Leveraging next generation sequencing in the study of myeloma somatic genetics

13th International Myeloma Workshop
May 6, 2011 Paris

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Wellcome Trust Sanger Institute


wellcome trust


Adenoid Cystic Carcinoma
Research Foundation


breakthrough
breast cancer


CANCER RESEARCH UK

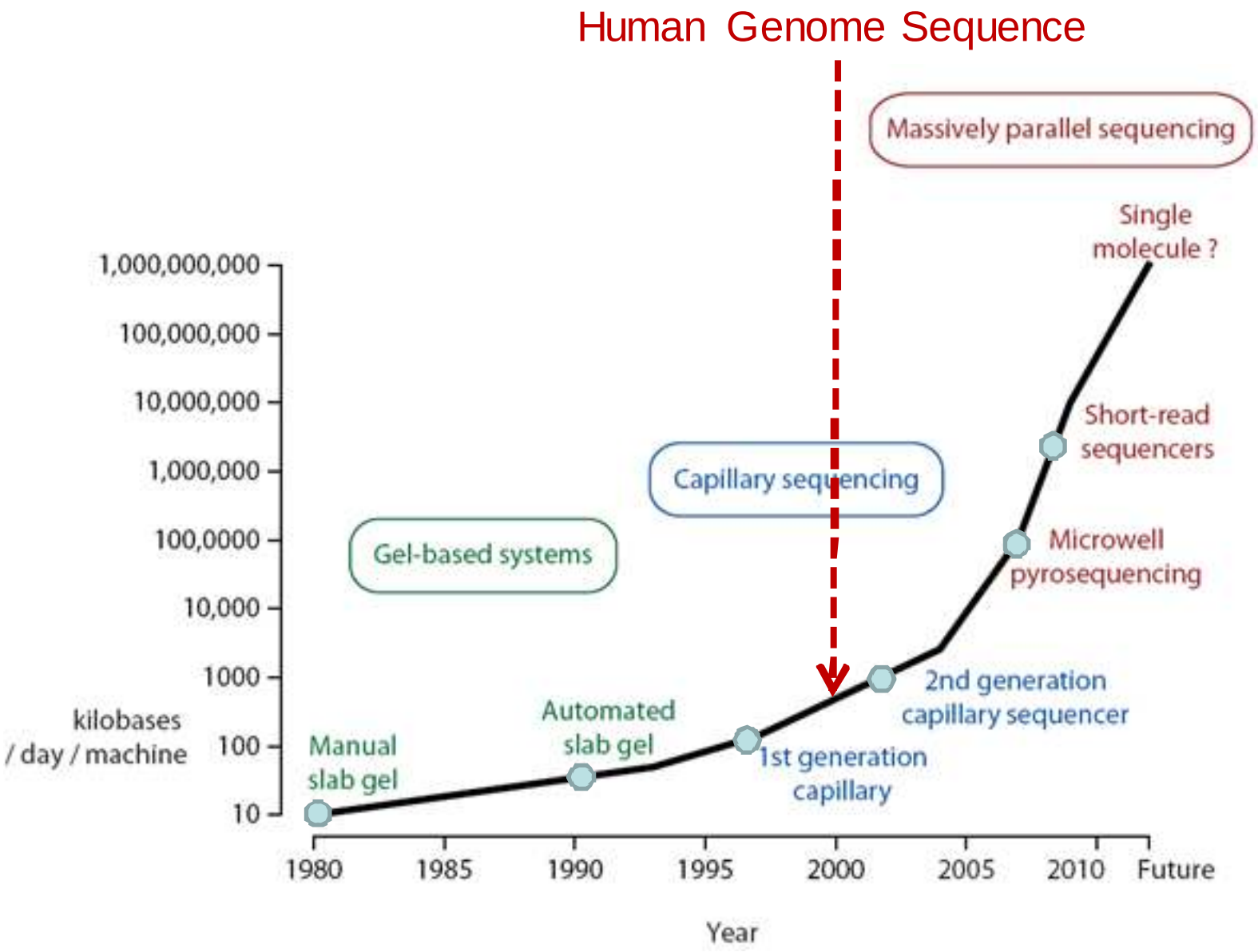

THE KAY KENDALL LEUKAEMIA FUND


The Institute
of Cancer Research


basis
COOPERATION


SEVENTH FRAMEWORK
PROGRAMME
PREDICT
CONSORTIUM

No financial interests or conflicts of interest to declare


Human Genome Sequence
Massively parallel sequencing
Single molecule ?
Short-read sequencers
Microwell pyrosequencing
2nd generation capillary sequencer
1st generation capillary
Capillary sequencing
Automated slab gel
Manual slab gel
Gel-based systems
1,000,000,000
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kilobases / day / machine
1980
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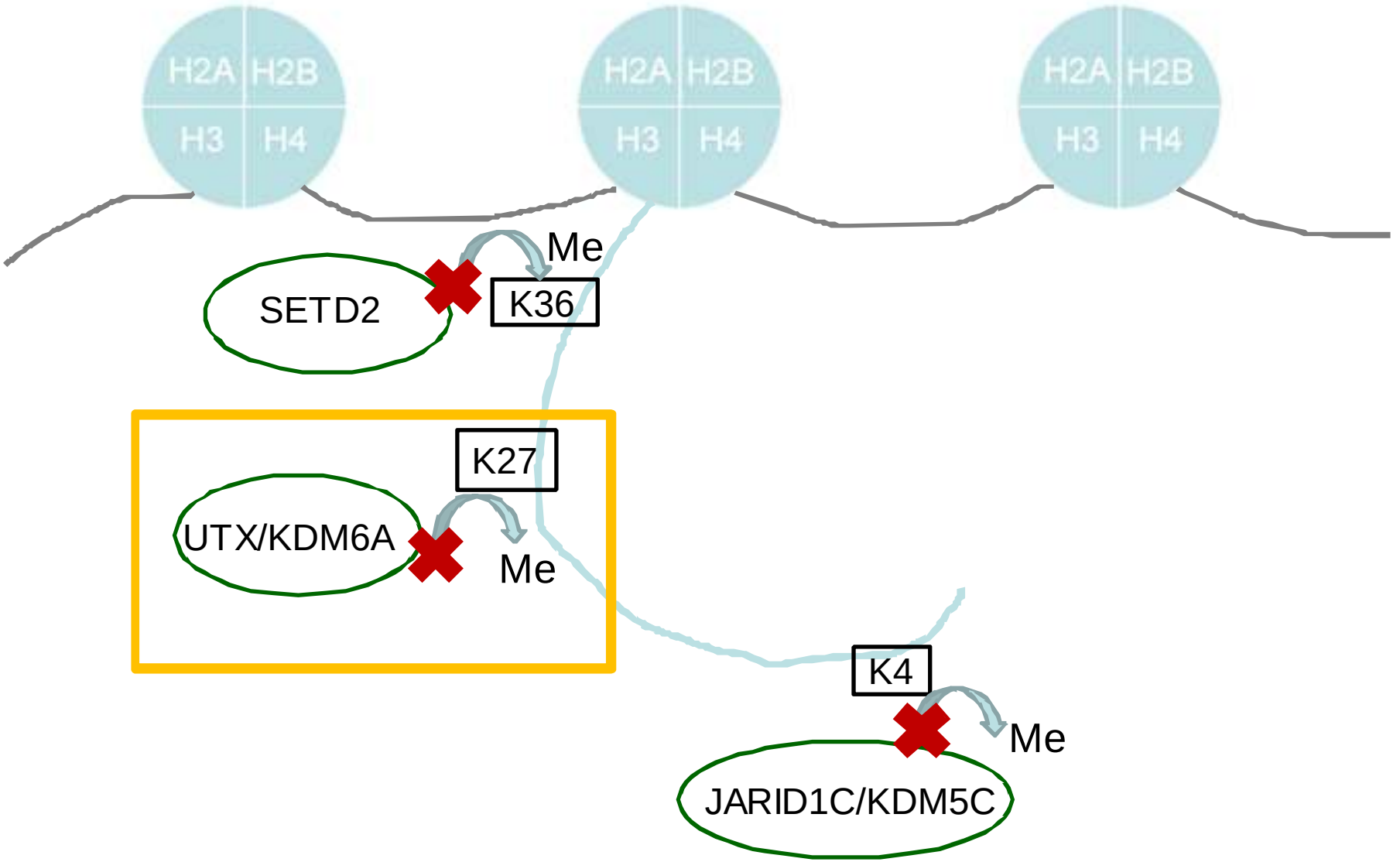
Future
Year

PCR based capillary sequencing

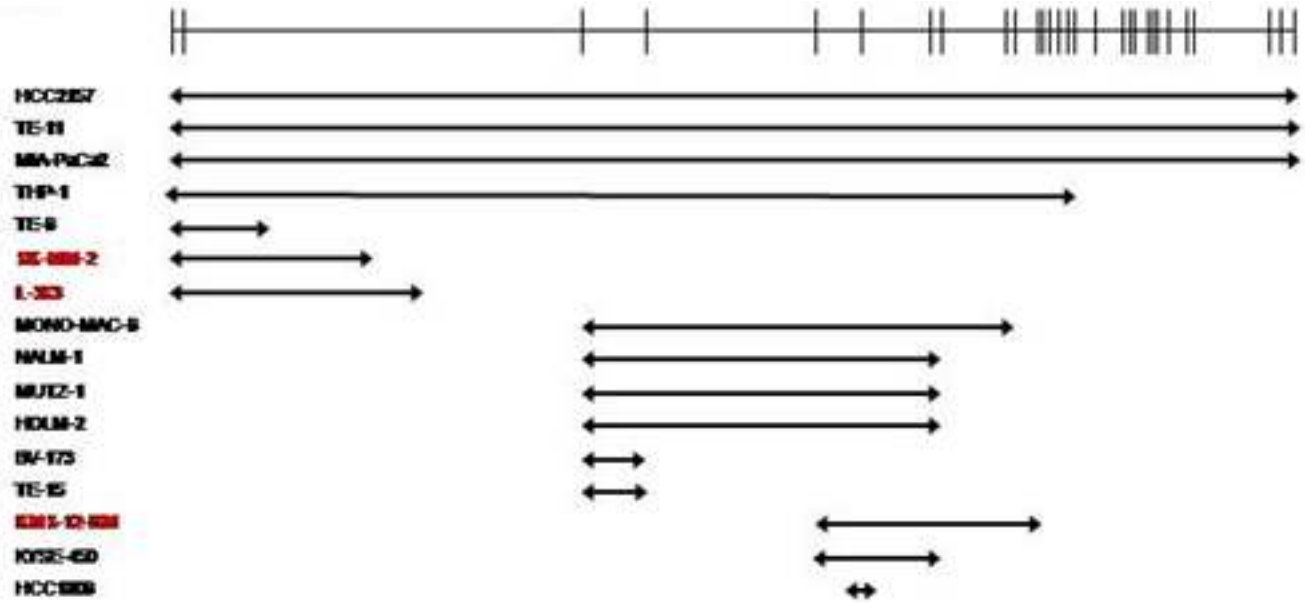
- 3544 genes exon by exon sequentially in 100 cases of clear cell renal carcinoma

- Followup of candidate new cancer genes in ~1000+ cancer samples of varying histology



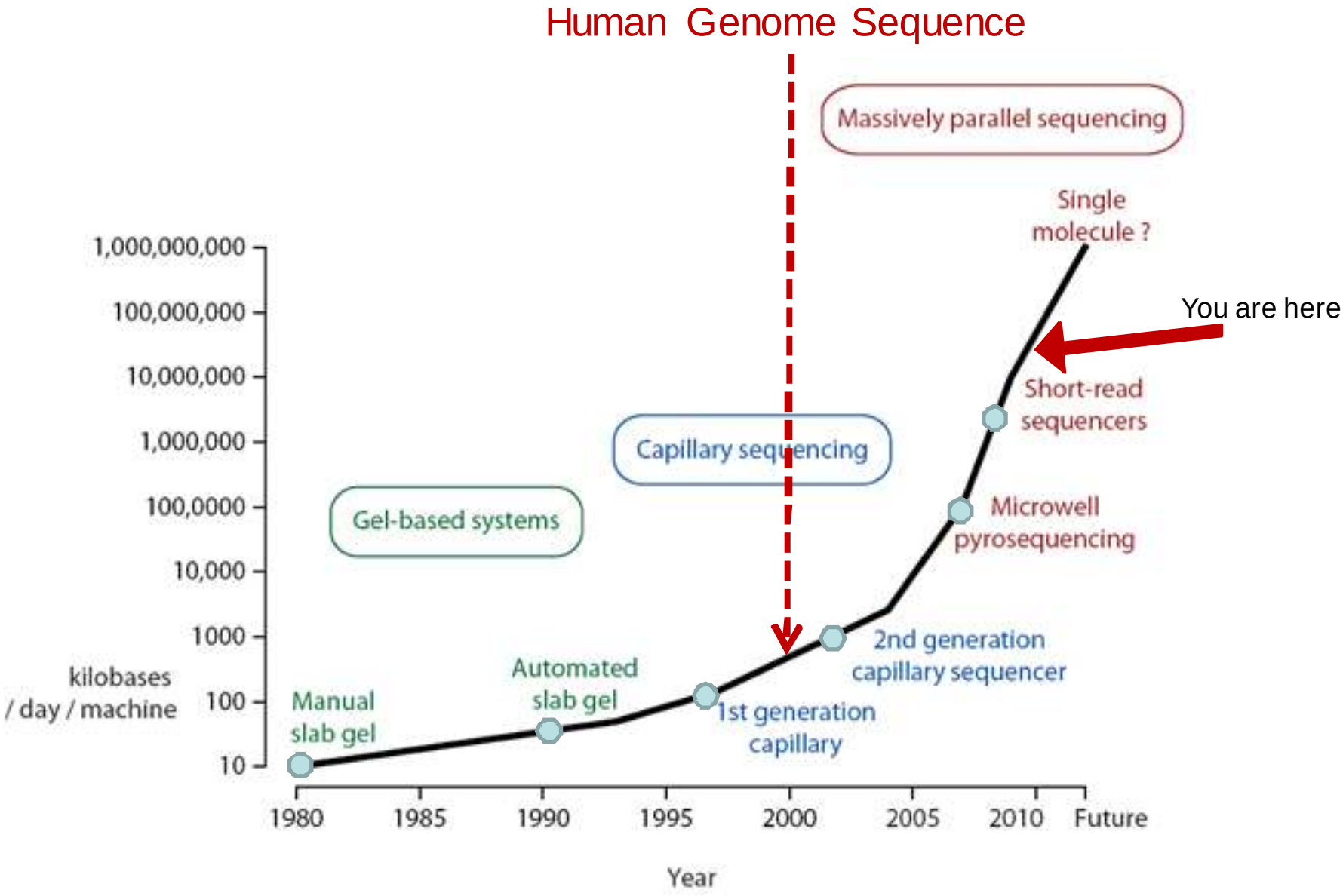
Van Haaften et al, Nature Genetics, 2009
Dalgliesh et al, Nature 2010

KDM6A/UTX mutations in Myeloma



Sample	Cancer type	Sex	mutation - DNA	mutation - Protein
L-363	MM	F	Hom del exons 1, 2	
LP-1	MM	F	Hom c.1621C>T	p.Q541X
PD2916a	MM	F	Het c.1787delA	p.N596fsX3
KMS-12-BM	MM	F	Hom del exons 5-12	
PD2933a	MM	M	Het c.3014delT	p.L1005fsX43
SK-MM-2	MM	M	Hom del exons 1, 2	

Van Haaften et al, Nature Genetics, 2009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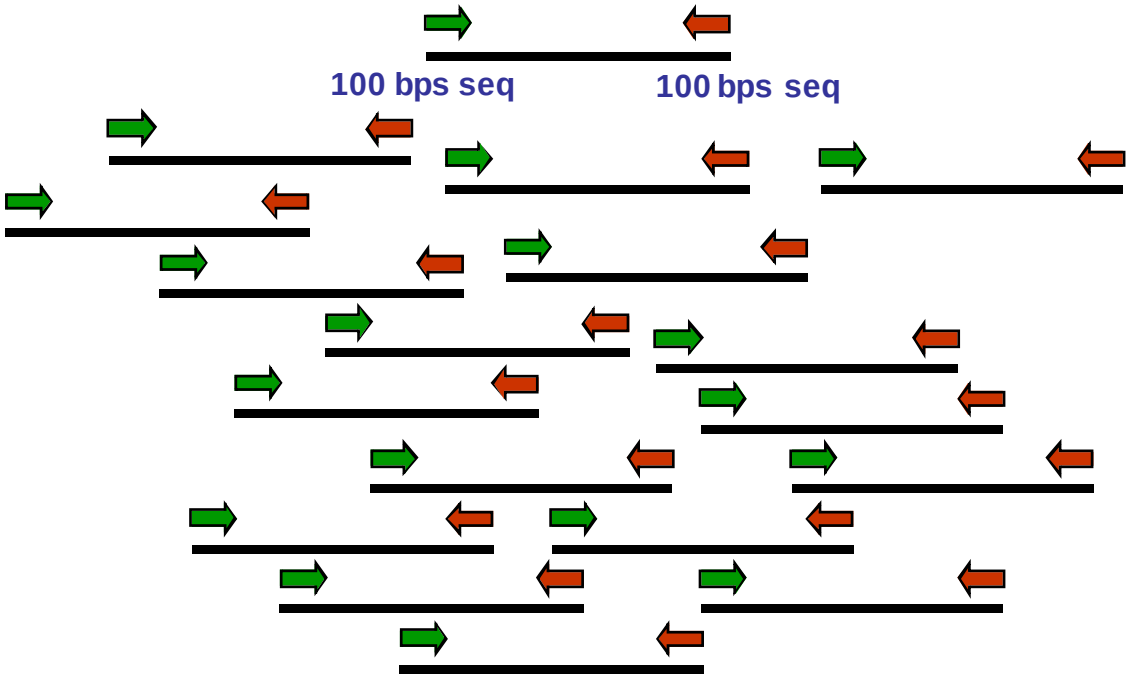
Paired-end Reads

Random 400bp fragments of matching cancer and normal genomes



Map paired sequences back to reference genome

Somatic Mutations in Cancer

Substitution

```
Reference: ATCCGTATCACGGTCACCAGATCAGTCCAGT
Sample:    ATCCGTATCACGGTCAGCAGATCAGTCCAGT
```

Deletion/Insertion

```
Reference: ATCCGTATCACGGTCACCAGATCAGTCCAGT
Sample 1:  ATCCGTATCACGGTCA-CAGATCAGTCCAGT
Sample 2:  ATCCGTATCACGGTCACTCAGATCAGTCCAGT
```

Rearrangement

```
Reference: ATCCGTATCACGGTCACCAGATCAGTCCAGT//TGCTTGTC
Sample:    ATCCGTATCACGGTCAGGTCGTACTTATGCA//CCGACATG
```

Copy Number Gain

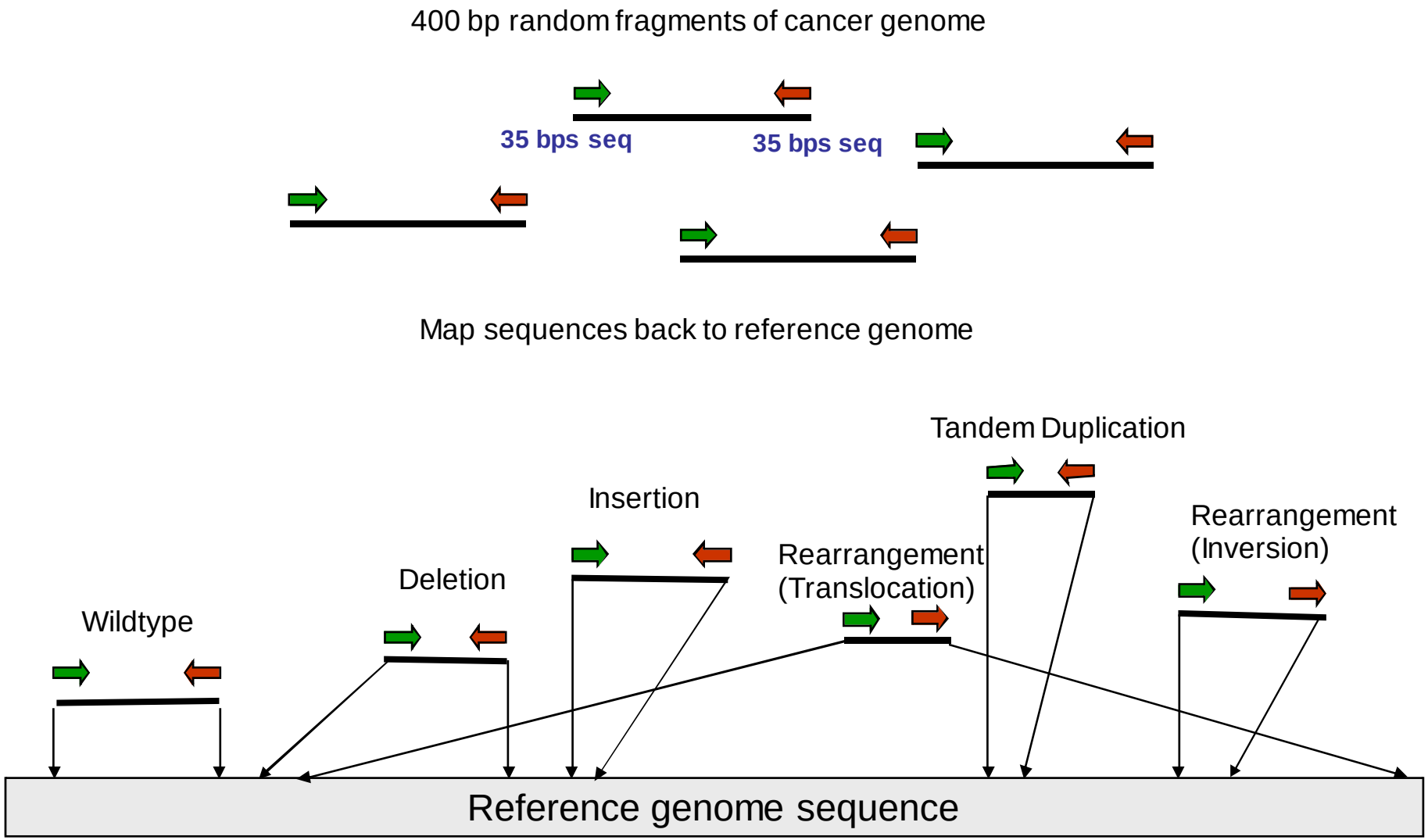
```
Reference: ATCCGTATCACGGTCACCAGATCAGTCCAGT
Sample:    ATCCGTATCACGGTCACCAGATCAGTCCAGT
           ATCCGTATCACGGTCACCAGATCAGTCCAGT
           ATCCGTATCACGGTCACCAGATCAGTCCAGT
           ATCCGTATCACGGTCACCAGATCAGTCCAGT
```

Rearrangement screens

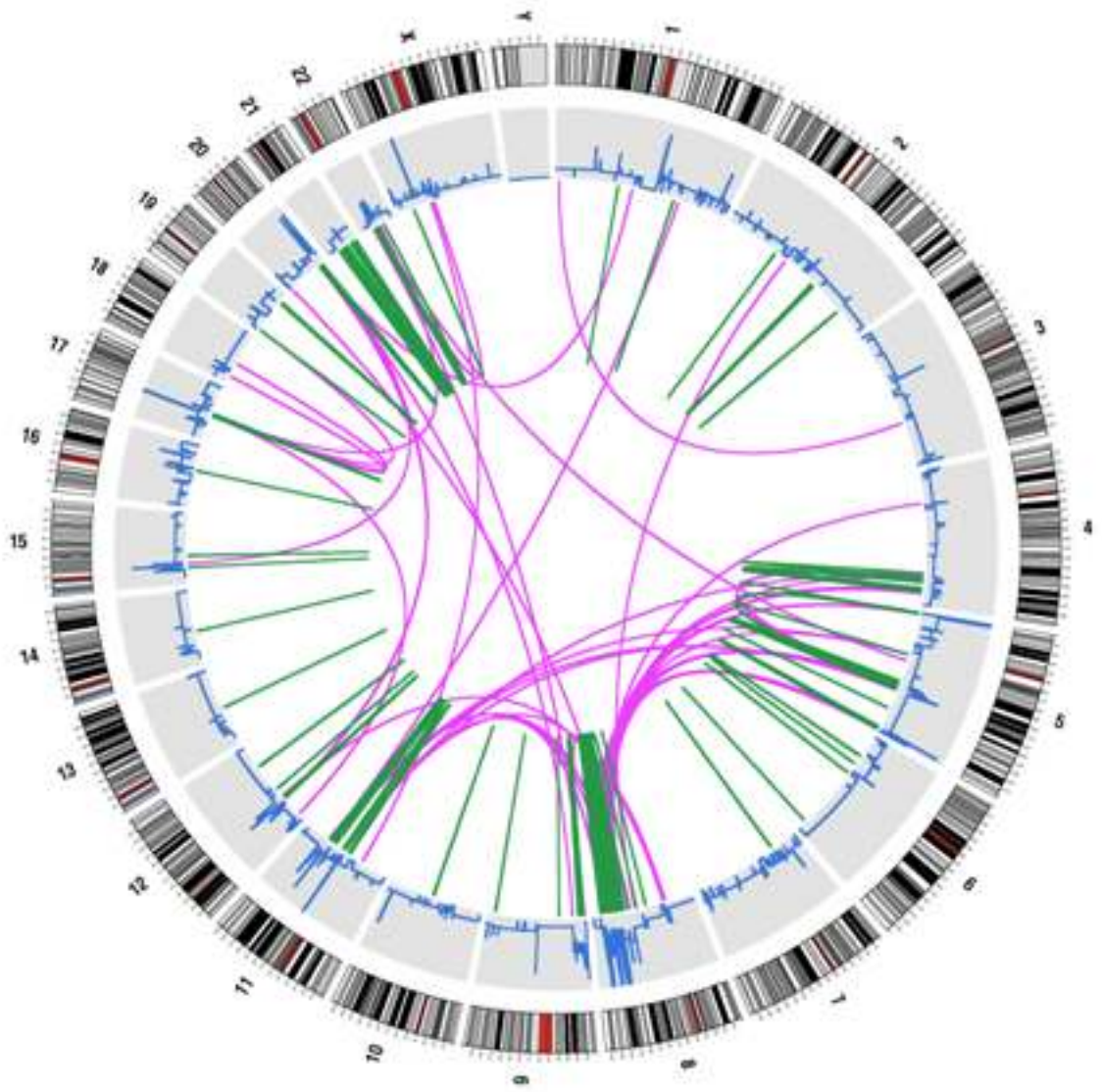
Whole ‘exome’ sequencing

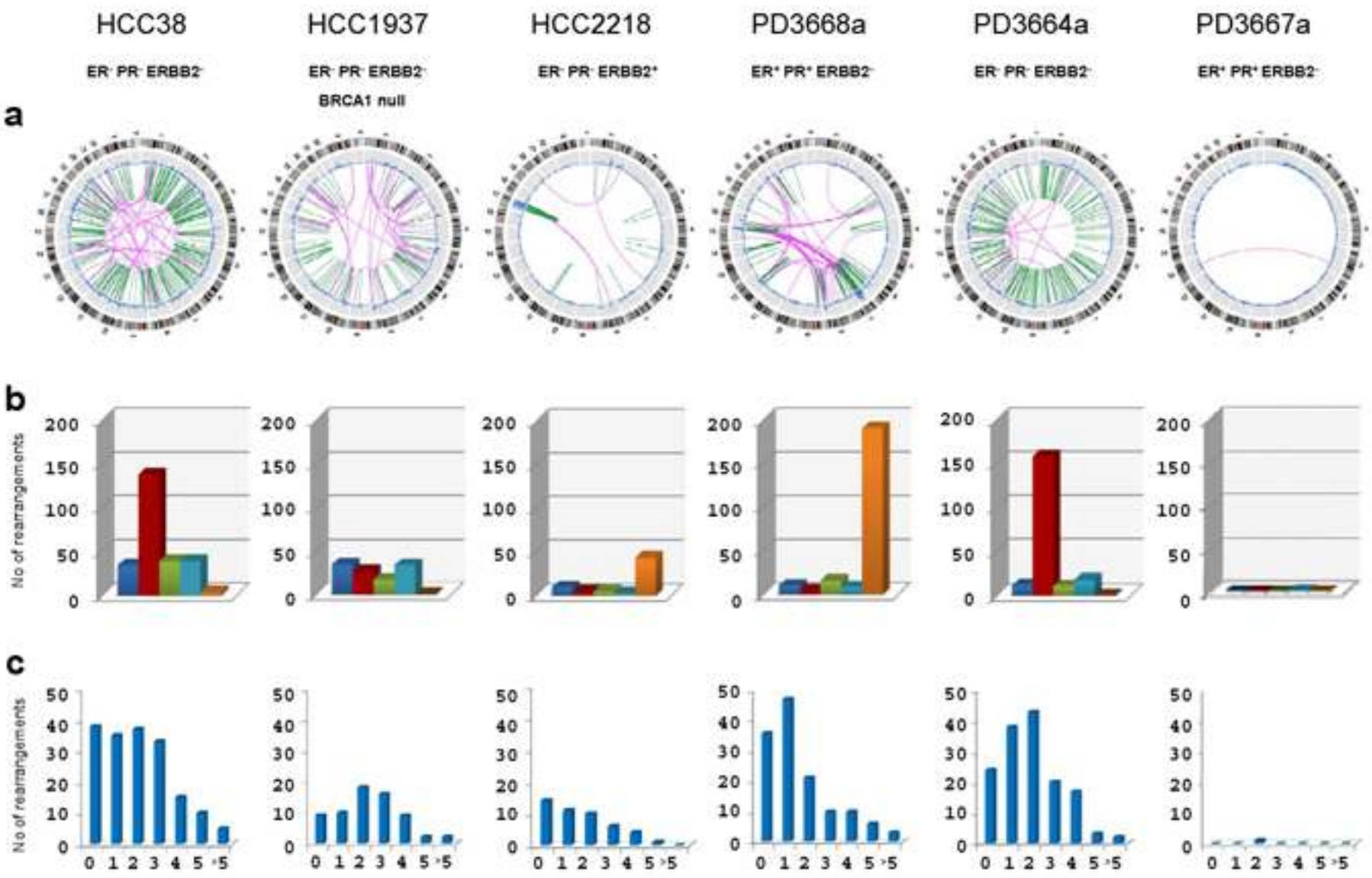
Whole genome shotgun sequencing

Illumina paired-end reads – rearrangements and exomes to whole genome shotgun



Genomic landscapes of rearrangement in human cancers



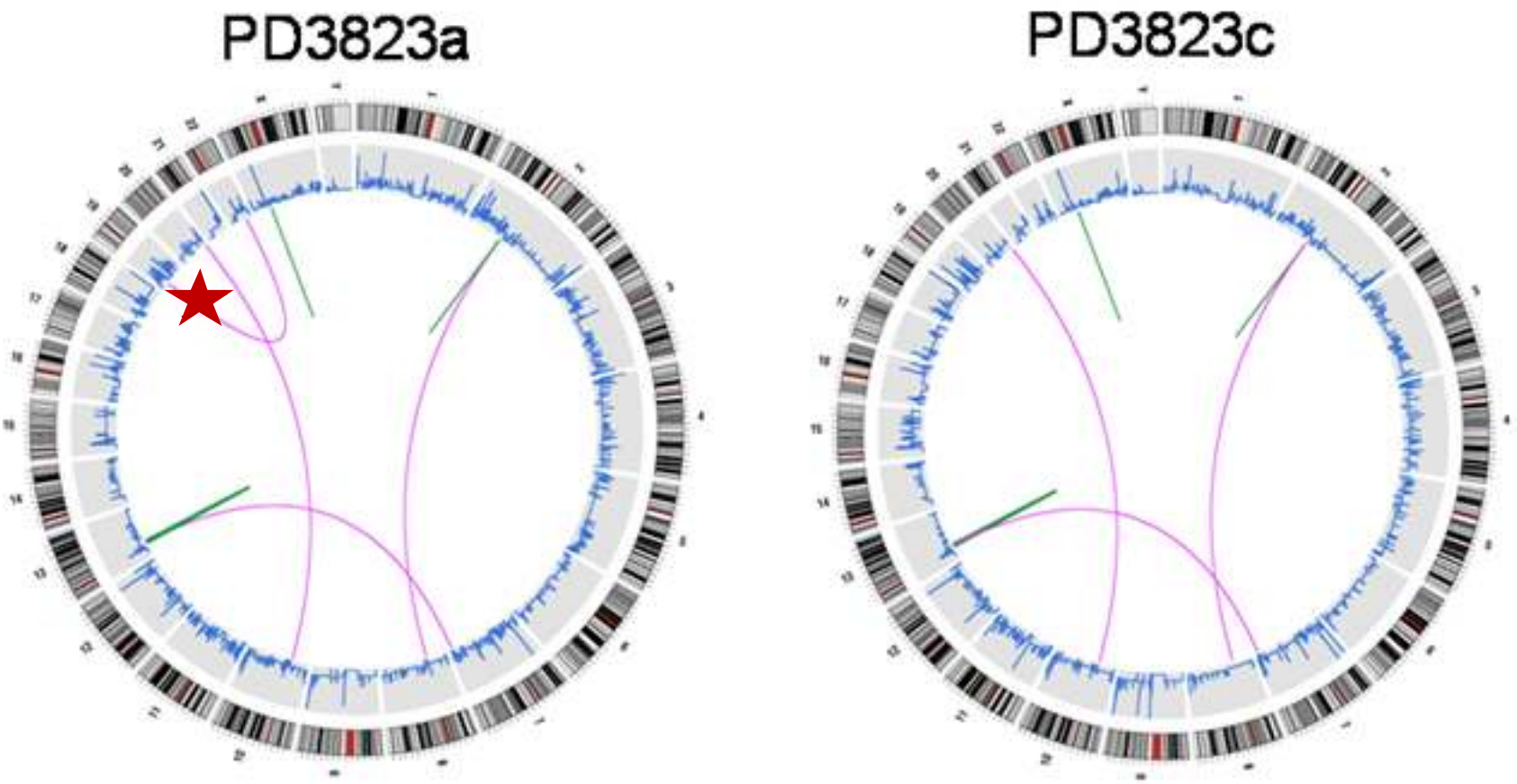


Stephens et al, Nature. 2009 Dec 24;462(7276):1005-10.

Pilot rearrangement screen

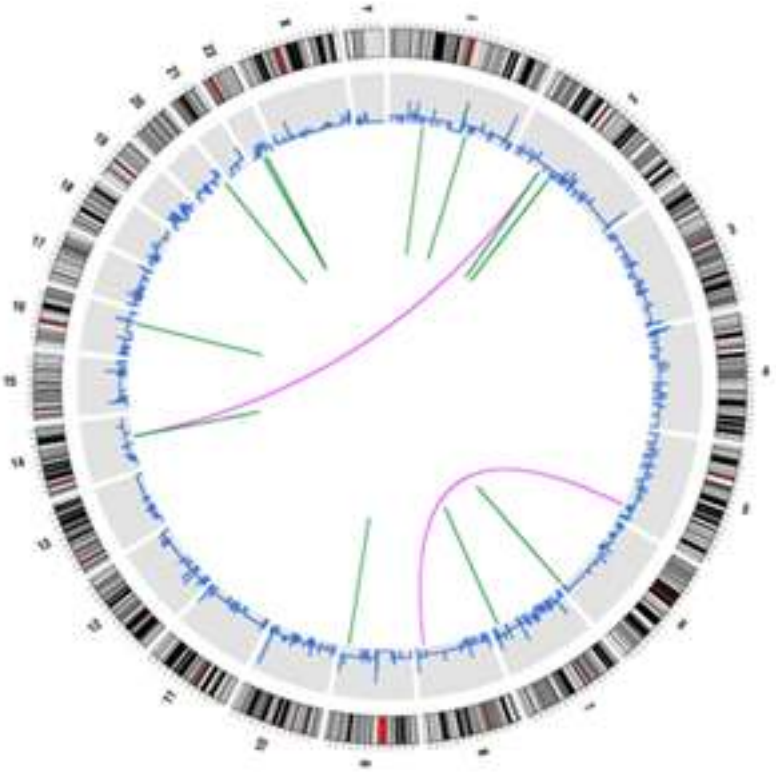
- Samples from patients at two time points during therapy
  - diagnosis/relapse
  - relapse/second relapse
  - DNA damaging or novel therapy

Ongoing genomic rearrangement during progression

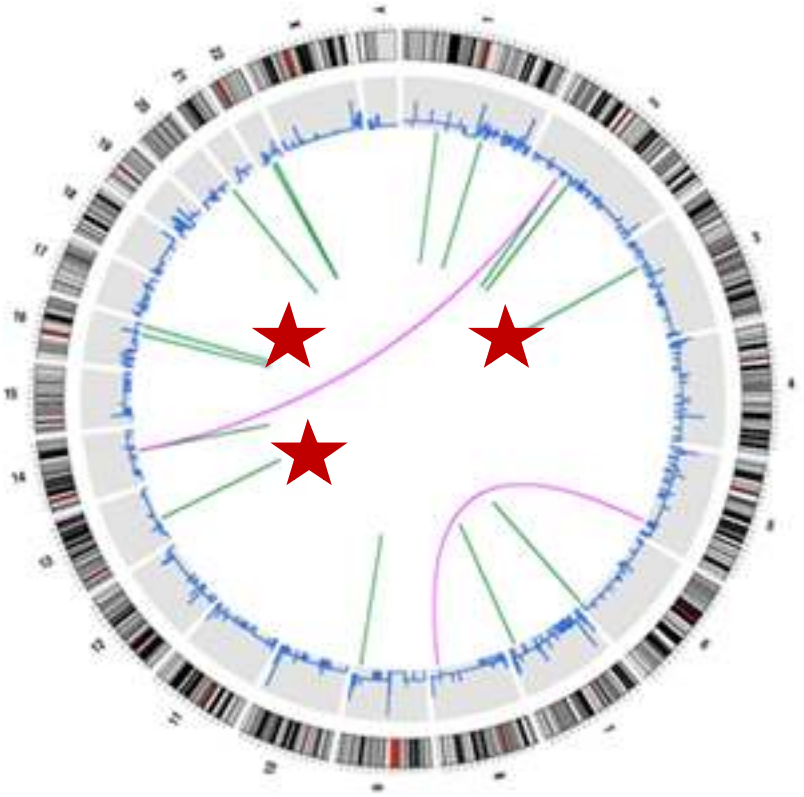


Ongoing genomic rearrangement during progression

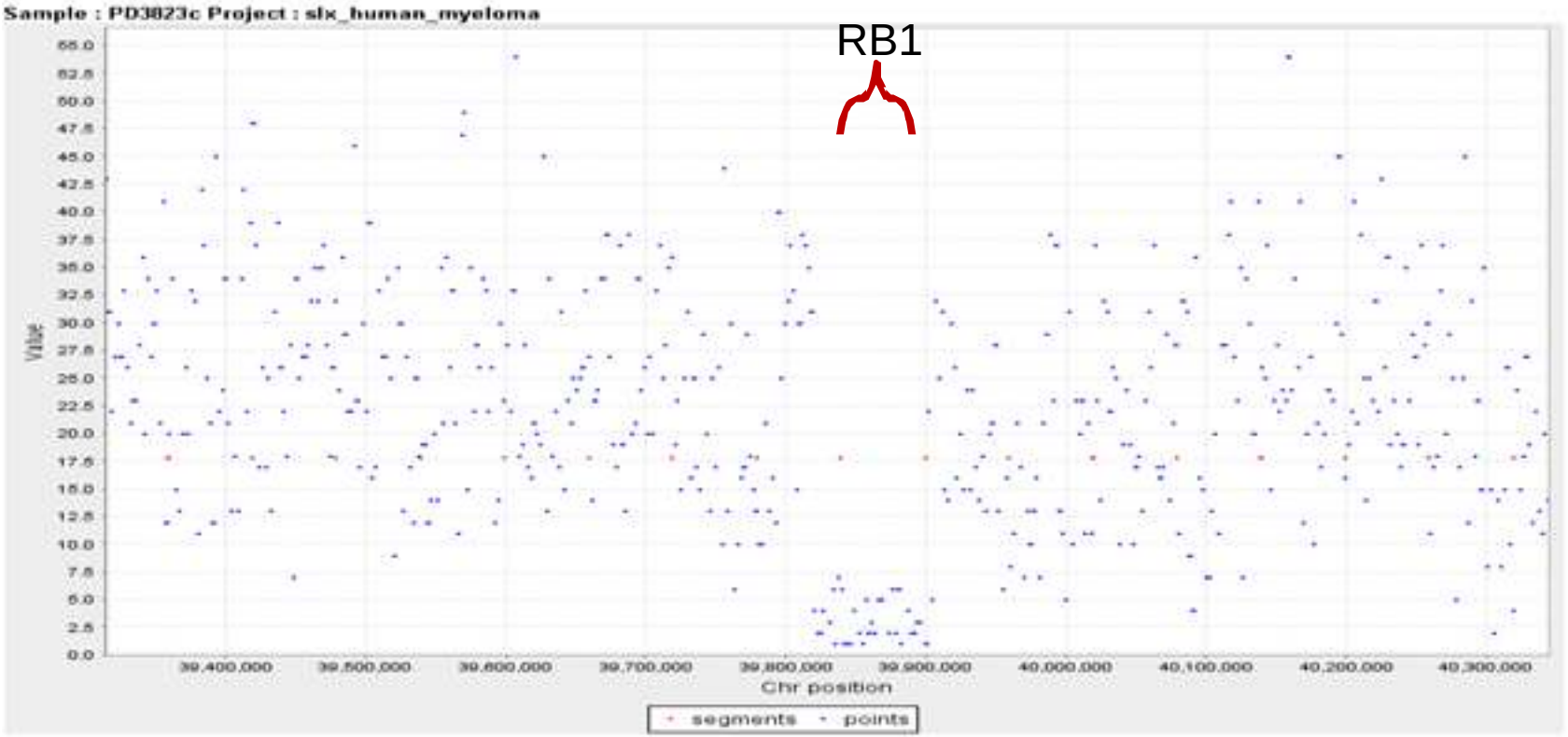
PD3825a



PD3825c



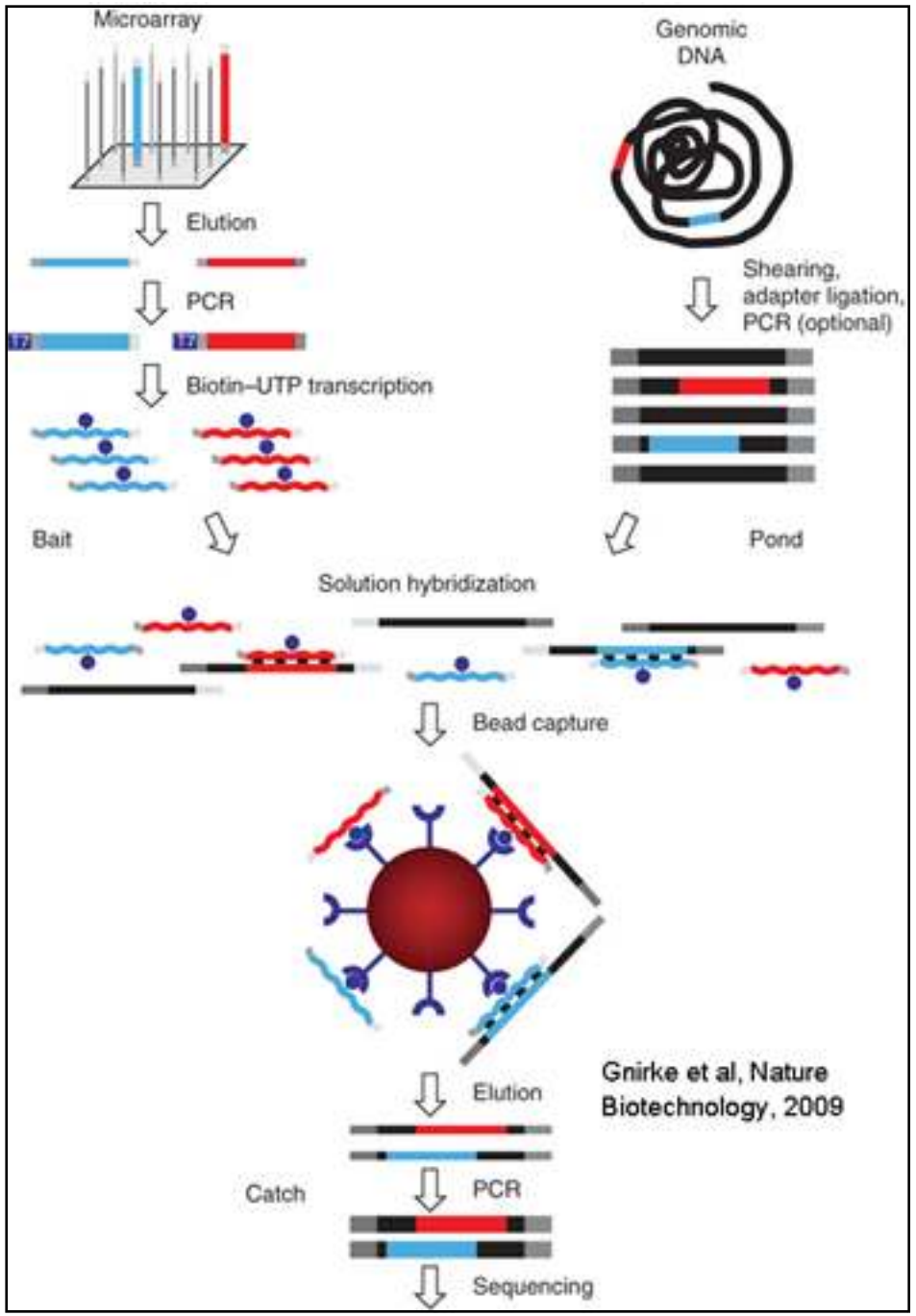
Chromosome 13q homozygous deletion detected in low-coverage paired-end Illumina sequencing read data



Rearrangement screens

Whole ‘exome’ sequencing

Whole genome shotgun sequencing



Solution Hybrid Capture of all coding exons and miRNA genes

Exome is GenCode/ICGC includes 21,416 protein coding genes + 1664 miRNA genes

Current ICGC Breast Cancer Working Group data sets

Exome sequencing — 21,416 protein coding genes, 1664 miRNAs
Copy number analysis

69 breast cancers: — 57 ER positive
12 ER negative

Call somatic base substitutions, indels and copy number changes

Identify driver mutations in known and new cancer genes

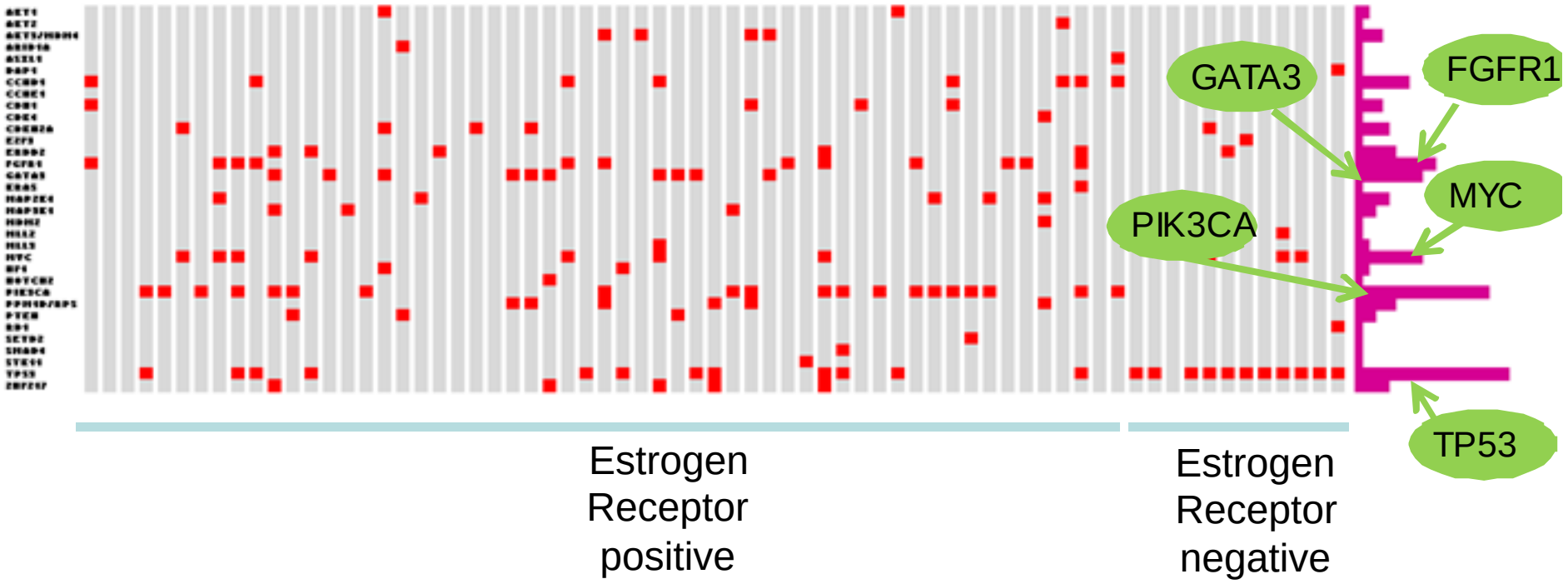
Follow-up in 300 cases

Mutated cancer genes in breast cancer

AKT1
AKT2
AKT3/MDM4
ARID1A
ASXL1
BAP1
CCND1
CCNE1
CDH1
CDKN2A
CDK4
ERBB2
E2F3
FGFR1
GATA3
KRAS
MAP2K4
MAP3K1
MDM2
MLL2
MLL3
MYC
NF1
NOTCH2
PIK3CA
PPM1D/RPS6KB1
PTEN
RB1
SETD2
SMAD4
STK11
TP53
ZNF217

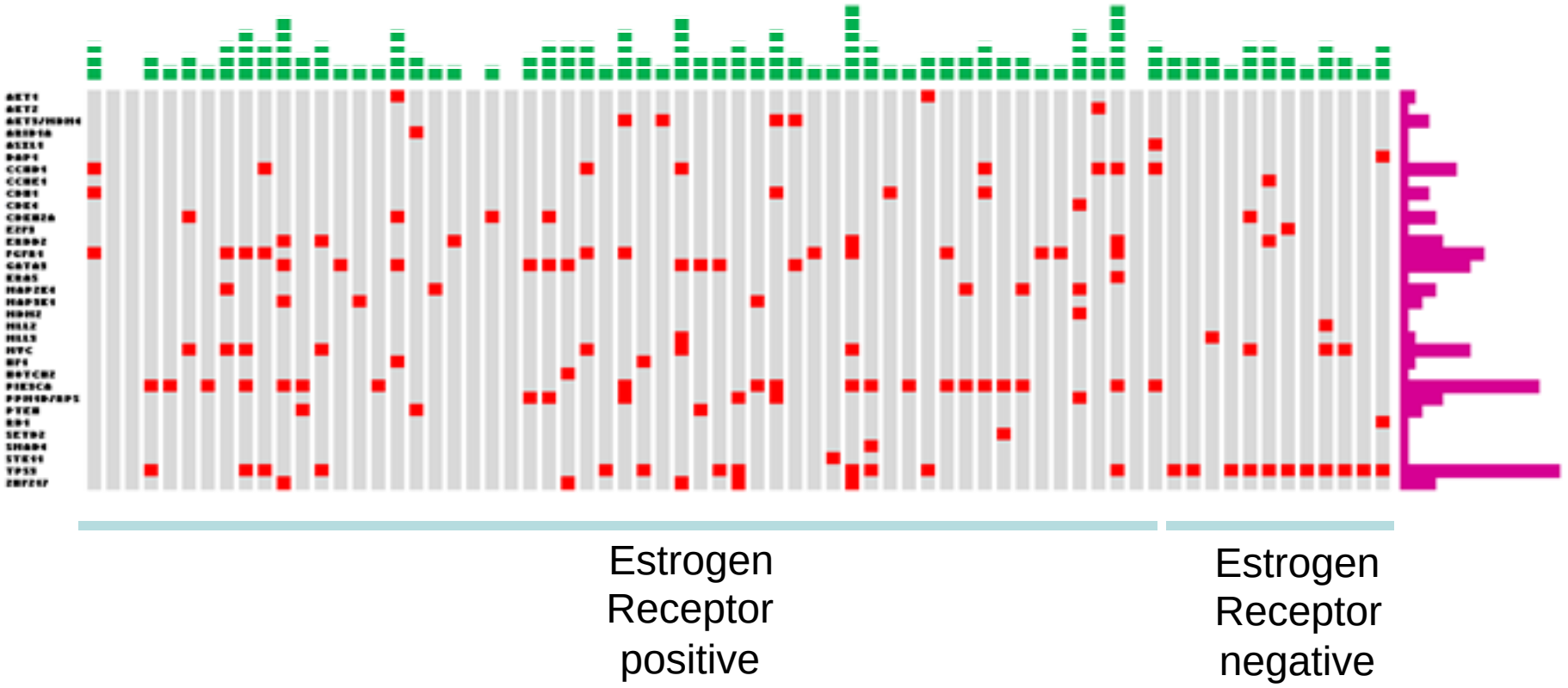
The landscape of driver mutations in breast cancer

Which Drivers?



The landscape of driver mutations in breast cancer

How many drivers/case?



Myeloma Exomes I

- Samples from patients at two time points during therapy
  - diagnosis/relapse
  - relapse/second relapse
  - DNA damaging or novel therapy

n = 9 patients (27 exomes)

Myeloma Exomes II

- Half of cases low risk – hyperdiploidy with trisomy 5
- Half cases high risk - defined by either del(17p) or del(12p)
- n = 53 patients

Rearrangement screens

Whole ‘exome’ sequencing

Whole genome shotgun sequencing to high coverage gets you both of the above, plus all ‘non-coding’ mutations

Cancer Genome Project

Sally Bamford
David Beare
Graham Bignell
Nidhi Bindal
Adam Butler
Angela Cheverton
Helen Davies
Charlotte Dunham
Simon Forbes
Christopher Greenman
Claire Hardy
John Hinton
Mingming Jia
David Jones
Chai Yin Kok
Calli Latimer
King Wai Lau
Kenric Leung
Meng-lay Lin
Mark Maddison
Claire Mahoney
John Marshall
Stuart McLaren
Andrew Menzies
Lina Chen
Juok Cho
Prasad Gunasekaran
Chris Lloyd
Catherine Leroy
Nancy Miller

Laura Mudie
Keiran Raine
Rebecca Shepherd
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Tony Webb
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Su-Kit Chew
Sari Ward
Adam Shlien
John Gamble
Ian Whitmore
Stacey Price
Reva Jayakumar
Elli Papaemmanuil
Serge Dronov
Elizabeth Murchison

Mathew Garnett
Anne McLaren-Douglas
Andrew Barthorpe
Patrick Brien
Laura Hirst
Frances Jewitt
Tatiana Mironenko
Jorge Soares
Ian Thompson
Sonja Heidorn

Serena Nik-Zainal
Jennifer Yen

Peter Campbell
Ultan McDermott
Mike Stratton

Sancha Martin
Angela Macharia
Wendy McLaughlin

Gillian Dalgliesh
Gijs Van Haaften

ccRCC work:
Bin Teh
Kyle Furge
Karl Dykema

Myeloma:
Nikhil Munshi
Ken Anderson
Herve Avet Loiseau