DuplexSeq™ Mutagenesis Assay

Consideration for use in a regulatory context

2023 ICCVAM Forum
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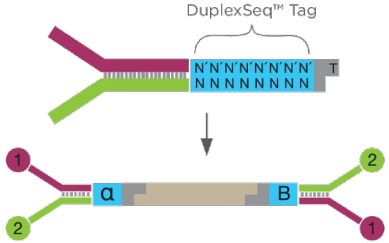
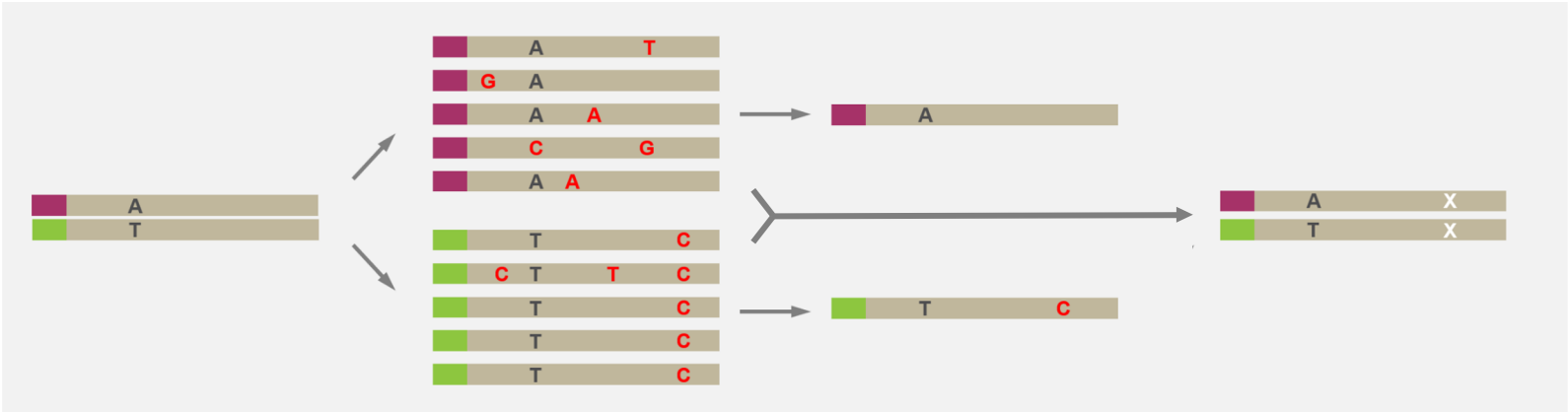
TwinStrand Biosciences

TwinStrand Duplex Sequencing® technology

- Biological systems rely on redundant information in two DNA strands to maintain genomic fidelity
- Duplex Sequencing (DS) recapitulates this solution *in vitro* with specialized biochemistry and informatics

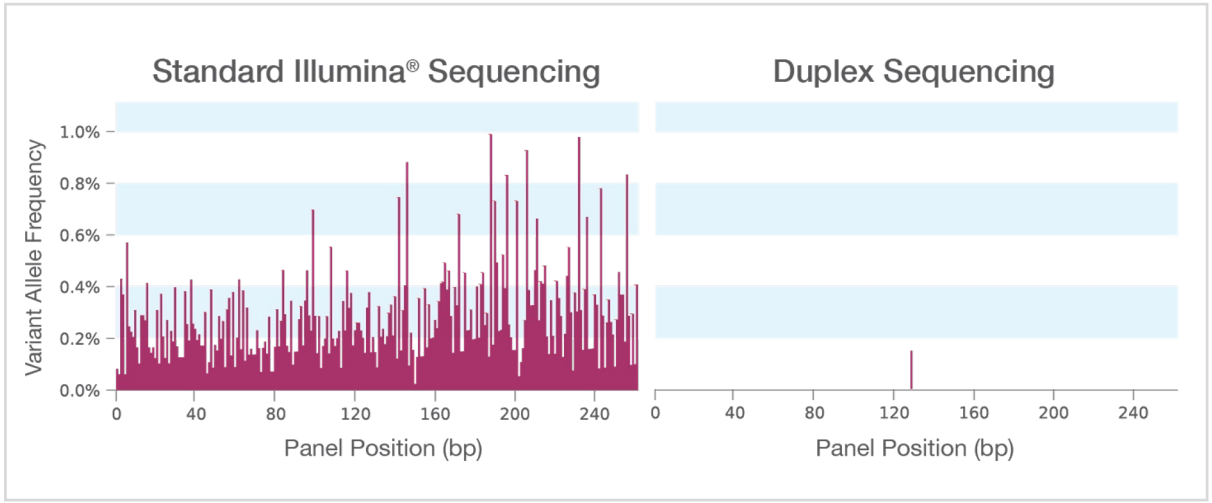




Average error rate: $\frac{1}{1000}$ $\frac{1}{25{,}000}$ $< \frac{1}{10{,}000{,}000}$

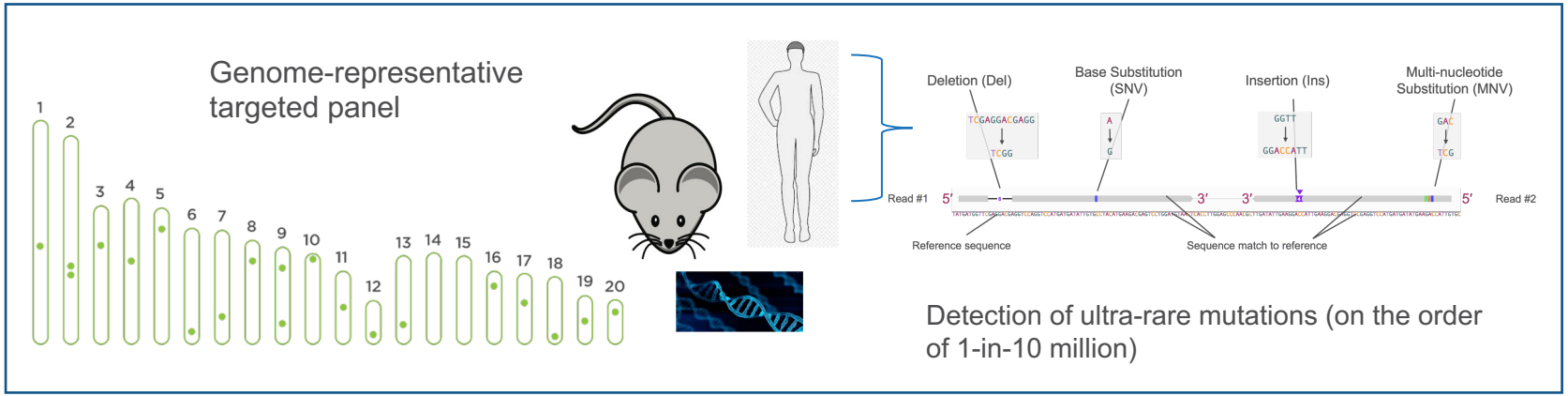
Duplex Sequencing reveals true, ultra-rare mutations *

- > 10,000-fold boost in accuracy vs. standard NGS
- Eliminates virtually all background errors, revealing ultra-low frequency variants
- Compatible with standard Illumina® sequencers
- Easy to adopt, minimal special equipment needed
- Cloud-based informatics, no programming skills required



DS as a new tool for genomic safety assessment *

- DS measures mutations *directly* in the DNA of almost any tissue or cell type from any organism $



- Yields quantitative mutation frequency (MF), mutation spectra and trinucleotide mutation signatures; the latter makes it possible to infer mutational processes that have been implicated in human cancers

- Enables mechanistic insight into the mutagenic mode-of-action (both *in vitro* and *in vivo*)

DS is broadly relevant to many forms of genomic safety *

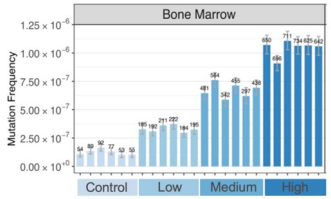
MUTAGENESIS

CARCINOGENESIS

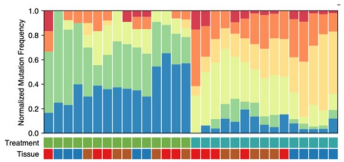
Classic

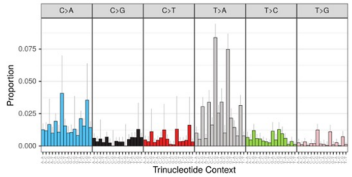
Neogenotoxins

Clonal expansions $

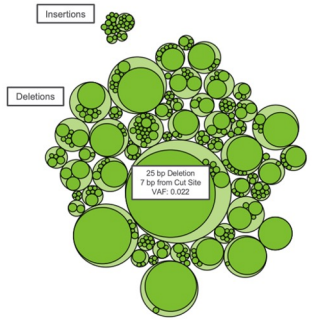


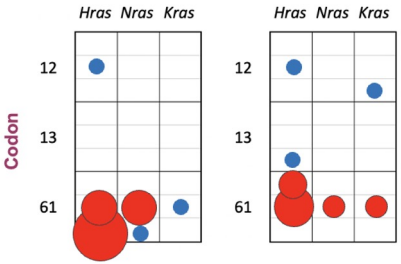
Quantitative mutant frequency





Data-rich mutant spectrum)





***In vivo*, *in vitro*, organoid, in human**

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Brief summary of evidence-generation to date *

2017

Phase 1: Equivalency (superiority) of DS to classic mutagenesis assays

- Qualify in vivo strains: MutaMouse, Big Blue, Sprague Dawley
- Bridging studies with Pig-a and TGR
- Inter-lab reproducibility & assay concordance (*in vitro* and *in vivo*)
- 3 WG at HESI: mutagenesis, neogenotoxins and carcinogenicity
- Tech transfer to Public Sector and Pharma
- Publication of mouse and rat data, bioinformatic pipeline
- Feasibility w/NAMs: cells, reconstructed tissue, organ-on-a-chip

2022

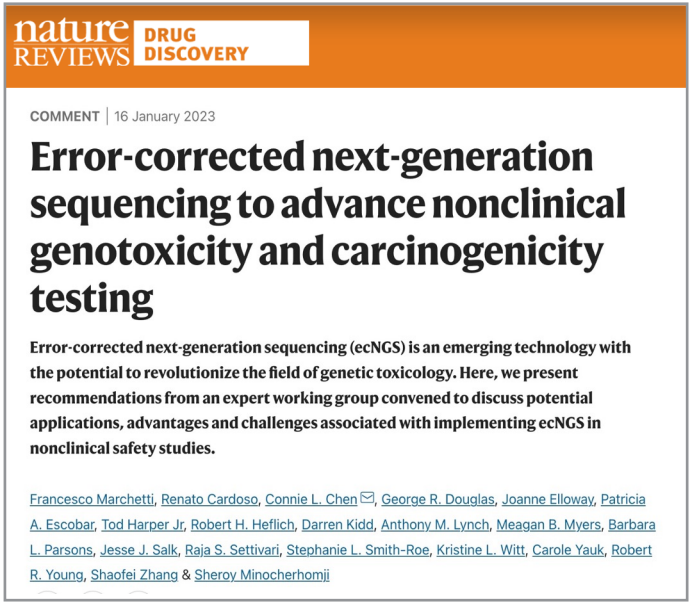
Phase 2: Provide confidence of ecNGS for regulatory use

- HESI position paper published in Nature Reviews
- Inter-lab protocol standardization, sensitivity testing (HESI)
- Tech transfer expanded to 7 labs (Pharma and Public Sector)
- New *in vitro* working group at HESI
- Pharma industry started using DS for nitrosamine testing

2023

Phase 3: Plan a regulatory path forward

2024



nature REVIEWS DRUG DISCOVERY

COMMENT | 16 January 2023

Error-corrected next-generation sequencing to advance nonclinical genotoxicity and carcinogenicity testing

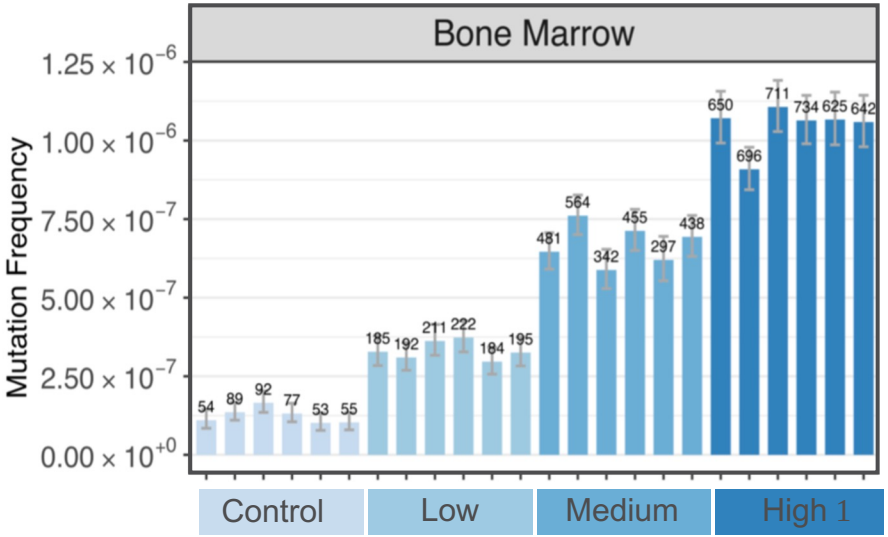
Error-corrected next-generation sequencing (ecNGS) is an emerging technology with the potential to revolutionize the field of genetic toxicology. Here, we present recommendations from an expert working group convened to discuss potential applications, advantages and challenges associated with implementing ecNGS in nonclinical safety studies.

Francesco Marchetti, Renato Cardoso, Connie L. Chen, George R. Douglas, Joanne Elloway, Patricia A. Escobar, Tod Harper Jr, Robert H. Heflich, Darren Kidd, Anthony M. Lynch, Meagan B. Myers, Barbara L. Parsons, Jesse J. Salk, Raja S. Settivari, Stephanie L. Smith-Roe, Kristine L. Witt, Carole Yauk, Robert R. Young, Shaofei Zhang & Sheroy Minocherhomji

HESI-consortium position paper describing many regulatory adoption pathways for ecNGS and calls for working toward TGs describing an analytical procedure to be used across test systems contributing to NAMs

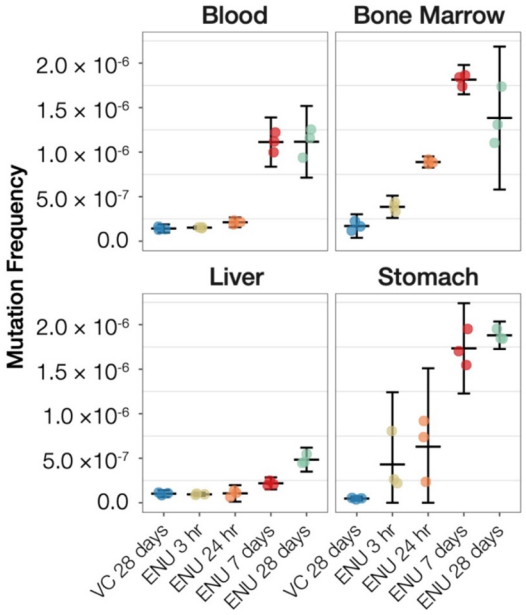
Mutant frequency (MF) *in vivo* mutagenesis in rodents

Benzo[a]pyrene in mice



(LeBlanc et al. 2022 BMC Genomics, PMID: 35902794 w/Health Canada)

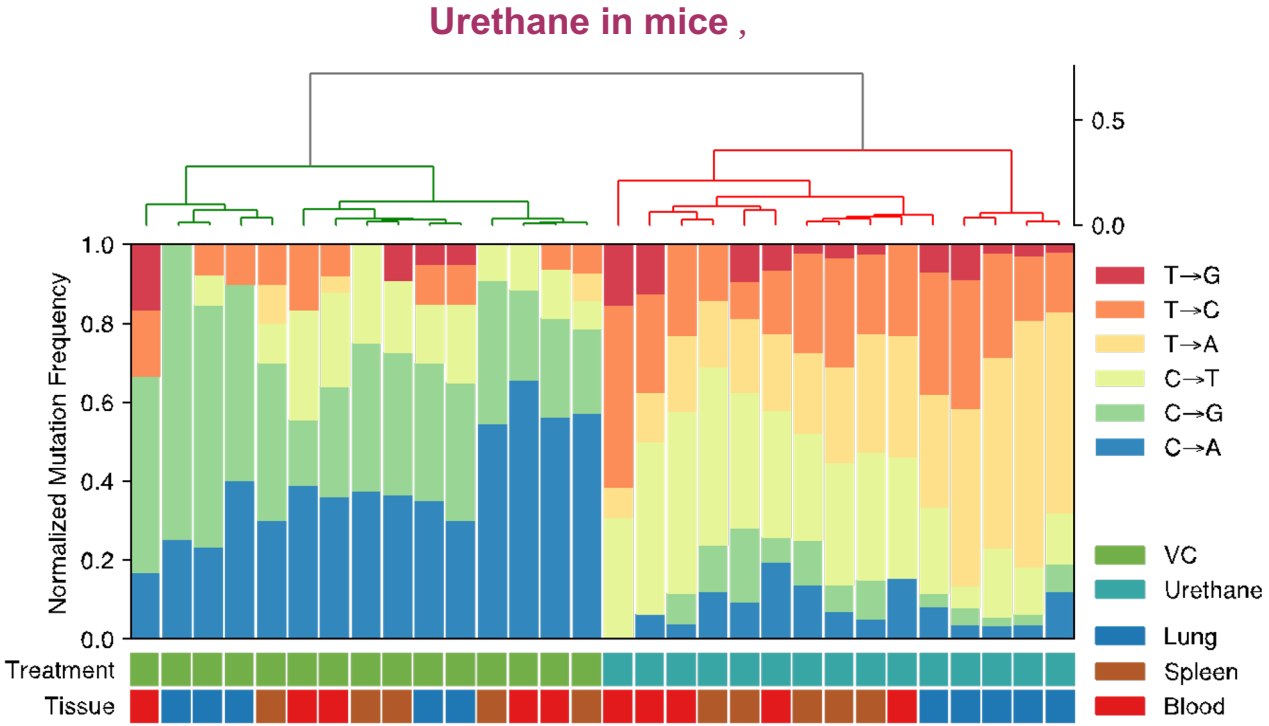
N-ethyl-N-nitrosourea in rats



(Publication in preparation, w/NIEHS/NTP, Inotiv)

Simple mutation spectra: *in vivo* mutagenesis in rodents

- Simple mutation spectra yields distinct patterns in normal exposed tissues
- Unsupervised hierarchical clustering correctly assigned exposure with 100% specificity & specificity

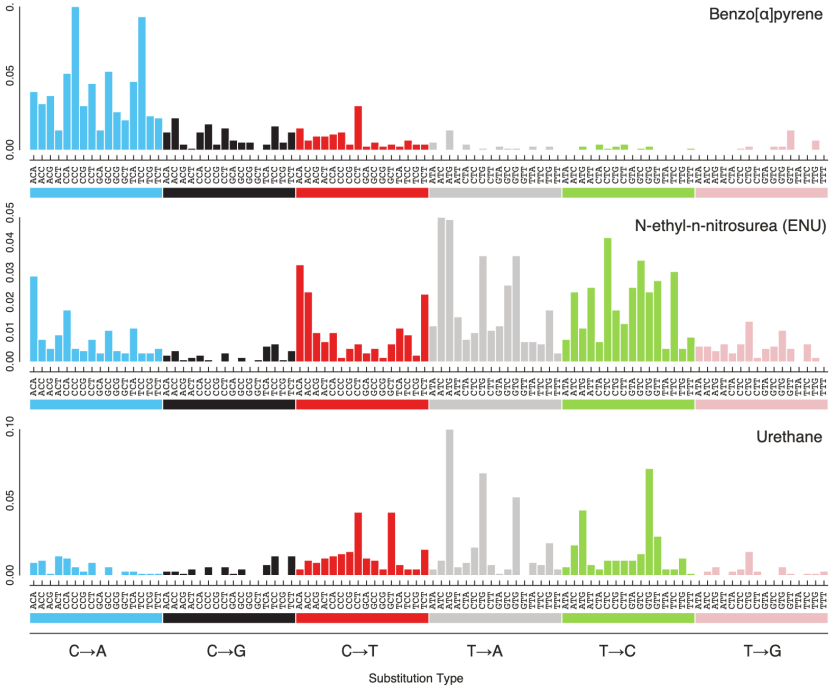


(Valentine et al. 2020 PNAS, PMID: 33318186 w/Amgen, MilliporeSigma)

Trinucleotides mutation signatures: rodents *in vivo*

- Trinucleotide mutation spectra yields even richer patterns of exposure
- Signal appears in less than 1 month in healthy-appearing tissues
- Patterns can be mathematically matched to databases with statistical confidence
- Pattern for B[a]P (major carcinogen in tobacco smoke) closely matches mutation patterns in human lung cancers of smokers

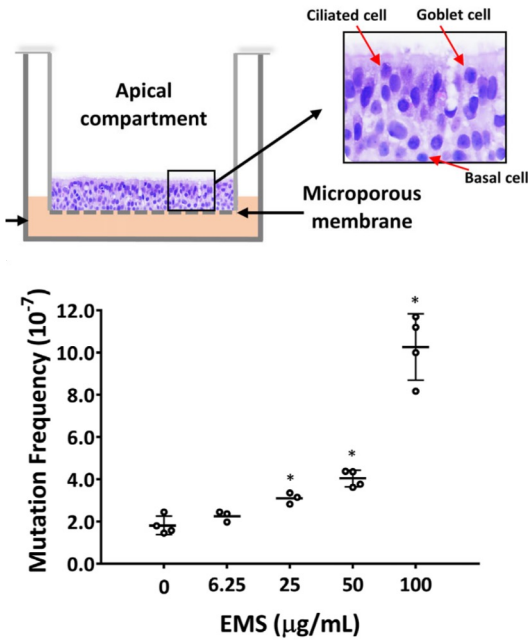
B[a]P, ENU, urethane in mice



(Valentine et al. 2020 PNAS, PMID: 33318186 w/Amgen, MilliporeSigma)

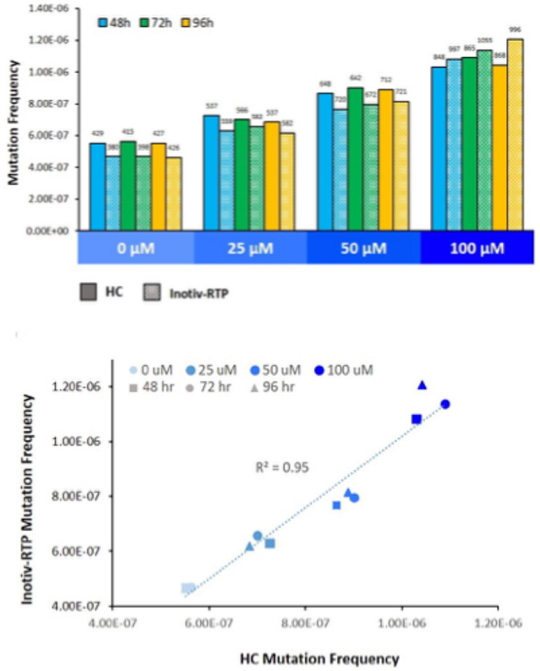
In vitro mutagenesis: reconstructed ALI tissue and TK6 cells

EMS in ALI model- 28 days exposure



(Wang et al. 2021 EMM, PMID: 34050964 w/NCTR/FDA)

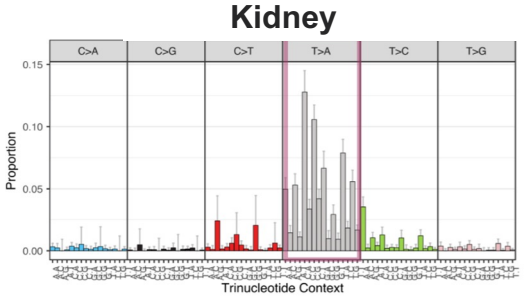
TK6 cells treated with ENU 48, 72 and 96h (

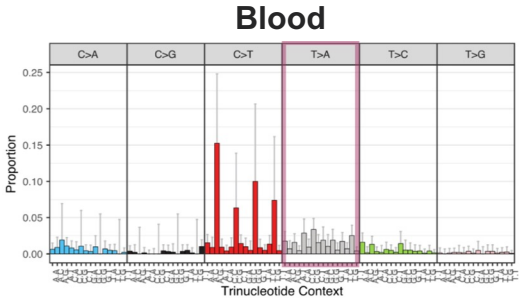


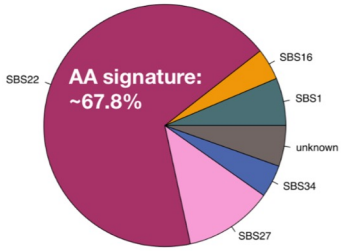
(Cho et al. 2023 bioRxiv,Health Canada and Inotiv)

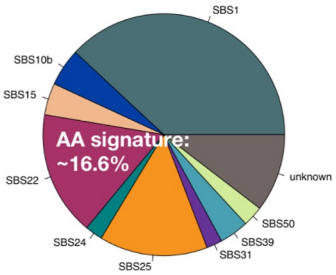
In human: non-invasive detection of aristolochic acid exposure

- Aristolochiaceae-containing traditional Chinese medicines and contaminated crops causes thousands of cases of liver and kidney cancer per year
- It is currently impossible to detect distant past exposures
- Duplex Sequencing identifies exposure signatures from blood and urine samples









(Publication in preparation, w/Rozen Group, Duke NUS)

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Asks of ICCVAM to evolve genomic safety assessment

Awareness

- Familiarity with the technology, types of data output and the community's evidence and activities to date

Help

- Guidance toward the right regulatory pathway that (expeditiously) meets the needs of many
- Qualify an endpoint that can be used to measure mutation in various biological platforms (*in vivo* and *in vitro*)
- A TG or IATA case study describing analytical procedures and a guidance document to support different applications

Ideas

- DS can be applied to genomic safety in many different ways; we want to hear what you think is most important

Partnership

- We want to work with all stakeholders including the various agencies you represent. Please reach out!

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