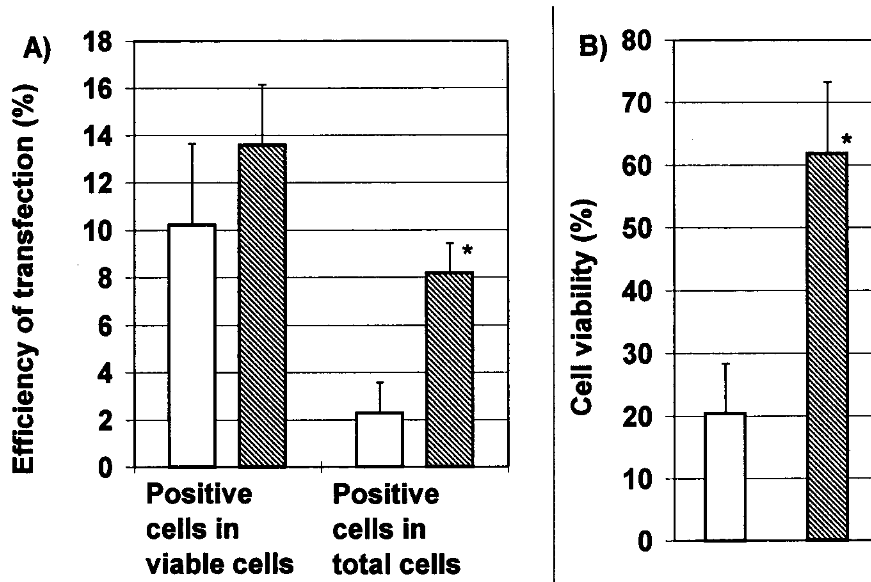

Fig. 3. Efficiency of transfection and cell viability after post-pulse separation of dead and viable cells. 24 h after electroporation with phGFP-S65T the HL-60 cells were overlaid onto a Ficoll-Paque discontinuous gradient and centrifuged at 350 x *g* for 30 min at R.T. The cells were collected and transferred to growth medium for continuous cultivation followed by FCM analysis. A) Efficiency of transfection before (void column) and after post-pulse separation (hatched column), B) cell viability before (void column) and after post-pulse separation (hatched column). The cell viability was determined as in Fig. 1. The data shown represent the mean from six independent experiments ± SEM.
*P < 0.01 as compared with not post-pulse separated cells (Student's t-test).

GFP on HL-60 cells was detected 48 h after electroporation as a decrease of transfection efficiency determined by GFP. These conclusions may also be supported by the corresponding appearance of blisters on the GFP-positive cell surface (data not shown).

One of the general problems of transient gene transfection of cells growing in suspension is a high percentage of dead cells in this suspension after the pulse. To increase the yield of viable cells in the sample we used gradient centrifugation with Percoll (according to the manufacturer's instruction for blood cells) or Ficoll-Paque (both from Pharmacia, Little Chalfont, Buckinghamshire, UK). Figure 3 compares the results of the analysis of crude and separated populations 24 h after the pulse. It is shown that separation using the Ficoll-Paque gradient increases the yield of transfection-positive (4 times; Fig. 3A) and viable (3 times; Fig. 3B) cells per total cells. However, using the Percoll gradient we did not obtain applicable results (data not shown).

In summary, in this report we studied the different electroporation conditions for HL-60 cells. We concluded that the best efficiency of transfection (about 12–14% of positive cells) could be reached in the following conditions: exponentially growing cells, 250–270 V and about 1000 μF in RPMI medium as the electroporation buffer, and 10–20 μg plasmid per pulse. Finally, an electroporated suspension of HL-60 cells may be efficiently separated into living and dead cells using the Ficoll-Paque reagent.

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