Figure S1  Development of MinION™ short-fragment library preparation method. (a) Evaluation of E5 detachment from Y-adapter in the adapter mixture. Gels showing levels of
detachment of E5 protein from the adapter mixture provided in kit (SQK-MAP004) under different conditions. Lane 1, 3, 5 show the untreated adapter mixture. Lane 2 and 4 show the results of adapter mixture incubated in T4 ligase buffer at 16 °C or 4 °C O/N respectively. Lane 6 and 7 show the results of adapter mix incubated in blunt/TA ligase master mix with extended 4 h incubation at 4 °C. Incubation of the adapter mixture at 16 °C or 4 °C O/N in 1X T4 ligation buffer results in detachment of the E5 protein (Lane 1 vs 2 and Lane 3 vs 4, respectively). Incubation of the adapter mixture in 1X blunt/TA ligase master mix at 4 °C for 2 h or RT 5min followed by 4 °C 2 h did not result in detachment of E5 protein (Lane 5 vs. Lane 6 and 7 respectively). (b) Ligation in 20 μL T4 ligation system. Gels showing ligation products using 20 μL T4 ligation system overnight incubation at 16 °C. 16 °C O/N incubation of adapter:fragment at 10:1 ratio results in ~75% control fragments ligated to adapters on both ends (Lane 4, 5 vs lane 1); 16 °C O/N incubation of adapter:fragment at 5:1 ratio results in ~65% control fragments ligated to adapters on both ends (Lane 2, 3 vs lane 1). Using the adapter:fragment at a 5:1 ratio enables 2 ligation reactions to be performed using the same kit reagents, resulting in ~130% (i.e., ~ 0.26 pmol ) fragments with adapters on both ends for downstream experiments (Lane 1, control fragment; lane 2-3, ligate control adapter and fragment at a 1/2 ratio; lane 4-5, ligate control adapter and fragment at the ratio suggested in manufacturer’s protocol. ) (c) Fragmentation and size-selection of a MinION™ short-fragment library preparation. (Lane 1, gDNA (> 15kb); lane 2, fragmented gDNA; lane 3, large fragments (> 650bp) discarded during size-selection; lane 4, size-selected DNA fragments (350-600bp)).