



# HOKKAIDO UNIVERSITY

Title	A DNA Microarray-based Analysis of the Host Response to a Nonviral Gene Carrier: A Strategy for Improving the Immune Response
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## **Supplementary Materials and Methods**

### **Materials**

Male ICR mice (5-6 weeks old) were purchased from CLEA (Tokyo, Japan). ELISA assay kits of Quantikine Immunoassay mouse INF- $\gamma$  was purchased from R&D systems (Minneapolis, MN, USA). [ $^3\text{H}$ ]cholesteryl hexadecyl ether (CHE), Soluene-350 and Hionic Flour were purchased from Perkin-Elmer Life Sciences, Japan (Tokyo, Japan). Other materials were purchased as described Materials and Methods section. Transaminase CII-test WAKO was obtained from Wako (Osaka, Japan).

### **Methods**

#### **Determination of serum biochemical value and cytokine**

ALT levels in serum were measured with test kits, and IL-6, IFN- $\gamma$ , TNF- $\alpha$ , and IFN- $\alpha$  levels in serum were determined with ELISA kits according to the manufacturer's instructions.

#### **Accumulation of systemically administered NPs in the spleen**

A lipid film was prepared in a glass test tube by evaporating a chloroform solution of lipids, containing DOTAP, DOPE and cholesterol (300 nmol total lipids in 3:4:3 molar

ratio). [<sup>3</sup>H]CHE was also added in lipid solution as a tracer (Stein Y, Halperin G, Stein O. Biological stability of [<sup>3</sup>H]cholesteryl oleyl ether in cultured fibroblasts and intact rat. *FEBS Lett* 1980; **111**: 104-106.). The modification of PEG-DSPE and chol-GALA and subsequent encapsulation of pDNA/PEI complex were performed as described in Materials and Methods section. NPs labeled with [<sup>3</sup>H]CHE were intravenously injected at a normal pressure (25 µg pDNA/mouse). At 2 hr after i.v. injection, spleen was collected and solubilized in 1 ml of Soluene-350 at 42°C for 12 hr. The radioactivities were determined by liquid scintillation counting, after adding 10 ml of Hionic Flour.