

## **Technical Points About Plasmid-Mediated Transfection or RNAi-Mediated Gene Knockdown in Methyl-BSA-Elicited Macrophages Using the Amaxa Nucleofector® System**

(Dr. Yankun Li, September, 2005)

- Use the cell dissociation buffer from Invitrogen. It takes 30-60 min to get the macrophages off the dish (and sometimes it doesn't work at all), but the cells remain healthy. NB: The Nucleofection method does not work on freshly harvested peritoneal cells in suspension.
- When spinning down the cells, use the table top centrifuge in the tissue culture room at a speed of 1200 rpm for 10 min. (With higher speed, the cells become aggregated.)
- While spinning, set the Nucleofector program to Y-001, label Eppendorf (Eppi) tubes, and aliquot the plasmid DNA or siRNA (Qiagen) into the tubes.
- Resuspend the cells into the appropriate amount of Amaxa macrophage transfection buffer (premixed with supplement buffer!). Use 1 million cells in 100  $\mu$ l buffer per reaction.
- Mix the cell suspension with the plasmid DNA or RNAi in the Eppi tube first, then transfer to the cuvette (do not discard the Eppi tube). Tap the cuvette gently to get rid of air bubbles.
- After electroporation, add 400  $\mu$ l warm full media to the cuvette, use the plastic bubble to transfer the suspension back to the Eppi tube, put the tube into the cell culture incubator for 10-15 min, and then plate the cells as usual. Change medium after 5-6 h, then every 24 h.
- You should conduct a dose curve of your construct to see how much gives you the maximum transfection efficiency.