D) RNA purification with spin column

Kit used:
https://www.neb.com/products/t2040-monarch-rna-cleanup-kit-50-ug#Product%20Information

Before you start:

Add 4 volumes of ethanol (≥ 95%) to the Monarch RNA Wash Buffer before use, as directed on the bottle.
All centrifugation steps should be carried out at 16,000 x g. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

Protocol steps of RNA purification:

1. **Add 100 µl RNA Cleanup Binding Buffer to the 50 µl sample.** A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µl, scale buffer volumes accordingly. Samples with a starting volume > 150 µl will require reloading of the column during Step 3.

2. **Add 150 µl (1 volume) of ethanol (≥ 95%) to your sample and mix by pipetting or flicking the tube. Do not vortex.** This will enable the binding of RNA ≥ 25 nt. If you wish to bind RNA as small as 15 nt, add 2 volumes (300 µl) of ethanol to your sample instead of 1 volume (150 µl). The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.

3. **Insert column into collection tube, load sample onto column and close the cap. Spin for 1 minute, then discard flow-through.** For diluted samples > 900 µl, load a portion of the sample, spin, and then repeat as necessary.

4. **Re-insert column into collection tube. Add 500 µl RNA Cleanup Wash Buffer and spin for 1 minute. Discard the flow-through.**

5. **Repeat wash (Step 4).**

6. **Transfer column to an RNase-free 1.5 ml microfuge tube (not provided**). Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

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7. **Elute in nuclease-free water according to the table below.** The eluted RNA can be used immediately or stored at -70°C. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.
8. Spin for 1 minute with 20–100 µl nuclease-free water

Now you have purified sgRNA for the Crispr-cas9 to cut at the designed location

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