGCCAGCCT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 538)
FLNA_101	TACCATCTCGTGTTCGTACC GGTACC ACCCTGTCTGGGTGGAAGTCCTGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 539)
FLNA_102	TACCATCTCGTGTTCGTACC GGTACC GCTGGCTTGTGACGGCCACACCTG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 540)
FLNA_103	TACCATCTCGTGTTCGTACC GGTACC GTCCTGGACTTGACCCGAAGTGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 541)
FLNA_104	TACCATCTCGTGTTCGTACC GGTACC GTAAGTGCCATTGCCGTTGTCCTTG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 542)
FLNA_105	TACCATCTCGTGTTCGTACC GGTACC GTAGGTGGGCTCGTGGGCCTTGAGC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 543)
FLNA_106	TACCATCTCGTGTTCGTACC GGTACC CGAAGTCGATGTCAGCTTCGGCGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 544)
FLNA_107	TACCATCTCGTGTTCGTACC GGTACC ACCATAATGGTGAGTGCCAGCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 545)
FLNA_108	TACCATCTCGTGTTCGTACC GGTACC AGGGCTCCACCTTGACTCGGATGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 546)
FLNA_109	TACCATCTCGTGTTCGTACC GGTACC CACCATGCGACTGAGGCCAGGGCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 547)
FLNA_110	TACCATCTCGTGTTCGTACC GGTACC ATGTCCACATCTCGCACTGCATCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 548)
FLNA_111	TACCATCTCGTGTTCGTACC GGTACC CCTGCTGGACAGGCGTGACTTGA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 549)
FLNA_112	TACCATCTCGTGTTCGTACC GGTACC AAAGGGCTCTTAGGGATGGGATCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 550)
FLNA_113	TACCATCTCGTGTTCGTACC GGTACC AGGCCAGACACCTTGATCTTGCTGA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 551)
FLNA_114	TACCATCTCGTGTTCGTACC GGTACC CCCTTTGATTGACTGTGAACTCCT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 552)
FLNA_115	TACCATCTCGTGTTCGTACC GGTACC ACCTCATAGGGCCCTTCTCACGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 553)
FLNA_116	TACCATCTCGTGTTCGTACC GGTACC TGGTGTCGATGGTGAAGCGGGCGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 554)
FLNA_117	TACCATCTCGTGTTCGTACC GGTACC GCGAACACCGTGGGCCTTGAATGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 555)
FLNA_118	TACCATCTCGTGTTCGTACC GGTACC TCCACTTGAATTGGCCACCTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 556)
FLNA_119	TACCATCTCGTGTTCGTACC GGTACC GGATGTACACCTCGGCCGGAAGCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 557)
FLNA_120	TACCATCTCGTGTTCGTACC GGTACC TACTTGATGGTGACGGTGAGGCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 558)
FLNA_121	TACCATCTCGTGTTCGTACC GGTACC ACCGCAGGTTCCACCTGCAGCTTGC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 559)
FLNA_122	TACCATCTCGTGTTCGTACC GGTACC AAGACACCCTGGCCCTCAATACCAG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 560)
FLNA_123	TACCATCTCGTGTTCGTACC GGTACC CCGGTCTGTGTCAGAGCCCGGGCGT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 561)
FLNA_124	TACCATCTCGTGTTCGTACC GGTACC AGGTCTCCGTCAGGTTGCCTGAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 562)
FLNA_125	TACCATCTCGTGTTCGTACC GGTACC CCTCGTAAGCGTGACTCCACTTT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 563)
FLNA_126	TACCATCTCGTGTTCGTACC GGTACC TGCAGGACATCTTGGCCTCGGAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 564)
FLNA_127	TACCATCTCGTGTTCGTACC GGTACC TGCCAGCCTCATAAGGGATGACTC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 565)
FLNA_128	TACCATCTCGTGTTCGTACC GGTACC TGAAAGGACTGCCTGGCACTTGATG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 566)
FLNA_129	TACCATCTCGTGTTCGTACC GGTACC CGGGCCAGAGCACTTGACCTTGA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 567)
FLNA_130	TACCATCTCGTGTTCGTACC GGTACC CCCTTGCACTTGACCTGCAATGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 568)

FLNA_131	TACCATCTCGTGTTCGTACC GGTACC CTGGGTGCCATCAGCGTTGTCTACC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 569)
FLNA_132	TACCATCTCGTGTTCGTACC GGTACC TAGGCAGCACCTTGACCTTGAAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 570)
FLNA_133	TACCATCTCGTGTTCGTACC GGTACC GCACGCCAGTGGTGTGAGCCCCGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 571)
FLNA_134	TACCATCTCGTGTTCGTACC GGTACC ATCTGGACAGCCAGCAGGCCCTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 572)
FLNA_135	TACCATCTCGTGTTCGTACC GGTACC CCGTCATGGTTGTCTTGATGTGTG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 573)
FLNA_136	TACCATCTCGTGTTCGTACC GGTACC TCCACAGTGATCACCGTCTCCTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 574)
FLNA_137	TACCATCTCGTGTTCGTACC GGTACC CCATCAGGCGTGACACGGTGACAG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 575)
FLNA_138	TACCATCTCGTGTTCGTACC GGTACC CAGATGACGTATTTGCCCGGCTGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 576)
FLNA_139	TACCATCTCGTGTTCGTACC GGTACC TTGCCTGAGGGCATCCGAACCTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 577)
FLNA_140	TACCATCTCGTGTTCGTACC GGTACC TACCGCACGGTCACGGTGCCGCTCT TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 578)
FLNA_141	TACCATCTCGTGTTCGTACC GGTACC CGTAATCCACATAGAACTGCAAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 579)
FLNA_142	TACCATCTCGTGTTCGTACC GGTACC TGGCAGGCTTGTCTACTACTCCATG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 580)
FLNA_143	TACCATCTCGTGTTCGTACC GGTACC GCCCTCAATGGCCAGAGACAGGCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 581)
FLNA_144	TACCATCTCGTGTTCGTACC GGTACC TACTTGACTAGAATGCTGTAGTCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 582)
FLNA_145	TACCATCTCGTGTTCGTACC GGTACC GATCCGTCTCTGAGATGTTGATGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 583)
FLNA_146	TACCATCTCGTGTTCGTACC GGTACC AACAGGGCTCCTCCCGGCCGAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 584)
FLNA_147	TACCATCTCGTGTTCGTACC GGTACC GAGACCGAACACGACTGGCATCCC TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 585)
FLNA_148	TACCATCTCGTGTTCGTACC GGTACC TCAATGATAAATCTGTCAGGCTCAA TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 586)
FLNA_149	TACCATCTCGTGTTCGTACC GGTACC TGATGTAGTTGCCCTGGCTCTGTGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 587)
FLNA_150	TACCATCTCGTGTTCGTACC GGTACC CGCCTGTCACCTTCACAGAGAAGGG TAT ATTTAACCG AATTC GTGGTGAGTAGCGGAACGAA (SEQ ID NO: 588)

FLNA split probe bridge sequence

Name	Bridge sequence
FLNA_bridge	ATTGTAAAGCGTGAGAAA GGTAATGGGCGGTAAAT ATTGTAAAGCGTGAGAAA (SEQ ID NO: 589)

FLNA split probe readout sequence

Name	Readout sequence
Cy5_Readout	/5Cy5/TTTCTACGCTTTACAAT (SEQ ID NO: 590)

Table 10 Reference FKPM values for AML12, mouse kidney, liver, frontal cortex and ovary for the 317 genes in the split library.

Reference FPKM for 317-genes split library

Gene name	Transcript ID	AML-12	Kidney	Liver	Frontal Cortex	Ovary
Blank1		0	0	0	0	0
Blank2		0	0	0	0	0

Blank3		0	0	0	0	0
Blank4		0	0	0	0	0
Blank5		0	0	0	0	0
Blank6		0	0	0	0	0
Blank7		0	0	0	0	0
Blank8		0	0	0	0	0
Ppfia2	ENSMUST00000029404.11	0.15192	0.04	0.02	15.425	0.02
Klf5a	ENSMUST00000099172.3	0.068099	1.295	1.235	342.67	3.45
Camk4	ENSMUST00000042868.4	0.16044	0.035	0.005	10.56	0.035
Cenpe	ENSMUST00000062893.7	18.8485	0.045	0.01	0.2	0.185
Akap6	ENSMUST00000095737.3	0.032227	0.14	0.025	15.605	0.365
Klf1a	ENSMUST00000171796.3	0.089827	0.64	0.07	120.305	4.315
Ptpnz1	ENSMUST00000090568.3	0.061093	0.06	0.01	35.63	1.46
Ccdc177	ENSMUST00000073251.6	0	0	0.005	2.39	0.105
Mki67	ENSMUST00000033310.7	37.822	0.135	0.03	0.235	1.435
Map1a	ENSMUST00000094639.5	0.035225	0.4	0.035	82.56	3.655
Hmcn2	ENSMUST00000113532.4	0.01155	0.18	0.01	0.155	7.08
Brip1	ENSMUST00000044423.3	12.0383	0.26	0.115	0.025	0.13
Aspm	ENSMUST00000053364.10	15.0238	0.02	0.01	0.07	0.755
Kntc1	ENSMUST00000031366.7	19.5018	0.115	0.015	0.11	0.885
Myof	ENSMUST00000041475.10	154.2339	4.67	0.15	0.97	4.735
Myom2	ENSMUST00000033842.3	0.081423	4.555	0.04	0.045	0.125
Ahrak	ENSMUST00000092956.2	236.0856	6.225	0.805	2.77	8.6
Cacna1e	ENSMUST00000187541.2	1.3159	0.065	0.02	18.545	0.19
Zfp831	ENSMUST00000059452.5	0.013624	0.105	0.04	2.68	0.03
Rims3	ENSMUST00000071093.4	0.16167	0.18	0.335	23.255	1.04
Adamts2	ENSMUST00000040523.8	0.14825	3.165	1.51	0.775	71.525
Dzank1	ENSMUST00000081982.7	1.0306	0.935	0.045	30.275	1.17
Plk3c2a	ENSMUST00000170430.1	74.0708	2.65	0.875	3.015	0.835
Prune2	ENSMUST00000087689.4	0.17921	0.13	0.005	9.47	0.86
Myh11	ENSMUST00000090287.3	0.003366	4.365	0.26	2.175	57.02
Nat8l	ENSMUST00000056355.8	0.016113	3.56	0.03	58.45	4.895
Myo5c	ENSMUST00000036555.6	38.7977	2.195	0.02	0.34	3.21
Erc2	ENSMUST00000090302.5	0.18012	0.805	0.74	17.365	0.085
Brca2	ENSMUST00000044620.7	6.4182	0.125	0.065	0.415	0.31
Tspan7	ENSMUST00000115526.1	19.8019	48.37	15.395	741.31	24.435
Col6a6	ENSMUST00000098441.5	0.008119	0.135	0.03	0.09	1.71
Atp1a2	ENSMUST00000085913.6	1.116	14.055	0.195	185.775	17.37
Galnt15	ENSMUST00000022460.6	0.050628	1.6	0.315	0.57	16.08
Trabd2b	ENSMUST00000094894.3	0.02677	18.39	0.06	0.555	2.84
Smchd1	ENSMUST00000127430.1	34.3279	1.195	0.345	3.54	1.165
Mysm1	ENSMUST00000075872.3	16.725	0.43	0.24	2.415	0.24
Spag17	ENSMUST00000164539.1	0.024643	0	0.005	0.01	0.21
Dock10	ENSMUST00000077946.7	0.20537	1.57	0.38	14.565	0.565
Srgap3	ENSMUST00000088373.6	0.73845	3.225	0.725	49.765	5.47

Atr	ENSMUST00000034980.7	18.0366	0.92	0.4	1.74	0.57
Armcx4	ENSMUST00000124226.2	0.043819	0.505	0.11	6.98	0.87
Trxb	ENSMUST00000087399.4	0.054933	4.07	1.98	0.635	36.685
Utp20	ENSMUST00000004470.7	33.4202	1.94	0.87	2.585	1.655
Gas2l3	ENSMUST00000099374.4	9.6628	0.11	0.22	0.47	1.325
Veph1	ENSMUST00000029419.7	0.93919	3.83	0.02	0.04	0.055
Col4a4	ENSMUST00000087050.6	0.072728	17.545	0.055	0.125	4.8
Ly75	ENSMUST00000112533.3	7.5712	0.325	0.375	0.44	0.43
Hivep2	ENSMUST00000187083.2	1.2425	2.81	0.98	37.99	3.995
Myh7b	ENSMUST00000092995.5	0.14153	0.015	0.005	0.905	0.08
Map3k9	ENSMUST00000035987.7	0.014592	1.37	0.015	7.035	0.565
Foxo1	ENSMUST00000053764.6	0.23454	8.175	4.485	3.645	77.605
Ntrk2	ENSMUST00000079828.5	0.20646	1.97	2.285	70.06	13.32
Scn10a	ENSMUST00000084787.5	0.010614	0	0.005	0.05	0.24
Finc	ENSMUST00000090474.6	5.9856	0.85	0.03	0.22	21.62
Pygo1	ENSMUST00000038489.5	0.051895	0.17	0.145	5.09	1.16
Pask	ENSMUST00000027493.3	8.6466	0.3	0.225	0.56	1.425
Sptbn2	ENSMUST00000008991.6	0.12332	6.575	8.4	78.255	1.355
Ksr2	ENSMUST00000180430.1	0.10047	0.99	0.04	3.68	0.1
Slk	ENSMUST00000026043.7	81.5086	5.51	2.59	11.565	3.79
Sptlc2	ENSMUST00000021424.4	58.8505	6.77	1.565	3.6	5.75
Nup210l	ENSMUST00000029548.4	1.483	0.075	0.01	0.165	0.27
Mrc2	ENSMUST00000100335.5	0.23292	13.505	0.49	2.135	40.645
Utp14b	ENSMUST00000053760.7	11.6554	0.325	0.555	2.755	0.265
Notch3	ENSMUST00000087723.3	0.18334	8.185	0.87	3.265	34.575
Cand1	ENSMUST00000020315.8	86.0633	5.045	3.075	12.2	8.135
Col6a5	ENSMUST00000190193.2	0.004947	0.16	0.06	0.12	1.075
Sdk2	ENSMUST00000041627.9	0.074561	0.255	0.05	2.91	0.725
Lgr6	ENSMUST00000044828.9	0.60692	0.33	0.015	8.17	19.76
Prtg	ENSMUST00000055535.8	0.028414	0.035	0.005	0.54	0.15
Dnah1	ENSMUST00000048603.7	0.069171	0.08	0.015	0.68	1.895
Zbtb41	ENSMUST00000039867.8	23.8026	2.995	0.885	3.395	1.465
Fbxo32	ENSMUST00000022986.6	1.2279	0.83	0.175	2.49	11.72
Caskin1	ENSMUST00000024958.7	0.091806	0.175	0.055	32.07	18.57
Zfp26	ENSMUST00000159569.3	19.7889	1.11	0.645	4.835	1.185
Cacna1c	ENSMUST00000186889.2	0.071178	0.47	0.025	10.03	4.625
Heatr1	ENSMUST00000059270.8	27.1337	1.79	1.335	3.25	3.395
Col4a6	ENSMUST00000101205.2	1.1748	0.905	0.01	0.275	5.41
Hspg2	ENSMUST00000030547.10	0.97227	10.445	2.14	0.975	37.47
Kprnb1	ENSMUST00000001479.4	193.7558	13.28	9.59	25.755	22.54
Zfyve16	ENSMUST00000022217.8	12.7013	1.315	0.375	2.515	0.885
Myh10	ENSMUST00000102611.5	1.1369	3.245	0.36	24	5.585
Slc4a7	ENSMUST00000057015.6	20.3773	0.735	0.57	5.095	2.165
Grik3	ENSMUST00000030676.7	0.030225	0.06	0.01	15.785	10.89
Rnf150	ENSMUST00000078525.5	0.13519	0.845	0.115	5.98	1.535

Sdk1	ENSMUST00000085774.6	0.017245	0.435	0.085	1.43	4.22
Ythdc2	ENSMUST00000037763.7	12.1174	0.545	0.375	4.255	0.365
Il17rd	ENSMUST00000035336.3	0.23613	2.01	0.1	0.84	7.02
Synm	ENSMUST00000074233.6	0.21727	0.41	0.035	4.135	8.365
Dnah11	ENSMUST00000084806.6	0.021572	0.315	0.06	2.335	0.68
Flna	ENSMUST00000114299.3	173.0184	37.345	5.2	21.145	470.345
Fry	ENSMUST00000087204.5	0.62454	2.6	0.145	13.31	2.815
Cacna1a	ENSMUST00000121390.3	4.1076	0.545	0.21	20.9	5.36
Chst2	ENSMUST00000036267.7	0.06983	1.705	0.29	15.45	6.065
Psd3	ENSMUST00000038959.11	18.5113	1.065	2.1	46.12	1.5
Gpr161	ENSMUST00000111450.2	0.43661	0.91	0.09	1.915	6.81
Plxnd1	ENSMUST00000015511.10	3.4124	30.905	10.29	15.525	144.685
Rsf1	ENSMUST00000042399.9	12.4442	0.82	0.275	4.82	0.79
Slc38a1	ENSMUST00000100262.2	0.95264	1.315	0.265	26.725	14.9
Cacna2d1	ENSMUST00000039370.9	20.2413	0.61	0.05	32.18	2.805
Ppl	ENSMUST00000035672.3	17.2285	2.04	2.3	0.085	2.15
Trove2	ENSMUST00000159879.1	13.2001	1.315	0.155	5.505	0.845
Sox11	ENSMUST00000079063.6	7.5783	0.04	0.005	4.92	0.81
Mlxip	ENSMUST00000068237.7	1.539	9.295	2.63	6.43	42.29
Pkd1l3	ENSMUST00000109242.3	0.23261	0.015	0.005	0.09	0.025
Igsf10	ENSMUST00000039419.7	0.008606	0.32	0.145	0.4	1.785
Eif5b	ENSMUST00000027252.7	17.3736	1.9	1.325	4.695	0.705
Nid1	ENSMUST00000005532.7	1.1068	28.78	4.265	4.25	65.63
Alms1	ENSMUST00000072018.5	4.1423	0.32	0.095	1.265	0.715
Csmd1	ENSMUST00000082104.6	0.092488	0.15	0.005	1.875	1.59
Zfp516	ENSMUST00000171238.3	3.3071	3.195	1.31	3.74	22.735
Shroom3	ENSMUST00000113054.4	0.7962	10.415	2.085	0.715	24.465
Ddr2	ENSMUST00000194690.1	0.31682	5.51	0.755	2.17	14.56
Bend4	ENSMUST00000169190.1	0.038403	0.135	0.08	1.435	1.93
Sned1	ENSMUST00000062202.9	0.83901	4.685	3.3	2.385	24.19
Piezo1	ENSMUST00000067252.9	6.7555	24.03	2.055	1.71	50.425
Adamts17	ENSMUST00000098382.5	0.033952	0.095	0.025	0.94	0.775
Rttn	ENSMUST00000023828.7	4.6342	0.675	0.175	0.925	0.97
Trpm2	ENSMUST00000105401.4	0.55543	0.11	0.215	1.705	0.035
Arhgap31	ENSMUST00000023487.4	0.91672	3.49	0.75	4.68	15.86
Mkl2	ENSMUST00000149359.1	1.6283	3.385	2.515	26.125	6.69
Scn4a	ENSMUST00000021056.7	0.073875	0.4	0.02	0.02	0.14
Col7a1	ENSMUST00000026740.5	5.4853	0.285	0.025	1.33	2.5
Trpo1	ENSMUST00000109401.3	86.9988	13.99	6.765	13.755	13.565
Adam19	ENSMUST00000011400.7	0.050168	1.81	0.465	3.72	8.965
Plxna3	ENSMUST00000004326.3	1.2799	1.27	0.315	7.82	12.95
Gpr179	ENSMUST00000093942.4	0.030874	0.045	0.005	0.14	0.295
Pkdrej	ENSMUST00000064370.4	0.006604	0.03	0.01	0.2	0.24
Mylk	ENSMUST00000023538.8	3.0176	20.63	12.18	3.11	74.695
Trim33	ENSMUST00000029444.8	20.9992	2.555	1.065	6.2	3.585

Pcdhb22	ENSMUST00000192409.1	0.11783	0.965	0.23	2.755	5.28
Pten	ENSMUST00000013807.7	73.0409	6.86	8.685	24.82	2.76
Pappa	ENSMUST00000084501.3	0.02177	0.825	0.01	0.19	0.795
Plxna1	ENSMUST00000163139.3	17.1436	17.79	4.15	11.83	79.48
Fancm	ENSMUST00000058889.4	5.6309	0.49	0.3	1.845	1.15
Arhgef28	ENSMUST00000109426.1	35.2521	10.59	0.005	4.455	12.16
Tmc3	ENSMUST00000039317.9	0.010088	0.51	0.06	0.045	0.22
Plxnc1	ENSMUST00000099337.3	0.11454	0.335	1.7	6.29	12.095
Reps2	ENSMUST00000112334.3	0.041798	2.75	2.3	11.375	0.78
Nin	ENSMUST00000085314.5	10.9574	0.995	0.23	6.64	1.835
Pdzd8	ENSMUST00000099274.2	34.8986	2.835	2.64	7.24	9.765
Pcdhb19	ENSMUST00000059571.6	0.080284	0.17	0.02	0.985	0.94
Tmem2	ENSMUST00000096194.4	18.6815	3.865	1.69	3.035	3.18
Lrrk2	ENSMUST00000060642.6	3.1741	6.29	0.115	1.6	0.62
Flrt2	ENSMUST00000057324.3	0.65841	1.185	0.06	5.69	5.1
Nvl	ENSMUST00000027797.8	20.151	2.045	1.285	7.545	3.97
Myo9a	ENSMUST00000135298.3	7.5305	1.775	0.16	7.275	0.625
Fat4	ENSMUST00000061260.7	0.010693	0.915	0.15	1.27	2.79
Milt4	ENSMUST00000139666.3	50.9298	7.65	3.3	16.61	9.3
Celsr1	ENSMUST00000016172.7	0.34221	5.835	3.21	0.645	16.14
Map4	ENSMUST00000035055.10	14.1386	15.42	6.95	90.515	26.885
Dock6	ENSMUST00000034728.7	0.43807	15.03	6.03	5.69	40.735
Spon1	ENSMUST00000046687.11	0.3401	11.85	0.195	15.68	26.4
Spag9	ENSMUST00000041956.9	25.645	8.915	3.555	52.235	4.53
Sh3pxd2b	ENSMUST00000038753.5	6.3427	2.135	0.15	4.8	14.915
1700020114Rik	ENSMUST00000153581.1	13.632	10.41	6	52.255	6.02
Gm29666	ENSMUST00000189185.1	0.87292	0.16	0.045	0.97	0.14
Abca4	ENSMUST00000013995.8	2.2317	0.895	0.01	0.245	1.095
Golga4	ENSMUST00000084820.4	23.0089	5.2	1.635	10.11	2.39
Nhs12	ENSMUST00000101339.6	0.90199	2.235	0.195	4.555	7.66
Tnrc18	ENSMUST00000151477.3	6.6458	9.085	4.195	6.745	36.665
Dnah8	ENSMUST00000170651.1	0.017458	0.055	0.015	0.065	0.185
Xirp1	ENSMUST00000111635.2	0.004795	0.315	0.075	0.02	0.115
Ptprm	ENSMUST00000037974.8	0.68155	4.66	0.39	11.16	5.88
RP23-383N15.1	ENSMUST00000192800.1	0	0.22	0.045	0.405	0.105
Mgam	ENSMUST00000071535.6	0.10509	8.81	11.215	0.095	0.065
Zfp334	ENSMUST00000103084.3	0.92428	0.66	0.325	4.01	4.58
Scn7a	ENSMUST00000042792.6	0.015723	0.605	0.015	0.48	0.755
Trpm6	ENSMUST00000040489.7	0.32383	1.085	0.055	0.12	0.725
2410089E03Rik	ENSMUST00000110617.1	9.8956	1.585	0.4	7.21	2.75
Polr1a	ENSMUST00000055296.8	35.0729	5.115	4.13	5.105	16.61
Dennd3	ENSMUST00000043414.7	2.2845	13.92	1.71	2.145	5.565
Abcc2	ENSMUST00000026208.5	13.3131	49.305	93.46	0.1	0.18
Cgnl1	ENSMUST00000072899.4	24.2009	47.5	7.815	3.565	6.01
Stab2	ENSMUST00000035288.10	0.055239	8.34	12.26	0.06	1.08

Akap11	ENSMUST00000022593.5	11.8471	4.295	1.945	21.08	2.875
Notch4	ENSMUST00000015612.9	1.1254	6.89	0.885	1.2	7.75
Ppm1l	ENSMUST00000029355.8	2.7363	2.245	1.43	12.14	3.625
Wdr90	ENSMUST00000079461.10	7.4088	2.07	1.625	1.115	12.305
Edaradd	ENSMUST00000179308.1	0.031618	0.265	0.02	0.19	0.07
Kcnq1ot1	ENSMUST00000185789.1	1.1162	0.16	0.085	0.76	0.265
RP24-338G10.1	ENSMUST00000193744.1	0	0.03	0.03	0.105	0.165
Crb2	ENSMUST00000050372.7	3.488	1.565	0.025	0.655	3.175
Arhgef10	ENSMUST00000084207.7	4.1728	4.565	0.61	9	16.8
Irs1	ENSMUST00000069799.2	2.5152	4.195	5.59	3.65	22.515
Crocc	ENSMUST00000102491.5	2.2642	5.925	0.335	10.785	13.925
Ehbp1l1	ENSMUST00000049295.10	14.4396	6.89	2.62	5.335	29.7
Ogt	ENSMUST00000044475.4	32.7175	21.29	9.1	102.26	32.17
Thsd7a	ENSMUST00000046121.8	3.4074	1.505	0.06	3.64	0.825
Dsp	ENSMUST00000124830.1	15.592	2.745	4.145	0.14	5.005
Daam2	ENSMUST00000057610.6	0.34713	10.75	0.975	15.99	12.57
Sestd1	ENSMUST00000102660.3	5.9251	20.865	0.375	11.73	7.215
Sart1	ENSMUST00000044207.4	20.3807	3.475	2.065	8.89	5.43
Dab2ip	ENSMUST00000145698.3	3.0777	19.31	5.265	27.27	49.775
Ago4	ENSMUST00000084289.4	0.35746	0.59	0.5	2.635	0.745
Fgd5	ENSMUST00000089334.4	0.088143	3.385	0.73	0.96	3.86
Pla2r1	ENSMUST00000112525.4	0.4315	1.62	0.25	0.095	0.97
Arfgef1	ENSMUST00000088615.6	22.058	6.265	2.855	11.25	2.435
Onecut2	ENSMUST00000175965.4	3.8344	0	4.59	0.155	0.77
Tbc1d32	ENSMUST00000099739.3	3.6428	1.145	0.4	1.815	0.515
Dpy19l4	ENSMUST00000084892.7	10.0448	2.995	1.12	5.315	1.42
Myom3	ENSMUST00000105854.1	0.24026	0.305	0.05	0.005	0.105
Gpr116	ENSMUST00000113599.1	0.17574	12.39	2.08	4.765	6.35
Tjp1	ENSMUST00000032729.6	42.1997	10.51	3.99	15.84	14.075
Plcb2	ENSMUST00000102524.3	0.045128	0.67	0.31	0.38	1.44
Aldh1l2	ENSMUST00000020497.9	0.34242	0.51	0.03	1.015	0.435
Arhgef5	ENSMUST00000031750.9	30.2495	17.605	5.43	0.89	9.855
Helz2	ENSMUST00000108831.3	1.5309	7.51	31.34	0.85	18.125
Klf21a	ENSMUST00000088614.7	7.6264	6.515	3.035	21.525	4.02
Itpr1	ENSMUST00000032192.6	6.1055	24.87	3.49	36.325	13.06
Rassf4	ENSMUST00000035842.4	0.27494	1.765	1.17	3.4	6.09
Abca3	ENSMUST00000079594.7	13.9371	125.74	48.585	17.55	20.73
Nipbl	ENSMUST00000052965.6	11.5482	2.985	1.35	4.99	3
Wdr7	ENSMUST00000072726.5	6.4182	3.8	2.795	16.735	3.345
Phlpp2	ENSMUST00000179721.3	1.4244	3.86	0.66	8.22	6.53
Zbtb1	ENSMUST00000042779.3	4.2827	0.9	0.445	1.55	2.02
Tmed8	ENSMUST00000037418.5	2.3264	4.05	0.505	8.305	4.08
Cnot1	ENSMUST00000098473.6	45.7584	12.535	8.545	13.015	10.565
Abca9	ENSMUST00000044850.3	0.006076	1.295	0.245	1.165	1.315
Slc12a7	ENSMUST00000017900.7	11.806	57.5	46.195	1.865	112.275

Svil	ENSMUST00000126977.3	8.6685	6.01	6.495	4.39	26.32
Prkaa2	ENSMUST00000030243.7	1.736	14.325	6.605	4.285	0.925
Myo6	ENSMUST00000113268.3	25.014	19.245	2.675	11.75	5.475
Btrc	ENSMUST00000065601.7	7.9632	5.42	2.41	17.965	5.55
Nfia	ENSMUST00000075448.8	1.9953	26.88	21.805	9.09	57.48
Cmah	ENSMUST00000167746.3	5.365	2.69	4.905	0.03	0.045
Abca1	ENSMUST00000030010.3	0.18044	5.2	12.885	6.225	24.35
Osbpl8	ENSMUST00000105275.3	15.1743	7.99	2.105	11.37	2.585
Cdyl2	ENSMUST00000109102.2	0.93834	0.88	0.285	2.095	0.605
Wdfy4	ENSMUST00000130509.4	0.010188	1.575	0.885	0.36	2.06
Plcx2	ENSMUST00000130481.1	7.246	0.89	13.905	12.515	0.335
4932438A13Rik	ENSMUST00000057272.10	13.0887	5.6	3.495	16.97	3.325
Dennd4a	ENSMUST00000038890.5	2.2408	1.585	2.565	7.945	2
Rnf213	ENSMUST00000093902.7	5.0865	9.635	9.51	3.635	25.88
Thada	ENSMUST00000047524.8	5.4838	2.135	0.905	1.455	3.105
Myo18a	ENSMUST00000000645.8	58.0374	24.075	11.82	19.285	15.54
Arid1a	ENSMUST00000145664.4	2.9113	22.035	12.38	22.855	46.105
Kdm7a	ENSMUST00000002305.8	2.6234	1.37	0.825	5.275	2.585
Acacb	ENSMUST00000102582.3	0.094988	9.805	9.89	0.695	12.765
Szt2	ENSMUST00000075406.7	1.9876	11.305	6.01	12.64	23.78
Itpr2	ENSMUST00000053273.10	2.8832	12.9	3.67	2.51	9.24
Lsm11	ENSMUST00000129820.3	4.8305	1.22	0.93	3.445	5.425
Mlec	ENSMUST00000112121.3	47.8277	118.53	28.07	20.58	55.865
Dock9	ENSMUST00000100299.5	8.1985	15.025	1.25	14.375	14.965
Nedd4l	ENSMUST00000080418.4	9.5737	7.08	2.96	13.655	2.04
Dock8	ENSMUST00000025831.6	0.77808	9.92	6.845	0.57	9.52
Nhs1	ENSMUST00000037341.9	1.7878	5.875	1.05	4.935	2.615
Ppp2ca	ENSMUST00000020608.2	68.756	19.555	13.075	39.965	26.905
Dicer1	ENSMUST00000041987.6	14.0004	4.275	2.83	7.6	4.7
Gtf3c4	ENSMUST00000171404.3	13.4732	3.705	2.995	5.125	5.525
Kmt2c	ENSMUST00000045291.9	4.7035	6.69	3.035	16	8.135
Brwd1	ENSMUST00000113829.3	20.4707	6.315	5.375	25.78	27.745
Nf1	ENSMUST00000071325.4	7.3464	5.055	2.545	14.415	7.08
Macf1	ENSMUST00000097897.6	33.1411	33.89	10.045	69.81	39.905
Rnf217	ENSMUST00000081989.7	5.7943	0.64	1.975	2.135	4.165
Luzp1	ENSMUST00000105849.4	13.8241	7.415	1.975	8.59	6.33
Cdc42bpb	ENSMUST00000041965.3	13.1609	35.79	24.09	34.105	80.285
Utrn	ENSMUST00000076817.4	12.3318	6.415	1.945	5.64	6.86
Ep400	ENSMUST00000112435.4	10.5929	12.095	7.385	10.795	29.565
Sec16a	ENSMUST00000114082.4	10.026	44.96	49.215	11.775	77.965
Dip2c	ENSMUST00000174552.3	4.5092	5.425	2.26	10.925	9.925
Itga4	ENSMUST00000099972.4	0.98389	0.745	0.22	1.455	0.86
Jrk	ENSMUST00000050234.3	3.0198	2.195	0.59	1.61	3.47
Elmsan1	ENSMUST00000110294.1	2.1229	3.835	2.27	3.215	8.14
Edem1	ENSMUST00000089162.3	26.1889	9.995	36.81	3.915	17.59

Son	ENSMUST00000114037.4	42.8623	41.73	30.405	90.75	116.81
Sox6	ENSMUST00000072804.6	1.7896	3.84	1.54	1.915	0.4
Ctif	ENSMUST00000165559.1	0.41227	3.855	6.235	10.86	8.125
Uhrf1bp1l	ENSMUST00000020112.5	14.9405	6.94	5.02	13.115	3.51
Sbf1	ENSMUST00000123791.3	24.207	43.31	30.32	37.735	92.27
Rfx7	ENSMUST00000093820.5	6.3641	3.42	1.79	7.645	3.875
Nbeal2	ENSMUST00000167320.3	22.7269	22.705	6.865	6.845	19.445
Dock4	ENSMUST00000037488.6	3.5462	2.305	3.745	9.67	7.92
Trim56	ENSMUST00000054384.5	5.4775	7.035	3.405	0.875	7.93
Dync1h1	ENSMUST00000018851.9	31.0827	20.345	7.335	34.97	30.03
Crebbp	ENSMUST00000023165.7	3.0552	10.25	5.13	12.09	15.08
Gm20342	ENSMUST00000185480.1	0.47756	0.995	0.375	1.425	1.04
Fign	ENSMUST00000131615.4	0.16505	1.48	1.08	0.455	0.885
Baz2a	ENSMUST00000170054.4	2.8055	12.265	7.03	13.83	18.38
Parp14	ENSMUST00000042665.8	0.34536	2.465	2.58	0.805	1.85
AU022252	ENSMUST00000141112.1	6.2459	3.7	10.075	3.765	2.62
Als2cl	ENSMUST00000155014.1	19.0563	9.665	9.545	1.675	12.565
Prdm15	ENSMUST00000095849.5	4.6767	1.93	1.36	3.86	4.475
Med12	ENSMUST00000117706.3	5.8026	6.195	3.095	6.785	11.99
Polr2a	ENSMUST00000058470.11	3.9096	17.89	6.765	12.235	15.215
Amer1	ENSMUST00000084535.5	0.99038	1.025	0.605	1.385	2.12
Prrc2c	ENSMUST00000182660.3	24.3771	14.05	6.365	21.175	21.51
Setd1b	ENSMUST00000163030.4	0.43086	4.095	4.36	4.635	6.965
Aars	ENSMUST00000034441.7	70.6912	17.64	35.415	25.155	33.645
Rapgef1	ENSMUST00000102872.6	8.902	23.235	16.515	30.53	39.895
Kif13a	ENSMUST00000056978.7	4.8551	4.675	1.84	3.495	6.69
Irgq	ENSMUST00000049020.7	4.0891	9.435	8.965	18.785	14.635
Ep300	ENSMUST00000068387.6	6.7401	11.735	4.805	8.765	15.095
Rc3h1	ENSMUST00000161609.3	12.3175	8.205	4.565	4.415	10.83
Abhd2	ENSMUST00000037315.8	54.3027	18.115	49.21	15.365	32.57
Usp36	ENSMUST00000106296.4	4.8823	4.755	4.495	6.93	11.825
Aim1	ENSMUST00000020017.8	3.1261	2.185	1.94	0.835	1.015
Impg2	ENSMUST00000069936.7	0.12107	0.105	0.17	0.215	0.04
Dock5	ENSMUST00000039135.4	2.3545	2.985	1.965	0.84	3.74
Dcaf5	ENSMUST00000054145.6	5.2977	9.62	11.82	16.135	19.975
Rprd2	ENSMUST00000090791.3	5.7879	4.5	2.295	6.405	5.69
Spg11	ENSMUST00000036450.7	5.166	3.78	2.47	3.825	6.405
Smg7	ENSMUST00000043560.10	11.9726	18.295	6.955	16.41	14.7
Wdr81	ENSMUST00000173320.3	6.61	10.725	14.17	5.59	15.5
F8	ENSMUST00000033539.8	1.036	1.03	0.725	0.255	0.87
Atxn1l	ENSMUST00000093162.3	6.9561	9.77	4.06	6.12	9.485
Ubn1	ENSMUST00000052449.5	8.431	10.295	5.455	9.415	14.125
Nav2	ENSMUST00000064395.8	2.7094	8.87	10.075	11.265	8.795
Rnf169	ENSMUST00000080817.4	7.4291	3.135	3.975	5.865	3.705
Atg2b	ENSMUST00000041055.7	7.2585	4.42	3.16	6.6	5.605

Qser1	ENSMUST00000117237.1	2.0168	2.665	1.34	2.645	2.585
Arhgef12	ENSMUST00000165665.3	20.3522	24.855	13.04	24.395	15.285
Letm1	ENSMUST00000005431.5	21.2177	24.655	14.625	14.805	12.795
Larp1	ENSMUST00000178636.1	14.0446	20.295	24.67	18.37	28.09
Arhgap35	ENSMUST00000075845.6	17.0046	15.72	14.565	19.845	25.1
Zfyve26	ENSMUST00000021547.6	3.9207	4.605	4.46	3.245	6.19
Prpf8	ENSMUST00000018449.6	51.1044	52.23	41.555	36.85	65.435
Nr6a1	ENSMUST00000112877.3	2.2584	1.3	2.1	1.94	2.25