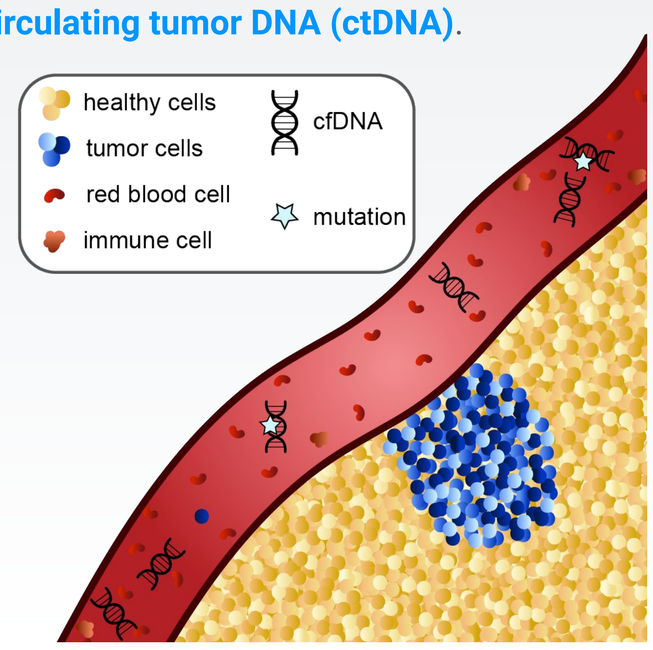
CyclomicsSeq a sensitive liquid biopsy genetic test

Real-time and cost-efficient cancer monitoring in blood

1. Circulating tumor DNA

Cancer genomes contain genetic variations, which are caused by a combination of mutation accumulation and selection during tumor evolution. When tumor cells die, their DNA is broken into small fragments of around 170 bp, which can be released into the bloodstream or other body fluids (liquid biopsies), and is known as **circulating tumor DNA (ctDNA)**.

Since normal cells also shed DNA into the bloodstream, a small proportion of the DNA in the blood of a cancer patient consists of ctDNA fragments. The amount of ctDNA in the blood and the mutation content form important biomarkers for tumor recurrence and response to cancer treatment.



2. Challenges in ctDNA sequencing

There are three **major challenges** with respect to the detection of genetic mutations in the circulating tumor DNA in blood:

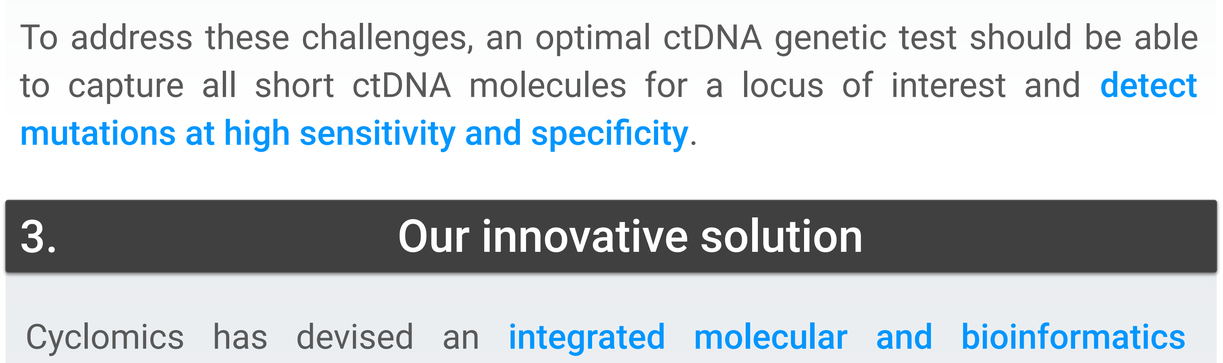
1. Only a small amount of free circulating DNA is present in a tube of blood.
2. Only a tiny fraction of the free circulating DNA in blood is coming from tumor cells, usually 1% or less. Therefore, cancer mutations at any genetic locus are only present at low frequency.
3. ctDNA fragments are small, typically around 170 bp.

To address these challenges, an optimal ctDNA genetic test should be able to capture all short ctDNA molecules for a locus of interest and **detect mutations at high sensitivity and specificity**.

3. Our innovative solution

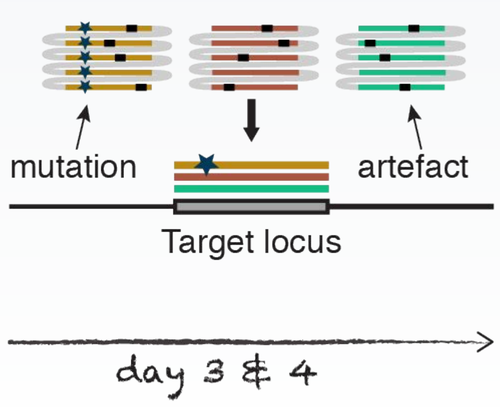
Cyclomics has devised an **integrated molecular and bioinformatics workflow** to capture and amplify ctDNA molecules from liquid biopsies for any genetic locus of interest.

The protocol consists of multiplexed and high-fidelity amplification of genetic loci, e.g. the *TP53* tumor suppressor gene. Amplified molecules are concatemerized in the presence of a backbone adaptor to long DNA strings with alternating backbone and target sequence, ready for direct sequencing on any long-read DNA sequencer.



The current protocol has been **optimized for use with the Oxford Nanopore sequencing systems**.

Data analysis comprises consensus mutation calling and reporting through our **proprietary raw signal processing software suite SquiggleProcessor**.

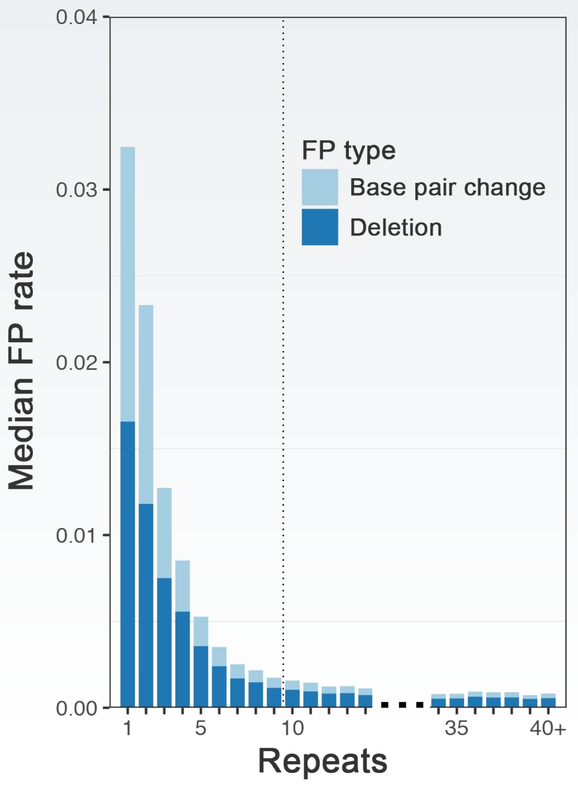


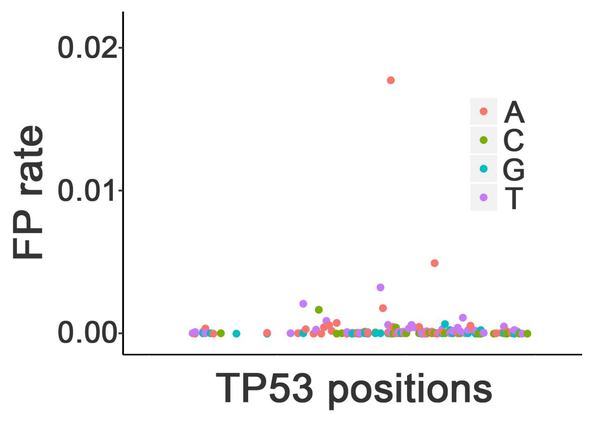
4. Real-time sequencing of blood

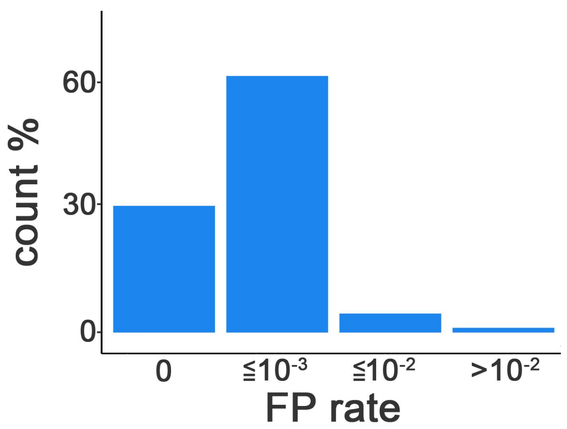
To evaluate performance, we applied CyclomicsSeq to several blood samples of patients with cancer, focusing on sequencing of the *TP53* gene. **CyclomicsSeq produces highly homogeneous** reads with alternating backbone and *TP53* insert copies, leading to more than 80% of reads being useful for subsequent analysis.

Consensus calling improves accuracy leading to detection sensitivities below $1/10^4$.

We have tested the false-positive rate for known *TP53* mutations from the Cosmic cancer mutation database, and show that >94% of the positions have no false-positives or a rate $<10^{-3}$.

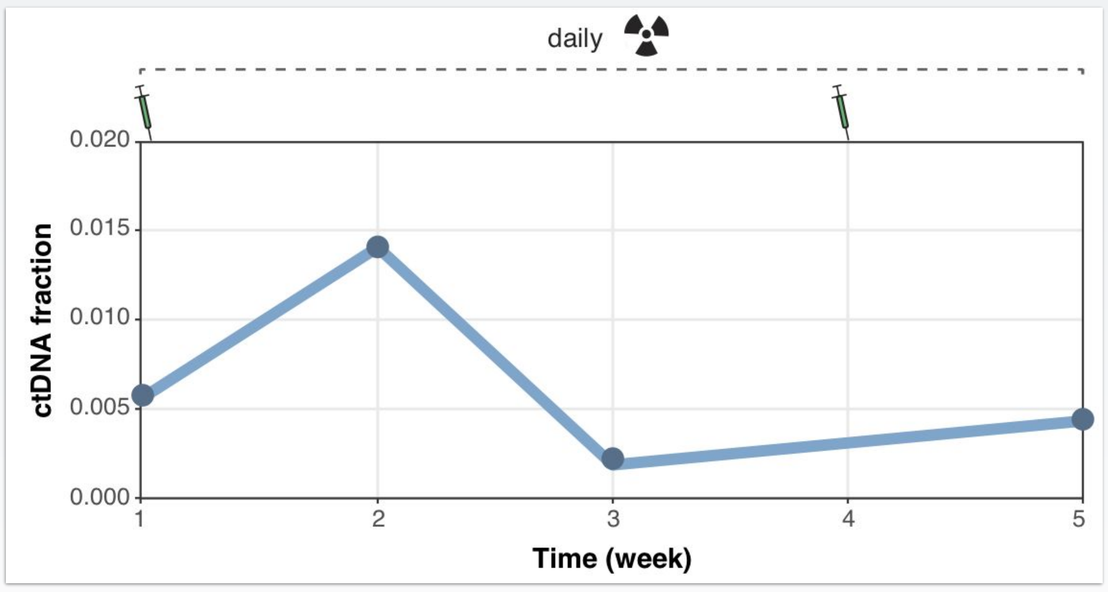


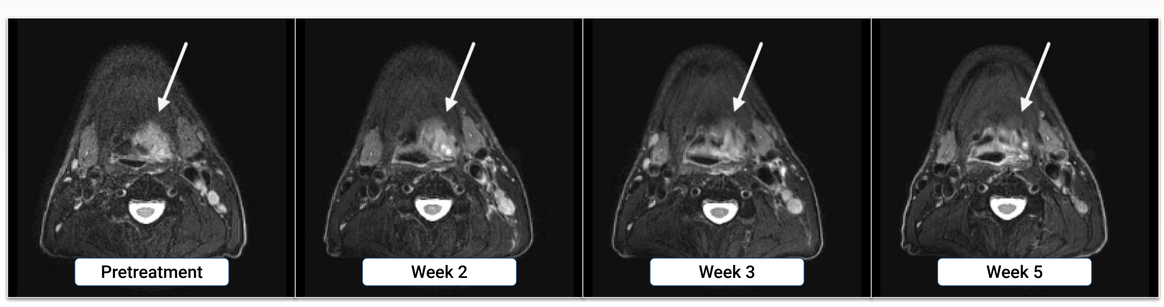




5. Use case: Head & Neck Cancer

Clinical use of CyclomicsSeq is demonstrated in the context of treatment response and recurrence monitoring for patients with head & neck cancer. **CyclomicsSeq was applied to a series of blood samples acquired during and after chemoradiation treatment.** Concurrent MRI images were used for comparison to *TP53* mutation frequencies. These data show how CyclomicsSeq combined with nanopore-sequencing has great potential for cancer monitoring.





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