Göteborg, 2022

Next generation molecular diagnostics using ultrasensitive sequencing

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Medicinaregatan 3, den 14 april 2022, klockan 9.00.

av Stefan Filges

Fakultetsopponent: Professor Ulf Landegren, Uppsala universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. A Ståhlberg, PM Krzyzanowski, M Egyud, **S Filges**, L Stein, TE Godfrey. *Simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing*. Nature protocols (2017)

II. **S Filges**, E Yamada, A Ståhlberg, TE Godfrey. *Impact of polymerase fidelity on background error rates in next-generation sequencing with unique molecular identifiers/barcodes*. Scientific reports (2019)

III. **S Filges**, P Mouhanna, A Ståhlberg. *Digital quantification of oligonucleotide synthesis errors*. Clinical Chemistry (2021)

IV. T Österlund, **S Filges,** G Johansson, A Ståhlberg, *UMIErrorCorrect and UMIVisualizer: Software for Consensus Read Generation, Error Correction and Visualization using Unique Molecular Identifiers*. (Manuscript)

V. G Johansson, D Andersson, **S Filges**, J Li, A Muth, T Godfrey, A Ståhlberg. *Considerations and quality controls when analyzing cell-free tumor DNA*. Biomolecular Detection and Quantification (2019)

VI. N Fredriksson, K Elliott, **S Filges**, J Van den Eynden, A Ståhlberg, E Larsson. *Recurrent promoter mutations in melanoma are defined by an extended context-specific mutational signature*. PLoS genetics (2017)

VII. K Elliott, M Boström, **S Filges**, M Lindberg, J Van den Eynden, A Ståhlberg, A Clausen, E Larsson. *Elevated pyrimidine dimer formation at distinct genomic bases underlies promoter mutation hotspots in UV-exposed cancers*. PLoS genetics (2018)

VIII. L Ny, H Jespersen, J Karlsson, S Alsén, **S Filges**, C All-Eriksson, B Andersson, A Carneiro, H Helgadottir, M Levin, I Ljuslinder, R Olofsson Bagge, V Sah, U Stierner, A Ståhlberg, G Ullenhag, L Nilsson, J Nilsson. *The PEMDAC phase 2 study of pembrolizumab and entinostat in patients with metastatic uveal melanoma*. Nature Communications (2021)

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

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Next generation molecular diagnostics using ultrasensitive sequencing

Stefan Filges

Avdelning för Laboratoriemedicin, Institutionen för Biomedicin, Sahlgrenska akademin, Göteborgs universitet, Sverige, 2022.

Abstract

Massively parallel sequencing enables the exploration of the genetic heterogeneity within microbial, viral and tumor cell populations. Detecting circulating tumor DNA in blood and other body fluids has the potential to revolutionize molecular diagnostics. However, these liquid biopsies typically contain only minute amounts of highly degraded DNA and standard sequencing approaches lack the resolution to detect rare genetic variants. The overall goal of this thesis was to develop an ultrasensitive sequencing approach with single molecule resolution that requires only minimal amounts of material. To this end, we developed the simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing protocol (SiMSen-Seq). SiMSen-Seq achieves ultrasensitive detection of nucleotide variants by attaching unique molecular identifiers to target DNA molecules using PCR primers. SiMSen-Seq is enabled by highly optimized reaction conditions and the use of a stem-loop structure that prevents the UMI from forming non-specific PCR products. We showed that ultrasensitive variant detection is attained mainly by using UMI, while gains in sensitivity from using high-fidelity polymerases were minor. We also demonstrated that oligonucleotide quality is essential in numerous molecular applications, including SiMSen-Seq. Next generation diagnostics tools also demand optimized preanalytical conditions to achieve the necessary variant detection sensitivity, while remaining fast, simple, and cost efficient. Therefore, we established a workflow for cell-free DNA analysis and developed quantitative PCR-based quality controls to evaluate each experimental step. We also developed a bioinformatics pipeline for processing any type of targeted sequencing data containing unique molecular identifiers, including barcode clustering, error correction, variant calling, and visualization. Next, we used SiMSen-Seq in applications requiring ultrasensitive mutant detection. We first employed SiMSen-Seq to experimentally confirm that UV light rapidly induces highly recurrent mutations within a specific promotor motif. These mutations remained sub-clonal even after weeks of cell culture, arguing against a tumor-driving role. Our results highlight the importance of sequence context for the interpretation of somatic variants in cancer. We also showed that ctDNA can be used as a clinical biomarker for tumor burden and to monitor treatment efficacy in uveal melanoma. Patients with high ctDNA levels had worse overall survival, demonstrating the clinical utility of circulating tumor-DNA-based liquid biopsy analysis. In conclusion, we showed that SiMSen-Seq is a simple, flexible, low-DNA input protocol that enables rare variant detection to address a multitude of clinical and basic research questions.

Keywords: Liquid biopsy, cell-free DNA, circulating tumor DNA, molecular diagnostics, next-generation sequencing, unique molecular identifiers, melanoma

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