Bisulfite-converted Duplexes for the Strand-specific Detection and Quantification of Rare Mutations

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Technology description

Unmet Need

One of the most important opportunities in the future of the fight against cancer is the detection of cancer-related genetic mutations in the blood or bodily fluids at early stages of cancer, at the early stages of recurrence, or to become aware of changing cancer genetics during a treatment course. Studies have shown that blood, saliva, pap smear, or pancreatic cyst fluid samples can be used to detect circulating tumor DNA (ctDNA) at early-mid stages of cancer progression. However, most approaches to analyzing the DNA samples depend on sequencing instruments with relatively high error rates, which limits the detection sensitivity to around 1 mutant among 100 wild-type molecules. Unfortunately, in liquid biopsies the incidence of ctDNA in normal DNA is often closer to 1 in thousands of molecules. In these cases, for the earliest possible detection of mutant sequences, a new, more sensitive approach is needed.

Technology overview

Johns Hopkins researchers have developed a method of greatly increasing the sensitivity of mutation detection from fluid patient samples by filtering out DNA mutations made in the process of analyzing the samples. The important new step is initial bisulfite treatment of the DNA, which converts all non-methylated C residues to U residues, allowing researchers to differentiate the two original strands of DNA that are input. Then, a molecular barcode is added to the strands, and they are amplified by PCR. These two steps allow scientists to separate mutations that get introduced in error by sequencing instruments or from PCR: If a mutation is from the original patient sample, it will be found in every sample with the same barcode andin both strands of the template DNA. This method identifies and removes errors from high-throughput sequencing and PCR, increasing sensitivity of detection to better than 0.02%, or 1 in 5000. Additionally, the inventors can barcode all DNA from a specific patient, allowing patient samples to be combined and analyzed more efficiently.

Stage of Development

The inventors have demonstrated the ability to detect and quantify cancer-related mutations at very low frequency in patient samples.

Publications

Mattox AK, et al. PNAS 114. 4733-4738, 2017

Application area

定量检测罕见突变
鉴别会复发的临床患者

Advantages

BiSeqS能够以一种高度敏感和特异性的方式准确地定量罕见突变
能准确地鉴别会复发的临床患者
BiSeqS可以有效地使用所有的模板分子，并保持高度的特异性检测出10毫升血浆中只有一个或两个突变的DNA分子

Institution

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