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


SBS3-PBLib-F acaccttttccctacacgacgctcttccgatct atggactatcatatgcttaccgt
SBS9-PBLib-R tgactggagttcagacgtgtgctcttccgatct gccataccaatgggcccgaa
SBS3 acaccttttccctacacgacgctcttccgatct
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:
   a. 2x Kapa Sybr Fast qPCR mix (or any other PCR mix): 6.5ul
   b. Diluted product from PCR1: 2ul
   c. NEB Forward Indexing primer (or other indexing primer): 0.2-0.4uM
   d. NEB Reverse Indexing primer (or other indexing primer): 0.2-0.4uM
   e. 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):
   a. 95°C for 3 min
   b. Repeat {95°C for 20 sec; 64°C for 25sec} 10 to 15 cycles
   c. 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.