Correlation of mutations detected in liquid and tissue biopsies

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1. Abstract

Circulating cell-free DNA (ccfDNA) in plasma can be used to detect biomarkers that show great promise for diagnosis and monitoring of cancer, giving rise to the possibility of liquid biopsies that obviate the need for invasive tissue collection. The low concentration and highly fragmented nature of ccfDNA, coupled with the low frequency of potential oncogenic biomarkers, present challenges. This will require a purification method that is efficient and highly reproducible.

Here, we describe a method for purifying nucleic acids based on novel surface and binding chemistries. The combination of these two approaches allows for increased binding of fragmented DNA. The method is automated to ensure highly reproducible results. Up to 4mls of plasma can be processed and eluted in as little as 50ul, yielding high DNA. This greatly facilitates use in Next Generation Sequencing.

Using the automated method, ccfDNA was purified from the plasma of 7 patients who had previously undergone surgical resection for malignancy. DNA was also purified from the FFPE malignant tissue off of slides, following macrodissection, from the same patients. NGS was used to interrogate both sample types for potentially oncogenic variants. Several laboratory developed tests, all including COLD-PCR, were also employed to verify the presence or absence of variants. The two types of samples showed excellent correlation on mutations, suggesting that use of a less invasive liquid biopsy has the potential to enable actionable mutation detection without using more invasive solid tumor biopsy means.

2. Areas of interest for ccfDNA in molecular oncology research

- Cancer Applications
  - ccfDNA often exhibits the same alterations as DNA from tumor tissues.
  - Quantitative changes in ccfDNA levels
    - Normal levels of ccfDNA are 10-30ng/ml plasma; in cancer patients levels can be significantly higher
  - Biomarkers for cancer
    - Mutations in oncogenes such as KRAS are detected in various cancers
    - Mutagenic KRAS can be detected in plasma of cancer patients
  - Microsatellite alterations
    - Microsatellite Instability and Loss of Heterozygosity are suggested to play a role in carcinogenesis
  - Epigenetic alterations
    - Changes in methylation patterns can be seen in ccfDNA

3. Materials and Methods

Samples were purchased from commercial vendors or residual plasma/FFPE tested at UWHC.

Promega's Maxwell® RSC instrument was used to purify ccfDNA from plasma and FFPE.

Concentration of DNA was determined using QuantiFluor® dsDNA dye and qPCR. Fluorescence quantitation was done using Promega's QuantiFluor® dsDNA system. Quantitation by qPCR was performed using Promega amplification chemistry.

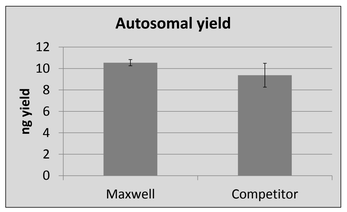
Next generation sequencing was performed at the University of Wisconsin Hospitals and Clinics using an Ion Torrent™ sequencer. Mutations were detected using the Ion AmpliSeq™ Cancer Hotspot Panel v2 from Life Technologies. A COLD-PCR assay was used to confirm results seen in NGS.

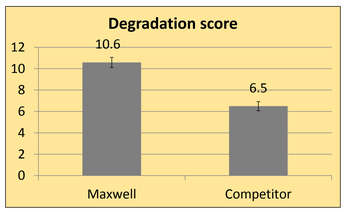
4. Protocol

- The Maxwell® RSC instrument is a small, magnetic particle-handling robot that allows efficient binding of ccfDNA to the paramagnetic particle in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge, mixing during processing.
- **There is no pre-processing.** Plasma is added directly to well 1 of the cartridge.
- 16 samples can be processed simultaneously.
- Elution volume is 50 to 100 uL.
- Run time is 80 minutes.

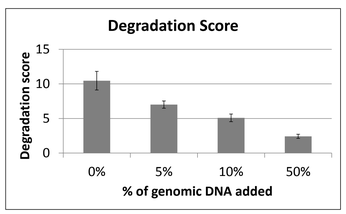
5. Use of longer target amplification as a measure of genomic DNA contamination

- Amplifiable yield can be determined for ccfDNA using a small, autosomal target.
- An amplicon of >280 is used to amplify species larger than 170bp including genomic DNA to develop a degradation score (short/long).





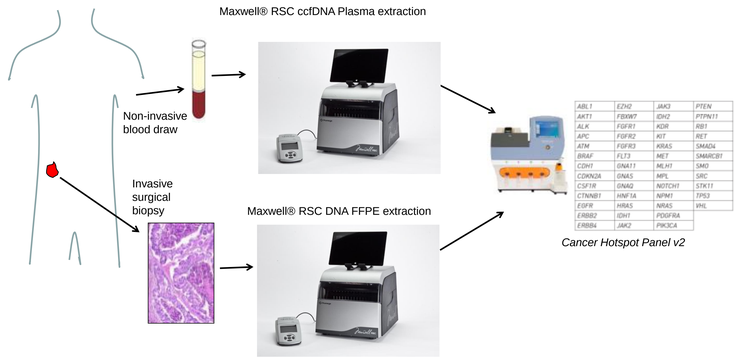
- The degradation score "improves" as contaminating genomic DNA is added.



- The degradation score can assay for genomic contamination.
- Useful when sizing or genomic contamination are important to assess.

* The Maxwell® RSC instrument, Maxwell® RSC ccfDNA plasma kit and Quantus™ fluorometer are for Research Use Only.

6. Study design – comparison of tissue and liquid biopsies



7. Correlation of FFPE and ccfDNA results

Sample	Diagnosis	Test Ordered	CHPv2 Result on FFPE	CHPv2 Result on Plasma
M-14-00195	Metastatic adenocarcinoma	**EGFR**	TP53 p.E339*, **EGFR p.E746-A750del, EGFR p.T790M**	TP53 p.E339*, **EGFR p.E746-A750del, EGFR p.T790M**
M-14-00916	Non-small cell lung carcinoma	**EGFR**	**EGFR Wt**, KDR p.G1333E, TP53 p.G226fs*21, CDKN2A p.G67S, Hras p.Q61K, Nras p.Q61K	**EGFR Wt**
M-14-00984	CRC	**Kras**	**Kras Wt**, APC p.E1317Q, TP53 p.R342*	**Kras Wt**, APC p.E1317Q
M-14-00986	Adenocarcinoma	**EGFR, Kras**	**Kras p.G12D**, **EGFR Wt,** TP53 p.H178fs*69	**Kras Wt**, **EGFR Wt**
M-14-01009	Adenocarcinoma	**EGFR**	**EGFR Wt**, Braf p.V600E, TP53 p.R248Q	**EGFR Wt**, SmarcB1 p.T63Nfs*1
M-14-01010	Adenocarcinoma	**EGFR**	**EGFR Wt**, Kras p.G12D, FBXW7 p.R479Q	**EGFR Wt**, APC p.S1364fs*11
M-14-01013	Melanoma	**Braf**	**Braf p.V600E**, CDKN2A p.E88K, TP53 p.S127F, TP53 p.P128fs*42	**Braf p.V600E**, CDKN2A p.E88K, TP53 p.S127F

- Subsequent COLD-PCR assay confirmed presence of the Kras p.G12D mutation in *986 plasma eluate.
- Good concordance of the FFPE and liquid biopsy sample shows promise for additional clinical research.

8. Conclusion

- Protocol is completely automated, decreasing the chance of user error or contamination
- Sixteen samples of up to 1ml can be processed in 80 minutes
- ccfDNA was purified from plasma from patients with cancer
- Next generation sequencing of the DNA detected mutations commonly associated with cancer, suggesting that this method could be used to screen plasma in a form of "liquid biopsy"
- Correlation study showed good concordance between ccfDNA purified from plasma and DNA purified from FFPE