UV Crosslinking (with IP step added)

1. Set up binding reaction.

Standard conditions:

4 µl	NE/protein or BC100
1 µl	BC400
1 µl	13% PVA
1 µl	tRNA/BSA/BC100 mix (0.25µg/µl tRNA+ 0.2µg/µl BSA in BC100)
0.4 µl	80 mM $MgCl_2$
0.4 µl	25 mM ATP
0.4 µl	0.5 M creatine phosphate (cp)
1 µl	H_20 or cold RNA
1 µl	radiolabeled RNA

Make pre-mix of all common items.
Always add radiolabeled RNA last.

2. Incubate binding reaction at 30 ºC for 20 minutes.
3. Pipet reactions into a 96 well U-bottom plate on ice. (keep top off plate)
4. Place handheld 254 nm lamp overtop and crosslink for 20 minutes
5. Pipet reactions into new eppendorf tubes that contain 20 U RNase T1 + 20 µg RNase A
6. Digest at 37 ºC for 20 minutes.
7. Add 13 µl 2x SDS sample buffer and 1.3 µl 2-ME
8. Boil samples 5 minutes and load on SDS-PAGE gel.

To Immunoprecipitate crosslinked sample, make following changes/additions:

1. After <u>crosslink</u> (step 4) or <u>digest</u> (step 6) pool 2-4 identical reactions in eppendorf tube
2. Bring volume to 195 µl with H_20
3. Add 4 µl antibody (plenty for 4D11 (anti-hnRNP L) or BB7 (anti-PTB))
   some other antibodies require more antibody and so decrease H_20 accordingly
4. Add 200 µl 2xRIPA buffer (see over for recipe)

5. Rotate 1 hr. at 4 ºC
6. Add 25 µl protein G-sepharose beads prewashed in 1x RIPA buffer

7. Rotate 1 hr. at 4 ºC

8. Spin down and wash 4 x 200 µl in 1x RIPA buffer

9. After last wash remove all but about 20 µl of buffer.

(9B. If IP was done <u>before</u> RNase digestion then add 20 U RNase T1 + 20 µg RNase A and digest 20 minutes at 37 ºC)

10. Add 20 µl 2xSDS sample buffer + 2 µl 2-ME

11. Boil samples 5 minutes and load on SDS-PAGE gel

<u>2x RIPA buffer</u>
20 mM Tris pH 8.0
2% NP40
10 mg/ml deoxycholate
4 mM EDTA
200 mM NaCl