Cloning & Libraries

SMARTer™ Ultra Low RNA Kit for Illumina® Sequencing FAQ

Starting Material

Q: What is the range of input starting material that has been tested using this kit?

A: From 10 pg to 10 ng of total RNA.

Q: What range of input starting material is supported?

A: From 100 pg to 10 ng of total RNA.

Q: Have you performed cDNA synthesis with laser-captured cells?

A: No, we have not performed cDNA synthesis with laser-captured cells.

Q: Have you tested RNA from plant or insect tissues?

A: No. However, there is no reason for us to believe that this kit will not work for any of those mentioned tissues based on data obtained from previous SMART™ kits. A major hurdle when using plant tissue is polysaccharide contamination. If you are planning to work with a plant sample, you need to make sure it is polysaccharide-free.

RNA Purification and Quality Assessment

Q: What methods can I use for assessing RNA quality?

A: You can use the following kits for assessing limited RNA quality from Agilent: the **Agilent RNA 6000 Pico Kit** (Cat. No. 5067-1513) or, if more total RNA is available, the **Agilent RNA 6000 Nano Kit** (Cat. No. 5067-1511). Alternatively, qRT-PCR methods can be used if you have specific primers; however, RNA integrity will not be assessed as easily.

Q: What RNA purification kits are recommended?

A: For total RNA purification we recommend choosing the best suitable RNA purification kit depending on the source of your starting material—plant, tissue, or cells. The following kits offer RNA isolation from small amounts of material, however, Clontech has not tested these kits with the SMARTer Ultra Low RNA Kit for Illumina Sequencing:

1. **NucleoSpin RNA XS**
   (Clontech/MACHEREY-NAGEL Cat. No. 740902.10)
   - Starting Sample: Small amounts (10^2 cells) of laser captured cells, cryosections, etc.
   - Elution from 10^2 HeLa cells: 0.1–1.5 ng in 5 µl
2. **ArrayPure Nano-scale RNA Purification Kit**
   (Epicentre Cat. No. MPS04050)
   - Starting Sample: 10 to 10,000 eukaryotic cells
3. **ZR Whole-Blood RNA MiniPrep**
   (Zymo Research Cat. No. R1020)
   - Starting Sample: ≤ 200 µl of blood (up to 1 ml with reloading)
   - Elution volume > 6 µl
4. **ZR Tissue & Insect RNA MicroPrep**
   (Zymo Research Cat. No. R2030)
   - Starting Sample: Small amounts (n ≥ 1 and ≤ 10 mg) of insect and arthropod specimens (e.g. mosquitoes, bees, lice, ticks, *Drosophila melanogaster)*
5. **ZR RNA MiniPrep** (Zymo Research Cat. No. R1064)
   - Starting Sample: Cells from culture or small amounts of solid tissue (10^2–10^7 cells in suspension or solid form)
   - Elution volume > 25 µl
6. **TurboCapture 96 mRNA Kit** (Qiagen Cat. No. 72251)
   - Starting Sample: 10,000 cells down to a single cell

Q: What media were tested for direct use of cells for SMARTer cDNA synthesis?

A: When working with cells it is important to pick cells using media/buffers that do not inhibit cell growth or suppress cDNA synthesis. The viability of the cells in the media/buffers should also be tested before use according to the guidelines in the user manual, Appendix B: Working with Cells. The following media were tested and found to be compatible with the SMARTer reaction:

1. Superblock (Pierce Cat. No. 37515)
2. 0.1 ml of [DMEM F12 + Glutamax (Invitrogen Cat. No. 10565)]
   +3.6 µl of 25% BSA (Invitrogen Cat. No. A10008-01)
3. PBS buffer (recipe for 1 L, 0.2 micron filtered):
   0.2 g of KCl
   0.24 g of KH_2PO_4 (anhydrous)
   8.00 g of NaCl
   1.44 g of Na_2HPO_4 (anhydrous)
   add dH_2O to 1 L

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PCR Cycle Optimization

Q: Why do I need to perform PCR cycle optimization?

A: Choosing the optimal number of PCR cycles ensures that the ds cDNA will remain in the exponential phase of amplification. When the yield of PCR product stops increasing with more cycles, the reaction has reached its plateau. Overcycled cDNA can result in a less representative sample. Undercycling, on the other hand, results in a lower yield of cDNA: The optimal number of cycles for your experiment is one cycle fewer than is needed to reach the plateau. Be conservative: when in doubt, it is better to use fewer cycles than too many.

Q: How can I optimize PCR cycles for my sample?

A: If you have a very limited amount of material or your sample is unique, use a similar source of RNA or cells to perform PCR cycle optimization prior to using the actual sample. To perform PCR cycle optimization, prepare several tubes containing an amount of RNA equal to your sample amount. Subject each tube to a different range of cycles. For example, if you have 1 ng of RNA, subject one tube to the recommended number of cycles. Subject the other two tubes to 2–3 cycles fewer or more than the first tube, e.g. 15, 12 (recommended for 1 ng), and 10 cycles.

Library Sequencing & Performance

Q: What are the main advantages of using Illumina's next-gen sequencing technology over other next-gen sequencing methods?

A: The main difference is depth of coverage, which gives you better sensitivity and quantification of transcripts. A single lane of Genome Analyzer *IIx* gives you over 40 million reads.

Q: In your in-house experiments, what percentage of libraries generated from SMARTer cDNA map to the genome?

A: On average, >95% of reads mapped to the genome.

General Protocol Questions

Q: For the purification of first-strand cDNA, is it right that EtOH wash is not needed? Why did you omit the ethanol wash step?

A: Yes, we omitted the ethanol wash step to reduce the loss of cDNA during handling.

Q: How long can one keep denatured RNA mix in the IsoFreeze PCR rack?

A: No longer than is necessary to set up the next step.

Q: Do I need to trim or remove SMART adaptor sequences?

A: No, it is not necessary to remove the SMART adaptor sequences.

Q: My magnetic stand is not the recommended brand, can I still use it?

A: We recommend using the MagnaBot II because of its design. The magnet is located on one side of the tube, therefore it attracts beads to one side, making it easy to remove buffer from the other side. We recommend taping a 10 µl pipette tip refill rack onto the top of the magnetic separation device to keep the tubes/strips in place. You can still use other separation devices, however, in most devices the magnet is in the form of a ring, which attracts beads to the bottom of the tube, making it difficult to remove buffer completely without touching the beads.

Q: Is it OK to use a single channel pipette instead of the recommended multichannel pipette?

A: Yes, it is OK to use a single channel pipette.

Q: Can I purchase some of the components separately?

A: No, the kit components are not sold separately.

Ordering Information

Product	Size	Cat. No.
SMARTer Ultra Low RNA Kit for Illumina Sequencing	10 rxns	634935

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