Mini gel filtration chromatography for the MCMs

Preparation of Sephacryl 300 HR

1. Bring S300 resin to room temp.
2. Gently resuspend and pour ~2 mL into a 10 mL plastic column. Drain off ethanol.
3. Add 2 mL room temp S/0.15 buffer, resuspend gently, drain. Repeat several times.
4. Resuspend resin to ~50% in S/0.15

Preparation of 1 mL pipette column

1. Trim a tiny bit of the tip of a 1 mL glass pipet so that a syringe cap fits. Trim the top so a round gel loading tip can reach the 0 mL mark.
2. Insert a small plug of silanized glass wool into the top of the pipette and force into tip with compressed air.
3. Place a piece of tubing (2 inches) on top to act as a reservoir.

Pouring the column

1. Run 1 mL S/0.15 through the pipette column, cap the bottom, and fill with S/0.15 to the reservoir.
2. Add 50% S300 to the reservoir and let slowly settle into the pipette. Add more resin as needed to bring the column volume up to ~1 mL.
3. Let several bed volumes of S/0.15 drip through the column to help pack the resin.
4. Always keep the column topped off with buffer when not in use, cap the bottom and use a binder clip to seal the reservoir on top.

Calibration

1. To find the void volume, dissolve a small amount of blue dextran in S/0.15, load 25 µL and let it enter the column. Chase with buffer to determine how much is needed to elute the blue dex peak (i.e. the void volume).
2. Next, load a 40 µL cocktail of protein standards (10 µL each of 8 mg/mL thyroglobulin, 10 mg/mL apoferritin, 1 mg/mL β-amylase, and 1 mg/mL BSA) and let it enter the column. Chase with buffer and collect one-drop (~21 µL/drop) fractions starting near the void volume.
3. Add 4 µL 6x loading dye to fractions, boil, and run 10 µL of each on one or two 12% SDS-PAGE gels at 200V.
4. Sypro stain and quantitate the bands to find the peak fractions of each.

Running the MCM samples

1. Run 1-2 bed volumes of S/0.15 through the column to clean it out.
2. Load 20-25 µL purified MCM sample and let it enter the column.
3. Add a bed volume of buffer and collect 20 one-drop fractions beginning near the void.
4. Add 4 µL 6x loading dye to each fraction, boil, and run 10 µL of each on 7% SDS-PAGE gels at 200V.
5. Sypro stain and quantitate the bands to find the peak(s).

MCM2-7 and 467 hexamers run slightly larger than thyroglobulin. MCM3 runs as a dimer.

Gel filtration chromatography

A 1 mL glass column was packed with Sephacryl 300 HR (Sigma) equilibrated in buffer S/0.15 and calibrated with a standard molecular weight markers including blue dextran (2,000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), and BSA (66 kDa). Small samples of purified protein (20-25 μL) were subjected to analytical gel filtration chromatography run by gravity flow at room temperature. 21 μL fractions were collected and analyzed by SDS-PAGE and staining with Sypro orange.