# In vivo DMS treatment of cultured cells for rG4 microarray

(For use by rG4 microarray project customers)

Estimated time: 2–3 h

**Required Materials**

- 1 mL syringe with hypodermic needle
- 95% Ethanol (ice cold)
- 1X PBS (ice cold)
- 2-mercaptoethanol (ice cold)
- Dimethyl sulfate (DMS) (Sigma Cat# 320293). **Warning:** *DMS is highly toxic and a probable carcinogen. It does not have strong odor or irritation to alert exposure. It should be handled in chemical hood only.*

The DMS treatment is used to methylate the RNA bases in the unfolded regions while leaving the bases within the folded rG4 structure unmodified. The covalent RNA methylation patterns persist during RNA purification and fragmentation under denaturing conditions, thereby "fixing" the rG4 landscape at the moment of DMS treatment.

1. Grow 10 million cells under your experimental condition.

   **CRITICAL:** *The last media replacement must be at least 6 h before the DMS treatment to avoid potentially induced stress response that may impact rG4 dynamics. Samples should be processed immediately by swift cell harvesting, flash freeze, and using pre-chilled buffers.*

   **Note:** *Pre-chill 25% 2-Mercaptoethanol in PBS on ice. Keep the syringe, needle, ethanol, and 2-mercaptoethanol at ready in the chemical hood.*

2. Harvest the adherent cultured cells.

   a. Wash cells once with 8 mL 1X PBS buffer.
   b. Apply 6 mL 0.05% trypsin per 15 cm diameter dish.
   c. Incubate the dish in a 37 °C incubator for 5 min and neutralize the trypsin with 5 mL of cell culture media.
   d. Resuspend and transfer the cells to 15 mL Falcon tubes. Collect the cells by centrifugation at 300 *g* for 3 min.
   e. Aspirate the supernatant.
   f. Use 5 mL cell culture media to resuspend pellets in the tube for DMS treatment.
   g. Quickly transfer samples for DMS treatment in the chemical hood.


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3. DMS treatment.

**Warning:** *DMS is highly toxic and a probable carcinogen. It does not have strong odor or irritation to alert exposure. It should be handled with gloves on in chemical hood only. DMS liquid waste could emit toxic fumes. Carefully dispose of the waste in a designated hazardous chemical waste container.*

**Note:** *DMS solution bottle is sealed with a safety cap. Use a needle and syringe to handle the solution.*

a. Apply 400 uL of 50% DMS in ethanol to the cells.
b. Tightly close the Falcon tube caps.
c. Hold the tubes with a gloved hand for 7 min, gently inverting the tubes to homogenize the solution. Place the tube on ice.
d. Add 1.3 mL 2-Mercaptoethanol to neutralize the DMS. Gently invert the tubes a few times.
e. Spin down cells at 400 *g* for 3 min at 4°C.
f. Discard the supernatant and resuspend the pellet with 1 mL ice-cold 25% 2-mercaptoethanol in PBS.
g. Collect cells by centrifugation at 400 *g* for 3 min at 4°C.
h. Repeat the wash steps e to f once.
i. Snap-freeze the cells on dry ice. The cell pellets can be stored at -80°C until RNA extraction.

4. Total RNA extraction

With the DMS treated cells, you may:

Proceed to extract the total RNA using standard TRIzol method, and send the total RNA to Arraystar for rG4 microarray.


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