

96 CHANNEL ACCURACY

MANUAL NEXT GENERATION SEQUENCING LIBRARY PREPARATION

HLA-Laboratory of the Stefan-Morsch-Stiftung in Birkenfeld, Germany

Introduction

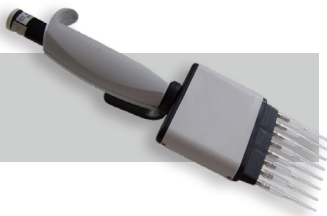
Next generation sequencing (NGS) became essential in routine HLA typing due to it's efficiency in context of resolution, costs and throughput. To get a grip on the required multiple pipetting steps during NGS library preparation, we integrated the manual 96-channel PlateWizard™ (Axon) pipetting system into our NGS workflow.

Methods

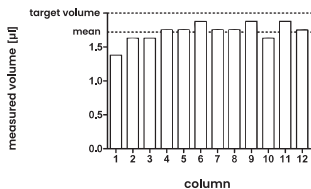
To qualify this manual 96 channel pipetting device, we compared its performance to a conventional 8 channel pipette in terms of:

- Pipetting accuracy (weighing transferred volumes)
- Reproducibility
- Totally required pipetting time within a HLA NGS workflow

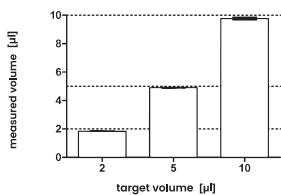
8 Channel Pipette



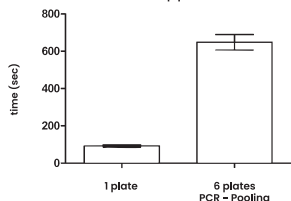
Reproducibility 8 channel pipette



Accuracy 8 channel pipette



Time Investment 8 channel pipette



NGS workflow

(192 samples, 6 loci)

Long range PCR

1104 sec

PCR-Pooling

1296 sec

Purification

Fragmentation

184 sec

Purification

DNA-End-Repair

184 sec

Adapter ligation

Purification

Index-PCR

184 sec

IPCR-Pooling

Quantification

184 sec

Library-Pool

MiSeq

204 sec

515 sec

34 sec

34 sec

34 sec

34 sec

52 min, 16 sec

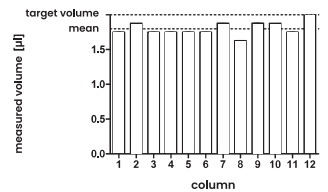
total pipetting time

14 min, 15 sec

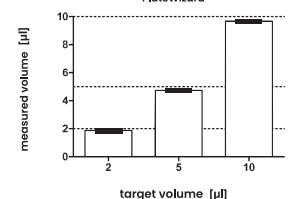
mPlateWizard™



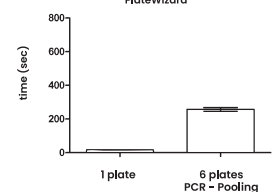
Reproducibility PlateWizard™



Accuracy PlateWizard™



Time Investment PlateWizard™



Conclusion:

Advantages of mPlateWizard™

- Higher reproducibility
- Same level of accuracy
- Significantly less time required
- Higher throughput is possible without the need of expensive robotic devices