

High-throughput automation of LC-MS sample preparation: The SP3-iST workflow on the Freedom EVO® 100 and the Resolvex® A200

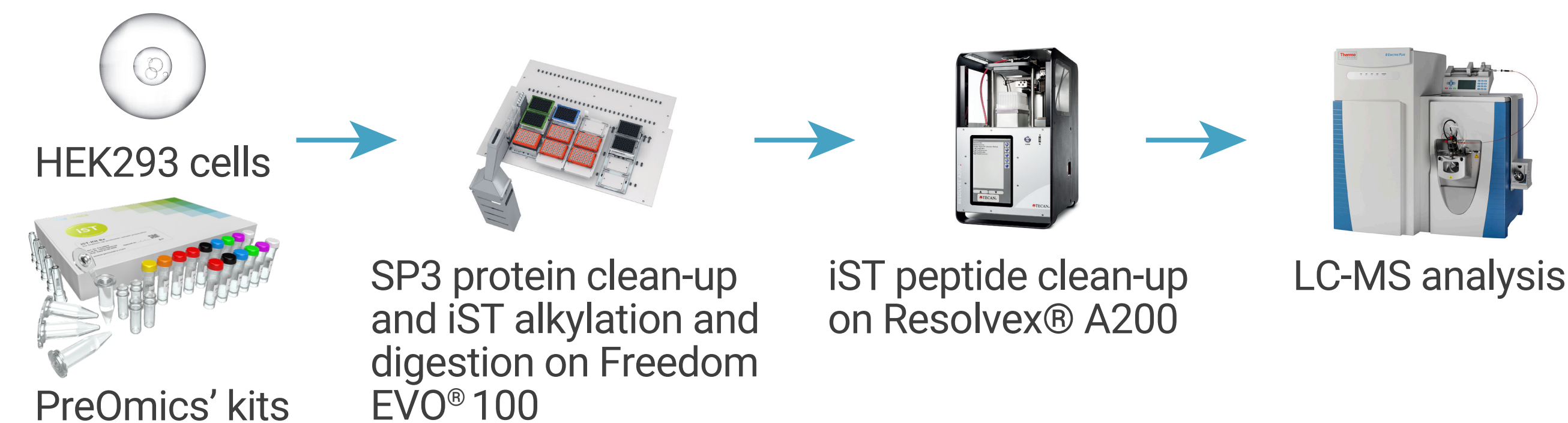
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Automation of SP3-iST workflow

- Automated solution for LC-MS sample preparation on Freedom EVO® 100 and Resolvex® A200
 - Combination of versatile SP3 workflow and proven, high-quality iST technology
- Reproducible, standardized and high-throughput LC-MS sample preparation

Materials & Methods



- Input:** 15 µg, 25 µg or 50 µg of previously lysed HEK293 cells.
- Workflow:** Sample preparation was done employing the iST-PSI and the SP3-iST Add-on kits (both PreOmics). Automated SP3 protein clean-up and iST alkylation and digestion was implemented on the Freedom EVO® 100 (Tecan) and automated iST peptide clean-up was performed on the Resolvex® A200 (Tecan; 'Automated SP3-iST'). As control for the automated protocol on the Freedom EVO® 100, SP3 protein clean-up and iST alkylation and digestion was performed manually in parallel with 50 µg protein input ('Manual SP3-iST').
- LC-MS analysis:** Easy nLC 1000 coupled to QEplus instrument.
- Data analysis:** Progenesis Q1 software and MASCOT database search.

Results

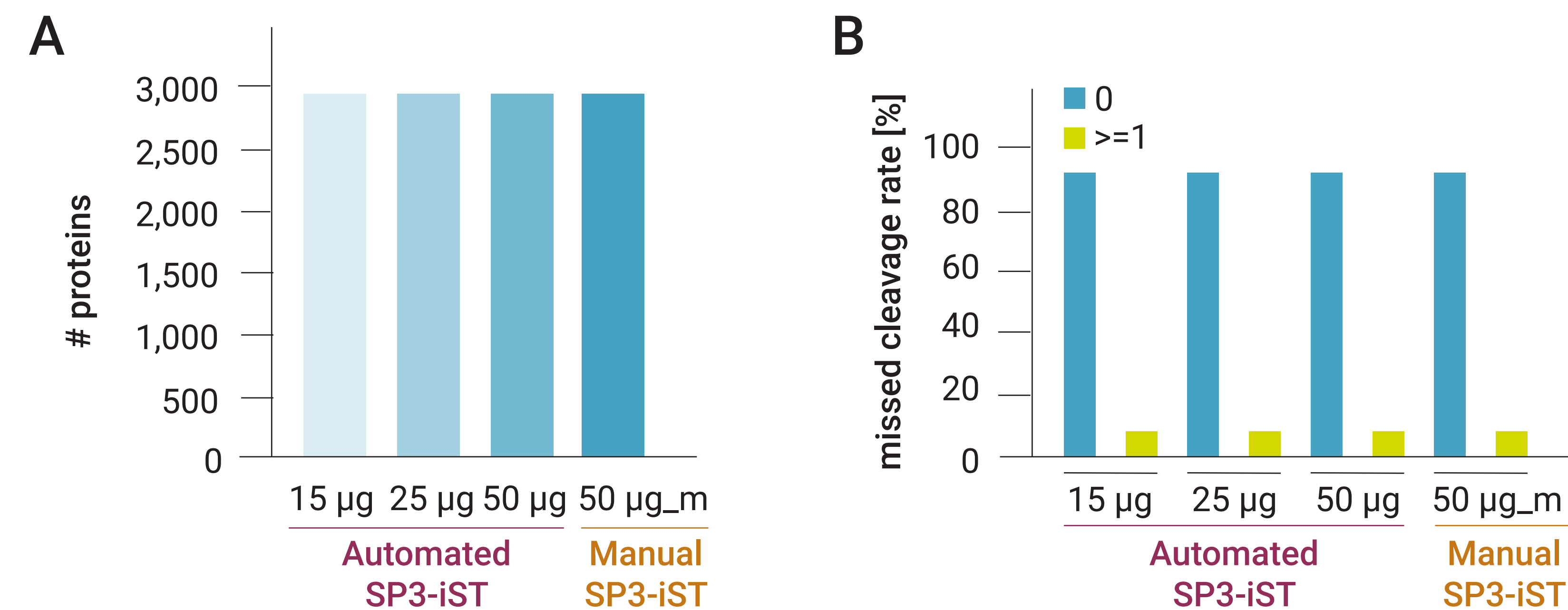


Fig. 1| Performance of the automated SP3-iST workflow with different input amounts (6 replicates each) and the manual SP3-iST workflow (3 replicates). High consistency in protein IDs (A) and percentage of missed cleavages (B).

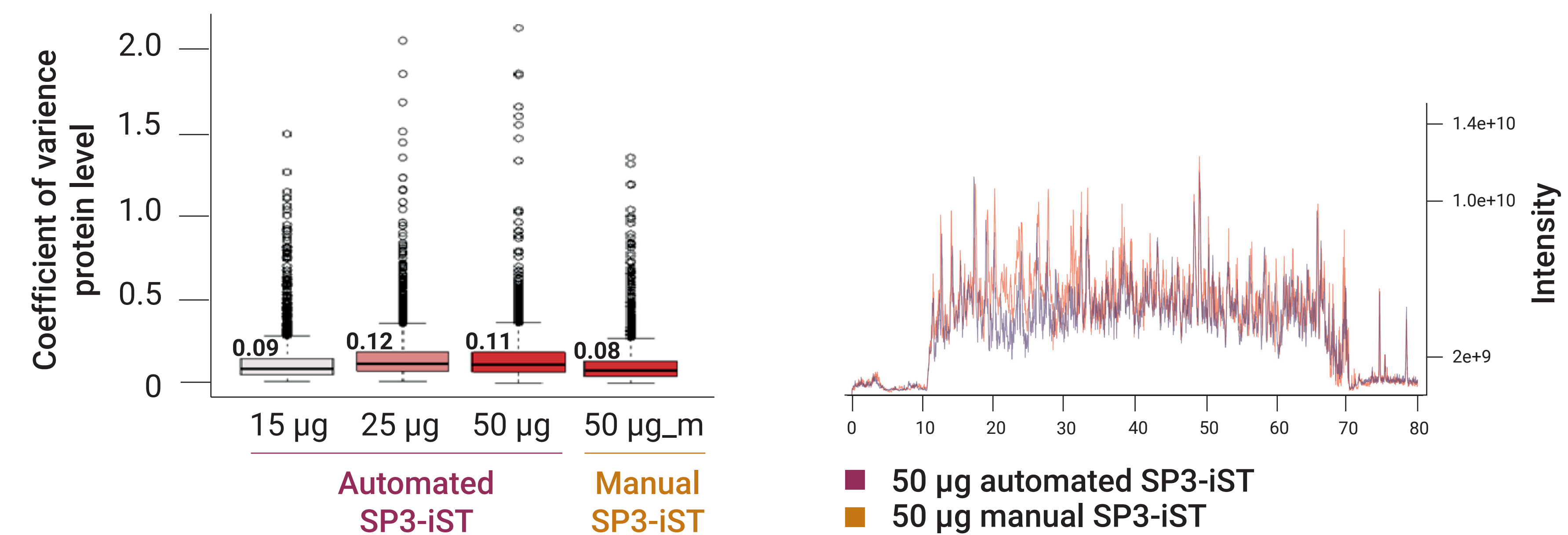


Fig. 2| Technical variability of replicates for automated SP3-iST workflow (6 replicates each) and for manual SP3-iST workflow (3 replicates each).

Fig. 3| High consistency in chromatograms for automated SP3-iST and manual SP3-iST workflow with 50 µg input each.

Discussion

- ~3000 proteins IDs from HEK293 cells
- Excellent digestion efficiency (< 10 % total missed cleavage)
- Low technical variability
- High consistency between different protein input amounts

Key features of workflow

- High-throughput application: 96 samples within 5 hours
- Highly automated: ~20 minutes hand-on time
- Compatible with large variety of lysis buffers
- Excellent sample quality
- Ease-of-use & script-based programming