Supporting Information

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SI Text

Preparation of Long Padlock Probes (LPP). The common spacer for all of the probes had two amplification primers (AP) that are used in the multiplex PCR and a sequence derived from bacteriophage lambda in between the primers. It was constructed by amplification using bacteriophage lambda as a template where the forward primer TGTCTATAGTGTCACCTAAATTAAT-GTGACGATAGCTG had an adapter sequence containing the AP I at the 5′ end, and the reverse primer TCCCTTTAGT-GAGGGTTAATAGTACGCTTACTTCCGCG that had and adaptor with the AP2 at its 5′end. The resulting double-stranded PCR product with the sequence TGTCTATAGTGTCACCTA AATTAATGTGACGATAGCTGAAAACTGTACGATA AACGGTACGCTGAGGGCGGAAAAAATCGTCGGG GACATTGTAAAGGCGGCGAGCGCGGCTTTTCCGCGC CAGCGTGAAAGCAGTGTGGACTGGCCGTCAGGTACC CGTACTGTCACCGTGACCGATGACCATCCTTTTGATC GCCAGATAGTGGTGCTTCCGCTGACGTTTCGCGGA AGTAAGCGTACTATTAACCCTCACTAAAGGGACA served as the template for all subsequent probe constructions.

Using one set of primers as an example, the forward primer has the sequence 5′GTACGAGGTCTCACTGTAAGCCCTG-CAATTTCCCCCCATCGATTCCCTTTAG, where the BsaI site is GGTCTC, the target-specific sequence that is underlined, and ClaI sequence ATCGAT. The sequence of the reverse primer is CATCGTGAGTCACTCGTCATGGGGTAA-GACGATCATAGAGGGGCGCGCCCTATAGTGT, where the MlyI site GAGTC, the target-specific sequence underlined, and the AscI site GGCGCGCC.

A list of primer pairs used for each probe and the targeted exons are shown in Table S1. One microgram of purified PCR product was digested with 10 units of BsaI (New England Biolabs) in buffer 3 (100 mM NaCl, 50 mM Tris·HCl pH 7.9, 10 mM MgCl$_2$, 1 mM DTT) at 50°C for 2 h, followed by digestion with 5 units of Shrimp Alkaline Phosphatase (USB Corporation) at 37°C for 60 min in the same reaction buffer. After heat inactivation at 80°C for 20 min, the dephosphorylated PCR products were digested with 10 units of MlyI (New England Biolabs) to generate the other target-specific end of the molecule. To generate the single-stranded probe the digested PCR product was treated with 0.1 units lambda exonuclease (New England Biolabs) at 37°C for 15 min in the same restriction enzyme buffer followed by heat inactivation at 80°C for 20 min. Because the molecule is unphosphorylated, the probes were treated with 5 units of T4 Polynucleotide Kinase (New England Biolabs) in 50 mM Tris·HCl, pH7.9, 10 mM MgCl$_2$, 1 mM ATP, and 1 mM DTT.
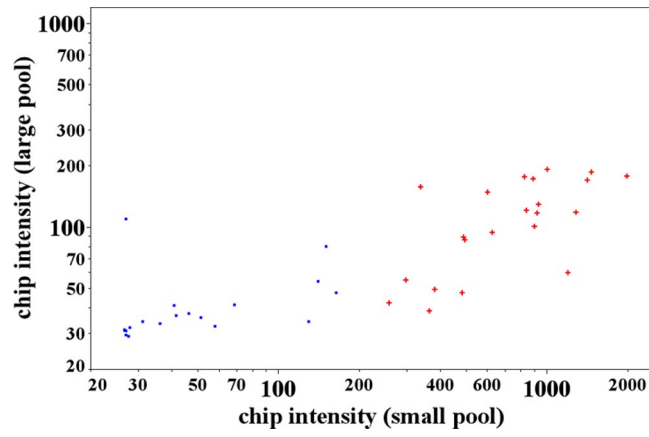
Fig. S1. A subset of probes whose amplicons had CG content >59% and chip intensity of <200 were pooled together and amplified as a smaller pool. The products were then hybridized to the resequencing chip. The probes that failed in both the experiments are shown in red, and the successful amplifications in the smaller pool are shown in blue.

Table S1. Comparison of amplification with MIP and SMART probes

Name	Gap-fill size, bp	MIPS* (C_T values)	SMARTS* (C_T values)	Genomic control[†] (C_T values)
SK319	161	23.7	21.2	22.5
SK317	186	25	27.4	23.6
SK307	200	20.113	23.2	23.4
SK320	220	26.5	24.9	23.4
SK314	240	21.6	22.5	23.0
SK301	273	28.4	21.9	23.8
SK302	301	28.4	20.9	23.1
SK310	383	30.5	23.5	23.3

*The template was 10^{-6} dilution of products after amplification and were amplified with primers specific for each product.
[†]The template was 1 ng of genomic DNA.

Table S2. Sequences of MIP, primers for SMART probes, and qPCR primers used in Table S1

Name	Size of gap fill, bp	Padlock probe sequence	qPCR primer F	qPCR primers R	SMART probe forward primer	SMART probe reverse primer
301	161	TGGAGTA CGTCTCGT AGTCCTTT AGTGAGG GTTAATAG TACGCAC AGTTATGA TTTAAAGC AAATGTAT CGCGTCAC ATTATTTA GGTGACA CTATAGTC ACCATTTG TTTTACTGG	TCTCTT TCCCACA TCCCAA ACTT	GGCATCTT TGGTGTCA TAAGAA	CATCGTGAGT CACTCGCCAA ACATCCCAGT AAAACAAATG GTGACTATAG TGTCACCTAAAT	GTACGAGGTCTCA ACCTCATGCAGAG CATCAACCAAAGT CCCTTTAGTGAGGG TTAAT
302	186	GAAATGA CAAATAT AGATGCCT TTAGTGAG GGTTAATA GTACGCA CAGTTATG ATTTAAAG CAAATGT ATCGCGTC ACATTATT TAGGTGA CACTATAG ATGACTTT GACACCA TGCC	GTTTGA GTGGGG CAAAAA TACA	GATGCAG ATGAGCA ATTTTCTG	CATCGTGAGT CACTCGTCAC AGATAGGCAT GGTGTCAAAG TCATCTATAGT GTCACCTAAAT	GTACGAGGTCTCA GAAATGACAAATA TAGATGGCAAAAG CCATCCCTTTAGTG AGGGTTAAT
307	200	AAACTCCC AGGTTTGT CTAGGCCT TTAGTGAG GGTTAATA GTACGCA CAGTTATG ATTTAAAG CAAATGT ATCGCGTC ACATTATT TAGGTGA CACTATAG CAAGGTA GAGAAGG AACTCACC	**TGGAGG AGATGA TGCTGGTG**	**ACTGAAG CCGTCCTC AATGC**	**CATCGTGAGT CACTCGCTCTG GGGTGAGTTC CTTCTCTACCT TGCTATAGTGT CACCTAAAT**	GTACGAGGTCTCA AAACTCCCAGGTTT GTCTAGGAAGGAG TTTCTCCCTTTAGT GAGGGTTAAT
310	220	TGTGAAG CTGAGTTA ATTTACCT TTAGTGAG GGTTAATA GTACGCA CAGTTATG ATTTAAAG CAAATGT ATCGCGTC ACATTATT TAGGTGA CACTATAG CAATCCTT TTTACTTTCA	CTGCAG GACATG ACAACTCATC	GACTGCA GGAACTC CTTAAAGC	CATCGTGAGT CACTCGTGAA GGAAAATGAA AGTAAAAAGG ATTGCTATAGT GTCACCTAAAT	GTACGAGGTCTCAT GTGAAGCTGAGTT AATTTATGTAAGTC ATCCCTTTAGTGAG GGTTAAT

Name	Size of gap fill, bp	Padlock probe sequence	qPCR primer F	qPCR primers R	SMART probe forward primer	SMART probe reverse primer
314	240	CTCAGTGT GGTAAGG TTCCCTTT AGTGAGG GTTAATAG TACGCAC AGTTATGA TTTAAAGC AAATGTAT CGCGTCAC ATTATTTA GGTGACA CTATAGCC TGGCAGA CTTGTATA TTTTC	AACTTC AGCCCT GGGAATCT	GCACAAA GGCATCTA TCTCCAT	CATCGTGAGT CACTCGACCA TAGACAGGAA GGAAAAGAGT TTTTCTATAGT GTCACCTAAAT	GTACGAGGTCTCAC TCAGTGTGGTAAG GTTCAGAGCCTTCC CTTTAGTGAGGGTT AAT
317	273	GGCCTTAA AAGCTGC CTCTCCTT TAGTGAG GGTTAATA GTACGCA CAGTTATG ATTTAAAG CAAATGT ATCGCGTC ACATTATT TAGGTGA CACTATAG ACTATCTC TGAGAGG GTGG	CTGGCC GTCCTG ACCATC	GGACCCA CCAGGTTG ATGA	CATCGTGAGT CACTCGCTGT GTCTCCACCCT CTCAGAGATA GTCTATAGTGT CACCTAAAT	GTACGAGGTCTCAT ACAATGTGGCCTTA AAAGCTGCCTCTTC CCTTTAGTGAGGGT TAAT
319	301	GAACAGG TACAAAT ACTGCACC TTTAGTGA GGGTTAAT AGTACGC ACAGTTAT GATTTAAA GCAAATG TATCGCGT CACATTAT TTAGGTGA CACTATAG CTCTGGAG ACGTACA AACATA	CTCCCC AGCACT TCCTACAC	CCTCTCTC TTTCGTGCTGC T	CATCGTGAGT CACTCGAATC TGGTATGTTTG TACGTCTCCA GAGCTATAGT GTCACCTAAAT	GTACGAGGTCTCAT CTAATGAAGAACA GGTACAAATACTG CATCCCTTTAGTGA GGGTTAAT

Name	Size of gap fill, bp	Padlock probe sequence	qPCR primer F	qPCR primers R	SMART probe forward primer	SMART probe reverse primer
320	383	CAGCAGG TGTTCCAA CCCTACAC CCTTTAGT GAGGGTT AATAGTA CGCACAG TTATGATT TAAAGCA AATGTATC GCGTCAC ATTATTTA GGTGACA CTATAGG AACGGGA TGTGGGTT GTTGGT	CACAAC CTGAGT CTGCAC AAG	CCCCTTCT CGCTCTCC AC	CATCGTGAGT CACTCGACCA ACAACCCACA TCCCGTTCCTA TAGTGTCACCT AAAT	GTACGAGGTCTCAC AGCAGGTGTTCCA ACCCTACACTCCCT TTAGTGAGGGTTAAT

Other Supporting Information Files

Dataset S1 (XLS)