



Figure S3. Validation of our method for enriching for motile sperm using Percoll. Sperm from two males, one DBA and one 6J, were mixed in equal numbers. Prior to mixing, DBA (left column) or 6J (right column) sperm were heat-shocked to kill them. Sperm were run through the Percoll method, then retrieved and DNA isolated. A region containing a diagnostic SNP (rs30481467, red arrow) was PCR-amplified (Forward primer: CGTATGTTGATGGCCAGCTC; Reverse primer: GGGTCAATGTGAGGTCACCA) and Sanger-sequenced. Top row: sperm mixture prior to loading onto Percoll column. Middle row: sperm isolated from the middle Percoll layer, which will contain motile and immotile sperm (note that the non-heat-shocked male will still produce some amount of immotile sperm). Bottom row: sperm isolated from the bottom Percoll layer, which should only contain motile sperm. We are showing the sequence generated with the reverse primer, identical results were observed with the forward primer (not shown). As expected, the diagnostic SNP appeared heterozygous in the artificial sperm mixtures prior to Percoll separation and in the middle layer of Percoll. However, sperm containing the non-heat-shocked allele were enriched in the bottom layer, validating our approach for separating motile from immotile sperm.