

High-throughput nucleic acid extraction in biobanking: performance assessment of the chemagic Prime instrument in large-scale population studies.

Introduction

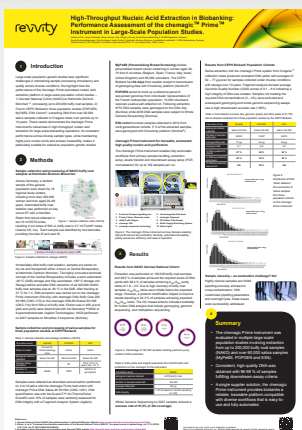
Large-scale population genetic studies face significant challenges in maintaining sample processing consistency and quality across diverse conditions. We highlight here the performance of the chemagic™ Prime™ automated nucleic acid extraction platform in large-scale population cohort studies – 1) German National Cohort (NAKO) at Helmholtz Zentrum München^{1,2}, processing up to 200,000 buffy coat samples, 2) French CEPH Biobank's three population studies (POPGEN, MyPeBS, E3N Cohort)³, extracting DNA from over 60,000 saliva samples collected in Oragene tubes over periods up to 10 years. These results demonstrate the chemagic Prime instrument's robustness in high-throughput nucleic acid extraction for large-scale biobanking operations. Its consistent performance across diverse sample types, while maintaining highly pure nucleic acids and process traceability, makes it particularly suitable for extensive population genetic studies.

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Special acknowledgements:

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Methods

Sample collection and processing of NAKO buffy coat samples at Helmholtz Zentrum Muenchen

Across Germany, a random sample of the general population were drawn by 18 regional study centers, including more than 200,000 women and men aged 20–69 years. Automated buffy coat isolation was performed on day one at RT with a Hamilton robot from blood collected in two 10 ml EDTA tubes, resulting in six tubes of 500 uL buffy coat in 0.7 ml FluidX tubes (Azenta US, Inc). Each sample was identified by two barcodes providing the tube ID and rack ID.



Figure 1: Sample collection sites (NAKO).

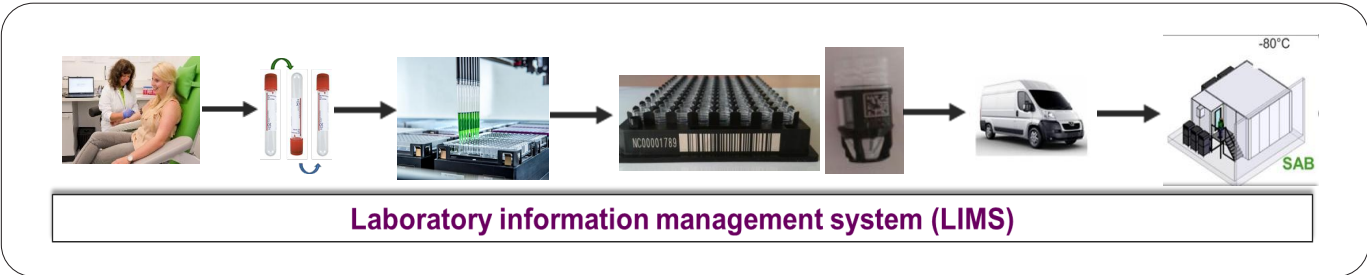


Figure 2: Sample collection to storage (NAKO).

Immediately after buffy coat isolation, samples are stored on dry ice and transported within 4 hours to Central Biorepository at Helmholtz Zentrum München. The highly innovative technical concept of the Central Biorepository includes a semi-automated -80 °C (SAB) storage and fully-automated -180 °C storage unit. Storage before and after DNA extraction of all 200,000 NAKO buffy coat samples was at -80 °C in the SAB. After thawing at 37 °C

for 1 h, DNA extraction was carried out on the chemagic Prime instrument (Revvity) with chemagic DNA Buffy Coat 200 Kit H96 (CMG-713) or the chemagic DNA BC/Saliva Kit H96 (CMG-714) from 500 µl of buffy coat. Elution was in 400 µl and yield and purity were determined with the Nanodrop 8000 or 8 spectrophotometer (Agilent Technologies). WGS performed on 2227 samples on NovaSeq X sequencer (Illumina).

Sample collection and processing of saliva samples for three population studies at CEPH Biobank

Table 2: Sample collection and storage conditions (CEPH).

Population study	MyPeBS	POPGEN	E3N
Place of collect	Prevention center/radiology clinic	Home	Home
Biological material collected	Saliva OG-600	Saliva OG-600	Saliva OG-500
Storage before extraction	-	RT	RT/4 °C for 10 years then -30 °C
Number of samples extracted	26959	10227	24570 (5 % genotyped)

Samples were collected as described and extraction performed on 4 ml of saliva with the chemagic Prime Instrument with chemagic Prime DNA Saliva 4K Kit H24 (CMG-1481). DNA quantification was with the Quant-iT kit (ThermoFisher Scientific) and 10% of samples were randomly assessed for DNA integrity with a Fragment Analyzer System (Agilent).

MyPeBS (Personalising Breast Screening) studies personalized breast cancer screening in women aged 40- 70 from 6 countries (Belgium, Spain, France, Italy, Israel, United Kingdom) with 56,000 volunteers. The CEPH Biobank had 84 days from sample receipt to transmission of genotyping data with OncoArray platform (Illumina®).

POPGEN aimed to build up a reference panel of sequenced genomes from individuals' representative of the French metropolitan population. 15,000 volunteers received a saliva self-collection kit. Following extraction, 9772 DNA samples were genotyped on the GSA chip (Illumina) while 4000 DNA samples were subject to Whole Genome Sequencing (Illumina).

E3N cohort involved samples collected in 2010 from multi-generational cohorts. 5 % of the extracted samples were genotyped with OncoArray platform (Illumina®).

chemagic Prime Instrument for traceable, automated high quality nucleic acid purification

The chemagic Prime Instrument enables fully automated workflows from primary sample handling, extraction setup, eluate transfer and downstream assay setup (PCR, normalization) for up to 192 samples per run.

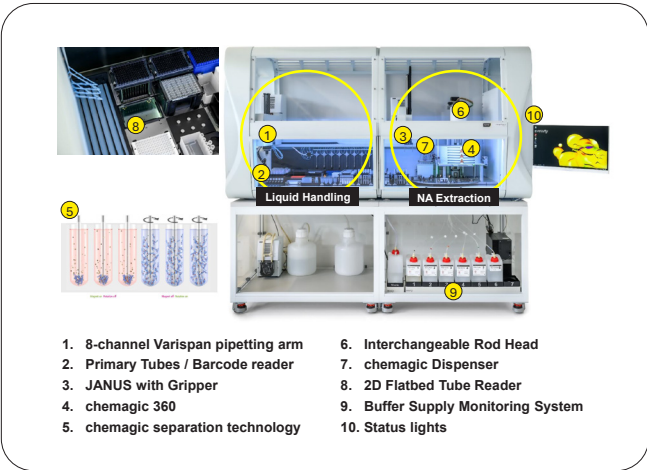


Figure 3: The chemagic Prime instrument and key features enabling high performance NA purification, flexibility, automated traceability, quality assurance, efficiency and ease of operation.

Results

Results from NAKO German National Cohort

Extraction was performed on 182,520 buffy coat samples and 99.5 % of samples achieved the required levels of DNA yields with 98.4 % of samples achieving A_{260}/A_{280} purity ratios of 1.8 – 2.0. Due to high viscosity of buffy coat samples, A_{260}/A_{230} ratios were initially below the expected range. However, a protocol modification helped to improve results resulting in 94.3 % of samples achieving expected A_{260}/A_{230} ratios. The QC measurements indicated suitability for further DNA analysis that include genotyping, genomic sequencing, and methylation sequencing.

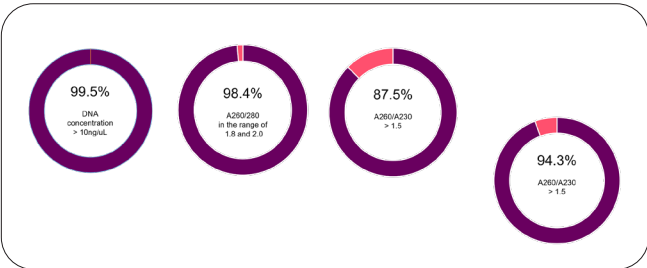


Figure 4: Percentage of 182,520 samples meeting yield and purity criteria of DNA extraction.

Table 3: DNA yields and integrity assessed from NAKO buffy coat extractions on the chemagic Prime instrument.

Population study	NAKO
Biological material collected	EDTA Buffy Coat
Number of samples extracted	182,520
Average DNA yield	88 μg
DNA integrity	> 20 kb based on random sampling (n=192)

Whole Genome Sequencing on 2227 samples showed a success rate of 97,2% ($\geq 30x$ coverage).

Results from CEPH Biobank Population Cohorts

Saliva extraction with the chemagic Prime system from Oragene collection tubes produced consistent DNA yields with averages of 62 – 77 μg even for samples collected under diverse conditions with storage over 10 years. Fragment analysis showed average Genomic Quality Number (GQN) scores of 6.1 – 6.6 indicating a high integrity of DNA was isolated. Samples not meeting the required DNA concentrations (3 – 4%) were excluded and subsequent genotyping and whole genome sequencing assays saw a high downstream success rate (~99%).

Table 4: Downstream success rate, genomic quality and DNA yields of 61,756 saliva samples extracted from three population studies by the CEPH Biobank

Population study	MyPeBS	POPGEN	E3N
Biological material collected	Saliva OG-600	Saliva OG-600	Saliva OG-500
Number of samples extracted	26959	10227	24570
Average DNA yield	77 µg	62 µg	66 µg
Average GQN	6.1	6.6	6.4
% DNA qualified for GWAS	97 %	96 %	96 %
% DNA successfully genotyped	99 %	99 %	99 %
% DNA successfully sequenced	NA	99 %	NA

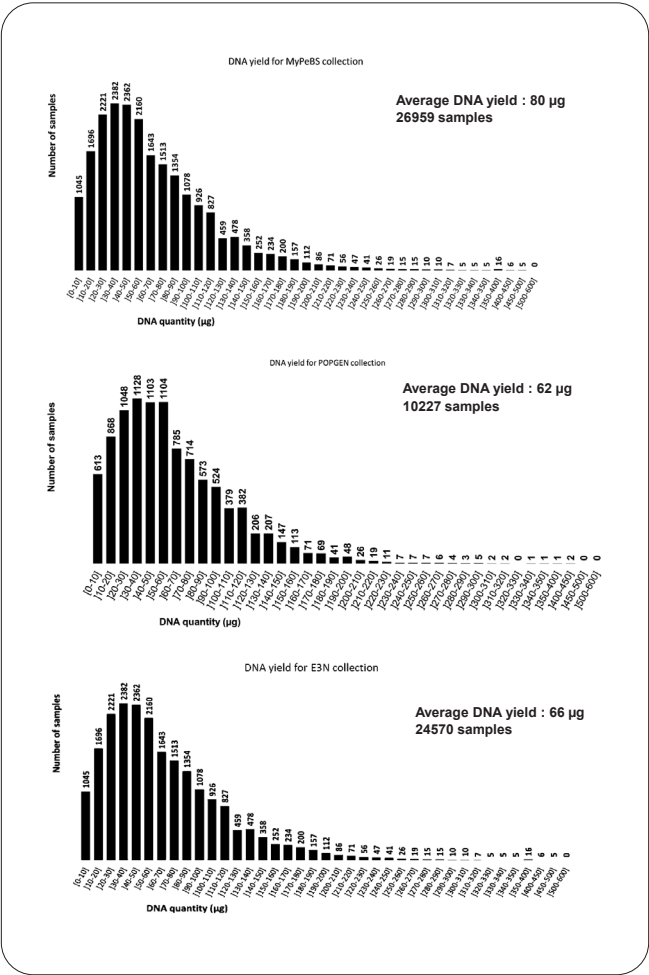
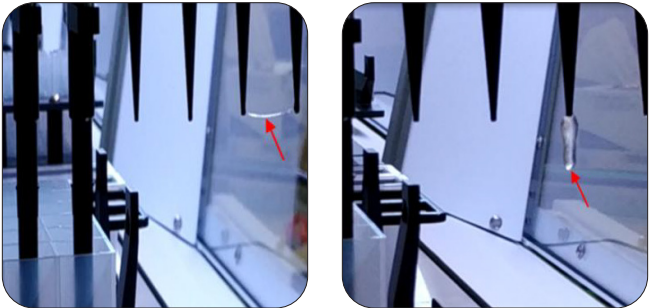


Figure 5: Distribution of DNA Yields obtained from extraction of Saliva samples from three population cohorts on the chemagic Prime instrument.

Sample viscosity – an automation challenge? No!

Highly viscous samples can inhibit pipetting accuracy and lead to cross-contamination. With customized pipetting parameters and overnight lysis, these issues were successfully addressed.

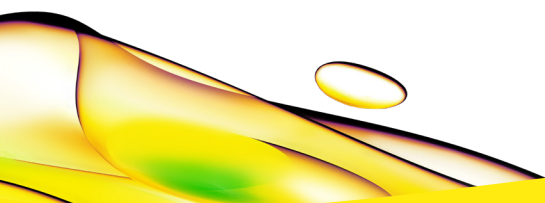


Summary

- The chemagic Prime instrument was evaluated in multiple large-scale population studies involving extraction from up to 200,000 buffy coat samples (NAKO) and over 60,000 saliva samples (MyPeBS, POPGEN and E3N).
- Consistent, high-quality DNA was obtained with 96-98 % of samples fulfilling downstream assay criteria.
- A single supplier solution, the chemagic Prime instrument provides biobanks a reliable, traceable platform compatible with diverse workflows that is easy-touse and fully automated.

References:

1. German National Cohort (NAKO Gesundheitsstudie) on Helmholtz München Website <https://www.helmholtz-munich.de/en/epi/cohort/nako>
2. Peters, A. et al. "Framework and baseline examination of the German National Cohort (NAKO)." European journal of epidemiology vol. 37,10 (2022): 1107-1124.
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3. Population cohort information on Foundation Jean Dausset-CEPH website <https://www.fjd-ceph.org/crb-du-ceph/projets-collaboratifs>



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