Supplementary Note 1: Protocol optimization using modified Taguchi methods

Based on the initial experiments, we determined that the tagmented nuclei needed additional purification. Specifically, we determined that the majority of cross-contamination was due to free transposase adapters in the tn5, as well as unconsumed tn5 transposase, acting as internal PCR primers during the 2nd round of indexing. However, initial experiments with washing by centrifugation resulted in very poor recovery of tagmented nuclei. Consequently, we performed a series of optimization experiments to a) increase overall nuclei recovery, and b) wash out both debris from lysed nuclei and unconsumed adapters using modified Taguchi methods [1, 2, 3]. Experiments were designed to test both pre-tagmentation nuclei isolation conditions, tagmentation conditions, and different washing methods. The latter included washing with centrifugation, conjugating the nuclei to solid phase for washing, bait hybridization capture of the adapter sequences, and a decoy tagmentation oligo to consume unused transposase. All of these methods were compared to the gold standard of flow-assisted nuclei sorting (FANS). We used L_8 arrays for nuclei recovery optimization, and an L_{16} array for the post-tagmentation purification [2, 3]. After performing the experiments, we used level average analysis as described in [3] to deconvolute both the main effects as well as interactions between various factors. As can be seen in Table SN2, we settled on a HEPES-based isolation buffer (NIB-H) for pre-tagmentation nuclei isolation, with the tagmentation buffer being TAPS based (for better high temperature buffering performance), with the addition of glucosamine (10mM), TMA-Acetate (2mM), and Pluronic f-127 (0.1%). Post-tagmentation, we determined optimal centrifugation conditions include >=15% Glycerol as a cushion, using a DMF-free version of the tagmentation buffer.

For washing, we determined that the bait hybridization was technically the superior method (Table SN1), however with the addition of 15% Glycerol in the centrifugation step, nuclear recovery was sufficient to allow washing by centrifugation. Since the time and consumable cost was much lower using centrifugation compared to using biotinylated capture baits, we chose to use the simpler method for the remaining experiments in this paper.

Supplementary Note Table 1: Comparing post-tagmentation clean-up methods

Condition	**Level 1**	**Level 2**	**Fold Change**
Decoy	0 uM*	2uM	1.45
Baits	10uM	20uM*	1.70
Hybridization Time	5:00	10:00	1.07
Hybridization Temperature	4°C	22°C*	1.25
Volume C1 Dynabeads	1uL*	2uL	1.35
Binding time	5:00	15:00*	1.325
Total Improvement			2.157

Supplementary Note Table 2: Improving nuclei recovery

Condition	Level 1	Level 2	Fold Improvement
Buffer	NIB-HEPES*	NIB-2.0 (NaOAc + MgOAc)	1.57
Glucosamine	10mM*	20mM	1.57
Tetramethylammomium Acetate (TMAOAc)	2mM*	10mM	1.67
Pluronic F-127	0%	0.1%*	1.23
Total Improvement			1.40

Supplementary Note References:

1. Taguchi, Genichi, Subir Chowdhury, and Yuin Wu. *Taguchi's quality engineering handbook*. John Wiley & Sons, 2004.
2. Taguchi, Genichi. "Table of orthogonal arrays and linear graphs." *Rep. Stat. Appl. Res., JUSE* 6 (1960): 1-52.
3. Peace, Glen Stuart. *Taguchi methods: a hands-on approach*. Addison Wesley Publishing Company, 1993.