QuantSeq-Flex Targeted RNA-Seq Library Prep Kit for Illumina platforms

Reference card

200 min Library Generation

First Strand cDNA Synthesis

- [ ] Mix 5 µl RNA, 5 µl **FS1x** ● and 5 µl **dT** ● **OR** 5 µl **Custom Targeted Primers**.
- [ ] Heat to 85 °C for 3 min, then cool to RT temperature. For targeted sequencing and FFPE samples skip this step.
- [ ] Pre-mix 4.5 µl **FS2x** ● and 0.5 µl **E1** ● per reaction.
- [ ] Add 5 µl **FS2x** / **E1** mix per reaction.
- [ ] Incubate for 15 min at 37 °C (or up to 50 °C for targeted primers with a high Tm).

RNA Removal

- [ ] Add 5 µl **RS1** ○, mix well.
- [ ] Incubate 10 min at 95 °C, then cool to 25 °C.
- [ ] Add 5 µl **RS2** ○, mix well.

2nd Strand Synthesis

- [ ] Pre-mix 13 µl **SS1x** ● and 2 µl **RSP** ● **OR** 2 µl **Custom Targeted Primers** per reaction, mix well.
- [ ] Add 15 µl **SS1x** / primer mix per reaction. Mix well.
- [ ] Incubate 1 min at 98 °C, slowly ramp down to anneal primers (25 °C - 50 °C).
- [ ] Incubate 30 min at 25 °C (or up to 50 °C for targeted primers with a high Tm).
- [ ] Pre-mix 4 µl **SS2** ● and 1 µl **E2** ● per reaction.
- [ ] Add 5 µl **SS2** / **E2** mix per reaction.
- [ ] Incubate 15 min at 25 °C (or 50 °C for targeted primers with a high Tm).

Purification

- [ ] Add 20 µl **PB** per reaction, mix well, incubate 5 min.
- [ ] Place on magnet for 2 - 5 min, remove and discard supernatant.
- [ ] Add 40 µl **EB**, mix well, incubate 2 min at RT.
- [ ] Add 72 µl **PS** (or 48 µl **PS** for low input, FFPE, or low quality RNA), mix well, incubate 5 min at RT.
- [ ] Place on magnet for 2 - 5 min, remove and discard supernatant.
- [ ] [ ] Wash the beads (**PB**) twice with 120 µl 80 % EtOH, 30 sec.
- [ ] Dry beads for 5 - 10 min.
- [ ] Add 20 µl **EB**, mix well, incubate 2 min at RT.
- [ ] Place on magnet for 2 - 5 min, transfer 17 µl of the supernatant into a fresh PCR plate.

70 min Library Amplification

PCR

- [ ] Pre-mix 7 µl **PCR** ● and 1 µl **E3** ● per reaction, mix well.
- [ ] Add 8 µl **PCR** / **E3** premix to 17 µl of each purified library.
- [ ] Add 5 µl **BC** (from the 96-well plate) for each reaction, mix well.
- [ ] PCR: 98 °C, 30 sec
  - 98 °C, 10 sec
  - 65 °C, 20 sec
  - 72 °C, 30 sec
  - } x (use qPCR to determine cycle number)
  - 72 °C, 1 min; 10 °C, ∞

ATTENTION: Increase cycle number for low input RNA and samples with low target RNA content!

Purification

- [ ] Add 30 µl **PB** (or 27 µl **PB** for low quality or FFPE RNA) per reaction, mix well, incubate 5 min.
- [ ] Place on magnet for 2 - 5 min, remove and discard supernatant.
- [ ] Add 30 µl **EB**, mix well, incubate 2 min at RT.
- [ ] Add 30 µl **PS**, mix well, incubate 5 min at RT.
- [ ] Place on magnet for 2 - 5 min, remove and discard supernatant.
- [ ] [ ] Wash the beads twice with 120 µl 80 % EtOH, 30 sec.
- [ ] Air dry beads for 5 - 10 minutes.
- [ ] Add 20 µl **EB**, mix well, incubate 5 min at RT.
- [ ] Place on magnet for 2 - 5 min, transfer 15 - 17 µl of the supernatant into a fresh PCR plate.

ATTENTION: Spin down solutions before opening tubes or plates!

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QUANT SEQ™
Simply counting 3'ends

Enabling complete transcriptome sequencing

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