Overview of Next Generation Sequencing technologies (and bioinformatics) in cancer

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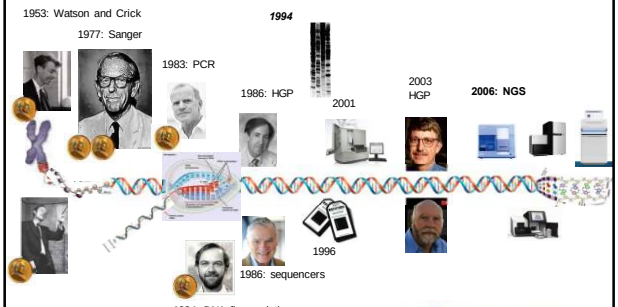
Agenda

- What is NGS
- Understanding sequencing: NGS technologies described
- Applications in Cancer
  - ctDNA analysis as a liquid biopsy
  - A genomic case report
  - Copy-number profiling by shallow whole genome sequencing
- The future of NGS

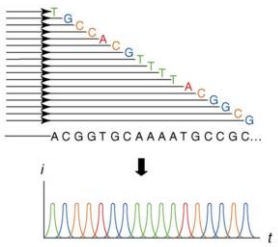
What is NGS: next-generation sequencing



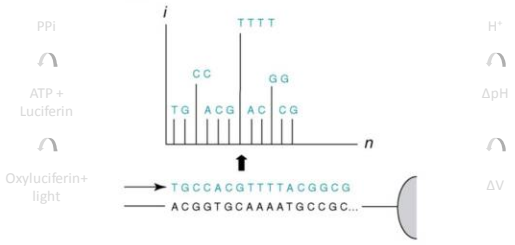
Understanding sequencing:

- A whistle stop tour of the major NGS platforms

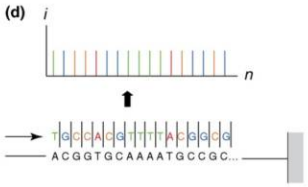
Understanding sequencing: Sanger sequencing



Understanding sequencing: 454/Ion Torrent

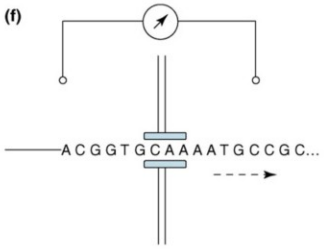


Understanding sequencing: Illumina SBS

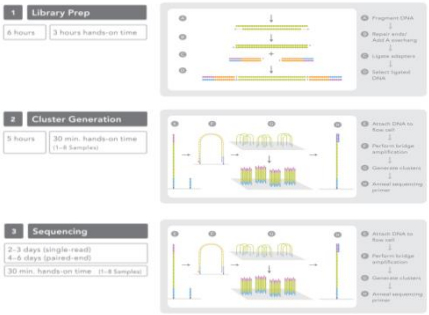


Understanding sequencing: Pacific Biosciences

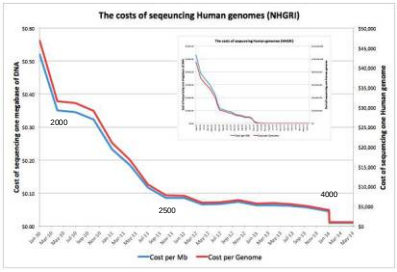
Understanding sequencing: Nanopore sequencing



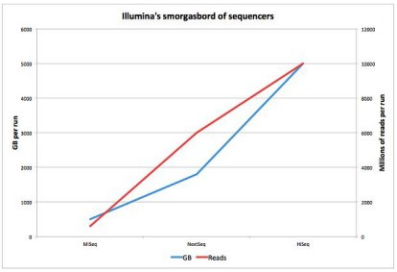
How Illumina works



The Moore's law slide



Which sequencer to use



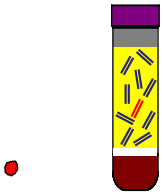
NGS applications in cancer

- ctDNA analysis as a liquid biopsy
- Copy-number profiling by shallow whole genome sequencing
- A genomic case report

ctDNA analysis as a liquid biopsy

- What is circulating tumour DNA
- Using ctDNA in personalised medicine
- ctDNA and tumour evolution
- What's the future for ctDNA analysis?

ctDNA: what is circulating tumour DNA



The use of ctDNA in personalised medicine

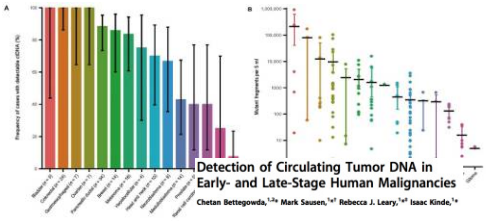
Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Sarah-Jane Dawson, F.R.A.C.P., Ph.D., Dana W.Y. Tsui, Ph.D.,

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

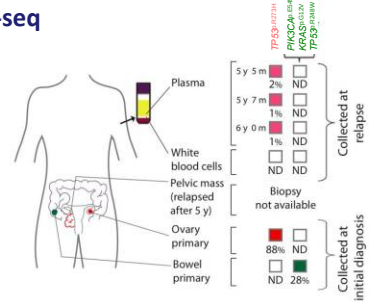
The use of ctDNA in personalised medicine



Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies

Chetan Bettegowda, Mark Sausen, Rebecca J. Leary, Isaac Kinde,

Retrospective confirmation of site-of-origin in relapse of synchronous ovarian & bowel cancer by TAM-seq



TAm-seq

Access Array

Monitoring tumour dynamics during treatment

Comparing ctBiomarkers to monitor tumour dynamics

Plasma ctDNA levels correlate with survival

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

ctDNA and tumour evolution: tumour evolution may contribute to therapeutic failure

Branched evolution

ctDNA and tumour evolution: exome-sequencing of ctDNA

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

ctDNA and tumour evolution: identifying genes involved in acquired drug resistance

Tamoxifen / Trastuzumab

Lapatinib / Capecitabine

GAS6 MED1 PDGFRA

Days of follow up

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

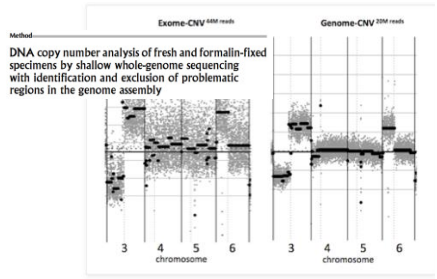
Copy-number profiling by shallow whole genome sequencing

Copy-number is a driving event in cancer

ARTICLE

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups

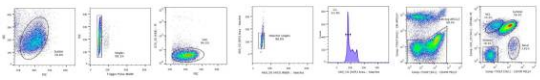
Low-coverage WGS provides excellent CNV calls



Single-cell CNV analysis – Flow-cytometry

FLOW-CORE

- Cells were stained with multiple markers: CD45,CD31, CD49f, EpCAM and CD24
- Viability and live cell cycle dyes (PI & Hoechst 3342) were used to exclude dead cells and to sort for G1 only
- Cells were sorted on a BD Influx directly into plates containing 2.5µl of lysis buffer at 4°C

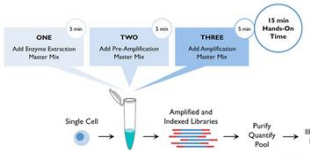


Single-cell CNV analysis - Genomics

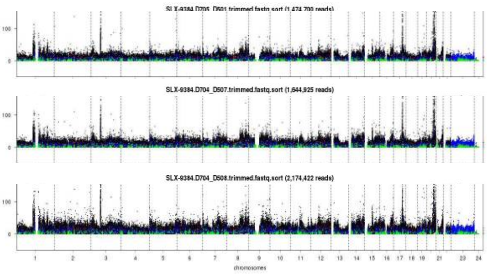
RUBICON PICOPLEX IS A SIMPLE AND ROBUST METHOD

- A quick and simple 3-step workflow
- Used in many PGD labs
- Single-tube processing provides a low risk of contamination
- Average 1-3M single-end 50bp reads per cell

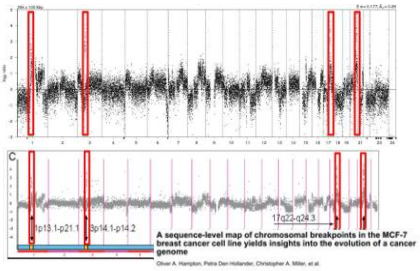
RUBICON GENOMICS



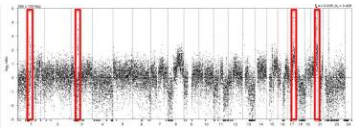
Single-cell read counts are very similar



Single-cell CNV-seq compares well to arrays

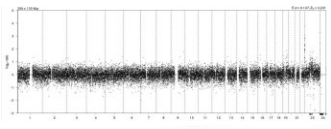


Controls have the expected CNVs



Commercial MCF7 (bulk DNA)

Normal luminal cells (10 cells)



Single-cell CNV analysis

CONCLUSIONS

- Single-cells can be successfully index sorted and amplified for CNV-seq
- Single-cell CNV-seq is a viable method for use in cancer genomics studies
- Sorting costs are negligible, sequencing costs were around £20 per cell – but library prep costs need to drop to make Single-cell CNV-seq economical

A genomic case report

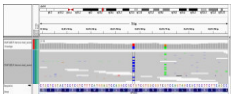
A genomic case report

- 3 year old with a life-threatening immunodeficiency
- Routine immunological testing was uninformative
- Presentation suggested genetic component
- Possible STAT1 mutation
  - Led to debate about clinical path
  - HSCT considered ineffective

Desperately ill toddler [redacted] needs bone marrow transplant to save her life

A genomic case report

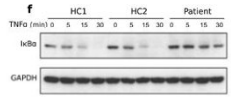
- Whole genome sequencing
  - Illumina TruSeq PCR-free
  - 30x coverage of trio
  - Interpretation of "exome"



A genomic case report

- Whole genome sequencing
  - Illumina TruSeq PCR-free
  - 30x coverage of trio
  - Interpretation of "exome"

- NFKB1A mutation
  - *de novo* het c.94A>G in NFKBIA
  - results in IκBα serine>glycine (p.S32G where serine usually phosphorylated)
  - Loss of IkBα phosphorylation prevents normal signaling via degradation
  - IkBα signaling is vital in the adaptive and innate immune response

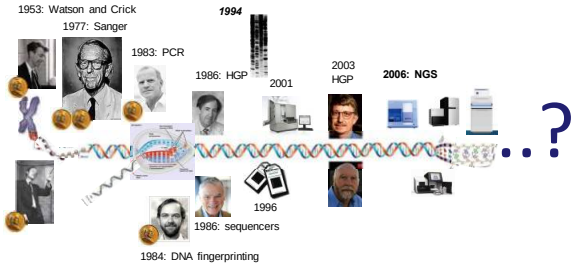


A genomic case report

- NFKB1A mutation detected by rapid WGS
- Transplant was likely to be curative
  - 9/10 HLA-A-mismatched unrelated donor
  - one of two patients worldwide alive with this condition

Disseminated *Mycobacterium malmoense* and *Salmonella* infections associated with a novel variant in *NFKBIA*. **JCI 2017**

The future of NGS



Turn-around times for oncology testing

CURRENT TESTING

- EGFR, KRAS – ASSESS, FLAURA, AURAex 3-11 days
- BRCA – Myriad 7 days (Sanger)
- TP53 – Foundation Medicine 10 days

FUTURE TESTING

- A personal story of a breast cancer patient
- GP, *wait*, biopsy, *wait*, Follow up, *wait*, surgery, *wait*, follow up, *wait*, radiotherapy, *wait*, *wait*, *wait*

Nanopore sequencing compared to Illumina

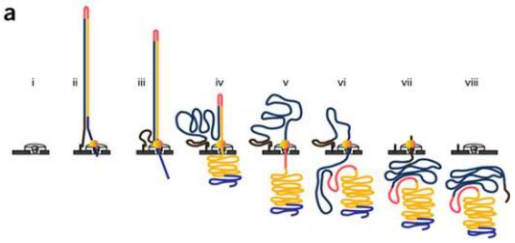
HiSeq *NextSeq*

- High-throughput NGS
- Excellent accuracy
- High investment costs
- Long turnaround time
- Core facilities/testing hubs

MinION Voltrax

- Disruptive technology
- Huge opportunities
- Point of care testing
- Non-invasive sample input
- Fast turn-around time

How does a nanopore sequencer work



Nanopore sequencing in ctDNA

THE VISION *"ONE HOUR FROM PLASMA TO MUTATION CALL"*

- ctDNA extraction without clean up
- Rapid PCR or Isothermal amplification
- MinION sequencing
- "Run-until" analysis

TESTING e.g. EGFR T790M

- Non-invasive same-day test
- 200,000 potential patients per year
- Saves 70,000 tests that currently fail on tissue biopsy

What next…for Oxford Nanopore

20 years "working" on *ERBB2*

20 years "working" on *ERBB2*: single gene

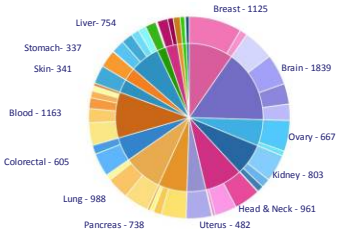
A differential PCR assay for the detection of c-erbB 2 amplification used in a prospective study of breast cancer

J Clin Pathol Mol Pathol 1997;50:254-256

Breast cancer is a heterogeneous disease. There may be many different mechanisms by which tumours grow, metastasise, and evade treatment response. Genetic markers that sub-classify these tumours could help identify those patients who would benefit most from adjuvant therapy.

Figure 1 The amplification products from the differential PCR of c-erbB 2 and the reference gene β globin from three normal DNA controls (lanes 1–3), from three breast tumours with amplification of c-erbB 2 (lanes 4–6), and from a dilution of SKBR3 DNA in normal DNA, equivalent to eight copies (lane 7), five copies (lane 8), and three copies (lane 9) of c-erbB 2. The 174, 102, and 80 base pair bands from the molecular weight marker (pUC18 DNA digested with HaeIII) are seen in lane 10.

20 years "working" on *ERBB2*: whole genomes



20 years "working" on *ERBB2*: NGS panels

Fig 6.3: The potential for trastuzumab treatment

Cancer	UK Cases	Percent ERBB2 amplified	Cases ERBB2 amplified
Breast	50285	12.6%	6311
Bowel	41581	3.1%	1289
Lung	43463	2.3%	1000
Bladder	10399	5.8%	607
Prostate	41736	0.9%	384
Pancreas	8773	2.0%	175
Skin	13348	0.6%	80
Brain	9365	0.2%	14
Kidney	10144	0.1%	13
NHL	12783		0

Acknowledgements

- CRUK and The University of Cambridge
- My lab (Paul, Hannah, Fatimah, Marta, Ros, Johanna and Joaquin)
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- ctDNA (**Nitzan Rosenfeld:** Tim, Muhammad, Dana, Davina. **Carlos Caldas**: Sarah-Jane Dawson. **James Brenton:** Christine Parkinson)
- NFKB1A (James Thaventhiran, Matt Eldridge, Emily Staples)
- Copy number (Sarah Leigh-Brown, Nina Lane)