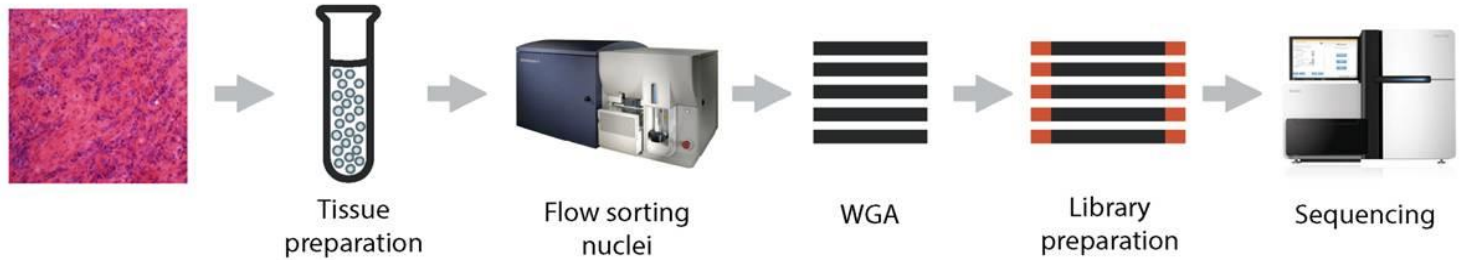A. Krasnitz, M. Wigler

# Computational Framework for Single-Cell Genomics of Tumors
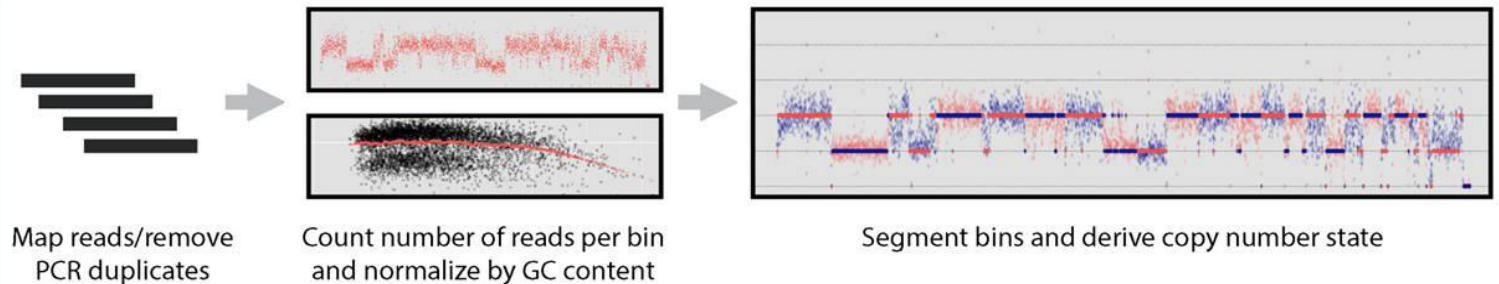
- Workflow from tissues to trees
- Viewer demo
- Patient cases
- Adoption
- Future

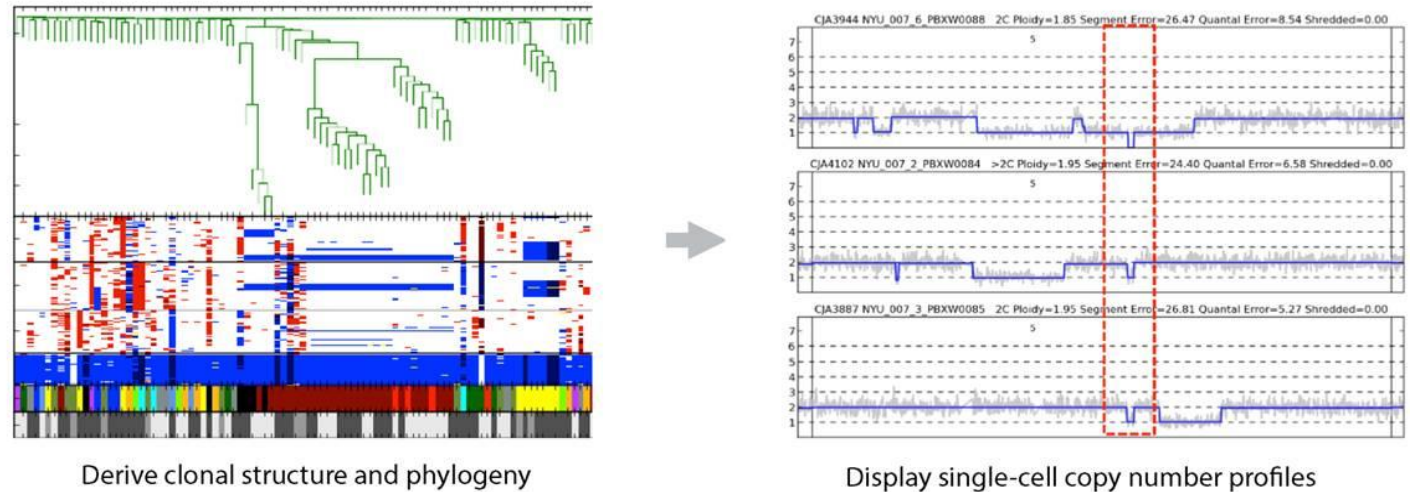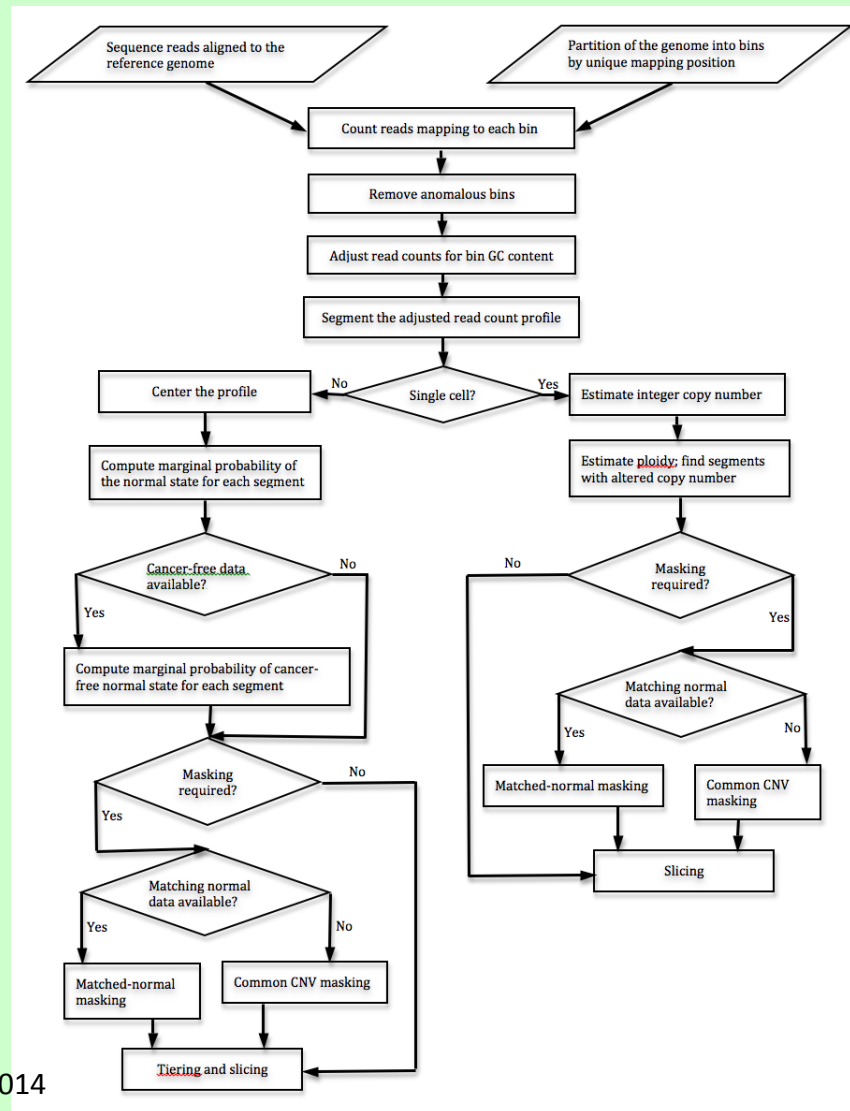# Sparse genomic analysis of individual nuclei by sequencing (s-GAINS)



J. Alexander, J. Kendall, A.Krasnitz, M. Wigler

# Informatics: from read sets to integer-valued CN profiles



J. Kendall, A. Krasnitz, Meth. Mol. Biol. 2014

# Break-point coincidence analysis
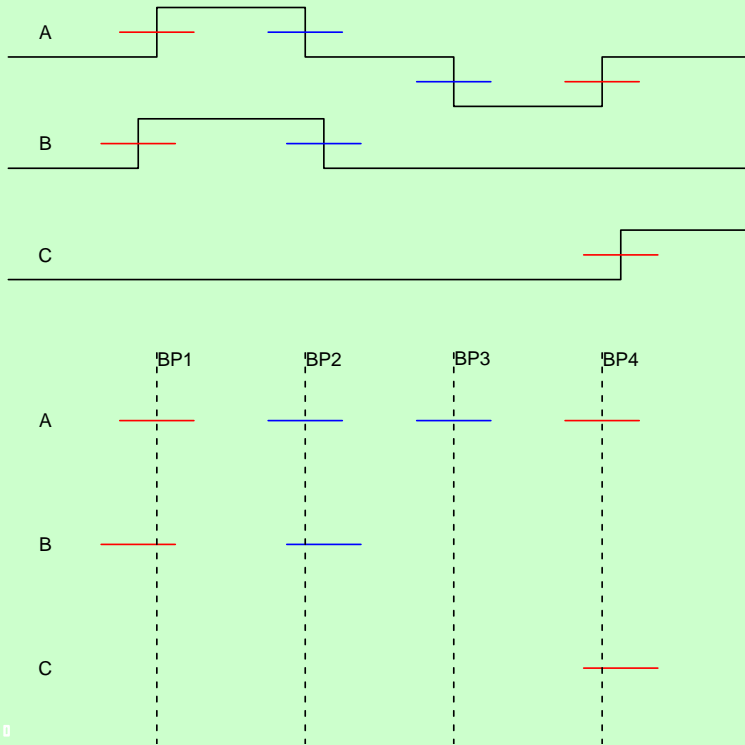
## Break-point incidence table

|  | A | B | C |
|---|---|---|---|
| BP1 | + | + | - |
| BP2 | + | + | - |
| BP3 | + | - | - |
| BP4 | + | - | + |

B

|  |  | + | - |
|---|---|---|---|
| A | + | 2 | 2 |
|  | - | 0 | 0 |

Use Fisher's p-values as pairwise dissimilarities

Fisher's exact test: is this table surprisingly diagonal?

```
0.91,0.06;0.08x0.04)
None 0.5 0.296875 912 46.0
button_press_event <matplotlib.backends.backend_tkagg.FigureCanvasTkAgg object at 0x7f
8b72833a90>
event.canvas.jknumber =  0
plot event

[96]
CJA1056 9727420 RPC0014 Area 3 4C
MPL MouseEvent: xy=(920,14) xydata=(0.604166666667,0.296875) button=1 dblclick=False i
naxes=Axes(0.91,0.01;0.08x0.04)
None 0.604166666667 0.296875 920 14.0
button_press_event <matplotlib.backends.backend_tkagg.FigureCanvasTkAgg object at 0x7f
8b72833a90>
event.canvas.jknumber =  0
[]
displaySectorOrder
fig.canvas.jknumber =  4
minnie2:Gleason9.2 krasnitz$ docker pull szhygulin/viwer:latest
latest: Pulling from szhygulin/viwer
b173ea589fe6: Pull complete
Digest: sha256:22e0d92550eafcafc88d28c6f00d89b84ed82cd51549f3023c72cee279a73f89
Status: Downloaded newer image for szhygulin/viwer:latest
minnie2:Gleason9.2 krasnitz$
```

# Single-cell genomic profiling of prostate cancer

Current practice

High PSA (>4ng/ml), suspicious DRE

↓

TRUS/CT/MRI

↓

Core or FNA biopsy

↙                                    ↘

Localized, Gleason<6
Or
Localized, Gleason≤6, >60 yrs
Surveillance

Else aggressive treatment,
RP if possible

# Shortcomings of conventional pathology:

- 65% probability that any 2 pathologists disagree by ≥ 1 unit of Gleason score.
- Differing scores on core vs. post-RP biopsies.
- Some of low-scoring cases may be aggressive due to subclonal cell populations that go undetected.

Wigler
Tewari
Lepor

| Case | Age | Sample$^$ | Sectors | Gleason Score Final** | Clonal | Subclones | Total # Clonal Cells | Total # Cells |
|------|-----|-----------|---------|------------------------|--------|-----------|----------------------|---------------|
| nyu003 C | 47 | PBXW | 13 | NA | no | no | 0 | 347 |
| nyu002 E | 72 | PBXW | 13 | NA | yes | no | 5 | 581 |
| cor002 E | 64 | TBXP | 5 | 6 (3+3) | yes | no | 4 | 402 |
| nyu005 C | 64 | PBXW | 14 | 6 (3+3)# | yes | no | 8 | 309 |
| nyu007 E | 65 | PBXW | 13 | 7 (3+4)^ | yes* | yes | 40 | 270 |
| nyu001 E | 63 | PBXW | 14 | 7 (3+4) | yes* | yes | 176 | 622 |
| nyu010 E | 79 | PBXW | 15 | 7 (3+4) | yes* | no | 22 | 341 |
| nyu011 C | 63 | PBXW | 10 | 7 (4+3) | yes | no | 15 | 221 |
| nyu004 C | 75 | PBXW | 14 | 7 (4+3)# | yes* | no | 48 | 314 |
| cor003 E | 80 | TBXP | 5 | 9 (4+5) | yes | yes | 139 | 347 |
| cor001 C | 77 | TBXP | 6 | 9 (5+4) | yes | no | 87 | 261 |
| Average Age | 67 | Total | 122 | ----- | ----- | ----- | 544 | 4015 |

$PBXW = Prostate Biopsy Washing, TBXP = Tissue Biopsy Prostatectomy

** Gleason score of radical prostatectomy specimen: # Downgraded, ^Upgraded

*Independent Clones of ≥ 2cells

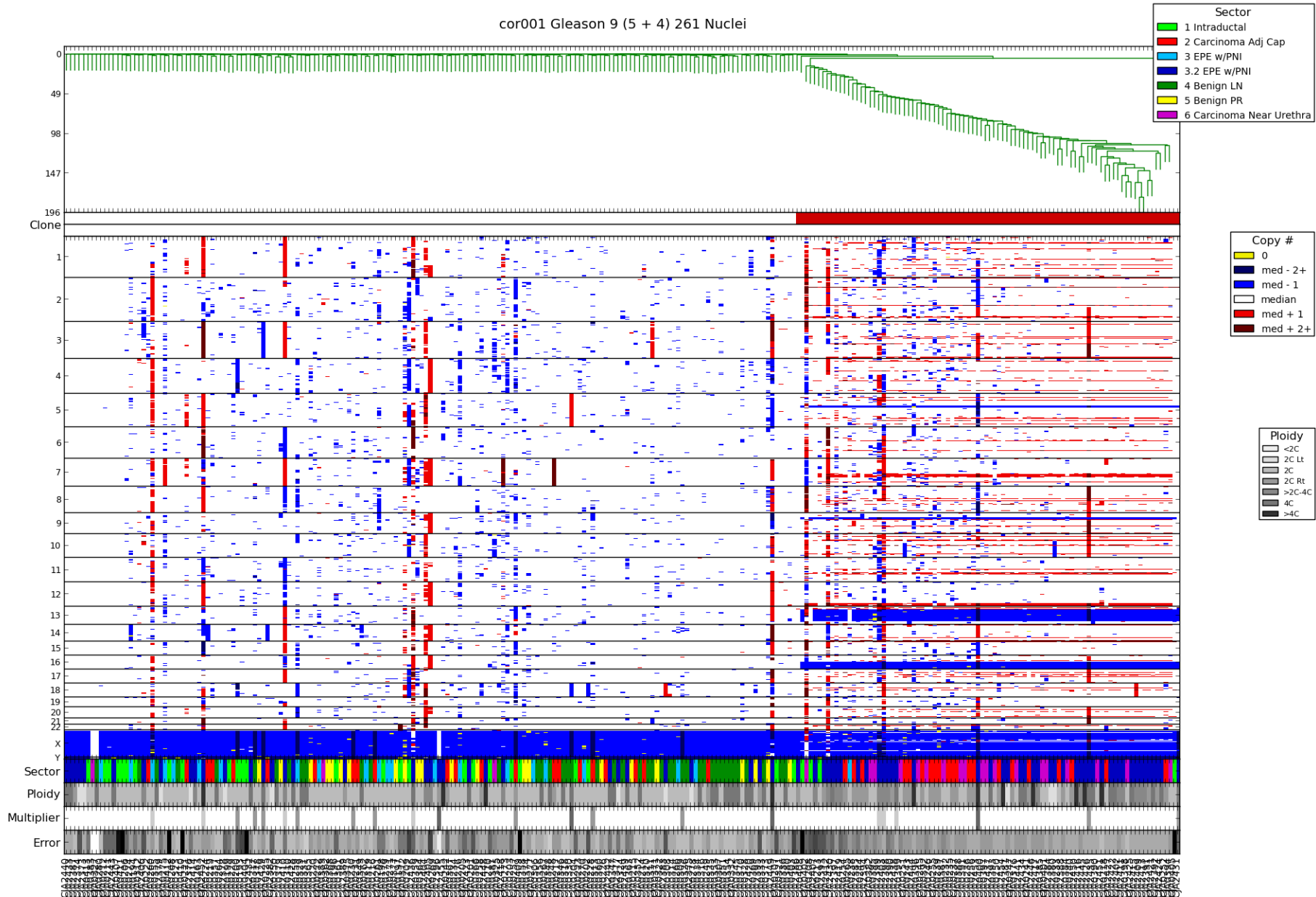C = Concise, E = Expanded (case reports in supplemental section)

Can single-cell genomic profiling help desambiguate pathology?
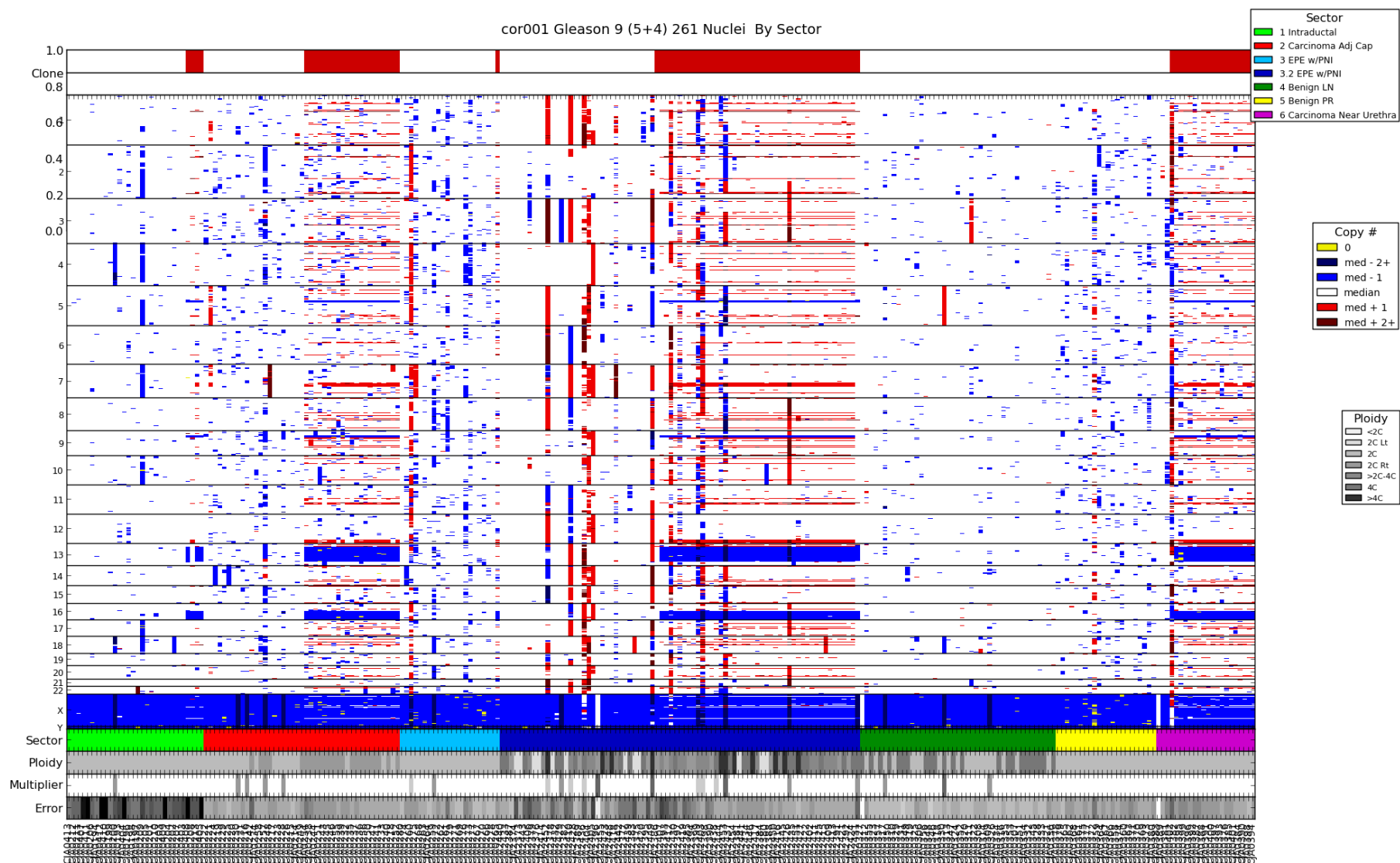In particular, clones of cells with major genomic alterations ➔ likely aggressive malignancy.
Can we detect them?

cor001 Gleason 9 (5 + 4) 261 Nuclei

Sector
1 Intraductal
2 Carcinoma Adj Cap
3 EPE w/PNI
3.2 EPE w/PNI
4 Benign LN
5 Benign PR
6 Carcinoma Near Urethra

Copy #
0
med - 2+
med - 1
median
med + 1
med + 2+

Ploidy
<2C
2C Lt
2C
2C Rt
>2C-4C
4C
>4C

Clone

Sector

Ploidy

Multiplier

Error

cor001 Gleason 9 (5+4) 261 Nuclei  By Sector

**Sector**
- 1 Intraductal
- 2 Carcinoma Adj Cap
- 3 EPE w/PNI
- 3.2 EPE w/PNI
- 4 Benign LN
- 5 Benign PR
- 6 Carcinoma Near Urethra

**Copy #**
- 0
- med - 2+
- med - 1
- median
- med + 1
- med + 2+

**Ploidy**
- <2C
- 2C Lt
- 2C
- 2C Rt
- >2C-4C
- 4C
- >4C

CJA2425      RPC0003.2 (57-3) Area 3.2    4C Ploidy=2 Segment Error=25.91 Quantal Error=17.19 Shredded=0.00

CJA0384      RPC0006 (54-6) Area 6    2C Ploidy=2 Segment Error=27.59 Quantal Error=19.80 Shredded=0.00

CJA0244      RPC0002 (57-2) Area 2    2C Rt Ploidy=1.95 Segment Error=32.78 Quantal Error=26.35 Shredded=0.00

CJA0218      RPC0002 (57-2) Area 2    2C Ploidy=2 Segment Error=26.03 Quantal Error=16.08 Shredded=0.00

NYU003 Benign 310 Nuclei

Sector
1 Benign RLB PBXW0028
2 Benign RLM PBXW0029
3 Benign RLA PBXW0030
4 Benign RB PBXW0031
5 Benign RM PBXW0032
6 Benign RA PBXW0033
7 Benign LLB PBXW0034
8 Benign LLM PBXW0035
9 Benign LLA PBXW0036
10 Benign LB PBXW0037
11 Benign LM PBXW0038
12 Benign LA PBXW0039
13 Benign RTMID PBXW0040

Copy #
0
med - 2+
med - 1
median
med + 1
med + 2+

Ploidy
2C
2C Rt
>2C
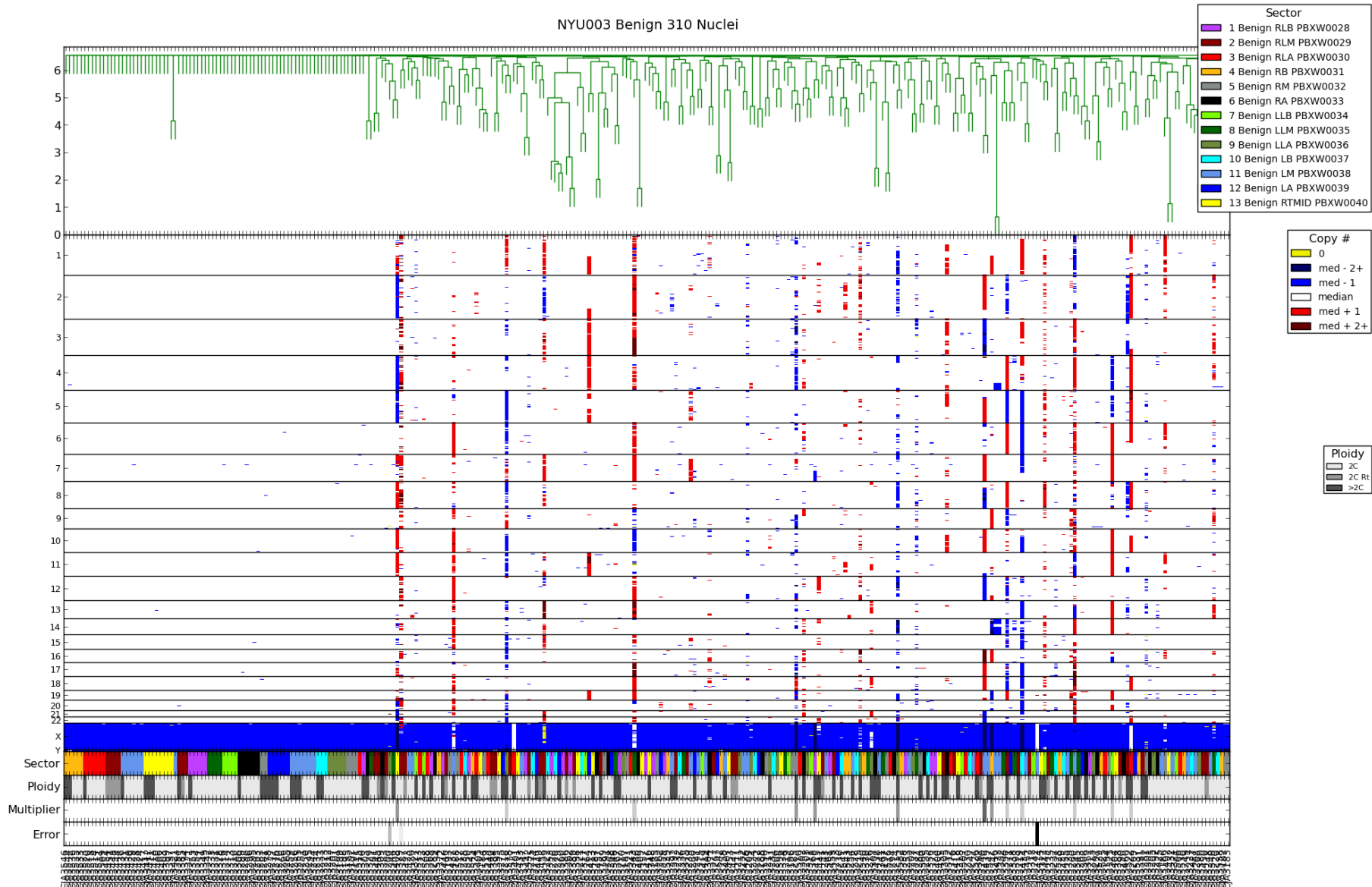
# Flat vs unstable cell genomes
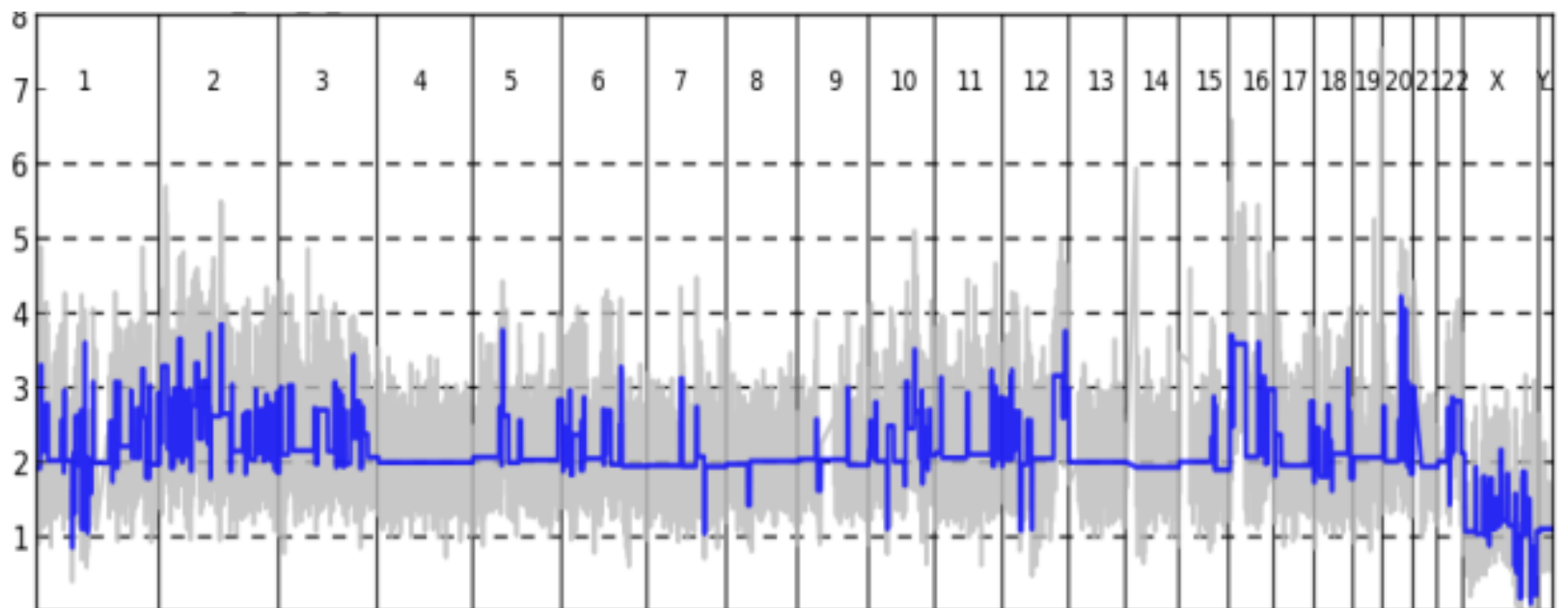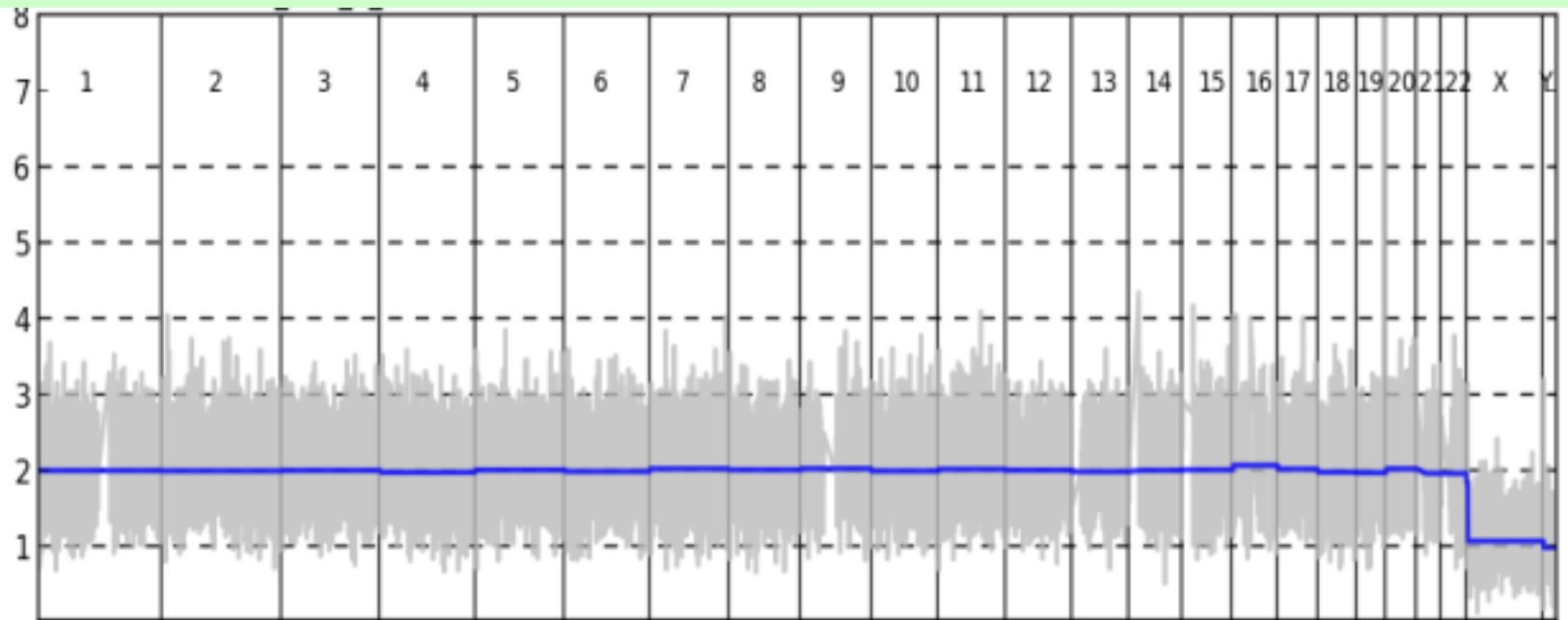
# NYU007 Gleason 7(3+4) 279 Nuclei



**Sector**
- 1 Benign LLB PBXW0083
- 2 GL6(3+3) LLM PBXW0084
- 3 HGPIN LLA PBXW0085
- 4 Benign LMB PBXW0086
- 5 Benign LMM PBXW0087
- 6 Benign LMA PBXW0088
- 7 Benign RMB PBXW0089
- 8 Inflam RMM PBXW0090
- 9 Benign RMA PBXW0091
- 10 Benign RLB PBXW0092
- 11 Benign RLM PBXW0093
- 12 Benign RLA PBXW0094
- 13 Benign LPL PBXW0095

**Copy #**
- 0
- med - 2+
- med - 1
- median
- med + 1
- med + 2+

**Ploidy**
- 2C
- 2C Rt
- >2C
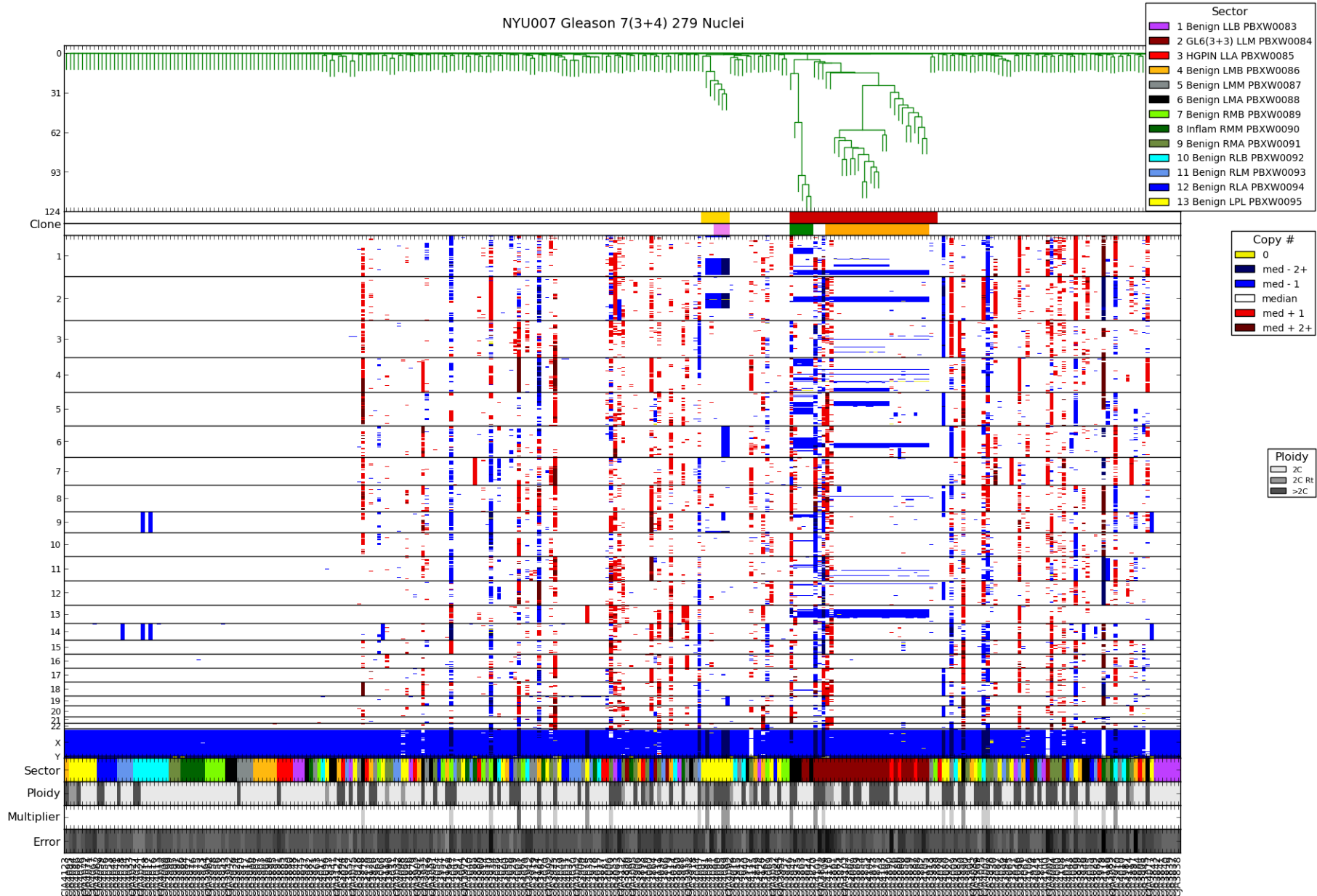
Prostate biopsy washings
NYU007.Gleason 7 (3 + 4)

RIGHT — LEFT

BASE — BASE
MID — MID
APEX — APEX

Lateral — Medial — Medial — Lateral

10  7   4   1
11  8   5   2
12  9   6   3

13

MRI-Targeted Biopsy
Left Posterolateral
Mid-Gland

nyu007.3:
Sector 13

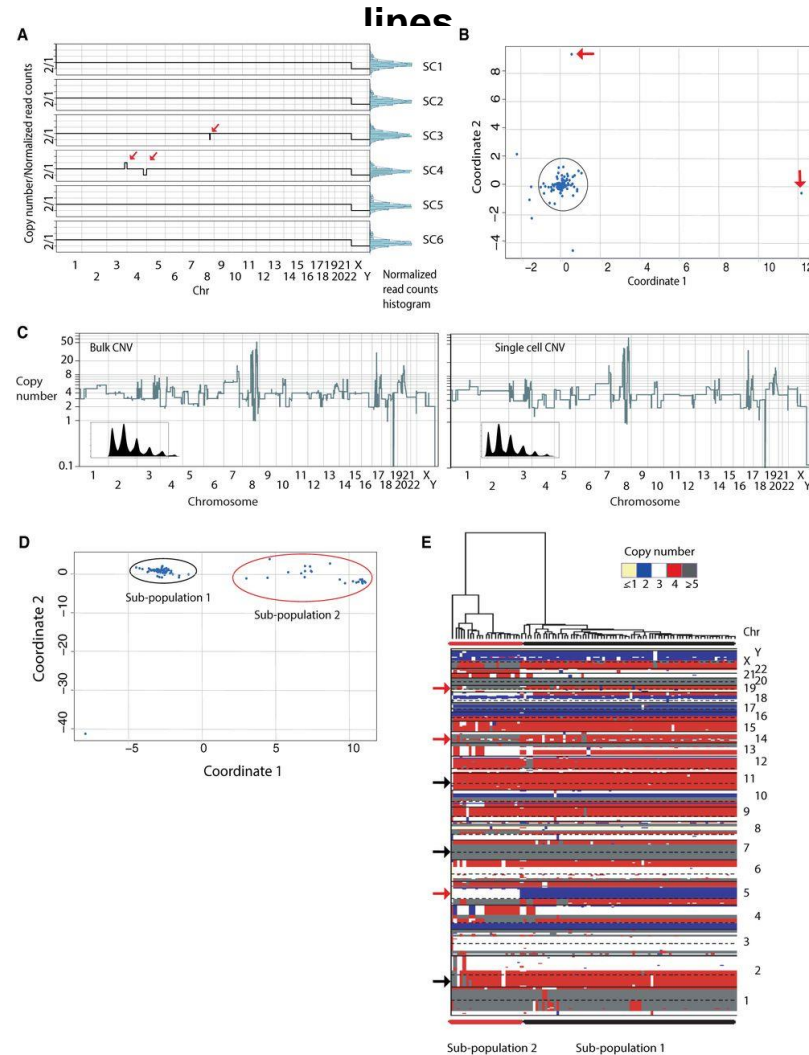nyu007.1:
Sectors - 2 and 6
nyu007.2:
Sectors - 2 and 3
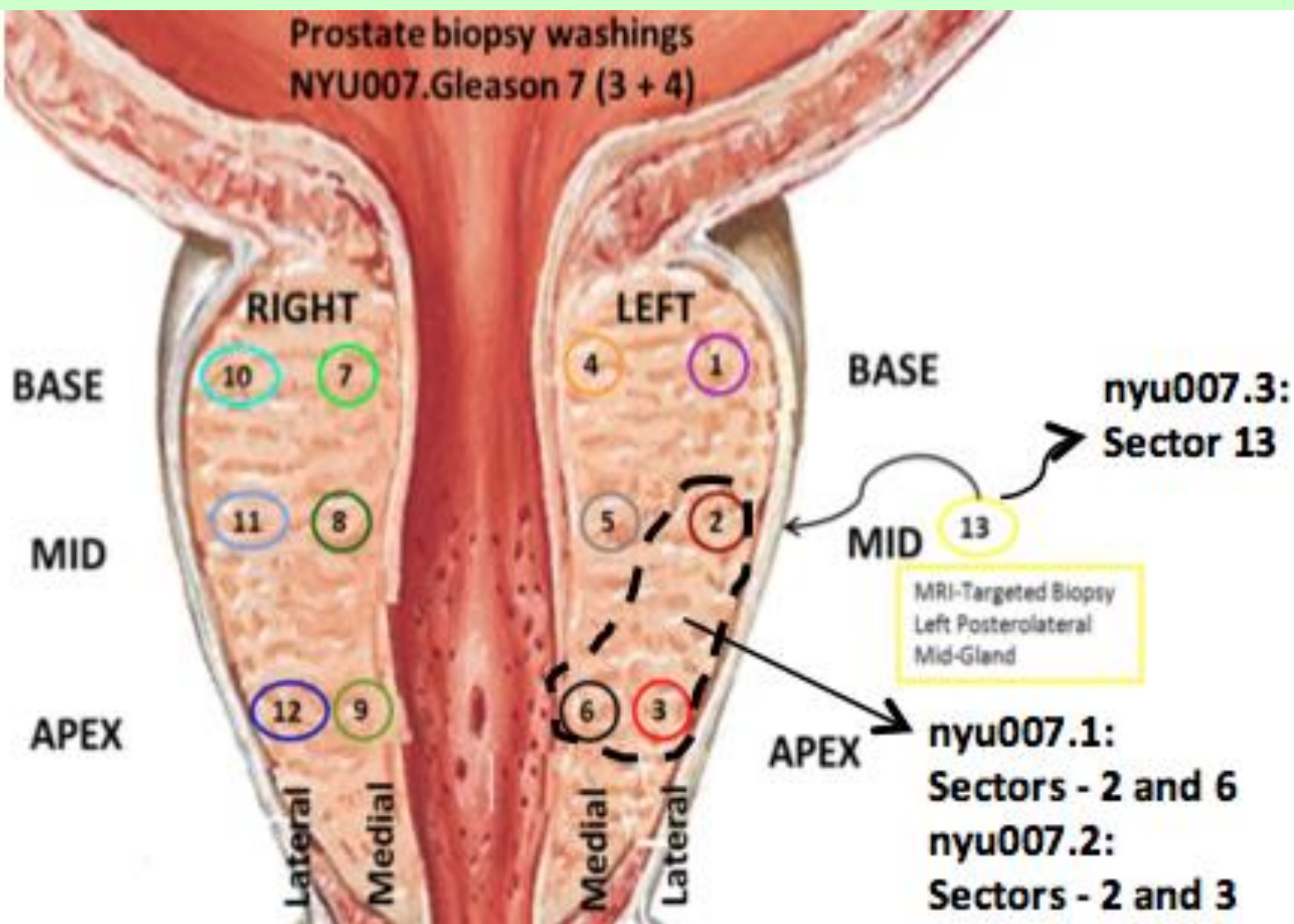
Adoption:

@MSKCC (T. Baslan, BRCA, single cells)
@NYGC (L. Muthuswamy, PAAD, organoids)

# C-DOP-L approach provides uniform, unbiased amplification of single-cell genomes, accurate determination of copy number states and reveals genomic heterogeneity in breast cancer cell lines



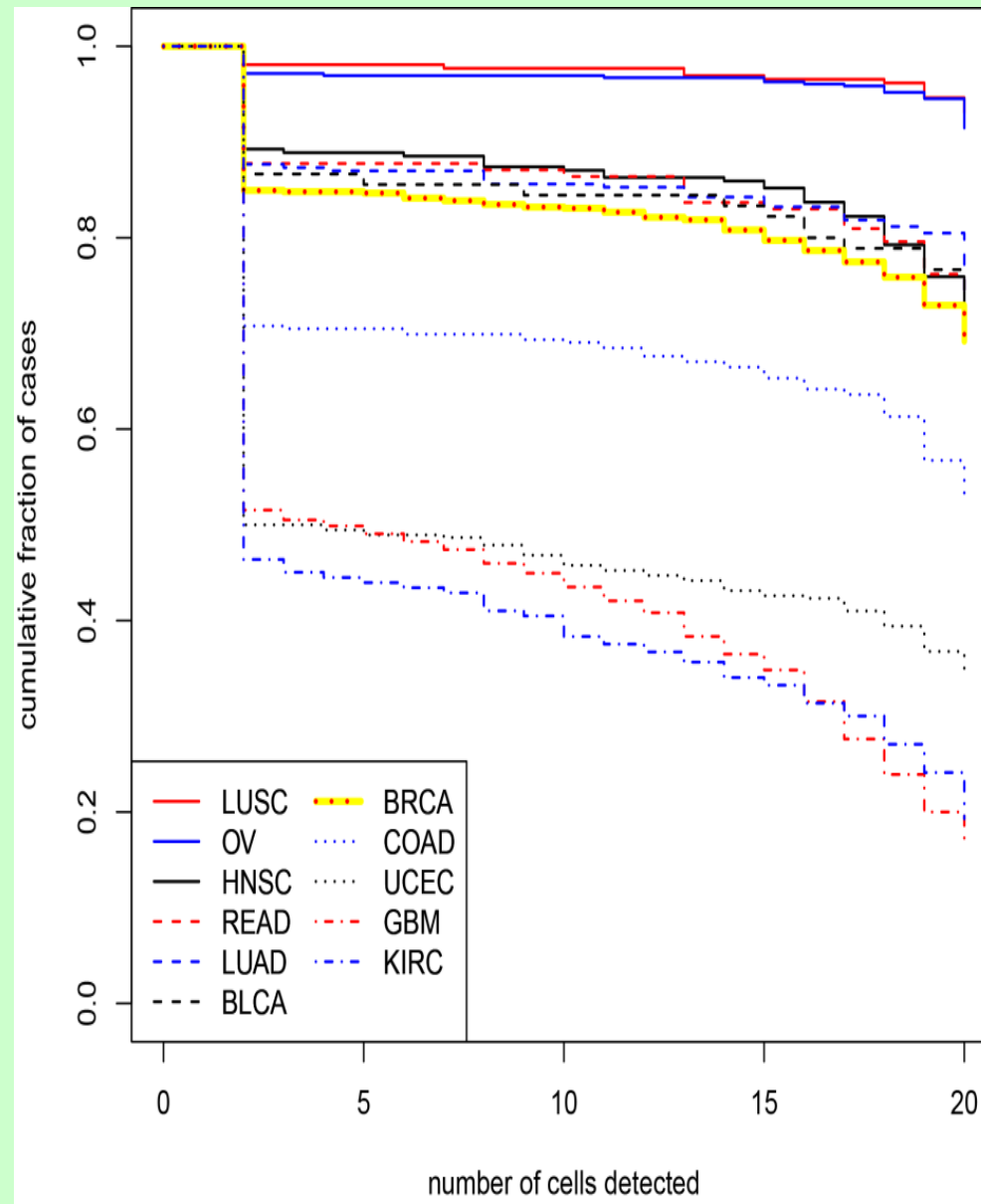**Timour Baslan et al. Genome Res. 2015;25:714-724**

# Next steps

## IT

- Finalize and publish the Viewer
- Dockerize the pipeline

## Early detection

Detect clonal populations among cells in circulation, following depletion of nucleated blood cells.

# Detecting 20

Collaborators & key players

ITCR

Cornell/Mt. Sinai
Abhishek Srivastava
Siobhan Gruschow
Alexandra Peyser
Shalini Singh Yadav
Brian Robinson
Ashutosh Tewari

NYU
Juliana Laze
Herbert Lepor

CSHL
Jude Kendall
Joan Alexander
Viacheslav Zhygulin