

Supplemental Protocol: Bacterial Lysis with PIXUL™ Multi-Sample Sonicator

Description: PIXUL Multi-Sample Sonicator makes bacterial lysis easy, to quickly check multiple cultures for recombinant protein expression.

IMPORTANT: PIXUL sonication requires using PIXUL 96-well round bottom plates (cat. no. 53139).

1. Nine MBP-target gene expression constructs in BL21(DE3) cells were grown in 2 mL LB medium with Ampicillin and agitation overnight at 37°C.
2. A 1:100 dilution of this overnight culture was then sub-cultured in 2 mL LB medium with Ampicillin and agitation for 2.5 hours at 37°C, then induced with 1 mM IPTG overnight at 25°C.
3. 750 µL of overnight culture were spun down, the supernatant discarded, and the pellet resuspended with 75 µL Bacterial Lysate Buffer.

Bacterial Lysate Buffer

50 mM Tris-HCl (pH 8.0)

100 mM NaCl

1 mM DTT

0.5 mM PMSF

1X Pepstatin (0.5 µg/mL)

1X Leupeptin (1.0 µg/mL)

0.1 µg/mL Lysozyme

3 U/mL Benzonase

4. Samples were incubated on ice for 20 minutes, then transferred to an Active Motif PIXUL™ 96-well Plate with Sealer (Cat. No. 53139), and sonicated with the PIXUL Multi-Sample Sonicator for 3 minutes at 10°C.
5. Samples were transferred into 1.5 mL centrifuge tubes and centrifuged at maximum speed for 15 minutes, then 75 µL of the supernatant were transferred to a fresh 1.5 mL centrifuge tube.
6. 75 µL 2X SDS-PAGE loading buffer were added into each supernatant sample, and 75 µL 1X SDS-PAGE loading buffer were added to each pellet samples. Samples were then heated for 5 minutes at 95°C.
7. 20 µL of supernatant samples and 10 µL of pellet samples were run on a 4-12% SDS-PAGE gradient gel at 200 V for 40 minutes, and the gel was stained with Instant Blue Coomassie Stain Solution.

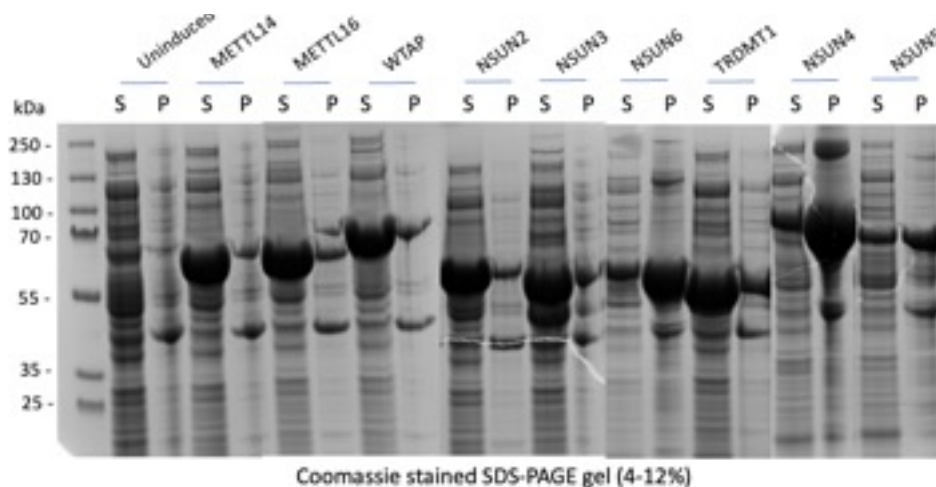


Figure 1. SDS-PAGE showing induction of various recombinant proteins in the supernatants (S) of nine different constructs. Lysis was performed by the PIXUL Multi-Sample Sonicator in 3 minutes.

Technical Support

If you need assistance at any time, please contact Active Motif Technical Support at the numbers or email for your region below.

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