mXBP-1 RT-PCR splicing assay with 19S & 14AS

(this protocol is for the mouse gene and is not predicted to work on human RNA)

A. RT Reaction : 20 ul in PCR tube

4 ul 2.5mM dNTPs
2 ug RNA
Oligo dT primer
4 ul 5X 1st strand buffer
ul DEPC H_2O ⟶ up to 17.5 ul

- Heat @ 70˚C 10 min
- Ice 1min
- Add 2 ul 0.1MDTT and 0.5 ul MLV-RT
- 42˚C 1.5 hr

- Add 30 ul H_2O and transfer to 1.5 ml tube
- 95˚C 10 min
- Ice 2min
- Spin

B. PCR :

- 5 ul diluted RT
- 10 pm mXBP1.19S (**5'** GGCCTTGTGGTTGAGAACCAGGAG 3')
- 10 pm mXBP1.14AS (**5'** GAATGCCCAAAAGGATATCAGACTC **3'**)
- 1.25 ul 2.5 mM dNTP
- 2.5 ul 10X rep buffer
- 0.25 ul Taq
- ul H_2O to 25 ul

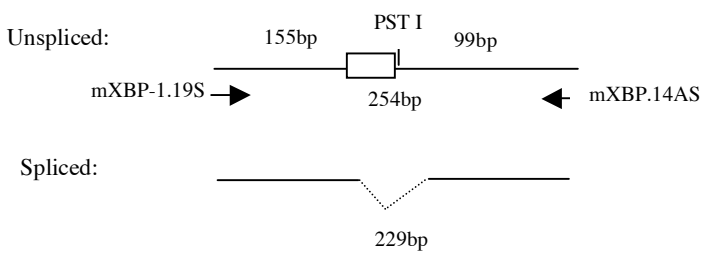
PCR program : 94˚C 4 min

94˚C 10 sec
68˚C 30 sec] X 35
72˚C 30 sec

72˚C 10 min

If products are run on a 2% 10-15cm gel the ligated and non-ligated products can be separated. Alternatively they can be digested with Pst I. (See figure below)

RT-PCR product



Mammalian XBP-1 RT-PCR splicing assay

(this protocol works for the human and mouse genes).

A. RT Reaction : 20 ul in PCR tube

4 ul 2.5mM dNTPs
2 ug RNA
10pM Oligo dT primer
4 ul 5X 1st strand buffer
ul DEPC H_2O ⟶ up to 17.5 ul

- Heat @ 70˚C 10 min
- 62˚C 2.5 min
- 42˚C 1.5 hr
  (once temperature has reached 42˚C, add 2 ul 0.1MDTT and 0.5 ul MLVRT)

- Add 30 ul H_2O and transfer to 1.5 ml tube
- 95˚C 10 min
- Ice 2min
- Spin

B. PCR :

- 5 ul diluted RT
- 10 pm hXBP1.3S (**5'** A AAC AGA GTA GCA GCT CAG ACT GC **3')**
- **or** mXBP1.3S (**5'** A AAC AGA GTA GCA GCG CAG ACT GC **3'**)
- 10 pm mXBP1.12AS* (**5'** TC CTT CTG GGT AGA CCT CTG GGA G **3')**
- 1.25 ul 2.5 mM dNTP
- 2.5 ul 10X rep buffer
- 0.25 ul Taq
- ul H_2O to 25 ul

*mXBP1.12AS works for both human and mouse

PCR program : 94˚C 4 min

94˚C 10 sec
63-68˚C 30 sec ⎫ X 35
72˚C 30 sec ⎭

72˚C 10 min

To distinguish the unspliced from the spliced band you can either digest with Pst I (which cuts only in the unspliced cDNA) or you can run a carefully prepared 2-3% gel that will resolve the two PCR products. (Note the mouse product is 3 BP longer than the human: