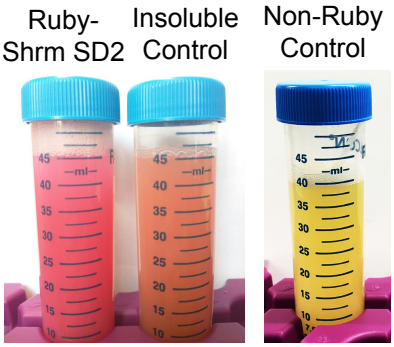
# Illustration of Sample Purification of $His_{10}$-mRuby2-Shroom SD2

**A**

**Step 2.3.1**

Protein Expression

1) Test many cell lines
2) Optimize media and growth conditions




Culture After Expression

**B**

**Step 2.3.2 thru 2.3.4**

Solubility

1) Resuspend in lysis buffer
2) Lyse with lysozyme/sonication
3) Centrifuge to remove insoluble protein

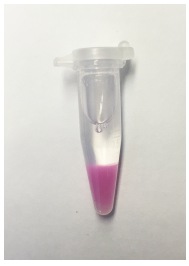

Soluble Protein

**C**

**Step 2.3.6**

Immobilize on Ni NTA beads

1) Incubate soluble fraction with beads
2) Wash extensively with lysis buffer
3) Pellet Beads



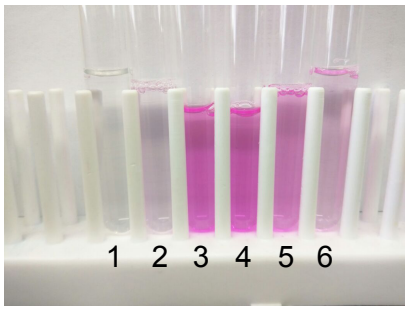
His-mRuby2-Shroom SD2 bound to Ni-NTA beads

**D**

**Step 2.3.7**

Elute from beads

1) Elute from beads
2) Optimize with Imidazole gradient if needed




1) Lysis buffer wash
2) 80mM Imidazole wash
3-6) Elution 1-4