serts in blue and white colonies is not completely clear, but one possibility is that the pZErO vector permits expression of LacZα-ccdB at non-lethal levels even with insertion of foreign DNA and that this "leakiness" is reduced with longer inserts.

To further verify that blue-white selection provides a higher yield of tags per concatemers, we analyzed data from an independent library in which concatemers from random colonies were sequenced with or without blue-white selection. For the 323 concatemers that were sequenced without prior blue-white selection, the number of recovered tags per concatemer averaged 3.9. In contrast, the yield rose by 3.5-fold to an average of 13.5 tags per concatemer for 328 concatemers that had been subjected to blue-white selection.

In summary, we find that the addition of X-Gal and IPTG to bacterial plates and the use of blue-white selection significantly enhance the yield of SAGE concatemers following transfection of DH10B bacteria with pZErO vector. This avoids the need for the more time-consuming prescreening of colonies by agarose gel electrophoresis and results in a substantial enhancement in the efficiency with which SAGE concatemer libraries can be sequenced.

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Rapid and Efficient Method for Suspending Cells for Neurotransmitter Uptake Assays

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Platelets are a convenient source of serotonin uptake transporters (SERTs) for studying the biochemical and pharmacological characteristics of serotonin transport across the plasma membrane (10). Serotonin accumulation by platelets depends on extracellular Na^+ and Cl^- and is inhibited by cold temperature and drugs such as fluoxetine and cocaine (7,9,12). Molecular cloning has revealed that the amino acid sequence of the human platelet SERT protein is identical to the neuronal SERT (8), making the platelet a good model for neuronal serotonin uptake and a useful material in which to study drugs that modify serotonin homeostasis. We have previously reported using human platelets to study psychoactive drugs that act at the SERT (2–4). Because of known differences in the efficacy and potency of drugs acting at the human SERT compared to the SERT obtained from other species such as rodents or insects, data obtained in the human platelet system are more relevant to human pharmacology.

Human platelets can be obtained fresh or, more commonly, as outdated blood bank donor platelets. While outdated platelets may not be equivalent in all respects to fresh platelets, they do retain the ability to accumulate serotonin in a temperature- and drug-sensitive manner (4,13,14). In a typical procedure, after pooling the platelets from several donors, the cells are pelleted by centrifugation. The P1 pellet is washed several times by floating the pellet in a balanced electrolyte buffer, followed by centrifugation. The final pellet is resuspended by manual pipetting. Manual pipetting involves the aspiration and ejection of buffer and cell pellet fragments through a pipet tip (11,15); this process is repeated as many times as needed to obtain a uniform suspension. The shear forces resulting from the movement of liquid through the pipet tip cause the cell pellet to break up into

smaller and smaller pieces until a homogeneous cell suspension is eventually obtained. Manual pipetting is also used for suspending cells, such as human embryonic kidney 293 (HEK 293) cells, grown in tissue culture (5). For some cells types, only a few passes through the pipet tip are sufficient to disperse the cells evenly. For other cells, including platelets, resuspension by manual pipetting can take several minutes and tens or hundreds of passes through the pipet tip to obtain a uniform suspension. The resulting suspension is usually not truly homogeneous by visual inspection, but generally contains small clumps of cells. If sufficiently large aliquots of this suspension are used in an uptake experiment, the aggregates are relatively evenly distributed and the variability of the data among replicates is not a concern. However, as the volume of the aliquots is decreased, variability among replicates increases because there can be a significant difference in the amount of biological material among replicate aliquots due to the presence of clumped cells. To solve this problem and to shorten the time required for resuspending cells, we have developed a method of resuspension using a motor-driven rotor/stator-type tissue homogenizer. Our method results in significant timesavings and ensures a uniform distribution of cells for neurotransmitter uptake experiments. When we compared data using manual suspension with suspension using the homogenizer, we found that low motor speeds and brief exposure times gave results that were less variable and just as robust as data obtained from cells suspended manually. Using a pharmacological approach, we also confirmed that the storage of serotonin by platelets prepared according to our method occurred via the SERT. Uptake was almost completely blocked by the SERT inhibitor fluoxetine, but not by the vesicle inhibitor reserpine.

Our protocol for the assay of serotonin uptake into platelets is as follows: outdated human donor platelets were obtained from the blood bank at Pitt County Memorial Hospital (Greenville, NC, USA). Platelets from 10 donors were pooled, DMSO was added to 10% volume, and 6-mL aliquots were frozen and stored at -80°C until use. On the day of an experiment, an aliquot of platelets was thawed and added to 20 mL ice-cold Krebs-Ringer-HEPES (KRH) buffer containing 124.0 mM NaCl, 2.9 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 5.2 mM *d*-glucose, 25.0 mM HEPES, 0.1 mM sodium ascorbate, 0.1 mM pargyline, pH 7.4. The pargyline and ascorbate are present to prevent degradation and metabolism of the [^{3}H]serotonin ([^{3}H]5-HT) used in the experiment. The initial platelet suspension was subjected to centrifugation at 4340× *g* for 10 min at 4°C, and the supernatant was discarded. The P1 cell pellet was washed twice by floating it in 20 mL ice-cold KRH, without breaking up the pellet. To compare the motor-assisted method to manual resuspension, the final P3 pellet was dispersed in 20 mL ice-cold KRH either manually or by using a motor-driven homogenizer at different speed settings and for various times. For manual resuspension, the P3 pellet was broken up by repeatedly pipetting the pellet and buffer through a 5-mL pipet tip using a Rainin P5000 Pipetman® (Rainin Instrument, Woburn, MA, USA). The procedure took 5–10 min before a visually uniform suspension was achieved. This suspension invariably contained small aggregates of cells. For resuspension with the motor-driven blender, we used a Brinkman/Kinematica benchtop model PT 10/35 (Polytron®) equipped with a model PTA-10S 12-mm diameter standard generator (Brinkmann Instruments, Westbury, NY, USA). We did not observe clumps of cells following resuspension with the homogenizer, even at the lowest speed setting and shortest time interval. After resuspending the platelets by either method, the suspensions were assayed for protein content using the procedure of Bradford (1). The ability of the cells to accumulate [^{3}H]5-HT was tested by adding a 495-µL aliquot of the platelet suspension to glass tubes in an ice bath; all assays were performed in triplicate. [^{3}H]5-HT creatinine sulfate (specific activity 25.5 Ci/mmol; New England Nuclear, Boston, MA, USA) was added to all tubes while they remained in the ice bath (5 µL stock solution in water; 10 nM final concentration), giving a

Table 1. Specific [3H]5-HT Uptake, fmol/min/mg Protein[a]

Resuspension Method	Motor Speed Setting 4	Motor Speed Setting 6
Homogenizer, 6 s	286.9 ± 71 (78%)	154.7 ± 27 (69%)
Homogenizer, 12 s	267.2 ± 63 (75%)	121.0 ± 26 (58%)
Homogenizer, 36 s	178.6 ± 35 (63%)	56.3 ± 6[b] (40%)
Manual pipetting	219.3 ± 67 (73%)	

[a]Platelets were suspended in buffer as described. Values are the mean ± SEM of 3–6 experiments, each done in triplicate. Numbers in parentheses indicate what fraction of total uptake is represented by the specific uptake values.
[b]$P < 0.05$ vs. manual pipetting.

final volume of 500 µL. Some tubes were then incubated in a 37°C shaking water bath for 5 min to initiate [3H]5-HT uptake. This condition defines "total" uptake. "Nonspecific" uptake was defined by leaving some assay tubes in the ice bath; "specific" uptake is defined as uptake at 37°C minus uptake at 0°C. Uptake was quenched by returning the "total" tubes to the ice bath. After adding 3 mL ice-cold 150 mM NaCl, each assay tube was immediately vacuum-filtered through glass fiber filters (Whatman GF/B; Whatman International, Kent, UK) pretreated with 0.1% polyethyleneimine. Filters were washed with 2 × 3 mL ice-cold saline, allowed to dry briefly under vacuum, and then placed in liquid scintillation vials. Scintillation cocktail was added (4 mL; ScintiSafe™ 30%; Fisher Scientific, Pittsburgh, PA, USA), and the vials were sealed, vortex mixed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard Tri-Carb 2200CA; Canberra Industries, Meriden, CT, USA).

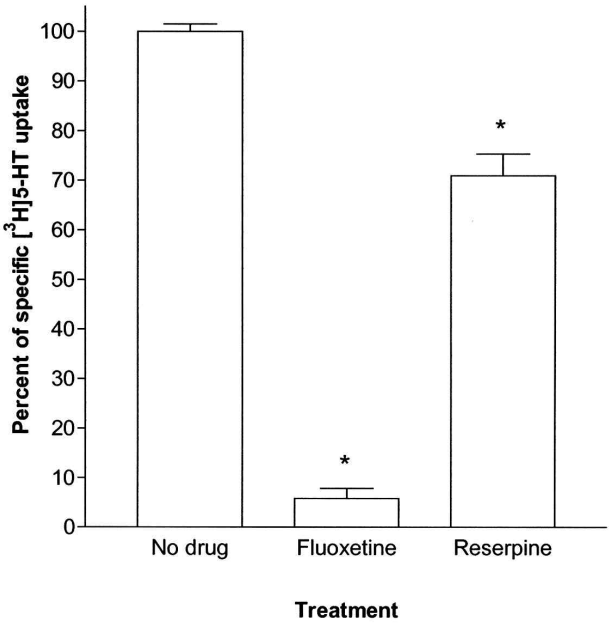

Figure 1. Effect of fluoxetine or reserpine on [3H]5-HT uptake. Platelets were prepared as described in the text and preincubated with no drug, 1 µM fluoxetine, or 1 µM reserpine before initiating [3H]5-HT uptake. Specific uptake was defined as uptake at 37ºC minus uptake at 0ºC, in the absence of drugs. Specific uptake was 82% of total radioactivity. *$P < 0.01$ versus no drug.

To verify that the accumulation of [3H]5-HT was due to typical SERT activity, we pharmacologically characterized the uptake process using the SERT uptake inhibitor fluoxetine and the synaptic vesicle monoamine transporter (VMAT) inhibitor reserpine. The ability of platelets to store [3H]5-HT in the presence of drugs was tested as follows: platelets were resuspended using the homogenizer at speed setting 6 for 10 s, and then 490-µL aliquots were dispensed into assay tubes containing 5 µL KRH (for total and nonspecific determinations) or 5 µL 100 µM test drug stock solution. The assay tubes were preincubated with the test drugs in a 37°C shaking water bath for 10 min and then returned to the ice bath. After 15 min, 5 µL [3H]5-HT stock solution were added, and the rest of the assay was performed as explained above. The final volume of the incubations was thus 500 µM, and the final concentrations of fluoxetine or reserpine were each 1 µM.

In experiments comparing manual with motor-assisted resuspension, data were normalized for protein content and transformed from dpm to fmol/min/mg protein. Data from 3–6 experiments were combined for statistical analysis and are presented as the mean ± SEM. Multiple comparisons between motor-driven homogenizer treatments and manual resuspension were made using one-way ANOVA followed by Dunnett's *t* test, with $P < 0.05$ considered significant.

Uptake in the presence of fluoxetine or reserpine is expressed as the percent of specific uptake and represents the mean ± SEM of triplicate assessments; multiple comparisons between drug treatments and specific uptake in the absence of drugs were performed as described above, with $P < 0.05$ considered significant.

Table 1 summarizes the results of experiments comparing motor-assisted with manual resuspension. Specific uptake ranged from a low of 40% of total uptake (speed setting 6 for 36 s) to a high of 78% of total uptake (speed setting 4 for 6 s). When cells were resuspended using the homogenizer for 36 s

at setting 6, there was a significant decrease in the amount of [^{3}H]5-HT accumulated ($P < 0.05$) compared to manual suspension (Table 1). None of the other motor speed settings or resuspension times were significantly different from the manual pipetting method, although there was a trend at both speed settings for decreased uptake as the platelets were exposed to the homogenizer for longer times. The uptake data obtained from cells that were resuspended manually were more variable (larger standard deviation) than the data obtained from motor-assisted resuspension. The variability likely arises from the difficulty in achieving a homogeneous suspension by manual pipetting, as discussed earlier.

Figure 1 shows drug effects on [^{3}H]5-HT uptake. In this experiment, specific uptake represented 82% of total uptake. Both fluoxetine and reserpine decreased the amount of [^{3}H]5-HT stored by the platelets ($P < 0.01$). However, the selective SERT uptake blocker fluoxetine decreased [^{3}H]5-HT accumulation by 94%, whereas the VMAT blocker reserpine decreased uptake by only 29%. The reserpine-sensitive [^{3}H]5-HT uptake is most likely due to storage of [^{3}H]5-HT in platelet dense granules. The dense granule storage mechanism exhibits the characteristics of VMAT-mediated transport, such as sensitivity to reserpine, dependence on ATP and a proton gradient, and Na^+ independence (6). Nevertheless, these data demonstrate that the accumulation of [^{3}H]5-HT by platelets processed as described here is largely due to SERT-mediated uptake, not uptake into platelet dense granules.

In summary, these results establish that resuspending washed platelets with a motor-driven homogenizer at moderate speeds for 6–12 s does not adversely affect SERT-mediated [^{3}H]5-HT accumulation. We now routinely use this procedure for resuspending platelets for [^{3}H]5-HT uptake experiments. We have also used the technique described here to suspend HEK 293 cells expressing the human SERT, the human dopamine transporter, and the human norepinephrine transporter with similar results. Our method results in a considerable savings in preparation time and does not noticeably impair transporter function, producing data that are less variable than data obtained with manually resuspended cells.

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