Memorial Sloan Kettering Cancer Center

2020 RPD Research Professional's Dav

# Identifying QA/QC Parameters Affecting Success in Downstream Applications for DNA Extracted from FFPE

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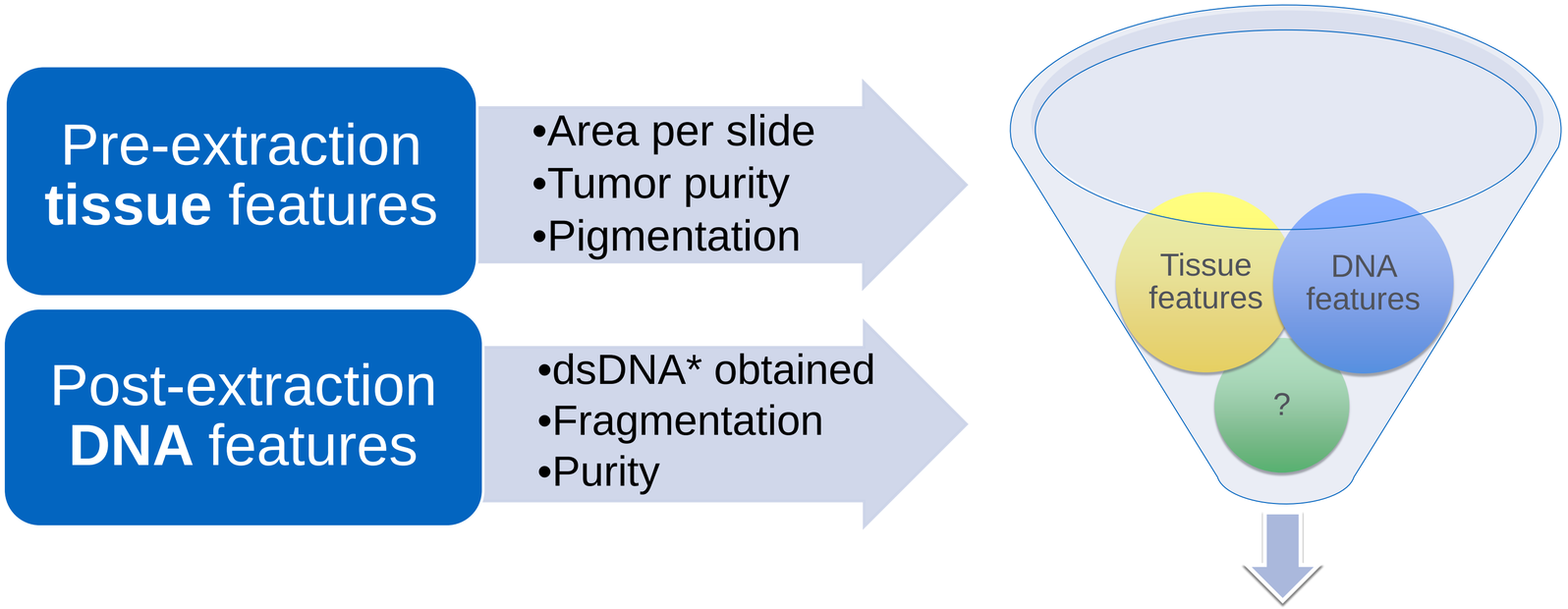
# Background

- As part of InterMEL, an ongoing study of melanoma-specific survival in patients with primary melanoma stages II-III, we are co-extracting RNA/DNA from limited archived formalin-fixed paraffin embedded tissue (FFPE) for use on multiple-omics platforms
- FFPE and small-sized pigmented tumors present a challenge for the isolation of nucleic acids
- Study design, logistics, and optimization of co-extracted nucleic acids are vital for qualification and optimal performance in downstream testing, defined as success

# Objectives

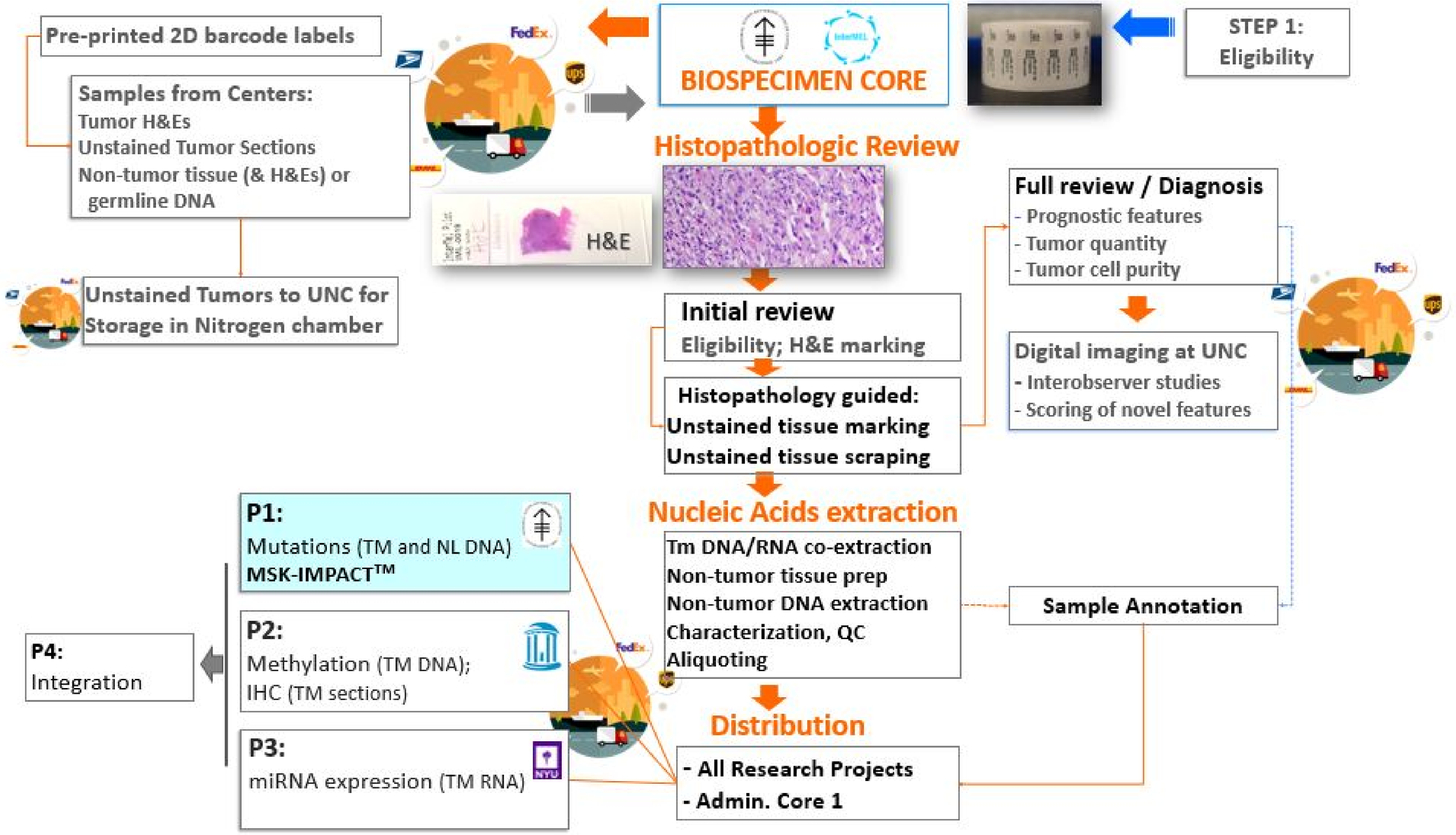
1. Identify the quality and quantity features that influence success of MSK-IMPACT™ in co-extracted tumor DNA

2. Evaluate pre-extraction tissue and co-extracted DNA characteristics and compare these to performance



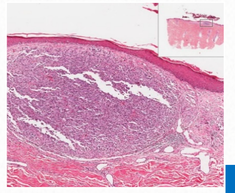

* dsDNA= double stranded DNA

# Overview of Specimens and related Data Flow in InterMEL



# Methods

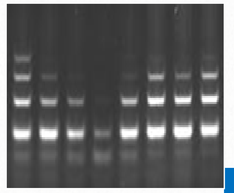
- Pathology review: diagnostic confirmation and qualifying features for project including tissue integrity and tumor purity
- Histopathology-guided marking and scraping of slides, estimation of tumor size (area), and nucleic acid co-extraction using Qiagen co-extraction kits following manufacturer's protocols with minor modifications
- DNA quality assessment and quantitation by the Molecular Epidemiology Lab and IGO
- Comparison of tumor- and -DNA-derived variables to downstream testing output
- Identification of features correlating with success using Pearson method



Pathology Review

Tissue features

- Microscopically:
  Tumor purity (%)
- Macroscopically
  Tumor tissue area
  Pigmentation



Post-extraction

DNA features

- DNA quantity:
  Nanodrop (total DNA)
  Qubit (dsDNA); %dsDNA**
- DNA quality:
  A260/A280 ratio
  A260/A230 ratio
  Fragmentation
  Sample identity/agreement

IGO

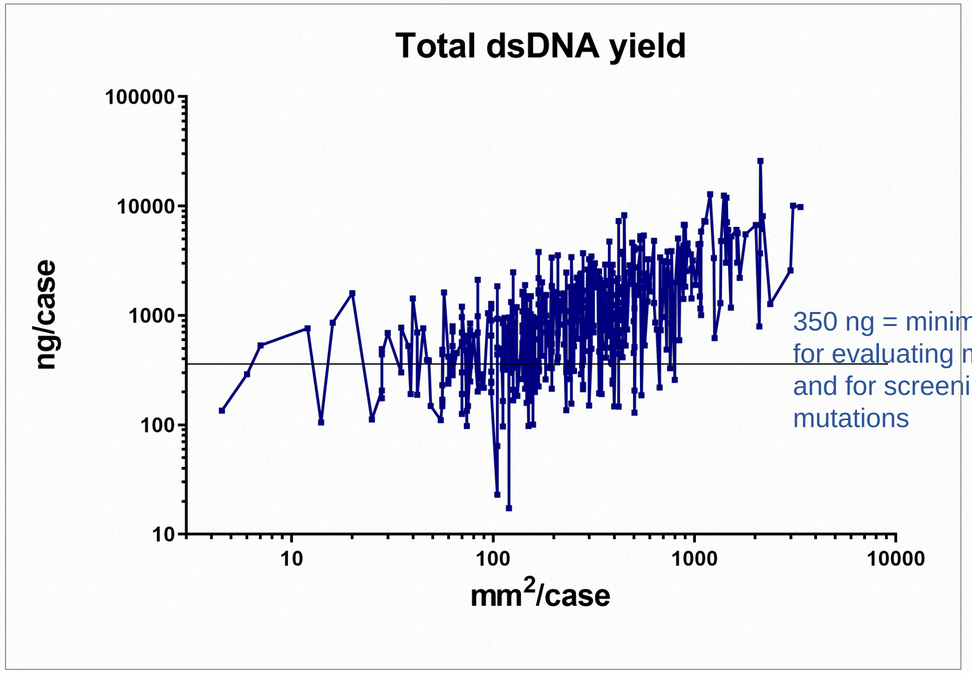
Testing

Mutation testing

- DNA quantity:
  Quant-iT (dsDNA)
- Library quantity:
  Quant-iT (dsDNA)
- Pass/Fail
- Coverage (# of reads)

**%dsDNA= 100* dsDNA/total DNA

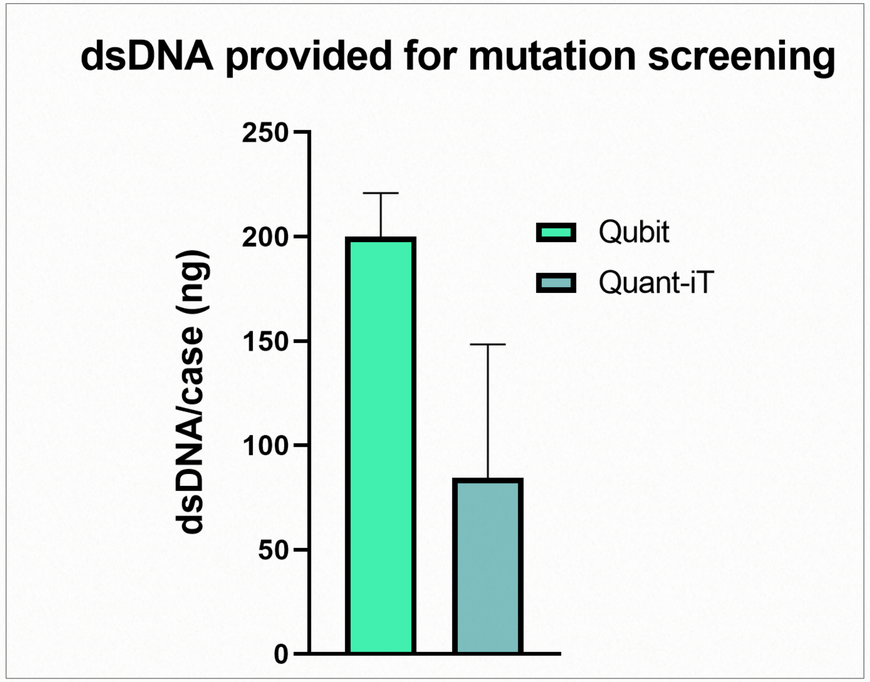
# Tumor samples for MSK-IMPACT™ testing: DNA features



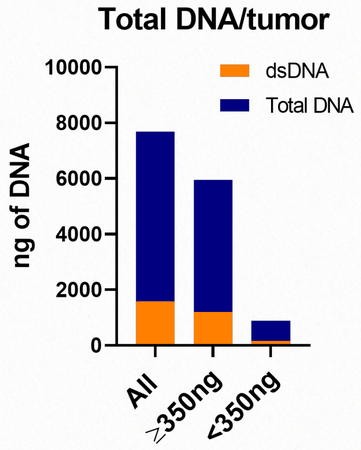

350 ng = minimum dsDNA for evaluating methylation and for screening of mutations

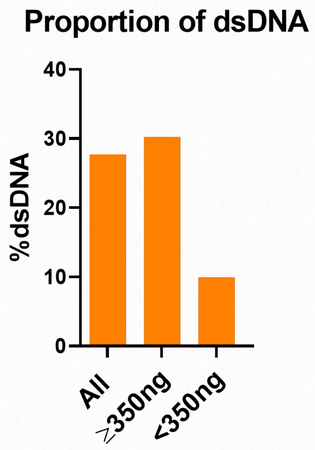
361 of 449 cases ≥350ng

Distribution for MSK-IMPACT™ assay




*Regardless of differences in the cc measured by the two methods (Qubit and Quant-iT), all samples were included in the Library prep step, and tested with the MSK-IMPACT™ assay*



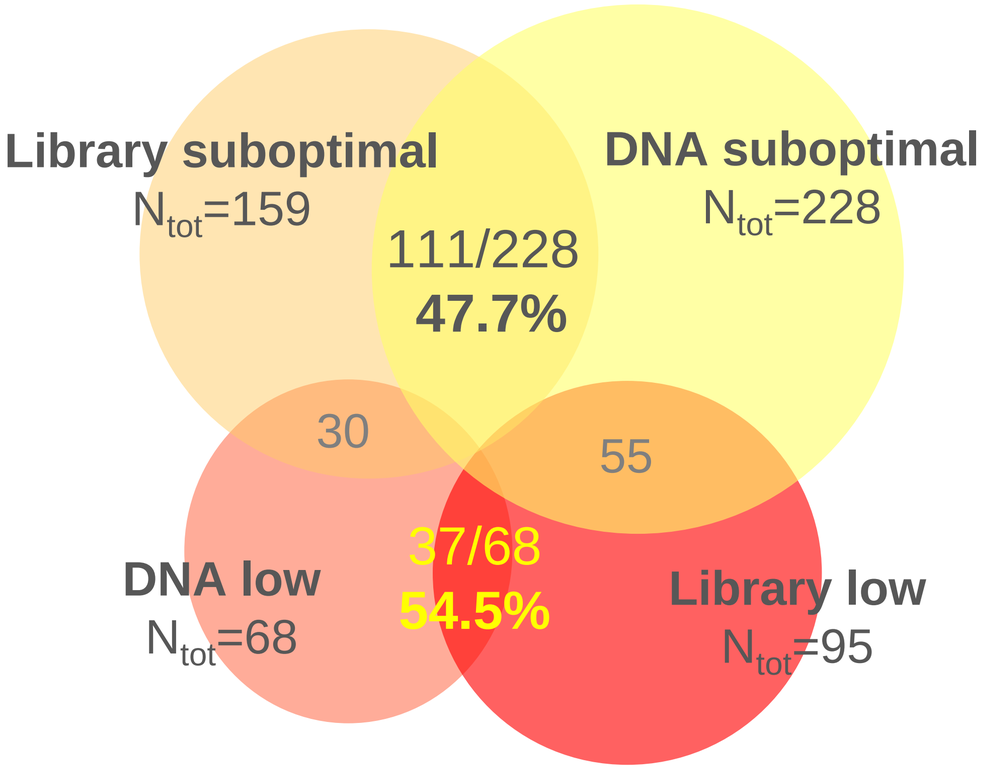

*Median values across 499 tumors are shown.*
*Total DNA includes both single and double stranded DNA.*

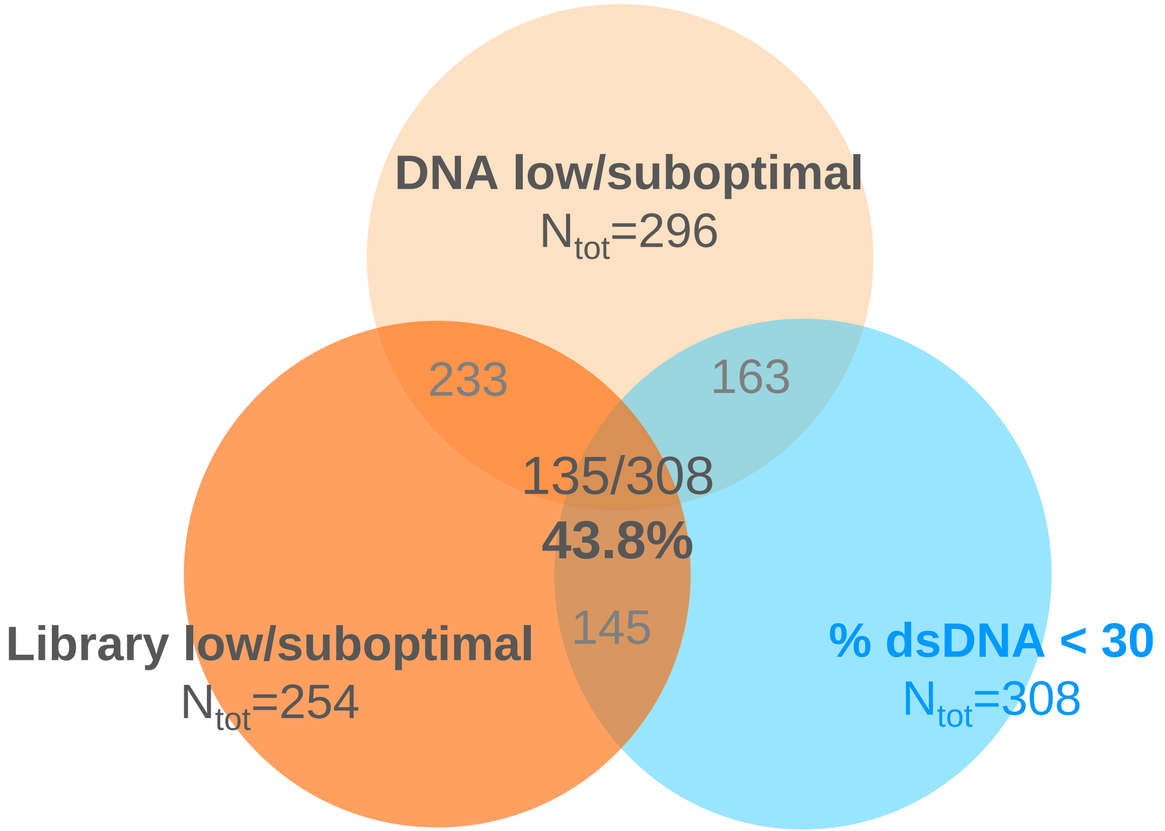
**Comparison of quality (measured by % dsDNA) and quantity of tumor DNA:**

- The % med dsDNA in cases with sufficient dsDNA for testing by two assays was 30.20.
- The % med dsDNA among cases with insufficient dsDNA for testing by two assays was 9.90.

In addition, there are 51 cases with insufficient non-tumor DNA; among these, the % med dsDNA for the tumor was 24.05.

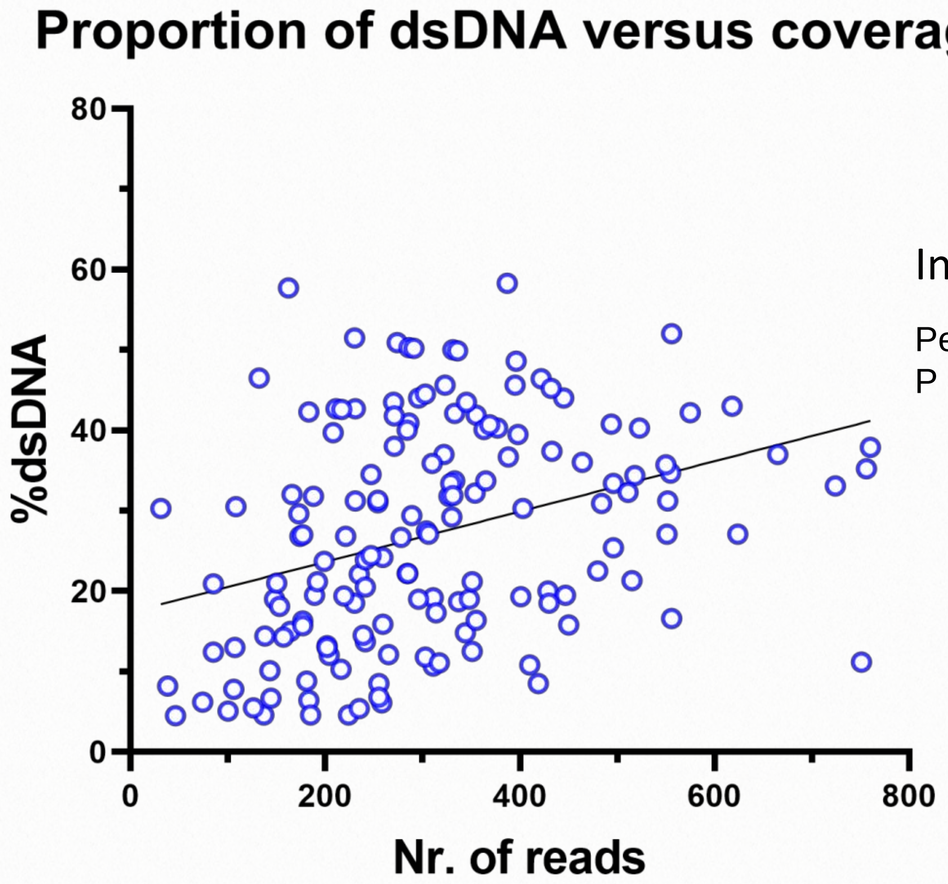
# Comparison of quantity of dsDNA & derived Libraries (Quant-iT) with input dsDNA quality (Qubit BR), in 335 InterMEL cases




DNAs and Libraries highlighted in yellow and light orange fall just below IGO's quantitative standards
DNAs and Libraries highlighted in pink and red fall below IGO's quantitative standards for capture
DNA highlighted in blue fall below Molecular Epi Lab's quality threshold for dsDNA

# Number of reads correlate with the proportion of dsDNA in samples screened for mutations with the MSK-IMPACT™ assay




InterMEL cases, n=154

Pearson's r = 0.3427

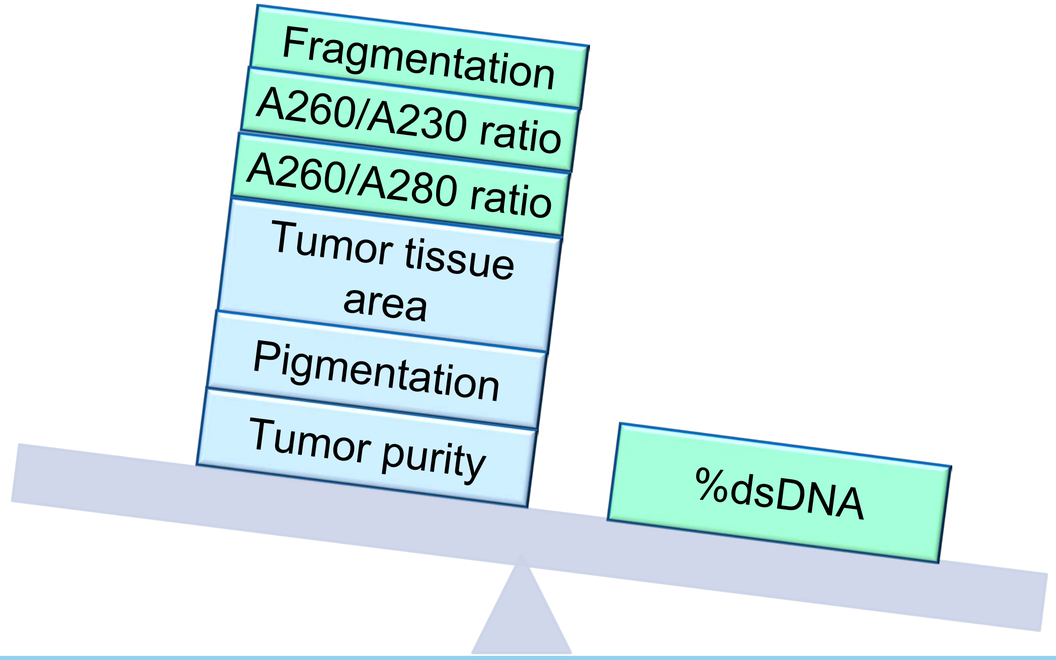
P = $1.452 \times 10^{-5}$

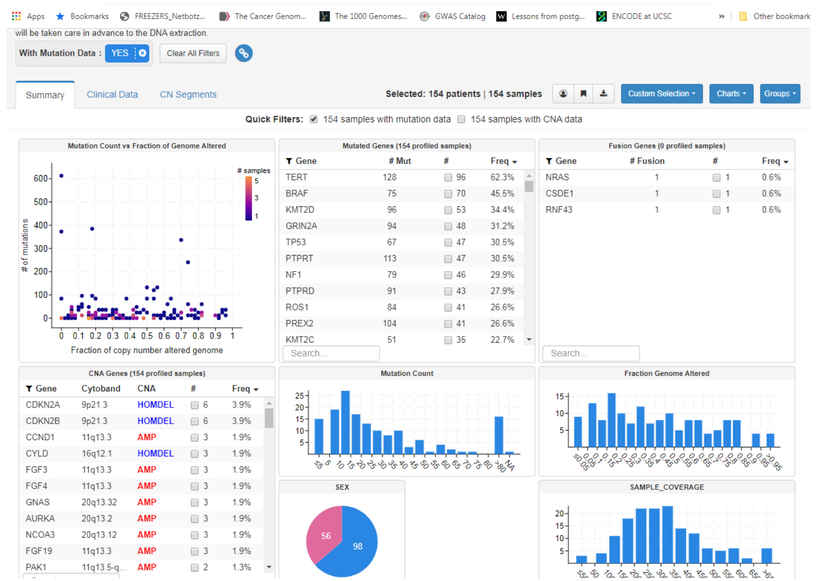
# Summary of Results

We found a positive correlation between:

- Proportion of dsDNA and MSK-IMPACT™ coverage

Variable that appears to matter the most for successful downstream testing:



# Conclusions

- Proportion of double stranded DNA, although not perfect, appears to be the best indicator of performance in downstream screening of mutations using the MSK-IMPACT™ assay

- Our design, approach, and observations could be useful in the study of other cancers in which limited and archived tumor tissues are tested on multiple genetic, epigenetic, and phenotypic platforms