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)

Single Nucleus Sequencing

- Tissue preparation
- Flow sorting nuclei
- WGA
- Library preparation
- Sequencing

Data Processing

- Map reads/remove PCR duplicates
- Count number of reads per bin and normalize by GC content
- Segment bins and derive copy number state

Visualization

Single-Cell Genomics Viewer (SCGV)

- Derive clonal structure and phylogeny
- Display single-cell copy number profiles

J. Alexander, J. Kendall, A. Krasnitz, M. Wigler
Informatics: from read sets to integer-valued CN profiles

Break-point coincidence analysis

Break-point incidence table

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BP2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BP3</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BP4</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Use Fisher’s p-values as pairwise dissimilarities

Fisher’s exact test: is this table surprisingly diagonal?
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.

Can single-cell genomic profiling help desambiguate pathology? In particular, clones of cells with major genomic alterations likely aggressive malignancy. Can we detect them?
Flat vs unstable cell genomes
NYU007 Gleason 7(3+4) 279 Nuclei

[Diagram showing a genetic tree with various branches and nodes labeled with different sectors and copy numbers, indicating copy number variations (CNVs).]

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

Ploidy:
- 2C
- 2C hi
- >2C

Sector:
- 1 Benign LLB PBXW0083
- 2 GL6(3+3) LLM PBXW0084
- 3 HGPNI 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 RLA PBXW0093
- 12 Benign RLA PBXW0094
- 13 Benign LPL PBXW0095

Multiplier:

Error:

[The diagram includes detailed visual representations of copy number variations across different sectors, indicating genomic instability or clonal evolution in the tissue sample.]
Prostate biopsy washings

NYU007. Gleason 7 (3 + 4)

nyu007.1: Sectors - 2 and 6

nyu007.2: Sectors - 2 and 3

nyu007.3: Sector 13

MRI-Targeted Biopsy
Left Posterolateral
Mid-Gland
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

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Alexandra Peyser
Shalini Singh Yadav
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