



PLATEMASTER®: HIGH THROUGHPUT, MANUAL NGS LIBRARY PREPARATION

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ABSTRACT

The Stefan-Morsch-Stiftung (Foundation) screens the human leucocyte antigen (HLA) gene complexes of potential stem cell donors to identify potential organ donors for leukemia patients. The larger the bone marrow registry and the quicker a donor can be identified, the better the chances for a successful transplant. Next-generation sequencing (NGS) protocols are essential in routine HLA typing due to their accuracy, cost, and throughput.^{1,2} To maximize the throughput of manual NGS library preparation, the 96-channel Gilson PLATEMASTER® pipetting system can be easily integrated into existing workflows.

METHODS

In this experiment, the accuracy, reproducibility, and total pipetting time required to complete an established HLA NGS workflow was compared between the Gilson PLATEMASTER and an 8-channel pipette.

ACCURACY AND REPRODUCIBILITY

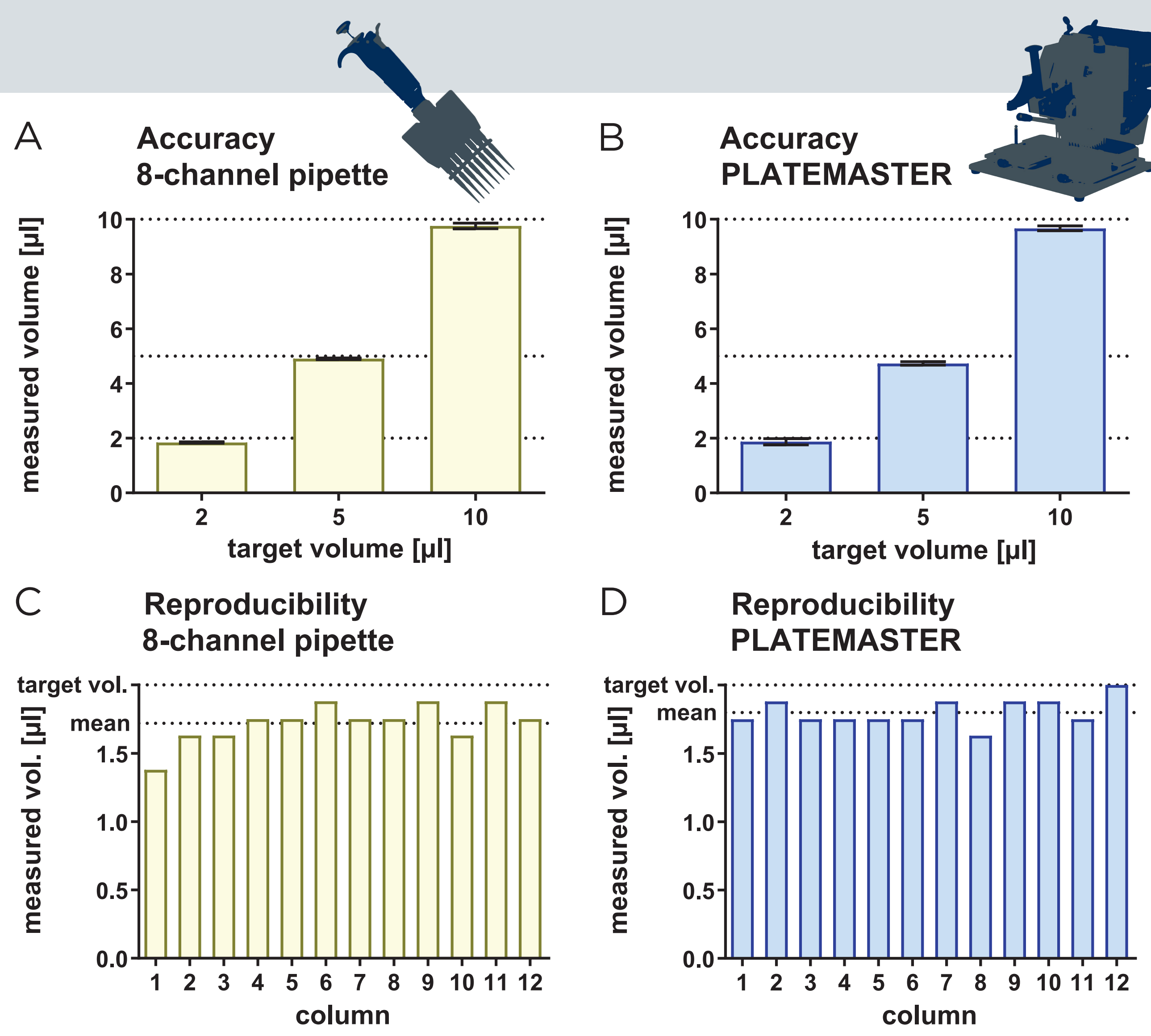


Figure 1 Pipetting accuracy was measured gravimetrically at three separate volumes in triplicate with the 8-channel pipette (A) and PLATEMASTER® (B). Pipetting reproducibility was tested by measuring a transferred volume of 2 µL across 12 columns of a 96-well plate.

CONCLUSION

In NGS library preparation workflows, when compared to an 8-channel pipette, PLATEMASTER:

- Maintains a high degree of accuracy and reproducibility
 - Saves a significant amount of time
 - Reduces tedious pipetting steps by a factor of 12, minimizing any possibility of cross-contamination
- ▶ PLATEMASTER provides the highest throughput possible, without the use of potentially expensive robotic devices



PIPETTING TIME COMPARISON

8-channel pipette	Time	Step	Time PLATEMASTER
1104 sec		Long range PCR	204 sec
1296 sec		PCR-Pooling	515 sec
		Purification	
184 sec		Fragmentation	34 sec
		Purification	
184 sec		DNA-End-Repair	34 sec
		Adapter Ligation	
		Purification	
184 sec		Index-PCR	34 sec
		IPCR-Pooling	
184 sec		Quantification	34 sec
		Library-Pool	
		MiSeq	
52 min, 16 sec	total pipetting time	14 min, 15 sec	
192	pipetting steps	16	

Figure 2 NGS workflow scheme (192 samples, 6 loci). Required time for pipetting steps (bold) was compared using an 8-channel pipette (left) or the PLATEMASTER® (right). Using PLATEMASTER® reduced the number of pipetting steps by a factor of 12, and decreased time spent pipetting by 73%, when compared to an 8-channel pipette.

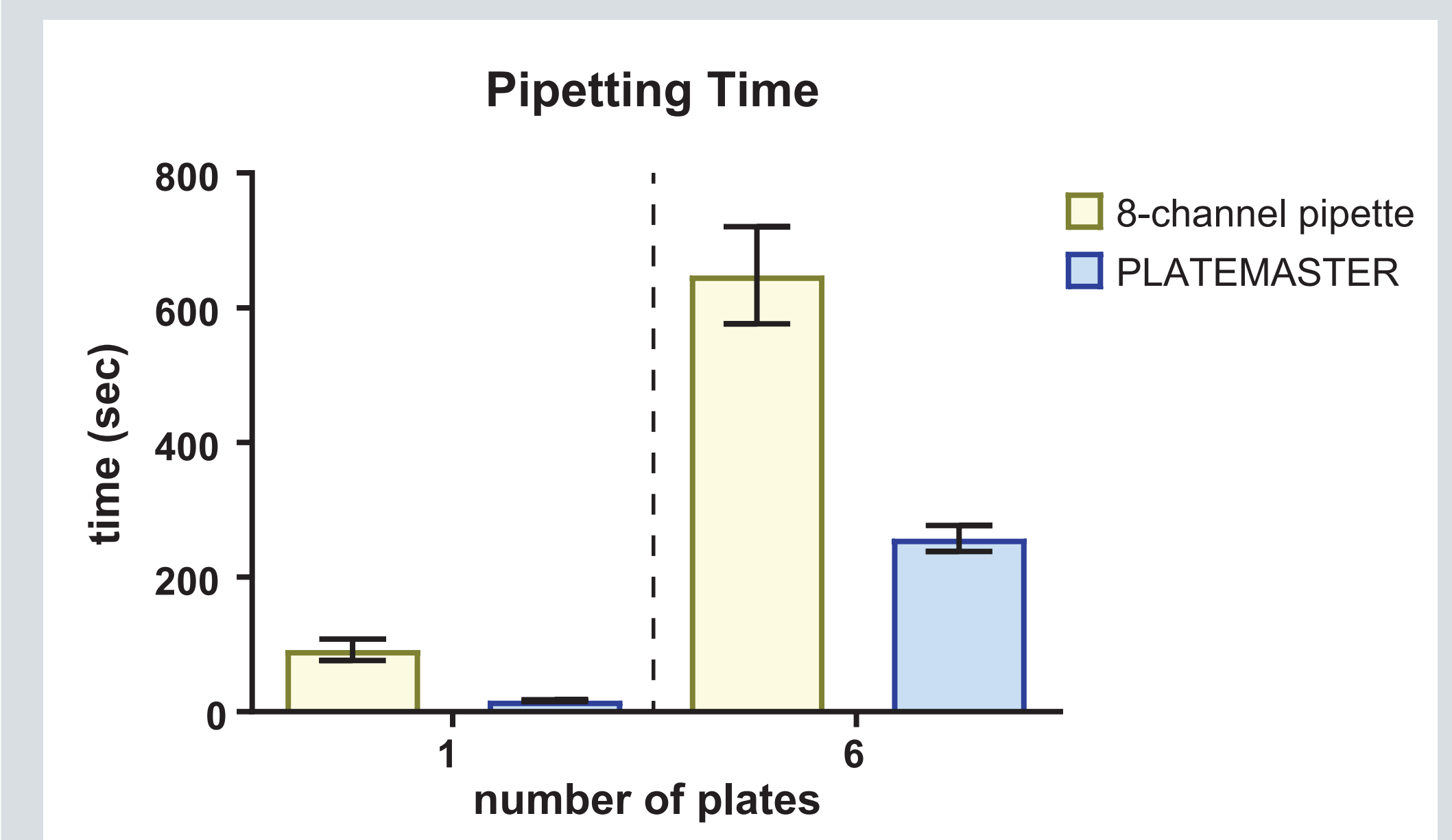


Figure 3 The time spent preparing one and six 96-well plates using an 8-channel pipette and PLATEMASTER®.

REFERENCES

Lan, J. H. and Zhang Q., Clinical application of next-generation sequencing in histocompatibility and transplantation. *Curr Opin Organ Transplant*, 20, 461 - 467 (2015).
Chapman, M., Warren, E. H. III, Wu, C. J., Application of Next Generation Sequencing to Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*, 18, S151 - S160 (2012).



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