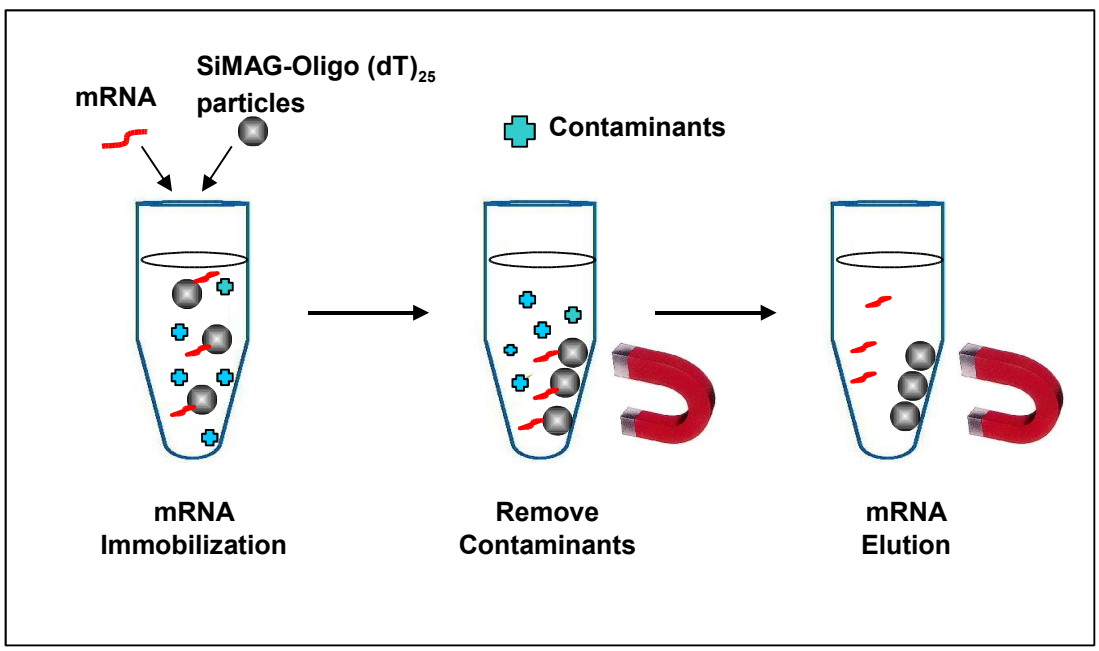
Purification of mRNA with SiMAG-Oligo $(dT)_{25}$

Introduction:

SiMAG-Oligo $(dT)_{25}$ particles are designed for the rapid isolation of highly purified, intact mRNA from eukaryotic total RNA.

The use of **SiMAG-Oligo $(dT)_{25}$** relies on base pairing between the poly-A tail of mRNA and the oligo dT sequences bound to the surface of the beads.

The isolated mRNA can be used directly in most downstream applications in molecular biology.



Equipment and reagents:

- **SiMAG-Oligo $(dT)_{25}$** (10 mg/ml in ddH_2O, 0.05% sodium azide)
- **Binding Buffer:** 20 mM Tris-HCl, pH 7,5
  1.0 M LiCl
  2 mM EDTA
- **Wash Buffer:** 10 mM Tris-HCl, pH 7.5
  0.15 M LiCl
  1 mM EDTA
- **Elution Buffer:** RNase-free water (DEPC-treated water)
- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- Disposable gloves should be used during handling of all materials and reagents, to avoid contamination with RNases from hands.
- Sterile, disposable plastic-ware should be used and glassware should be sterilized by heat (autoclave).
- It is recommended to use diethyl pyrocarbonate (DEPC) treatment of water and salt solutions. The DEPC inactivates RNases by covalent modification. DEPC is added to the solution to a concentration of 0.1%.

Note: DEPC is a suspected carcinogen and should be handled with care!

This protocol describes the purification of mRNA from total RNA samples. The procedure can be scaled up or down to suit the experimental design.

Protocol: Purification of mRNA from total RNA

1. Resuspend the **SiMAG-Oligo (dT)$_{25}$** particles by vortexing for 5-10 seconds and transfer 50 µl SiMAG-Oligo (dT)$_{25}$ into a clean 1.5 ml microcentrifuge tube.

2. Wash the particles 2 x with 500 µl Binding Buffer before use to remove sodium azide by using the magnetic separator and completely remove and discard the supernatant.

3. After the second wash step resuspend the particles in 250 µl Binding Buffer.

4. Add 100 µl of total RNA (75 µg total RNA) to the particles and mix the suspension on a shaker for 5 minutes at room temperature.

5. Wash the bead / mRNA complex 2 x with 500 µl Wash Buffer and collect by using the magnetic separator.
Note: *Remove the Wash Buffer carefully between wash steps.*

6. Resuspend the bead / mRNA complex in Elution Buffer for the desired down-stream application. The volume depends on the yield of mRNA and the desired concentration of the mRNA, 30-50 µl is recommended.

7. If elution of the mRNA from **SiMAG-Oligo (dT)$_{25}$** beads is wanted, incubate at 65°C for 5 minutes.

8. Place the tube in the magnetic device to collect the beads and transfer the supernatant containing mRNA in a new tube.