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hgRNA Illumina Library Prep PCR Protocols for MARC1 models distributed from the MMRRC
RRID:MMRRC_065812-UCD, STOCK MARC1-PB3/Mmucd
RRID:MMRRC_065424-UCD, STOCK MARC1-PB7/Mmucd

Protocol provided by Dr. Reza Kalhor. The PB7 founder line is published, and the publication is pending for founder line PB3. *Kalhor R, Kalhor K, Mejia L, Leeper K, Graveline A, Mali P, Church GM. Developmental barcoding of whole mouse via homing CRISPR. Science. 2018 Aug31;361(6405). pii: eaat9804. doi: 10.1126/science.aat9804. Epub 2018 Aug 9. (Medline PMID: 30093604)*

SBS3-PBLib-F	acactctttccctacacgacgctcttccgatct atggactatcatatgcttaccgt
SBS9-PBLib-R	tgactggagttcagacgtgtgctcttccgatct gccataccaatgggcccgaa
SBS3	acactctttccctacacgacgctcttccgatct
SBS9	tgactggagttcagacgtgtgctcttccgatct

1. Obtain genomic DNA from each mouse using one of the following strategies:
   - Clean: using any genomic DNA purification protocol or kit (such as Qiagen DNeasy blood & tissue kit) extract genomic DNA and measure its concentration.
   - Dirty: Obtain two ear notches from each mouse in an Eppendorf tube and dissolve them using 25-30ul of Qiagen Buffer ATL with proteinase K (or similar solution) by shaking at 56C for 15-60min. Inactivate proteinase K with PMSF or other appropriate protease inhibitor. Dilute 1ul of this dissolved mixture 100 times in TE with 0.1% Triton-X100. Use 1-2ul of this diluted mixture in the next step without measuring concentration.

2. Set PCR1 for each sample as follows (use a mastermix for multiple samples):

2x Kapa Sybr Fast qPCR mix	5ul
Template DNA	0.1-10ng
SBS3-PBLib-F primer	0.05uM
SBS9-PBLib-R primer	0.05uM
SBS3 primer	0.2uM
SBS9 primer	0.2uM
Water:	To 10ul total volume

3. Cycling conditions in a realtime PCR machine:
   - 95°C for 3 min
   - Repeat {95°C for 20 sec; 64°C for 20sec} 20 to 30 cycles
   - Stop the reaction after amplification starts but before it reaches the mid-exponential phase

4. Dilute each PCR product 10-100 folds. It is helpful to dilute samples that amplified more in PCR1 accordingly at this stage to obtain similar concentrations for all samples.

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5. Set PCR2 (indexing) for each sample as follows:
    - 2x Kapa Sybr Fast qPCR mix (or any other PCR mix): 6.5ul
    - Diluted product from PCR1: 2ul
    - NEB Forward Indexing primer (or other indexing primer): 0.2-0.4uM
    - NEB Reverse Indexing primer (or other indexing primer): 0.2-0.4uM
    - Water: To 13ul total volume

6. Cycling conditions in a realtime PCR machine (dependent on the PCR mix used, here for Kapa Sybr Fast mix):
    - 95°C for 3 min
    - Repeat {95°C for 20 sec; 64°C for 25sec} 10 to 15 cycles
    - Stop the reaction when most plates reach mid-exponential phase

7. Combine indexed PCR products into a library and purify it using Zymo or Qiagen kit.
8. Run a sample of the library on the gel. Correct product is 400-450bp (should be a narrow smear). If a smaller primer dimer band exists, gel-extract the correct size range (300-500bp range).
9. Quantify and sequence with Illumina (MiSeq is adequate) using 190 by 60 paired end reads.

barcode	classification
AAACCCCGGG	Slow
ACATTCGGTT	Slow
ACCACTGCTG	Slow
ACCCTGGGAC	Slow
ACTCCATGTT	Slow
AGCACTGTAC	Slow
AGTCTGCCTC	Slow
ATGCTTAGCT	Slow
ATGGCGCCTA	Slow
CATCGTCGTC	Slow
CCTTTACCGC	Slow
CGAATCCTTT	Slow
CGACAGTTAT	Slow
CGTGTTGTCT	Slow
CTGAGTTTTA	Slow
CTTTTGTCGG	Slow
GCCAAGATGG	Slow
GCCAGCCGCT	Slow
GCTCTACGCC	Slow
GGCCCCTACA	Slow
GGGTGACACG	Slow
GTCAAATACC	Slow
TAACTTATAC	Slow
TAGCCATGCA	Slow
TCTATCGAGG	Slow
TTTGGCACAC	Slow
AACGCCCTAC	Inactive
AACTATCGGC	Inactive
AACTCACCTA	Inactive
AAGACTTCAT	Inactive
ACTCGGTTAC	Inactive
AGACCCTCGC	Inactive
AGCCCAAATC	Inactive
AGTTTCCGAA	Inactive
CCCATCACCC	Inactive
CCCCTCACTT	Inactive
CCTCACCCCA	Inactive
CGCATGATGC	Inactive
CGCCGTAGTA	Inactive
GACCTCCAAT	Inactive
GCCCCATTCC	Inactive
GCGAAGTCCC	Inactive
GGCACCCTCC	Inactive
TAACTGCTCT	Inactive
TCTCTAGATC	Inactive
AAGCCGCGCG	Intermediate
CATTGGAGGT	Intermediate
CCCAAAACAC	Intermediate
CGTAGGGCCC	Intermediate

CTACTCGGCC	Intermediate
CTGCTATCGA	Intermediate
GAAGACCCGC	Intermediate
GACACAGACA	Intermediate
GATACCCCCA	Intermediate
GTACACAATT	Intermediate
CACAACGCCC	Fast
CACTCTCAAG	Fast
GTGGAGCCTC	Fast
TTAGCTATGT	Fast
TTGAGCATAA	Fast
CTCGTAAGTC	Fast
TGGTGATGGT	Fast
AGCACACCCA	Intermediate
AGGTGTTCAA	Intermediate
ATGGAGGGAC	Fast
ATGTCATCGA	Intermediate
CAAGGGCCCT	Intermediate
CACCTCGGAC	Intermediate
CATATATTCC	Intermediate
CCGTACTTCA	Intermediate
CGGGGGTTCC	Intermediate
GACCCTTCCT	Intermediate
GCGTCGCCCT	Intermediate
TACACAGATA	Intermediate
TACACCCCAC	Intermediate
TAGAACCATG	Intermediate
TCATCAGGCC	Intermediate
TCTTATAACC	Intermediate
ATGACCCACC	Slow
CCACCATCCG	Slow
CCTACAACAG	Slow
CGGTCTGTAC	Slow
GACGAAGACA	Slow
GACGGTTCCA	Slow
GCCAACGAGC	Slow
GCCCAGATAC	Slow
GGCGACCGCA	Slow
TTATCGTTCA	Slow
TTGTGACATA	Slow
AAGGCGGATC	Inactive
ACAAATCCTG	Inactive
ACGTCGCGGA	Inactive
AGGATACGCC	Inactive
ATAGCTGATG	Inactive
CAATCCGACT	Inactive
CACGGGTAAT	Inactive
CACTTAATTC	Inactive
CATACCCCAC	Inactive
CCACTAACTT	Inactive

CCTTAAGCGC	Inactive
GAACCGCTGG	Inactive
GACTCTGGCC	Inactive
GAGCGCTGGG	Inactive
GCCCACCACA	Inactive
GGCCTTTGAC	Inactive
GTATCCCGAG	Inactive
TCATACGGGC	Inactive
TCTCCGGGCA	Inactive
TGACGGTTCT	Inactive
TGCCAGCTGA	Inactive
TGGTTTCTTT	Inactive
TTACCACGAA	Inactive