

satisfy the requirements of SBS sequence base calling. The sample codes are intentionally positioned next to genomic DNA fragments. The driver for this design is ligation bias, meaning that there are base preferences for DNA ligation efficiency in the ~2 bases upstream and the 1-2 bases downstream of the ligation junction. By placing the sample code at the ligation junction, all fragments within a specific sample experience the ligation influences/biases.

* - Without wishing to be bound to any particular theory, it is contemplated that the sequence labels and sample labels could be created as inter-digitated nucleotide sequences.

(3) The “read start point” within genomic fragments is one of two key elements that define a “unique sequence read.” As discussed in section (1) above, the unique identifying “tag” for each read is comprised of the sequencing label AND the read start point. As considered in more detail below, the collection of unique [(1)+(3)] sequence tags is essential for determination of large scale CNV. Here “large scale CNV” is defined as any CNV that involves the entirety of at least one probe binding region plus some adjacent sequence. Large scale CNV can be as large as gain or loss of entire chromosomes.

[(1) + (2)] The sequence label and sample label are embedded in adaptor sequences that are ligated to end-repaired genomic fragments in the initial stage of library construction process where a whole genome library is created.

(4) The sequencing read. The sequence information from genomic fragments is, of course, a central focus of the genomic assays. Each read is considered in the context of multiple, overlapping reads produced within the same assay.

(5) The probe level – “genomic indexing.” The overall genomics assay strategy is to combine multiple sequence labels into a composite “molecular annotation” that places each sequencing read into a larger framework of genomic analysis. Within this operational paradigm, Read 1 reveals elements (1-4) of each annotated clone. Read 2 reveals the probe sequence that retrieved each clone by hybridization based capture and subsequent enzymatic processing. The probe sequence information is central to the genomics strategy because all reads are initially clustered according to the probe that

captured them. This clustering of information on a probe-by-probe basis is termed “genomic indexing” because each read is indexed to a genomic probe prior to analysis.

One of the interesting features of the probe label is that the constellation of all probe sequences within a capture reaction is well-defined (we know which probes went into the capture reaction). This implies that Read 2 does not necessarily need to cover the entire 60 nt probe sequence. Rather, Read 2 only needs to be of sufficient length to enable unambiguous identification of all probes within a specific reaction. As one non-limiting example, the probe set discussed in Example 15 consists of 192 probes that can be differentiated based on only 7 nt of 5' probe sequence (two of the probes with identical 7 nt 5' termini were tagged with dinucleotide codes so they could be informatically differentiated).

(6) The capture label. The composition of the libraries is determined by the intimate molecular interaction between probes and target sequences. The performance of each unique probe sequence can be monitored using the capture label, which can be as simple as a string of several (4 – 6) random bases. The diversity and statistical distribution of capture labels detected in sequencing is a direct measure of probe performance. By way of example, imagine a case where very few sequences are associated with a particular probe sequence. It may be tempting to attribute this deficit of sequences to poor probe performance, and therefore to initiate iterative cycles of probe redesigns. However, sequence under-representation may also be a consequence of sequences that do not ligate well to adaptors and or sequences that do not amplify well with the particular PCR regimen that is used. The use of capture labels allows differentiation of these failure modes. With poor probe performance, the very few capture events that do occur will manifest as very few capture labels that show up multiple times. In contrast, poor representation for reasons upstream of the actual capture reaction (ligation, PCR, end-repair, etc.) will result in a large constellation of capture labels that will be, by and large, uniquely represented. In particular embodiments, as one transitions into automated designs of thousands of probes, the ability to informatically QC probe performance will become increasingly important.

EXAMPLE 17

PROBE SELECTION AND IMPLEMENTATION

SUMMARY

Probe sequence selection and the methods to use them have, necessarily, been developed in concert. This example describes probe selection criterion in Section I and the laboratory methods that make them most effective in Section II. See, *e.g.*, Figure 30.

SECTION I. SELECTION OF TARGETING PROBES.

In the most general terms, target enrichment probes are 60 nt in length. Probes are generally directional, meaning they capture sequences on one side (generally the 3' side) of their position. Tail sequences that add additional functionality (*e.g.*, PCR primer binding site, binding site for complementary oligo that enables biotin pull-out, *etc*) are added in addition to the core targeting 60-mer. Sixty nucleotide targeting sequences are chosen with the following constraints and criterion: (1) The probe is positioned from -100 to +50 nt relative to the start of the target sequence. In Figure 30, the “start” of the target sequence is the intron:exon junction; (2) Probes are designed with redundancy, as illustrated, such that the resulting sequences from a pair of probes are overlapping in opposite orientations; (3) Probes are selected (where possible) to possess GC content not less than 33% (>20 G's or C's per 60 mer) and not more than 67% (<40 G's or C's per 60 mer); (4) Probes are selected to avoid repeats wherever possible. This is done with the help of REPEATMASKER and/or unique alignability criterion, both of which can be viewed on the UCSC genome browser; and (5) In case the position requirement, GC requirement, and the uniqueness requirement cannot be met, selection rules are relaxed in the following order (GC >position>uniqueness). In other words, GC and positioning are flexible; the uniqueness criterion is not.

SECTION II. LABORATORY METHODS.

The input to target enrichment is probes, a gDNA library, and buffers, which have been described elsewhere herein. The first step in targeted enrichment is melting of the gDNA library, which begins in a form as double-stranded PCR fragments. This

is achieved by denaturation of the gDNA, preferably at a concentration of 100 ng/μl in a total volume of 10 μl, at 98°C for 2 min followed by immediate transfer to ice. The gDNA library is suspended in a low salt buffer that contains 10 mM Tris pH 8.0 and 0.1 mM EDTA. The second step is to add 5 μl of concentrated binding buffer (4M NaCl, 40 mM Tris pH 8.0, 0.4 mM EDTA and 0.4% Tween20). While these conditions are specific, the overarching concept is that the concentration of salt must be increased to 2N osmolarity to achieve rapid kinetic association of complementary DNA strands. Five microliters of probe is also added such that the final concentration of probe is 250 pM in each probe. The mixture of gDNA library, buffer and probe is heated to 98°C for 2 min and cooled in 1°C increments for four min each down to 68°C. In the third step, probe:gDNA complexes (the probe has a biotin associated with it) are bound to magnetic beads that are coated with streptavidin. In the fourth step, stringent washing is used to remove unwanted associations between probe and non-target sequences that may occur because of, for example, short matches of nucleotide sequence between probe and gDNA. Stringency is achieved by using low-salt, high-formamide wash buffer as, for example, a buffer containing 30%-35% formamide (v/v), 10 mM Tris pH 8.0, 0.1 mM EDTA and 0.5% Tween 20). Several washes of the beads are used to achieve the desired purity of target sequence (*e.g.*, four). The washed beads possess target sequences bound to probe that are processed, amplified, and sequenced. In summary, low salt melting of the gDNA library, high salt probe annealing, and high formamide washes are used in concert with probe designs to achieve high levels of target sequence enrichment.

EXAMPLE 18

EXEMPLARY SEQUENCES

OVERVIEW

Exemplary genome tags, sample codes and library information are shown in Tables 32-34 below.

Table 32: Exemplary Genome tags

Genome tags			
AAC	CAA	GAT	TAG
ACA	CCC	GCG	TCT
AGT	CGG	GGC	TGA
ATG	CTT	GTA	TTC

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Table 33: Exemplary Sample Codes

Sample codes			
GAA	TCA	CGA	ATA
TAC	GCC	AGC	CTC
AAG	CCG	TGG	GTG
CAT	ACT	GGT	TTT

Table 34: Exemplary Library

Genome tags	Sample codes	Sample ID	Input
AAC	ATA	NA18917	50 genomes
ACA	CCG	NA18917	100 genomes
AGT	GGT	NA18917	200 genomes
ATG	TAC	NA18917	400 genomes
CAA	AGC	NA12878	50 genomes
CCC	CAT	NA12878	100 genomes
CGG	GTG	NA12878	200 genomes
CTT	TCA	NA12878	400 genomes
GAT			
GCG			
GGC			
GTA			
TAG			
TCT			
TGA			
TTC			

EXAMPLE 19

CONSTRUCTION OF TAGGED, TARGETED, GENOMIC LIBRARY

SUMMARY

Several ways to construct tagged, targeted genomic sequencing libraries are contemplated herein. In this embodiment, DNA repair is used to attach probe-associated sequences to captured genomic fragments. This approach worked well for creating sequence-ready targeted genomic libraries.

CONCEPT

An important principle of the library construction is that the sequence ready clones are comprised of DNA sequences derived from both genomic fragments and capture probes. This “recombination” of parts greatly enriches for those genomic fragments that are in direct contact with probe, and it enables focused sequencing reads on one side of a probe sequence. In this design, the tripartite complex formed between the target genomic library fragment, the capture probe and the common partner oligo possesses a structure reminiscent of a DNA replication fork. Such forks occur during normal DNA replication, but they also occur during DNA repair processes. In the latter case, it is often necessary to trim 5' displaced strands to enable joining of the newly polymerized strand to the adjacent 3' sequence. This repair process requires two enzymes and three enzymatic activities. DNA polymerase holoenzymes like *E. coli* DNA polymerase or *Bst* DNA polymerase possess two of these activities, a 5' to 3' endonuclease activity that removes these 5' displaced flaps and, of course, DNA polymerase activity.

In particular embodiments, *Bst* polymerase is preferred because it also lacks 3' to 5' nuclease activity that is often associated with DNA polymerase holoenzymes. See, e.g., Figure 31. This feature is useful because it suggests that the single-stranded 3' DNA protrusions of the target genomic clones do not require protection. The other enzyme and activity required is a nick-closing DNA ligase such as the NAD⁺-requiring *Taq* DNA ligase. Following processing, processed fragments are amplified by PCR to allow for size selection and quantification prior to sequencing.

PROOF OF PRINCIPLE OLIGONUCLEOTIDES

For this experiment, eight target regions were chosen that correspond to eight genomic regions for which we have qPCR assays. The forward and reverse primers for these eight regions are shown in Table 35. The capture probes are the exact reverse complement of capture probes that were used and validated elsewhere herein. These probes span a %GC range from 22% to 73% as noted in Table 37.

Table 35: Proof of principle oligonucleotides

Table 1. Proof of principle oligonucleotides			
SEQ ID NO:	qPCR Assay	Name	Sequence
585	17	SRY_F	CTGGTGCTCCATTCTTGAGTGTGT
586		SRY_R	GGACAACAGGTTGTACAGGGATGA
587	18	VHL_F	TACAGGAGACTGGACATCGTCAGG
588		VHL_R	CTTTCTGCACATTGGGTGGTCTT
589	19	UGT_F	GGTGATCAGATGGACAATGCAAAG
590		UGT_R	TCATTTCAGAACATTCAGGGTCA
591	20	TNF_F	ACCTCAATGGCCTAAGCAAGTGC
592		TNF_R	GCCTCTTACCTGGGTCACACATTT
593	21	RUNX_F	ATCTTGCAACCTGGTTCTTCATGG
594		RUNX_R	GCTGGCAATGATGAAACTACTCG
595	22	RHD_F	CCAAGTTTCAACTCTGCTCTGCTG
596		RHD_R	GTTGAACACGGCATTCTTCCTTTC
597	23	PTEN_F	GGAAAGGGACGAACTGGTGTAAATG
598		PTEN_R	AAATCTAGGGCCTCTTGTGCCTTT
599	24	EP300_F	AGCCCCATGGATCTTCTACCATT
600		EP300_R	ATACTGCCAGGGCTCCTGATACTG
	Capture probe	Name	Sequence
601	17	SRY_r1f_V3	AATCGGGTAACATTGGCTACAAAGACCTACCTAGATGCTCCTTTTTACGATAACTTACAGGTGAAAACCAGGATCAACTCCCGTGCCAGTCACATCTCGT
602		SRY_r1r_V3	ATTATAAGTATCGACCTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCGTGAAAACCAGGATCAACTCCCGTGCCAGTCACATCTCGT

Probes were synthesized as ultramers by IDT, rehydrated to 100 uM, and pooled; each probe in the pool is present at 6.25 uM. To create a 100X stock solution in which each probe was present at 100 nM, 10 uM of pool and 10 µl of 100 uM common, biotinylated partner oligo were combined in 605 µl of TEzero + 0.05% Tween 20 (TT). The 100X stock was further diluted 100-fold (10 µl into 990 µl TT) to give a working solution in which each probe is present at a concentration of 1 nM.

CAPTURE/PROCESSING PROTOCOLS

One objective of the proof of principle study was to validate probe performance and test the efficiency of processing on sequence-ready library yield. The genomic library pool was derived from a 16 sample set library. For probe annealing, four of 10 µl library aliquots in separate PCR strip tubes were heated to 98°C for 2 min and cooled on ice. Five µl of 4X bind buffer and five µl of probe were added to each tube, and the solution was annealed using a 1°C step for four min thermal cycler program from 98°C to 69°C. Annealed complexes were bound to streptavidin-coated magnetic beads, washed four times with 25% formamide-containing wash buffer and one time with TEzero. The final complexes were suspended in 2 µl of TEzero.

Four treatments of the four complexes were investigated: (1) No processing, amplification with ACA2 primer alone to determine raw capture efficiency; (2) No processing, amplification with AF and CR to determine unprocessed amplification and capture efficiency; (3) PreCR processing in 10 µl prior to AF and CR amplification to explore low volume processing; and (4) PreCR processing in 50 µl prior to AF and CR amplification to establish high volume processing effects.

PreCR processing was accomplished by adding the manufacturer's recommended solution that contained per 100 µl:

- 82 µl of water
- 10 µl of Thermopol buffer
- 1 µl of 100X NAD⁺
- 1 µl of 10 mM dNTPs
- 2 µl of PreCR enzyme mix

Ten µl of PreCR cocktail was added to tube 3 and 50 µl was added to tube 4. These were incubated at 37C for 20 min.

Following PreCR treatment, all four samples were resuspended to 50 µl by the addition of TEzero, and Q5 PCR cocktail with the appropriate PCR primers was added to a final volume of 250 µl. Each aliquot of PCR cocktail contained:

- 125 µl water
- 50 µl 5X Q5 reaction buffer
- 25 µl of 10 uM primer (either ACA2 or a 1:1 blend of AF and CR)
- 5 µl of 10 mM dNTPs
- 2.5 µl of Q5 hot start enzyme

50 µl of each PCR reaction mix was aliquoted to a tube containing 1.25 µl EvaGreen and 1 µl ROX dyes, mixed, and quadruplicate 10 µl aliquots were added to a qPCR optical PCR plate. The remaining 200 µl was split into to 100 µl aliquots. Both the qPCR and conventional PCR reaction were cycled as:

- 98°C – 30 sec
- 98°C – 10sec, 69°C – 10 sec, 72°C-10sec for 40 cycles (qPCR) and plateau cycle (conventional).

The real-time PCR reaction was monitored to determine the optimal stopping point for the conventional PCR reactions. For the ACA2 reaction, the stopping point was at 21 cycles. For the remaining reactions, the stopping point was at 28 cycles. These qPCR reactions are further described in the Results section, *infra*.

Ten µl of raw PCR was collected for gel analysis and a remaining aliquot of 100 µl was purified 1:1 with beads. The purified PCR product was eluted with 50 µl TEzero and quantified by Qubit. The DNA yields were: (1) 7.44 ng/µl; (2) 10.6 ng/µl; (3) 12.1 ng/µl; and (4) 15.7 ng/µl.

QPCR ANALYSIS OF CAPTURE/PROCESSING

A single Eco qPCR plate containing an array of eight assays – Assays 17 – 24 (Table 37) by six samples was used to assess capture efficiency. The six samples were:

1. 10 ng/µl of original gDNA library
2. NTC
3. 0.01 ng/µl of sample 1
4. 0.01 ng/µl of sample 2
5. 0.01 ng/µl of sample 3

6. 0.01 ng/μl of sample 4

The Q5 hot start assay mixture contained:

- 237.5 μl H₂O
- 100 μl 5X Q5 reaction buffer
- 10 μl dNTPs
- 12.5 μl EvaGreen
- 10 μl ROX
- 5 μl Q5 hot start enzyme

This cocktail was distributed in 48 μl aliquots and 3 μl of Assay primer (10 uM in both F and R primer) was added. This was distributed in columns. Two μl of sample was added in rows and the plate was cycled as described above.

RESULTS

Amplification of complexes. While the fluorescence profile of amplifying complexes is used primarily to identify the amplification plateau (which occurs much sooner for single primer than dual primer amplicons), the C_q value can be used to look at the content of amplicon between samples. In this experiment, the observed C_q values were:

Sample	Conditions	C _q
1	no PreCR, ACA2 single primer	15
2	no PreCR, AF + CR	21
3	10 μl PreCR, AF + CR	20
4	50 μl PreCR, AF + CR	19

These data demonstrated that PreCR treatment increased the abundance of P1 + P2 (AF + CR) amplicons.

Gel images of the post-processing PCR products shown in Figure 33 show that PreCR treatment supported the amplification of a larger size distribution of clones. The untreated, sample 2 amplicon is primarily a cluster of small size fragments. Sample 3 and to a greater extent sample 4 are more broadly distributed smears.

The qPCR results showing target enrichment are shown in Table 36 below. The raw sequence capture in sample 1 was surprisingly high. At least two factors may have

contributed to such an unexpected improvement over historical data sets: (1) The core annealing process (pre-melt, higher temps, low salt binding to strep beads) has been optimized; and (2) A longer partner oligo (40 nt vs 35 nt) was used.

Even with no PreCR treatment, P1+P2 (AF+CR) amplifiable material was made (sample 2) and substantial enrichment of target signal over gDNA (and/or NTC) was observed.

PreCR treated complex also yielded enrichment levels comparable to unprocessed (sample 1) control. This is a fantastic demonstration of the fact that PreCR processing can stimulate the recombination of the probe-based partner oligo with the genomic library-based target clones. While the levels of enrichment are not remarkable, the majority of clone material is small and falls outside the range of the qPCR assay. As noted elsewhere herein, judicious bead enrichment can dramatically increase the proportion of the library that covers the qPCR site.

In addition, the results indicated that more PreCR is not necessarily better. Sample 3 (10 μ l PreCR treatment) outperformed Sample 4 (50 μ l PreCR treatment) with respect to enrichment specific activity in 6 of 8 assays.

Table 36. qPCR enrichment data

Table 36. qPCR enrichment data for V3 experimental samples								
Cqs	Assay 17	Assay 18	Assay 19	Assay 20	Assay 21	Assay 22	Assay 23	Assay 24
gDNA lib	24	21	21	22	22	22	22	22
NTC	35	31	34	26	33	N/A	30	29
sample 1	16	13	13	12	14	13	15	14
sample 2	21	18	19	16	18	18	22	20
sample 3	16	14	15	13	14	15	17	15
sample 4	16	14	15	13	15	15	17	15
Abs value	Assay 17	Assay 18	Assay 19	Assay 20	Assay 21	Assay 22	Assay 23	Assay 24
gDNA lib	5	40	36	32	20	22	34	29
NTC	0	0	0	1	0		0	0
sample 1	1918	14374	9372	20243	8444	10189	2820	8133
sample 2	59	382	172	1526	295	403	34	87
sample 3	1085	5090	3051	10275	5213	4144	1068	2822
sample 4	1492	5381	2751	8770	3866	2777	777	2233
Adj value	Assay 17	Assay 18	Assay 19	Assay 20	Assay 21	Assay 22	Assay 23	Assay 24
gDNA lib	5	40	36	32	20	22	34	29
NTC	0	0	0	1	0	0	0	0

sample 1	1918016	14374082	9372333	20243011	8443797	10188837	2820166	8132920
sample 2	59292	381694	171590	1526000	295038	403467	33575	87344
sample 3	1085442	5089739	3050781	10275412	5212989	4143563	1068184	2821950
sample 4	1491501	5380882	2751029	8770152	3865764	2777330	777035	2232604
Fold enrich	Assay 17	Assay 18	Assay 19	Assay 20	Assay 21	Assay 22	Assay 23	Assay 24
sample 1	378673	356798	262412	641805	415341	453405	84090	281573
sample 2	11706	9475	4804	48382	14513	17954	1001	3024
sample 3	214298	126339	85418	325782	256421	184389	31850	97700
sample 4	294466	133566	77025	278058	190152	123592	23169	77296

DISCUSSION

The capture and processing methods disclosed in this example performed well using untreated complexes. Without wishing to be bound to any particular theory, it is contemplated that one reason that the untreated complexes performed so well was because both the capture probe and genomic fragments possess primer binding sites.

APPENDIX TO EXAMPLE 19

The primer and amplicon designs for this example are shown below in Table 37.

Table 37. Sequences used to perform Example 19

SEQ ID NO:	Target	Name	Sequence	%GC
603	SRY_r1, Assay 17	F qPCR primer	CTGGTGCTCCATTCTTGAGTGTGT	
604		R qPCR primer	GGACAACAGGTTGTACAGGGATGA	
605		qPCR Amplicon	CTGGTGCTCCATTCTTGAGTGTGTGGCTTTTCGTA CAGTCATCCCTGTACAACCTGTTGTCC	
606		F-probe	CTGTAAGTTATCGTAAAAAGGAGCATCTAGGTA GGTCTTTGTAGCCAATGTTACCCGATT	40%
607		R-probe	GAAGCAAACCTGCAATTCTTCGGCAGCATCTTCG CCTTCCGACGAGGTCGATACTTATAAT	47%

608		F-probe reverse complement	AATCGGGTAACATTGGCTACAAAGACCTACCTA GATGCTCCTTTTTACGATAACTTACAG	
609		R-probe reverse complement	ATTATAAGTATCGACCTCGTCGGAAGGCGAAGA TGCTGCCGAAGAATTGCAGTTTGCTTC	
610	VHL_r3 Assay 18	F qPCR primer	TACAGGAGACTGGACATCGTCAGG	
611		R qPCR primer	CTTTCTGCACATTTGGGTGGTCTT	
612		qPCR Amplicon	TACAGGAGACTGGACATCGTCAGGTCGCTCTAC GAAGATCTGGAAGACCACCCAAATGTGCAGAA AG	
613		F-probe	CTTGTTTCGTTTCCTTGTACTGAGACCCTAGTCTGC CACTGAGGATTTGGTTTTTGGCCCTTC	48%
614		R-probe	ATCAAGACTCATCAGTACCATCAAAAGCTGAGA TGAAACAGTGTAAGTTTCAACAGAAAT	35%
615		F-probe reverse complement	GAAGGGCAAAAACCAAATCCTCAGTGGCAGAC TAGGGTCTCAGTACAAGGAACGAACAAG	
616		R-probe reverse complement	ATTTCTGTTGAAACTTACACTGTTTCATCTCAGC TTTTGATGGTACTGATGAGTCTTGAT	
617	UGT1A 1_r4, Assay 19	F qPCR primer	GGTGATCAGATGGACAATGCAAAG	
618		R qPCR primer	TCATTTCCAGAACATTCAGGGTCA	

619		qPCR Amplicon	GGTGATCAGATGGACAATGCAAAGCGCATGGA GACTAAGGGAGCTGGAGTGACCCTGAATGTTCT GGAAATGA	
620		F-probe	TGTGTCCAGCTGTGAAACTCAGAGATGTAAGTG CTGACATCCTCCCTATTTTGCATCTCA	45%
621		R-probe	ATTTGAAACAATTTTATCATGAATGCCATGACC AAAGTATTCTTCTGTATCTTCTTTCTT	28%
622		F-probe reverse complement	TGAGATGCAAAATAGGGAGGATGTCAGCAGTTA CATCTCTGAGTTTCACAGCTGGACACA	
623		R-probe reverse complement	AAGAAAGAAGATACAGAAGAATACTTTGGTCAT GGCATTTCATGATAAAATTGTTTCAAAT	
624	TNFRS F14_r3, Assay 20	F qPCR primer	ACCTCAATGGCCTAAGCAAGTGTC	
625		R qPCR primer	GCCTCTTACCTGGGTCACACATTT	
626		qPCR Amplicon	ACCTCAATGGCCTAAGCAAGTGTCTGCAGTGCC AAATGTGTGACCCAGGTAAGAGGC	
627		F-probe	TGATGGGTGGGCTCCCGAAGGGGCCTCCCGCAG ACTTGCGAAGTTCCCACTCTCTGGGCG	67%
628		R-probe	CAGGGTGCGGGGGCATCCAGGCTGCCCAAGCG GAGGCTGGGCCGGCTGTGCTGGCCTCTT	73%
629		F-probe reverse complement	CGCCCAGAGAGTGGGAACTTCGCAAGTCTGCGG GAGGCCCTTCGGGAGCCCAACCATCA	
630		R-probe reverse complement	AAGAGGCCAGCACAGCCGGCCAGCCTCCGCTT GGGCAGCCTGGATGCCCCCGCACCTG	

631	RUNX_ r4, Assay 21	F qPCR primer	ATCTTGCAACCTGGTTCTTCATGG	
632		R qPCR primer	GCTGGCAATGATGAAAACACTCG	
633		qPCR Amplicon	AATCTTGCAACCTGGTTCTTCATGGCTGCGGTAG CATTTCTCAGCTCAGCCGAGTAGTTTTCATCATT GCCAGC	
634		F-probe	TTTTGAAATGTGGGTTTGTGTCATGAAACGTGT TTCAAGCATAGTTTTGACAGATAACG	37%
635		R-probe	TGCCCTAAAAGTGTATGTATAACATCCCTGATG TCTGCATTTGTCCTTTGACTGGTGTTT	40%
636		F-probe reverse complement	CGTTATCTGTCAAAACTATGCTTGAAACACGTTT CATGGCAACAAACCCACATTTCAAAA	
637		R-probe reverse complement	AAACACCAGTCAAAGGACAAATGCAGACATCA GGGATGTTATACATACACTTTTAGGGCA	
638	RHD_r5 Assay 22	F qPCR primer	CCAAGTTTCAACTCTGCTCTGCTG	
639		R qPCR primer	GTTGAACACGGCATTCTTCCTTTC	
640		qPCR Amplicon	CCAAGTTTCAACTCTGCTCTGCTGAGAAGTCCA ATCGAAAGGAAGAATGCCGTGTTCAAC	
641		F-probe	AACCCCTCGAGGCTCAGACCTTTGGAGCAGGAG TGTGATTCTGGCCAACCACCCTCTCTG	58%
642		R-probe	CATAAATATGTGTGCTAGTCCTGTTAGACCCAA GTGCTGCCCAAGGGCAGCGCCCTGCTC	55%

643		F-probe reverse complement	CAGAGAGGGTGGTTGGCCAGAATCACACTCCTG CTCCAAAGGTCTGAGCCTCGAGGGGTT	
644		R-probe reverse complement	GAGCAGGGCGCTGCCCTTGGGCAGCACTTGGGT CTAACAGGACTAGCACACATATTTATG	
645	PTEN_r 5, Assay 23	F qPCR primer	GGAAAGGGACGAACTGGTGTAATG	
646		R qPCR primer	AAATCTAGGGCCTCTTGTGCCTTT	
647		qPCR Amplicon	GGAAAGGGACGAACTGGTGTAATGATATGTGCA TATTTATTACATCGGGGCAAATTTTAAAGGCA CAAGAGGCCCTAGATTT	
648		F-probe	TACTTGTTAATTAAAAATTCAAGAGTTTTTTTTT CTTATTCTGAGGTTATCTTTTACCA	22%
649		R-probe	CCAAAATCTGTTTTCCAATAAATTCTCAGATCCA GGAAGAGGAAAGGAAAAACATCAAAA	33%
650		F-probe reverse complement	TGGTAAAAAGATAACCTCAGAATAAGAAAAAA AAACTCTTGAATTTTAAATTAACAAGTA	
651		R-probe reverse complement	TTTTGATGTTTTTCCTTTCCTCTTCCTGGATCTGA GAATTTATTGGAAAACAGATTTTGG	
652	EP300_r 18, Assay 24	F qPCR primer	AGCCCCATGGATCTTTCTACCATT	
653		R qPCR primer	ATACTGCCAGGGCTCCTGATACTG	

654		qPCR Amplicon	AGCCCCATGGATCTTTCTACCATTAAGAGGAAG TTAGACACTGGACAGTATCAGGAGCCCTGGCAG TAT	
655		F-probe	ATACTCCATCTCCCGTAAAAATAGTGAGACTTG AGTAATGTTTGATGTCACTTGTCTTTC	37%
656		R-probe	CAGTCACCACTATATTATTCTAGGTATCCCAGA AAAGTTAAAGTCAAATCTGAAACACAT	33%
657		F-probe reverse complement	GAAAGACAAGTGACATCAAACATTACTCAAGTC TCACTATTTTTACGGGAGATGGAGTAT	
658		R-probe reverse complement	ATGTGTTTCAGATTTGACTTTAACTTTTCTGGGA TACCTAGAATAATATAGTGGTGACTG	
659	Addition al oligos	Tail sequence for capture probes	GTGAAAACCAGGATCAACTCCCGTGCCAGTCAC ATCTCGT	
660		Common partner oligo sequence	/5BioTEG/ACGAGATGTGACTGGCACGGGAGTTG ATCCTGGTTTTTCAC	
661		Forward amplification primer AF	AATGATACGGCGACCACCGAGATCTACACGTCA TGCAGGACCAGAGAATTCGAATACA	
662		Reverse amplification primer CR	CAAGCAGAAGACGGCATAACGAGATGTGACTGG CACGGGAGTTGATCCTGGTTTTTCAC	

EXAMPLE 20

LIBRARY-FREE TARGETED GENOMIC ANALYSIS

SUMMARY

This example demonstrates a library-free genomic analysis. The goals were to identify the most useful parameters for implementing such methods in a reliable, reproducible, low-cost, and high-throughput format. In particular, it was discovered that T4 polymerase can copy many and diverse genomic sequences provided it is supplemented with T4 gene 32 protein in the presence of PEG8000 – a molecular crowding agent. In addition, it was found that suppression PCR just upstream of sequence library construction is a powerful method to enrich for long insert sequencing clones.

BACKGROUND

The molecular concepts behind library-free methods include:

(1) fragmenting gDNA to ~400bp or performing 1st strand cDNA synthesis with random 15-mers in the presence of ddNTPs (Figure 33);

(2) melting the gDNA or cDNA with labeled capture probes and purify the end-repaired gDNA/cDNA. For gDNA, the genomic sequence is repaired with a sequence tag that comprises a random hexamer sequence contained within the tail portion (Figure 33);

(3) processing the DNA complex in a single reaction at 20°C. The Buffer used is NEB CutSmart (NEB#4 and BSA), ATP, dNTPs, and PEG8000. The complex is processed with T4 DNA polymerase, T4 gene 32 protein (SB), and T4 DNA ligase. The adapter ligation strand is 5' phosphorylated and the partner strand comprises a 3' ddC. The opposite end of the adapter is staggered and can be blocked. A blunt configuration makes no self-dimer, is extremely efficiency and attaches the P1 containing ligation strand to the P2 containing target. (Figure 34);

(4) PCR amplification to add flow cell compatible sequences and to introduce sample-specific index sequence to each reaction (Figure 35); and

(5) DNA sequencing (Figure 35).

One potential artifact that may occur in particular embodiments is associated with the abundance of unoccupied probes. The 3' to 5' exonuclease activity of T4 DNA polymerase is capable of generating a blunt end on these molecules, which then becomes a substrate for ligation to the P1 adaptor sequence (Figure 36). These short “oligo-dimer” products will, without intervention, overwhelm the subsequent PCR reaction. To circumvent the potential artifact, a suppressive PCR design was used, in which a 25 nt segment of P2 was included in the P1 adaptor. Following suppression PCR amplification with this segment, forward and reverse primers with P1 or P2-specific extensions are used to add the index sequence and the flow cell-compatible extensions.

The oligonucleotides that enable post-processing suppressive PCR, full-length amplification and sequencing are shown in the Table 38 below.

Table 38.

SEQ ID NO:	Name	Sequence	Description
663	LF_part strand	AGTTGATCCTGGTTATACA/3ddC/	Adaptor partner strand
664	LF_lig strand	/5Phos/GTGTATAACCAGGATCAACTCCC GTGCCAGT	Adaptor ligation strand
665	LF_I1P	GTGAAAACCAGGATCAACTCCCGTGCCA GTCAC	Index 1 sequencing primer
666	LF_FSP	GTCATGCAGGAGTTGATCCTGGTTATAC AC	Library-free Forward sequencing primer
667	LF_Single primer sequence	ACTGGCACGGGAGTTGATCCTGGTT	Post-processing amplification primer
668	LF_FLFP	AATGATACGGCGACCACCGAGATCTAC ACGTCATGCAGGAGTTGATCCTGGTTAT ACAC	Library-free forward amplification primer
669	LF_FLRP_N701	CAAGCAGAAGACGGCATAACGAGATTTCG CCTTAGTGACTGGCACGGGAGTTGATCC TGGTTTTCAC	Index N701 reverse primer
670	LF_FLRP_N702	CAAGCAGAAGACGGCATAACGAGATCTA GTACGGTGACTGGCACGGGAGTTGATCC TGGTTTTCAC	Index N702 reverse primer
671	LF_FLRP_N703	CAAGCAGAAGACGGCATAACGAGATTTC TGCCTGTGACTGGCACGGGAGTTGATCC TGGTTTTCAC	Index N703 reverse primer
672	LF_FLRP_N704	CAAGCAGAAGACGGCATAACGAGATGCT CAGGAGTGACTGGCACGGGAGTTGATC CTGGTTTTCAC	Index N704 reverse primer

MATERIALS

Genomic DNA samples were collected from 4 subjects and purified using the Oragene saliva collection kit. The samples that were sequenced in this study were:

Genomic DNA samples and Illumina Indexes used in this study		
Coriel ID #	Sample description	Illumina Index ID
NA19249	Yoruba female	Index N701 reverse primer
GM03623	Aneuploid XXX and chr18 trisomy	Index N702 reverse primer
GM11226	XXXX	Index N703 reverse primer
GM18917	Yoruban/Nigerian - harbors deletion variants 1,2,4,9	Index N704 reverse primer

The probes used in these experiments are provided in Table 39 below. Hexamer tags are required to establish independent capture events with the same sequencing start site from sibling clones that arise during post-capture amplification.

Table 39.

SEQ ID NO:	Name	Sequence
673	CYP2D6_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAAGCACCTAGCCCCATTCTGCTGAGCAGGAGGTGGCA GGTACCCCAGACTGGGAGGTAA
674	CYP2D6_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGTCGGTGGGGCCAGGATGAGGCCAGTCTGTTACAC ATGGCTGCTGCCTCTCAGCTCT
675	AMY1_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NACCTGAGTAGCATCATTGTAGTTCTCGATATCTCCACTT CCAGTTTTACATTACCATCA
676	chrX_15_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCCTGGCCCTCAGCCAGTACAGAAAGTCATTTGTCAAGG CCTTCAGTTGGCAGACGTGCTC
677	chrX_15_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGAATTCATTGCCAGCTATAAATCTGTGGAAACGCTGCC ACACAATCTTAGCACACAAGA
678	chrX_477_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGACTTCAAAGAAATTACAAGTTGACATCTTGGACTCTAC CCCTCGTACTTTATCTCCTAT
679	chrX_477_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTCTCTTTGGGGTCAAGAAAGAATCCCTAGTGGATTTGGG ATTCTAGAGGAGGTGTTATAA
680	chrX_478_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTGCGATAACCATGCTGAAGATGAGCTAACCCAACCCAGCC AAGCAGGCAGGGCTGCGAAGGA

681	chrX_478_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NNGGGTAGGTGGAAAACCCAAGTAATGTGATTTTGTAAC ATCCACTGCTGCATTTGTTTGC
682	chrX_69_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTTACTTCCCTCCAGTTTTGTTGCTTGCAAAACAACAGAA TCTTCTCTCCATGAAATCATG
683	chrX_69_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCAGGGGTATCTATTATCCCCATTTTCTCACAAAGGAAAC CAAGATAAAAGGTTTAAATGG
684	PLP1_ex1_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGAAATTCTCTTGTGAATTCCTGTGTCTCTTGAATCTTCA ATGCTAAAGTTTTTGAACT
685	PLP1_ex2_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NNGGTTTGAGTGGCATGAGCTACCTACTGGATGTGCCTGA CTGTTTCCCTTCTTCTTCCC
686	PLP1_ex2_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCTATCTCCAGGATGGAGAGAGGGAAAAAAGATGGGT CTGTGTGGGAGGGCAGGTACTT
687	PLP1_ex3_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGAAAGAAGCCAGGTCTTCAATTAATAAGATTCCCTGGT CTCGTTTGTCTACCTGTTAATG
688	PLP1_ex3_M	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCAGACTCGCGCCCAATTTTCCCCACCCCTTGTTATTGC CACAAAATCCTGAGGATGATC
689	PLP1_ex3_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTCTTTCTTCTTCTTTATGGGGCCCTCCTGCTGGCTGAGG GCTTCTACACCACCGGCGCA
690	PLP1_ex4_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGTTTGTGTTTCTACATCTGCAGGCTGATGCTGATTCTA ACCACCCCATGTCAATCATTT
691	PLP1_ex4_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAACCAAATATATAGTGCTTCCATAGTGGGTAGGAGAGC CAAAGCACCCGTACCCTAACTC
692	PLP1_ex5_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGTCTCCATGTGGCCCCGTAAGTCCATAAAGCTTACCCT GCTTGCTTTTTGTGTCTTACT
693	PLP1_ex5_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCCATGGGTGTAATTTGTATGGTATTAGCTACTCCCTTGT AAAATAACCCAAATAACCCAC
694	PLP1_ex6_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTTTACAGTGGAGCATATTACTGCTGTTGCAAGAAACAGT TCTTCCTCTTTCATTTTCCTG
695	PLP1_ex6_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NATAGCTGTACCCACACTATCTCAGGCCTATTTACTTGCC AAGATCATTCAAAGTCAACTC

696	PLP1_ex7_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGATTTGAGGAGGGAGTGCTTTCTTTCTACTCTCATTCA CATTCTCTCTTCTGTTCCCTA
697	PLP1_ex7_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCAGCATTGTAGGCTGTGTGGTTAGAGCCTCGCTATTAGA GAAAGGGGGATTCTACGGGG
698	KRAS_ex1_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTGTTACCTTTAAAAGACATCTGCTTTCTGCCAAAATTAA TGTGCTGAACTTAACTTACC
699	KRAS_ex1_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTTCCCAGTAAATTACTCTTACCAATGCAACAGACTTTAA AGAAGTTGTGTTTTACAATGC
700	KRAS_ex2_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTAAATGACATAACAGTTATGATTTTGCAGAAAACAGAT CTGTATTTATTTCAAGTGTTACT
701	KRAS_ex2_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGACAGGTTTTGAAAGATATTTGTGTTACTAATGACTGTG CTATAACTTTTTTTCTTTCC
702	KRAS_ex3_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NACTCAAAAAATAAAAACTATAATTACTCCTTAATGTCAG CTTATTATATTCAATTAAAC
703	KRAS_ex3_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAACACCTTTTTTTGAAGTAAAAGGTGCACTGTAATAATCC AGACTGTGTTTCTCCCTTCTC
704	KRAS_ex4_F	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGAAACCTTTATCTGTATCAAAGAATGGTCCTGCACCAGT AATATGCATATTAAACAAGA
705	KRAS_ex4_R	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGTGTATTAACCTTATGTGTGACATGTTCTAATATAGTCA CATTTTCATTATTTTTATTAT
706	MYC_r1_F1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NCCCCAGCCAGCGGTCCGCAACCCTTGCCGCATCCACGA AACTTTGCCCATAGCAGCGGGC
707	MYC_r1_R1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN Ncgactcatctcagcattaaagtataaaaaataaataaaaggcaagtggacttcggtg
708	MYC_r2_F1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN Nctgtggcgcgcactgcgcgctgcgccaggtttccgcaccaagaccctttaactcaagac
709	MYC_r2_F2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTTCTACTGCGACGAGGAGGAGAACTTCTACCAGCAGCA GCAGCAGAGCGAGCTGCAGCCC
710	MYC_r2_F3	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NACCGAGCTGCTGGGAGGAGACATGGTGAACCAGAGTTT CATCTGCGACCCGGACGACGAG

711	MYC_r2_F4	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGCCGCCGCCTCAGAGTGCATCGACCCCTCGGTGGTCTTC CCCTACCCTCTCAACGACAGC
712	MYC_r2_R1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGGCGGCTAGGGGACAGGGGCGGGGTGGGCAGCAGCTC GAATTTCTTCCAGATATCCTCGC
713	MYC_r2_R2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGACGAGCTTGGCGGCGGCCGAGAAGCCGCTCCACATA CAGTCCTGGATGATGATGTTTT
714	MYC_r2_R3	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGGAGAGCAGAGAATCCGAGGACGGAGAGAAGGCGCT GGAGTCTTGCGAGGCGCAGGACT
715	MYC_r2_R4	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN Ntaagagtggcccgtaaataagctgccaatgaaatgggaaaggtatccagccgccact
716	MYC_r3_F1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN Nttgtattgtacagcattaatctggtaattgattatttaataatgtaaccttgctaaaggag
717	MYC_r3_F2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGAGGCCACAGCAAACCTCCTCACAGCCCACTGGTCCTC AAGAGGTGCCACGTCTCCACAC
718	MYC_r3_F3	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NAGAGGAGGAACGAGCTAAAACGGAGCTTTTTTGCCCTG CGTGACCAGATCCCGGAGTTGG
719	MYC_r3_R1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NTCCAACCTGACCCTCTTGGCAGCAGGATAGTCCTTCCGA GTGGAGGGAGGCGCTGCGTAG
720	MYC_r3_R2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGCTTGGACGGACAGGATGTATGCTGTGGCTTTTTTAAGG ATAACTACCTTGGGGGCCTTT
721	MYC_r3_R3	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNN NGCATTGATCATGCATTTGAAACAAGTTCATAGGTGATT GCTCAGGACATTTCTGTTAGA

METHODS, RESULTS AND DISCUSSION

PART I. Four gDNAs (F, S, C and L) were diluted to 20 ng/μl in 150 μl final volume. The samples were sonicated to 500 bp and 125 μl was purified with 125 μl of beads. The starting material and purified, fragmented gDNA are shown in Figure 37. The concentrations of gDNA were: (1) F - 137 ng/μl; (2) S - 129 ng/μl; (3) C - 153 ng/μl; and (4) L - 124 ng/μl.

For capture, 10 μ l of gDNA sample was heated to 98°C for 2 min (to achieve strand dissociation) and cooled on ice. 5 μ l of 4X bind and 5 μ l of the 49 probe pool (SEQ ID NOs: 150-198) (1 nM in each probe combined with 50 nM universal oligo 61, were added and the mix was annealed (98°C 2 min followed by 4 min incubations at successive 1°C lower temperatures down to 69°C). The complexes were bound to 2 μ l of MyOne strep beads that were suspended in 180 μ l TEzero (total volume 200 μ l) for 30 min, washed four times, 5 min each with 25% formamide wash, washed once with TEzero, and the supernatants were withdrawn from the bead complexes.

For processing and adaptor ligation, 100 μ l of T4 mix was made that contained: 60 μ l water; 10 μ l NEB “CutSmart” buffer; 15 μ l 50% PEG8000; 10 μ l 10 mM ATP; 1 μ l 1 mM dNTP blend; 1 μ l T4 gene 32 protein (NEB); and 0.5 μ l T4 DNA polymerase (NEB). 25 μ l of mix was added to each of the four samples and incubated at 20°C for 15 min followed by a 70°C incubation for 10 min to heat inactivate the T4 polymerase. Following the inactivation step, 1.25 μ l of adaptor (10 μ M) and 1.25 μ l of HC T4 DNA ligase were added. This mixture was further incubated at 22°C for 30 min and 65°C for 10 min.

One attractive feature of the library free methods is that processed complexes are still attached to beads. Beads were pulled from the ligation buffer and washed once with 200 μ l of TEzero. The complexes were then resuspended in 2 μ l. For amplification, single primer amplification in a 20 μ l volume was used to both amplify target fragments and to enrich for long genomic fragments over probe “stubs”. Following the amplification, a larger volume PCR reaction with full length primers were used to create a “sequence-ready” library.

A Q5-based single primer PCR amplification buffer was made by combining 57 μ l water, 20 μ l 5X Q5 reaction buffer, 10 μ l of single primer 117 (see Table 38), 2 μ l of 10 mM dNTPs, and 1 μ l of Q5 hot start polymerase. Eighteen μ l was added to each tube followed by amplification for 20 cycles (98°C – 30sec; 98°C – 10 sec, 69°C – 10 sec, 72°C – 10 sec for 20 cycles; 10°C hold). Following the PCR, the beads were pulled out and the 20 μ l of pre-amp supernatant was transferred to 280 μ l of PCR mix that contained 163.5 μ l water, 60 μ l 5X Q5 buffer, 15 μ l of forward primer 118 (10 μ M), 15 μ M of reverse primer 119 (10 μ M), 6 μ l of 10 mM dNTPs, 13.5 μ l of EvaGreen+ROX

dye blend (1.25 parts EG to 1 part ROX), and 3 µl of Q5 hot start polymerase (adding the dye to all reactions was unintended). Two 100 µl aliquots were amplified by conventional PCR (98°C- 10sec, 69°C – 10 sec, 72°C – 10 sec) and quadruplicate ten µl aliquots were amplified under qPCR conditions. Figure 38 shows the amplification plot that was observed for all four samples. The reaction seems to go through an inflection/plateau reminiscent of PCR and the conventional reactions were stopped at 20 cycles (now 40 total cycles of PCR). Figure 39 shows a 2% agarose gel containing the products of these amplification reactions. Figure 40 shows a 2% agarose gel containing the amplification products following bead purification.

The library-free samples were assayed to determine if gene specific targets were captured and selectively amplified using the well-validated qPCR capture assays described elsewhere herein. The target regions for Assays 1 – 16 are shown in Table 40.

Table 40. Target regions for assays 1-16.

Assay	Sequence
Assay 1	PLP1 exon 2
Assay 2	PLP1 exon 2
Assay 3	PLP1 exon 2
Assay 4	PLP1 upstream of exon 2
Assay 5	PLP1 downstream of exon 2
Assay 6	PLP1 200 bp downstream of exon 2
Assay 7	PLP1 exon 3
Assay 8	Chr 9, off target
Assay 9	CYP2D6
Assay 10	Chr X: 154376051
Assay 11	Chr X: 154376051
Assay 12	Chr X: 6929264
Assay 13	KRAS Region 1
Assay 14	KRAS Region 2
Assay 15	Myc Region 2
Assay 16	Myc Region 2

For qPCR analysis, genomic DNA from sample F at 10 ng/μl (2 μl is added to 8 μl of PCR mix to give a final volume and concentration of 10 μl and 2 ng/μl, respectively) was used as control. Purified processed material from the F and S samples was diluted to 0.01 ng/μl = 10 pg/μl and 2 μl was added to each 8 μl PCR reaction to give a final concentration of 2 pg/μl. The results are shown in Table 41.

Table 41. qPCR results

For	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8
gDNA	23	22	25	22	22	23	23	19
F-Bb	13	13	13	13	13	30	12	55
S-Bb	14	14	13	13	14	20	13	N/A
	Assay 9	Assay 10	Assay 11	Assay 12	Assay 13	Assay 14	Assay 15	Assay 16
gDNA	23	21	23	22	22	23	23	21
F-Bb	11	13	13	15	16	17	14	13
S-Bb	11	13	14	17	17	16	13	14
Adj	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8
gDNA	16	24	4	17	22	15	8	146
F-Bb	12150	13296	9374	16729	13728	79	22495	0
S-Bb	6142	7714	10410	10093	6475	132	14548	0
	Assay 9	Assay 10	Assay 11	Assay 12	Assay 13	Assay 14	Assay 15	Assay 16
gDNA	60	39	18	30	17	43	49	37
F-Bb	45447	11671	11034	1268	440	352	6373	16448
S-Bb	66935	13477	7634	678	1677	1203	10171	19305
Adj	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8
gDNA	16	24	4	17	22	15	8	146
F-Bb	12250333	11296286	9874203	16735130	13737864	79462	22495321	2
S-Bb	6341637	7703563	10410406	10093356	6424647	131376	14548060	0
	Assay 9	Assay 10	Assay 11	Assay 12	Assay 13	Assay 14	Assay 15	Assay 16
gDNA	60	39	18	30	17	43	49	37
F-Bb	45447222	11670722	11034288	1188163	440136	352219	6373326	16447972
S-Bb	66934509	13476684	7634492	677562	1677493	1203428	10170993	19304903
Adj	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8
F-Bb	778620	562325	2393251	987542	627702	5247	2657354	0
S-Bb	253671	325934	2808248	595465	293551	8706	1730754	0
	Assay 9	Assay 10	Assay 11	Assay 12	Assay 13	Assay 14	Assay 15	Assay 16
F-Bb	757299	300823	627284	42799	25665	19716	130461	439563
S-Bb	1348229	321597	434810	22512	62817	27819	208198	515812

The qPCR data indicated that the library-free technology is very effective at retrieving the targeted genomic regions and at leaving off-target regions behind (e.g., Assays 6, 8). The fold purifications, often >500,000-fold, were directly comparable to data from earlier experiments generated with libraries as disclosed elsewhere herein.

Part II – Leave-one-out analysis: The enzymatic requirements for complex processing were evaluated: the design of experiment is shown in Table 42 below.

Table 42. Experimental design

EXPERIMENT:	1	2	3	4	5
T4 DNA polymerase	no	no	yes	yes	yes
T4 gene 32 protein	no	yes	no	yes	yes
T4 DNA ligase	no	yes	yes	no	yes

To make capture complexes for analysis, twelve identical reactions were created. Ten μl of 135 ng/ μl sonicated gDNA was melted, annealed with a tagged, bound to streptavidin coated beads, washed and resuspended in TEzero as described *supra*. Five hundred μl of processing master mix was prepared by combining 270 μl water, 50 μl 10X CutSmart buffer, 50 μl of 10 mM ATP, 75 μl of 50% PEG8000, and 5 μl of 10 mM dNTPs. This buffer was divided into 10 90 μl aliquots (duplicate tests were performed) and enzyme was added in the amounts described above (per 90 μl of master mix was added 1 μl of T4 gene 32 protein, 0.5 μl of T4 polymerase, 5 μl of adaptor and/or 5 μl of HC T4 ligase). Following T4 fill-in and ligation as described *supra*, the complexes were washed free of processing mix in TEzero and resuspended in 2 μl TEzero. Complexes were resuspended in 20 μl final volume each of single primer amplification mix and amplified for 20 cycles as described *supra*. The beads were then pulled aside using a magnet and the 20 μl clarified amplification was diluted into 180 μl of full-length F+R (118+119) PCR amplification mix. Fifty μl was pulled aside for qPCR analysis and the remaining 150 μl was split in two and amplified by conventional PCR. The 50 μl qPCR samples were mixed with 2.5 μl of dye blend and 10 μl aliquots were monitored by fluorescence change. The traces of this experiment are shown in Figure 41.

One of the two conventional PCR aliquots was pulled at 10 cycles and the other at 16 cycles of PCR. Aliquots of these raw PCR reactions (5 μl of each reaction) were analyzed on 2% agarose gels. The results are shown in Figure 42. The surprising result is that all three enzymes are required for the efficient production of amplifiable library material. A more subtle observation is that the size distribution of all-three-enzyme-material at 10 cycles is significantly larger than the size distribution of P+L alone that appears at 16 cycles.

These data together with the qPCR from the initial investigation support the interpretation that T4 DNA polymerase in conjunction with T4 gene 32 protein in the

presence of the molecular crowding agent PEG8000 (the latter contribution has not been evaluated) is capable of efficiently copying captured genomic material onto capture probes.

Part III - Generation of a library-free sequencing library: The methods described above were used to produce a DNA sequencing library with the four Coriel samples shown in the “Materials” section of this report. Each one of the four samples was coded with an individual index code in the final PCR step. The final library constituents (shown separately prior to pooling) are shown in the gel image in Figure 43. The “normal” library smear usually stretches from 175 bp upward. Here, the smallest fragments are > 300 bp. Similarly, the largest fragments appear to be 750 bp or larger. Larger fragments do not give rise to optimal libraries. These samples were all twice purified on 80% bead:sample ratios. These samples were pooled into a 16.9 ng/μl pool that, with an estimated average insert size of 400 bp, is ~65nM. The samples were sequenced.

The library-free methods worked well for CNV analyses. Unique read counts for the X-linked gene *PLP1* were normalized to the autosomal loci *KRAS* and *MYC* and the plot of these data is shown in Figure 44. The data illustrate that absolute copy number is lost with the library-free procedure (the “copies” of *KRAS* relative to *MYC* are no longer comparable). However, relative copy number (the change of *PLP1* relative to the autosomal normalizers) is robustly detected. The sequencing results also showed striking features related to read start sites relative to probe. Figure 45 shows that reads are detected as far as 900 bp from the probe; and between coordinates 1100 and 1300 every single start point is used multiple times. These data indicated that reads start at every single possible base position and that there is little ligation/processing bias. In addition, there are very few reads that start within 100 bp of the probe, consistent with the very large size distribution of the library that was observed on gels.

EXAMPLE 20

TARGETED GENE EXPRESSION ANALYSIS

OVERVIEW

This example demonstrates the development of targeted gene expression libraries. The input is RNA, not DNA, and therefore double-strand cDNA synthesis step is required. A preferred method is 1st strand synthesis using an RNase H⁻ reverse transcriptase or a kit that exhibits RNase H⁻ like activity (*e.g.*, Promega's GoScript) and priming with random hexamers. A preferred method for 2nd strand synthesis is to use a kit that includes *E. coli* DNA polymerase holoenzyme, NAD⁺-dependent ligase and RNase H (*e.g.*, New England Biolabs 2nd strand cDNA synthesis module).

Because there is a very wide range of transcript copies, there must be a correspondingly wide range of random tags that are introduced on adaptors to sheared and end-repaired cDNA. Accordingly, random 8-mers (65,536 possible sequences) were used. The adaptor was engineered with a random 8-mer sequence followed by 10-12 fixed bases that can both serve as an annealing site for a complementary 10-12 that facilitates ligation and that is used as a sample identifier in the case of multiplexed samples.

The actual number of unique versus duplicated reads – in other words the statistical distribution of reads - is one important factor in determining expression levels. One potential source of error are reads that are duplicated after the capture event. To identify these errors, a random tag was added to the capture probe such that each capture event is labeled.

The treatment and sequencing of targeted RNA-seq libraries follows the same procedure as treatment of genomic libraries.

The informatics analysis starts with the removal of post-capture duplicate reads and alignment to the target transcriptome. The unique read counts among aligning reads are then determined. While the data can then be fit to a statistical distribution, it was found that the raw unique read counts are a very close approximation to actual expression levels.

PURPOSE

The purpose of these experiments was to make targeted expression sequencing libraries from heart and liver total RNAs and to make both total RNA and targeted RNA libraries from the same starting material a direct comparison could be made.

SUMMARY

The RNA counts from total RNA libraries and from targeted RNA libraries showed good agreement along two parameters. First, the expression ratios between heart vs liver samples were well correlated. Second, the measurement of absolute abundance of different transcripts within a given sample was in good agreement when total RNA counts were compared to targeted RNA counts. These first-pass data indicated that quantitative targeted nucleic acid methods can be extended beyond genomic DNA into the analysis of cDNA libraries.

STRATEGY

To create a reasonable total RNA library that is depleted of rRNA, dT priming was used. To create targeted RNA libraries, total RNA samples were initially primed with IDT-supplied random hexamers. Random hexamer priming likely provides the most comprehensive coverage of the transcriptome. The total RNA libraries were sequenced after amplification with PCR primers that introduce P1 and P2 flow cell sequences. For targeted analysis, the capture, washing, processing and amplification steps were performed as contemplated elsewhere herein. The targeted clones were then sequenced.

METHODS

Oligonucleotides: For total RNA libraries the poly-dT primer: TTTTTTTTTTTTTTTTTTVN (SEQ ID NO: 722) was used. For targeted RNA-seq, an adaptor design was created in which the first 8 bases were random and the next 12 served as a “code” and an anchor sequence for a 12 mer partner strand oligo that could therefore form a ligatable duplex.

The sequences of these adaptors were:

SEQ ID NO:	Name	Sequence
723	RNA_L_1	TGCAGGACCAGAGAATTCTGAATACANNNNNNNNGACTCCGATCCC
724	RNA_P_1	GGGATCGGAGTC
725	RNA_L_2	TGCAGGACCAGAGAATTCTGAATACANNNNNNNNCGGAACTCGGAG
726	RNA_P_2	CTCCGAGTTCCG

cDNA library construction: The following methods were used to synthesize the four following cDNA libraries: (1) Heart total RNA (dT primed); (2) Heart targeted RNA (N₆ primed); (3) Liver total RNA; and (4) Liver targeted RNA (N₆ primed). One µg/µl total RNA was diluted 10-fold to 100 ng/µl in TEz. The following components were combined in a total volume of 10 µl, heated to 65°C and transferred to ice: 2 µl of diluted total RNA (100 ng); 2 µl of 5 uM poly-dTVN primer or 2 µl of 50 uM N₆ (IDT); and 6 µl of water.

The mix was combined with 10 µl of 1st strand cocktail (4 µl 5X GoScript buffer; 1.6 µl 25 mM Mg⁺⁺ (2 mM final concentration); 1.0 µl 10 mM dNTPs (500 uM final concentration); 1.0 µl GoScript enzyme; and 2.4 µl water) and incubated at 42°C for 30 min, then 70°C for 10 min. Sixty microliters of 2nd strand synthesis reagents (48 µl water, mix; 8 µl 10X second strand synthesis buffer; 4 µl second strand enzyme mix) were added to each reaction and incubated at 16°C for 2 hours.

Following the second strand synthesis 55 µl TEz was added to each reaction and reactions were transferred to glass Covaris sonication tubes and sonicated to about 500bp. 125 µl of the sonicated sample was transferred to a PCR strip tube and 125 µl beads were added. Following purification, the sample was resuspended to a final volume of 20 µl

End repair was carried out for 19 µl of the reaction using the methods contemplated herein. The end repaired fragments were then ligated to adaptors at 22°C for 30 min and heat inactivated at 65°C for 10 min. Ligation was carried out in a 40 µl final volume: 25 µl of repaired fragments; 2 µl of 10 uM adaptor (10 uM in L strand, 20 uM in P strand); 4 µl 10X buffer; 6 µl 50% PEG8000; 1 µl water; and 2 µl HC T4 ligase. 60 µl TEz and 100 µl beads were added to each reaction and the sample was purified to a final volume of 20 µl.

Library amplification was monitored using qPCR and each library was amplified by PCR by combining 20 µl of purified ligation mix with 130 µl PCR mix (75 µl 2X NEBNext master mix, 15 µl ACA2-20, 40 µl water). 50 µl was aliquoted into a well that contains 2.5 µl EvaGreen+ROX and further aliquoted to 10 µl in qPCR plate. The remaining 100 µl was kept in PCR strip tube. The PCR amplification was carried out at 72°C – 30 sec, 98°C – 30 sec, and variable cycles of 98°C – 10sec, 60°C – 10 sec, 72°C – 10 sec.

For dT libraries, 100 µl PCR reactions were purified with 120 µl beads. ACA2-20 (a 20 nt PCR primer) amplified material was diluted 20-fold – 5 µl into 95 µl of PCR mix – that contains 50 µl 2X NEBNext master mix, 5 µl of F primer, 5 µl of R-primer and 35 µl of water. The F primer is oligo #8, ACA2_FLFP
AATGATACGGCGACCACCGAGATCTACACGTCATGCAGGACCAGAGAATTC
GAATACA (SEQ ID NO: 69) and the reverse primer is oligo #63, exome
CAC3_FLRP
CAAGCAGAAGACGGCATAACGAGATGTGACTGGCACGGGACCAGAGAATTC
GAATACA (SEQ ID NO: 74). Amplification was carried out for 8 cycles. This step was included to grow the shorter, 20 bp ACA2 terminal sequences into longer P1 and P2, flow cell-compatible and sequencable sequences. Constructs that pick up the two different primers will amplify while those that have only one sequence will be suppressed. The resulting DNA was purified by adding 100 µl beads to the 100 µl PCR rxn and resuspended in a final volume of 50 µl.

DNA was quantified by Qubit and examined by gel electrophoresis. The DNA was sequenced using the forward primer oligo #7, ACA2_FSP
ACACGTCATGCAGGACCAGAGAATTCGAATACA (SEQ ID NO: 68) and the reverse primer oligo #62, exome CAC3_RSP
GTGACTGGCACGGGACCAGAGAATTCGAATACA (SEQ ID NO: 73). dT-primed RNA was sequenced in runs_48 & 49.

The DNA gel of samples is shown in Figure 46. The large fragment size distribution of dT-primed total RNA libraries was somewhat surprising.

For targeted RNA sequencing, N₈-primed libraries were resuspended in 40 µl of TEz. Fragment content in heart and liver libraries was quantified: 153 fg/µl of heart

sample cDNA and 760 fg/μl of liver sample cDNA. Based on these data, 40 μl of the heart ligation sample and 8 μl of the liver ligation sample were carried into downstream PCR amplification.

Progress of library amplification was monitored using qPCR and the library was amplified by PCR combining 40 μl of purified ligation mix (heart) or 8 μl of ligation mix + 32 μl of TEz (liver) with 210 μl PCR mix (125 μl 2X NEBNext master mix, 25 μl ACA2, 60 μl water). 50 μl was aliquoted into a well that contains 2.5 μl EvaGreen+ROX and further aliquoted into 10 μl aliquots in a qPCR plate. The remaining 100 μl was placed in a PCR strip tube. The PCR amplification was carried out at 72°C – 30 sec, 98°C – 30 sec, and variable cycles of 98°C – 10sec, 60°C – 10 sec, 72°C – 10 sec. 200 μl of PCR products were purified with 200 μl beads and resuspended in a final volume of 25 μl. The concentration of PCR products was 41 ng/μl for the heart library and 42 ng/μl for the liver library.

For capture, heart and liver samples were combined and two of “2x” capture reactions were performed with tagged RNA-seq-specific probes (see Appendix, *infra* for sequences), washed, processed C+P (final yield = 40 μl of 23 ng/μl), and size selected 240 – 600 bp fragments on a Pippin automated DNA size selector. 5.4 ng/μl of fragments were recovered from the Pippin = 20.8 nM. A flow cell was loaded with the fragments and 51 nt first reads and 24 nt second reads were collected.

RESULTS AND DISCUSSION

To determine useful RNA-seq data, a heart versus liver sample was chosen for comparison. 21 transcripts were parsed out of transcripts reported in the RNA-seq Atlas (medicalgenomics.org/rna_seq_atlas) based on their absolute abundance in one or the other tissue (RPKM values of about 100, 10, 1 *etc.* in heart or liver) and on their ratios between tissues (again about 100, 10, 1, 0.1, 0.01 for heart vs liver ratios). The list of candidate transcripts and their reported RPKM values are shown in Table 43.

Table 43. Candidate RNA-seq transcripts

hgnc_symbol	transcript	transcript_length	heart	liver	h/l	l/h
MYH7	NM_000257	6030	2137.51	0.007	305358.6	0.0
NEBL	NM_006393	9213	115.617	0.097	1191.9	0.0
MGP	NM_000900	661	1059.49	11.155	95.0	0.0
DKG3	NM_013253	2746	118.464	1.136	104.3	0.0
BVES	NM_147147	5505	11.27	0.11	102.5	0.0
PDE4DIP	NM_001002812	4824	108.053	10.053	10.7	0.1
HAND2	NM_021973	2368	10.069	0.992	10.2	0.1
RP9P	NR_003500	1297	1.279	0.125	10.2	0.1
SOD2	NM_000636	1593	492.028	500.782	1.0	1.0
ATP5E	NM_006886	417	101.927	93.268	1.1	0.9
TRAPPC1	NM_021210	819	10.197	9.646	1.1	0.9
TAF1A	NM_005681	1879	1.101	1.035	1.1	0.9
MGST1	NM_145764	910	43.368	429.307	0.1	9.9
PDIA4	NM_004911	2952	14.701	154.615	0.1	10.5
STARD10	NM_006645	1988	1.163	11.147	0.1	9.6
TMEM14A	NM_014051	1014	0.11	1.119	0.1	10.2
APOB	NM_000384	14121	5.822	765.522	0.0	131.5
SERPINF2	NM_001165921	2092	1.034	102.91	0.0	99.5
TFR2	NM_003227	2888	0.142	13.762	0.0	96.9
FGL1	NM_147203	1337	1.102	1305.36	0.0	1184.5
FGA	NM_000508	3655	24.14	9012.08	0.0	373.3

The targeted RNA-seq library was compared to an untargeted, total RNA library made from the same total RNA sample. Poly dT priming was used to convert total RNA, which is primarily rRNA, into non-rRNA transcript libraries. For targeted RNA-seq, random hexamers were used. For the dT primed total RNA libraries, reads can be derived along the entire length of a transcript, some of which are quite long. As an example, the distribution of reads along MYH7 in heart was examined and reads derived from near the 5' end of this long transcript were found. To compare one (long) transcript to another (short) transcript, counts were normalized by transcript length (often referred to as the reads per million per Kb or RPKM method). Following this 1st degree normalization, counts were also normalized between total and targeted samples. The final read count data set is shown in Table 44.

Table 44. Read Count Data for Figures 49 and 50.

HEART	Atlas	Total	Targeted	LIVER	Atlas	Total	Targeted
MYH7	50188	71792	40164	MYH7	0	374	721
NEBL	2715	936	3460	NEBL	1	27	24
MGP	24876	1315	13162	MGP	90	42	393
DKK3	2781	18092	20763	DKK3	9	36	211
BVES	265	179	650	BVES	1	5	62
PDE4DIP	2537	850	5029	PDE4DIF	81	88	294
HAND2	236	479	1052	HAND2	8	42	279
RP9P	30	121	63	RP9P	1	20	12
SOD2	11553	780	5437	SOD2	4031	1503	8802
ATP5E	2393	1607	2363	ATP5E	751	451	2173
TRAPPC1	239	536	1970	TRAPPC1	78	80	397
TAF1A	26	22	34	TAF1A	8	7	48
MGST1	1018	1312	2862	MGST1	3455	5514	9281
PDIA4	345	603	1310	PDIA4	1244	1197	2144
STARD10	27	908	1214	STARD10	90	1857	3338
TMEM14A	3	74	57	TMEM14A	9	110	74
APOB	137	56	107	APOB	6162	5243	17030
SERPINF2	24	119	89	SERPINF2	828	7474	7795
TFR2	3	20	11	TFR2	111	3395	3489
FGL1	26	30	44	FGL1	10507	21763	11420
FGA	567	131	160	FGA	72538	50772	32014

Visual inspection revealed a good correlation between all three types of data (Atlas, Total and Targeted). One important comparison was between that total RNA-seq sample prepared herein and the targeted RNA-seq sample prepared herein, because both data sets were derived from the same total RNA samples. Two important points of comparison include: (1) The correlation of the actual heart versus liver expression ratio; and (2) the correlation of the absolute abundances of transcripts within a specific sample between total and targeted counts.

The first point addresses the preservation of expression profiles, but ignores the actual magnitude of counts being compared. An expression ratio heart versus liver comparison plot for total versus targeted expression ratios is shown in Figure 47. This plot shows an exceptional correlation ($r^2 = 0.95$) between “expression profiles” generated by the two methods.

The second point can be more rigorous because it asks for an absolute comparison between the two methods. A comparison of absolute expression levels as

measured in total RNA-seq or targeted RNA-seq is shown in Figure 48, where $\log_{10}(\text{count})$ values are plotted against one another. This comparison was sensitive not only to targeting, but also to the fact that the RNA-seq libraries were prepared by fundamentally different methods (dT priming for total, random hexamer priming for targeted). Despite the different methods of preparation there was an excellent correlation between the two methods.

This study demonstrated that the core methods of labeling with random tags combined with sequence-specific capture can produce target-specific RNA transcript data that preserves absolute expression abundance, reveals transcript-specific sequence information, and dramatically reduces the complexity of transcriptome data.

APPENDIX: PROBE SEQUENCES USED FOR RNA-SEQ ANALYSIS.

SEQ ID NO:	Name	Sequence
727	APOB_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGCCGAAG AGGAAATGCTGGAAAATGTCAGCCTGGTCTGTCCAAAAGATGCGACCC GATTC
728	APOB_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCAGCTGAC CTCATCGAGATTGGCTTGAAGGAAAAGGCTTTGAGCCAACATTGGAA GCT
729	ATP5E_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNTACTGGAG ACAGGCTGGACTCAGCTACATCCGATACTCCAGATCTGTGCAAAAGC AGTG
730	ATP5E_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCTGAAGA CAGAATTCAAAGCAAATGCTGAGAAGACTTCTGGCAGCAACGTAAAAA TTGTG
731	BVES_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNACAGAGT CCAGCCCATTGAGAGAATCAACTGCCATAGGTTTTACACCTGAGTTAGA AAGT
732	BVES_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAGCCAGCT CCAGTGACAGTGACGACGGCTTGACCAGTTTCTTCGGGGTACCTCCAG

		CA
733	DKK3_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNGACACGC AGCACAAATTGCGCAGCGCGGTGGAAGAGATGGAGGCAGAAGAAGCT GCTGC
734	DKK3_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNTGGTGTAT GTGTGCAAGCCGACCTTCGTGGGGAGCCGTGACCAAGATGGGGAGATC CTG
735	FGA_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNATGAGGA TCGTCTGCCTGGTCCTAAGTGTGGTGGGCACAGCATGGACTGCAGATAG TGGT
736	FGA_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNATTTTTTC TGTTTATTGCGATCAAGAGACCAGTTTGGGAGGATGGCTTTTGATCCAG CA
737	FGL1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNGTGTTTCAG TTTCATCCTTGTTACCACCGCTCTGACAATGGGCAGGGAAATTCGGCG CTC
738	FGL1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNTGCAAACC TGAATGGTGTATACTACAGCGGCCCTACACGGCTAAAACAGACAATG GGA
739	HAND2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCATCCCCA ACGTACCCGCCGACACCAAACCTCTCCAAAATCAAGACCCTGCGCCTGG CCA
740	HAND2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCGACCAG AATGGCGAGGCGGAGGCCTTCAAGGCAGAGATCAAGAAGACCGACGT GAAAG
741	MGP_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCTGATCCT TCTTGCCATCCTGGCCGCCTTAGCGGTAGTAACTTTGTGTTATGAATCAC AT
742	MGP_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCGAGCTCA

		ATAGGGAAGCCTGTGATGACTACAGACTTTGCGAACGCTACGCCATGG TTT
743	MGST1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAAGAAGT ATCTTCGAACAGATGACAGAGTAGAACGTGTACGCAGAGCCCACCTGA ATGA
744	MGST1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNTATTCCTT GAGTGGTCCCGACCCCTCTACAGCCATCCTGCACTTCAGACTATTTGTC GG
745	MYH7_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCAGACCA GGCCTTTTGACCTCAAGAAGGATGTCTTCGTGCCTGATGACAAACAGGA GTTT
746	MYH7_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAGAGCTCT TCAAACCTCAAGAACGCCTATGAGGAGTCCCTGGAACATCTGGAGACCT TCA
747	NEBL_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCAGGTCTT CTATAAGCCTGTTATTGAAGACTTAAGCATGGAATTGGCCAGAAAATGC ACG
748	NEBL_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAAAGAGC AAAACTACAAGGCCACTCCGGTAAGCATGACCCCGGAGATAGAGAGAG TGAG
749	PDE4DIP_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNGGATATCG CACTCTGTCCCAGCACCTCAATGACCTGAAGAAGGAGAACTTCAGCCTC AAG
750	PDE4DIP_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNGAAAGCC AAGCTGCTGCAGAGAAGTTGGTGCAAGCCTTAATGGAAAGAAATTCAG AATT
751	PDIA4_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCCGGACG AGGATTCTTCTAACAGAGAAAATGCCATTGAGGATGAAGAGGAGGAGG AGGAG

752	PDIA4_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCGTCAAG GTCGTGGTGGGAAAGACCTTTGACTCCATTGTGATGGACCCCAAGAAG GACG
753	RP9P_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGATCAC GAGTTATGAAAAACCTCCTCCTGGGCTTATCAAGGTTGGCATTGCAAAT GGT
754	RP9P_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAAAGTTTC TTCATCGTTGTCTCCCTGCTGGTCACATGAGTTTACGATTCCTTAGAAG TG
755	SERPINF2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCCCGGCGC GTTCCGACTGGCTGCCAGGATGTACCTGCAGAAAGGATTTCCCATCAAA GA
756	SERPINF2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGCCTGAA AGGCTTCCCCCGCGGAGACAAGCTTTTCGGCCCTGACTTAAAACTTGTG CCC
757	SOD2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAAACAACCT GAACGTCACCGAGGAGAAGTACCAGGAGGCGTTGGCCAAGGGAGATGT TAC
758	SOD2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGGAGAA GCTGACGGCTGCATCTGTTGGTGTCCAAGGCTCAGGTTGGGGTTGGCTT GGT
759	STARD10_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGACCAAG ACTTTCGCAGCTTCCGGTCAGAGTGTGAGGCTGAGGTGGGCTGGAACCT GACC
760	STARD10_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGATGTAC AAGGCGTGCCTCAAGTACCCCGAGTGGAACAGAAGCACCTGCCTCAC TTCA
761	TAF1A_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNTTCAGTGA AGAATTAAGGGCCTGTGACAGATGATGAAGAAGTGGAACATCTGT

		GCTC
762	TAF1A_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNGAAACCA CCTTGCGTGGGTTCAAGAAGAGTGGAAGTCCAGGAAAACTGGTGGCC AGGC
763	TFR2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCTTTGGGG TCTATTCCAGAGAGCGCAACAAGTGTCCCAAGATCCTCTCAGACCGTC TAC
764	TFR2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGGCCTAC CCATTCTGCACACAAAGGAGGACACTTATGAGAACCTGCATAAGGTG CTG
765	TMEM14A_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNATCGGTTT TGGTTATGCAGCCCTCGTGACATTGGAAGCATTTTGGATATAAGCGG AGA
766	TMEM14A_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGCTTTCTT CCTGGCTACCATAATGGGTGTGAGATTTAAGAGGTCCAAGAAAATAAT GCC
767	TRAPPC1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCACAACCT GTACCTGTTTGACCGGAATGGAGTGTGTCTGCACTACAGCGAATGGCAC CGC
768	TRAPPC1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNATCCGAG ATGTGCTGCACCACATCTACAGTGCCTGTATGTGGAGCTGGTGGTGAA GAA
769	APOB_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGCCGAAG AGGAAATGCTGGAAAATGTCAGCCTGGTCTGTCCAAAAGATGCGACCC GATTC
770	APOB_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCAGCTGAC CTCATCGAGATTGGCTTGGAAGGAAAAGGCTTTGAGCCAACATTGGAA GCT
771	ATP5E_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNTACTGGAG

		ACAGGCTGGACTCAGCTACATCCGATACTCCCAGATCTGTGCAAAAGC AGTG
772	ATP5E_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCTGAAGA CAGAATTCAAAGCAAATGCTGAGAAGACTTCTGGCAGCAACGTAAAAA TTGTG
773	BVES_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNACAGAGT CCAGCCCATTGAGAGAATCAACTGCCATAGGTTTTACACCTGAGTTAGA AAGT
774	BVES_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGCCAGCT CCAGTGACAGTGACGACGGCTTGACCAGTTTCTTCGGGGTACCTCCAG CA
775	DKK3_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGACACGC AGCACAAATTGCGCAGCGCGGTGGAAGAGATGGAGGCAGAAGAAGCT GCTGC
776	DKK3_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNTGTTGTAT GTGTGCAAGCCGACCTTCGTGGGGAGCCGTGACCAAGATGGGGAGATC CTG
777	FGA_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNATGAGGA TCGTCTGCCTGGTCCTAAGTGTGGTGGGCACAGCATGGACTGCAGATAG TGGT
778	FGA_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNATTTTTTC TGTTTATTGCGATCAAGAGACCAGTTTGGGAGGATGGCTTTTGATCCAG CA
779	FGL1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGTGTTTCAG TTTCATCCTTGTTACCACCGCTCTGACAATGGGCAGGGAAATTCGGCG CTC
780	FGL1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNTGCAAACC TGAATGGTGTATACTACAGCGGCCCTACACGGCTAAAACAGACAATG GGA

781	HAND2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCATCCCCA ACGTACCCGCCGACACCAAACCTCTCCAAAATCAAGACCCTGCGCCTGG CCA
782	HAND2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCGACCAG AATGGCGAGGCGGAGGCCTTCAAGGCAGAGATCAAGAAGACCGACGT GAAAG
783	MGP_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCTGATCCT TCTTGCCATCCTGGCCGCCTTAGCGGTAGTAACTTTGTGTTATGAATCAC AT
784	MGP_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCGAGCTCA ATAGGGAAGCCTGTGATGACTACAGACTTTGCGAACGCTACGCCATGG TTT
785	MGST1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAAGAAGT ATCTTCGAACAGATGACAGAGTAGAACGTGTACGCAGAGCCACCTGA ATGA
786	MGST1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNTATTCCTT GAGTGGTCCCACCCCTCTACAGCCATCCTGCACTTCAGACTATTTGTC GG
787	MYH7_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCAGACCA GGCCTTTTGACCTCAAGAAGGATGTCTTCGTGCCTGATGACAAACAGGA GTTT
788	MYH7_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAGAGCTCT TCAAACCTCAAGAACGCCTATGAGGAGTCCCTGGAACATCTGGAGACCT TCA
789	NEBL_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNCAGGTCTT CTATAAGCCTGTTATTGAAGACTTAAGCATGGAATTGGCCAGAAAATGC ACG
790	NEBL_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNAAAGAGC AAAACCTACAAGGCCACTCCGGTAAGCATGACCCCGGAGATAGAGAGAG

		TGAG
791	PDE4DIP_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNGGATATCG CACTCTGTCCCAGCACCTCAATGACCTGAAGAAGGAGAACTTCAGCCTC AAG
792	PDE4DIP_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNGAAAGCC AAGCTGCTGCAGAGAAGTTGGTGCAAGCCTTAATGGAAAGAAATTCAG AATT
793	PDIA4_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCCGGACG AGGATTCTTCTAACAGAGAAAATGCCATTGAGGATGAAGAGGAGGAGG AGGAG
794	PDIA4_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCGTCAAG GTCGTGGTGGGAAAGACCTTTGACTCCATTGTGATGGACCCCAAGAAG GACG
795	RP9P_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNAGATCAC GAGTTATGAAAAACCTCCTCCTGGGCTTATCAAGGTTGGCATTGCAAAT GGT
796	RP9P_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNAAAGTTTC TTCATCGTTGTCTCCCTGCTGGTCACATGAGTTTACGATTCCTTAGAAG TG
797	SERPINF2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNCCCGGCGC GTTCCGACTGGCTGCCAGGATGTACCTGCAGAAAGGATTTCCCATCAAA GA
798	SERPINF2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNGCCTGAA AGGCTTCCCCCGCGGAGACAAGCTTTTCGGCCCTGACTTAAAACTTGTG CCC
799	SOD2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNAACAACCT GAACGTCACCGAGGAGAAGTACCAGGAGGCGTTGGCCAAGGGAGATGT TAC
800	SOD2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTTCACNNNNNNNAGGAGAA

		GCTGACGGCTGCATCTGTTGGTGTCCAAGGCTCAGGTTGGGGTTGGCTT GGT
801	STARD10_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGACCAAG ACTTTCGCAGCTTCCGGTCAGAGTGTGAGGCTGAGGTGGGCTGGAACCT GACC
802	STARD10_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGATGTAC AAGGCGTGCCTCAAGTACCCCGAGTGGAACAGAAGCACCTGCCTCAC TTCA
803	TAF1A_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNTTCAGTGA AGAATTA AAAAGGGCCTGTGACAGATGATGAAGAAGTGGAACATCTGT GCTC
804	TAF1A_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGAAACCA CCTTGCGTGGGTTCAAGAAGAGTGGAAGTCCAGGAAAACTGGTGGCC AGGC
805	TFR2_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCTTTGGGG TCTATTCCAGAGAGCGCAACAACTGTCCCCAAGATCCTCTCAGACCGTC TAC
806	TFR2_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNAGGCCTAC CCATTCTGCACACAAAGGAGGACACTTATGAGAACCTGCATAAGGTG CTG
807	TMEM14A_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNATCGGTTT TGGTTATGCAGCCCTCGTGACATTTGGAAGCATTTTTGGATATAAGCGG AGA
808	TMEM14A_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNGCTTTCTT CCTGGCTACCATAATGGGTGTGAGATTTAAGAGGTCCAAGAAAATAAT GCC
809	TRAPPC1_1	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNCACAACCT GTACCTGTTTGACCGGAATGGAGTGTGTCTGCACTACAGCGAATGGCAC CGC

810	TRAPPC1_2	ATGTGACTGGCACGGGAGTTGATCCTGGTTTTACNNNNNNNATCCGAG ATGTGCTGCACCACATCTACAGTGCGCTGTATGTGGAGCTGGTGGTGAA GAA
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In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A method for generating a tagged genomic library comprising:
 - (a) treating fragmented genomic DNA with end-repair enzymes to generate fragmented end-repaired genomic DNA; and
 - (b) ligating a random nucleic acid tag sequence, and optionally a sample code sequence and/or a PCR primer sequence to the fragmented end-repaired genomic DNA to generate the tagged genomic library.
2. The method of any of the preceding claims, wherein the random nucleic acid tag sequence is from about 2 to about 100 nucleotides.
3. The method of any of the preceding claims, wherein the random nucleic acid tag sequence is from about 2 to about 6 nucleotides.
4. The method of any of the preceding claims, wherein the fragmented end-repaired genomic DNA contains blunt ends.
5. The method of any of the preceding claims, wherein the blunt ends are further modified to contain a single base pair overhang.
6. The method of any of the preceding claims, wherein the ligating comprises ligating a multifunctional adaptor module to the fragmented end-repaired genomic DNA to generate the tagged genomic library, wherein the multifunctional adaptor molecule comprises:
 - (i) a first region comprising a random nucleic acid tag sequence;
 - (ii) a second region comprising a sample code sequence; and
 - (iii) a third region comprising a PCR primer sequence.
7. The method of any of the preceding claims, further comprising hybridizing a tagged genomic library with a multifunctional capture probe module to form a complex,

wherein the multifunctional capture probe module hybridizes to a specific genomic target region in the genomic library.

8. The method of any of the preceding claims, further comprising isolating the tagged genomic library-multifunctional capture probe module complex.

9. The method of any of the preceding claims, further comprising 3'-5' exonuclease enzymatic processing of the isolated tagged genomic library-multifunctional capture probe module complex to remove the single stranded 3' ends.

10. The method of any of the preceding claims wherein the enzyme for use in the 3'-5' exonuclease enzymatic processing is T4 DNA polymerase.

11. The method of any of the preceding claims, further comprising performing PCR on the 3'-5' exonuclease enzymatically processed complex from the preceding claims, wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence.

12. A method for targeted genetic analysis comprising:

- (a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module selectively hybridizes to a specific genomic target region in the genomic library;
- (b) isolating the tagged genomic library-multifunctional capture probe module complex from a);
- (c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;
- (d) performing PCR on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is copied in order to generate a

hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; and

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

13. The method of claim 12, wherein steps a) through d) are repeated at least about twice and the targeted genetic analysis of e) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

14. The method of claim 13, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

15. The method of claim 14, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

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

(a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module complex selectively hybridizes to a specific genomic target region in the genomic library;

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

(c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;

(d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule

comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence;

(e) performing PCR amplification of the hybrid nucleic acid molecule in d);

and

(f) quantitating the PCR reaction in e), wherein the quantitation allows for a determination of copy number of the specific genomic target region.

17. The method of claim 16, further comprising obtaining the sequences of the hybrid nucleic acid molecules from step e).

18. The method of claim 17, wherein steps a) through e) are repeated at least about twice and a sequence alignment is performed using the hybrid nucleic acid molecule sequences obtained from the at least two e) steps.

19. The method of claim 18, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

20. The method of claim 19, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

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

(a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module complex selectively hybridizes to a specific genomic target region in the genomic library;

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

(c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;

(d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence; and

(e) performing PCR amplification of the hybrid nucleic acid molecule in d).

22. The method of claim 21, further comprising obtaining the sequences of the hybrid nucleic acid molecules from step e).

23. The method of claim 22, wherein steps a) through e) are repeated at least about twice and a sequence alignment is performed using the hybrid nucleic acid molecule sequences obtained from the at least two e) steps.

24. The method of claim 23, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

25. The method of claim 24, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

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

(a) hybridizing a tagged genomic library with a multifunctional capture probe module complex, wherein the multifunctional capture probe module comprises selectively hybridizes to a specific genomic target region in the genomic library;

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

(c) performing 3'-5' exonuclease enzymatic processing on the isolated tagged genomic library-multifunctional capture probe module complex from b) using an enzyme with 3'-5' exonuclease activity to remove the single stranded 3' ends;

(d) performing a PCR reaction on the enzymatically processed complex from c) wherein the tail portion of the multifunctional capture probe molecule is replicated in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the genomic target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence;

(e) performing PCR amplification of the hybrid nucleic acid molecule in d);
and

(f) performing targeted genetic analysis on the hybrid nucleic acid molecule from e).

27. The method of claim 26, wherein steps a) through e) are repeated at least about twice and the targeted genetic analysis of f) comprises performing a sequence alignment of the hybrid nucleic acid molecule sequences from the at least two e) steps.

28. The method of claim 27, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

29. The method of claim 28, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

30. A method for targeted genetic analysis comprising:

(a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library;

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

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

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

31. The method of claim 30, wherein steps a) through c) are repeated at least about twice and the targeted genetic analysis of d) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

32. The method of claim 31, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

33. The method of claim 32, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

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

(a) hybridizing a tagged genomic library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific genomic target region in the genomic library;

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

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

(d) performing PCR amplification of the hybrid nucleic acid molecule in c);
and

(e) quantitating the PCR reaction in d), wherein the quantitation allows for a determination of copy number of the specific genomic target region.

35. The method of claim 34, further comprising obtaining the sequences of the hybrid nucleic acid molecules from step d).

36. The method of claim 35, wherein steps a) through d) are repeated at least about twice and a sequence alignment of the hybrid nucleic acid molecules from the at least two d) steps.

37. The method of claim 36, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

38. The method of claim 37, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

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

40. The method of any of the preceding claims wherein the tagged genomic library is amplified by PCR to generate an amplified tagged genomic library.

41. The method of any of the preceding claims wherein the genomic DNA is from a biological sample selected from the group consisting of blood, skin, hair, hair follicles, saliva, oral mucous, vaginal mucous, sweat, tears, epithelial tissues, urine, semen, seminal fluid, seminal plasma, prostatic fluid, pre-ejaculatory fluid (Cowper's fluid), excreta, biopsy, ascites, cerebrospinal fluid, lymph, and tissue extract sample or biopsy sample.

42. A tagged genomic library comprising tagged genomic sequences, wherein each tagged genomic sequence comprises:

- (a) fragmented end-repaired genomic DNA;
- (b) a random nucleotide tag sequence;
- (c) a sample code sequence; and
- (d) a PCR primer sequence.

43. A hybrid tagged genomic library comprising hybrid tagged genomic sequences for use in targeted genetic analysis, wherein each hybrid tagged genomic sequence comprises:

- (a) fragmented end-repaired genomic DNA;
- (b) a random nucleotide tag sequence;
- (c) a sample code sequence;
- (d) a PCR primer sequence;
- (e) a genomic target region; and
- (f) a multifunctional capture probe module tail sequence.

44. A multifunctional adaptor module comprising:

- (a) a first region comprising a random nucleotide tag sequence;
- (b) a second region comprising a sample code sequence; and
- (c) a third region comprising a PCR primer sequence.

45. A multifunctional capture probe module comprising:
- (a) a first region capable of hybridizing to a partner oligonucleotide;
 - (b) a second region capable of hybridizing to a specific genomic target region; and
 - (c) a third region comprising a tail sequence.
46. The multifunctional capture probe module of any of the preceding claims wherein the first region is bound to a partner oligonucleotide.
47. A multifunctional adaptor probe hybrid module comprising:
- (a) a first region capable of hybridizing to a partner oligonucleotide and capable of functioning as a PCR primer and
 - (b) a second region capable of hybridizing to a specific genomic target region.
48. The multifunctional capture probe hybrid module of any of the preceding claims, wherein the first region is bound to a partner oligonucleotide.
49. The method of any of the preceding claims, wherein the partner oligonucleotide is chemically modified.
50. A composition comprising a tagged genomic library, a multifunctional adaptor module and a multifunctional capture probe module.
51. A composition comprising a hybrid tagged genomic library according to any of the preceding claims.
52. A reaction mixture for performing the method of any one of the preceding claims.
53. A reaction mixture capable of generating a tagged genomic library comprising:
- (a) fragmented genomic DNA; and

(b) DNA end-repair enzymes to generate fragmented end-repaired genomic DNA.

54. The reaction mixture of any of the preceding claims, further comprising a multifunctional adaptor module.

55. The reaction mixture of any of the preceding claims, further comprising a multifunctional capture probe module.

56. The reaction mixture of any of the preceding claims, further comprising an enzyme with 3'-5' exonuclease activity and PCR amplification activity.

57. A method for DNA sequence analysis comprising:

(a) obtaining one or more clones, each clone comprising a first DNA sequence and a second DNA sequence, wherein the first DNA sequence comprises targeted genomic DNA sequence and the second DNA sequence comprises a capture probe sequence;

(b) performing a paired end sequencing reaction on the one or more clones and obtaining one or more sequencing reads; and

(c) ordering or clustering the sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

58. A method for DNA sequence analysis comprising:

(a) obtaining one or more clones, each clone comprising a first DNA sequence and a second DNA sequence, wherein the first DNA sequence comprises targeted genomic DNA sequence and the second DNA sequence comprises a capture probe sequence;

(b) performing a sequencing reaction on the one or more clones in which a single long sequencing read of greater than about 100 nucleotides is obtained, wherein the read is sufficient to identify both the first DNA sequence and the second DNA sequence; and

(c) ordering or clustering sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

59. The method of claim 57 or claim 58, wherein the sequences of the one or more clones are compared to one or more human reference DNA sequences.

60. The method of claim 59, wherein sequences that do not match the one or more human reference DNA sequences are identified.

61. The method of claim 60, wherein non-matching sequences are used to create a *de novo* assembly from the non-matching sequence data.

62. The method of claim 61, wherein the *de novo* assemblies are used to identify novel sequence rearrangements associated with the capture probe.

63. A method for genomic copy number determination analysis comprising:

- (a) obtaining one or more clones, each clone comprising a first DNA sequence and a second DNA sequence, wherein the first DNA sequence comprises a random nucleotide tag sequence and a targeted genomic DNA sequence and the second DNA sequence comprises a capture probe sequence;
- (b) performing a paired end sequencing reaction on the one or more clones and obtaining one or more sequencing reads; and
- (c) ordering or clustering the sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

64. A method for genomic copy number determination analysis comprising:

- (a) obtaining one or more clones, each clone comprising a first DNA sequence and a second DNA sequence, wherein the first DNA sequence comprises a random nucleotide tag sequence and a targeted genomic DNA sequence and the second DNA sequence comprises a capture probe sequence;
- (b) performing a sequencing reaction on the one or more clones in which a single long sequencing read of greater than about 100 nucleotides is obtained, wherein the read is sufficient to identify both the first DNA sequence and the second DNA sequence; and

(c) ordering or clustering sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

65. The method of claim 63 or claim 64, wherein the random nucleotide tag sequence is about 2 to about 50 nucleotides in length.

66. The method of claim 63 or claim 64, further comprising analyzing all sequencing reads associated with a second read sequence by:

- (a) determining the distributions of unique and redundant sequencing reads;
- (b) counting the number of times a unique read is encountered;
- (c) fitting a frequency distribution of the unique reads to a statistical distribution;
- (d) inferring a total number of unique reads; and
- (e) normalizing the total number of inferred unique read to an assumption that most human genetic loci are generally diploid.

67. The method of claim 66, wherein an inferred copy number of one or more targeted loci are determined.

68. The method of claim 67, wherein the one or more target loci that deviate from an expected copy number value are determined.

69. The method of claim 67, wherein the one or more targeted loci of a gene are grouped together in a collection of loci and the copy number measurements from the collection of targeted loci are averaged and normalized.

70. The method of claim 67, wherein the inferred copy number of a gene is represented by the normalized average of all the target loci representing that gene.

71. A method for generating a tagged RNA expression library comprising:
(a) fragmenting a cDNA library;

(b) treating the fragmented cDNA library with end-repair enzymes to generate fragmented end-repaired cDNA; and

(c) ligating a multifunctional adapter molecule to the fragmented end-repaired c DNA to generate a tagged RNA expression library.

72. A method for generating a tagged RNA expression library comprising:

(a) preparing a cDNA library from the total RNA of one or more cells;

(b) fragmenting the cDNA library;

(c) treating the fragmented cDNA with end-repair enzymes to generate fragmented end-repaired cDNA; and

(d) ligating a multifunctional adapter molecule to the fragmented end-repaired c DNA to generate a tagged RNA expression library.

73. The method of claim 71 or claim 72, wherein the cDNA library is an oligo-dT primed cDNA library.

74. The method of claim 71 or claim 72, wherein the cDNA library is primed by random oligonucleotides comprising about 6 to about 20 random nucleotides.

75. The method of claim 71 or claim 72, wherein the cDNA library is primed by random hexamers or random octamers.

76. The method of claim 71 or claim 72, wherein the cDNA library is fragmented to a size of about 250 bp to about 750 bp.

77. The method of claim 71 or claim 72, wherein the cDNA library is fragmented to a size of about 500 bp.

78. The method of any one of claims 71 to 77, wherein the multifunctional adaptor module comprises:

- (i) a first region comprising a random nucleic acid tag sequence, and optionally;
- (ii) a second region comprising a sample code sequence, and optionally
- (iii) a third region comprising a PCR primer sequence.

79. The method of any one of claims 71 to 78, wherein the multifunctional adaptor module comprises a first region comprising a random nucleic acid tag sequence, a second region comprising a sample code sequence, and a third region comprising a PCR primer sequence.

80. The method of any one of claims 71 to 78, further comprising hybridizing a tagged cDNA library with a multifunctional capture probe module to form a complex, wherein the multifunctional capture probe module hybridizes to a specific target region in the cDNA library.

81. The method of any one of claims 71 to 78, further comprising isolating the tagged cDNA library-multifunctional capture probe module complex.

82. The method of any one of claims 71 to 78, further comprising 3'-5' exonuclease enzymatic processing of the isolated tagged cDNA library-multifunctional capture probe module complex to remove the single stranded 3' ends.

83. The method of claim 82, wherein the enzyme for use in the 3'-5' exonuclease enzymatic processing is T4 DNA polymerase.

84. The method of claim 82 or claim 83, further comprising performing PCR on the 3'-5' exonuclease enzymatically processed complex, wherein a tail portion of the multifunctional capture probe molecule is copied in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the cDNA target region capable of hybridizing to the multifunctional capture probe module and the complement of the multifunctional capture probe module tail sequence.

85. A method for targeted gene expression analysis comprising

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

86. A method for targeted gene expression analysis comprising:

- (a) hybridizing a tagged RNA expression library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific target region in the RNA expression library;
- (b) isolating the tagged RNA expression library-multifunctional capture probe hybrid module complex from a);
- (c) performing 5' to 3' DNA polymerase extension of the multifunctional capture probe on the complex from b) to replicate a region of the captured, tagged target region that is 3' of the multifunctional capture probe in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the multifunctional capture probe hybrid module and the complement of the tagged target region that is located in the 3' direction from the location where the multifunctional capture probe hybrid module hybridizes to the target region; and

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

87. A method for targeted gene expression analysis comprising:

(a) hybridizing a tagged cDNA library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific target region in the cDNA library;

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

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

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

88. The method of any one of claims 85 to 87, wherein at least two different multifunctional capture probe modules are used in the at least two (a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

89. The method of claim 88, wherein at least one multifunctional capture probe module hybridizes downstream of the target region and at least one multifunctional capture probe module hybridizes upstream of the target region.

90. A method for cDNA sequence analysis comprising:

(a) obtaining one or more clones, each clone comprising a first cDNA sequence and a second cDNA sequence, wherein the first cDNA sequence comprises targeted

genomic cDNA sequence and the second cDNA sequence comprises a capture probe sequence;

(b) performing a paired end sequencing reaction on the one or more clones and obtaining one or more sequencing reads; and

(c) ordering or clustering the sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

91. A method for cDNA sequence analysis comprising:

(a) obtaining one or more clones, each clone comprising a first cDNA sequence and a second cDNA sequence, wherein the first cDNA sequence comprises targeted genomic DNA sequence and the second cDNA sequence comprises a capture probe sequence;

(b) performing a sequencing reaction on the one or more clones in which a single long sequencing read of greater than about 100 nucleotides is obtained, wherein the read is sufficient to identify both the first cDNA sequence and the second cDNA sequence; and

(c) ordering or clustering sequencing reads of the one or more clones according to the probe sequence of the sequencing reads.

92. The method of claim 90 or claim 91, further comprising analyzing all sequencing reads associated with a second read sequence by:

(a) determining the distributions of unique and redundant sequencing reads;

(b) counting the number of times a unique read is encountered;

(c) fitting a frequency distribution of the unique reads to a statistical distribution;

(d) inferring a total number of unique reads; and

(e) converting unique read counts into transcript abundance using normalization to the total reads collected within each cDNA library sample.

93. A method for targeted genetic analysis comprising:

(a) hybridizing a tagged DNA library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific target region in the DNA library;

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

(c) performing a concerted enzymatic processing of the tagged DNA library-multifunctional capture probe hybrid module complex from b) that includes 5' FLAP endonuclease activity, 5' to 3' DNA polymerase extension, and nick closure by a DNA ligase to join the complement of the multifunctional capture probe to the target region that is 5' of the multifunctional capture probe binding site in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the complement of the multifunctional capture probe hybrid module and a region of the tagged target region that is located 5' of the location where the multifunctional capture probe hybrid module hybridizes to the genomic target region; and

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

94. The method of claim 93, wherein steps a) through c) are repeated at least about twice and the targeted genetic analysis of d) comprises a sequence alignment of the hybrid nucleic acid molecule sequences obtained from the at least two d) steps.

95. The method of claim 94, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

96. The method of claim 95, wherein at least one multifunctional capture probe module hybridizes downstream of the target region and at least one multifunctional capture probe module hybridizes upstream of the target region.

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

(a) hybridizing a tagged DNA library with a multifunctional capture probe hybrid module complex, wherein the multifunctional capture probe hybrid module selectively hybridizes to a specific target region in the genomic library;

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

(c) performing a concerted enzymatic processing of the tagged DNA library-multifunctional capture probe hybrid module complex from b) that includes 5' FLAP endonuclease activity, 5' to 3' DNA polymerase extension, and nick closure by a DNA ligase to join the complement of the multifunctional capture probe to the target region that is 5' of the multifunctional capture probe binding site in order to generate a hybrid nucleic acid molecule, wherein the hybrid nucleic acid molecule comprises the complement of the multifunctional capture probe hybrid module and a region of the tagged target region that is located 5' of the location where the multifunctional capture probe hybrid module hybridizes to the target region; and

(d) performing PCR amplification of the hybrid nucleic acid molecule in c);
and

(e) quantitating the PCR reaction in d), wherein the quantitation allows for a determination of copy number of the specific target region.

98. The method of claim 97, further comprising obtaining the sequences of the hybrid nucleic acid molecules from step d).

99. The method of claim 98, wherein steps a) through d) are repeated at least about twice and a sequence alignment of the hybrid nucleic acid molecules from the at least two d) steps.

100. The method of claim 99, wherein at least two different multifunctional capture probe modules are used in the at least two a) steps, wherein the at least two a) steps employ one multifunctional capture probe module each.

101. The method of claim 100, wherein at least one multifunctional capture probe module hybridizes downstream of the genomic target region and at least one multifunctional capture probe module hybridizes upstream of the genomic target region.

102. The method of any one of claims 93 to 101, wherein the targeted genetic analysis is sequence analysis.

103. The method of any one of claims 93 to 102, wherein the target region is a genomic target region and the DNA library is a genomic DNA library.

104. The method of any one of claims 93 to 103, wherein the target region is a cDNA target region and the DNA library is a cDNA library.

CLFK_001_02WO_ST25.TXT
SEQUENCE LISTING

<110> Clearfork Bioscience, Inc.
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<120> METHODS FOR TARGETED GENOMIC ANALYSIS

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 <210> 39
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<210> 55
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<400> 67
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cagggagca aagggggccc ctaccagaca tcttgacag cactctaaca agcctgggga	300
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aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt	300

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 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
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<220>
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<210> 106
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<223> dideoxycytosine

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<221> misc_feature

<222> (34)..(34)

<223> n is a, c, g, or t

<400> 106

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<211> 400

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<213> Homo sapiens

<400> 107

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ctgtgcaaga tgtctggtag gggccccctt tgcttccctg gtggccactg gattgtgttt 180

ctttgggggtg gcactgttct gtggctgtgg acatgaagcc ctactggca cagaaaagct 240

aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300

acctgccctc ccacacagac ccatcttttt tttccctctc tccatcctgg agatagagaa 360

ctcttcagta ccttagtaac tagcagggga ctgggggtgga 400

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<212> DNA

<213> Artificial Sequence

<220>

<223> Ultramer targeted to PLP1 exon 2

<400> 108

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<210> 109

<211> 114

<212> DNA

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<220>

<223> Ultramer targeted to PLP1 exon 2

<400> 109

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<223> Ultramer targeted to PLP1 exon 2

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 ctgtgcaaga tgtctggtag gggccccctt tgcttccctg gtggccactg gatttgtgtt 180
 ctttgggggtg gcactgttct gtggctgtgg acatgaagcc ctactggca cagaaaagct 240
 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
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 ctcttcagta ccttagtaac tagcagggga ctgggggtgga 400

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 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
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 ctgtgcaaga tgtctggtag gggccccctt tgcttccctg gtggccactg gattgtgttt 180
 ctttgggggtg gcactgttct gtggctgtgg acatgaagcc ctactggca cagaaaagct 240
 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
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 <212> DNA
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 ctgtgcaaga tgtctggtag gggccccctt tgcttccctg gtggccactg gattgtgttt 180
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 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
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 aattgagacc tatttctcca aaaactacca agactatgag tatctcatca atgtgtaagt 300
 acctgccctc ccacacagac ccatcttttt tttccctctc tccatcctgg agatagagaa 360
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<210> 123
 <211> 22
 <212> DNA
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 <220>
 <223> Primer

 <400> 123
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<210> 124
 <211> 25
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 <220>
 <223> Primer

 <400> 124
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<210> 125
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 <220>
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 <400> 125
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<210> 126
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 <400> 126
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<210> 127
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 <400> 127
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<210> 128
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 <400> 128
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 <210> 133
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 <210> 138
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 <210> 139
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<210> 141
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 <400> 141
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<210> 142
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<210> 143
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 <400> 143
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<210> 144
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 <400> 144
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<210> 145
 <211> 24
 <212> DNA
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 <220>
 <223> Primer

 <400> 145
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 <210> 146
 <211> 60
 <212> DNA
 <213> Homo sapiens

 <400> 146
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 <210> 147
 <211> 60
 <212> DNA
 <213> Homo sapiens

 <400> 147
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 <210> 148
 <211> 60
 <212> DNA
 <213> Homo sapiens

 <400> 148
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 <210> 149
 <211> 60
 <212> DNA
 <213> Homo sapiens

 <400> 149
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 <210> 150
 <211> 95
 <212> DNA
 <213> Homo sapiens

 <400> 150
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 gcaggaggtg gcaggtagcc cagactggga ggtaa 95

 <210> 151
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 <212> DNA
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 <400> 151
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agtctgttca cacatggctg ctgcctctca gctct 95

<210> 152
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 <212> DNA
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<400> 152
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 cgatatctcc acttccagtt ttacattttac catca 95

<210> 153
 <211> 95
 <212> DNA
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<400> 153
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 tcatttgtca aggccttcag ttggcagacg tgctc 95

<210> 154
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 <212> DNA
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<400> 154
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 gtggaaacgc tgccacacaa tcttagcaca caaga 95

<210> 155
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 <212> DNA
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<400> 155
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 atcttggact ctaccctcg tactttatct cctat 95

<210> 156
 <211> 95
 <212> DNA
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<400> 156
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 ctagtggatt tgggattcta gaggaggtgt tataa 95

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 <212> DNA
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<400> 157
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 aacccaacca gccaaagcagg cagggtgctg aagga 95

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<210> 158
<211> 95
<212> DNA
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<211> 95
<212> DNA
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<400> 159
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<212> DNA
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<212> DNA
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 <212> DNA
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<400> 167
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 <212> DNA
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ttgcaagaaa cagttcttcc tctttcattt tcctg                                   95

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<400> 172
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<212> DNA
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<400> 174
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<400> 175
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 gcagaaaaca gatctgtatt tatttcagtg ttact 95

<210> 178
 <211> 95
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<400> 182

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gccgcatcca cgaaactttg cccatagcag cgggc	95
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aaaaaaataa attaaaaggc aagtggactt cggtg	95
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tctaccagca gcagcagcag agcgagctgc agccc	95
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 <213> Artificial Sequence

 <220>
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 <220>
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 <212> DNA
 <213> Homo sapiens

 <400> 652
 agcccatgg atctttctac catt 24

 <210> 653
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 <212> DNA
 <213> Homo sapiens

 <400> 653
 atactgccag ggctcctgat actg 24

 <210> 654
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 tggcagtat 69

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 <211> 40
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 <220>
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<220>
<223> Primer

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<210> 662
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<220>
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<220>
<221> misc_feature
<222> (20)..(20)
<223> n is a, c, g, or t

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<220>
<223> Adaptor ligation strand oligonucleotide

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<210> 665
<211> 33
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<220>
 <223> Index 1 sequencing primer

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 <210> 666
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 <220>
 <223> Library-free Forward sequencing primer

 <400> 666
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 <210> 667
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 <220>
 <223> Post-processing amplification primer

 <400> 667
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 <220>
 <223> Library-free forward amplification primer

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 ttcac 65

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 <220>
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ttcac 65

<210> 671
 <211> 65
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 <213> Artificial Sequence

<220>
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ttcac 65

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ttcac 65

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<220>
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 <223> n = any nucleotide

<220>
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 <223> n is a, c, g, or t

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<220>
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<222> (36)..(41)
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<210> 678
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<210> 680
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<220>
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<220>
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<220>
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<223> n is a, c, g, or t

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agtaatgtga ttttgaaca tccactgctg catttgtttg c                             101

<210> 682
<211> 101
<212> DNA
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<220>
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<223> n is a, c, g, or t

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<210> 683
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

<400> 684

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<210> 685
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 tacctactgg atgtgcctga ctgtttcccc ttcttcttcc c 101

<210> 686
 <211> 101
 <212> DNA
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<220>
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<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

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 attaataaga ttccctgggtc tcgtttgtct acctgttaat g 101

<210> 688
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<223> n is a, c, g, or t

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ccccacccc ttgttattgc cacaaaatcc tgaggatgat c 101

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<211> 101

<212> DNA

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<223> n is a, c, g, or t

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<210> 690

<211> 101

<212> DNA

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<222> (36)..(41)

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caggctgatg ctgatttcta accaccccat gtcaatcatt t 101

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<211> 101

<212> DNA

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<221> misc_feature

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<223> n is a, c, g, or t

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<211> 101

<212> DNA

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<211> 101

<212> DNA

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<223> n is a, c, g, or t

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<210> 697
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<220>
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ctgcttttctg ccaaaattaa tgtgctgaac ttaaacttac c                          101

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gattttgcag aaaacagatc tgtattttatt tcagtgttac t                          101

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<223> n is a, c, g, or t

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atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ntaagagtgg cccgttaa
60
aatgctgcaa tgaaaatggg aaaggtatcc agccgccac t
101

<210> 716
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 716
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nttgtatttg tacagcatta
60
atctggtaat tgattatttt aatgtaacct tgctaaagga g
101

<210> 717
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
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<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 717
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ngaggccaca gcaaacctcc
60
tcacagccca ctggtcctca agaggtgcca cgtctccaca c
101

<210> 718
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 718

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atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nagaggagga acgagctaaa 60
acggagcttt ttgcccctgc gtgaccagat cccggagttg g 101

<210> 719
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 719
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ntccaacttg accctcttgg 60
cagcaggata gtccttccga gtggagggag gcgctgcgta g 101

<210> 720
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 720
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ngcttggacg gacaggatgt 60
atgctgtggc ttttttaagg ataactacct tgggggcctt t 101

<210> 721
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 721
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aaacaagttc ataggtgatt gctcaggaca tttctgtag a 101

<210> 722
<211> 20
<212> DNA

<213> Artificial Sequence

<220>

<223> poly- dT primer

<220>

<221> modified_base

<222> (20)..(20)

<223> n = any nucleotide

<220>

<221> misc_feature

<222> (20)..(20)

<223> n is a, c, g, or t

<400> 722

tttttttttt ttttttttvn

20

<210> 723

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide aptamer

<220>

<221> misc_feature

<222> (26)..(33)

<223> n is a, c, g, or t

<220>

<221> modified_base

<222> (36)..(43)

<223> n = any nucleotide

<400> 723

tgcaggacca gagaattcga atacannnnn nnngactccg atccc

45

<210> 724

<211> 12

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide aptamer

<400> 724

gggatcggag tc

12

<210> 725

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide aptamer

<220>

<221> misc_feature

<222> (26)..(33)

<223> n is a, c, g, or t

<220>
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 <222> (36)..(43)
 <223> n = any nucleotide

 <400> 725
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 <210> 726
 <211> 12
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide aptamer

 <400> 726
 ctccgagttc cg 12

 <210> 727
 <211> 101
 <212> DNA
 <213> Artificial Sequence

 <220>
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 <220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

 <400> 727
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 aaaatgtcag cctgggtctgt ccaaaagatg cgacccgatt c 101

 <210> 728
 <211> 100
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide probe

 <220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

 <400> 728
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncagctgacc tcatcgagat 60
 tggcttggaaggaa ggaaaaggct ttgagccaac attggaagct 100

 <210> 729
 <211> 101
 <212> DNA
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 <220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 729

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ntactggaga caggctggac 60

tcagctacat ccgatactcc cagatctgtg caaaagcagt g 101

<210> 730

<211> 101

<212> DNA

<213> Artificial sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 730

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caaatgctga gaagacttct ggcagcaacg taaaaattgt g 101

<210> 731

<211> 101

<212> DNA

<213> Artificial sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 731

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nacagagtcc agcccattga 60

gagaatcaac tgccataggt tttacacctg agttagaaag t 101

<210> 732

<211> 100

<212> DNA

<213> Artificial sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 732
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 gacgacgggt tgcaccagtt tcttcgggggt acctccagca 100

<210> 733
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 733
 atgtgactgg cacgggagtt gatcctgggtttttcacnnnnn ngacacgcag cacaattgc 60
 gcagcgcggt ggaagagatg gaggcagaag aagctgctgc 100

<210> 734
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 734
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 gaccttcgtg gggagccgtg accaagatgg ggagatcctg 100

<210> 735
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 735
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 tcctaagtgt ggtgggcaca gcatggactg cagatagtgg t 101

<210> 736
 <211> 100

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<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
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<222> (36)..(41)
<223> n is a, c, g, or t

<400> 736
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nattttttct gtttattgcg      60
atcaagagac cagtttgga ggatggcttt tgatccagca                                100

<210> 737
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 737
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ngtggttcagt ttcaccttg      60
ttaccaccgc tctgacaatg ggcagggaaa tttcggcgct c                            101

<210> 738
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 738
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ntgcaaacct gaatgggtga      60
tactacagcg gccctacac ggctaaaaca gacaatggga                                100

<210> 739
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>

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<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 739
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncatcccca cgtaccgcc      60
gacaccaaac tctccaaaat caagaccctg cgcctggcca                             100

<210> 740
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 740
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncgaccagaa tggcgaggcg      60
gaggccttca aggcagagat caagaagacc gacgtgaaag                             100

<210> 741
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 741
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nctgacctt cttgccatcc      60
tggccgcctt agcggtagta actttgtgtt atgaatcaca t                             101

<210> 742
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 742
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncgagctcaa tagggaagcc      60
tgtgatgact acagactttg cgaacgctac gccatgggtt                             100

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<210> 743
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 743
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn naagaagtat cttcgaacag 60
 atgacagagt agaacgtgta cgcagagccc acctgaatga 100

<210> 744
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 744
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ntattccttg agtgggtccc 60
 acccctctac agccatcctg cacttcagac tatttgtcgg 100

<210> 745
 <211> 101
 <212> DNA
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<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 745
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncagaccagg ccttttgacc 60
 tcaagaagga tgtcttcgtg cctgatgaca aacaggagtt t 101

<210> 746
 <211> 100
 <212> DNA
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<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 746

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nagagctctt caaactcaag 60

aacgcctatg aggagtcctt ggaacatctg gagaccttca 100

<210> 747

<211> 101

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 747

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncaggctctt tataagcctg 60

ttattgaaga ctttagcatg gaattggcca gaaaatgcac g 101

<210> 748

<211> 100

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 748

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn naaagagcaa aactacaagg 60

ccactccggt aagcatgacc ccggagatag agagagtgag 100

<210> 749

<211> 101

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

CLFK_001_02WO_ST25.TXT

<400> 749
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 agcacctcaa tgacctgaag aaggagaact tcagcctcaa g 101

<210> 750
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 750
 atgtgactgg cacgggagtt gatcctgggt ttcacnnnnn ngaaagccaa gctgctgcag 60
 agaagttggt gcaagcctta atggaaagaa attcagaatt 100

<210> 751
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 751
 atgtgactgg cacgggagtt gatcctgggt ttcacnnnnn nccggacgag gattcttcta 60
 acagagaaaa tgccattgag gatgaagagg aggaggagga g 101

<210> 752
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 752
 atgtgactgg cacgggagtt gatcctgggt ttcacnnnnn ncgtcaaggt cgtggtggga 60
 aagacctttg actccattgt gatggacccc aagaaggacg 100

<210> 753
 <211> 100

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<212> DNA
<213> Artificial Sequence

<220>
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<220>
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<222> (36)..(41)
<223> n is a, c, g, or t

<400> 753
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nagatcacga gttatgaaaa      60
acctcctcct gggcttatca aggttggcat tgcaaatggt                               100

<210> 754
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 754
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn naaagtttct tcatcgttgt      60
cctccctgct ggtcacatga gtttacgatt ccttagaagt g                               101

<210> 755
<211> 100
<212> DNA
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<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 755
atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ncccggcgcg ttccgactgg      60
ctgccaggat gtacctgcag aaaggatttc ccatcaaaga                               100

<210> 756
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>

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<221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 756
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ngcctgaaag gcttcccccg 60
 cggagacaag cttttcggcc ctgacttaaa acttggtgcc 100

<210> 757
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 757
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 aggagaagta ccaggaggcg ttggccaagg gagatgttac 100

<210> 758
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 758
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn naggagaagc tgacggctgc 60
 atctgttgggt gtccaaggct cagggtgggg ttggcttggt 100

<210> 759
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 759
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn ngaccaagac tttcgagct 60
 tccggtcaga gtgtgaggct gaggtgggct ggaacctgac c 101

<210> 760
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 760
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nagatgtaca aggcgtgcct 60
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<210> 761
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 761
 atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn nttcagtga gaattaaaag 60
 ggcctgtgac agatgatgaa gaagtggaaa catctgtgct c 101

<210> 762
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide probe

<220>
 <221> misc_feature
 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 762
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 tcaagaagag tggaactcca ggaaaaactg gtggccaggc 100

<210> 763
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 763

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gagcgcaaca actgtcccca agatcctctc agaccgtcta c 101

<210> 764

<211> 100

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 764

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn naggcctacc cattcctgca 60

cacaaaggag gacacttatg agaacctgca taagggtgctg 100

<210> 765

<211> 101

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

<400> 765

atgtgactgg cacgggagtt gatcctgggtt ttcacnnnnn natcggtttt ggttatgcag 60

ccctcgtgac atttgaagc atttttggat ataagcggag a 101

<210> 766

<211> 100

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide probe

<220>

<221> misc_feature

<222> (36)..(41)

<223> n is a, c, g, or t

CLFK_001_02WO_ST25.TXT

<400> 766
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<210> 767
 <211> 101
 <212> DNA
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<220>
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<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 767
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 accggaatgg agtgtgtctg cactacagcg aatggcaccg c 101

<210> 768
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 <222> (36)..(41)
 <223> n is a, c, g, or t

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 acatctacag tgcgctgtat gtggagctgg tggatgaagaa 100

<210> 769
 <211> 101
 <212> DNA
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<220>
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<220>
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 <222> (36)..(41)
 <223> n is a, c, g, or t

<400> 769
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 aaaatgtcag cctgggtctgt caaaagatg cgacccgatt c 101

<210> 770
 <211> 100

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<212> DNA
<213> Artificial Sequence

<220>
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<220>
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<222> (36)..(41)
<223> n is a, c, g, or t

<400> 770
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tggtcttgaa ggaaaaggct ttgagccaac attggaagct                               100

<210> 771
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<220>
<221> misc_feature
<222> (36)..(41)
<223> n is a, c, g, or t

<400> 771
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tcagctacat ccgatactcc cagatctgtg caaaagcagt g                               101

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<211> 101
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caaatgctga gaagacttct ggcagcaacg taaaaattgt g                               101

<210> 773
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<220>

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<221> misc_feature
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<223> n is a, c, g, or t

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gagaatcaac tgccataggt ttacacctg agttagaaag t                             101

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<211> 100
<212> DNA
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<220>
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<220>
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<222> (36)..(41)
<223> n is a, c, g, or t

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gacgacgggt tgcaccagtt tcttcggggg acctccagca                             100

<210> 775
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gcagcgcggg ggaagagatg gaggcagaag aagctgctgc                             100

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<211> 100
<212> DNA
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<223> n is a, c, g, or t

<400> 776
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<220>
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<220>
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 <211> 100
 <212> DNA
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<220>
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 <223> n is a, c, g, or t

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<210> 780
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<222> (36)..(41)

<223> n is a, c, g, or t

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<212> DNA

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<222> (36)..(41)

<223> n is a, c, g, or t

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gacaccaaac tctccaaaat caagaccctg cgcctggcca 100

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<211> 100

<212> DNA

<213> Artificial Sequence

<220>

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<222> (36)..(41)

<223> n is a, c, g, or t

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gaggccttca aggcagagat caagaagacc gacgtgaaag 100

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<211> 101

<212> DNA

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<222> (36)..(41)

<223> n is a, c, g, or t

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<220>
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<220>
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 <223> n is a, c, g, or t

<400> 784
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<210> 787
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<212> DNA
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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aacgcctatg aggagtcctt ggaacatctg gagaccttca                                100

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<223> n is a, c, g, or t

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ttattgaaga cttagcatg gaattggcca gaaaatgcac g                                101

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<220>
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<220>

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<221> misc_feature
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 <223> n is a, c, g, or t

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<210> 791
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 <212> DNA
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<220>
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<210> 792
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 <222> (36)..(41)
 <223> n is a, c, g, or t

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 acctcctcct gggcttatca aggttggcat tgcaaatggt 100

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<210> 797
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<223> n is a, c, g, or t

<400> 797

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<211> 100

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<223> n is a, c, g, or t

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<222> (36)..(41)

<223> n is a, c, g, or t

<400> 799

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<220>

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<223> n is a, c, g, or t

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<210> 803
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<212> DNA
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gagcgcaaca actgtcccca agatcctctc agaccgtcta c                             101

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cacaaaggag gacacttatg agaacctgca taagggtgctg                             100

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