RUTGERS
School of Graduate Studies

Office of Student Affairs and Admissions
SGS at New Jersey Medical School
Rutgers, The State University of New Jersey
185 South Orange Avenue, MSB C-696
Newark, NJ 07103

http://njms.rutgers.edu/gsbs/
p. 973-972-4511
f. 973-972-7148

YOU ARE INVITED TO ATTEND THE DEFENSE OF THE DOCTORAL DISSERTATION

"Detection of *KRAS* Mutations Associated With Cancer Using SuperSelective Primers in Real-Time PCR and Digital PCR Assays"

By

Emma Tova Gerber

Molecular Biology, Genetics, and Cancer Program

BS, 2015, Brandeis University

Thesis Advisor: Fred Russell Kramer, Ph.D.
Professor
Department of Microbiology, Biochemistry, and Molecular Genetics

Thursday, November 3rd, 2022
10:00 AM
ICPH Auditorium

Join Zoom Presentation:
https://rutgers.zoom.us/j/93833113385?pwd=WFJOcFV2RmpyNHN4djFlWUZWemFpdz09

Meeting ID: 938 3311 3385
Password: 139656

ABSTRACT

Molecular profiling of a tumor is crucial for personalized cancer treatment. A tumor's genetic profile provides information concerning diagnosis, prognosis, and treatment. Circulating tumor DNA containing cancer-associated mutations reflecting this profile are present in blood plasma. There is a need for a sensitive method of detecting these rare mutant fragments, which occur among an abundance of wild-type DNA fragments originating from healthy cells. "SuperSelective" PCR primers have been developed that selectively amplify mutant target fragments, while ignoring related wild-type fragments. These primers can potentially enable the detection of ten mutant target fragments among one million wild-type fragments.

Therefore, we designed a set of SuperSelective primers, and an accompanying conventional reverse primer, that amplify clinically relevant mutations in codon 12 of the KRAS oncogene: Gly12Ala, Gly12Asp, Gly12Ser or Gly12Cys, and Gly12Val, which are mutated in a high percentage of all tumor types. We also designed a set of primers that amplify the β-Actin gene as a control to confirm that the assay is working, and to determine the amount of DNA present. We used molecular beacon probes to detect and quantitate each of the mutant amplicons.

These primers were studied in real-time PCR assays, and their designs were modified to improve the resulting sensitivity and selectivity. We then carried out experiments to determine the most efficient conditions for their use in digital PCR assays. We utilized three different digital PCR platforms: the Bio-Rad QX200 system, the Qiagen QIAcuity system, and the Stilla Technologies Naica System.

Assays were performed with each primer set, and then with all primer sets together, to amplify varying amounts of plasmid or genomic DNA fragments containing relevant KRAS mutations. We tested these systems with samples that mimic those found in blood using small amounts of mutant DNA in the presence of an excess of wild-type genomic DNA. The results demonstrate that SuperSelective PCR primers can amplify mutant DNA sensitively, while ignoring wild-type DNA, in digital PCR assays. This result serves as a model design for the sensitive multiplex detection of mutant ctDNAs in blood samples from cancer patients. Hopefully, such assays will significantly improve personalized cancer treatment.