IP protocol for *Ashbya*

- Make lysate as for Western blot and aim for 1-5 µg/µl protein concentration.

- If desired and time permitting, pre-incubate lysate with protein A or G-sepharose beads to eliminate non-specific binding to beads. Not necessary if you have an untagged control lysate.

- Take 500µg of lysate and incubate with 1 µl anti-Myc antibody. Incubate rotating at 4 degrees for 2-3 hours.

- Add 30 µl protein A or protein G sepharose beads to your lysate. Incubate rotating at 4 degrees for one hour.

- Centrifuge tubes at about 2000 rpm, at 4 degrees for 2 minutes. Remove supernatant from beads. Wash beads 3X with lysis buffer + protease inhibitors, rotating for 3 minutes before centrifuging to wash.

- After final wash resuspend in hot SDS sample buffer and boil for 5 minutes-mix every now and then during the incubation with sample buffer to help release from beads.

Preparation of sepharose beads

- Wash enough for all IPs with 3X10 ml dH20 or with PBS. Spin at 700rpm for 2 minutes to pellet beads during washing (higher and the beads will break)

- Resuspend so 50% beads/50% NP40 LB (ex 3 ml beads, 3 ml LB).

- Cut the ends off some yellow tips with a razor blade to facilitate pipetting of beads