# ONtrack: chimerism monitoring employing third-generation sequencing

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## Introduction

- Post-haematopoietic stem cell transplantation (HSCT) chimerism monitoring is pivotal for early detection of relapse and guiding timely therapeutic interventions.
- ONtrack is based on NGStrack (GenDx) for chimerism monitoring using Oxford Nanopore Technologies (ONT) sequencing (Figure 1), enabling a fast and flexible workflow (Table 1).
- The performance of an ONtrack prototype was assessed by testing chimerism accuracy, both at GenDx and at external sites.

**Table 1.** ONtrack characteristics

| | |
|---|---|
| Markers | 34 in 2 mixes |
| Sample processing | 1-96 samples/run |
| Sensitivity | Up to 0.5% |
| Turnaround time (single sample) | 3 hours |
| Turnaround time (28 samples) | 24 hours |
| Hands-on time | <2 hours |

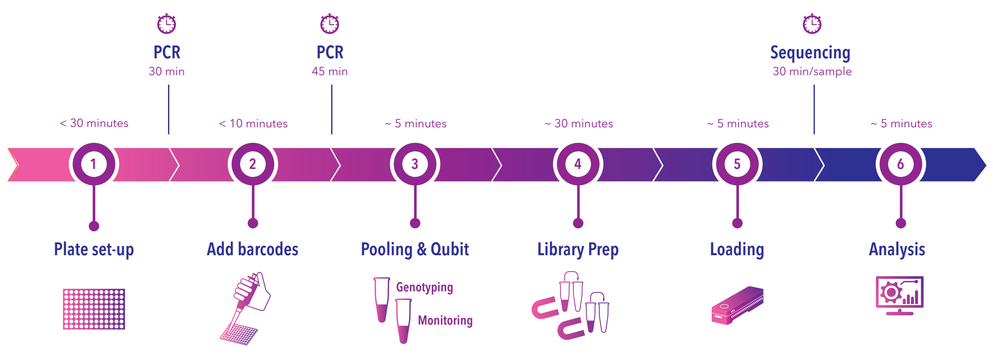


**Figure 2. ONtrack workflow.** 1. Markers are amplified with two mixes; 2. unique barcodes are added per sample; 3. separate amplicon pools are created for genotyping and for monitoring; 4. both pools are prepared as ONT-ready libraries; 5. libraries are mixed and sequenced on an ONT flow cell. 6. genotyping and monitoring analysis is performed in TRKengine.

## Trueness

- ONtrack chimerism (%) results are highly comparable to UKNEQAS robust mean and NGS reference method (Figure 3), as shown for External Proficiency Testing samples as well as patient samples.
- ONtrack quantifies true chimerism percentages in samples isolated from blood, bone marrow and T cell fractions.
- For double transplants, ONtrack facilitates determination of chimerism% of both recipient as well as the1st donor (Figure 3C-D).

## Reproducibility

- ONtrack shows high reproducibility in chimerism levels for a low- and high-chimerism sample, showing the reliability of the assay (Figure 4A).
- Noise patterns between sites match the associated chimerism patterns (Figure 4B-C), indicating that site-specific noise levels are the main factor of variation in measured chimerism.

## Conclusion

- ONtrack is a fast and flexible method for post-HSCT monitoring.
- The ONtrack prototype generates accurate chimerism results:
  - ONtrack is highly comparable to chimerism monitoring assays currently used in clinical practice.
  - ONtrack results are reproducible between sites.


**Figure 1. Principle of ONtrack.** Bi-allelic indel markers are amplified and sequenced. First, pre-transfer samples are typed as long-long, short-short (homozygous) or long-short (heterozygous) for every marker. Next, the chimerism percentage in post-transfer samples is determined using the informative markers.

## Methods

- For all studies ONtrack was performed according to the workflow in Figure 2.
- Trueness was studied by measuring three sample sets with ONtrack and comparing to reference results:
  1. UKNEQAS samples (n=10); reference: UKNEQAS robust mean.
  2. SFHI EQA samples (n=15); reference: NGS-based IVD; both methods performed at EFS Marseille (Immunogenetics Laboratory, Établissement Français du Sang PACA-Corse, Marseille, France).
  3. Patient samples (n=11 recipient chimerism and n=3 first-donor chimerism after second transplant): five blood samples, four bone marrow samples, two CD3 samples; collected from patients with acute myeloid leukemia and granulomatous disease; reference: NGS-based IVD; both methods performed at EFS Marseille.
- Reproducibility of ONtrack was studied using two artificially mixed samples measured at GenDx and four external sites.

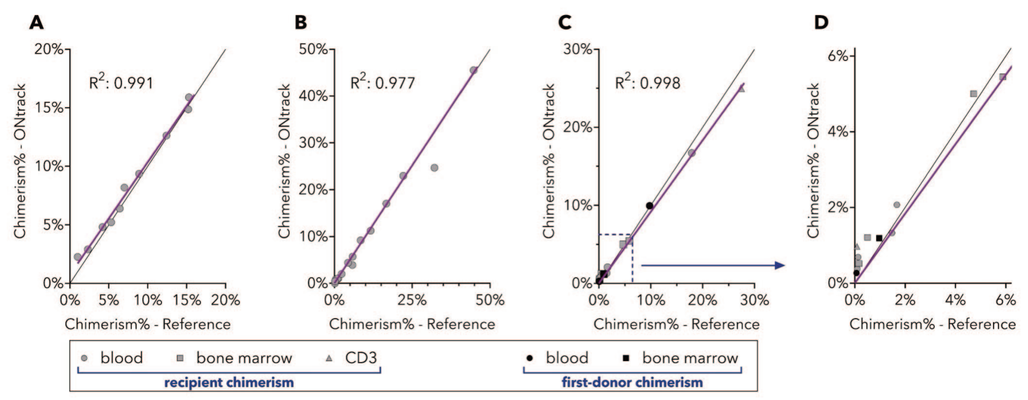


**Figure 3. Trueness.** ONtrack results were highly comparable to reference results. Purple line: Passing& Bablok linear regression fit; black line: y=x diagonal. A) UKNEQAS samples; B) SFHI EQA samples; C) Patient samples; D) zoom-in of C.

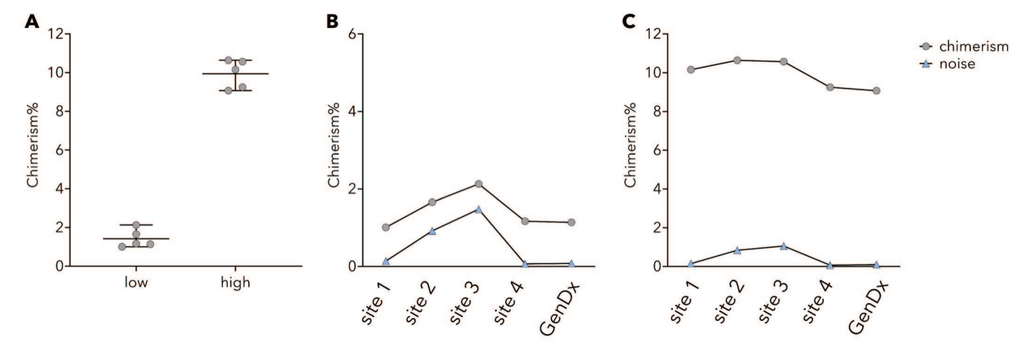


**Figure 4. Reproducibility.** A) small variation in measured chimerism (%) results between sites for "low" and "high" sample. Similar levels of chimerism (gray) and associated noise (blue) measured by each site for the low (B) and high (C) samples.

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