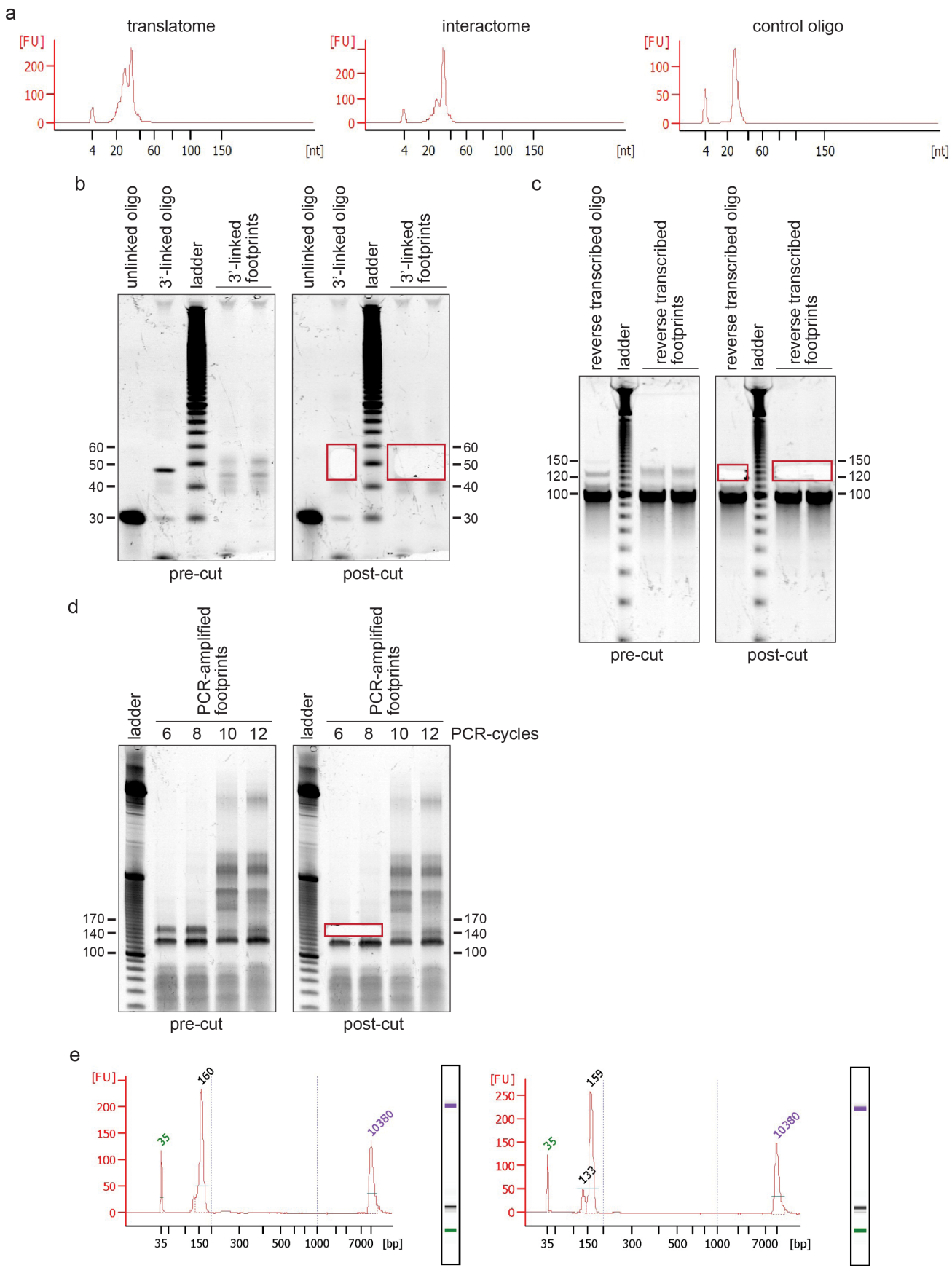
a
translatome
interactome
control oligo
[FU]
[nt]
b
unlinked oligo
3'-linked oligo
ladder
3'-linked footprints
pre-cut
post-cut
c
reverse transcribed oligo
ladder
reverse transcribed footprints
pre-cut
post-cut
d
ladder
PCR-amplified footprints
6 8 10 12 PCR-cycles
pre-cut
post-cut
e
[FU]
[bp]
35
160
10380
159
133

Gel purification steps and Bioanalyzer results during the deep sequencing library preparation after size selection.

(a) Quantification of dephosphorylated RNA on a Bioanalyzer Small RNA chip (step **38** of the Supplementary Methods). Depicted are samples of translatome, interactome and control oligonucleotide. In all three samples a marker peak with a length of 4 nt is included. (b–d) RNA or DNA samples were loaded on polyacrylamide gels. Gels were stained with SYBR gold and shown before (pre-cut) and after (post-cut) the region of interest (marked with the red box) was excised. (b) Separation of 3'-linked mRNA footprint fragments as well as 3'-linked RNA control oligonucleotide from unligated linker on a 10% TBE-Urea polyacrylamide gel (step **45** and following of the Supplementary Methods). The phosphorylated RNA control oligonucleotide was loaded for size comparison. (c) Purification of the reverse transcribed ssDNA of footprint fragments and control oligonucleotide after the second ligation step on a 10% TBE-Urea polyacrylamide gel (step **60** and following of the Supplementary Methods). (d) Purification of dsDNA after PCR on an 8% TB polyacrylamide gel (step **90** and following of the Supplementary Methods). The PCR was run for 6, 8, 10 and 12 cycles, but only products after 6 and 8 cycles were excised due to the occurrence of higher molecular weight products in later cycles indicating non-linear amplification. (e) Quantification of the dsDNA library for deep sequencing on a Bioanalyzer High Sensitivity DNA chip (step **95** of the Supplementary Methods). Markers with a size of 35 and 10380 nt were included. A peak with the size of around 160 nt (containing footprint fragments with an average of 31 nt in length) shows the expected PCR product. The occurrence of an additional smaller peak with around 130 nt (right panel only) indicates circularization and PCR amplification of free linker L1'L2' lacking a footprint fragment and should, therefore, be avoided.