METHODS FOR SINGLE CELL RNA_{SEQ}

Technical Journal Club, Sandra Ivic, 17th March '15

Introduction

„RNA sequencing (RNA-seq) is the application of any of a variety of next-generation sequencing techniques (also known as deep sequencing because of their potential for high coverage) to study RNA.“

(Chu et al. Nucleic Acid Ther. 2012 Aug; 22(4): 271–274)

- High-throughput sequencing and RNA-seq used extensively to profile bulk tissues
- Growing demand for whole transcriptome analysis of single cells
- Direct analysis of:
  - Rare cells types
  - Primary cells
  - Desire to profile interesting subpopulations from larger heterogeneous populations

1st article

Quantitative assessment of single-cell RNA-sequencing methods

NATURE METHODS | VOL.11 NO.1 | JANUARY 2014 | 41

Angela R Wu[1], Norma F Neff[1], Tomer Kalisky[1,8], Piero Dalerba[2–4], Barbara Treutlein[1], Michael E Rothenberg[5], Francis M Mburu[1,6], Gary L Mantalas[1], Sopheak Sim[3], Michael F Clarke[2–4] & Stephen R Quake[1,6,7]

Aim

They wanted to investigate RNAseq for single cells for its:

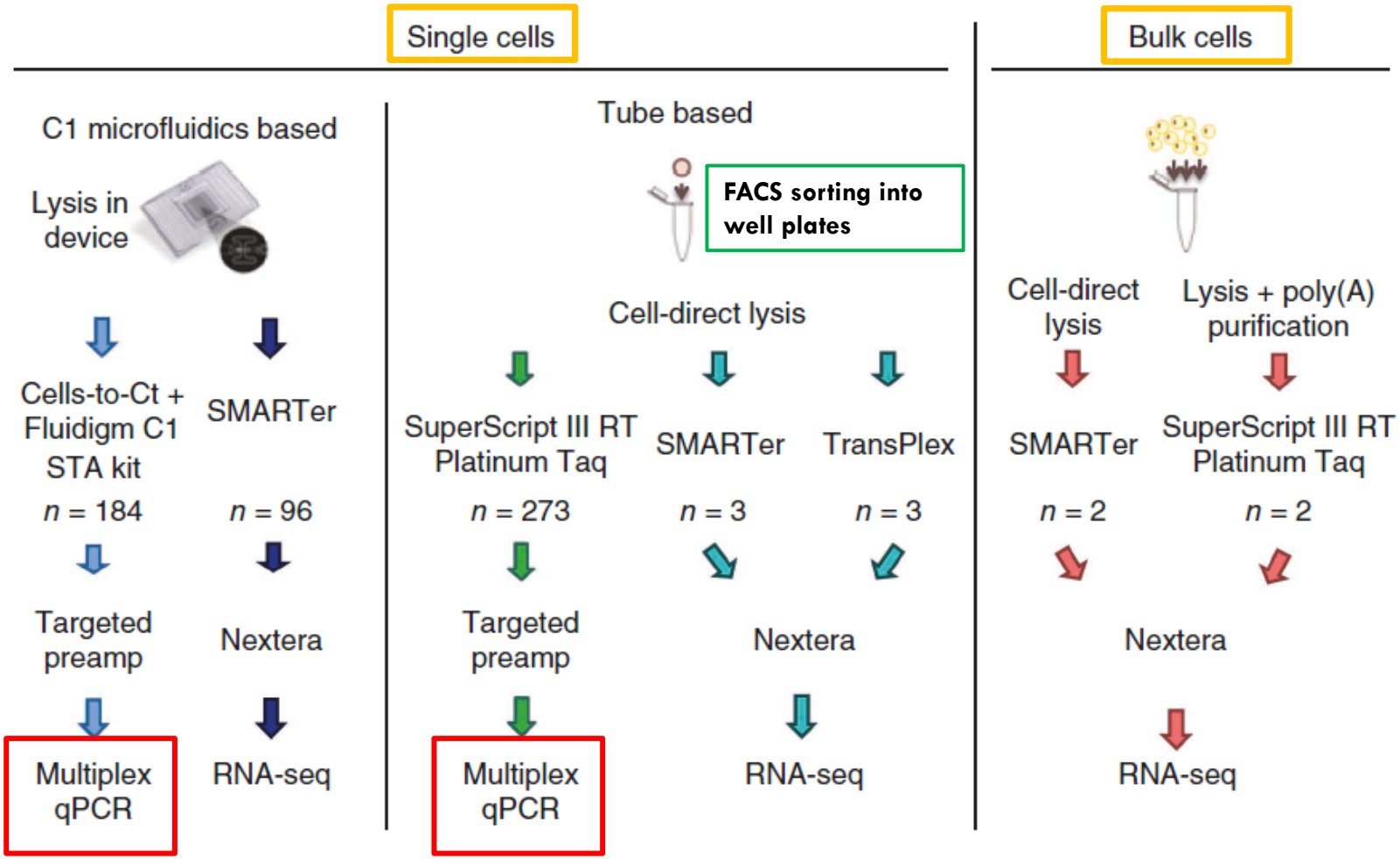
- Throughput
- Quantitative vs. qualitative value

They tried to asses:

- Sensitivity
- Precision

Preparation of samples

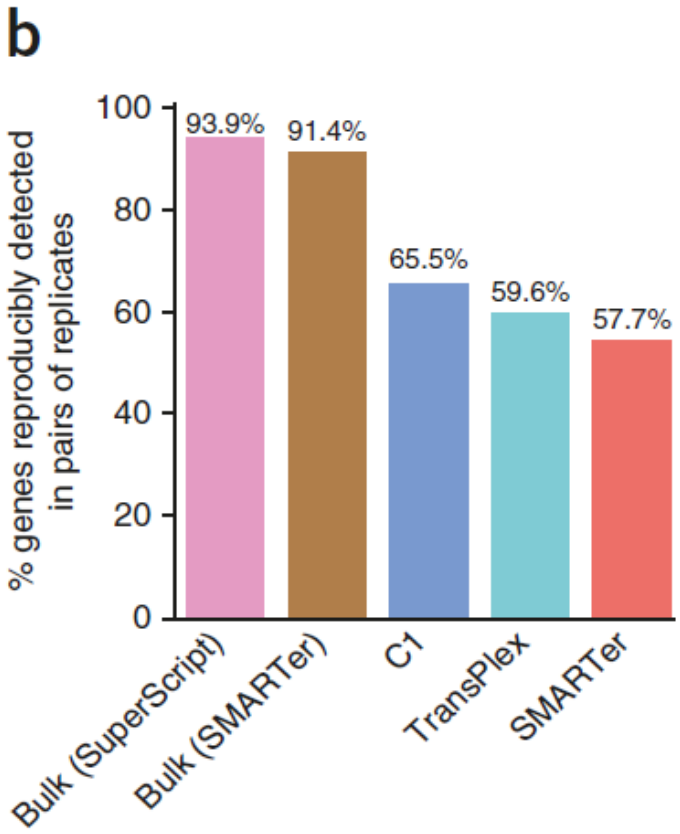
Sample: cultured **HCT116 cells**

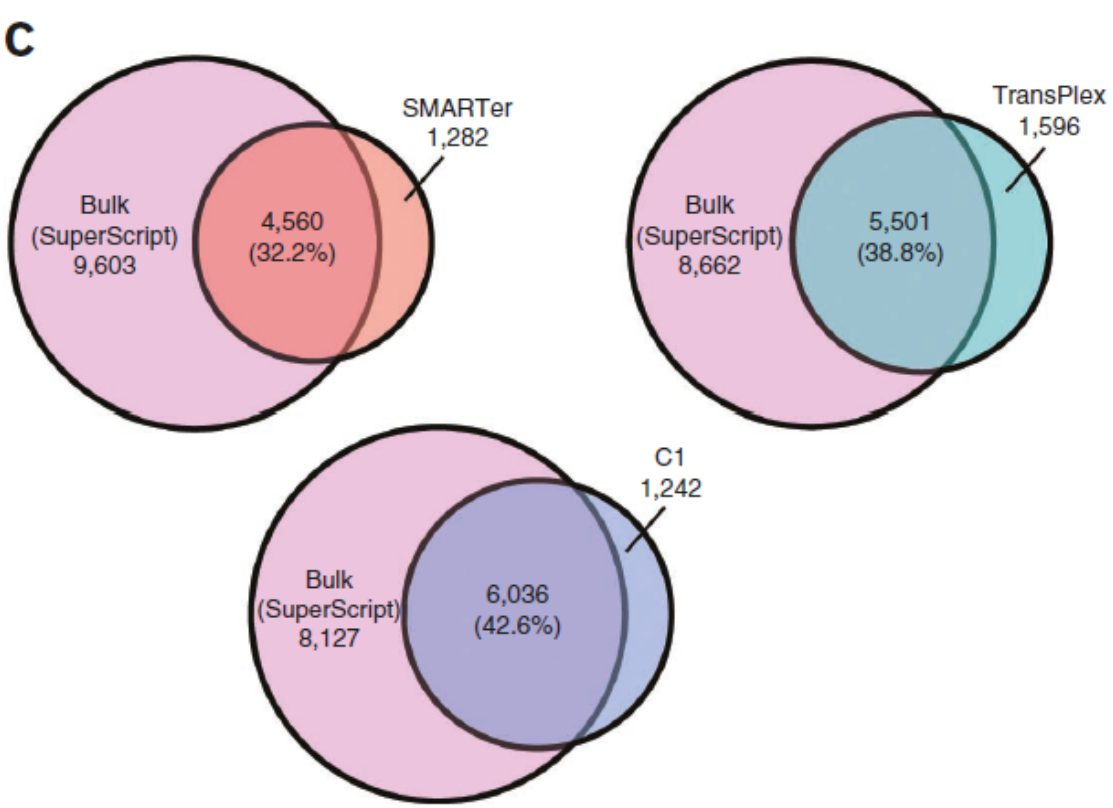


Kits used

Method	cDNA synthesis	Library construction	# Samples
Bulk RNA	1) Magnetic bead-based oligo dT priming extraction of mRNA from cell lysates, followed by Superscript II cDNA synthesis 2) SMARTer Ultra Low RNA kit – oligo dT priming	Nextera – tagmentation using transposase enzymes	1) $n = 2$ 2) $n = 2$
Clontech SMARTer	SMARTer Ultra Low RNA Kit – oligo dT priming	Nextera	$n = 3$
Sigma TransPlex	Sigma-Aldrich TransPlex WTA kit – random priming for both first and second strand synthesis, with a universal 5' priming sequence for subsequent PCR amplification.	Nextera	$n = 3$

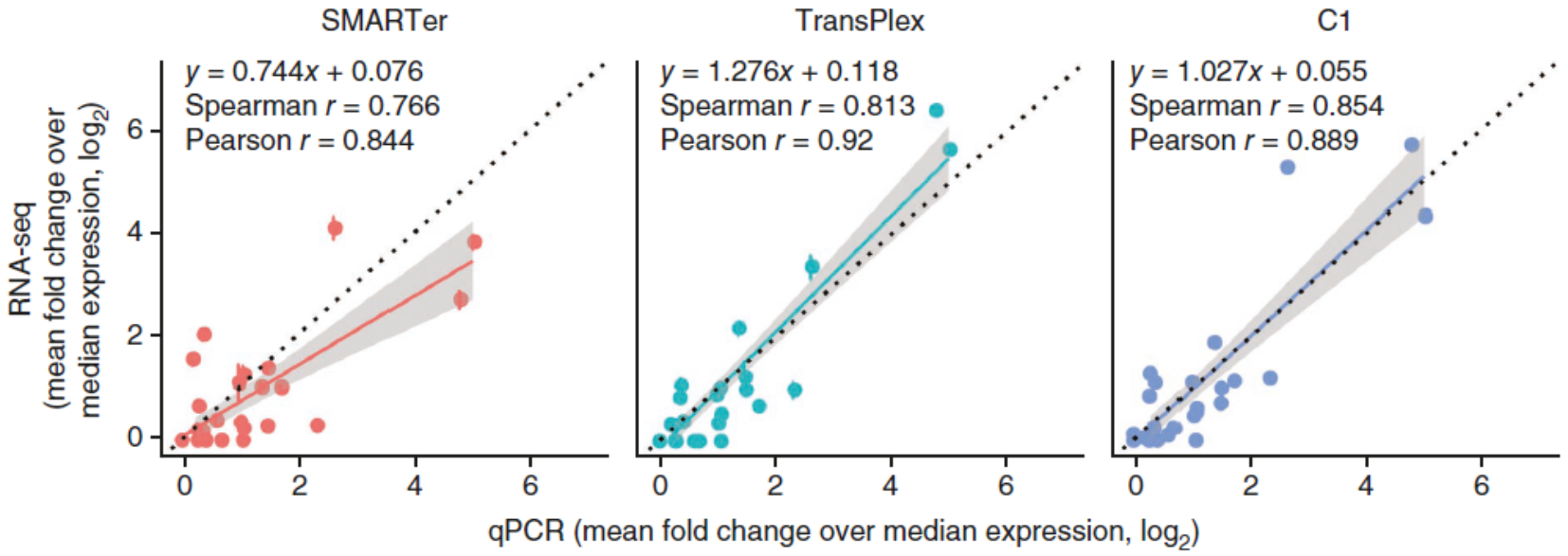
Results – Reproducibility/Sensitivity





- Reproducibility, as evaluated by the percentage of genes detected in pairs of replicate samples out of the mean total number of genes detected in this pair of samples.
- Sensitivity, as evaluated by overlap between genes detected by single-cell and bulk RNA-seq measurement.

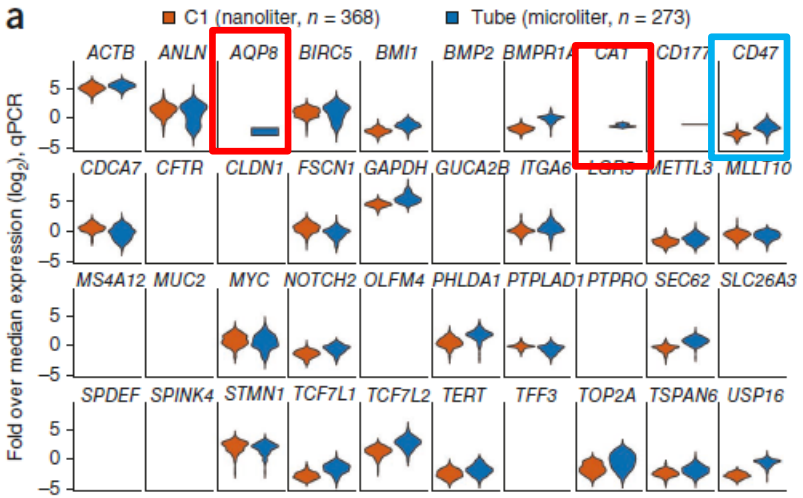
Results – Correlation



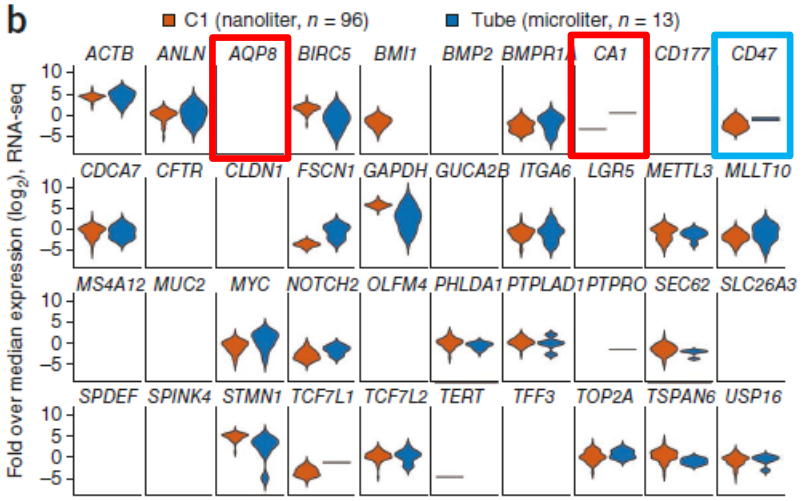
- qPCR - GoldStandard
- Good correlation between RNA-seq and qPCR for all single-cell preparations

Results – Comparison of gene expression distribution

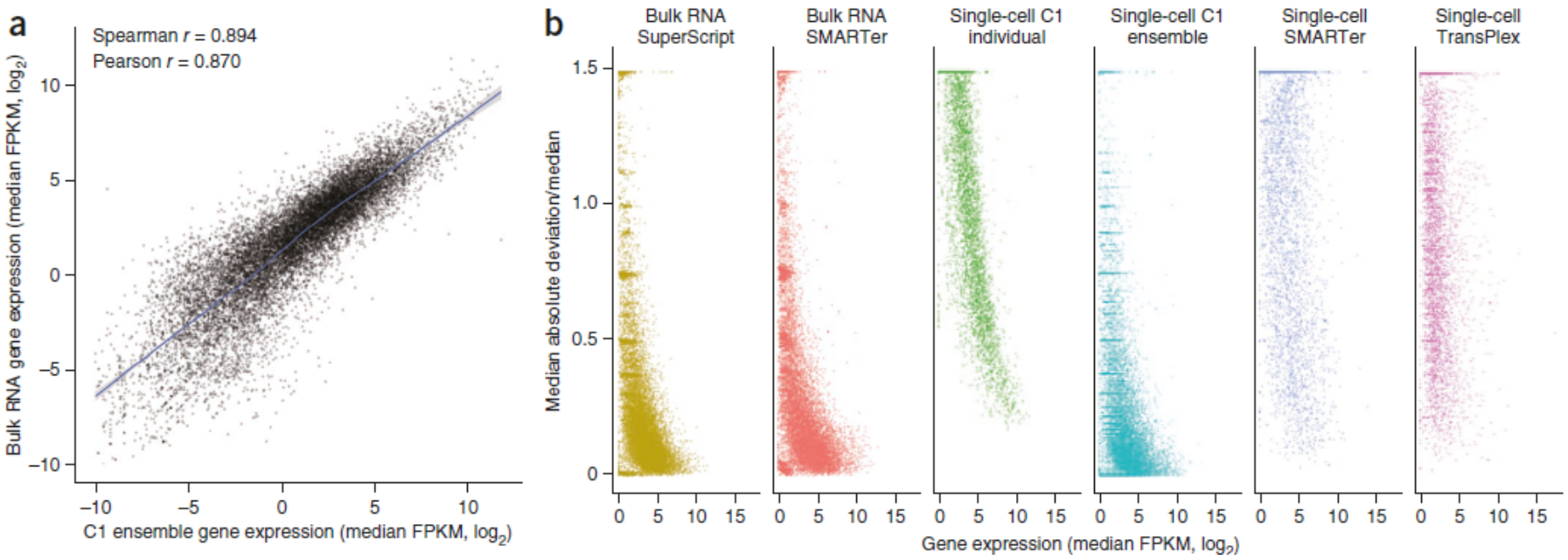
Single-cell qPCR



Single-cell RNA-seq



Results



In general, the microfluidic single-cell data had a more well-defined relationship, with less scatter, between expression level and variation than the single cells measured in tubes.

Summary

- RNA-seq results quantitatively comparable to qPCR
  - Especially when prepared in a nanoliter scale
  - reduced bias and improved correlation

2nd article

RESEARCH ARTICLE

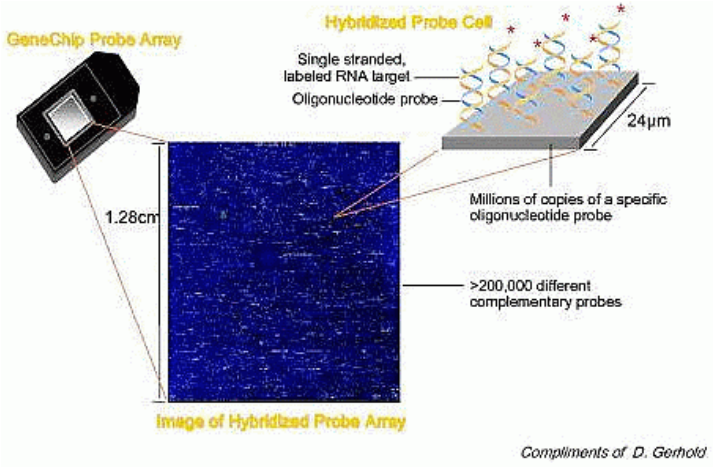
EXPRESSION PROFILING

Combinatorial labeling of single cells for gene expression cytometry

H. Christina Fan, Glenn K. Fu, Stephen P. A. Fodor*

SCIENCE sciencemag.org 6 FEBRUARY 2015 • VOL 347 ISSUE 6222

Just a few words about the last author…



Stephen P. A. Fodor:

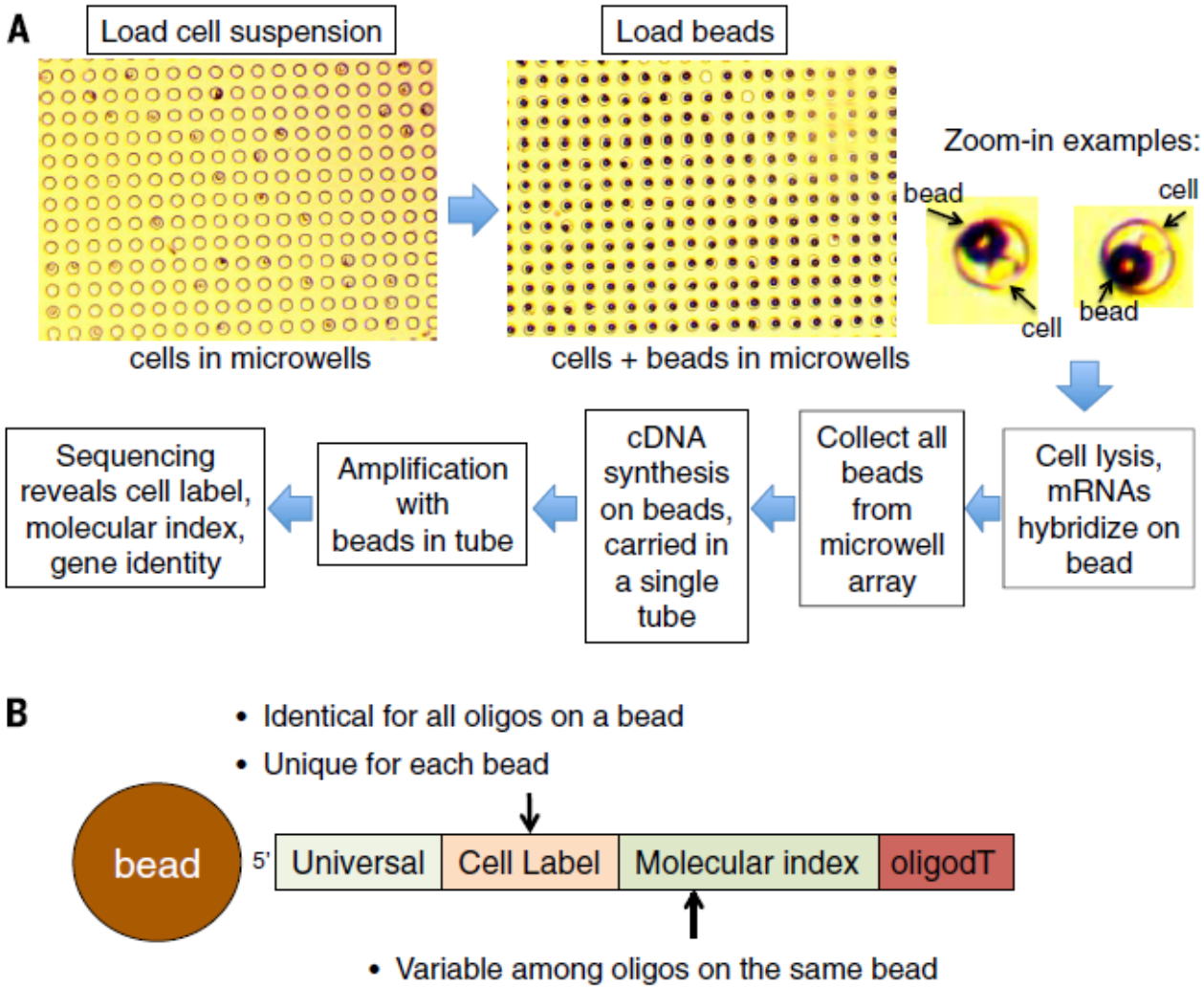
- Co-founder of Affymetrix
- Developed microarray technology
- Founder & CEO of Cellular Research Inc. (since 2011)

Introduction - Aim

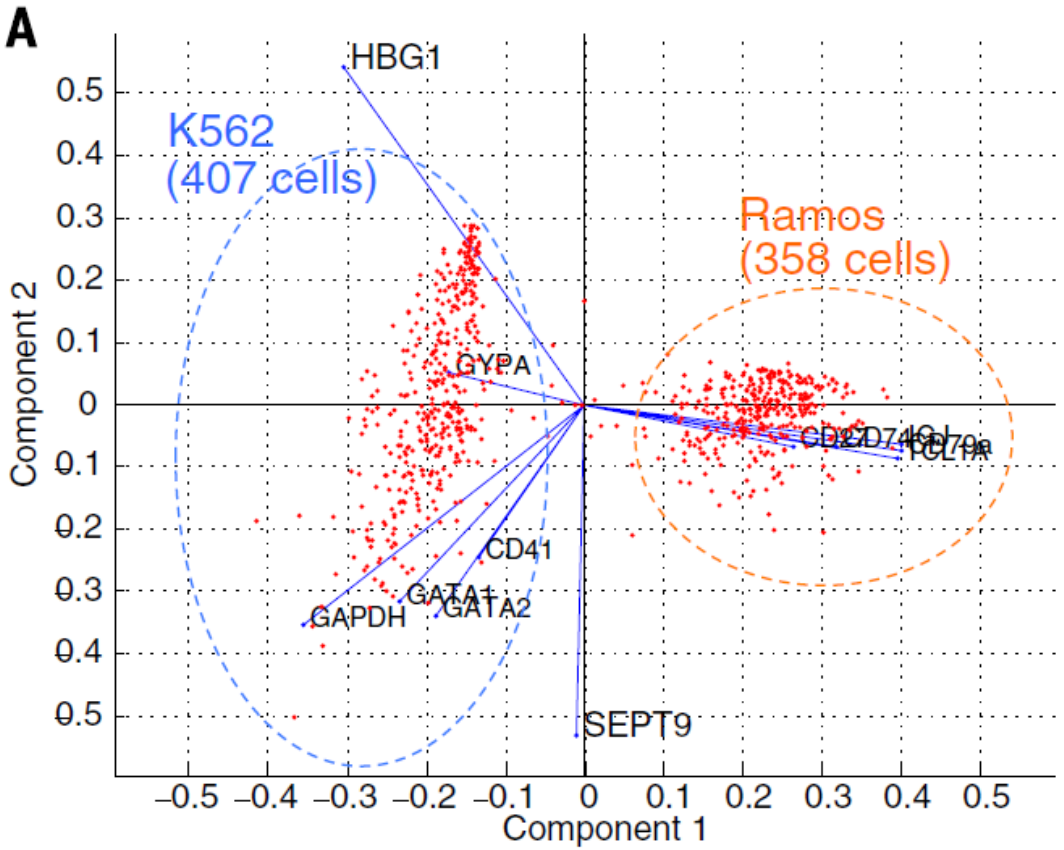
- To have a inexpensive system with no need for highly elaborated instrumentation
- Scalable approach
- Profiling of thousands of single cells across an arbitrary number of genes

"This technology, which we term **CytoSeq**, enables the equivalent of **protein flow cytometry for gene expression.**"

Introduction - Principle

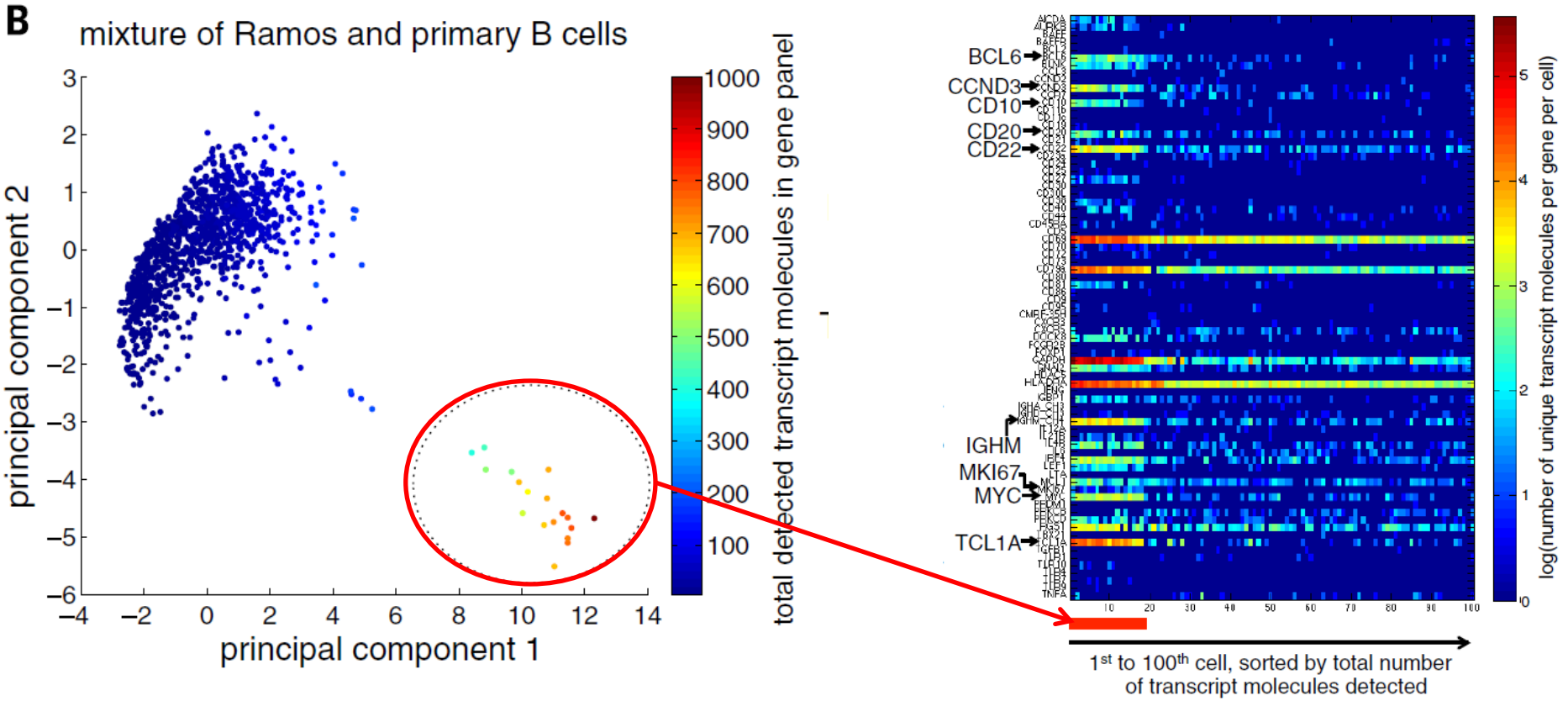


Results – identifying cell types in cell mixtures



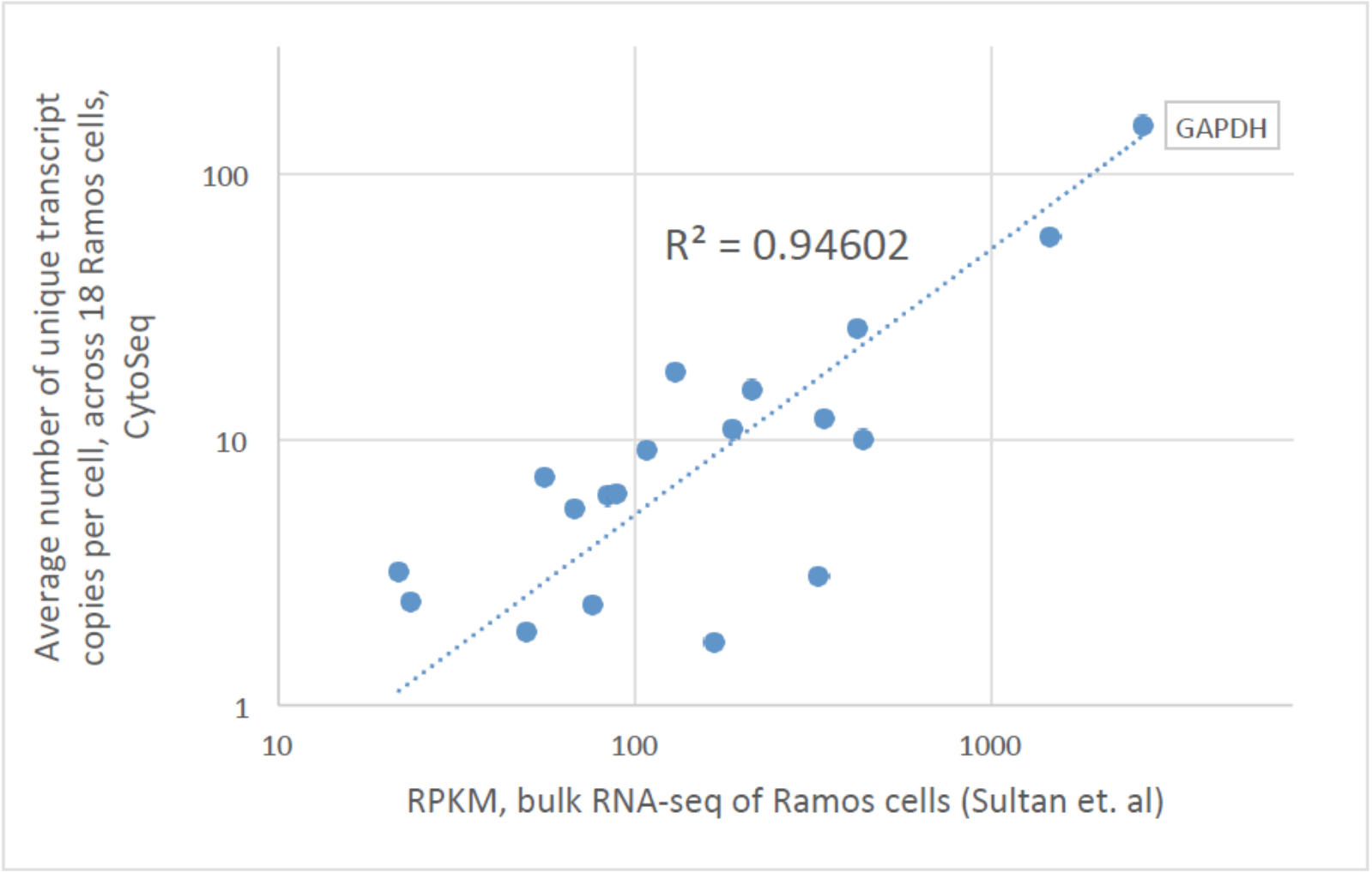
- Principal component analysis
- First component puts genes into two clusters, corresponding either to Ramos or K562 cells
- 2nd component → HBG1 variability

Results – B cells / Ramos cells

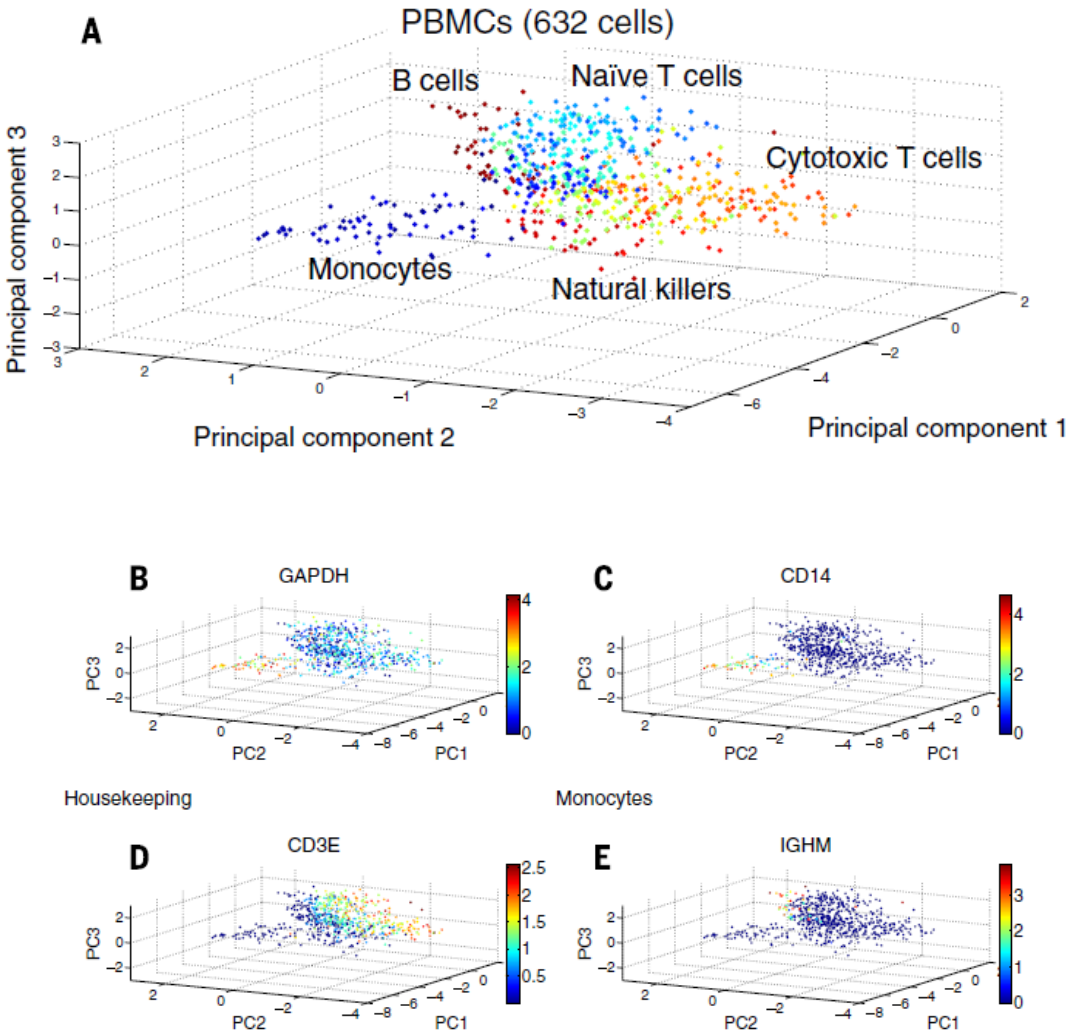


- A panel of 111 genes known for B cell function was analyzed
- Small number of Ramos spiked into B cells
- 18 cells transcriptionally more active

Results – Correlation with a bulk sample



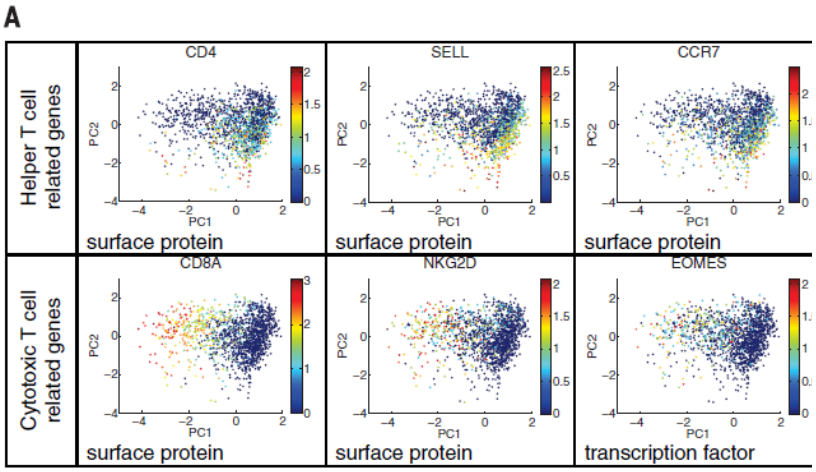
Results – Major cell type analysis via CytoSeq

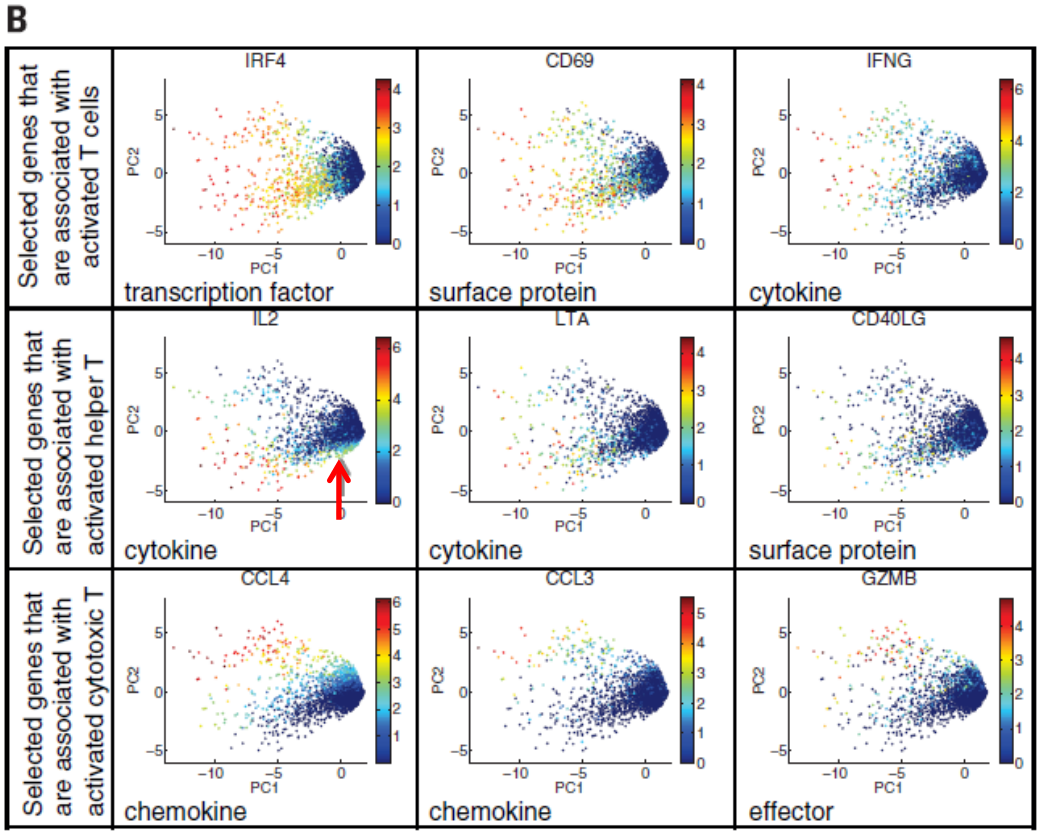


Results – T cell subsets after stimulation

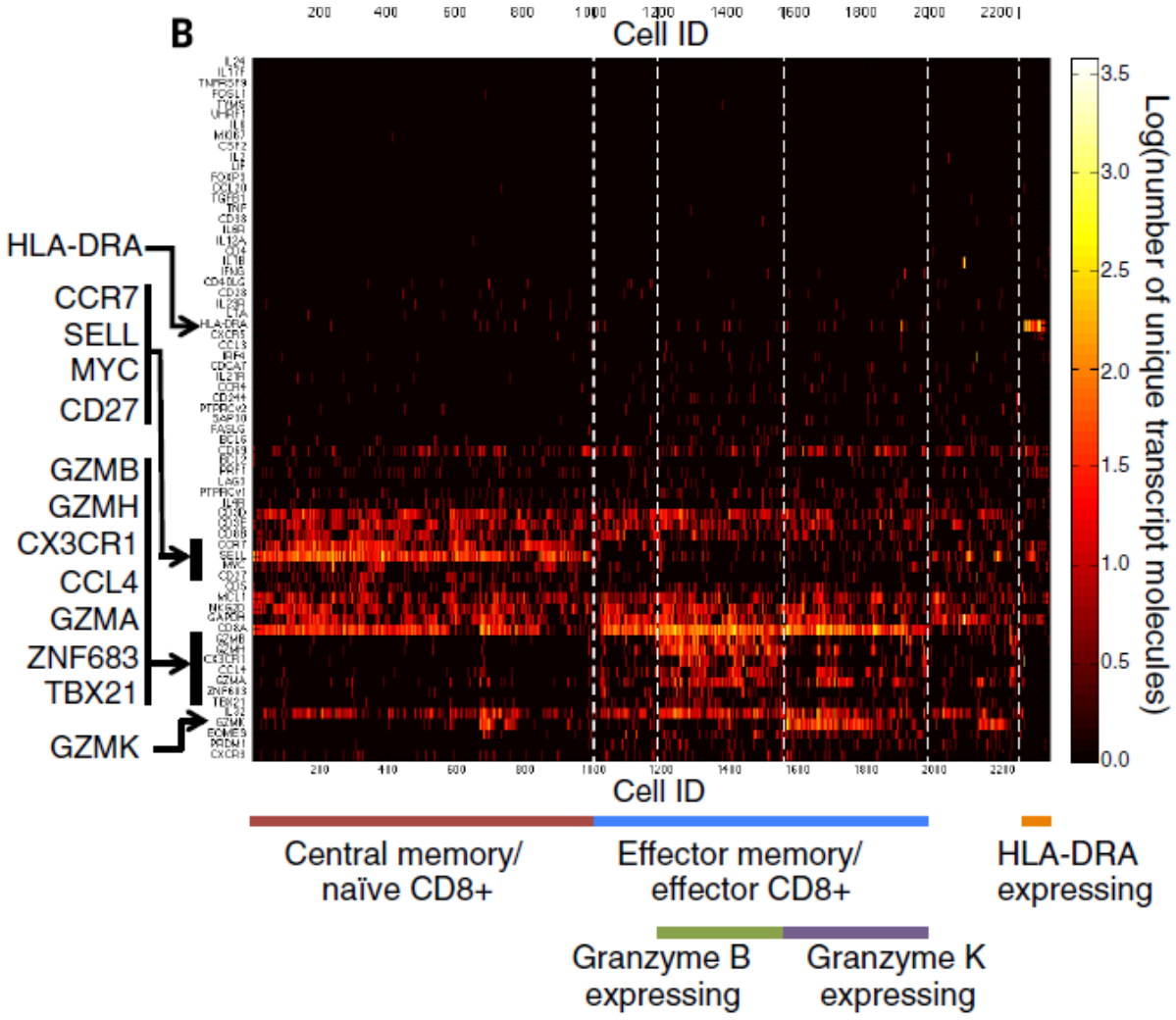
unstimulated

Stimulated with CD3/CD28 – Abs for 6 hours



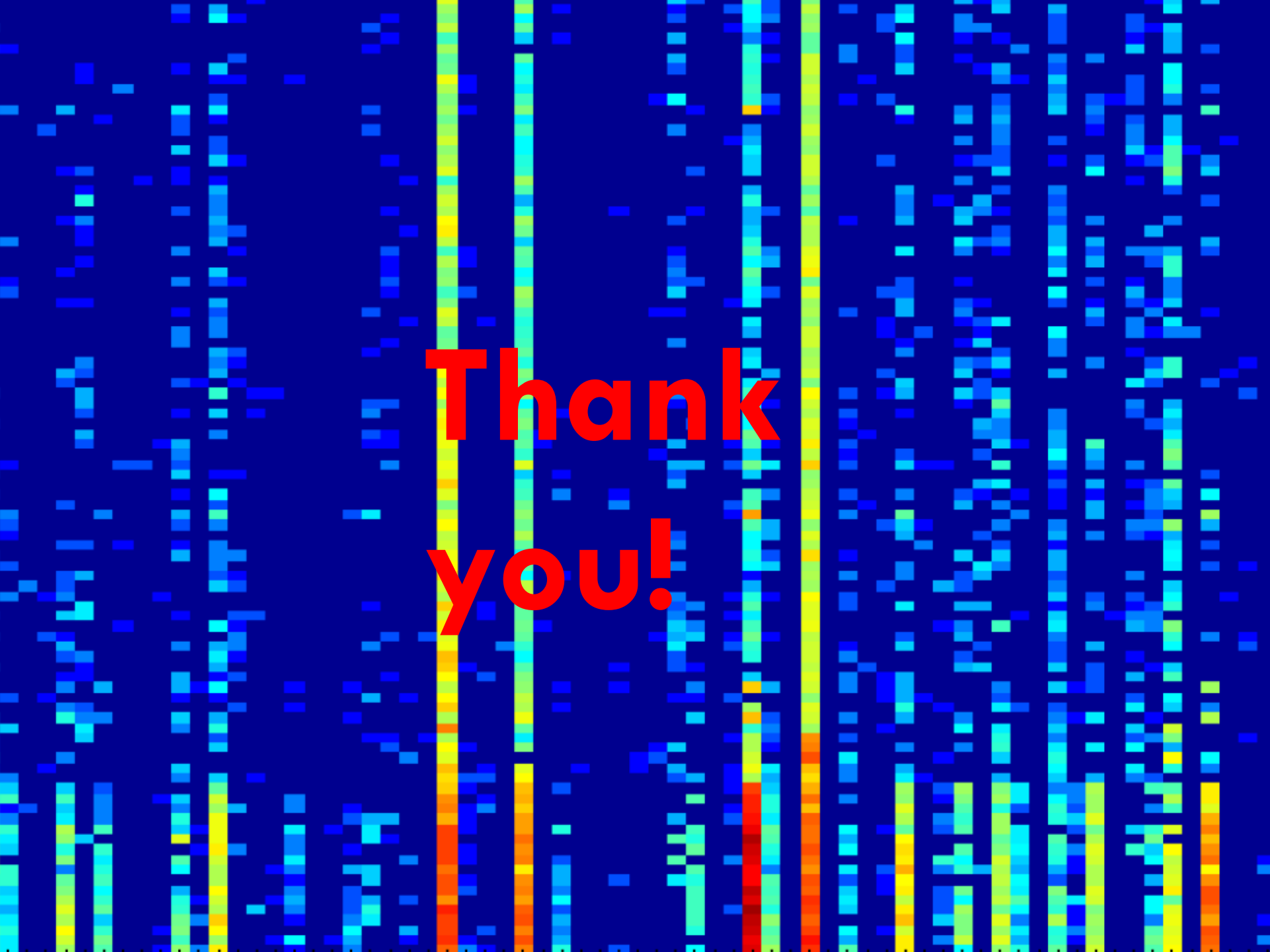


Results – Identification of rare antigen specific T cells



Summary

- Identification and counting of transcript molecules in a sample from a single cell in thousands
- Identification of expression profiles of single cells in a heterogeneous population
- Detection of rare cells in large background
- No expensive instrumentation needed, scalable
- Extension of Mass-Spec and FACS
- Could be used for circulating tumor analysis, immune disorder, infectious diseases, etc…


Thank
you!