Enabling Biomarker Testing in Clinical Trials

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Canadian Cancer Trials Group &
Queen's Department of Pathology and Molecular Medicine
Kingston, Ontario
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What is a biorepository or biobank?

"a large collection of biological or tissue samples amassed for research purposes"

- population based, disease specific, site specific
- real or virtual

A powerful tool in research and over the last two decades has provided support for genomics and personalised or precision medicine

If you collect specimens for research purposes, you are a biobank

A biorepository associated with a clinical trial is a composite of…

Tumour - associated with the clinical trial
Tissue - whole blood, plasma, serum, urine, bone marrow, CTC, cfDNA, buccal smears, microbiome
Derivatives - TMA, DNA, RNA, proteins, PBMC
Data - well described and validated clinical data including demographic information, patient and disease characteristics, therapy, outcome measures, adverse event profiles, quality of life

Tumour Tissue Data Repository (TTDR) of the CCTG

A national resource of clinical trial associated tissue on more than 120 trials providing material to the research community

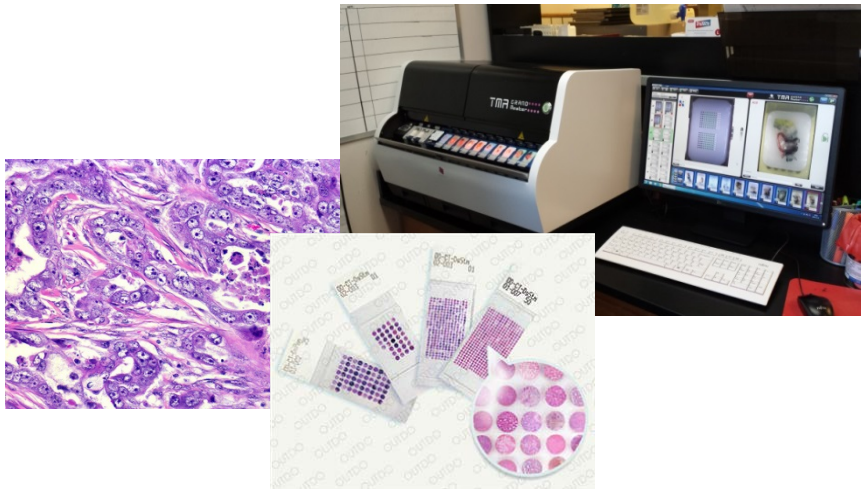
- Tumour tissue > 18,500 patients
- Plasma >8,500 patients
- Serum >13,500 patients
- ~ 300,000 individual samples banked and matched with quality clinical data

Housed within the Queen's Laboratory for Molecular Pathology (QLMP) in the Department of Pathology and Molecular Medicine

The actual bank…

- Administrative office: receipt, logging, inventory management, tracking of tissue and maintenance of TTDR database
- Management of correlative science projects, pulling and sending samples to researchers
- Storage for paraffin embedded formalin fixed material:
  - RT for blocks/TMAs
  - 4^{o}C refrigerated storage for cut sections
  - -80^{o}C freezers for whole blood
  - plasma, serum, urines, derived products

- TMA Design and Construction.



- DNA/RNA extraction and associated quality assurance.
- Cytogenetics, FISH, molecular diagnostic services
- NanoString
- Gene expression profiling platforms: Next Gen Sequencing
- Flow cytometry

- Kit production for sample collection (specific studies)
- Histological services for cutting and routine H&E staining of sections for quality assurance and digital imaging.
- Ventana Automated Immunohistochemistry.
- Quantitative Immunofluorescence.
- Whole section (Aperio) digital imaging and archival facility with web based access:
  - Review on line
  - Annotations
  - Image Management System
  - Marking for TMA construction
  - Image analysis
  - Brightfield and Fluorescence

Quality and consistency across trials

- Specific protocol language and generic sections
- Templated consent – mandated/optional conforming to TCPS guidelines and harmonized with US NCI language
- Laboratory Correlative Manuals
- Kit production
- Standardized MTAs
- Correlative Science Study Leads and committees for individual protocols

Overall Process / Safeguards for CCTG Collections

- Supervision by the CSTB committee and operational subcommittee
- Patient consent must be signed before requests for tissue to be sent to the bank are made
- Material is received, catalogued, de-identified and appropriately stored
- Database is maintained, secure, and regularly updated
- Defined procedures for approval of release of tissue to investigators
- Standardized SOPs are followed consistent with national and international guidelines

Confidentiality

De-identification of tissue

- All samples received are assigned a unique Tumour Bank ID
- Link is in the TTDR/PCO/Statistical support
- Samples released to investigators are only identified by the unique TBID
- Clinical database remains with the CCTG

Consent

- Informed consent as per TCPS is obtained and checked from every participant
- More complex in the era of genomics

Mission

To develop and conduct clinical trials aimed at improving the treatment and prevention of cancer with the ultimate goal of reducing morbidity and mortality from this disease.

About Us

The Canadian Cancer Trials Group is a cooperative oncology group which carries out clinical trials in cancer therapy, supportive care and prevention across Canada and internationally. It is one of the national programmes and networks of the Canadian Cancer Society Research Institute (CCSRI), and is supported by the Canadian Cancer Society (CCS).

Read More

Faculty Researchers

Meet the Faculty Researchers

Information

The information contained in this website is intended for use by Canadian Cancer Trials Group members at participating centres.

Recently Activated

CE7
Stereotactic Radiosurgery compared with Whole Brain

Planned Trials

ALC5
Novel Therapeutics vs Azaciticine in Patients with AML or High Risk

Quick Links

- CCTG Connection
- Central Office Staff
- Password Management
- Tissue Bank
- Inventory
- Application for Tissue Research
- Accrual Utility

Correlative Science / Tumour Bank

The Canadian Cancer Trials Group established the Tumour Tissue Data Repository (TTDR) in 1997 to support Correlative Science with the assistance of Rhone Poulenc Rorer (now Sanofi-Aventis). The Canadian Cancer Trials Group TTDR contains unique disease specific collections which are linked to an associated clinical dataset. This represents a "real" tumour bank in that tissue is collected from institutions across Canada and the world, catalogued and housed centrally in the Department of Pathology and Molecular Medicine at Queen's University.

Expand All

- + Our Mission
- + Profile
- + Inventory
- + About Us
- + What We Do
- Canadian Cancer Trials Group Correlative Science and Tissue Banking Procedures - 2013JAN31
- + Access To Our Collection
- Inventory
- + Our Facility
- + Links
- + Contact Us

Quick Links

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News

MA31 Details

Status: Closed
Activation Date: 2008JUL17
Closing Date: 2011DEC01
Phase: III

Description: A Randomized, Open-Label, Phase III Study of Taxane Based Chemotherapy with Lapatinib or Trastuzumab as First-Line Therapy for Women with HER2/neu Positive Metastatic Breast Cancer

Eligibility: Women with documented evidence of HER2/neu positive breast cancer (by local or central laboratory testing) which is metastatic, and with no prior chemotherapy and/or anti-HER2/neu targeted therapy in the metastatic setting.

Objective: Primary - Progression-Free Survival Secondary - Overall Survival - Time to CNS metastases at the time of progression - Incidence rates of CNS metastases at the time of progression - Overall objective response rate, time to response and duration of response - Clinical benefit response rate - Adverse event profile - Quality of Life (using the EORTC QLQ-C30 and a Trial Specific Checklist) - Clinical outcomes using biomarker changes in biological samples - Economic Evaluation: health utilities (using the EQ-5D questionnaire) and healthcare utilization

Participation: Open to member centres

Lay Description: This is a study for women with documented HER2/neu positive breast cancer which is metastatic and with no prior chemotherapy and/or anti-HER2/neu targeted therapy in the metastatic setting. HER2/neu status must be confirmed prior to randomization using either a local or the central laboratory [CTAG at the BCCA, Vancouver, Canada]. Eligible women will be assigned (open label) to one of two arms: ARM 1 - Taxane chemotherapy plus lapatinib; ARM 2 - Taxane chemotherapy plus trastuzumab. Taxane chemotherapy will include either q weekly paclitaxel or q 3-weekly docetaxel. The choice of taxane will be up to the treating physician and must be specified at the time of randomization. Patients on arm 1 will receive oral daily lapatinib concurrently with the chosen taxane for 24 weeks, and single-agent daily lapatinib thereafter, until PD. Patients on arm 2 will receive IV trastuzumab concurrently with the chosen taxane for 24 weeks, and single agent 3-weekly trastuzumab thereafter until PD.

Primary Publication Show

Other Publications Show

Inventory

Hide **Tissue Samples**

Disease Site	Trial Code	Patients Accrued	Patients - Blocks	Patients - Slides	Patients - Blocks and/or Slides
BREAST	MA31	652	272	645	645

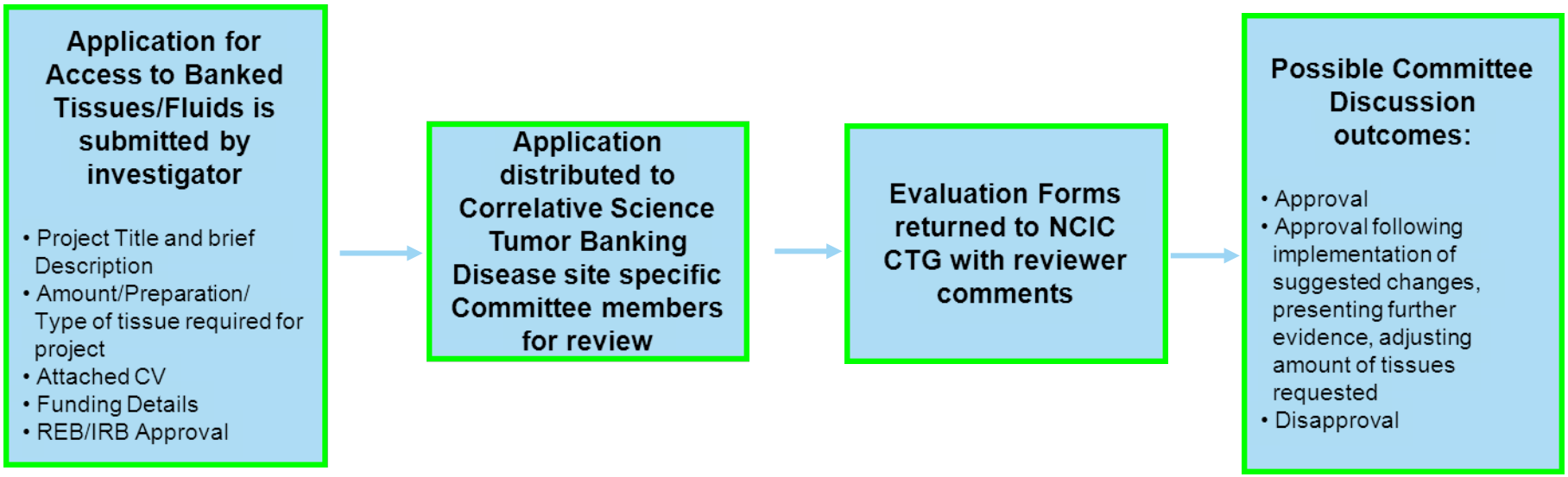
Hide **TMA Samples**
(Core size is 0.6 mm)

Disease Site	Trial Code	Patients Accrued	TMA Blocks	Patients on TMA Blocks
BREAST	MA31	652	10	486

Hide **Fluid Samples**

Disease Site	Trial Code	Patients Accrued	Patients - Whole Blood	Patients - Cellular Component of Blood	Patients - DNA extracted from Blood	Patients - RNA extracted from Blood	Patients - Plasma	Patients - Serum	Patients - Urine	Patients - Buccal
BREAST	MA31	652	18	0	359	0	491	495	0	0

Access to Banked Tissues Correlative Science DSS Committees



Specimens will be released upon verification of Funding, REB/IRB Approval, and Contract signing

Publication Standards for Biomarker Studies

- REMARK, JNCI, August 2005

"Reporting Recommendations for Tumour Marker Prognostic Studies"

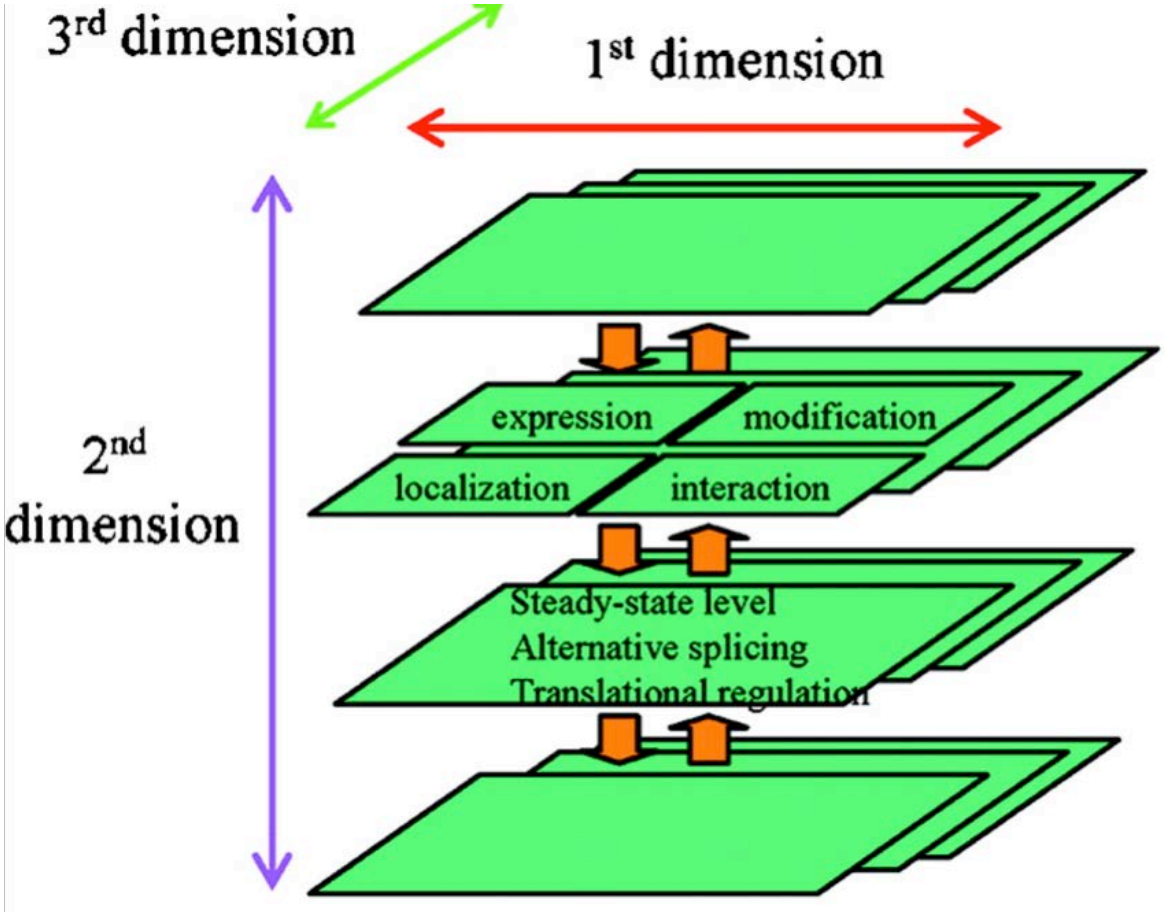
- REMARK BJC and PLoS Medicine, 2012

- Use of Archived Specimens for Prognostic and Predictive Markers, JNCI, November 2009

What is the value of the Tumour/Tissue Bank?

- Molecular genetic information on tumours is a critical component of basic, applied, and drug development research
- Access to this tissue permits the assessment of prognostic factors, predictive factors to therapeutic agents and treatment regimens
- Facilitates the understanding of the basic biological and genetic mechanisms of cancer.
- Crucial in the development of "targeted" and specific therapy. This information is vastly enriched, more valuable, and powerful when associated with a clinical trials database.

What kind of biomarker?



Other
- lipids
- metabolites
- physiological

Protein
- where
- which version
- functionality

RNA
- which type
- where
- when

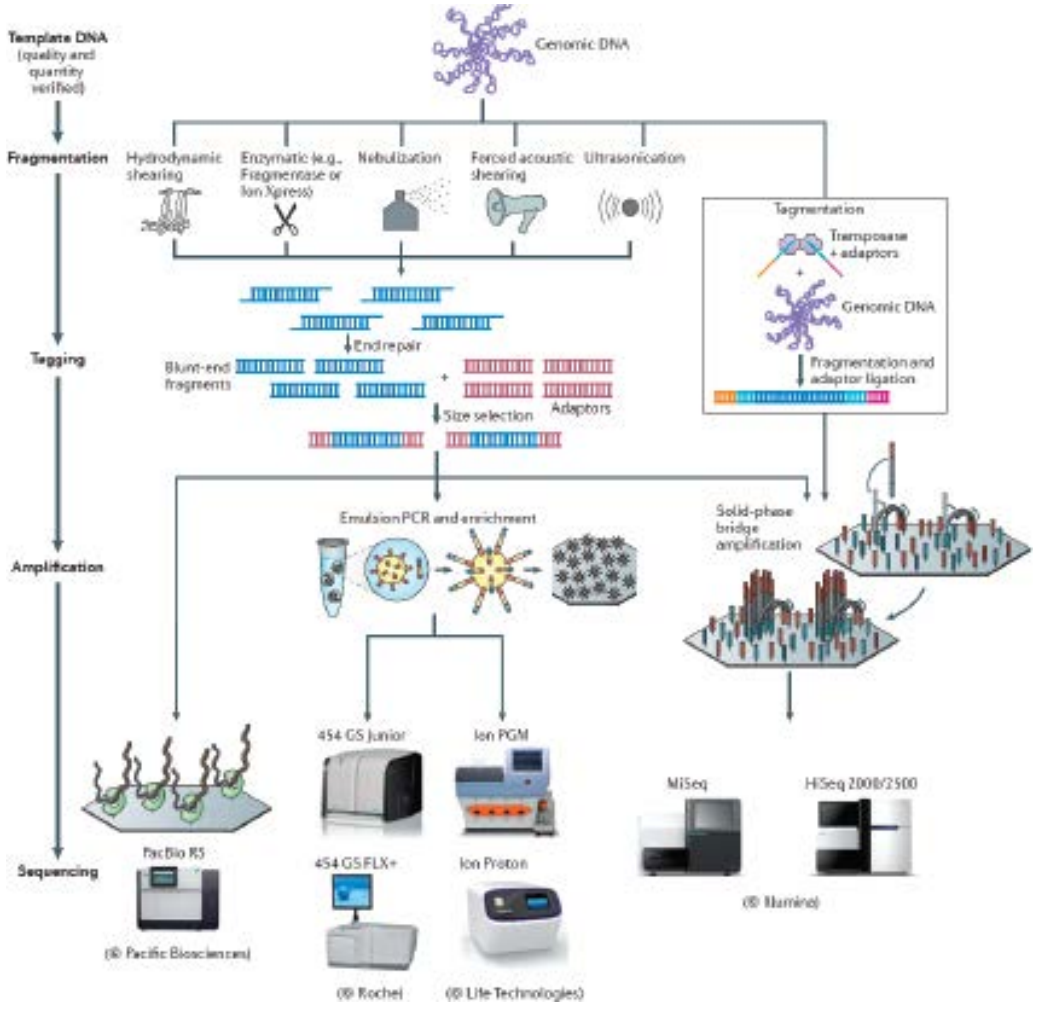
DNA
- single nucleotide changes
- copy number changes
- methylation

Considerations

- The type of sample you have access to makes a difference in what you can measure
- The collection of the sample can influence the behaviour of a biomarker
- The processing of a sample will influence the behaviour of a dynamic biomarker
- Biomarkers don’t necessarily behave the same in different sample types

Generic MPS

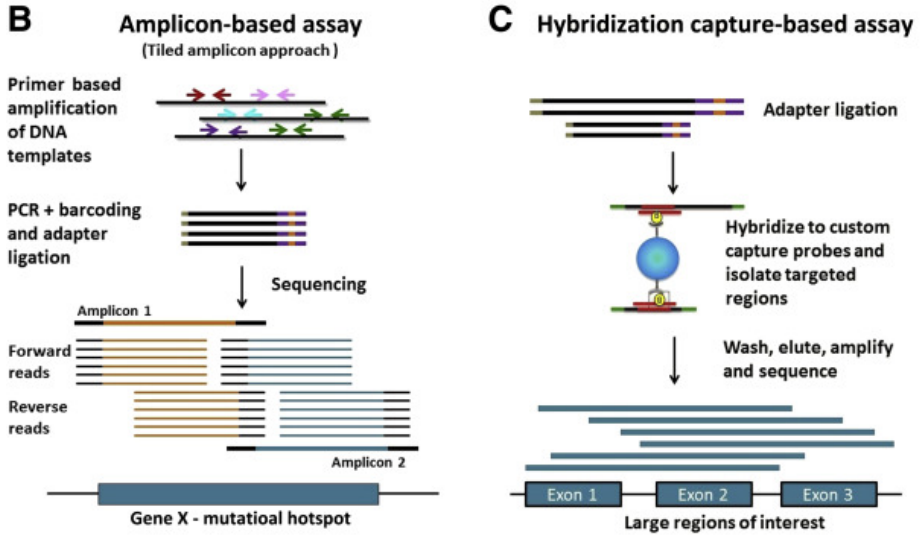

Library Preparation
Clonal Amplification
Sequencing


Template DNA (quality and quantity verified)
Genomic DNA
Fragmentation
Hydrodynamic shearing
Enzymatic (e.g., Fragmentase or Ion Xpress)
Nebulization
Forced acoustic shearing
Ultrasonication
Tagmentation
Transposase + adaptors
Genomic DNA
Fragmentation and adaptor ligation
Tagging
End repair
Blunt-end fragments
Size selection
Adaptors
Amplification
Emulsion PCR and enrichment
Solid-phase bridge amplification
454 GS Junior
Ion PGM
MiSeq
HiSeq 2000/2500
Sequencing
PacBio RS
454 GS FLX+
Ion Proton
(© Pacific Biosciences)
(© Roche)
(© Life Technologies)
(© Illumina)

Main MPS methods in use in clinical labs

Thermo Fisher

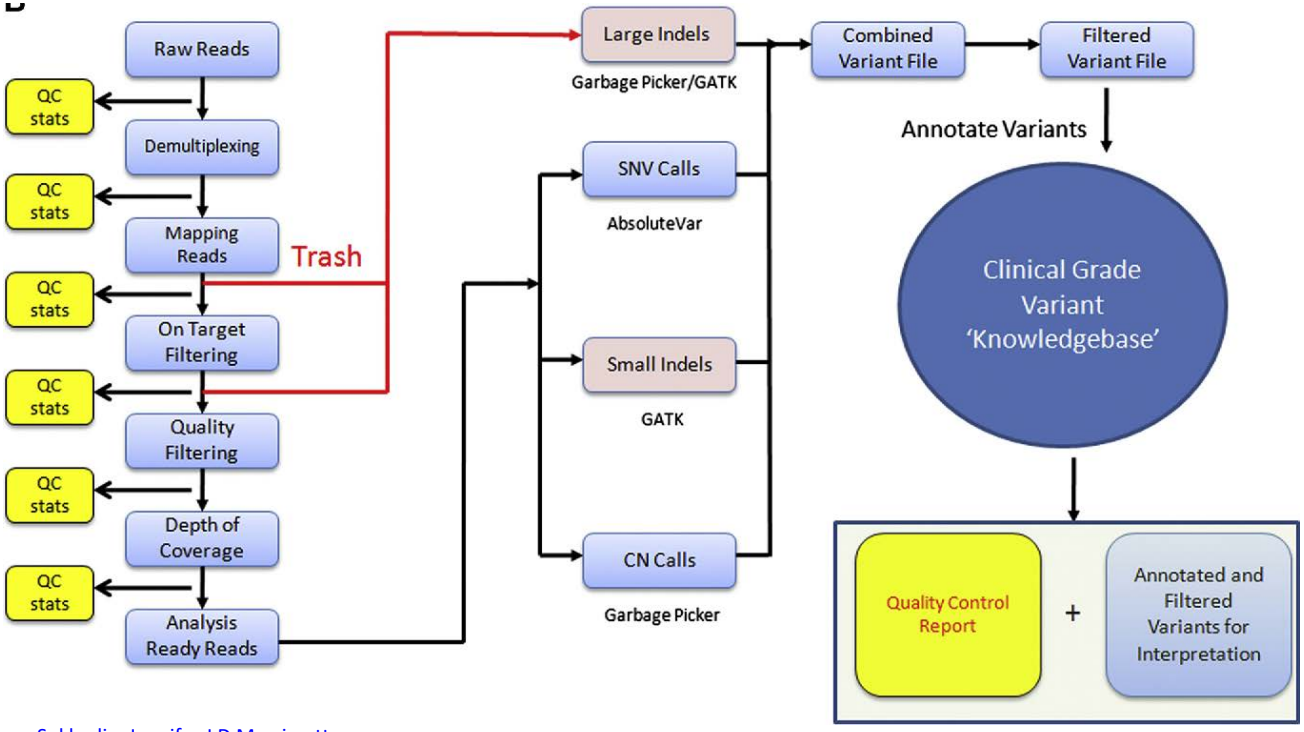
PGM
S5/S5-XL
Proton



Illumina

MiniSeq
MiSeq
NextSeq
HiSeq
HiSeqX Ten

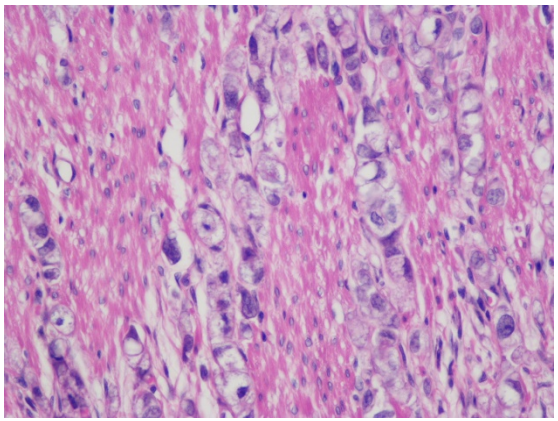
Analysis and interpretation



Taken from Robert D. Daber, Shrey Sukhadia, Jennifer J D Morrissette
Published in Cancer genetics 2013
DOI:10.1016/j.cancergen.2013.11.005

Estimating tumour %- a pre-analytic conundrum

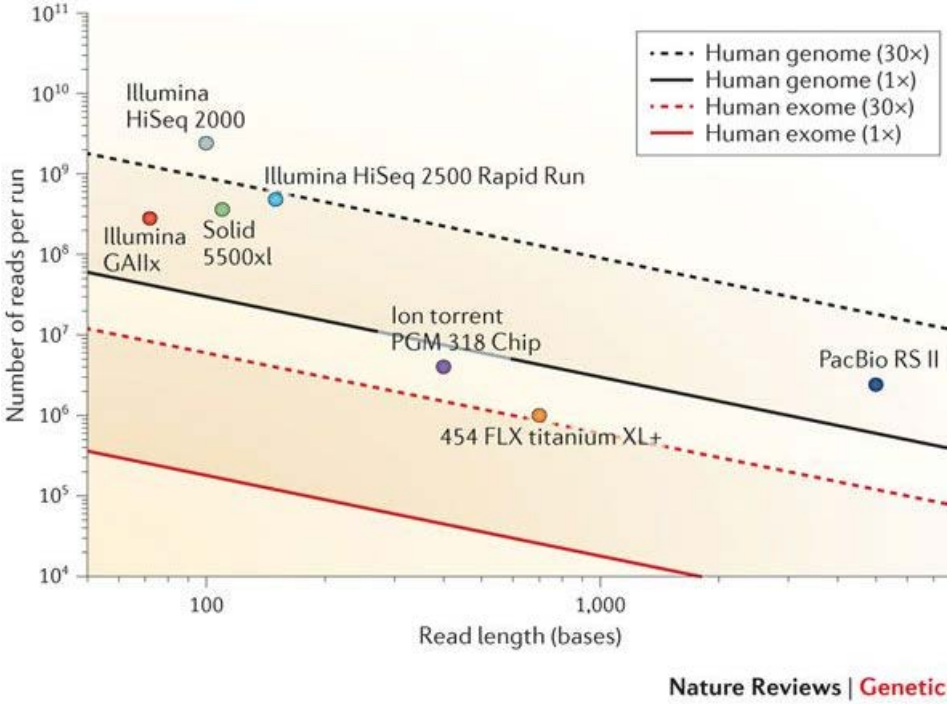
- Tumors are not diploid
  - If tetra-ploid: 2x 2N DNA content
    - 50% tumor DNA and 50% normal DNA
  - If octo-ploid: 4x 2N DNA content
    - 66% tumor DNA and 33% normal DNA
  - If hypodiploid (34 chromosomes, 0.75x 2N DNA content in tumor)
    - 27% tumor DNA 63% normal DNA



Estimate 33% tumour content?

- Tumour nuclei are different sizes compared to normal
  - Tumor nuclei at least 3x longer in one dimension than normal (and more variable in shape)
- How close to the H&E slide are the slides received in the molecular lab?

Depth of sequencing



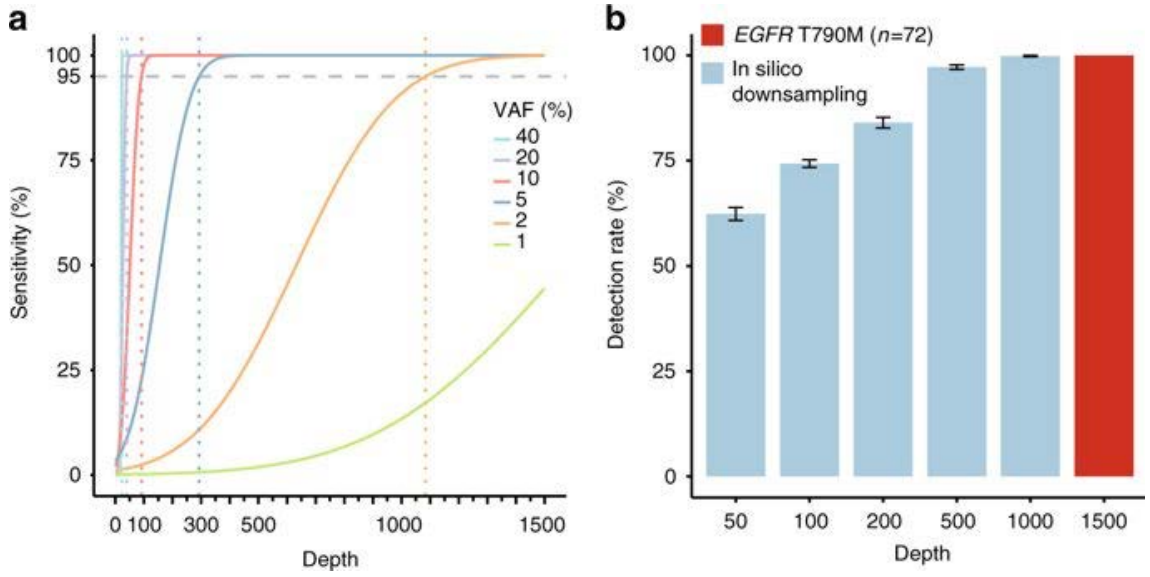
Taken from Nature Reviews Genetics **volume 15**, pages 121–132 (2014)

Depth of sequencing refers to the number of reads that cover any particular base or region of interest (ROI)

Each sequencing platform has a maximum number of bases that can be sequenced for any given size chip or flow cell

Can increase depth of sequencing by using more input nucleic acid or reducing the number of samples per assay

Limit of detection- related to depth



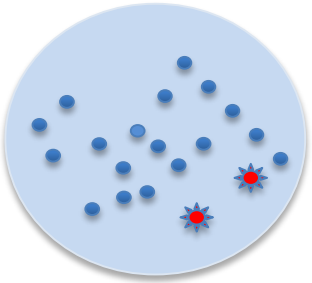
Nature Communications **8**, Article number: 1377 (2017)

Variant allele fraction (VAF): the % of molecules in the sequencing mix that carry the allele of interest

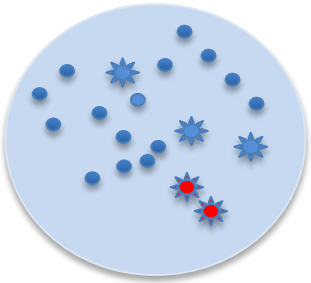
Limit of Detection (LoD): Variant allele fraction for which detection reaches 95% sensitivity at a given depth

Must be established for each type of variant one wishes to detect

LoD in real life- does it tell the whole story?



10% tumour cells
All are heterozygous for variant of interest
Variant is present at 5%



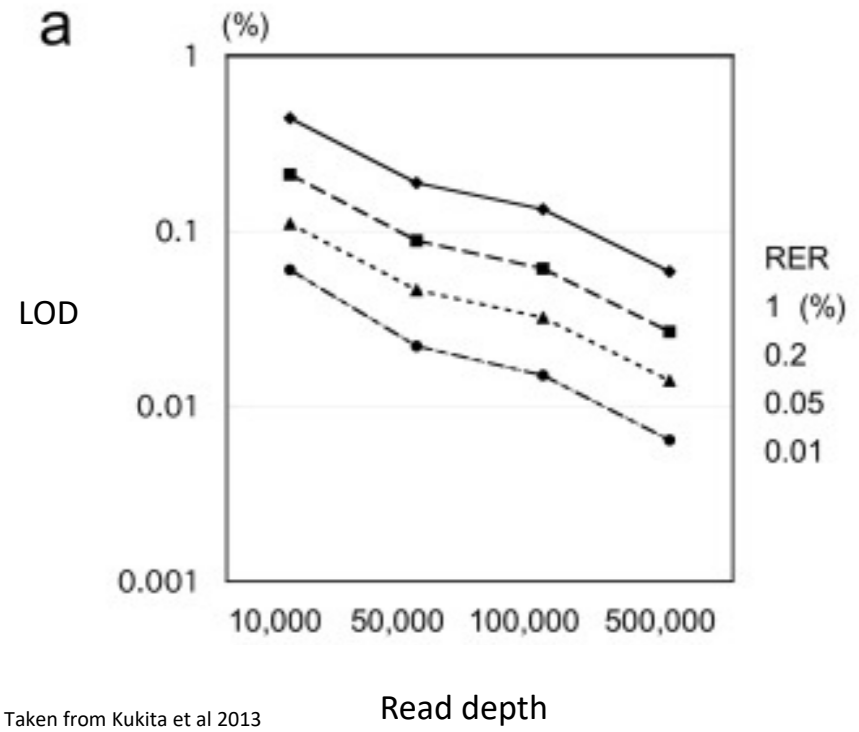
25% tumour cells
40% of them are heterozygous for variant of interest
Variant is present at 5%

Established the assay with a limit of detection for an SNV of 5% at a depth of 400

Identify an EGFR L858R variant present at 5% VAF

Both patients eligible for targeted therapy, but will both respond equally well? If information not conveyed, missing opportunity to look for other strategies? If tumour % estimate way off, is this predictive information less useful?

Error rates



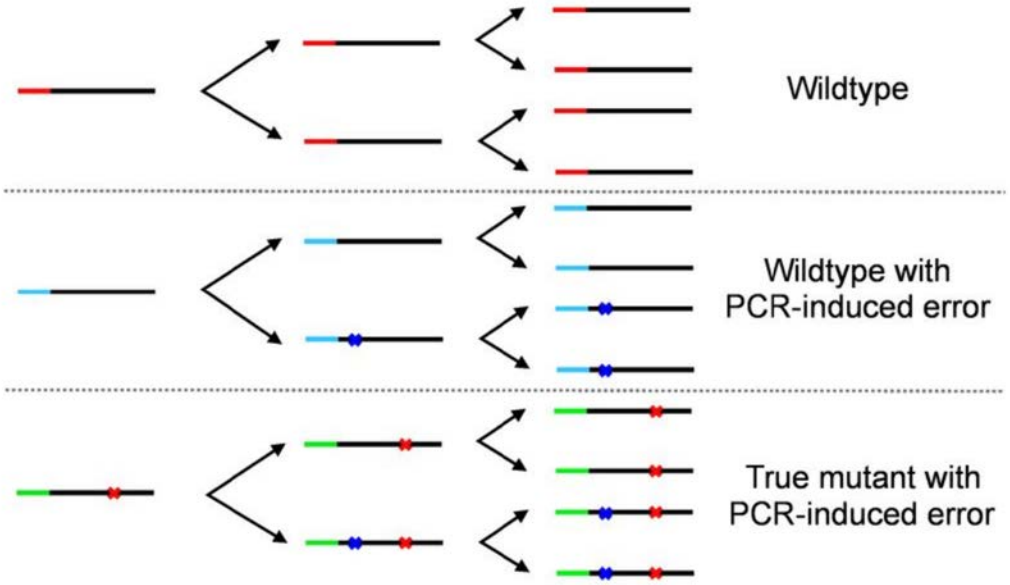
Taken from Kukita et al 2013
PLoS One 8:e81468

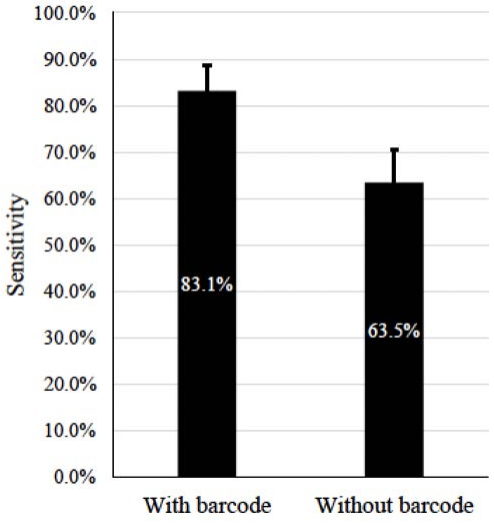
Estimating error rates for MPS is difficult because the errors come from multiple places, some specific to the platform, some specific to the library prep and others random

- Library preparation
- Amplification
- Sequencing
- Alignment
- DNA quality

If you have a 0.1% error, and generate 10M bases of sequence, that means that 10,000 of them are incorrect

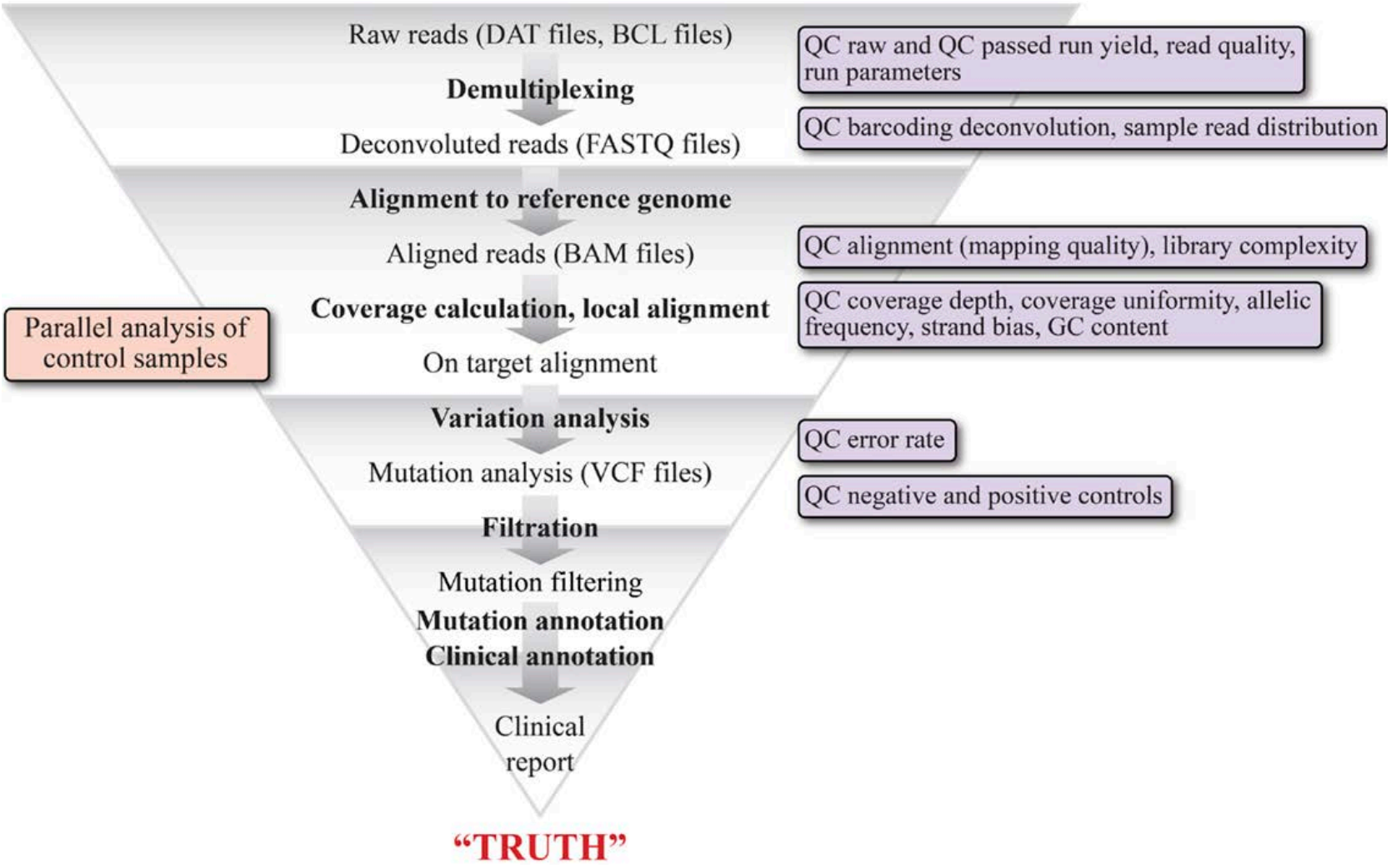
Improved accuracy- molecular barcodes





Taken from Yang et al
2018 BMC Cancer 18:319

Before you get it…



Front. Oncol., 17 April 2014 | http://dx.doi.org/10.3389/fonc.2014.00078