Single-cell Analysis and its Application in Virology

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Joint Graduate Seminar

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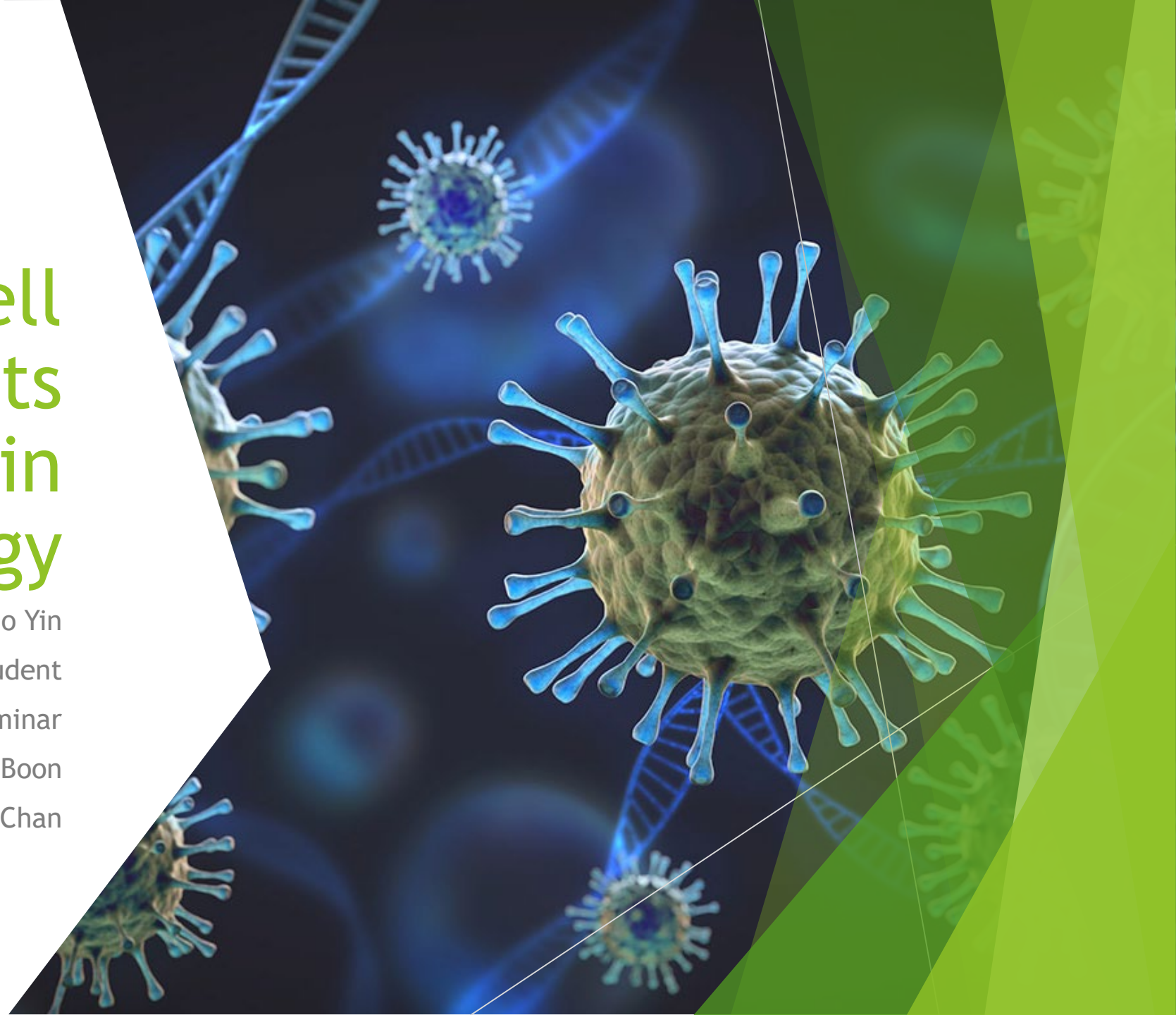
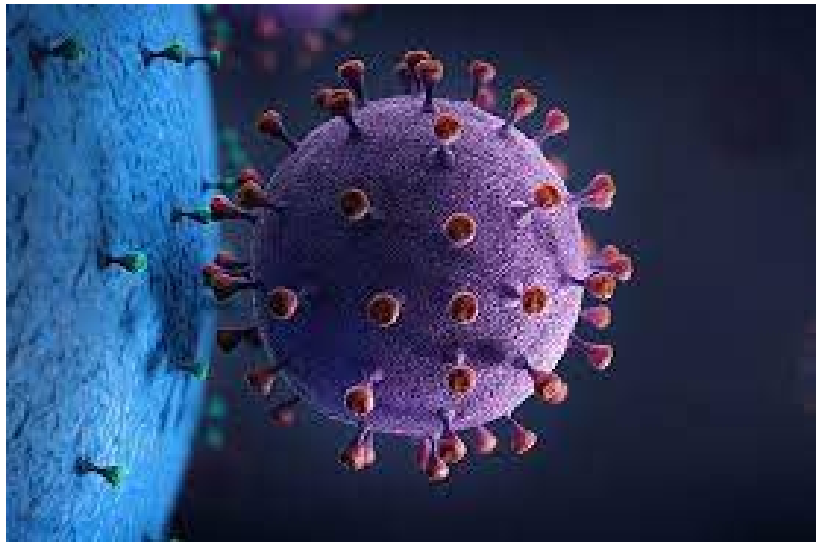
Co-supervisor: Prof. Paul Chan





Outline

- Cell heterogeneity
- Isolation of single cells
- Amplification of genetic material
- Application of single cell in virology
- Conclusion

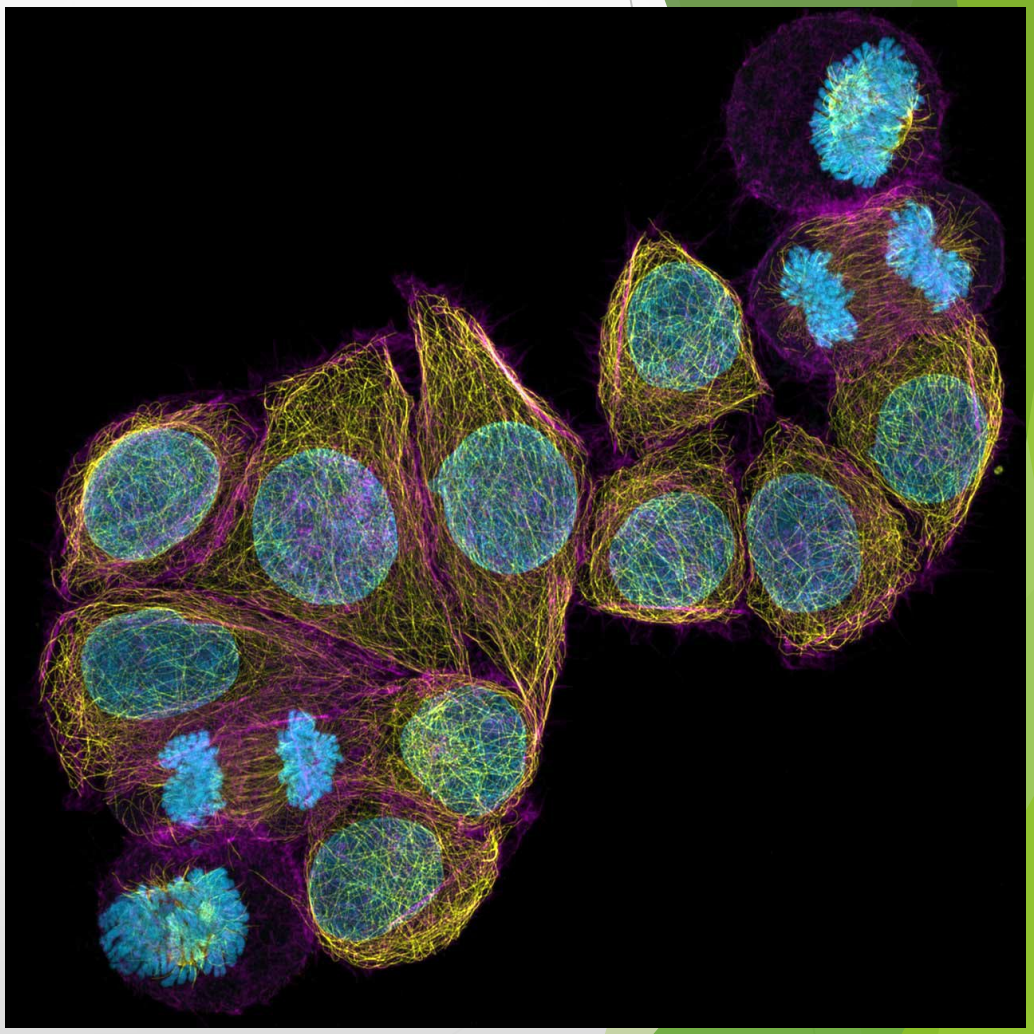
Cellular Heterogeneity

- Most of our current biological knowledge based on ensemble measurements
- Cell-to-cell in a population differs:

-Identity (Cell types, subpopulation/ lineage)

-state/process (Cell cycle, circadian rhythm)

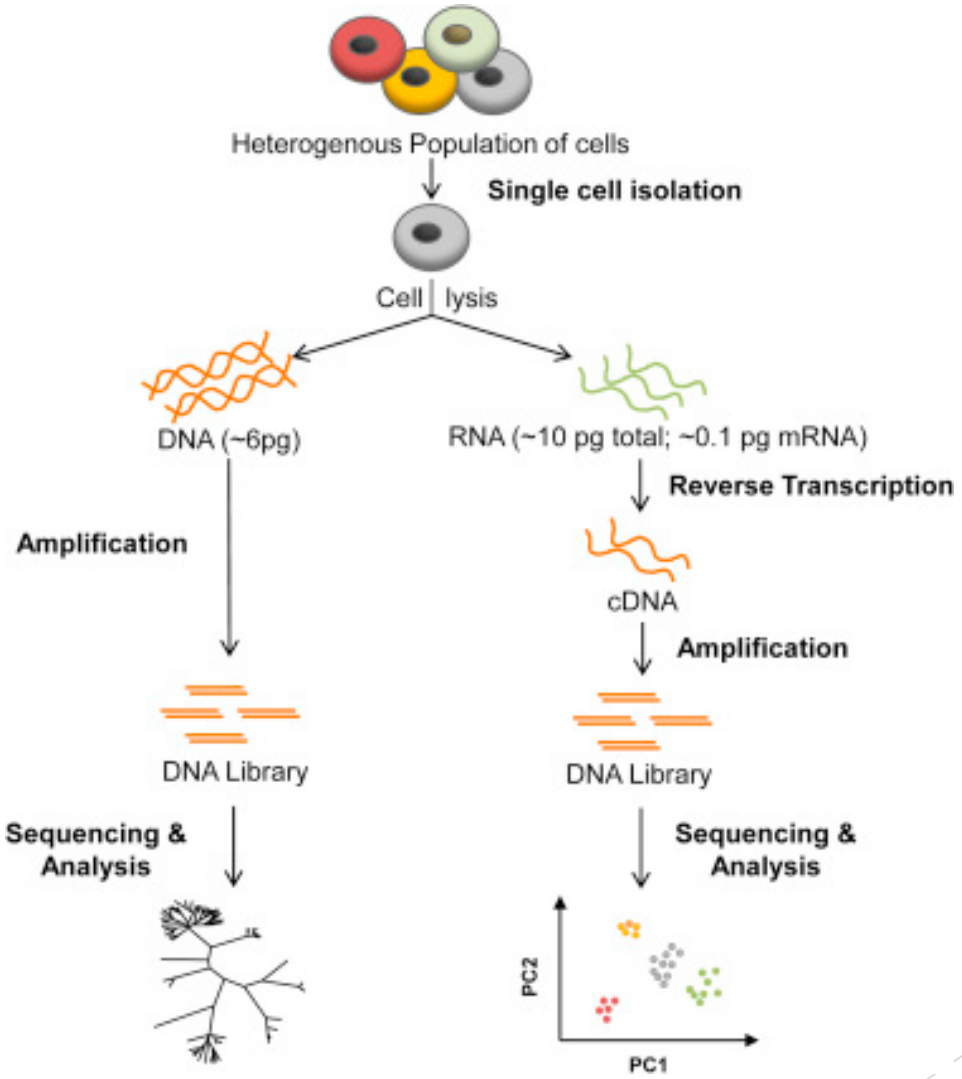
-stochastic variation



ZEISS, (2022)

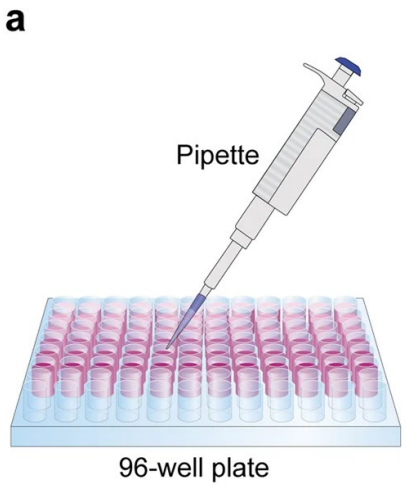
Single-cell (Sc) analysis

- Powerful tool to study cell heterogeneity through analysis of whole genome and transcriptome of individual cell
- Identify minority sub-population
- Discover unique characteristics of individual cells
- Proven useful in cancer, immunology, embryology and microbiology

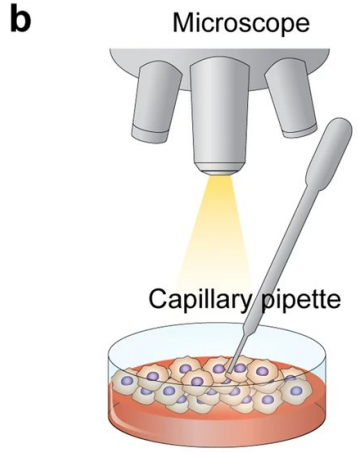


S Rato et al., Virus Research(2017)

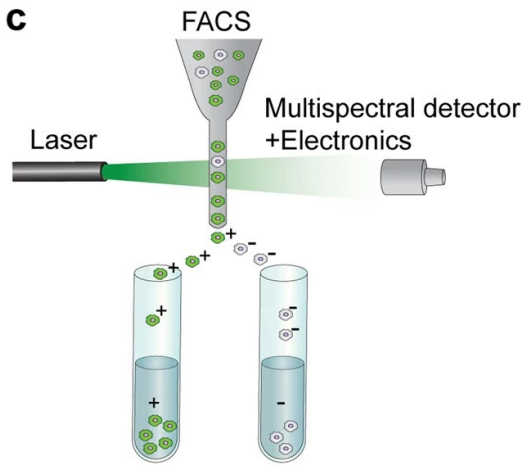
Single-cell isolation techniques



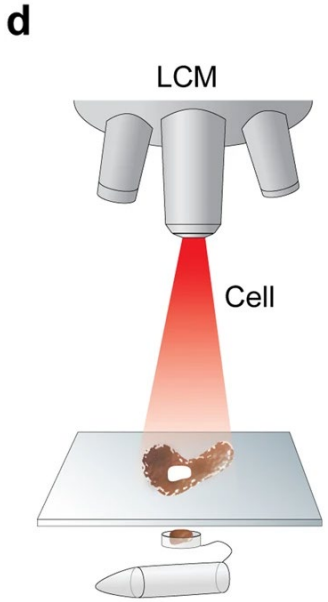
Limiting dilution



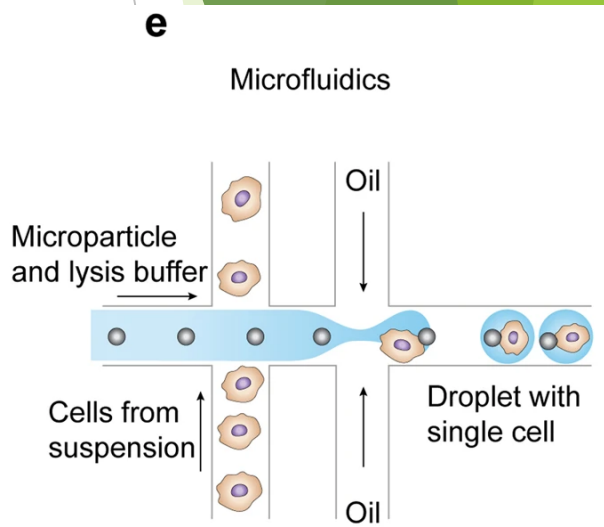
Micromanipulation



Fluorescence-activated cell sorting (FACS)

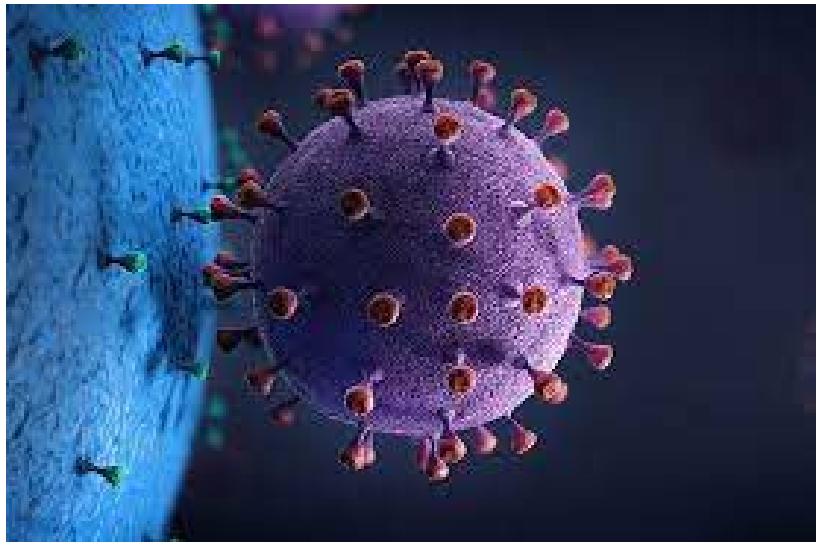


Laser capture microdissection (LCM)



Microdroplet-based microfluidics

Hwang, B et al., Exp Mol Med (2018)



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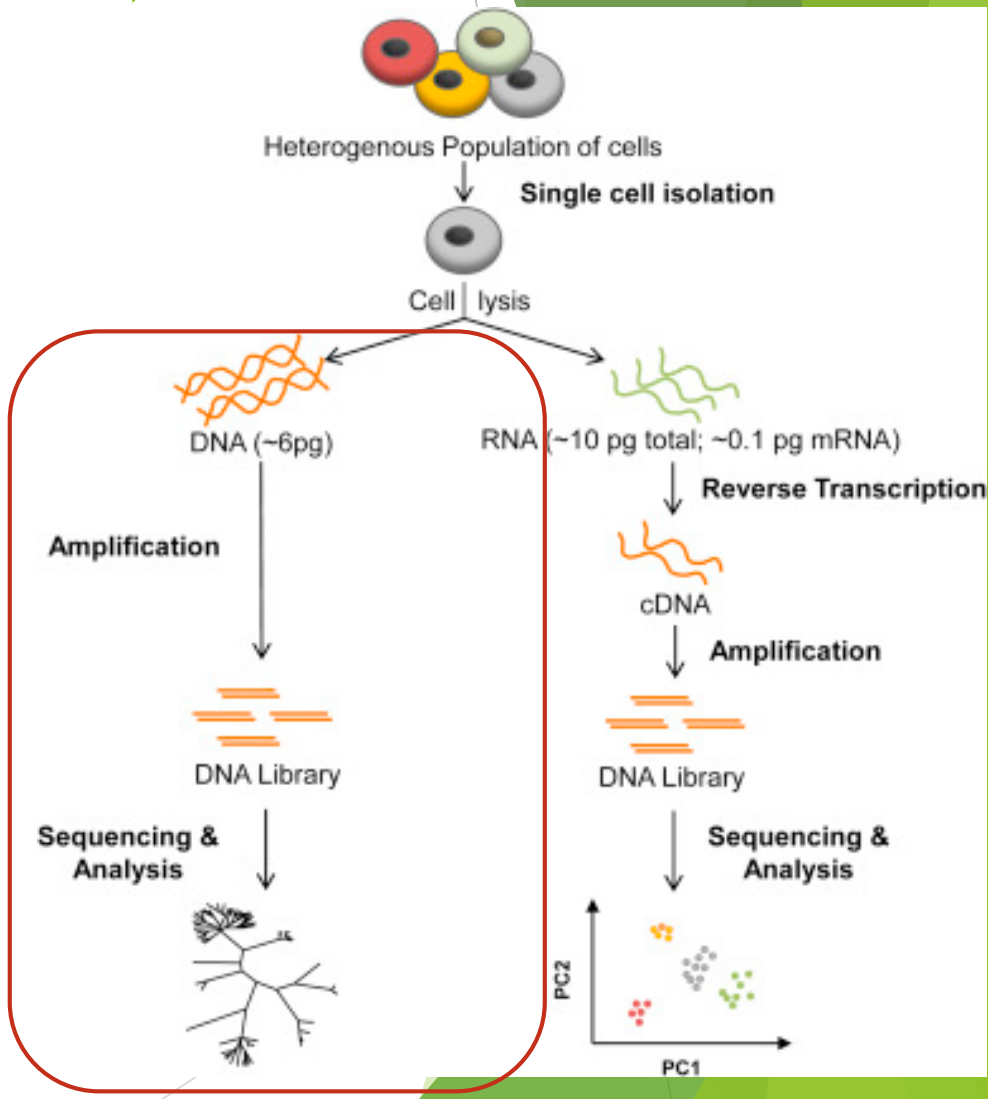
Whole genome amplification (WGA)

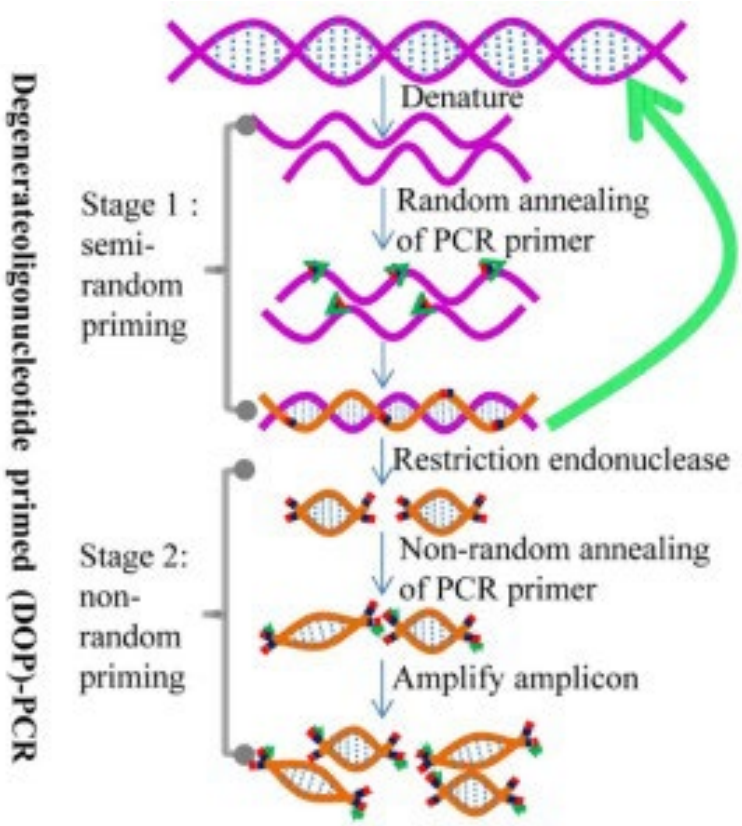
- Normal diploid human cell: 6-7 pg DNA, inadequate for genomic sequencing

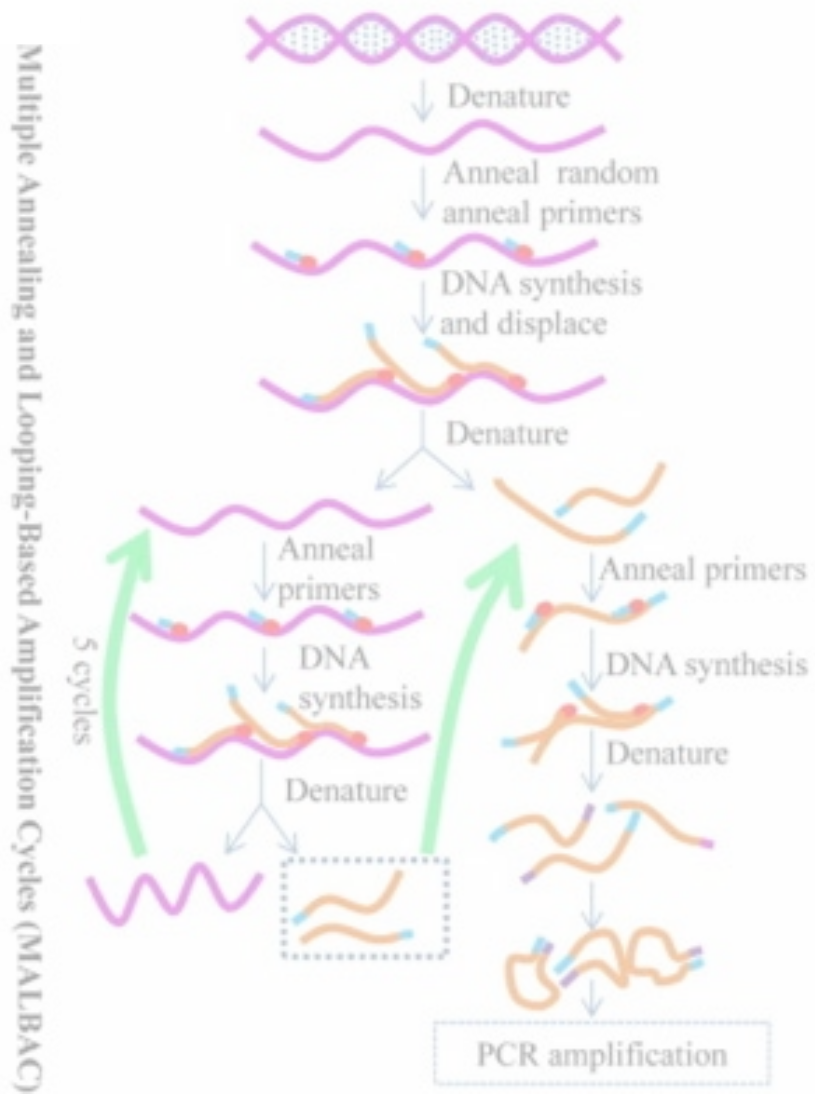
Amplification step:

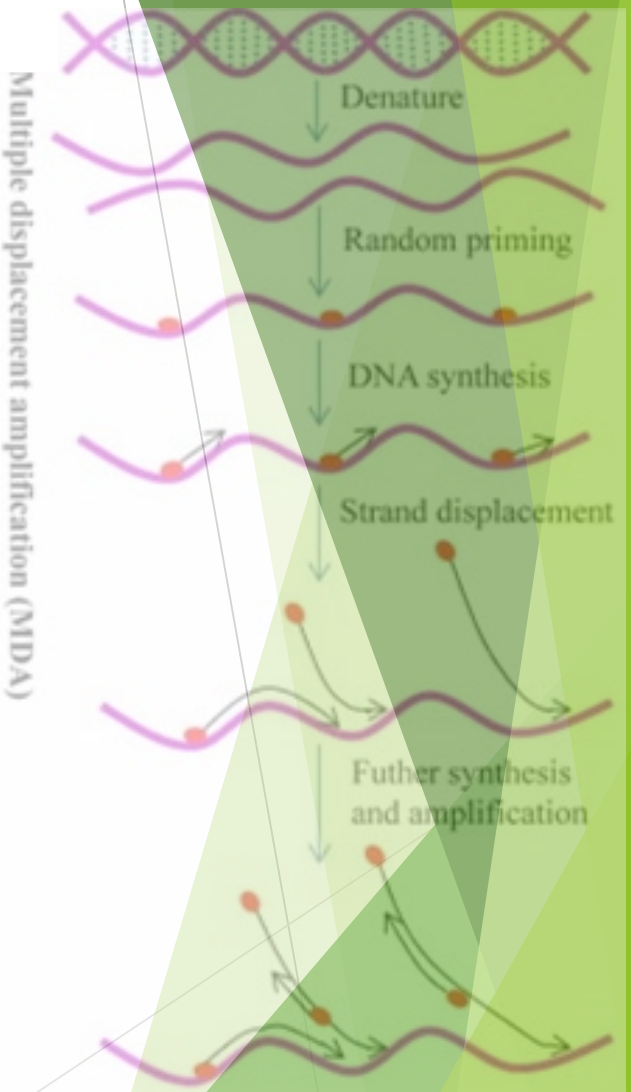
- degenerate-oligonucleotide-primed PCR (DOP-PCR)
- multiple annealing and looping-based amplification cycles (MALBAC)
- multiple-displacement-amplification (MDA)



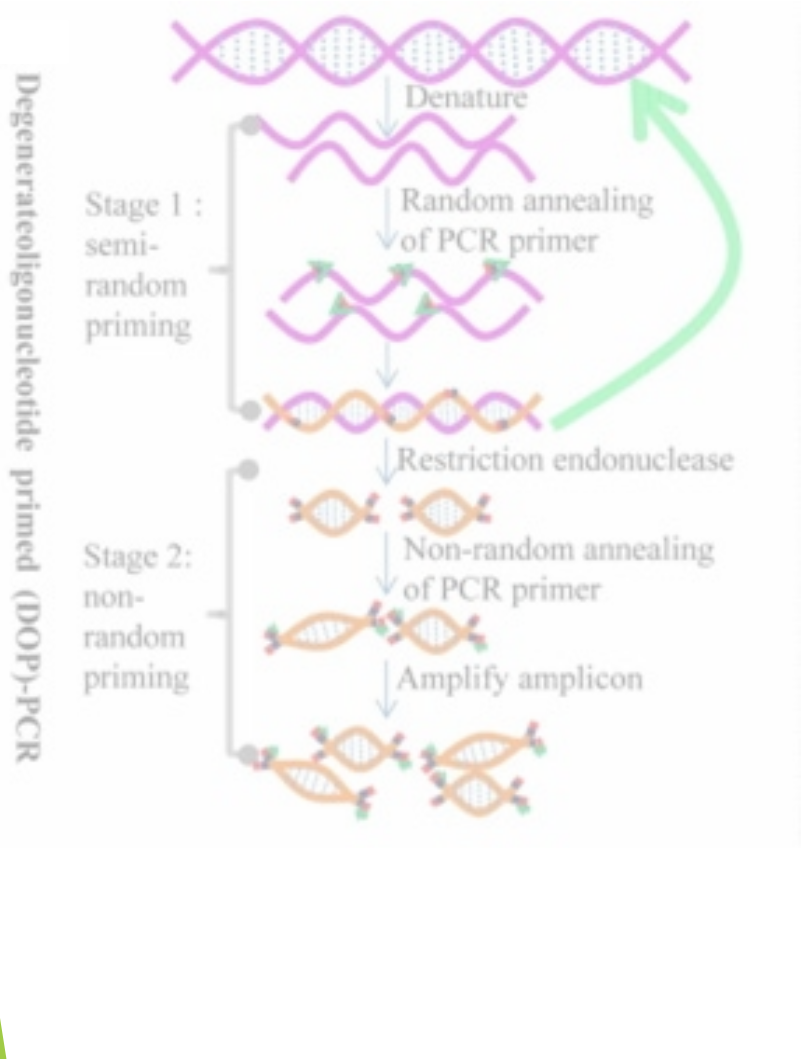
S Rato et al., Virus Research(2017)

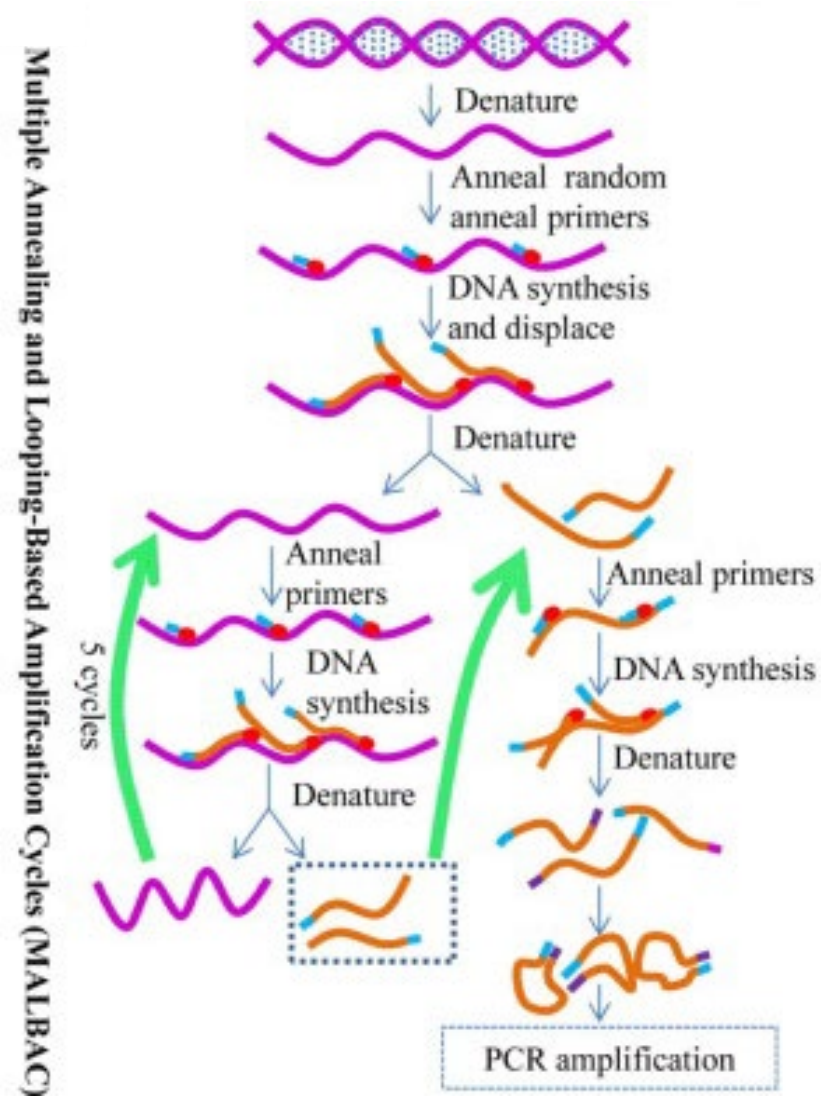


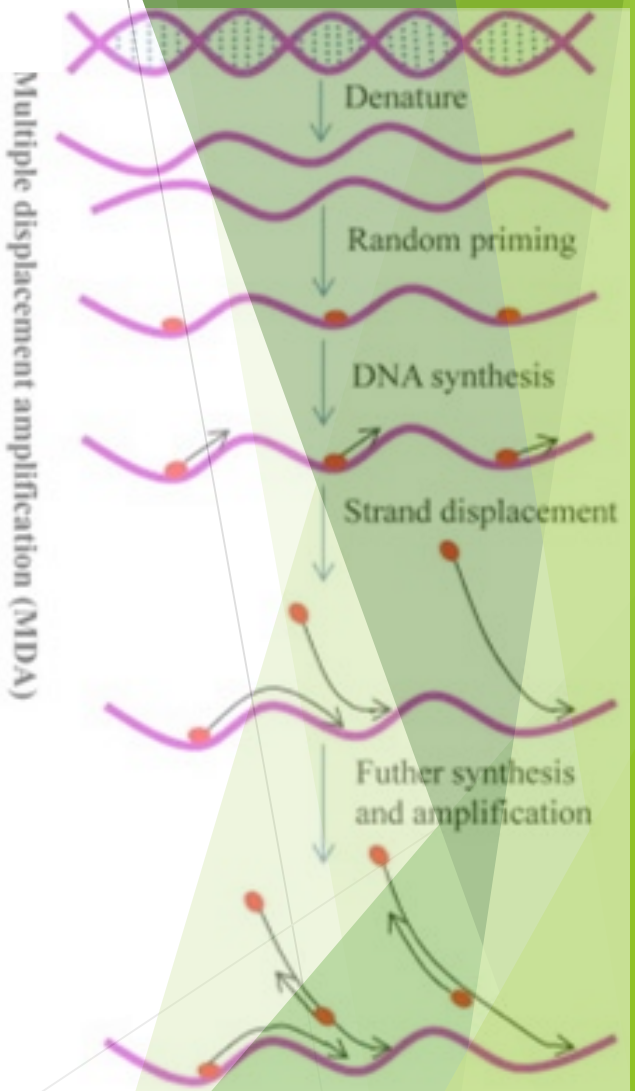




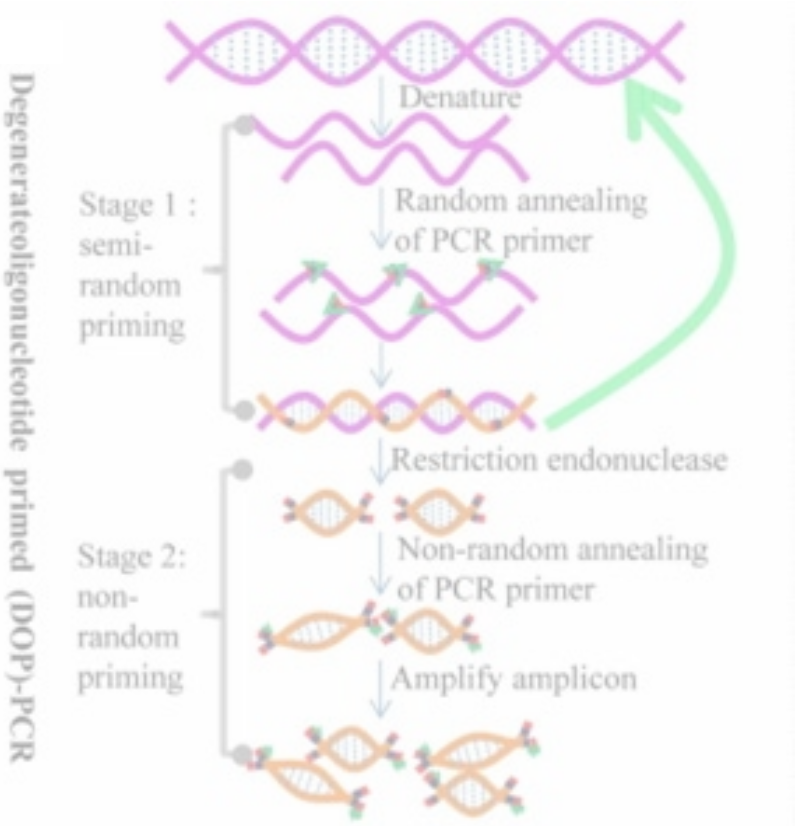
HJ Sun et al., Caner Letters (2015)

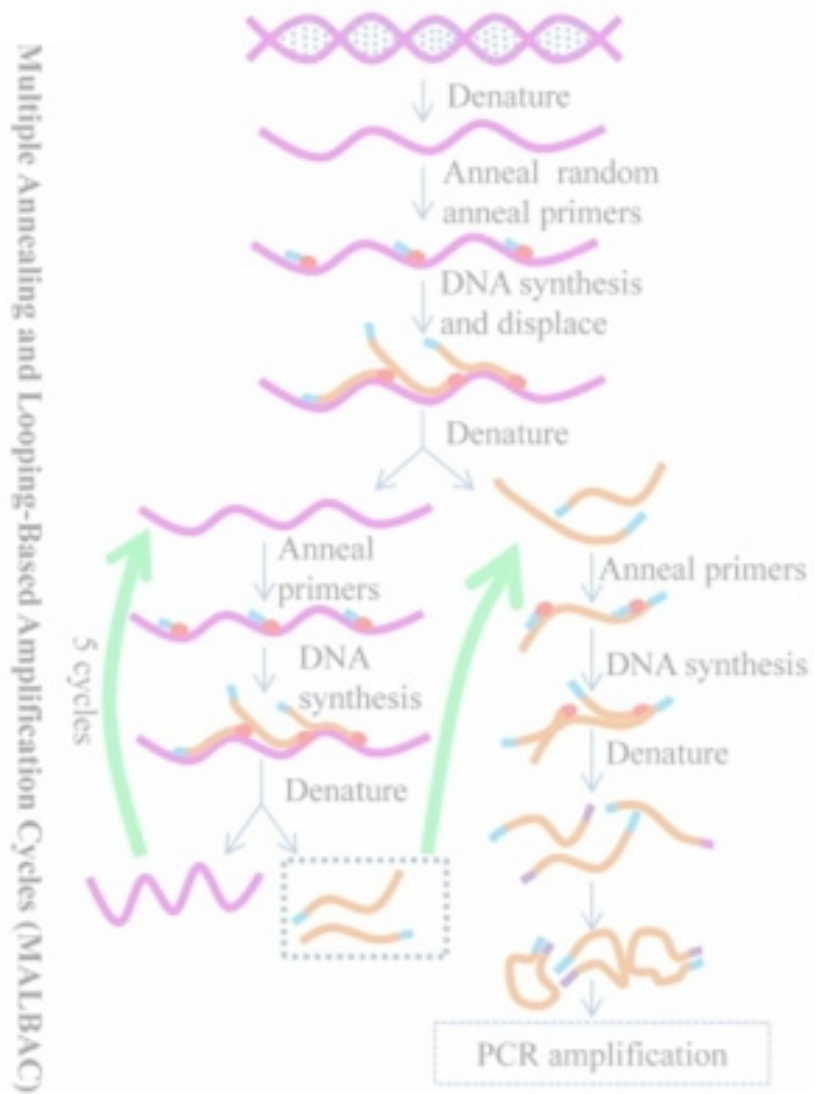


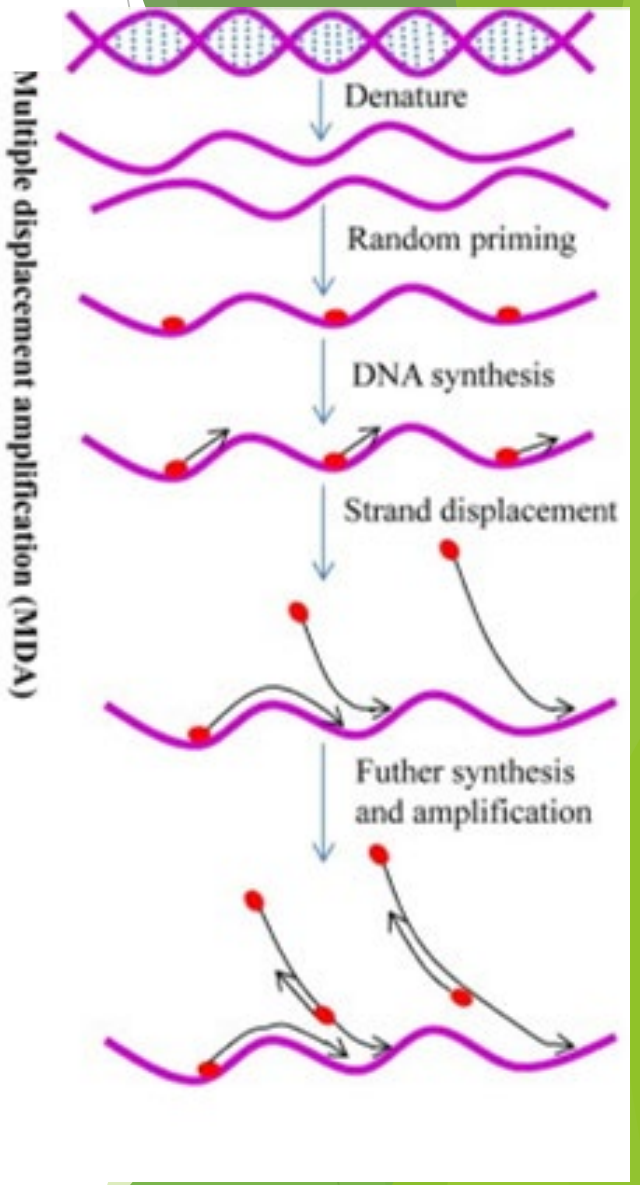




HJ Sun et al., Caner Letters (2015)



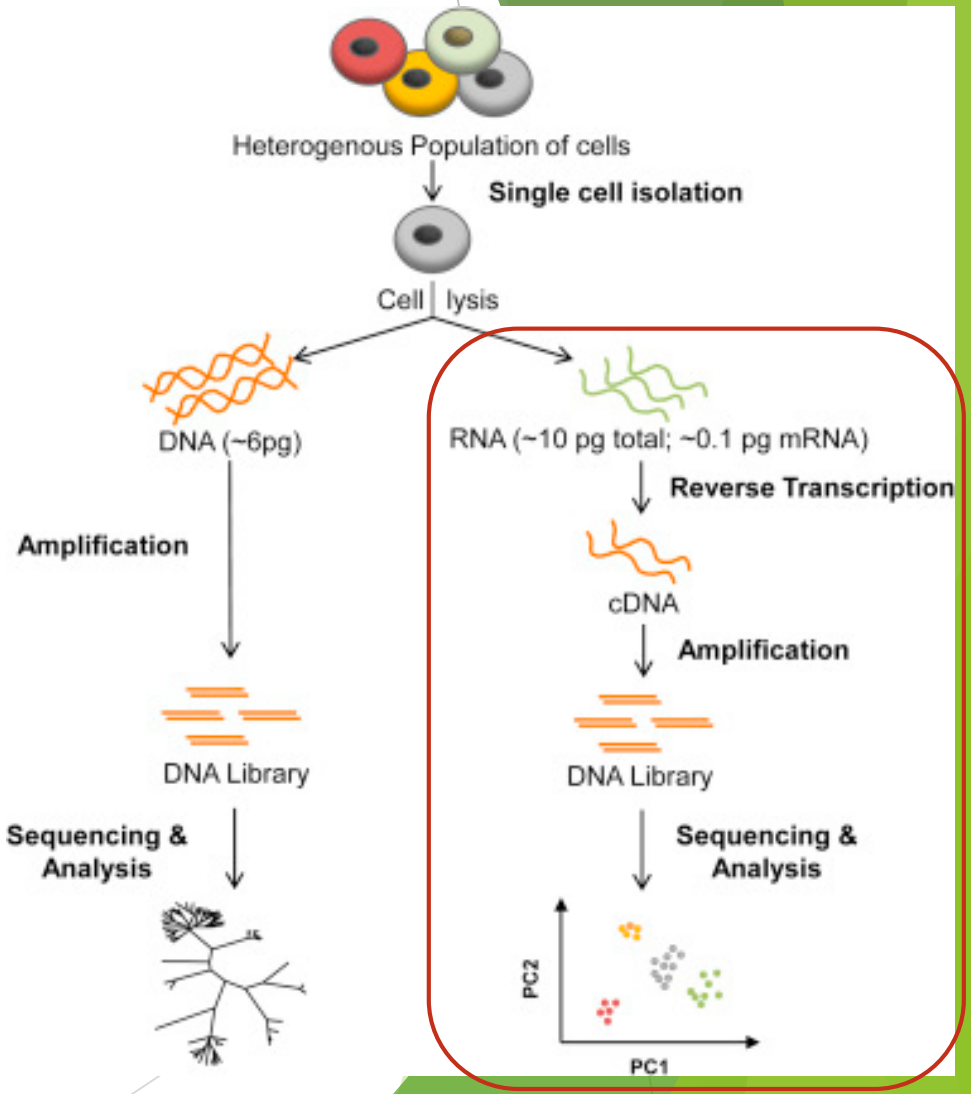




HJ Sun et al., Caner Letters (2015)

Whole transcriptome amplification (WTA)

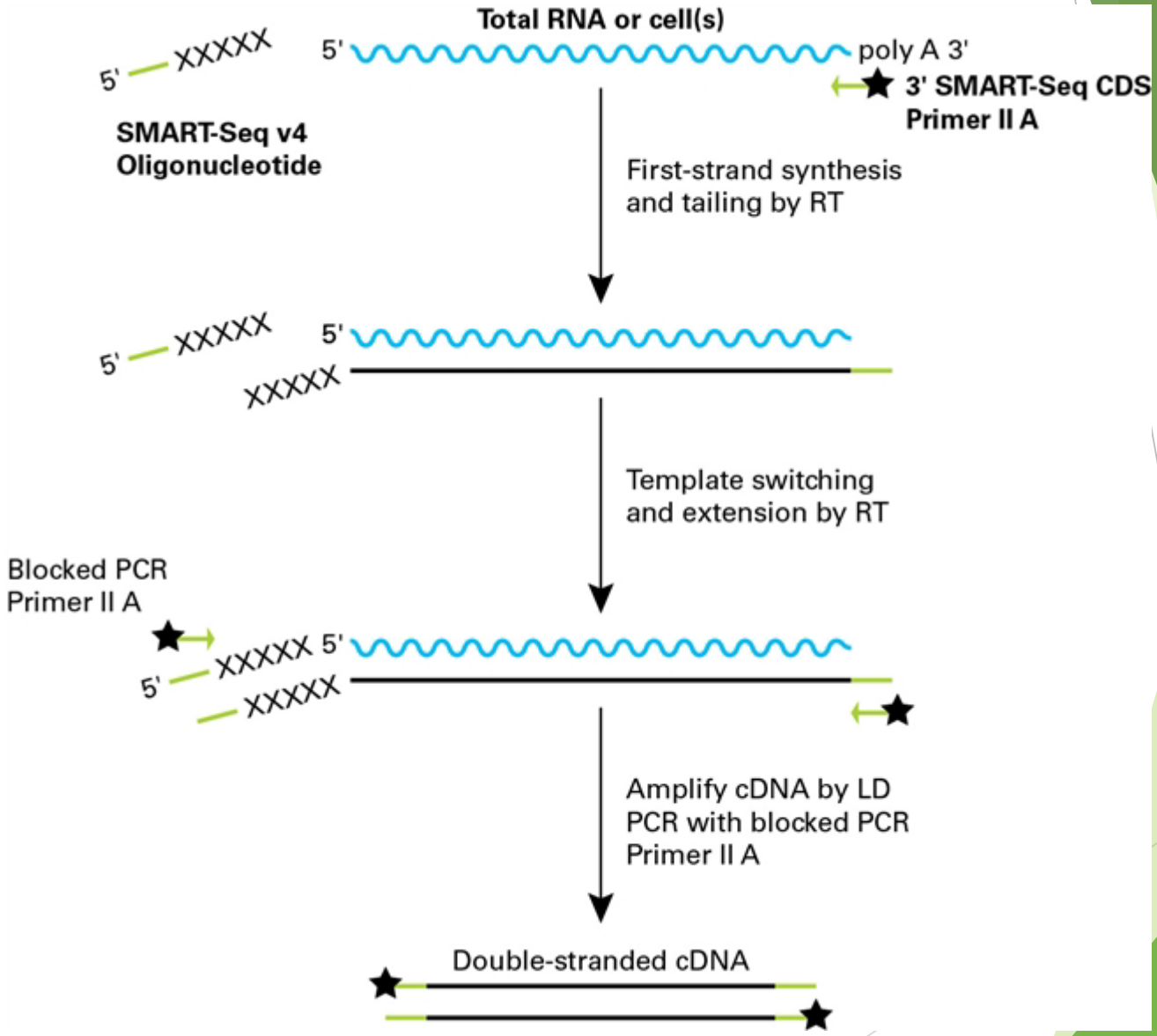
- Amount of RNA in single cell inadequate
- WTA generate cDNA library for single cell transcriptome sequencing
- Traditional/ modified PCR
- T7-*in vitro* transcription (IVT)
- Phi29 DNA polymerase-mediated RNA amplification (PMA)



S Rato et al., Virus Research(2017)

Modified PCR

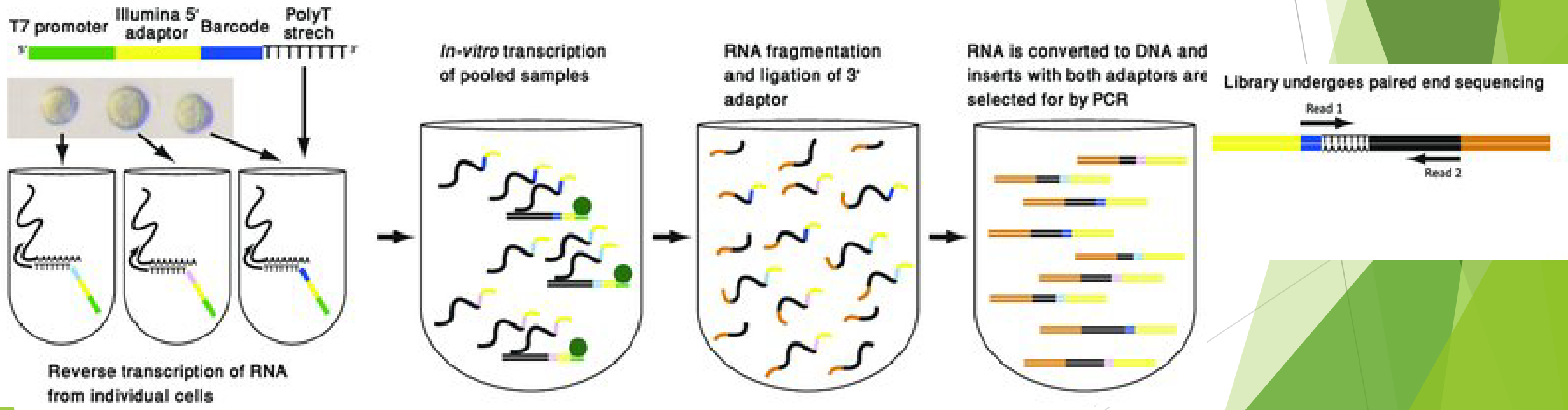
SMART techniques
"Switching Mechanism At the 5´ end of the RNA Transcript"



TAKARA, (2022)

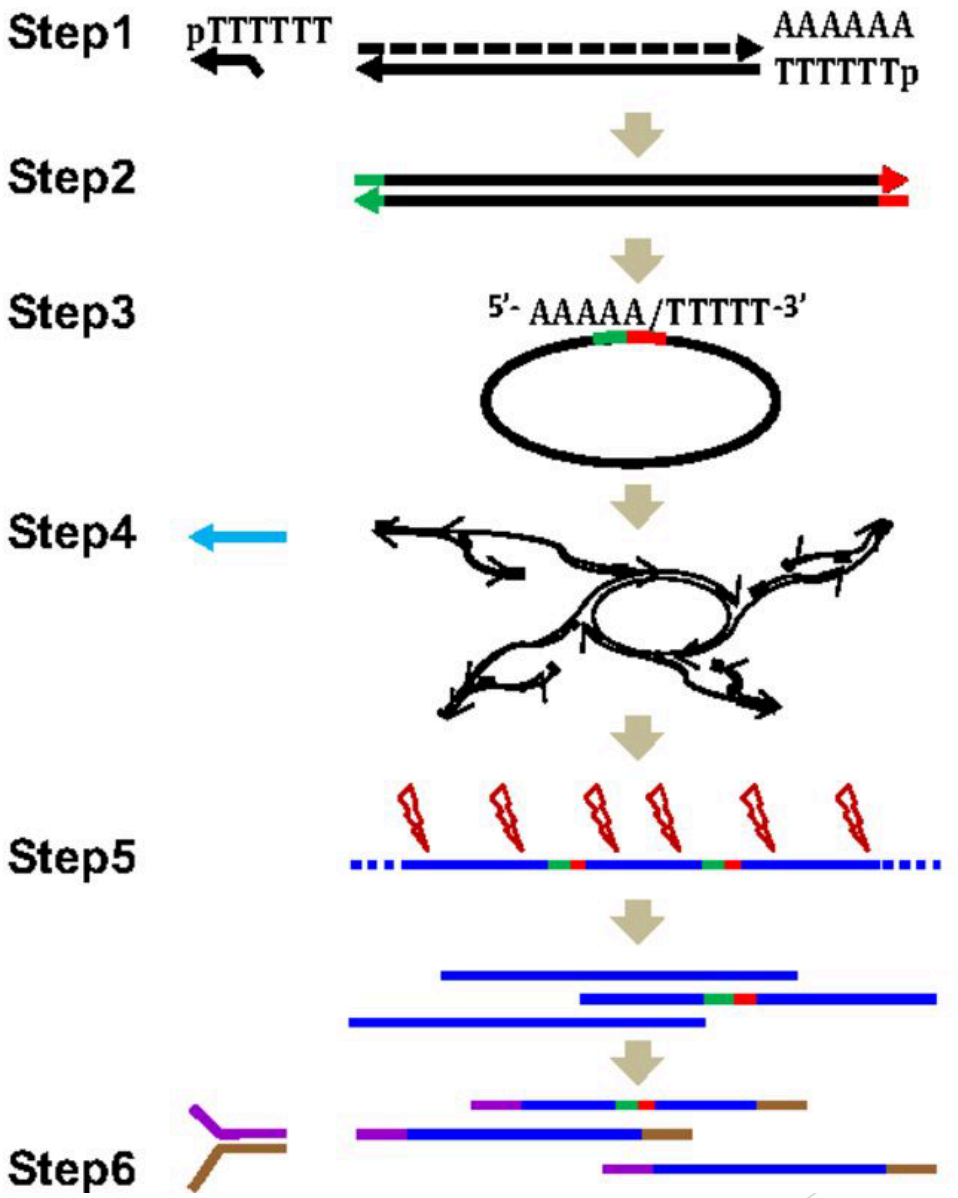
T7-*in vitro* transcription (IVT)

- Different than PCR-based method (different design of reverse transcription)
- CEL-seq (cell expression by linear amplification and sequencing)



XT Huang et al,, Front Oncol. (2018)

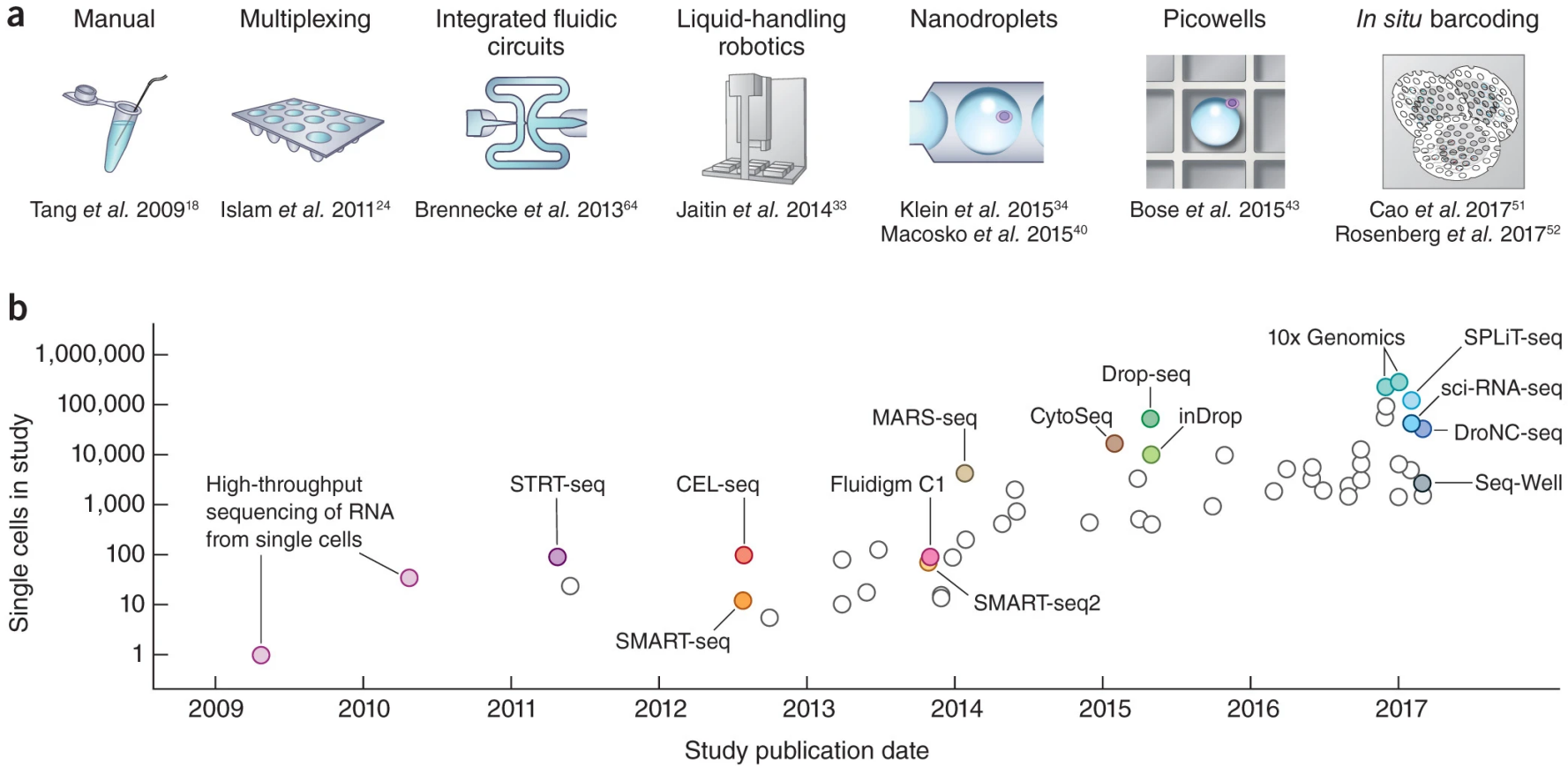
Phi29-mRNA amplification (PMA)



X Pan et al,. PNAS (2013)

scRNA-seq platforms

- Tremendous growth of commercial scRNA-seq platforms
- ↑ cell number profiled, accuracy, sensitivity
- ↓ reagents, cost



V Svensson et al., Nature Protocols (2018)

Single-cell technology in Virology

- Virus dependent on host cell to replicate
- Heterogeneity of host cell reflects in viral infection outcome
- 100% infected cells is difficult
- (cellular heterogeneity/ virus particles)
- SCs allows joint analysis of virus replication and host cell environment
- Virus/Cell-based: Viral replication, Virus-induced cellular response/ transcriptome, infection outcome

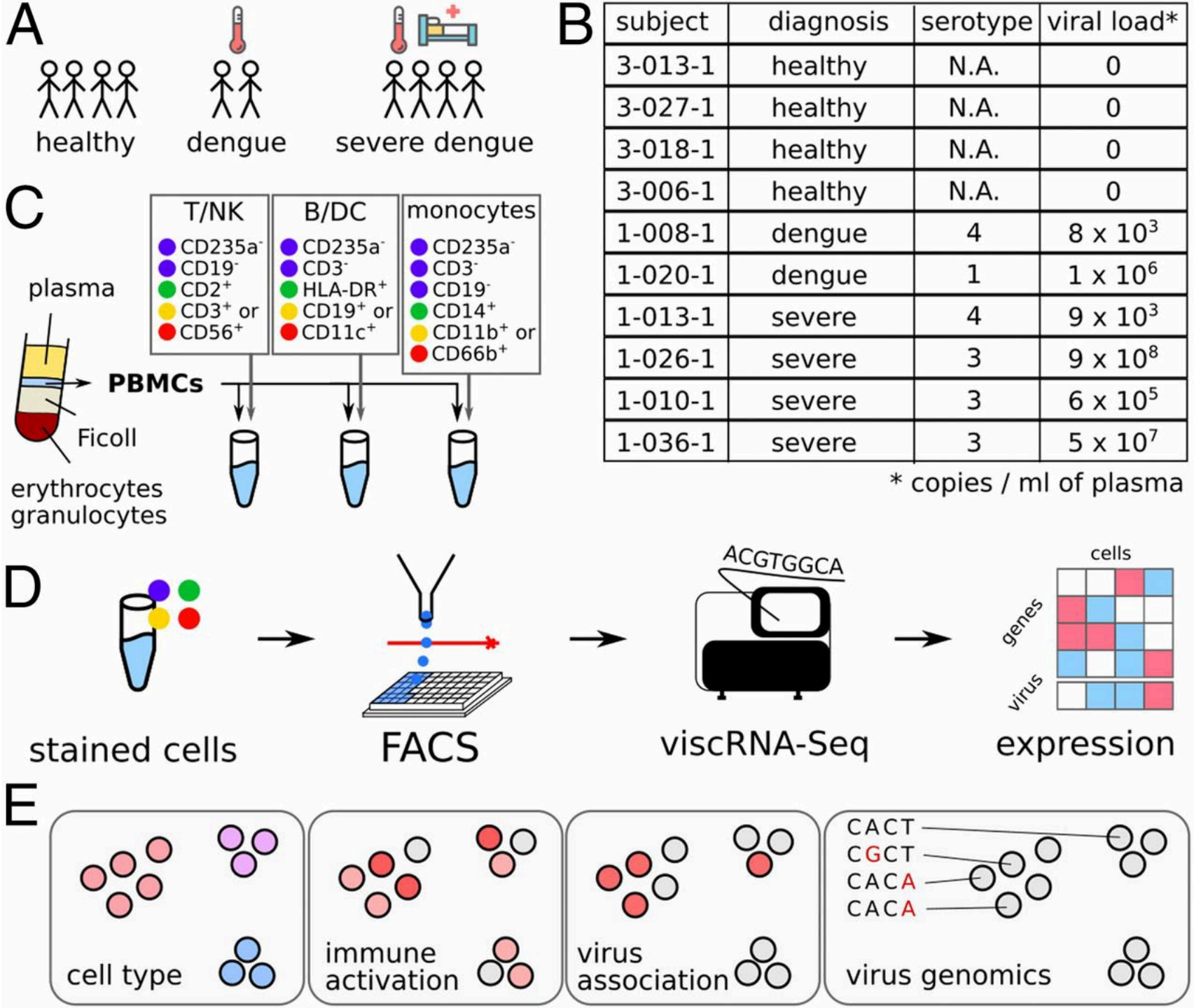
Virus-inclusive single-cell RNA sequencing reveals the molecular signature of progression to severe dengue

Fabio Zanini[a,1], Makeda L. Robinson[b,c,1], Derek Croote[a], Malaya Kumar Sahoo[d], Ana Maria Sanz[e], Eliana Ortiz-Lasso[f], Ludwig Luis Albornoz[f], Fernando Rosso[e,g], Jose G. Montoya[c], Leslie Goo[h], Benjamin A. Pinsky[c,d], Stephen R. Quake[a,h,i,2], and Shirit Einav[b,c,2]

[a]Department of Bioengineering, Stanford University, Stanford, CA 94305; [b]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305; [c]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305; [d]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94304; [e]Clinical Research Center, Fundación Valle del Lili, Cali 760026, Colombia; [f]Pathology and Laboratory Department, Fundación Valle del Lili, Cali 760026, Colombia; [g]Division of Infectious Diseases, Department of Internal Medicine, Fundación Valle del Lili, Cali 760026, Colombia; [h]Chan Zuckerberg Biohub, San Francisco, CA 94158; and [i]Department of Applied Physics, Stanford University, Stanford, CA 94305

Contributed by Stephen R. Quake, October 24, 2018 (sent for review August 10, 2018; reviewed by Katja Fink and Alex K. Shalek)

Zanini F, Robinson ML, Croote D, Sahoo MK, Sanz AM, Ortiz-Lasso E, Albornoz LL, Rosso F, Montoya JG, Goo L, Pinsky BA, Quake SR, Einav S. Virus-inclusive single-cell RNA sequencing reveals the molecular signature of progression to severe dengue. Proc Natl Acad Sci U S A. 2018 Dec 26;115(52):E12363-E12369. doi: 10.1073/pnas.1813819115. Epub 2018 Dec 7. PMID: 30530648; PMCID: PMC6310786.

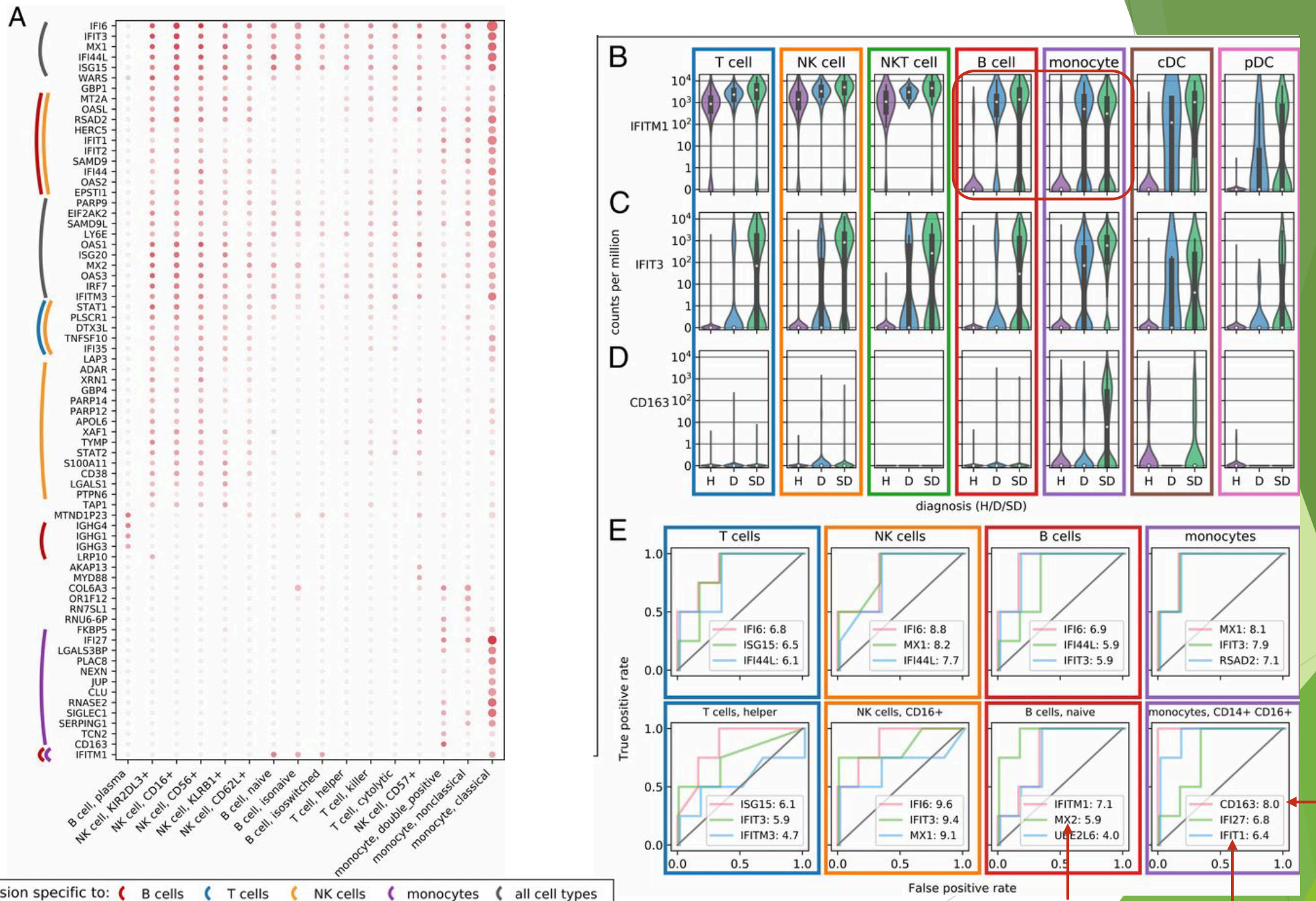


B

subject	diagnosis	serotype	viral load*
3-013-1	healthy	N.A.	0
3-027-1	healthy	N.A.	0
3-018-1	healthy	N.A.	0
3-006-1	healthy	N.A.	0
1-008-1	dengue	4	8×10^3
1-020-1	dengue	1	1×10^6
1-013-1	severe	4	9×10^3
1-026-1	severe	3	9×10^8
1-010-1	severe	3	6×10^5
1-036-1	severe	3	5×10^7

* copies / ml of plasma

Zanini F et al., PNAS (2018)



Zanini F et al., PNAS (2018)

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-27619-4 OPEN

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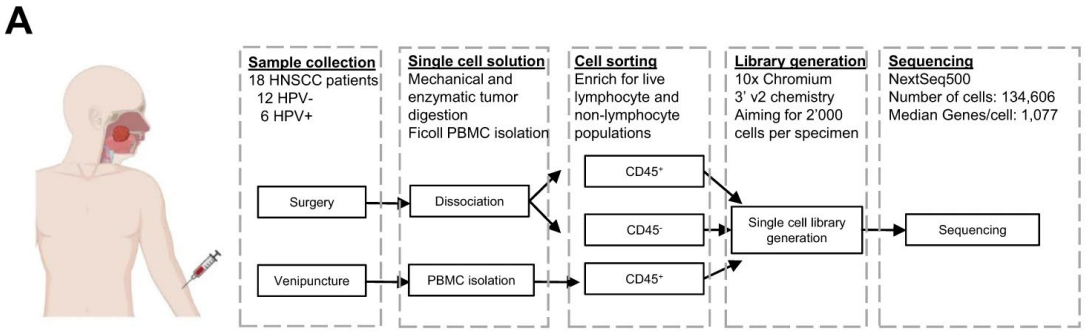
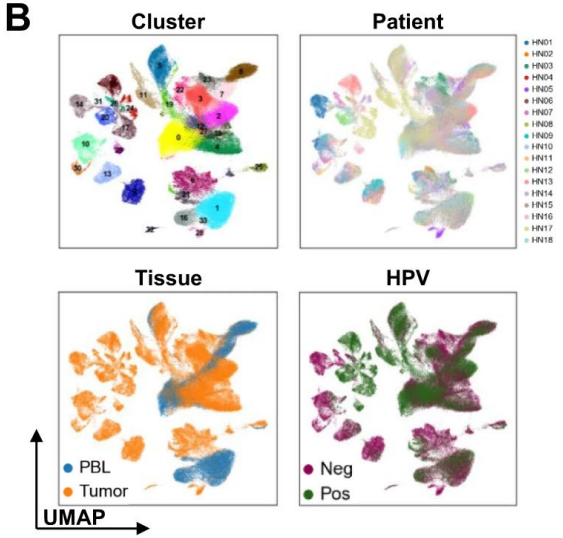
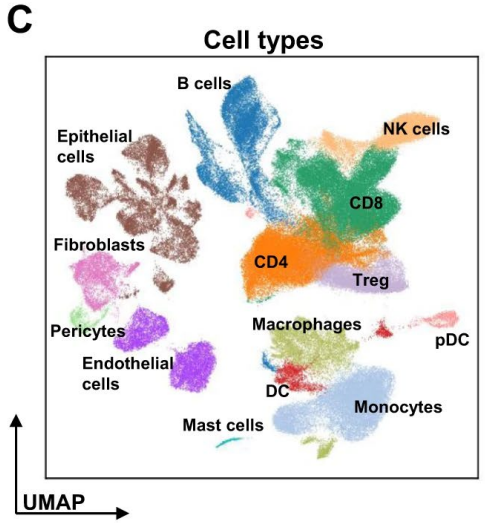
Investigating immune and non-immune cell interactions in head and neck tumors by single-cell RNA sequencing

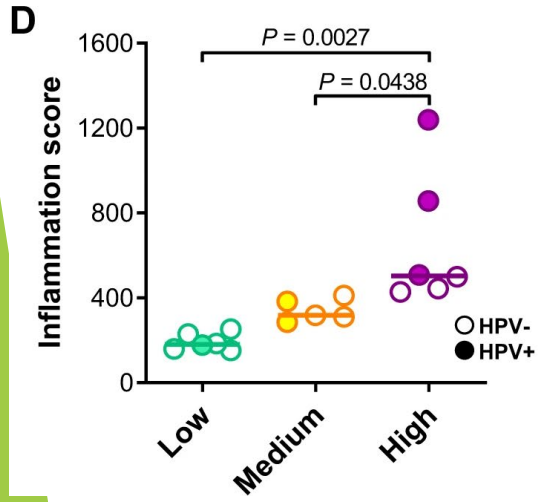
Cornelius H. L. Kürten[1,2,3,4,13], Aditi Kulkarni[2,3,4,13], Anthony R. Cillo[2,3,5], Patricia M. Santos[2,3,4], Anna K. Roble[2,3,4], Sayali Onkar[2,3,5,6], Carly Reeder[2,3,4], Stephan Lang[1], Xueer Chen[7], Umamaheswar Duvvuri[4], Seungwon Kim[4], Angen Liu[4,8], Tracy Tabib[9], Robert Lafyatis[9], Jian Feng[2,10,11], Shou-Jiang Gao[2,10,11], Tullia C. Bruno[2,3,5], Dario A. A. Vignali[2,3,5], Xinghua Lu[7], Riyue Bao[2,12], Lazar Vujanovic[2,3,4,13] & Robert L. Ferris[2,3,4,5]✉

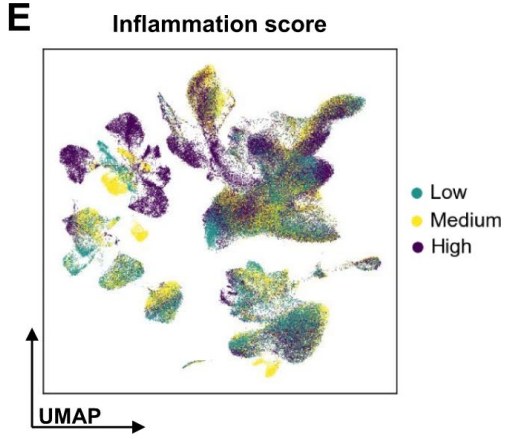
Kürten, C.H.L., Kulkarni, A., Cillo, A.R. *et al.* Investigating immune and non-immune cell interactions in head and neck tumors by single-cell RNA sequencing. *Nat Commun* 12, 7338 (2021). https://doi.org/10.1038/s41467-021-27619-4

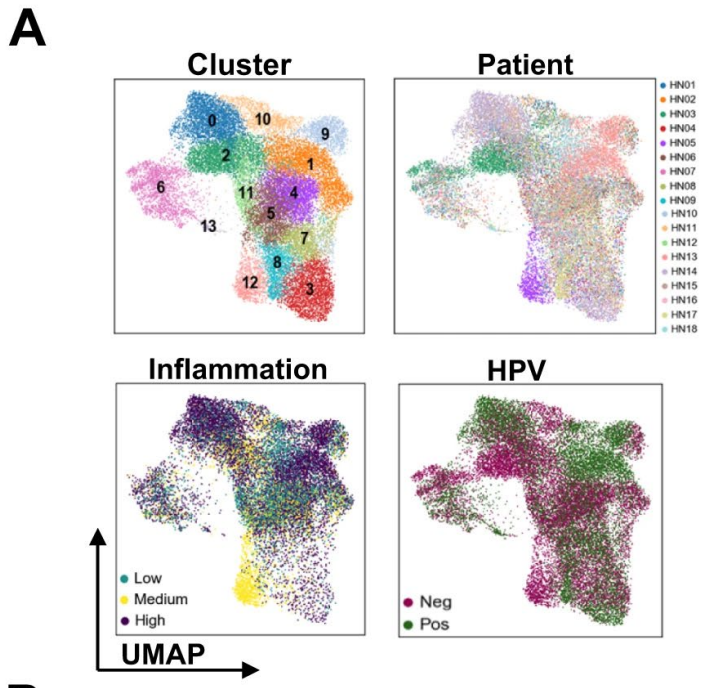


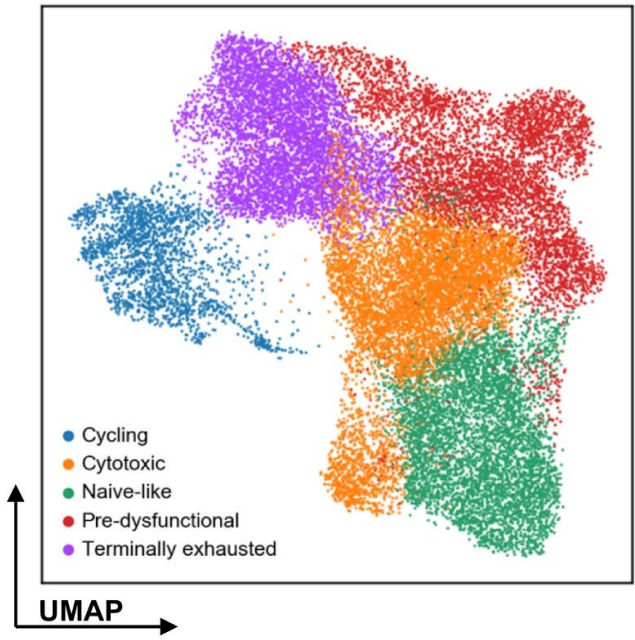


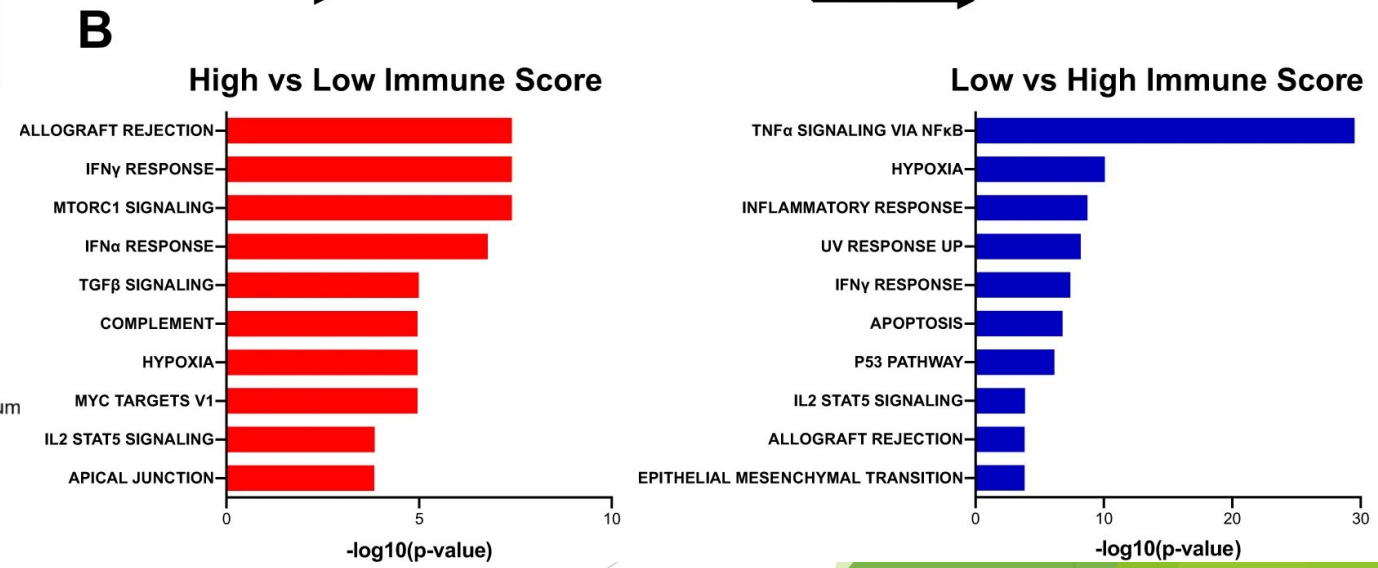




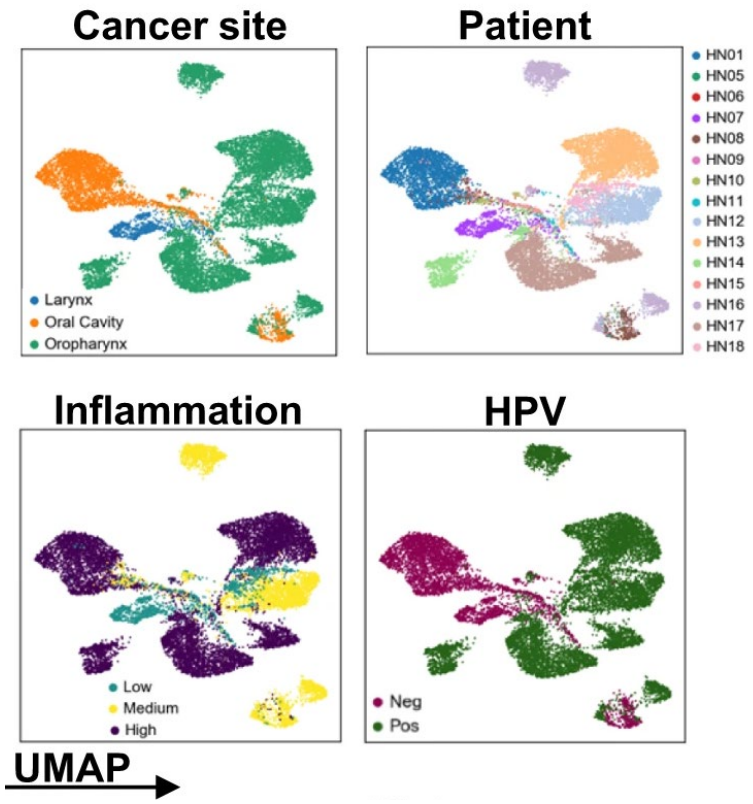


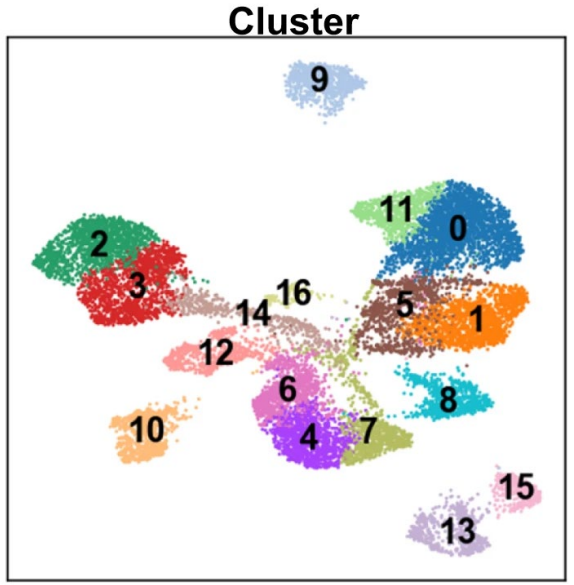


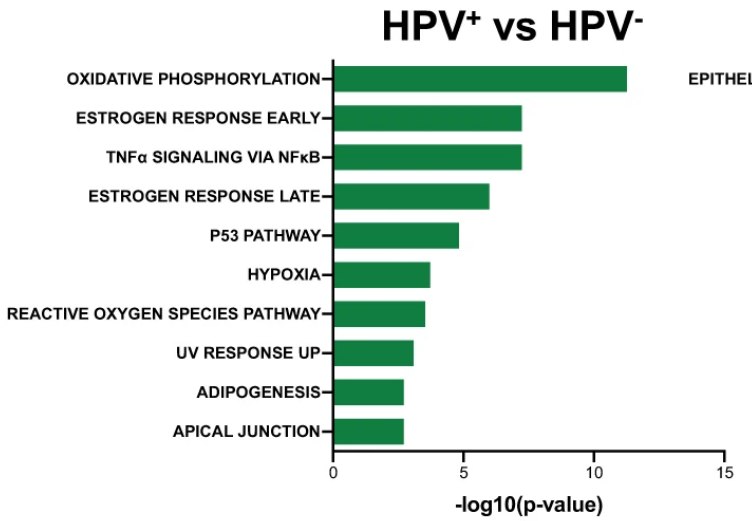


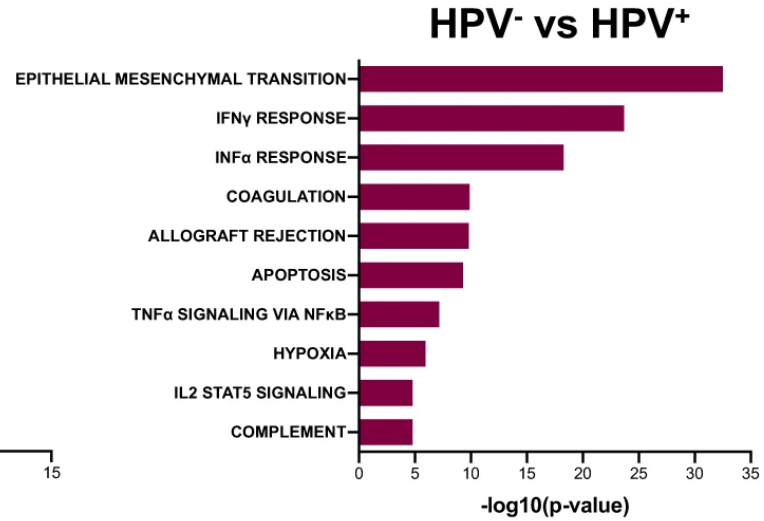


Kürten, C.H.L. et al, Nat Commun (2021)

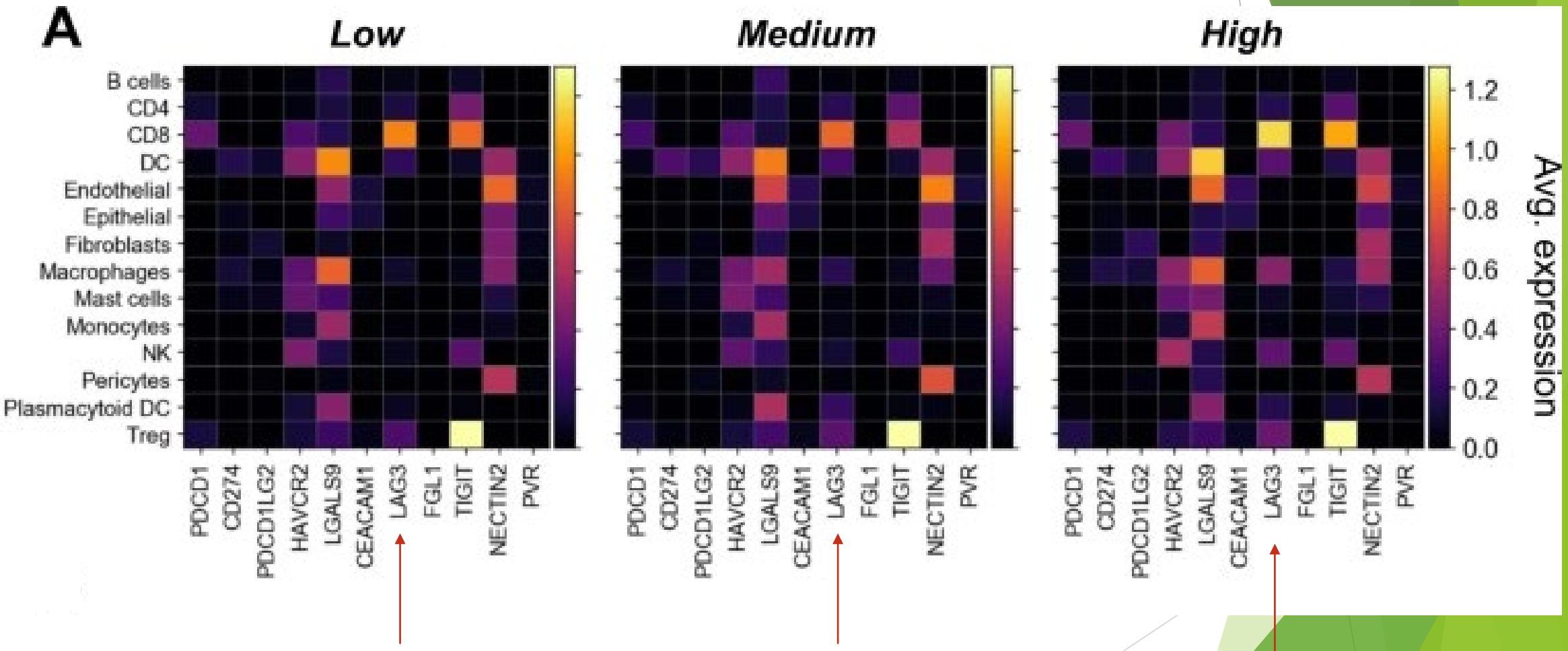








Kürten, C.H.L. et al, Nat Commun (2021)



Kürten, C.H.L. et al, Nat Commun (2021)

Conclusion

- Viral and cellular heterogeneity remain a challenge in Virology
- SC technologies allows examination of cell-to-cell variability on the outcome of viral infection
- Can capture viral diversity and evolution of sequence variation
- Better understanding of virus infection
- Allow, drug refinement, personalized medicine

Reference

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Thank you!

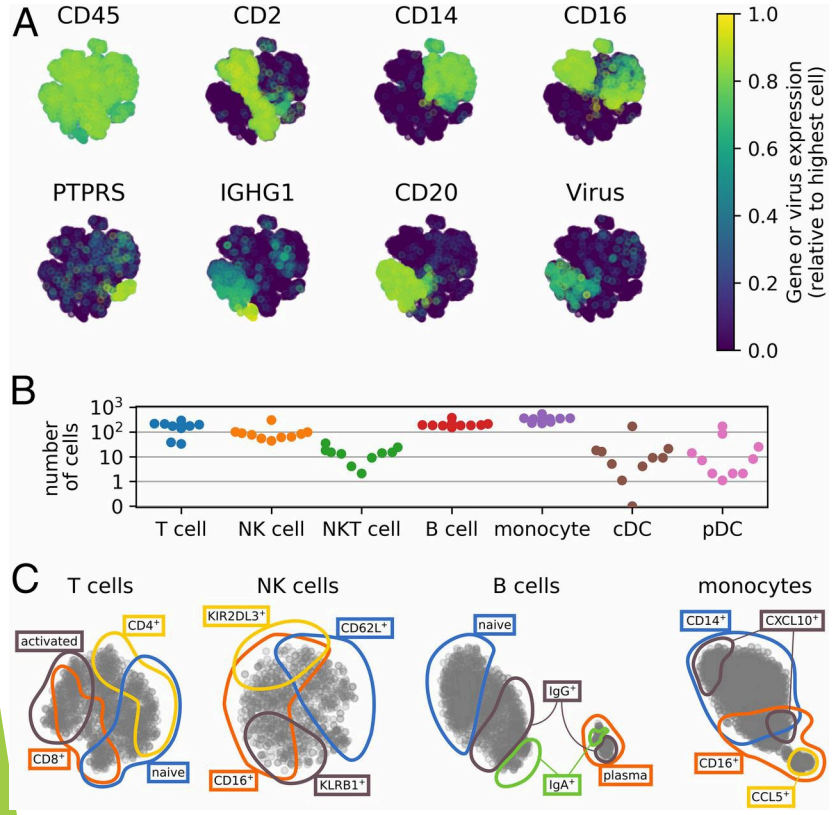
Supplementary (1)

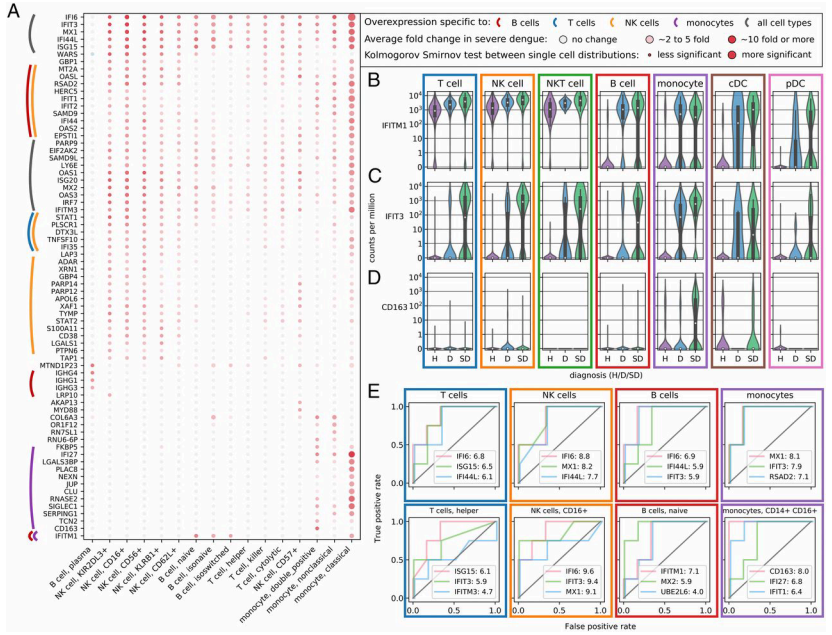
- DOP-PCR lies in its simplicity and cost-effectiveness, being no more complex than ordinary PCR and requiring little investment in kits or reagents.
- MALBAC Can sequence large templates, perform single-cell sequencing or sequencing for samples with very limited starting material, Full-amplicon looping inhibits over-representation of templates, reducing PCR bias, but Polymerase is relatively error prone compared to Phi 29, Temperature-sensitive protocol, Genome coverage up to ~90%, but some regions of the genome are consistently underrepresented
- PMA method allows for full-length transcript coverage to be obtained, but Slight 5′ end bias

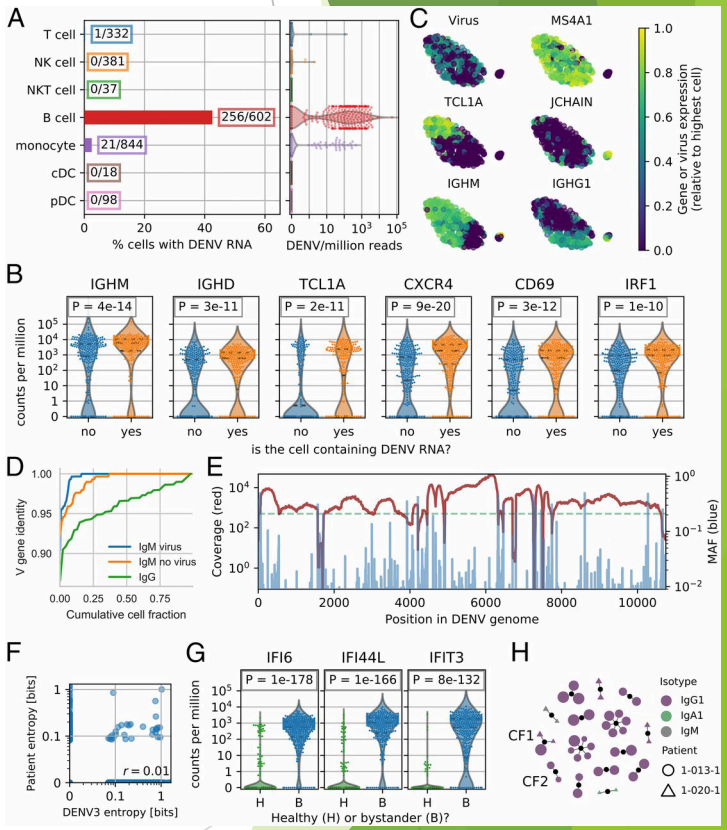
Supplementary (2)

Method	Principle	Coverage	Transcript lengths	Limitaion
CEL-seq	In vitro transcription	49%	Average 1.0 kb	Strong 3′ end bias, usually targets the last exons highly
Smart-seq	Modified PCR	Nearly full-length	Average 1.5 kb	Cannot capture partially reverse-transcribed mRNA
PMA	Phi29 DNA polymerase	Full-length	All sizes	Slight 5′ end bias

Supplementary (3)







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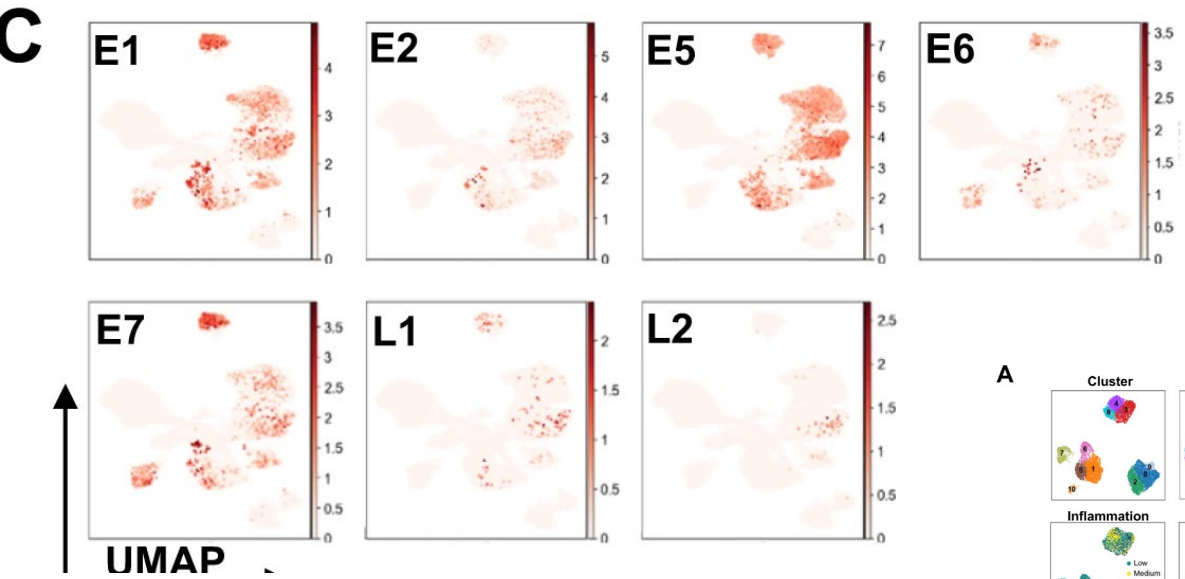

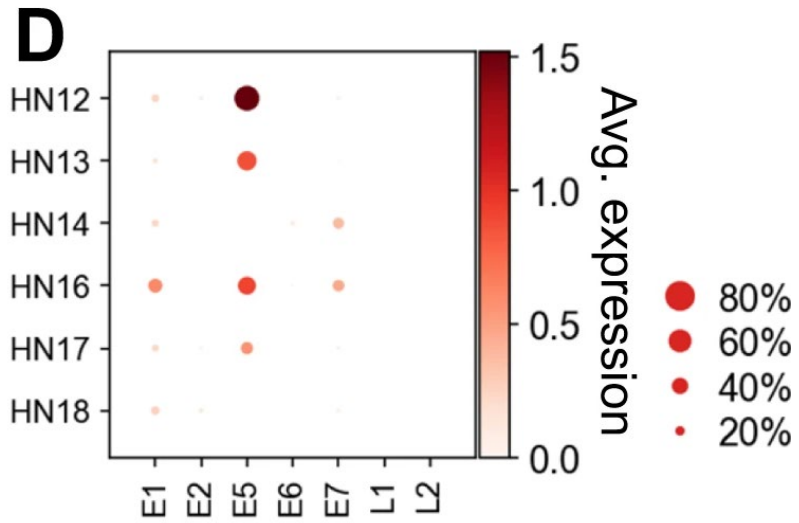

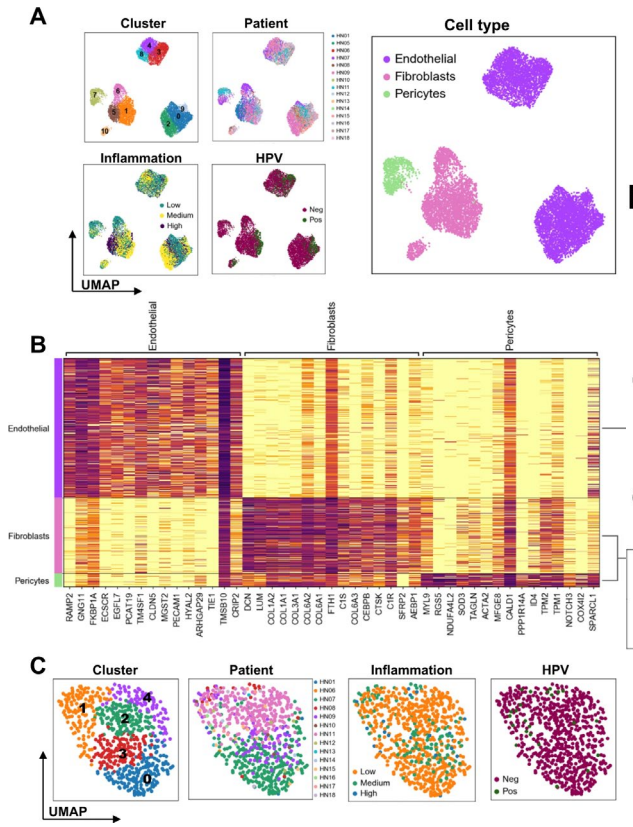

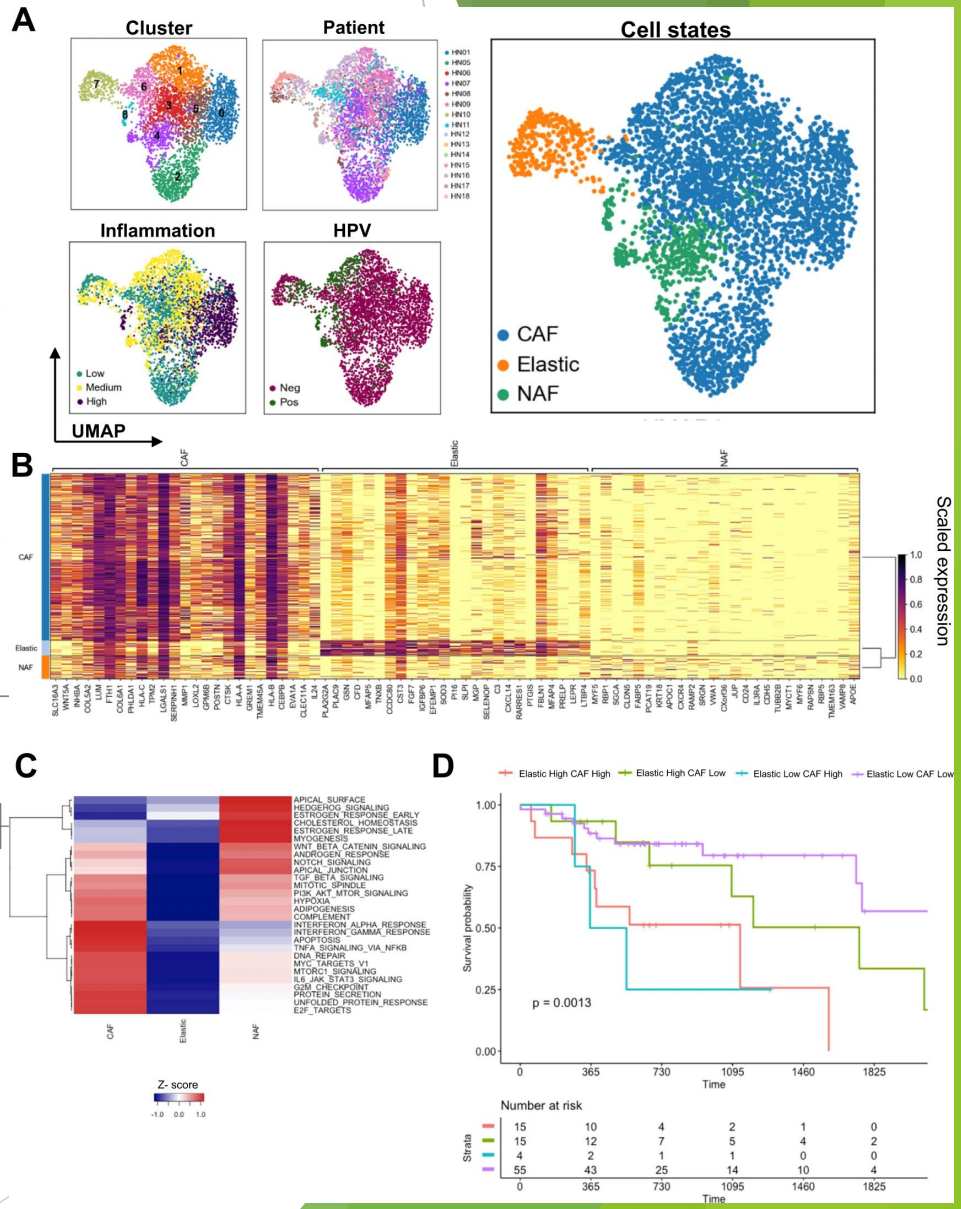
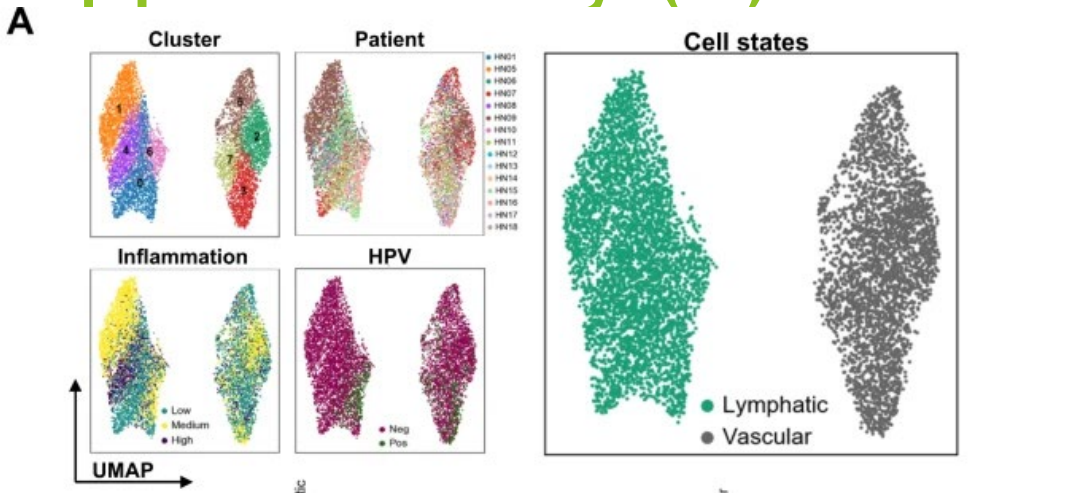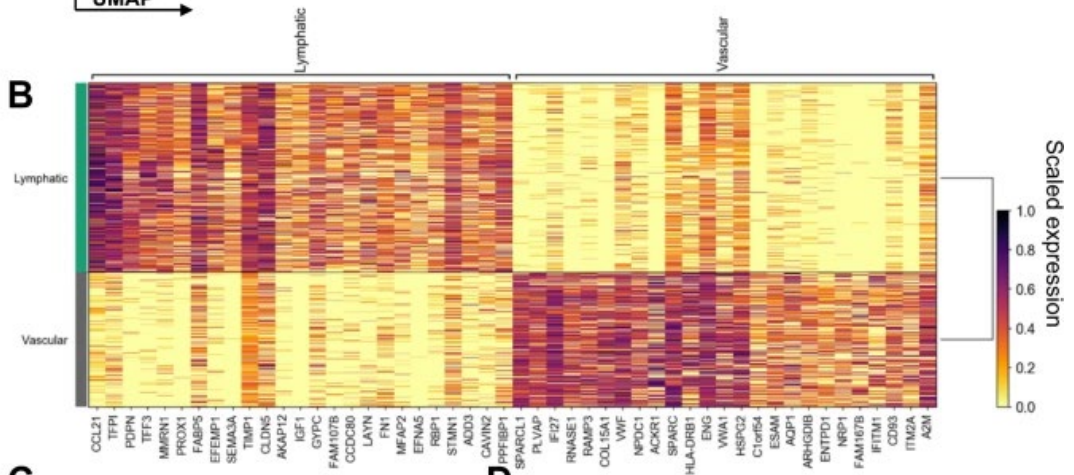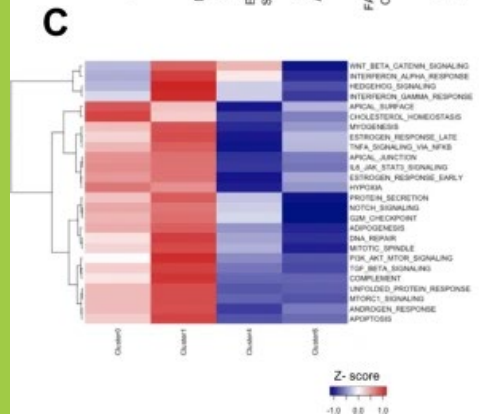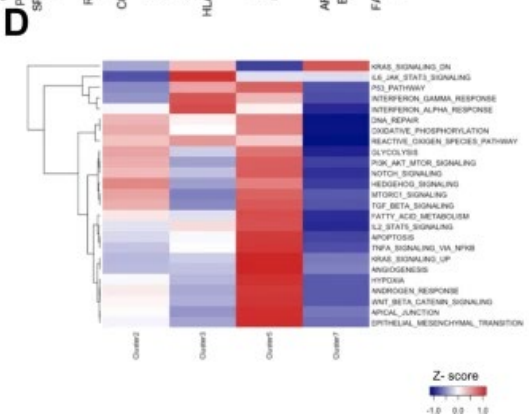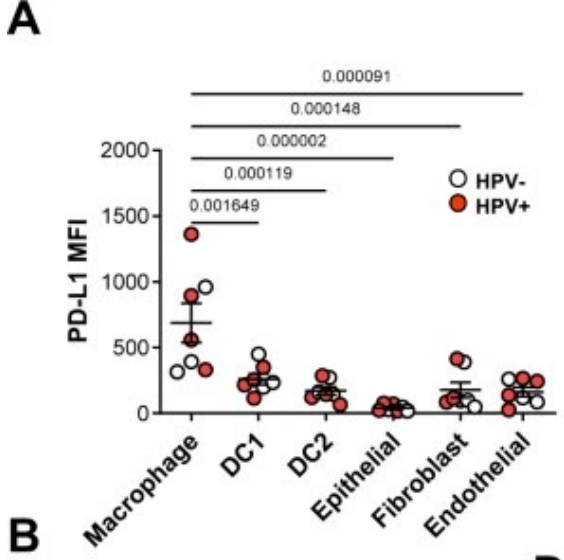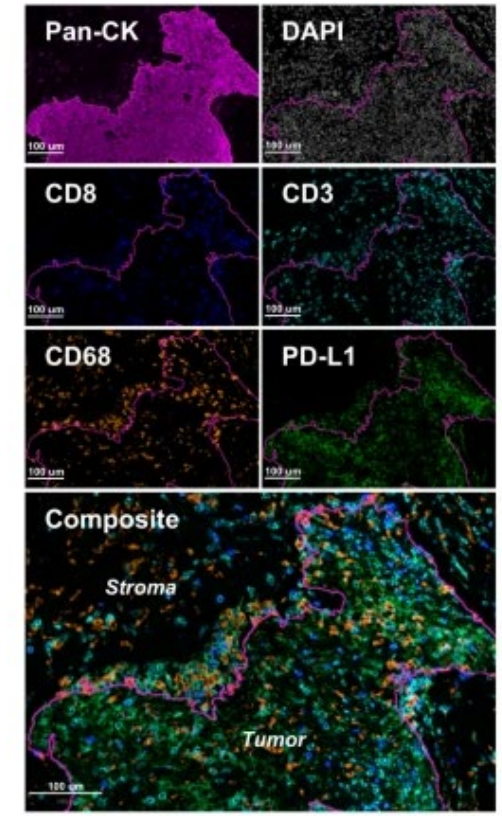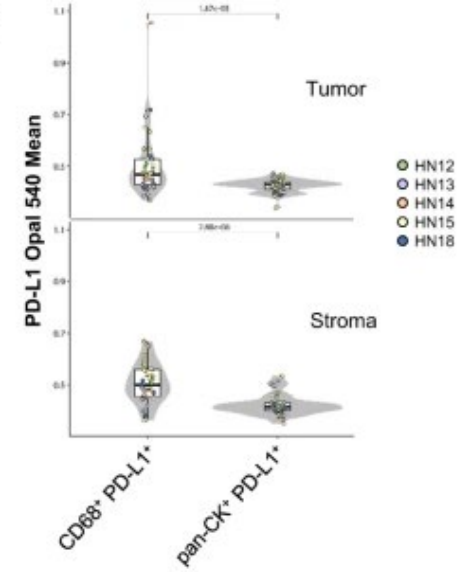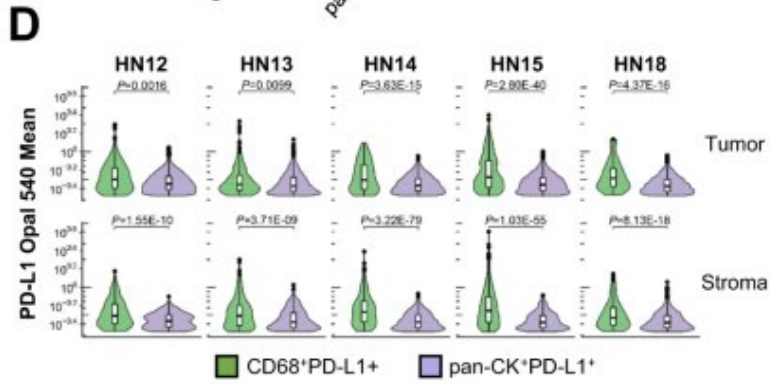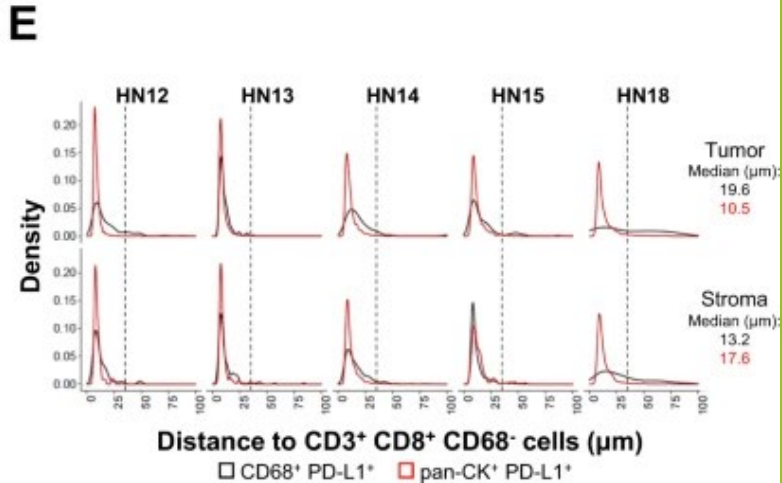
Supplementary (5)