

Protocol

Integrating readout of somatic mutations in individual cells with single-cell transcriptional profiling



In many biological applications, the readout of somatic mutations in individual cells is essential. For example, it can be used to mark individual cancer cells or identify progenies of a stem cell. Here, we present a protocol to perform single-cell RNA-seq and single-cell amplicon-seq using 10X Chromium technology. Our protocol demonstrates how to (1) isolate CD34+ progenitor cells from human bone marrow aspirate, (2) prepare single-cell amplicon libraries, and (3) analyze the libraries to assign somatic mutations to individual cells.

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Highlights

Isolation of CD34+ cells from human bone marrow aspirates

Enrichment of target somatic mutations from single-cell cDNA

Protocol enables single-cell RNA sequencing alongside single-cell amplicon sequencing

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Integrating readout of somatic mutations in individual cells with single-cell transcriptional profiling

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SUMMARY

In many biological applications, the readout of somatic mutations in individual cells is essential. For example, it can be used to mark individual cancer cells or identify progenies of a stem cell. Here, we present a protocol to perform single-cell RNA-seq and single-cell amplicon-seq using 10X Chromium technology. Our protocol demonstrates how to (1) isolate CD34+ progenitor cells from human bone marrow aspirate, (2) prepare single-cell amplicon libraries, and (3) analyze the libraries to assign somatic mutations to individual cells. For complete details on the use and execution of this protocol, please refer to Van Egeren et al. (2021).

BEFORE YOU BEGIN

Design of locus-specific amplicon primers

© Timing: 15 min per locus

- 1. Identify somatic mutations of interest and the mRNA in which they occur (Figure 1A).
- 2. Design locus-specific primers 1, 2, 3 to be roughly 300 bp, 150 bp and 50 bp upstream (5') of the somatic mutation (Figures 1B, 1C, and 1D)

Note: 1: 10× Genomics Chromium 3' pipeline captures the polyadenylated (polyA) transcripts. The directionality of the three locus-specific primers reverses and become reverse primers that anneal to the 3' end of cDNA during Polymerase chain reactions.

 \triangle CRITICAL: All three primers should have a length of around 25 bp and have a melting temperature of around 65°C to reduce experimental complexity.

3. Design locus-specific primer 4 by adding Illumina Read 2 sequence to the 5' end of the first 18–22 nucleotides of locus-specific primer 3 (Figures 1B and 1C, and Table 1).

Preparation of bone marrow aspirates from patients and healthy donors

 \odot Timing: \sim 1 h

- 4. Use a sterile syringe and tube with no heparin coating to aspirate the sample.
- 5. Collect bone marrow aspirate (BMA) and place it in EDTA-coated tubes.

Check for updates





Figure 1. Primer designs, directions, locations, sequences of target mutation; accurate identification of the mutated cells from the amplicon libraries

(A) Illustration of primer direction against target mRNA and the change of primer directionality during amplification of sc-cDNA. (B) Schematic diagrams of the nested PCR with locus-specific primers 1-4 (denoted as primers 1-4) from steps 1 to 5, respectively. (C) Oligonucleotide sequences and localization of common primers and adaptors.

(D) Example primer positions and sequences of targeted mutation. Red indicates primer sequences and blue indicates mutation nucleotides. (Figure reprinted with permission from Van Egeren et al., 2021).

Protocol



INTERNAL FORWARD AATGATACGGCGACCACCGAGAATCTCTCCCTCACACGACGCCC SHORT_INT_FOR AATGATACGGCGACCACCGAGAATCT JAR2_PRIMER1 ACCAACCTCACCAACATTACAGAGGCCT JAR2_PRIMER2 AGGAGCACGGTCAACTGCAACGGCTAACTGCAGCAAGAACAGA JAR2_PRIMER3 GCAGCAGATTGATGAGCAAGCTTTGCCAACGACAGCAAGTATGATGAGGACA ASH1_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGCAGCAAGTATGATGAGGAA ASH1_PRIMER1 GCATCTCACTCCTATCTGAAAAGTTGGACAGC ASH1_PRIMER2 TGGCCACAAGAAAAACCTAGACGATGTGCA ASH1_PRIMER3 GGAAATGTCCCCTCAGGCGTGCGTATCAA ASH1_PRIMER4 GTGACTGGAGTTCAGACGTGTGGACCAGG GOLIM4_PRIMER3 GGAAATGTCCCCTTCAGGCAGCAGGAGCAG GOLIM4_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGAAATGCCCCTTCAGGCT GOLIM4_PRIMER3 GGCACTTACTACGGCAGCAGGAGCAG GOLIM4_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGAATAGGATATGGATATGG ABHD2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGACCTTGGATAGGATAATGATATCG ABHD2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGACCTTGGATAGGATAATGATATCG ABHD2_PRIMER8 TGTATGGAGTGCAGCCGCCCTCCCATCTGCT ABHD2_PRIMER8 GTGACTGGAGGTCGACCCCCCTCTCTCAAATT ABHD2_PRIMER8 GTGACTGGAGGTGCGCCCCCCCATCTGCTTGGAGACACAAATACTGGC FHIT_PRIMER8 GTGACTGGAGTTCGAGCGTGGCCTCTCCGATCTTGGAGACACAAATACTGGC FHIT_PRIMER8 GTGACTGGAGTTCGAGCGTGGCCTCTCCGATCTTGGAGACACAAATACTGGC FHIT_PRIMER8 GTGACTGGAGTTCGAGCGTGGCCTCTCCGATCTTGGAGGACACAAATACTGGC FHIT_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTCTCGGATCTGGAGCACAAATACTGGC FHIT_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTTCCGATCTGGGGG FFYL_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTTCCGATCTGGGGCACACAATACTGCC FFYL_PRIMER8 GTGACTGGAGTTCGAACGTGTGCTCTCGGATCTGGGGTGTGTTTCTCATT FFYL_PRIMER8 GTGACTGGAGTTCGAACGTGTGCTCTTCGGATCTGGGCTGTCGTATCATA MAML3_PRIMER8 TGCCTAACAGTCGTACCACGTGTGCCTTCGGATCTGGGCTGTAGCCCAGCTGT FFYL_PRIMER8 GTGACTGGAGTTCGAACGTGTGCCTTCCGATCTGGGCTGTAGGCCGTGTGCCTGTCGATCTGGGCTGTAGGCCGTGGCCTGTCGACCAGCTT FFYL_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTTCCGATCTGGGCTGTAGGCCCGCGCACAT PFIL3_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTTCCGATCTGGGCGTGTTGAGCCCGACCAA SFF1_PRIMER8 GTGACTGGAGTTCGAACGTGGCCTTCCGATCTGGGCGGTTGAACGCCAAGCA SFF1_PRIMER8 GTGACTGGAGTTCGAACGTGTGCCTTCCGATCTGGGCGGTTTGAACGCCTAAACCCCTAGGCA SFF1_PRIMER8 GTGACTGGAGTTGAACGCTGGCCTTCCGATCTGGGCGTTTGAGGCAC
SHORT.INT.FOR AATGATAGGGGGACACCGAGAGT JAK2_PRIMER1 ACCAACCTCACCCAACATTACAGAGGCCT JAK2_PRIMER2 AGGAGACTACGGTCAACTGCATGAAAGAGAG JAK2_PRIMER3 GCAACCAACGTATGATGAGGAAGACGTTCCCAACAGA JAK2_PRIMER4 GTGACTGGAGTTCAGACGAGCTTCCGATCTGAAAGTAGAGGAGA JAK2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTTTCCGATCTGCGAAGGAAG
JAK2_PRIMER1 ACCAACCTCAACCAACATTACAGAGGCCT JAK2_PRIMER2 AGGAGCTACGGCAACTGCATGAAACAGA JAK2_PRIMER3 GCAGCAAGTATGATGAGCAAGCTCACTCCACA JAK2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTCCGACTGGCAAGTATGATGAGGCAA ASH1L_PRIMER2 TGGCCACAAGAAAAACCTGAGCCATGTCA ASH1L_PRIMER2 GGCACCTACAGACGTGTGCTCTCCGATCTGGAAAGTGCCCCTTCAGGCT ASH1L_PRIMER2 GGCACCTACGACGGTGGCGTCTCCGACTGGAAAGTGCCCCTTCAGGCT ASH1L_PRIMER3 GGAATGTCCCCTTCAGGCGTGGCGTCTCCGACTGGAAAGTGCCCCTTCAGGCT GOLIM4_PRIMER4 GTGACTGGAGTTCAGGCAGCGGGGCACAG GOLIM4_PRIMER1 TCACCCTATGAGGACAGTGGAACAG GOLIM4_PRIMER3 TGCTAGGAGTTCAGGCAGGGGAACAG GOLIM4_PRIMER3 TGCTAGGAGTTCAGACGTGGCCTTCCGATCTGGATATGGATATGGT ABHD2_PRIMER1 GGGGTGACACAGCAGGGGCACAGACAG ABHD2_PRIMER1 GGGGTGACACAGCAGGGCGCAGGAGAACAG ABHD2_PRIMER3 TGGAGGCACAGACCAGGCGGGCACAGAATG ABHD2_PRIMER4 GTGACTGGAGTTCAGGCGTGGCCTTTCCGATCTTGGAGACACAAATACTGC FHIT_PRIMER3 TGGGAGGCACAGACCAGGCGGGGG HIT_PRIMER3 TGGGAGGCACAGACAGAGGCCTTTCCCAATTG ABHD2_PRIMER4 GTGACTGGAGTTCAGGCGTGTCTTCCGATCTGGGGA HIT_PRIMER3 CCAGTGGAGTCAGACAGTGGGCTCTTCCGATCTGGGAGCACAAATACTGCC FHIT_PRIMER3 CCAGTGGGGTTCTTCCGATCTGGGGG HIT_PRIMER3 CCAGTGGGGTTCTTCCGATCTGGGGGG FHIT_PRIMER3 CCAGTGGGTTCTTCCGATCTGCGAGTGCGT HIT_PRIMER4 GTGACTGGAGTTCAGACGTGGGCCTTTCCCGATCTGCAGTGTGTTTCCATTT FRYL_PRIMER3 CCAGTGGAGTTCAGACGTGGTGCTCTTCCGATCTGCGGTGGCTTTCCATT FRYL_PRIMER3 GGATTCAGACGAGTGTGGCCTCTTCCGATCTGGGGTCAACAGGTGTGTCTTCCTTT FRYL_PRIMER3 GGACTGAACGACGTGTGCCTCTTCCGATCTGGGTCAACAGGTGTGTCTTCCATT FRYL_PRIMER3 GGCCTGAACAACAGTGTGCCCTCTTCCGATCTGGGCTGAGACGAGTGTGTCTTCC FRYL_PRIMER3 TGCCCTAACTGCTCTTCCGACCTGGGCTACACAGGTCGTGTCTTCCGATCTTGGCCTGGGCTGTCTGCCGAGTCGACCCCTTTCC FRYL_PRIMER3 TGCCTGAGCTGGAGTCCAGCCGGGGCAACAT PPIL3_PRIMER3 TGCCTGAGCTGGGCTCTTCCGACTGGGCTGCTGGCCTGGGCTGCCAGGCTG MAML3_PRIMER3 TGGCCTGAGCCGGGGGCGCCTTCGGGCTGGCGCTGTAGCCCAGGCTA SF1_PRIMER3 GGCGCGTGCTTACCCCCTAAGGCGGGGCGCCTTGGGCCGCTGTAGCCCCGAGG SF1_PRIMER3 GGCGCGGTGTATACCCGCCGGGGCGGCGCGCTTGAGCCCGAGGAG TRAPPC11_PRIMER3 ACCGGGAGTTGAACCCAGCGGGGGCGCGCGCTTGAGCCCGAGGAGTGAAGACCACACAGCG SF1_PRIMER4 GTGACTGGAGTCGACGCGGGGCGCTGCTCCCGACGCGGGGCGCTTGAGCCCCTAAG TRAPPC11_PRIMER3 ACCG
JAK2_PRIMER2 AGGAGACTACGGTCAACTGCATGCAACAGA JAK2_PRIMER3 GCAGCAAGTATGATGAGCAAGCTTTCTCACA JAK2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCAAGTATGATGAGAGAA ASH1L_PRIMER1 GCATCTCACTCCTATCTGAAAAGCTGGACCATGCA ASH1L_PRIMER2 TGGCCCACAAGAAAAACCTGGACCATGTCA ASH1L_PRIMER8 GGAATGTCCCCCTTCAGACAGCTGTGCATCTACA ASH1L_PRIMER8 GGAATGTCCCCCTTCAGACGGTGGCTCTTCCCAACTGGAAATGTCCCCCTTCAGGCT GOLIM4_PRIMER1 TCACCCCTATGAGAACAGTGGAACAGCAG GOLIM4_PRIMER2 GGGCACTTACTACTAGCGAGGAACAG GOLIM4_PRIMER2 GGGCACTTACTACTAGCGAGGACACG GOLIM4_PRIMER2 GGGCACTATCATCAGGCAGGAGACAG GOLIM4_PRIMER2 GGGCACTACTACTACGGCAGGGACACG GOLIM4_PRIMER2 AAATGGACAGACGCCTCTTCACAAATT ABHD2_PRIMER3 TGCTATGGAATACGACCACGCCTCTCCAAAATT ABHD2_PRIMER1 GGGTGCACCACACGCAGGCCCTTCTCAAAATT ABHD2_PRIMER1 GGGTGCACCACACGCAGGCCCTTCCCAAATT ABHD2_PRIMER2 AAATGGACAGAGAGCCCTCTTCCCATCTTGGAGACACAAATACTGCC FHIT_PRIMER1 GGCTGCACCACGCAGGCCCTTCCCAACTGGGGG FHIT_PRIMER2 GGGACTCCACACGCAGGCCCTTCCCGATCTTGGAGACACAAATACTGCC FHIT_PRIMER3 CGGAGTCCAGACGAGGTGGCTCTTCCGATCTTGGAGACACAAATACTGCC FHIT_PRIMER4 GTGACTGGAGTTCCAGCGTGGCTCTTCCGATCTTGGAGACACAAATACTGCC FHIT_PRIMER2 GGGATCCACAAAAGTGGAGCTGGGCTCTTCCGATCTTGGAGACACAAATACTGCC FFIL_PRIMER3 CGCATTGGAGTTCCAGCGTGGCTCTTCCGATCTTGGAGACACAAATACTGCC FFIL_PRIMER3 GGGATCACAAAAGTGGAGGTGGCTCTTCCGATCTGGAGGCACACAATACTGCC FFIL_PRIMER3 GGGCTCAACAGGTGTGCTCTTCCGATCTCGAGCGGGG FFIL_PRIMER3 GGGCTGCAACAGGTGTGTCTTCCCGACTGGGGCAACAG FFIL_PRIMER3 GGGCTGCAACAGGTGTGTCTTCCCGATCTGCGAGCAGGGG FFIL_PRIMER3 GGGCTGCAGCAGTGTGCTCTTCCGATCTGGGCTGACACAGTCTGTCATA MAML3_PRIMER3 GGGCTGCAGACAGTGTGGCTCTTCCGATCTGGCTGGCACCAGCTGTTCCCA FFIL_PRIMER4 GTGACTGGAGTTCAGACGTGGGCTCTTCCCGACCAGCGGGG FFIL_PRIMER3 GGGCTGCGGGGTCCAGCAGCGTGGCCTCTCCGATCTGGCCTGTAGTCCCAGCT MAML3_PRIMER3 GGGCTGCGGGGTCCAGCCGTGGGCTCTTCCGATCTGGGCTGTAGCCCAGGCA MAML3_PRIMER4 GTGACTGGGAGTTCAGACCGTGGGCTCTTCCGATCTGGGCTGTAACCCACGTG RSF1_PRIMER3 GGGCTGCTTGACGCCGTGGGCCTTCCCGACTGGCGTGTTAACCCCCAGGA TRAPPC11_PRIMER4 GTGACTGGAGTTCAGACCGTGGTGCTTCCCGACTGCGGCTGTTAACCCCCTAGG RSF1_PRIMER3 CGGGGCTGCTTCAGCACGTGGGCCTTCCGACCGTGGGCTGTTAACCCCCAAA TRAPPC11_PRIMER4 GT
JAK2_PRIMER3 GCAGCAGTATGATGAGCAAGCTTTCTCACA JAK2_PRIMER4 GTGACTGCAGTCTGGACGTGTGCTCTCCGATCTGCAGGAAGTATGATGAGAGAA ASHIL_PRIMER1 GCACTCACTCCTCATGCTGAAAGTTGACAAGC ASHIL_PRIMER2 TGGCCGCACAAGAAAAACCTAGACCATGTCA ASHIL_PRIMER2 GGAACTGCGCTGTCGGTGCGTCTCCGGACTGGAAATGTCCCCTTCAGGCT GOLIM4_PRIMER1 TCACCCTATGAGGAACAGTTGGAACAGCAG GOLIM4_PRIMER1 TCACCCTATGAGGAACAGTTGGAACAGCAG GOLIM4_PRIMER2 GGGCACTTACTACGGCAGCAGGAACAG GOLIM4_PRIMER3 TGCTATGGATATGGATAATGGTCAGGGAGGAG GOLIM4_PRIMER3 TGCTATGGAATATGATATCGTCAGGGAGCAG GOLIM4_PRIMER4 GTGACTGGAGTTCAGACCGTGTGCTCTCCGATCTGGATAATGATATCG ABHD2_PRIMER1 GGGTGACACAGCAAGACCCTTTCCAAATT ABHD2_PRIMER2 AAATGGACAAGAGCCCTCTTACTTCGGGGCA ABHD2_PRIMER3 TGGAGGGAGCACAGCCAGGCAGGGAGCAG ABHD2_PRIMER3 TGGAGGCACAAATACTGCCCCACTTCATCA ABHD2_PRIMER3 GGGTGACACAGCAAGTGGACTGCCTCTCCGATCTGGGAGACACAAATACTGCC FHIT_PRIMER3 GCCTACTAAAAGTGGAGCCGCTTTCCGATCTGTGAGACACAAATACTGCC FHIT_PRIMER3 GCCTACTAAAAGTGGAGCCGCTCTTCCGATCTGTGGAGACACAAATACTGCC FHIT_PRIMER3 CCAGTTGGTTTCCATCTCCGTGGGGGG FRYL_PRIMER4 GTGACTGGAGTCCAGACGTGTGCTCTCCCGATCTGTGTGTTTCCATTT FHIT_PRIMER3 CCAGTTGGAGTCCAGACGTGTGCTCTCCGATCTGCGGTGTGTTTCCATTT FFYL_PRIMER3 GGGTCCACAACGTCGTGTCCTCTCGGAGTGCAACAGTGTGTCCTCCG FFYL_PRIMER3 GGGTCCACACAGTCGTGTCCTCTCCGATCTGGGGGG FFYL_PRIMER4 GTGACTGGAGTTCCAGCCGTGTGCCTTTCCCATCTGGGGGG FFYL_PRIMER4 GTGACCGAGTTCCAGCCGTGTGCCTTTCCCA FFYL_PRIMER3 GGGTCCAACAGTCGTGTCCTCCCGATCTGGGGTCAACAGTCTGTCCATA MAML3_PRIMER1 GCCAATTGCTTTCCAGTCCTGCGATCTAGCGAGTCCAGCGTGTCCTTCCA FFYL_PRIMER4 GTGACTGGAGTTCCAGCGGTGGCTCTTCCCGATCTGGGCTGATAACCATCTTTC PPIL3_PRIMER4 GTGACTGGAGTTCCAGCCGTGGGCTCTTCCCGATCTGGCCTGTAGTCCCAGCT MAML3_PRIMER4 GTGACTGGAGTTCAGACGGTGGCTCTTCCCGATCTGGGCTGTAACCCATCTTTC PPIL3_PRIMER4 GTGACTGGAGTTCAGACGGTGGCTCTTCCGATCTGGCGTGTAACCCATCTTC PPIL3_PRIMER4 GTGACTGGAGTTCAGACGGTGGCTCTTCCGATCTGGGCTGTTAACCCCAGCT RSF1_PRIMER3 GGGCTGTTAACCCCCTAAACCGCTGTGGCTCTTCCGATCTGGGCTGTTAACCCCCTAGA SF1_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGTTAACCCCAAA SF51_PRIMER4 GTGACTGGAGTTCAGACCGTGTGCTCTCCGATCTGGGGTGTTGAGAGTTCAGACGTGTGTTAAGCAACCAAC
JAK2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGCAAGTATGATGAGGCAA ASH1L_PRIMER2 GGCACCTCCTATCTGAAAAGTGGACCGAG ASH1L_PRIMER2 GGGAATGTCCCCTTCAGGCTGTGCA ASH1L_PRIMER3 GGAAATGTCCCCTTCAGGCTGTGCACAG ASH1L_PRIMER3 GGAAATGTCCCCTTCAGGCTGTGCTCTCCCGATCTGGAAATGTCCCCTTCAGGCT GOLIM4_PRIMER1 GTGACTGGAGTTGAGCAGTGGGCTCTTCCCGATCTGGAAATGTCCCCTTCAGGCT GOLIM4_PRIMER1 GCCATATGAGAGCAGGGAGCAG GOLIM4_PRIMER1 GCCTATGGATAATGATATCGTTCAGGGAGCAG GOLIM4_PRIMER1 GGGTGACACGAGCAGGACAG GOLIM4_PRIMER1 GGGTGACACGACGAGGACAG ABHD2_PRIMER1 GGGTGACACGACGAGGACCAG ABHD2_PRIMER1 GGGTGACACGACGAGGACCAGCCTCTCCAAAATT ABHD2_PRIMER3 TGCTATGGAGTCAGACGTGTGCTCTCCGATCTTGGTAGGATAATGATATCG ABHD2_PRIMER3 GGGACACAGACACACAATCGCCCCCTCTCATCAT ABHD2_PRIMER3 GGGACCACAGACACGACGTGGCCTCTTCCGATCTTGGAGACACAAAATACTGCC HIT_PRIMER3 GGGACCACAAACGTGGGCCTCTTCCGATCTTGGAGACACAAAATACTGCC HIT_PRIMER3 GGGACCACAAACGTGGAGTCCTCCCGATCTTGGAGACACAAAATACTGCC HIT_PRIMER3 GGGACCACAAACGTGGGCCTCTTCCGATCTGGGAGACACAAAATACTGCC HIT_PRIMER3 GGGACCACAAACGTGGAGTCCTCTCCGATCTGGAGACACAAAATACTGCC HIT_PRIMER3 GGGACCACAAACGTGGGCCTCTTCCGATCTGCGGGG HIT_PRIMER4 GTGACTGGAGTTCAGACGTGGTCCTCTCCGATCTGCGGTGTTTTCTCATTT FRYL_PRIMER3 GGGTCCACACAGTCGTGGCCTCTTCCGATCTGCGGGG FFYL_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGTCAACAGTCTGTCT
ASHIL_PRIMERI GCATCTCACTCCTATCTGAAAAGTTGACAAGC ASHIL_PRIMER2 TGGCCACAAAGAAAAACCTAGACCATGTACA ASHIL_PRIMER3 GGAAATGCCCTTCAGGCTGTCGTACCA ASHIL_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAAAGTCCCCTTCAGGCT GOLM4_PRIMER1 TCACCCTATGAGGAACAGCTGGACACGCAG GOLM4_PRIMER2 GGGCACTTACTACGCGCAGCAGCAGACAG GOLM4_PRIMER2 GGGGCACAAGCACGCCGTCACACGCAGGACG GOLM4_PRIMER2 GGGGCACACAGCACGCCGTCACACGCAGCACGACG ABHD2_PRIMER4 GTGACTGGACTTCAGACGTGTGCCTTCCGATCTGGCATGGGATAATGATATCG ABHD2_PRIMER2 AAATGGACACAAAAAGTGCACCCCTCTACTTCA ABHD2_PRIMER2 AAATGGACACAAAAAGTGCACGTGTGCCTTCCGATCGGGGCACACAAATACTGCC FHIT_PRIMER3 TGTGACTGACGTGTGCTCTCCGATCGGGGGCACCACAAAATCGCCC FHIT_PRIMER4 GGGATCACAAAAGTGAAGATTGGATGCCGT FHIT_PRIMER3 CCAGTTGTTTTTTCTCATTTCCCTCTGAGGCGCACCACATTTTCCCATTT FHIT_PRIMER4 GGGCCACTACGAGGTGTGCCTTTCCGATCCCAGTTGTGTTTTCCATTT FRYL_PRIMER3 GGGCCACTACCAACGTGTGTCTTTCGCACCCCC FRYL_PRIMER4 GTGACTGGAGTTCAGACGTGTGCCTTTCCCACCTTGGGCTACACAGCTGTGTCCTCTGAGTCACACGTGTGTCTTCCA MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGCCTTTCCCACCC MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGCCTTTCCCACCC MAML3_PRIMER4 GTGACTGGAGTTC
ASHIL_PRIMER2 TGGCCACAAAGAAAACCTAGACCATGTCA ASHIL_PRIMER3 GGAAATGTCCCCTTCAGGCTGTCGTATCAA ASHIL_PRIMER4 GTGACTGGAGTTCAGCGGTGTGCTTCCGATCTGCGAACGGCCC GOLIM4_PRIMER1 TCACCCTATGAGGAACAGTTGGAACAGCAG GOLIM4_PRIMER2 GGGCACTTACTACGGCAGCAGGAGCAG GOLIM4_PRIMER2 GGGCACTTACTACGGCAGCAGGAGCAG GOLIM4_PRIMER2 GGGCACTAGTAGGATAATGGATAATGGTCAGGGAGCAG GOLIM4_PRIMER3 TGCTATGGATAATGGATACGGTCAGGGGAGCAG GOLIM4_PRIMER3 GTGACTGGAGTTGACAGCGTGTGCTCTCCGATCTTGATGGATAATGATATCG ABHD2_PRIMER1 GGGTGACACGACAGCCAGCTACTTCGCAACTTGGTGAGACACAAAATGGACGA ABHD2_PRIMER1 GGGTGACACAGACACTGCCCCCTTCACTTAC ABHD2_PRIMER3 TGTGAGACACAAAACTGCCCCCCTTCACTTCA ABHD2_PRIMER3 TGTGGAGCACCAAAACTGCCCCCCTTCCTATGCAGGAGGA FHIT_PRIMER3 GGGTCACACAAAACGTGTGCCTTTCCTAGTGGGGG FHIT_PRIMER3 CCCGTGTGACTGGGAGTTGGGCGT FHIT_PRIMER3 CCCGTGTGGTGTCTTCCGATCTGGGGGG FHIT_PRIMER3 CCCGGTGGGTCCAGCGGTGGCCTTCCGATCTCCGGTGGTTTTCCCATT FRY_PRIMER3 GGGTCACACAGTGGGTGTCATCGGGG FRY_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTTTCCGATCTCAGGTGGTTTTCCCATT FRY_PRIMER2 TGGCCCAACGTGTGTCATACCGGG FRY_PRIMER3 GAGTCAACAGTCGTGTCATACTGGGG FRY_PRIMER4 GTGACTGGAGTTCAGCGGTGTCCTTCCGATCTCAGGTCACACGGTCTGTCATA MAML3_PRIMER2 TGGCCCAACGTGTGTCATACCCCC FRY_PRIMER3 GAGTCAACAGTCTGTTCATACCCCCC FRY_PRIMER3 GGCCCAATGGTGTCATACGCGACT MAML3_PRIMER4 GTGACTGGAGTTCAGACGGTGTGCTCTCCGATCTGGGCCAACAGTCTGTTCATA MAML3_PRIMER3 TGCCCAAGTCGTGTCAACAGCTGTGTCTTCCGATCTGGATCAACGGCTGTTCCAACGGCTGTGCCAACGGCTGTGCCTTCCGATCTGGCCTGGGCAACAT PPIL3_PRIMER3 TGTGCCTGAAAACCAACTTTCTCCCCACCC MAML3_PRIMER3 TGTGCCTGGAGGTTCAGACGTGTGCCTTCCGATCTTGGCCTGGAGTCCCAGCTA RSF1_PRIMER4 GTGACTGGAGGTTCAGACGTGTGCCTTCCGATCTTGGCCTGGAGTCCCAGCTA RSF1_PRIMER3 GGGCTGCTTACACCACCTCTGCCTCCGGGCTGTGGTCTTGGCCTGGGCTGTCAGCCGGGTG RSF1_PRIMER4 GTGACTGGAGGTTCAGACGTGTGCCTTCCGATCTGGGCTGTCAGCCGGTG RSF1_PRIMER3 GGGCTGCTTAACCCCACACCTCGGCTGTCGGCTGTGGGCGTTTAACCCCAAACGCAT RSF1_PRIMER3 GGGCTGCTTAACCCCGCTGCCTTCCCGATCTGGGGCGGCTGTTAACCCCCAAA RSF1_PRIMER4 GTGACTGGAGGTTCAAACGCGGTGGTGTTTAACCCCAAACGCTGT TRAPPC11_PRIMER3 ACCCGGAGTTGAAGCAGGTGTGCTTCCGAACGGCGGTGTTTAGGGAGTTAC HSPA9_PRIMER3 GTGGGGGGTTGAAGAGCTTAACCCCCCGCAACGCTG CTAGGCGGG
ASHIL_PRIMER3 GGAAATGTCCCCTTCAGGCTGTCGTATCAA ASHIL_PRIMER4 GTGACTGGAGTTCAGACGTGGCTCTCCGAACTGGAAATGTCCCCTCAGGCT GOLIM4_PRIMER1 TGCTATGGAGATTCAGACGTGGCTCTAGGCACCAGCAG GOLIM4_PRIMER2 GGGCACTTACTACGGCAGCGGGGCACGAG GOLIM4_PRIMER3 TGCTATGGATAATGATACGTTCAGGGGGACAGG GOLIM4_PRIMER4 GTGACTGGAGTCGGCGCTGCTCCGACATGTGGGATAATGATATGG ABHD2_PRIMER1 GGGTGACACAGCAGAGCCCTTTACTGAGAGCAGCTGCTGTGCGATAGGATAATGATACG ABHD2_PRIMER1 GGGTGACACAGAAGCCCTTTACTGCGGGGG ABHD2_PRIMER4 GTGACTGGAGTGCAGCTGCGCTTTCCCAACTTGCAGGAGCACAAATACTGCC FHIT_PRIMER3 GCCACTTAATCCTTTTCCTACTTCGGGGGG HIT_PRIMER4 GGGATCACAAAAGTGAAGATTGGATGCGT FHIT_PRIMER3 CCAGTTGTGTTTCTCATTCCCTTAGAGCCA HIT_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCCAGTTGTGTTTTCCATTT FRYL_PRIMER4 GGGCCAACACGTCGTGTTCATACTCCCTTTGCACACGGGG FRYL_PRIMER4 GGGCCCAACAGTCTGTTCATACCCCCTATCCCCC FRYL_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTTTCCGATCTGCGAGTCAACAGGTCTGTTCCAA MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTTTCCCGATCTGCGAGCTACCTCTTCC MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGCCTTCCCACCC MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGCCTTCCCAACCTCTTCC PPIL3_PRIMER4 GGGCCTGACTCACCA
ASHIL_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAATGTCCCCTTCAGGCT GOLIM4_PRIMER1 TCACCCTATGAGGAACAGTTGGAACAGCAG GOLIM4_PRIMER2 GGGCACTTACTACGACAGCAGGAGCAG GOLIM4_PRIMER3 TGCTATGGATAATGATACGTCAGGGAGCAG GOLIM4_PRIMER4 GTGACTGGAGTTCAGACGAGTGTCCTTCCGAACAT ABHD2_PRIMER4 GTGACTGGAGTTCAGACGAGCCTCTTCAAAATT ABHD2_PRIMER2 AAATGGACAGAGCCCTTTACTTTGGGGCA ABHD2_PRIMER2 AAATGGACAGAAGCCCTTTACTTGGGGGGA ABHD2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGACACAAATACTGCC FHIT_PRIMER2 GGGATCACAAAAGTGAACGTGTGCTCTTCCGATCTGGGAGCACAAATACTGCC FHIT_PRIMER2 GGGATCACAAAAGTGAACGATGGACCCAC FHIT_PRIMER2 GGGATCACAAAAGTGAACGAGTGGCCGT FHIT_PRIMER3 CCAGTTGTGTTTTCCATTTCCCTTAGGAGCCA FHIT_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTGTGTTTCTCATTT FRYL_PRIMER3 GAGTCAACAGCTGTTTCAATCACTTCCCC FRYL_PRIMER4 GTGACTGGAGTTCAGACGTGTGTCTTTCCCACTTGGTCAACAGTCTGTTCATA MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGTCTTTCCCACTTGCTAGGTCAACAGTCTGTTCATA MAML3_PRIMER4 GTGACTGGAGTTCAGACGTGTGTCTTTCCCACCTTGCCTAGATAACCATCTTTC MAML3_PRIMER4 GTGACTGGAGTTCAGACCGTGTGCTCTTCCGACTTGCCTAGATAACCATCTTTC PPIL3_PRIMER4 GTGACTGGAGTT
GOLIM4_PRIMER1TCACCCTATGAGGAACAGGTTGGAACAGCAGGOLIM4_PRIMER2GGGCACTTACTACGGCAGCAGGAACAGGOLIM4_PRIMER3TGCTATGGATAATGATACGTTCAGGCAGCAGGAACAGGOLIM4_PRIMER4GTGACTGGAGTCTAGGCAGCAGGAGCAGABHD2_PRIMER1GGGTGACACAGCAAGACCCTTCCCAAAATTABHD2_PRIMER2AAATGGACAGAGCTCTTACTTTCGGGCAABHD2_PRIMER3TGTGAGACCCAAATACTGCCCCCTTCCAATTCAABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGTGAGACACAAATACTGCCFHIT_PRIMER2GGGATCACAAAAGTGAAGATGGAGGCCGTFHIT_PRIMER2GGGATCACAAAAGTGAGAGTGGCCTCCCGGTCTCCGATTGTGTGTTTCCATTTFRYL_PRIMER3CCAGTTGTGTTTTCTCATTCCCTTCGGAGGCCAFHIT_PRIMER2GGGATCACAAAGTGAGATGCCGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATGGATGCCGTFHIT_PRIMER3CCAGTTGGTGTTTCTCATTCCCTCCGATCTCCGATTGGTTTTCCATTTFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTTCCGATCTCCAGTTGGTTTTCCATTTFRYL_PRIMER3GAGTCAACAGTCTGTTAGTCCCTATCCCCCFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTCCAAGACGTGTGCCTTCCGATCTGAGTAAACCATCTTTCPPIL3_PRIMER1GCCCAGTGGAGTTCAGACCACCTTTTCCCCCCCCMAML3_PRIMER3TGGCCTGAGATCCAGCACGTGTGCCTTCCGATCTGGGCTAGATAACCATCTTTCPPIL3_PRIMER3GGGCTGCTTACCCCCCCCCCCCCCCTGTAGTCCCAGCTAPPIL3_PRIMER4GTGACTGAAGTCCACCACCTTCCGACCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER3GGGCTGCTTACCCCCTCCCCCCCCGGGTRSF1_PRIMER4GTGACTGAAGTTCCAACCCCTCTGGGGCACCATPPIL3_PRIMER4GTGACTGAACTCCTTCCGACCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER3GGGCTGCTTTACCCCCCCCCCTGGGCCTCTCCGATCTGGGCTGTTTAACCCCTAAA
GOLIM4_PRIMER2 GGGCACTTACTACGGCAGGCAGGAACAG GOLIM4_PRIMER3 TGCTATGGATAATGATATCGTTCAGGGAGCAG GOLIM4_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCGCATCTGGATAATGATATCG ABHD2_PRIMER1 GGGTGACACAGCAGAGCCCTTTCTCAAAATT ABHD2_PRIMER2 AAATGGACACAGAAGCCTCTTCTCAAAATT ABHD2_PRIMER3 TGTGAGACACAAAATACTGCCCACTTCATTCA ABHD2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCGTGGGGG HIT_PRIMER1 GCCTACTTAATCTTTCTCTTCGTGGGGG FHIT_PRIMER1 GCCTACTTAATCCTTTCCTACTTCGTGGGGG FHIT_PRIMER3 CCAGTTGTGTTTTTCCTACTTCGTGGGGG FHIT_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTTTCGATCCCAGTTGTGTTTTCTCATTT FRY_PRIMER3 CGACTGGAGTTCAGACGTGTGCCTTTCGATCCCGGG FRY_PRIMER4 GGGCTCAACAGCTGTTCATGTCCCTTCTGACTACTGGG FRY_PRIMER3 GAGTCAACAGCTGTTCTAGTCCCCCC FRY_PRIMER4 GTGCATGGAGTTCAGACCGCTGTGCCTTTCCGATCTGGGTCAACAGGTCGTTCCATA MAML3_PRIMER1 GCCAATGCTTTTCCAGCCAGGGT MAML3_PRIMER3 TGCCTGGAGTTCAGACCAGCTGTGCCTTTCCGATCTGGCCTGTAGTCCCAGCTCTTC PPIL3_PRIMER4 GTGCCTGGAGTTCAGACCAGCTGTGCCTTCCGACCC MAML3_PRIMER4 GTGCCTGTAGCCCAGCTGGCCCCCC MAML3_PRIMER1 TRAPPC11_PRIMER1 PPIL3_PRIMER3 GGGCTGCTTACCCCCCCCCTGGGCACA
GOLIM4_PRIMER3TGCTATGGATAATGATATCGTTCAGAGAGCAGGOLIM4_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTATGGATAATGATATCGABHD2_PRIMER1GGGTGACACAGCAGCAGCACGCTCTTCATTGGGGCAABHD2_PRIMER2AAATGGACAGAGGCTCTTACTTTGGGGCAABHD2_PRIMER3TGTGAGACACAGATCGCCCCCTTCATTCCGABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGACACAAATACTGCCFHIT_PRIMER1GCCTACTTAATCCTTTTCCTACTTCGTGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER2GGGATCACAAAAGTGAAGGTTGCATTCCGATGTGGTGTTTCTCATTTFRYL_PRIMER2GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGATGTGTGTTTCTCATTTFRYL_PRIMER2TGGCCCATACTGTCTTAGCCCTATCCCCFRYL_PRIMER3GAGTCAACAGTCTGTTCATACCCTCTTTCCGATCTGGGTGACAACAGTCTGTTCATAMAML3_PRIMER3GGCACCTGGGTGAAAACAAGTAAAACCAAGTAMML3_PRIMER4GTGACTGGAGATCAGCAGTGTGCTCTTCCGATCTGGAGTCAACAGTCTGTTCATAMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCCCMAML3_PRIMER4GTGACTGGAGATCAGCAGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTGAGTCCCAGCTAPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTGAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAARAPPC11_PRIMER1CCTGCACAACCTCCTTCCATAACCGCTTTCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGGGTGTTTTGGAGATTACHSPA9_PRIMER4GTGAC
GOLIM4_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCATGGATAATGATATCGABHD2_PRIMER1GGGTGACACAGCAAGACCCTTTCTCAAAATTABHD2_PRIMER2AAATGGACAGGACGTCTTACTTTGGGGCAABHD2_PRIMER3TGTGAGACACAAAATACTGCCCATTCATTCAABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGAGACACAAATACTGCCFHIT_PRIMER1GCCTACTTAATCCTTTTCCTACTTGGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER3CCAGTTGTGTTTTCTCATTTCCTATGAGCCGFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGTGTTTCTCATTTFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGTGTTTCTCATTTFRYL_PRIMER4GGATCACAGGGTTCAGACGTGTGCTCTTCCGATCTGAGCAACAGTCTGTTCATAMAML3_PRIMER2TGGCCCATACCTGTTCATATCACTTCCTTCCGAMAML3_PRIMER3GCCAATGCGTGGAAAACAAGTAAAACCAGGGTMAML3_PRIMER4GTGACTGGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGAACAGTCTTTCCMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER3TGGCCTGGGAGACACAGTGTGCTCTCCGATCTGGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGTGCCTGTAGTCCAGCGTGTGCTCTTCCGATCTGGCCTGAGATCCCAGCTAPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCCTGTGCCTTCCGATCTGGCCTGTAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGGATCTGGGCTGCTTAACCCCCAAARSF1_PRIMER3GGCGCGCTTTAACCCCTAAACCCCTCGGGTRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGGATTACHSPA9_PRIMER3ACCTGGAGTTGAGACTCAACACGGGAGAHSPA9_PRIMER4GTGGGGGAGTTGAAGCACCCTAGGCAA
ABHD2_PRIMER1GGGTGACACAGCAGCACGCCTTCTCAAAATTABHD2_PRIMER2AAATGGACAGAGCCTCTTACTTTGGGGCAABHD2_PRIMER3TGTGAGACACAAATACTGCCCCATTCAABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAGACACAAATACTGCCFHIT_PRIMER1GCCTACTTAATCCTTTTCCTACTTCGTGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER3CCCAGTTGTGTTTTCTCATTTCCCTAGGGCAFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGTGTGTTTTCTCATTTFRVL_PRIMER3CCAGTTGGACTGGAGCTCAGACGTGGCCTCTCCGATCTCCGGTGGTGTGTTTCTCATTTFRVL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTGCGATCGGGGCAACAGTCGTTCATAFRVL_PRIMER3GAGTCACACAGTCTGTTCATATCACTTCCTTTCCGAFRYL_PRIMER4GTGACTGGAGGTGAGACGTGGTGCTCTTCCGATCTGGGTCAACAGTCTGTCATAMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAACAGTCGTTCATAMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER3TGCCTAGATAACCAGCTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAGAGGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTGTAGTCCCAGCTARSF1_PRIMER3GGCTCCTTATCCCCTCCCTCCTCCCTCGCTGTGGCCTGGCTGG
ABHD2_PRIMER2 AAATGGACAGAGCCTCTTACTTTGGGGCA ABHD2_PRIMER3 TGTGAGACACAAATACTGCCCACTTCATTCA ABHD2_PRIMER4 GTGACTGAGACCAAATACTGCCCACTTCATTCA ABHD2_PRIMER1 GCCTACTTAATCCTTTTGCCACTTCGGGGGG FHIT_PRIMER2 GGGATCACAAAAGTGAACTTGAGTGCCGT FHIT_PRIMER3 CCAGTTGTGTTTTCTCATTTCCTTAGAGCCA FHIT_PRIMER4 GTGACTGGAGGTTCAGACGGTGGCTCTTCCGATCTCCGGTTGTGTTTTCTCATTT FRY_PRIMER3 CCAGTTGGTGTTCATCCTCTCTGACTACTGGG FRY_PRIMER4 GGACTCAACAGTCGTTCATACTGCCC FRY_PRIMER2 TGGTCCCATACTGTCTTAGTCCCTTTCCGATCTGAGTCAACAGTCGTGTCATA MAML3_PRIMER2 GGACTCAACAGTCGTGTCATACCCCC FRY_PRIMER3 GAGTCAACAGTCGTGTCAACACCCCCTTTCCGATCTGAGTCAACAGGTCGTCATACAGATGGTTCAGAAACAAAGTAAAACCGAGT MAML3_PRIMER4 GTGACTGGAGTTCAGAACCAGCTGTGGCTCTCCCGATCTGCCTAGATAACCATCTTTC PPIL3_PRIMER3 TGCCTGGAGTTCAGACCAGCCTGGGCAACAT PPIL3_PRIMER1 TRAPPC11_PRIMER1 PPIL3_PRIMER2 CAAGAGGTTTAGACCAGCGTGTGCTCTTCCGATCTGTGGCCTGTAGTCCCAGCTA RSF1_PRIMER3 GGGCTGCTTTAACCCCCCCCCTGAGACACT PPIL3_PRIMER4 GTGACTGGAGTTCAGACCGGTGTGCTCTTCCGATCTGTGGCTGTTAACCCCAGCTA RSF1_PRIMER3 GGGCTGCTTTAACCCCCCCCTGTAGTCCCGGCTGCTTTAACCCCCAGCTA RSF1_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCT
ABHD2_PRIMER3TGTGAGACACAAATACTGCCCACTTCATTCAABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGAGACACAAATACTGCCFHIT_PRIMER1GCCTACTTAATCCTTTCCTACTTGTGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER3CCAGTTGTGTTTCTCATTTCCCTTAGAGCCAFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTGTGTTTTCTCATTTFRVL_PRIMER1GGATTTGTCATTCCCTCTCTGACTACTGGGFRVL_PRIMER2TGGTCCCATACTGTCTAGTCCTATCCCCTATCGAGTCAACAGTCTGTTCATATFRVL_PRIMER3GAGTCAACAGTCTGTTCATACTCCTTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATGCTTTTTCAAGTACACCCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACCGTGTGCTCTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGCCTGGAGAACCAGCTGGGCCTCTCCGATCTGGCCTAGATAACCATCTTTCPPIL3_PRIMER4GTGACTGGAGTTCAGACCGCTGGGCAACATPPIL3_PRIMER3TGTGCCTTAGTCCCAGCCAGCTAGAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACCGTGTGCTCTCCGATCTGTGCCTGTAGTCCCAGCTARSF1_PRIMER3GGGCTGCTTTAACCCCAGCAGCGGGTRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGTTAACCCCTAAARSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCACTCTTCCCATACCGCTTTCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTTCCGATCACCTGGGACTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCACTCTTCCCATACCGGTTTRAPPC11_PRIMER2GTGGGGCATCAGACGTGTACCCCTAGGCAHSPA9_PRIMER1ACCTGACAGGAGTTCAAGACCGACACAAAGCA
ABHD2_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGAGACACAAATACTGCCFHIT_PRIMER1GCCTACTTAATCCTTTTCCTACTTCGTGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER3CCAGTTGTGTTTTCTCATTTCCCTTTGAGCCAFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTCCAGTTGTGTTTTCTCATTTFRYL_PRIMER1GGATTTGTTCATTCCCTCTGTGACTACTGGGFRYL_PRIMER2TGGTCCCATACTGTCTTAGTCCCTATTCCCCFRYL_PRIMER3GAGTCAACAGTCTGTTCATATCACTTCCTTCCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGACTCAGGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER1GCCAATGGAGTTCAGACGTGTGCTCTTCCGACTCAGACACAGTCTGTTCATAMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCCGACCTAGATAACCATCTTTCPPIL3_PRIMER8TGCCTGGAGTTCAGACGTGTGCTCTTCCGACTCTGCCTAGATAACCATCTTTCPPIL3_PRIMER4GTGACTGGAGTTCAGACGCTGGGCAACATPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CACACTCCTTATCTCCCTTCCGATCTTGGCCTGTAGTCCCAGCTARSF1_PRIMER3TGGCCTGTAGTCCCAGCTACCACGGGGRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCCTAAARSF1_PRIMER2GACACTCCACTCTTCCCTTCCCTCCGCGCTGCGCTTAACCCCCTAAARSF1_PRIMER3GGGCTGCTTTAACCCCTAAACCCCTCCTCCGGCTGCTTTAACCCCCTAAARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCCTAAARSF1_PRIMER3GGGCTGCTTTCCGCACCAGGGTGCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTCCGATCTGGGCTGCTTTAACCCCCTAAARAPPC11_PRIMER1CCTGCCGCATCTTCCCCTTCCGATCTGGGGCGCTTTAACCCCCTAAARAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGGCCTTACCCAAAGGACHSPA9_PRIMER2
FHIT_PRIMER1GCCTACTTAATCCTTTCCTACTTCGTGGGGGFHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER2GGACTGGAGTTCAGACGTGGCCCTTCCCGATCCCGTTGTGTTTCTCATTTFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCCGTTGTGTTTCTCATTTFRYL_PRIMER1GGATTTGTCATTCCCTCTTGACACAGGGFRYL_PRIMER2TGGCCCATACTGTCTTAGTCCCTTTTCCAFRYL_PRIMER3GAGTCAACAGTCTGTTCATACCCTCTTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTCCAGGAGTCAGCAGGTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCAGGTMAML3_PRIMER3TGCCTAGATACCATCTTTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGCCTAGATAACCATCTTTCCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACCTGGGCTACTCAGAGGGPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAGAGGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER3GGGCTGCTTTACCCCTCAAACTCCTTCCRSF1_PRIMER4GTGACTGAAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTCTTCCGATCTGGGATTTGGAGATTACHSPA9_PRIMER4GTGACTGACGACTCAACACTGTGCCTTACCCGAGCAHSPA9_PRIMER3ACCTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGAGTTTGAGAGATTACHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTACCCAAAGCAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTACCCAAAGCAHSPA9_PRIMER4GTGGGGAGTTCAAGACGTGTGC
FHIT_PRIMER2GGGATCACAAAAGTGAAGATTGGATGCCGTFHIT_PRIMER3CCAGTTGTGTTTTCTCATTTCCCTTAGAGCCAFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTCCAGTTGTGTTTTCTCATTTFRYL_PRIMER1GGATTTTGTCATTCCCCTCTTGACTACTGGGFRYL_PRIMER2TGGTCCCATACTGGCTATCCCCCFRYL_PRIMER3GAGTCAACAGTCTGTTCATATCACTTCCTTTCTCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTCAAGTACACCCACCTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGCCTGGAGTTCAGACGGTGTGCTCTCCGATCTGCCTAGATAACCATCTTCPPIL3_PRIMER4GTGACTGGAGTTCAGACCAGCTGGGCAACATPPIL3_PRIMER5TGTGCCTGTAGTCCCAGCTGGGCCACCATPPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCCACCATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTAGCAGGGTGGCTCTTCCGATCTGGGCCGCTGAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGGCCTGTAGTCCCAGCTARSF1_PRIMER3GGGCTGCTTACCCCTCACCTGGGGCTGCTTTACCCCCTAAARSF1_PRIMER3GGGCTGCTTAACCCCTCTCCCAAACCCTGGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGGGCTGCTTTAGGAGATTACHSPA9_PRIMER1ACCTGGACATCAGAGGAGCGTGACACAAGCAHSPA9_PRIMER2GTGGGGACTGAAACCAAGCAGAGCAHSPA9_PRIMER3GTGGGGGCTGTAAGCCACACACTGHSPA9_PRIMER4GTGACTGGAAGTTCAGACCGTGGCCTTCCCGATCACCTGGAGATTACAHSPA9_PRIMER4GTGGGGGCTGTAAGCACCACACTGHSPA9_PRIMER4GTGGGGGCTGAAGCGTGTAACCCACACTGHSPA9_PRIMER4<
FHIT_PRIMER3CCAGTTGTGTTTTCTCATTTCCCTTAGAGCCAFHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGTGTTTTCTCATTTFRYL_PRIMER1GGATTTTGTTCATTCCCTCTTGACTACTGGGFRYL_PRIMER2TGGTCCCATACTGTCTTAGTCCCTATTCCCCFRYL_PRIMER2GAGTCAACAGTCTGTTCATACACTTCCTTTCCAFRYL_PRIMER3GAGTCAACAGTCTGTTCAAGTACACTCCTTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCATTGCTGTGAAAACAGAGTAAAACCGAGTMAML3_PRIMER2ACCCTGGTGGAAAACACAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGTGCCTGTAGTCCAGCCGGGCAACATPPIL3_PRIMER4GTGACTGGAGTTCAGACGGTGTGCTCTTCCGATCTGGCCTGTAGTCCCAGCTARSF1_PRIMER3TGTGCCTGTAGTCCCAGCCAGGGGCAACATPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGCCTGTAGTCCCAGCTARSF1_PRIMER3TGTGCCTGTAGTCCCAGCAGGTGGCCTTTCCGATCTGGCCTGTAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER3GGGCTGCTTTACCCCTCCCTCGGGTRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGATCTAACCGCTAAATRAPPC11_PRIMER3ACCTGGAATTCATGGAGTTCAGACGTGTGCTGTTRAPPC11_PRIMER4GTGACTGGAAGTTCAGACGTGTGCCTTCCGATCACCTGGAGTTTTGGAGATTACHSPA9_PRIMER2GTGGGCACTGAAGCCTTAAGCAACAAGCAHSPA9_PRIMER2GTGGGGCTGTAAGCCAGAGCTGTAGCCAACATTGHSPA9_PRIMER4GTGACTGGAAGTTCAAGCACCTTAGGCAAHSPA9_PRIMER2GTGGGGACTGAAGCTTAAGCAACCAAGCAHSPA9_PRIMER3GTGGGGAGTTCAGACGTGTAA
FHIT_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGTGTTTTCTCATTTFRYL_PRIMER1GGATTTTGTCATTCCCTCTTGACTACTGGGFRYL_PRIMER2TGGTCCCATACTGTCTTAGTCCCTATTCCCCFRYL_PRIMER2GGAGTCAACAGTCTGTTCATATCACTTCCTTTCTCAFRYL_PRIMER3GAGTCAACAGTCTGTTCATACCACTCCTTTCTCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTTCAAGTAACACCACCTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAGAGGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCCTGTAGTCCCAGCTARSF1_PRIMER3TGTGCCTGTAGTCCCCTCCTTCAGGGTRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACAGGGTGTGCTCTTCCGATCTACCTGGATTTAGGAGTTACHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTAGGAGATTACHSPA9_PRIMER1ACCTGGACTGAGAGTCAAAGCAHSPA9_PRIMER2GTGGGCAGCTGTAATCCCAACACAGGCAHSPA9_PRIMER4GTGGGCTCATGCCTGTAATCCCAACACAGGAHSPA9_PRIMER2GTGTGGGAGTTGAAGCCTTAAGCAACAAAGCAHSPA9_PRIMER4GTGGGCAGTGGAGTTGAAGCACCTAGGCAAHSPA9_PRIMER4GTGGGGCAGTGAGCTTAACCCACAAGGCAHSPA9_PRIMER4GTGGGGCAGTGAGCTCTAGCCCTAGGCAAHSPA9_PRIMER4GTGGGGAGTGAGCT
FRYL_PRIMER1GGATTTTGTTCATTCCCTCTTTGACTACTGGGFRYL_PRIMER2TGGTCCCATACTGTCTTAGTCCCTATTCCCCFRYL_PRIMER3GAGTCAACAGTCTGTTCATATCACTTCCTTTCTCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCCCCCACCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGCCTAGATAACCATCTTTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTCAGACCAGCTGGGCAACATPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER2GACACTCCCTTACCCCTCCCTCGTGTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCATCTCTCCTTCCCATACCGGACTACCGGAGTTACAGAGATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER1ACCTGGACTGAGAGTCTAAGCAGAGAGACAHSPA9_PRIMER2GTGGGTGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGGTGGGAGTTGAAGACTCACCTAGGCAAHSPA9_PRIMER4GTGGGTGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER4GTGGGTGGATTGAAGATCACCCTAGGCAAHSPA9_PRIMER2GTGGGTGGATTGAAGAC
FRYL_PRIMER2TGGTCCCATACTGTCTTAGTCCCATTCCCCFRYL_PRIMER3GAGTCAACAGTCTGTTCATATCACTTCCTTTCCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTAGATAACCATCTTTCPPIL3_PRIMER3TGCCTAGATAACCAGCTGGGCAACATPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTGTGTCCTCCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER2GACACTCCACTCTCCCTCCTCTGTGGRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CCTCCCCGCATCTCTCCTTCCCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CCTGCATCGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER1ACCTGGACTTGAGACGTGGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACATTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER4GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTG
FRYL_PRIMER3GAGTCAACAGTCTGTTCATATCACTTCCTTTCTCAFRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER3TGTGCCTGTAGTCCCAGCTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAARSF1_PRIMER2GACACTCACACTCTCCCTCCTCTTGTGGRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTCCCGCATCTTCCCATACCGCTTTCRAPPC11_PRIMER3ACCTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGATCTAGCAGAGATTACHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGATTTGGAGATTACHSPA9_PRIMER3GTGGGGCATGCCTGTAAGCCCTAGACAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACCTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGAGTTGAAGATCACHSPA9_PRIMER4GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGGCCATGCCTGTAATCCCAACACCAAGCAHSPA9_PRIMER3GTGGGGAGTTGAAGATCACCCTAGGCAA<
FRYL_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCAACAGTCTGTTCATAMAML3_PRIMER1GCCAATTGCTTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAGGGGRSF1_PRIMER4GGGCTGCTTTAACCCCTCACTGTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGGGGCATGCAGGTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGGGGCAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGGGGCAGTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGGGGGCAGTGAAGATCACCCTAGGCAA </td
MAML3_PRIMER1GCCAATTGCTTTTCAAGTACACCCACTTTTACTMAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER4GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCCTGTTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTGTTRAPPC11_PRIMER3ACCTGGATTTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGGAGTTGAAGATCACHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4<
MAML3_PRIMER2ACCCTGGTGTGAAAACAAAGTAAAACCGAGTMAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCTTGCTGAAGATRAPPC11_PRIMER2CTCCCCGCATCTTCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTAGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER1ACCTGGCAGTGTAATCCCAACCATGHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGTGGTGAGAGTCAACC
MAML3_PRIMER3TGCCTAGATAACCATCTTTCTCCCCACCCMAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCCTCGTTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER2GTGGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGGAGTTGAAGATCAC
MAML3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTAGATAACCATCTTTCPPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCACCTCCTCAAAACTCCTTCCRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTAAGCAGCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCACHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
PPIL3_PRIMER1TRAPPC11_PRIMER1PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCACCCTAAAACTCCTTCCRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCCATCACCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGAGTTTAGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCACHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCACC
PPIL3_PRIMER2CAAGAGTTTGAAACCAGCCTGGGCAACATPPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCAAAACTCCTTCCRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTGGAGATTACHSPA9_PRIMER1ACCTGCACAAGAGTCTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
PPIL3_PRIMER3TGTGCCTGTAGTCCCAGCTACTCAAGAGGPPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCCTCTGTTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCTGCTGAAGATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTAGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGAGTTTTGGAGATTACHSPA9_PRIMER1ACCTGCACAGAGTCTAAGCAGTGTGCTCTTCCGATCTACCTGGAGTTTAGAGATTACHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
PPIL3_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCCTGTAGTCCCAGCTARSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCCTTGTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCTGCTGAAGATRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTAGAGAGTTCAGACGTGTGCTCTTCCGATCTAGCAGATTACHSPA9_PRIMER1ACCTGCACGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER2GTGGGGCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
RSF1_PRIMER1GACACCTCCTTATCTCCCTTCACCTGGGTRSF1_PRIMER2GACACTCAACTCTCCCTCCTTGTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCAATACCGCTTTCTRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGGGCTGCTGTTRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
RSF1_PRIMER2GACACTCAACTCTCCCTCCTTGTTGGRSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCAATACCGCTTTCTRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
RSF1_PRIMER3GGGCTGCTTTAACCCCTAAAACTCCTTCCRSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCAATACCGCTTTCTRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
RSF1_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTGCTTTAACCCCTAAATRAPPC11_PRIMER1CCTGCATCACCTCTTCCAATACCGCTTTCTRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
TRAPPC11_PRIMER1CCTGCATCACCTCTTCCAATACCGCTTTCTRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
TRAPPC11_PRIMER2CTCCCCGCATCTCTTCCTTGCTGAAGATRAPPC11_PRIMER3ACCTGGATTTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
TRAPPC11_PRIMER3ACCTGGATTTGGAGATTACATGGTGCTGTTRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
TRAPPC11_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGATTTTGGAGATTACHSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
HSPA9_PRIMER1ACCTGACAAGAGTCTTAAGCAACCAAAGCAHSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
HSPA9_PRIMER2GTGGGTCATGCCTGTAATCCCAACACTTGHSPA9_PRIMER3GTGTGGGAGTTGAAGATCACCCTAGGCAAHSPA9_PRIMER4GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGGAGTTGAAGATCAC
HSPA9_PRIMER3 GTGTGGGAGTTGAAGATCACCCTAGGCAA HSPA9_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGAGTTGAAGATCAC
HSPA9_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGAGTTGAAGATCAC
MEF2_PRIMER1 TGTCTCAGTCACATTTCTCCAGGTTTCCGT
MEF2_PRIMER2 TGAGATATCGATGTCATTTTCAATGCAGAGGCA
MEF2_PRIMER3 ACTGCAGTTCACTATTGGCATAACAAGTAACCA
MEF2_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGCAGTTCACTATTGGCA
JAK2_EXON12_PRIMER1 ACCAGATGGAAACTGTTCGCTCAGACAAT
JAK2_EXON12_PRIMER2 TGTCCCCCAAAGCCAAAAGATAAATCAAACCT
JAK2_EXON12_PRIMER3 ACCAACCTCACCAACATTACAGAGGCCTAC
JAK2_EXON12_PRIMER4 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAACCTCACCAACATTAC

(Continued on next page)





Table 1. Continued	
INTERNAL_FORWARD	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC
LMO7_PRIMER2	ACTGAAGTACAGTCTTATCATATGAGCAGAATGACG
LMO7_PRIMER3	TCCATTTGTTTAAGACTGTTAAACACAAGCACATTGC
LMO7_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCATTTGTTTAAGACTGTT
CES5A_PRIMER1	GGGTTAGGCATTGTAGTGGAGATAGGCATGGAA
CES5A_PRIMER2	AGGCTGGAGTGCAGTGGCATGATCTT
CES5A_PRIMER3	GGGCCTGTGCCACTACACTCAGCTAATTT
CES5A_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCCTGTGCCACTACACTC
CDC42SE2_PRIMER1	TGAGGATATGTGCAAGTGATGGTGCTGGAGTT
CDC42SE2_PRIMER2	GATGGTGCTGGAGTTGCCACAGTGAA
CDC42SE2_PRIMER3	TCAAGGGAAGTGGTTGAGGAAACGGAGT
CDC42SE2_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAGGGAAGTGGTTGAGGA
NRROS_PRIMER1	GAATCCATCTGTCTCCTTTCCTCAGCTTTGCCT
NRROS_PRIMER2	AGTCCCGGAGCTGGTGGCAAAGA
NRROS_PRIMER3	TCTCACGGGCCCAGCCTTACTCA
NRROS_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCACGGGCCCAGCCTTAC
NSFP1_PRIMER1	GGCGTTCATTTTGTGACAGTTCA
NSFP1_PRIMER2	AGCATTCACTGAGAAAAACAATAATGA
NSFP1_PRIMER3	TGGGGAAGATGGTAGGGAGTTTG
NSFP1_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGGAAGATGGTAGGGAGT
PIWIL2_PRIMER1	GGAGTTTCACTCTTGTTGCCCAGGCT
PIWIL2_PRIMER2	GTGCAATCTCAGCTCACCGCAACCT
PIWIL2_PRIMER3	TCTCCTGCCTCAGCCTCCCAAGT
PIWIL2_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCCTGCCTCAGCCTCCCA
ZNF22_PRIMER1	AACCTACAATTTACACACCTCCCTGCCTTCA
ZNF22_PRIMER2	GCCAAGTGTCAGACTCTAATGAGCCCTCA
ZNF22_PRIMER3	GCTCAGGTTTTAATTTCTATTGAATGCTA
ZNF22_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCAGGTTTTAATTTCTAT
UPF1_PRIMER1	TTCCCATTGCTCTAGGGCTTTCGGTTTCC
UPF1_PRIMER2	GGGTAGGTTTCCGCGGTGACCCC
UPF1_PRIMER3	TCTGCTTCGCCCTGTGCTGTGTTCTC
UPF1_PRIMER4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGCTTCGCCCTGTGCTGT

Note: Cryopreservation of BMA is not recommended for the protocol because the impact of cryopreservation on transcription profile of BMA is not known.

Preparation before experiment

 \odot Timing: \sim 1 h

- 6. See key resources table for preparation of needed materials.
- 7. Set centrifuge to room temperature ($20^{\circ}C-22^{\circ}C$).
- 8. Prepare 2% FBS/PBS and acclimate to room temperature (20°C–22°C).

Note: 2% FBS/PBS should be stored at 4°C for up to 6 months before and after usage.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
EasySep™ Human CD34 Positive Selection Kit II	STEMCELL Technologies	Cat# 17856

(Continued on next page)

Protocol



Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Whole bone marrow samples	Massachusetts General Hospital; Dana-Farber Cancer Institute	N/A
Chemicals, peptides, and recombinant proteins		
Q5 High-Fidelity 2X Master Mix	New England Biolab	M0492S
UltraPure Distilled Water	Invitrogen	10977
1X RBC Lysis Buffer	eBioscience	Cat# 00-4333-57
Fetal Bovine Serum	VWR	Cat# 89510-186
SPRIselect	Beckman Coulter	Cat# B23318
Lymphoprep	STEMCELL Technologies	Cat# 07801
EasySep Buffer	STEMCELL Technologies	Cat# 20144
1× Dulbecco's phosphate-buffered saline	Thermo Fisher Scientific	Cat# 14040133
eBioscience 1X RBC Lysis Buffer	Thermo Fisher Scientific	Cat# 00-4333-57
Critical commercial assays		
Chromium Single Cell 3′ GEM, Library & Gell Bead Kit v3, 16 rxns	10× Genomics	Cat# 1000075
Chromium Chip B Single Cell Kit, 48 rxns	10× Genomics	Cat# 120262
High Sensitivity D5000 ScreenTape	Agilent	Cat# 5067-5592
High Sensitivity D5000 Reagents	Agilent	Cat# 5067-5593
Qubit dsDNA HS Assay Kit	Invitrogen	Cat # 32854
Chromium i7 Multiplex Kit, 96 rxns	10× Genomics	Cat# 120262
Oligonucleotides		
Primers	Hormoz Lab	Table 1
Software and algorithms		
Amplicon analysis script	Hormoz Lab	N/A
Other		
SepMate™-15/50 tubes	STEMCELL Technologies	Cat# 85450
Plastic whole blood tube with spray-coated K2EDTA	Becton, Dickinson and Company	Cat# 366643
Alternatives		
Any DNA ScreenTapes and Reagents (for step 38)	Agilent	N/A
Any Bioanalyzer DNA Chips and Reagents (for step 38)	Agilent	Cat# 366643
Red Blood Cell Lysis Buffer	Sigma-Aldrich	Cat# 11814389001

STEP-BY-STEP METHOD DETAILS

Density gradient centrifugation

() Timing: 60–100 min

This section describes how to isolate mononuclear cells from BMA. Lymphoprep/Ficoll with a density of 1.077g/mL is used in this protocol (Figure 2).

- 1. Add 22 mL Lymphoprep/Ficoll to the 50 mL SepMate Tube by carefully pipetting it through the central hole of the SepMate insert without creating bubbles.
- 2. Dilute BMA with an equal volume of 2% FBS/DPBS and mix gently with wide-bore pipette.

Note: The maximum volume of diluted BMA is 8 mL for SepMate-15 and 34 mL for SepMate-50.

3. Overlay diluted BMA to Lymphoprep/Ficoll in the SepMate tube by slowly pipetting it down the side of the tube slightly above liquid level.

△ CRITICAL: Mixing diluted BMA and density gradient may result in incomplete separation or loss of cells and recovery will decrease.







Figure 2. Lymophoprep/Ficoll separation demonstration and expected layering after centrifugation (A) Illustration of layering of Lymophoprep/Ficoll and diluted bone marrow before and after centrifugation in a SepMate tube.

(B) Picture of separated layers of bone marrow after centrifugation.

- 4. Centrifuge tube at 1200 × g for 20 min at room temperature ($20^{\circ}C-22^{\circ}C$), with brake off.
 - △ CRITICAL: It is important that the brake is off otherwise the buffy layer of mono-nuclear cells (MNCs) will be disrupted and recovery will decrease.
 - ▲ CRITICAL: Polycythemia vera samples with high red blood cell (RBC) counts may require additional RBC lysis. The enriched cell layer (layer above the SepMate barrier) should be poured off into a new tube before lysing the RBCs using eBioscience 1X RBC Lysis Buffer. The manufacturer's protocol can be found here: https://www.thermofisher.com/ document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher. com%2FTFS-Assets%2FLSG%2Fmanuals%2F00-4333.pdf&title=VGVjaG5pY2FsIERhdGEgU 2hIZXQ6IDFYIFJCQyBMeXNpcyBCdWZmZXI=
- Remove the top layer of plasma/platelet and move the buffer layer containing MNCs to a new 50 mL tube.
- 6. Top up the MNCs to 45 mL with 2% FBS/PBS and mix well with a wide-bore pipette.
- 7. Centrifuge MNCs at 300 × g for 12 min at room temperature ($20^{\circ}C-22^{\circ}C$), with low brake.
- 8. Remove and discard supernatant.
- 9. Top up the MNCs again until 45 mL with 2% FBS/PBS and mix well with a wide-bore pipette.
- 10. Centrifuge MNCs at 120 × g for 12 min at room temperature ($20^{\circ}C-22^{\circ}C$), with no brake

▲ CRITICAL: If excess platelets remain (common when samples are from patients with essential thrombocythemia), repeat steps 9 and 10 once. Excess platelets can be identified by visual inspection of the supernatant after step 10. If the supernatant appears cloudy, we identify the sample as having excess platelets.

- 11. Discard supernatant and resuspend MNC in 1 mL of EasySep buffer on ice, mix with a wide-bore pipette.
- 12. Measure cell concentration with a hemocytometer and an automatic cell counter.

Magnetic-beads cell enrichment

© Timing: 45 min

The subsequent steps are performed to obtain a single-cell suspension of CD34+ cells.

- 13. Add MNCs (at concentration of $>10^8$ cells/mL) to 5 mL polystyrene round-bottom tube.
- 14. Add 100 μL EasySep Human CD34 Positive Selection Cocktail to the sample.

Protocol



- \triangle CRITICAL: If sample volume is greater than 1 mL, add the selection cocktail at a ratio of 100 μ L per 1 mL of sample.
- 15. Incubate the sample at room temperature (20°C–22°C) for 10 min.
- 16. Vortex EasySep Dextran RapidSpheres for 30 s immediately before use.
- 17. Add 75 μ L of EasySep Dextran RapidSpheres to the sample (Van Egeren et al., 2021).

 \triangle CRITICAL: If sample volume is greater than 1 mL, add the RapidSpheres at a ratio of 75 μ L per 1mL of sample.

- 18. Mix the sample and incubate at room temperature (20°C–22°C) for 5 min.
- 19. Top up the sample to 2.5 mL with EasySep Buffer.
- 20. Place the tube containing the sample in an EasySep magnet and incubate at room temperature (20°C-22°C) for 3 min.
- 21. Discard supernatant by inverting the magnet with the tube inside.
- 22. Rinse the tube with EasySep buffer.

Note: Rinse the side of the tube to achieve maximum recovery.

- 23. Repeat steps 19 to 22 four more times.
- 24. Resuspend MNC in 1 mL of EasySep buffer on ice, mix with a wide-bore pipette.
- 25. Measure cell concentration and cell viability with a hemocytometer and an automatic cell counter. The target final concentration is 700–1,200 cells/μL. The typical percent CD34+ cells viability obtained by following this procedure ranges from 85%–95% based on trypan blue staining.

Note: Measuring cell concentration with both a manual hemocytomter and an automatic cell counter is recommended to minimize the counting error.

26. Keep isolated cells on ice and proceed immediately to the 10× Genomic Chromium Single Cell protocol.

Chromium single cell 3' GEM cDNA and library construction

© Timing: 45 min

 Generate the full transcriptomic libraries according to 10× Genomic Chromium Single Cell v3 manufacturer's protocol. The manufacturer's protocol can be found at https://support. 10xgenomics.com/single-cell-gene-expression/library-prep/doc/user-guide-chromium-singlecell-3-reagent-kits-user-guide-v3-chemistry.

II Pause point: The cDNA can be stored in a freezer at -20° C for 1–2 months until the following procedures.

Locus-specific single-cell amplicon libraries

© Timing: 4–8 h

The five-step locus-specific PCR amplification first amplifies the somatic mutation of interest (PCR 1–3) in a nested fashion. Illumina sequencing adaptors are subsequently added to the amplified products by PCR (PCR 4–5). Libraries are then quantified on an Agilent Tapestation or Bioanalyzer. (Tapestation traces can be found in Figure 3)





Figure 3. Step-by-step tapestation traces for locus-specific amplicon library construction (A–E) Step by step tapestation traces from locus-specific PCR 1 to SI PCR. (F) Collapsed view of tapestation of PCR steps 1–5.

28. Nested locus specific PCR 1.

a. Prepare master mix in a 0.2 mL thin-wall PCR tube.

Reagent	Final concentration	Amount
Internal_forward (10 μM)	0.5 μΜ	1.25 μL
Locus-Specific Primer 1 (10 μM)	0.5 μΜ	1.25 μL
Single-cell cDNA	0.08 ng/µL	2 ng
2× Q5 master mix	1x	12.5 μL
ddH ₂ O	n/a	Variable
Total	n/a	25 μL

Note: Internal_forward (5'-AATGATACGGCGACCACCGAGATCTACAC-TCTTTCCCTA CACGACGCTC) completes Illumina Read 1 and adds partial Illumina P5 sequence to the 5' end of the cDNA library.

- b. Mix by pipetting 10 times or gently vortexing, centrifuge briefly.
- c. Insert tubes into a thermocycler and incubator with the following protocol.

Locus-specific PCR 1				
Steps	Temperature	Time	Cycles	
Initial Denaturation	98°C	45 s	1	
Denaturation	98°C	20 s	10 cycles	
Annealing	67°C	30 s		
Extension	72°C	180 s		
Final extension	72°C	60 s	1	
Hold	4°C	hold		



- 29. Remove primer-dimers using SPRIselect beads.
 - ▲ CRITICAL: SPRIselect beads should be acclimated to room temperature (20°C-22°C) before continuing.
 - a. Add 25 μL nuclease-free water to each sample.
 - b. Vortex to resuspend the SPRIselect reagent. Add 40 µL SPRIselect reagent (0.8×) to each sample and pipette mix (15×) or gently vortex mix.
 - c. Incubate 5 min at room temperature (20°C–22°C).
 - d. Place on the 10× magnetic separator (high orientation) until the solution clears.

Note: The high orientation is used when the tube contains more than 50 μ L of liquid and the low orientation is used when the tube contains less than 50 μ L of liquid for easy liquid handling.

- e. Remove the supernatant.
- f. Add 200 μL 80% ethanol to the pellet. Wait 30 s.

△ CRITICAL: 80% ethanol should be prepared fresh for best wash performance and yields.

- g. Remove the ethanol.
- h. Repeat steps f and g for a total of 2 washes.
- i. Centrifuge briefly and place on the magnet (low orientation).
- j. Remove any remaining ethanol and air dry for 2 min to avoid ethanol carryover.
- k. Add 26 μL nuclease-free water. Pipette mix (15×) or gently vortex mix.
- I. Incubate 2 min at room temperature (20°C–22°C).
- m. Place the tube strip on the magnet (low orientation) until the solution clears.
- n. Transfer 25 μ L sample to a new tube strip.
- o. Store at 4°C for up to 72 h or at -20°C for up to 4 weeks, or proceed to the next step immediately.

II Pause point: The PCR product can be stored in a freezer at -20° C for 1–2 months or in a fridge at 4°C for 24 h until the following procedures.

30. Nested locus specific PCR 2.

- a. Prepare master mix in a 0.2 mL thin-wall PCR tube.
- b. Mix by pipetting 10 times or gently vortexing, centrifuge briefly.
- c. Insert tubes into a thermocycler and incubator with the following protocol.

Reagent	Final concentration	Amount
Short_Int_For (10 μM)	0.5 μΜ	1.25 μL
Locus-Specific Primer 2 (10 µM)	0.5 μΜ	1.25 μL
PCR product from PCR 1	n/a	5 μL
2× Q5 master mix	1x	12.5 μL
ddH ₂ O	n/a	5 μL
Total	n/a	25 μL

Note: Short_Int_For consists of partial Illumina P5 (5'-AATGATACGGCGACCACCGAGATCT).

Locus-specific PCR 2				
Steps	Temperature	Time	Cycles	
Initial Denaturation	98°C	45 s	1	
Denaturation	98°C	20 s	10 cycles	
Annealing	67°C	30 s		
Extension	72°C	180 s		
Final extension	72°C	60 s	1	
Hold	4°C	hold		





31. Repeat step 30 to remove primer-dimers.

II Pause point: The PCR product can be stored in a freezer at -20° C for 1–2 months or in a fridge at 4°C for 24 h until the following procedures.

- 32. Nested locus specific PCR 3.
 - a. Prepare master mix in a 0.2 mL thin-wall PCR tube.
 - b. Mix by pipetting 10 times or gently vortexing, centrifuge briefly.
 - c. Insert tubes into a thermocycler and incubator with the following protocol.

Reagent	Final concentration	Amount
Short_Int_For (10 μM)	0.5 μΜ	1.25 μL
Locus-Specific Primer 3 (10 µM)	0.5 μΜ	1.25 μL
PCR product from PCR 2	n/a	5 μL
2× Q5 master mix	1×	12.5 μL
ddH ₂ O	n/a	5 μL
Total	n/a	25 μL

Locus-specific PCR 3				
Steps	Temperature	Time	Cycles	
Initial Denaturation	98°C	45 s	1	
Denaturation	98°C	20 s	10 cycles	
Annealing	67°C	30 s		
Extension	72°C	180 s		
Final extension	72°C	60 s	1	
Hold	4°C	hold		

33. Repeat step 30 to remove primer-dimers.

II Pause point: The PCR product can be stored in a freezer at -20° C for 1–2 months or in a fridge at 4°C for 24 h until the following procedures.

- 34. Nested locus specific PCR 4.
 - a. Prepare master mix in a 0.2 mL thin-wall PCR tube.
 - b. Mix by pipetting 10 times or gently vortexing, centrifuge briefly.
 - c. Insert tubes into a thermocycler and incubator with the following protocol.

Reagent	Final concentration	Amount
Short_Int_For (10 μM)	0.5 μΜ	1.25 μL
Locus-Specific Primer 4 (10 µM)	0.5 μΜ	1.25 μL
PCR product from PCR 3	n/a	5 μL
2× Q5 master mix	1×	12.5 μL
ddH ₂ O	n/a	5 μL
Total	n/a	25 μL

Note: Locus-Specific Primer 4 contains Illumina Read 2 (GTGACTGGAGTTCAG ACGTGTGCTCTTCCGATCT) and 18–22 nucleotides from the 5' end of locus-specific primer 3.

Protocol



Locus-specific PCR 4				
Steps	Temperature	Time	Cycles	
Initial Denaturation	98°C	45 s	1	
Denaturation	98°C	20 s	10 cycles	
Annealing	67°C	30 s		
Extension	72°C	180 s		
Final extension	72°C	60 s	1	
Hold	4°C	hold		

- 35. Repeat step 30 to remove primer-dimers.
- 36. Sample Index PCR.
 - a. Prepare master mix in a 0.2 mL thin-wall PCR tube.
 - b. Mix by pipetting 10 times or gently vortexing, centrifuge briefly.
 - c. Insert tubes into a thermocycler and incubator with the following protocol.

Reagent	Final concentration	Amount
SI Primer	n/a	0.5 μL
Chromium i7 Sample Index	n/a	2.5 μL
PCR product from PCR 4	n/a	2 μL
10× Amp Mix	1×	12.5 μL
ddH ₂ O	n/a	7.5 μL
Total	n/a	25 μL

Note: SI Primer, Chromium i7 Sample Index, and 10× Amp Mix are included in the 10× Chromium 3' kit. Record the i7 Sample Index for each library for downstream analysis.

Sample index PCR				
Steps	Temperature	Time	Cycles	
Initial Denaturation	98°C	45 s	1	
Denaturation	98°C	20 s	10 cycles	
Annealing	54°C	30 s		
Extension	72°C	180 s		
Final extension	72°C	60 s	1	
Hold	4°C	hold		

- 37. Repeat step 30 to remove primer-dimers.
- 38. Quantify the locus-specific library using Tapestation.

II Pause point: The PCR product can be stored in a freezer at -20° C for 1–2 months or in a fridge at 4°C for 24 h until the following procedures.

Note: High Sensitivity D5000 ScreenTapes and reagents are recommended dur to the library size. However, other Agilent ScreenTapes or Agilent Bioanalyzer reagents can be used for quantifying the libraries. If a TapeStation or Bioanalyzer is not available, quantification can be done by running a 2%–3% agarose gel with appropriate ladder. Quantification with NanoDrop and Qubit is not sufficient due to the lack of the fragment size information.

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Table 2. Expected outcomes for each step		
Outcome	Result	
Post Ficoll recovery	MNC with low RBC and platelet contamination	
Post CD34+ magnetic beads enrichment	0.2%–0.8% of the post-Ficoll cell count, 85%–95% viability on the post-column hemocytometer count	
10× Genomics Chromium 3' library construction	Single-cell cDNA, and single-cell transcriptome library	
Locus-specific nested PCR	Specific somatic mutation enriched cDNA library	
Bioinformatic processing	csv file for wildtype/mutation calling visualization on UMAP	

- 39. Sequence the locus-specific library using the following cycles:
 - a. Read 1 28 cycles
 - b. 17 Index 8 cycles
 - c. 15 index 0 cycles
 - d. Read 2 91 cycles

Note: The locus-specific library can be pooled with the transcriptome library for sequencing or be sequenced alone. We sequence the pooled transcriptome and locus-specific libraries on a NovaSeq SP Flowcell (800 million reads) and the locus specific libraries are usually allocated with 2% of the total reads (16 million reads). The libraries can also be sequenced on MiSeq Flowcells for testing and troubleshooting. Please take into account the multiple peaks in fragment size distribution when calculating library concentration.

EXPECTED OUTCOMES

See Table 2 for step-by-step expected outcomes.

QUANTIFICATION AND STATISTICAL ANALYSIS

© Timing: Variable, depending on processing power

This section describes the usage and logic of the bioinformatic script for analysis of the single-cell amplicon library sequences. The script maps the somatic mutation of interest to 10× single-cell barcodes using MATLAB. The bioinformatic analysis scripts are available at https://gitlab.com/hormozlab/scamplicon-library-analysis.

- 1. The computational pipeline consists of 1. the bioinformatic analysis MATLAB script and 2. two fastq files (read 1 and read 2) of locus specific amplicon libraries
- 2. Move the MATLAB script to the folder containing fastq files of locus specific amplicon libraries.
- 3. Change 'GeneName' and 'filename' to match R1, and R2 fastq files of the library.
- 4. Change 'corrGene2' to th sequence 91 bp downstream of the locus-specific primer 3 including the primer sequence.
- Change 'posMut' (denotes the position of the mutation from primer 3) and 'baseMut' (denotes the nucleotide of the mutation) to appropriate values. For JAK2V617F, posMut = 61 and base-Mut = 'T'.
- 6. Optional: Change 'barcode_inputfile' to match with barcode tsv file from 10× Chromium computational pipeline.
- 7. Run the MATLAB script. We recommend running the script on a server with at least 100 GB of RAM for 12 h. The script will do the following:
 - Parse Read 1 (containing single-cell barcodes and unique molecular identifier, UMI), and Read
 2 (containing mRNA information) along with their QC score and index into arrays.
 - b. Filter out reads with quality control (QC) score lower than 30 and calculate fraction of reads that passed QC threshold.

Protocol





Rank: 1

Figure 4. Accurate identification of the mutated cells from the amplicon libraries

(A–C) (A) In a control experiment MOLT4 (WT cells) were mixed with UKE-1 cells (homozygous JAK2-V617F mutation) and ran through the experimental and analysis pipeline. The two cell populations could be distinguished based on their transcriptional profiles: two distinct clusters were seen when transcriptomes of the cells were visualized using UMAP. Marker genes (TCF7 shown here) were used to identify the clusters as either MOLT4 or UKE-1 cells. Cells in which a mutated JAK2 transcript (B) or a WT JAK2 transcript (C) were detected in the amplicon libraries are shown as colored points. All other cells are shown in gray.

(D) All cells in which either a WT or mutated JAK2 transcript was detected in the amplicon libraries. JAK2 transcript were detected in ~ 4% of cells (249 out of 6563 cells). The rate of erroneously detecting a mutated transcript in a MOLT4 cell or a wildtype transcript in a UKE-1 cell in less than 1%. (E–G) Output plots from MATLAB analysis script. (E) Rank of unique indices. Index sequence can be found in MATLAB cell array 'uniqueindices'. (F) Number of reads vs rank of unique molecules and the threshold for calling the detected molecules as either wildtype or mutated. (G) Number of reads vs rank of unique cells. (H) Example of top 200 most common Read 2 and its align results. Related to Figure 1

Note: The expected output at this step are the sequencing reads with QC scores higher or equal to 30.

- c. Count and plot the most frequent unique indices (Figure 4E).
- d. Extract 11 bp around the mutated nucleotide with wildtype and mutated nucleotide substitutions for use in step m.
- e. Extract 10× cell barcodes from Read 1 and either use: Option 1, raw barcode from the sequencing files or Option 2, Collapse the extracted raw barcodes to the list of barcodes generated by the 10× Chromium computational pipeline. If Option 2 is used, the script will do the following:
 - i. Read and extract barcodes from 10× barcode tsv file.





- ii. Compare raw barcode to 10× barcode and compute distance between the two strings.
- iii. Collapse the raw barcode to the 10× barcode if the distance is equal to or smaller than 2 for barcode correction.
- f. Calculate the occurrences of each cell by counting the single-cell barcodes.
- g. Calculate the occurrences of each unique molecule by counting the unique-molecular-identifier (UMI).
- h. Plot the ranked ordered number of reads detected for each unique molecular identifier (Figure 4F), and the ranked ordered number of reads detected in each cell (Figure 4G).

Note: In a successfully amplified library with 5–20 million reads generated from \sim 10,000 cells, the number of Jak2 molecules with more than 1,000 reads should range from 200–500.

- i. Calculate the occurrences of each unique Read 2 sequence and plot the occurrences of each unique Read 2 versus the corresponding number of reads.
- j. Align top 200 most common Read 2 sequences to 'corrGene2' and print the alignment results and the number of reads to a txt file. (Figure 4H)

Note: In a successfully amplified library, the top two reads should align perfectly (wild type) or have one mismatch (mutated) compared to the 'corrGene2'.

- k. For each unique cell barcode, go through every Read 2 sequence for each unique UMI. Record and count the number of Read 2 sequences aligned to 'corrGene2' with an alignment score greater or equal to 150.
- I. For each unique cell barcode, go through all UMIs detected in that cell. Calculate the distance between the selected UMI and the most common UMI (UMI with highest number of reads). If the difference between the two UMI sequences is smaller or equal to two, merge UMI with the most common UMI and remove the redundant UMI. Repeat with the second most common UMI and so on until no further mergers are possible for UMI correction.
- m. For each unique cell barcode, align every Read 2 sequences with wildtype and mutated sequence for each unique UMI.
- n. Call a UMI as either wildtype or mutated when the number of wildtype or mutated Read 2 sequences exceed 50% of the total number of reads for that UMI.
- o. Store the UMI information and its wildtype/mutation call if the UMI contains more reads than a predetermined threshold.

Note: The thresholds are typically picked by selecting the middle point of the "knee" in number of reads versus the rank (occurrence) of molecules plot (see Figure 4F).

p. Store mutation calling information in a csv file by assigning two columns to each cell barcode where the first column indicates the number of wildtype transcripts detected in that cell and the second columns the number of mutated transcripts.

8. Visualize wildtype and mutated population on UMAP.

LIMITATIONS

Here, we presented a protocol to apply amplicon sequencing to single-cell cDNA. A disadvantage of this protocol is the high number (50) of PCR cycles required to successfully amplify specific somatic mutations. For lowly expressed genes, even larger amplification needs to be performed which presents a significant challenge in terms of PCR recombination and crossover.

TROUBLESHOOTING

Problem 1

Inadequate separation of layers during Ficoll/Lymphoprep isolation (steps 4 and 5).



Potential solution

Make sure that the acceleration and brake settings of the centrifuge are set correctly. At high speed, the phases might mix and disturb the gradient. Make sure that the amount of blood per tube does not exceed the recommendations. The FicoII/Lymphoprep solution should be at room temperature $(20^{\circ}C-22^{\circ}C)$.

Problem 2

Too much clumping of the cells during the separation. (steps 12, 20, and 23)

Potential solution

Make sure you use the EasySep buffer supplied from NEB. The EasySep buffer contains EDTA which will reduce the clumping.

Problem 3

Low cell viability. (steps 11, 25, and 26)

Potential solution

Spin the cells at low RPM (120 rpm) for 2–3 min. The centrifugation is enough to pellet live cells, but most dead cells will still be in suspension. Make sure to always leave the cells on ice.

Problem 4

No/low peak below 700 bp after locus-specific amplicon library generation. (steps 25-39)

Potential solution

Perform a Tapestation measurement after each PCR. For lowly expressed genes, add 3–5 cycles to the first locus-specific PCR. If the Tapestation result is still not satisfactory, add 3–5 cycles to the subsequent cycles.

Problem 5

Overclustering during amplicon library sequencing. (step 40)

Potential solution

If the Tapestation trace of the library shows multiple peaks, it is difficult to accurately calculate the molar concentration of the library after qPCR or qubit quantification. We generally assume that at least half of the reads generated in the fastq files come from library strands smaller than 700 bp. It is also important to mix in > 10% of PhiX control to identify the cause of the overclustering and decrease the homogeneity of the amplicon libraries.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Sahand Hormoz (sahand_hormoz@hms.harvard.edu).

Materials availability

Oligonucleotides could be purchased from manufacturers.

Data and code availability

Amplicon analysis code is available at https://gitlab.com/hormozlab/scamplicon-library-analysis.

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AUTHOR CONTRIBUTIONS

S.L., M.N., and S.H. developed and optimized the experimental and computational pipeline. S.L., and M.N. performed experiments. S.H. and S.L. analyzed and interpreted the data. S.L. and S.H. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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