hgRNA Illumina Library Prep PCR Protocols for MARC1 models distributed from the MMRRC


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

1. Obtain genomic DNA from each mouse using one of the following strategies:
   o Clean: using any genomic DNA purification protocol or kit (such as Qiagen DNeasy blood & tissue kit) extract genomic DNA and measure its concentration.
   o 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 56°C 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):
   o 2x Kapa Sybr Fast qPCR mix 5ul
   o Template DNA 0.1-10ng
   o SBS3-PBLib-F primer 0.05uM
   o SBS9-PBLib-R primer 0.05uM
   o SBS3 primer 0.2uM
   o SBS9 primer 0.2uM
   o Water: To 10ul total volume

3. Cycling conditions in a realtime PCR machine:
   o 95°C for 3 min
   o Repeat {95°C for 20 sec; 64°C for 20sec} 20 to 30 cycles
   o 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.
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
AAACCCGGG Slow
ACATTCGGTT Slow
ACCACTGCTG Slow
ACCCCTGGGAC Slow
ACTCCATGTT Slow
AGCACTGTAC Slow
AGTCTGCCTC Slow
ATGCTTAGCT Slow
ATGCGGCCTA Slow
CATCGTCGTC Slow
CCTTTACCGC Slow
CGAAATCCTTT Slow
CGACAGTTAT Slow
CGTGGTTGCT Slow
CTGAGTTTTA Slow
CTTTTGTCCG Slow
GCCAAGATGG Slow
GCCAGCCGCT Slow
GCTCTACGCC Slow
GGCCCCTACA Slow
GGGTGACACG Slow
GTCAAATACC Slow
TAACTTATAC Slow
TAGCCATGCA Slow
TCTATCGAGG Slow
TTTTGCACAC Slow
AACGCCCTAC Inactive
AACTATCGGC Inactive
AATCTACCTA Inactive
AAGACTTCAT Inactive
ACTCGGTTC Inactive
AGACCCCTGC Inactive
AGCCCAATAC Inactive
AGTCATCAGAA Inactive
CCCATCACCC Inactive
CCCCCTCATT Inactive
CCTCACCCCA Inactive
CGCATGATGC Inactive
CGCCGCTAGTA Inactive
GACCTCAATT Inactive
GCCCAATTCC Inactive
GCGAAGTCCC Inactive
GCGACCCTCC Inactive
TAACTGCTCT Inactive
TCTCTAGATC Inactive
AAGCCGCGCG Intermediate
CATTGAGGAT Intermediate
CCCAAAACAC Intermediate
CGTGGGCCCA Intermediate
CTACTCGGCC  Intermediate
CTGCTATCGA  Intermediate
GAAAGCCCGC  Intermediate
GACACAGACA  Intermediate
GATACCCCA  Intermediate
GTACACAATT  Intermediate
CACAACGGCC  Fast
CAACTCAAGG  Fast
GTGGAGCCTC  Fast
TTAGCTATGT  Fast
TGGAGCTAA  Fast
CTCTGTAAGTC  Fast
TGTTGATGGT  Fast
AGCACAACCA  Intermediate
AGGTGTCTAA  Intermediate
ATGGAGGGAC  Fast
ATGTCACGGA  Intermediate
CAAGGGCCT  Intermediate
CACCTCGGAC  Intermediate
CATATATTCC  Intermediate
CGGTCTTCA  Intermediate
CGGGGGTCC  Intermediate
GACCCTTCCT  Intermediate
GCGTCGCCCT  Intermediate
TACACAGATA  Intermediate
TACACCCCA  Intermediate
TAGAACCATG  Intermediate
TCACAGGCCC  Intermediate
TCTTATAACC  Intermediate
ATGACCCACC  Slow
CCACCATCCT  Slow
CCTACAACAG  Slow
CGGTCTGTAC  Slow
GACGAAGACA  Slow
GACGGTTCCA  Slow
GCAACCCAGC  Slow
GCCCACTACA  Slow
GGCGACCGCA  Slow
TTATCTTTCA  Slow
TTTGACACATA  Slow
AAGGCGGATC  Inactive
ACCAATACCT  Inactive
ACGTCGGGA  Inactive
AGGATACGCC  Inactive
ATAGCTGATG  Inactive
CAATCCCGACT  Inactive
CAGGGTGATA  Inactive
CAACTTAATCC  Inactive
CATACCCCA  Inactive
CCACTAACTT  Inactive
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCTTAAGCGC</td>
<td>Inactive</td>
</tr>
<tr>
<td>GAACCGCTGG</td>
<td>Inactive</td>
</tr>
<tr>
<td>GACTCTGGCC</td>
<td>Inactive</td>
</tr>
<tr>
<td>GAGCGCTGGG</td>
<td>Inactive</td>
</tr>
<tr>
<td>GCCCACCACA</td>
<td>Inactive</td>
</tr>
<tr>
<td>GGCCTTTGAC</td>
<td>Inactive</td>
</tr>
<tr>
<td>GTATCCCGAG</td>
<td>Inactive</td>
</tr>
<tr>
<td>TCATACGGGC</td>
<td>Inactive</td>
</tr>
<tr>
<td>TCTCCGGGCA</td>
<td>Inactive</td>
</tr>
<tr>
<td>TGACGTTTCT</td>
<td>Inactive</td>
</tr>
<tr>
<td>TGCCAGCTGA</td>
<td>Inactive</td>
</tr>
<tr>
<td>TGTTTCTTTT</td>
<td>Inactive</td>
</tr>
<tr>
<td>TTACCACGAA</td>
<td>Inactive</td>
</tr>
</tbody>
</table>