Commercially available lipid-based transfection reagents are widely used to deliver DNA to cells. However, these lipid-based transfection reagents show poor gene transfer efficiency in primary cells. Here, we demonstrate a simple method to improve gene transfer efficiency in primary fibroblasts and hepatoblasts using a combination of lipid-based transfection reagents. Our data show that combined use of Lipofectamine LTX and FuGENE HD increases the efficiency of gene transfer compared with the use of either reagent alone, and this combination achieves the best result of any pairwise combination of Lipofectamine LTX, FuGENE HD, TransFectin, and Fibroblast Transfection Reagent.
transfer efficiency of Lipofectamine LTX is increased by the addition of Plus reagent to diluted DNA in serum-free DMEM (Figure 1B). In our experiments, addition of Plus reagent also up-regulated the efficiency of gene transfer induced by transfection with a combination of Lipofectamine LTX and FuGENE HD (Figure 1B). Comparing pairwise combinations of transfection reagents, we confirmed that the best efficiency of gene transfer was achieved through the combined use of Lipofectamine LTX and FuGENE HD (Figure 1C).

We also tested the combination of Lipofectamine LTX and FuGENE HD for transfection of primary hepatoblasts. Although the efficiency of gene transfer in primary hepatoblasts was elevated by the reagent combination (Figure 2A), this increase was not as dramatic as that seen in primary fibroblasts (Figure 1A). We observed that the reagent combination reduced viability more than transfection with FuGENE HD alone in primary hepatoblasts (Figure 2A), unlike in primary fibroblasts (Figure 1A). According to manufacturers’ instructions and previous reports (7,8), cell density (cell confluency) can influence cytotoxicity and gene transfer efficiency when using lipid-based reagents. Cell density may affect cellular processes such as growth rate and endocytosis. Therefore, we tried the combination of Lipofectamine LTX and FuGENE HD for transfection under the different experimental conditions by increasing the density of primary hepatoblasts on Day -1 from 1 × 10⁴ to 2 × 10⁴ cells/well in a 96-well dish. Under these conditions, cell viability was minimally reduced, and gene transfer efficiency was further elevated by transfection with the reagent combination (Figure 2B).

Primary cells are more suitable for investigations into normal cellular physiology than transformed or immortalized cell lines since cell lines often show genetic and phenotypic differences from the cells that they originated from (9,10). Although the transfer of foreign genes in cells is important for analyzing the biological functions of the genes, primary cells are difficult to transfect with DNA when using lipid-based reagents (1-3). Previous studies have presented several methods for gene transfer in primary cells, such as recombinant viral vectors, electroporation, and nucleofection (11,12). Gene delivery using viral vectors is efficient in most mammalian cell types, but viral vectors are randomly integrated into the host genome, resulting in undesired effects such as insertional mutagenesis (11). Moreover, the need to harvest recombinant viral vectors from packing cell lines takes substantial time and involves safety risks (1,2,11). Electroporation and nucleofection (i.e., improved electroporation with cell type-specific solutions) use electrical pulses to create transient pores in plasma membranes (1,12). Although these methods can directly deliver DNA through the transient pores into cells, special devices are required to generate the electrical pulses (1,12).

The transfection protocol for this study uses a combination of two commercially available lipid-based reagents, Lipofectamine LTX and FuGENE HD, to increase the efficiency of gene transfer in primary fibroblasts and hepatoblasts without the need for any specialized equipment.

The use of a lipid/DNA complex for transfection was introduced by Felgner et al. (13): a single plasmid is surrounded by cationic lipids to neutralize the negative charge of DNA so that the lipid/DNA complex can

Figure 1. Effects of transfection of primary fibroblasts with different reagents. (A) pRL-CMV (0.5 μg) was mixed with 0–2 μl Lipofectamine LTX and/or FuGENE HD for transfection of primary fibroblasts. Renilla luciferase activity or cell viability was determined after 24 hours (n = 3). (B) pRL-CMV (0.5 μg) was mixed with 0 or 0.5 μl Plus reagent and then with 0–2 μl Lipofectamine LTX alone or with FuGENE HD for transfection of primary fibroblasts. Renilla luciferase activity was determined after 24 hours (n = 3). (C) pRL-CMV (0.5 μg) was mixed with 0–2 μl Lipofectamine LTX, FuGENE HD, TransFectin or Fibroblast Transfection Reagent (in the amounts and combinations indicated on the x-axis) for transfection of primary fibroblasts. Renilla luciferase activity was determined after 24 hours (n = 3). P-values were determined using Student’s t-test.

Figure 2. Effects of transfection of primary hepatoblasts with different reagents. On Day -1, primary hepatoblasts were seeded in wells of a 96-well dish: (A) 1 × 10⁴ cells/well and (B) 2 × 10⁴ cells/well. On Day 0, 0.5 μg of pRL-CMV was mixed with 0–2 μl Lipofectamine LTX and/or FuGENE HD for transfection. Renilla luciferase activity or cell viability was determined 24 hours later (n = 3). P-values were determined using Student’s t-test.
associate with the negatively charged surfaces of cells. Lipid-mediated gene transfer depends on several factors such as net charge and the structure of the lipid/DNA complex (7). Lipid-based transfection reagents are composed of three domains: a positively charged head group, a hydrophobic region, and a linker that tethers the polar and non-polar regions. Formation of the lipid/DNA complex is a dynamic event because of the unstable interactions resulting from (i) the electrical attraction of DNA to the polar region of lipid; (ii) hydrophobic attraction of lipid to lipid via the non-polar region; and (iii) electrical repulsion of DNA by DNA (7,14). Therefore, the lipid/DNA complex is presumed to form several different multilamellar lipid-DNA structures (7,14). There is much interest in designing novel reagents carrying DNA or to improve the application of already available reagents for better gene transfection efficiency in a variety of cells by modifying the interactions between DNA and its carriers (15). We hypothesized that lipid/DNA complex formation may be optimized for transfection in primary fibroblasts and hepatoblasts using the combination of Lipofectamine LTX and FuGENE HD. However, the precise mechanism remains unknown because the chemical structures of these reagents have not been published by their manufacturers.

In conclusion, we present a simple method for efficient gene transfer in primary fibroblasts and hepatoblasts based on the combined use of Lipofectamine LTX and FuGENE HD. Addition of the Lipofectamine LTX Plus reagent is a useful option to further increase the efficiency of gene transfer.

Author contributions
This study was designed by K.I., H.G., and Y.H. The experiments using primary fibroblasts were performed by K.I., O.W., M.N., and Y.T. The experiments using primary hepatoblasts were performed by K.I. and M.M. K.I. wrote the paper. All authors approved the final manuscript.

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Competing interests
All authors declare no conflict of interest.

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