**Figure S1 related to Figure 1. Autoradiographic visualization showing non-crosslinked control *vs* crosslinked sample.** The representative U2AF1 eCLIP-seq negative control, obtained from non-crosslinked U2AF1 S34F cells, is characterized by a faint, almost absent, RNA signal in comparison to the RNA signal in crosslinked U2AF1 S34F cells.

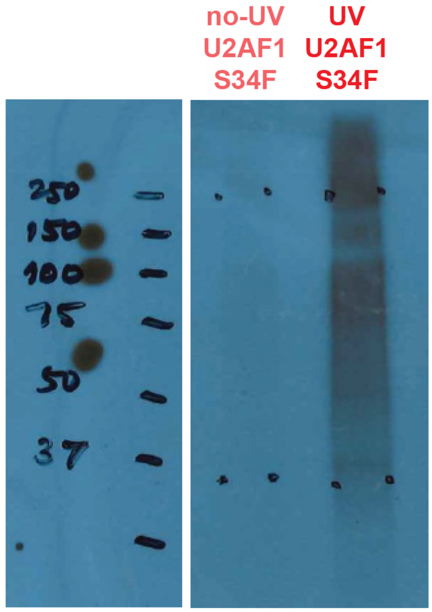

**Table S1 related to Step 21.** Illumina Pooling Guidelines.

**Pooling Guidelines for 8-Plex Combinations**

| 1 |  | 2 |  | 3 |  | 4 |  | 5 |  | 6 |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D501 | D705 | D502 | D706 | D503 | D701 | D505 | D702 | D506 | D704 | D507 | D703 |
| D502 | D704 | D501 | D702 | D505 | D703 | D503 | D706 | D507 | D705 | D506 | D701 |
| D503 | D703 | D505 | D705 | D506 | D706 | D507 | D701 | D504 | D702 | D508 | D704 |
| D505 | D701 | D503 | D704 | D507 | D702 | D506 | D703 | D508 | D706 | D504 | D705 |
| D506 | D710 | D507 | D712 | D504 | D707 | D508 | D708 | D501 | D709 | D502 | D711 |
| D507 | D709 | D506 | D708 | D508 | D711 | D504 | D712 | D502 | D710 | D501 | D707 |
| D504 | D711 | D508 | D710 | D501 | D712 | D502 | D707 | D503 | D708 | D505 | D709 |
| D508 | D707 | D504 | D709 | D502 | D708 | D501 | D711 | D505 | D712 | D503 | D710 |
| 7 |  | 8 |  | 9 |  | 10 |  | 11 |  | 12 |  |
| D501 | D710 | D502 | D712 | D503 | D707 | D505 | D708 | D506 | D709 | D507 | D711 |
| D502 | D709 | D501 | D708 | D505 | D711 | D503 | D712 | D507 | D710 | D506 | D707 |
| D503 | D711 | D505 | D710 | D506 | D712 | D507 | D707 | D504 | D708 | D508 | D709 |
| D505 | D707 | D503 | D709 | D507 | D708 | D506 | D711 | D508 | D712 | D504 | D710 |
| D506 | D705 | D507 | D706 | D504 | D701 | D508 | D702 | D501 | D704 | D502 | D703 |
| D507 | D704 | D506 | D702 | D508 | D703 | D504 | D706 | D502 | D705 | D501 | D701 |
| D504 | D703 | D508 | D705 | D501 | D706 | D502 | D701 | D503 | D702 | D505 | D704 |
| D508 | D701 | D504 | D704 | D502 | D702 | D501 | D703 | D505 | D706 | D503 | D705 |

**Pooling Guidelines for 6-Plex Combinations**

| 1 |  | 2 |  | 3 |  | 4 |  | 5 |  | 6 |  | 7 |  | 8 |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D501 | D705 | D501 | D710 | D502 | D704 | D502 | D709 | D503 | D703 | D503 | D711 | D505 | D701 | D505 | D707 |
| D502 | D706 | D502 | D712 | D501 | D702 | D501 | D708 | D505 | D705 | D505 | D710 | D503 | D704 | D503 | D709 |
| D503 | D701 | D503 | D707 | D505 | D703 | D505 | D711 | D506 | D706 | D506 | D712 | D507 | D702 | D507 | D708 |
| D505 | D702 | D505 | D708 | D503 | D706 | D503 | D712 | D507 | D701 | D507 | D707 | D506 | D703 | D506 | D711 |
| D506 | D704 | D506 | D709 | D507 | D705 | D507 | D710 | D504 | D702 | D504 | D708 | D508 | D706 | D508 | D712 |
| D507 | D703 | D507 | D711 | D506 | D701 | D506 | D707 | D508 | D704 | D508 | D709 | D504 | D705 | D504 | D710 |
| 9 |  | 10 |  | 11 |  | 12 |  | 13 |  | 14 |  | 15 |  | 16 |  |
| D506 | D710 | D506 | D705 | D507 | D709 | D507 | D704 | D504 | D711 | D504 | D703 | D508 | D707 | D508 | D701 |
| D507 | D712 | D507 | D706 | D506 | D708 | D506 | D702 | D508 | D710 | D508 | D705 | D504 | D709 | D504 | D704 |
| D504 | D707 | D504 | D701 | D508 | D711 | D508 | D703 | D501 | D712 | D501 | D706 | D502 | D708 | D502 | D702 |
| D508 | D708 | D508 | D702 | D504 | D712 | D504 | D706 | D502 | D707 | D502 | D701 | D501 | D711 | D501 | D703 |
| D501 | D709 | D501 | D704 | D502 | D710 | D502 | D705 | D503 | D708 | D503 | D702 | D505 | D712 | D505 | D706 |
| D502 | D711 | D502 | D703 | D501 | D707 | D501 | D701 | D505 | D709 | D505 | D704 | D503 | D710 | D503 | D705 |

**Table S2 related to Pre-processing of next-generation sequencing data.** 3’ RNA linker sequences.

| |
|---|
| >X1A_linker<br>ATATAGGNNNNNAGATCGGAAGAGCGTCGTGTAG |
| >X1B_linker<br>AATAGCANNNNNAGATCGGAAGAGCGTCGTGTAG |
| >X2A_linker<br>AAGTATANNNNNAGATCGGAAGAGCGTCGTGTAG |
| >X2B_linker<br>AGAAGATNNNNNAGATCGGAAGAGCGTCGTGTAG |
| >RiL19_linker_input<br>AGATCGGAAGAGCGTCGTG |

**Table S3 related to Pre-processing of next-generation sequencing data.** Sequencing metrics of U2AF1 freCLIP-seq and AGO2 eCLIP-seq.

| Sample | Raw reads | Deduplicated reads | Duplicates % | Post-trimming reads | Post-trimming reads % | Unique-mapped reads | Multi-mapped reads | Total-mapped reads | Mapping % | Average mapping length |
|---|---|---|---|---|---|---|---|---|---|---|
| U2AF1_WT_heavy_1 | 87045558 | 47552328 | 45.37% | 46912474 | 98.65% | 29967676 | 10265306 | 40232982 | 85.76% | 62 |
| U2AF1_WT_heavy_2 | 81620509 | 43373037 | 46.86% | 42700777 | 98.45% | 25925359 | 9340502 | 35265861 | 82.59% | 56 |
| U2AF1_WT_light_1 | 105709524 | 28975384 | 72.59% | 28171271 | 97.22% | 12220951 | 10186492 | 22407443 | 79.54% | 49 |
| U2AF1_WT_light_2 | 87500617 | 16315206 | 81.35% | 15656052 | 95.96% | 6326571 | 5920149 | 12246720 | 78.22% | 55 |
| Ago2_rep1 | 82698837 | 37723336 | 54.38% | 36769823 | 97.47% | 9442408 | 4586541 | 14028949 | 38.15% | 70 |
| Ago2_rep2 | 51389385 | 28419922 | 44.70% | 27911154 | 98.21% | 6224739 | 2790042 | 9014781 | 32.30% | 73 |