# Empowering Genetic Discovery through the Illumina NextSeq System

**Brian J. Henson, PhD**
***SR. Sequencing Specialist***



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# Agenda Focus on NextSeq Applications

- **Our Background and Mission**
- **Instrumentation**
- **WGS-large genomes, small genomes, metagenomics**
- **WES**
- **RNA-Seq**
- **Single-Cell**
- **Data Storage and Analysis**
  - BaseSpace Sequence Hub

# Who We Serve

*Innovation drives expanding market opportunities*

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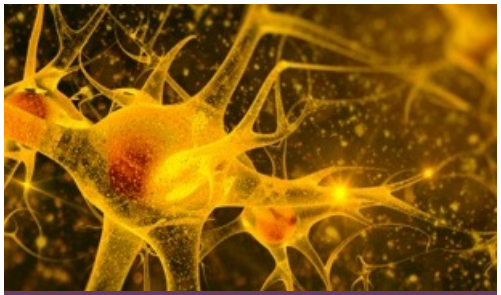
Reproductive Health

Oncology

Population Sequencing

Research



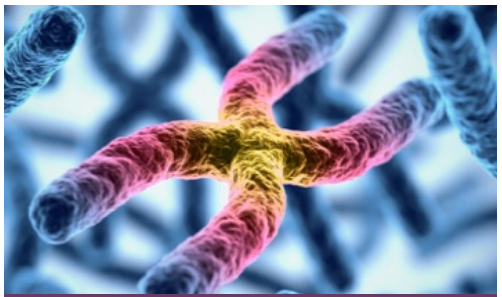
Complex Disease

Consumer

Infectious Disease

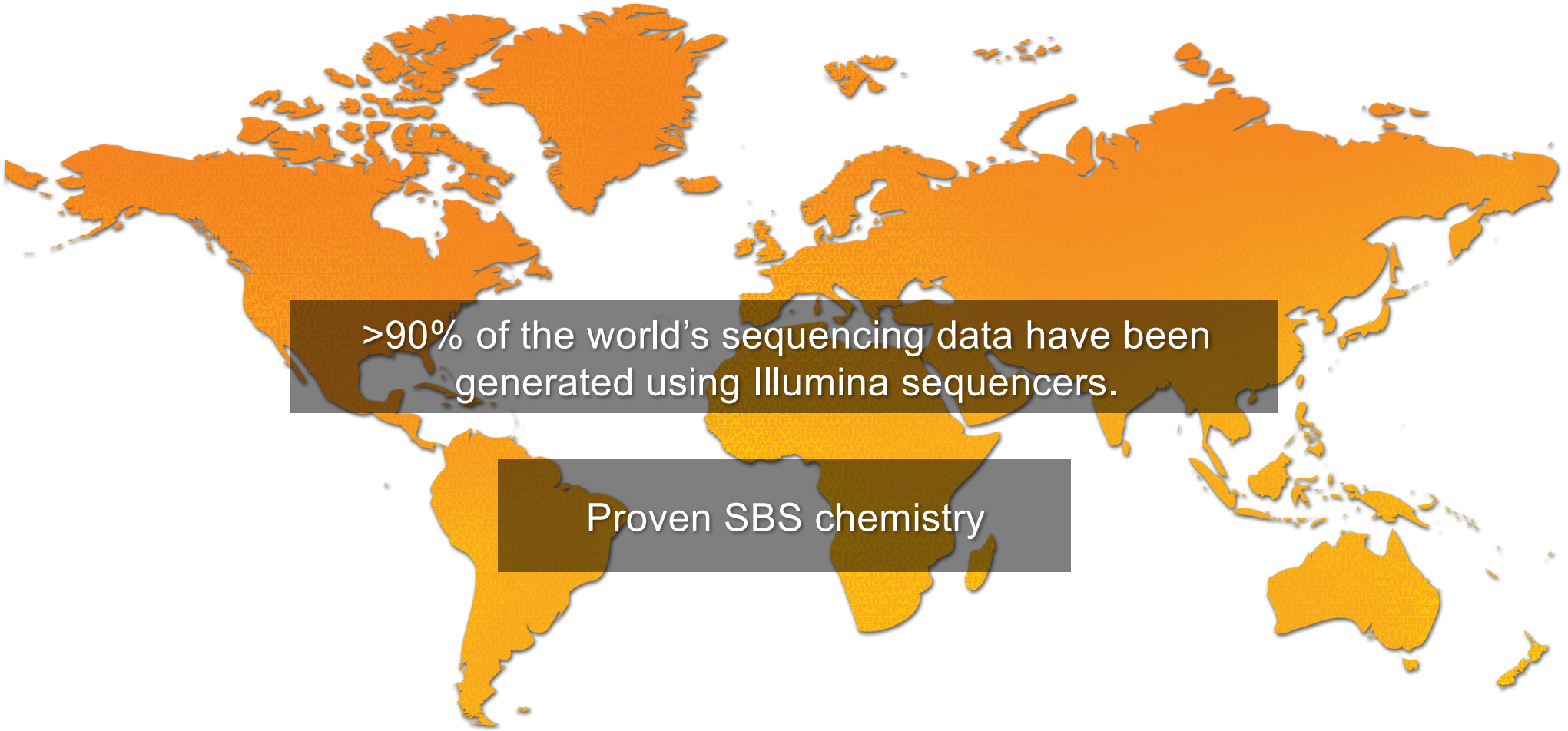
Forensics

Agriculture



Genetic Health

BioPharm


>90% of the world's sequencing data have been generated using Illumina sequencers.
Proven SBS chemistry



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# Sample to Answer Integration

*From library prep to downstream informatics & knowledge generation*


**Library Prep**

**Sequence**

**Answer**



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# NextSeq 500-550

20–120 | Gigabases

130–400 million | reads

$1040–4260 | run*

Up to 300 cycles
(2 x 150 bp) 29hr run

**Genomes, Exomes, Transcriptomes**


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# Whole Genome




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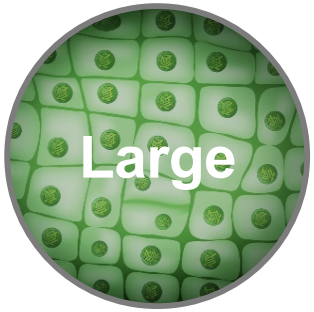



| | Human WGS | Complex Genomes | Small Genomes |
|---|---|---|---|
| **Applications** | • Cancer Genomics<br>• Variant Detection<br>• Genetic Risk Studies<br>• Population Genetics | • Agrigenomics (maize, wheat, bovine, etc.)<br>• Model Organisms (fruit fly, mouse, zebrafish, etc.)<br>• Plant/Animal Research | • Human Microbiome<br>• Microbiology<br>• Public Health Research<br>• Amplicon Sequencing<br>• Metagenomics |
| **Sequencing Examples** | • Human Genome (3.2 Gb), NovaSeq™ System, S2 Kit, 30x, 8 samples/flow cell<br>• Human Genome (3.2 Gb), HiSeq™ X System, v2.5, 30x, 8 samples/flow cell | • Fruit Fly Genome (175 MB), NextSeq™ 550 System, v2 Kit, 30x, 22 samples/flow cell<br>• Mouse Genome (2.7 Gb), HiSeq™ 4000 System, v1 Kit, 30x, 8 samples/flow cell | • *E.Coli* (4.6 Mb), MiniSeq™ System, 30x, 50 samples/flow cell<br>• Plasmids/Amplicons (650 kb), MiSeq™ System, 1000x, 11 samples/flow cell |





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# Introducing Nextera™ DNA Flex Library Prep

*gDNA, Blood, Saliva, Microbes*

**gDNA**

User guide & kit supported process

**Blood**

User guide & kit supported process

Blood input requires Illumina Flex lysis reagent

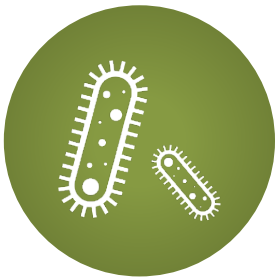
**Blood punch card**

Demonstrated protocols available at Illumina.com

**Saliva**

User guide & kit supported process

Saliva input requires Oragene saliva collection kit



**Microbial colony**

Demonstrated protocols available at Illumina.com

**A** **Isolate and purify DNA**

gDNA, blood, saliva, or microbe

**B** **Add DNA to bead-linked transposomes (BLT)**

Transposome attached to magnetic beads

**C** **DNA is tagmented and remains bound to the bead**

**No additional tagmentation can occur after bead saturation**

Allowing a large DNA input range (1–500ng)

Resulting in consistent insert size and normalized libraries





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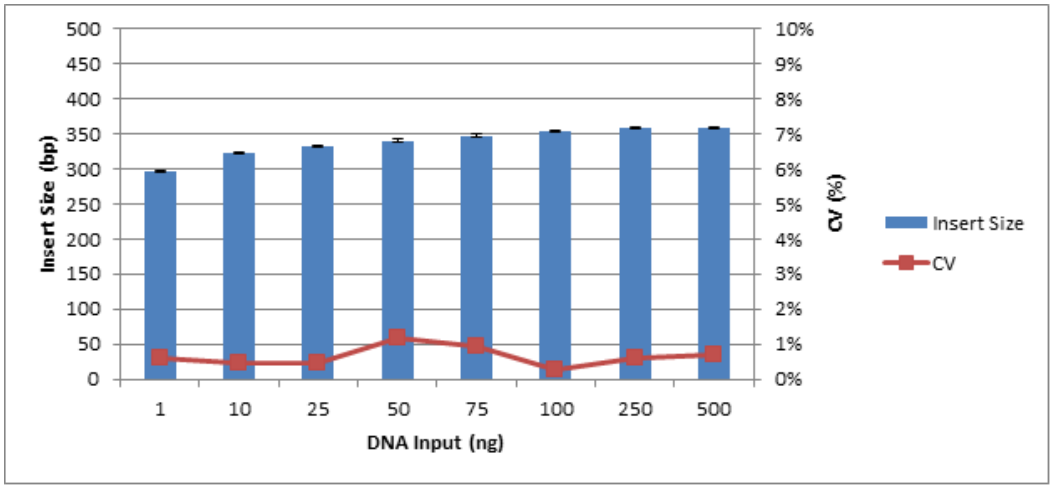
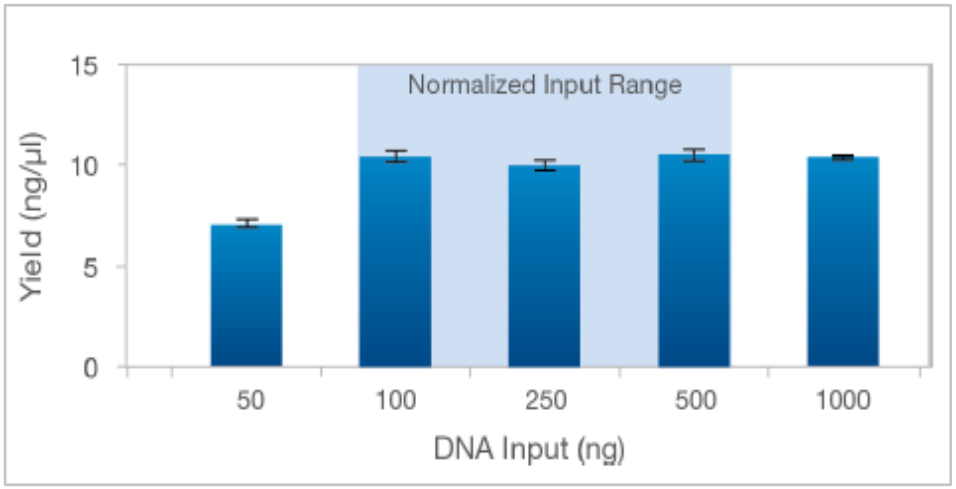
# Wide DNA Input and Normalized Libraries

## Consistent insert sizes and Normalization




- **Consistent insert size obtained with the use of a wide DNA input range**

- **Normalized libraries are be obtained with:**
  - 100ng-500ng gDNA input
  - Use of the liquid blood, saliva, dried blood, or bacterial colony protocol

With Nextera DNA Flex, precise input quantification is not required to yield consistent DNA insert sizes and normalized libraries

*Data maintained in Illumina internal files 2017



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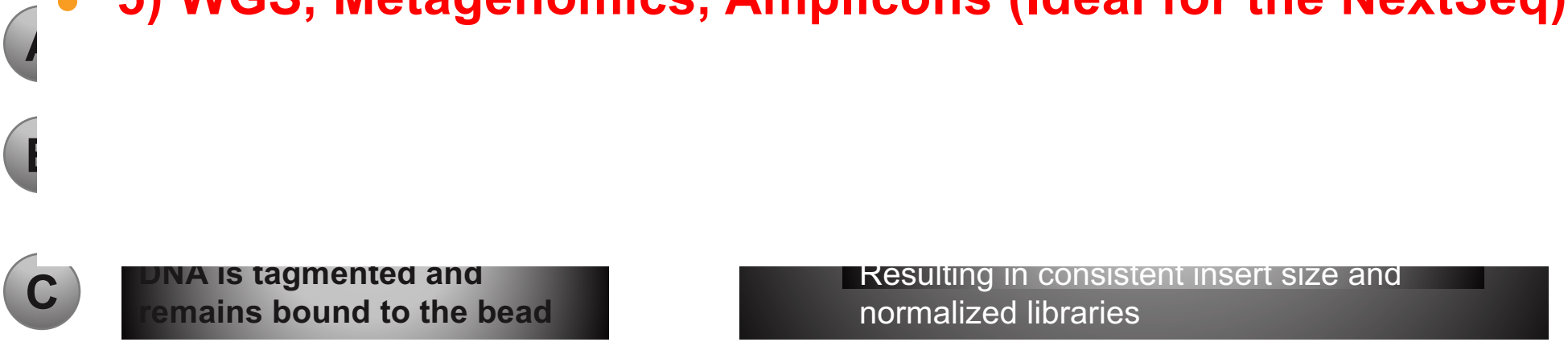
# Nextera™ DNA Flex Library Prep Kit

*gDNA, Blood, Saliva, Microbes*

- **Three Key Points**
- **1) Wide input range with consistent library sizes**
- **2) Normalized ready to sequence libraries**
- **3) 3.5 to 4 hours from sample to sequence ready libraries**
- **4) Biological samples**
- **5) WGS, Metagenomics, Amplicons (Ideal for the NextSeq)**





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# Whole Exome




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# Enabling High Performance Workflows for Exome Enrichment

## Current

Order 1
Catalog Number

Illumina components

## New

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Decoupled Kit

**Library Prep**

**Indexed Adapters**

*Purchase library prep and indexed adapters separately from Illumina*

IDT® INTEGRATED DNA TECHNOLOGIES

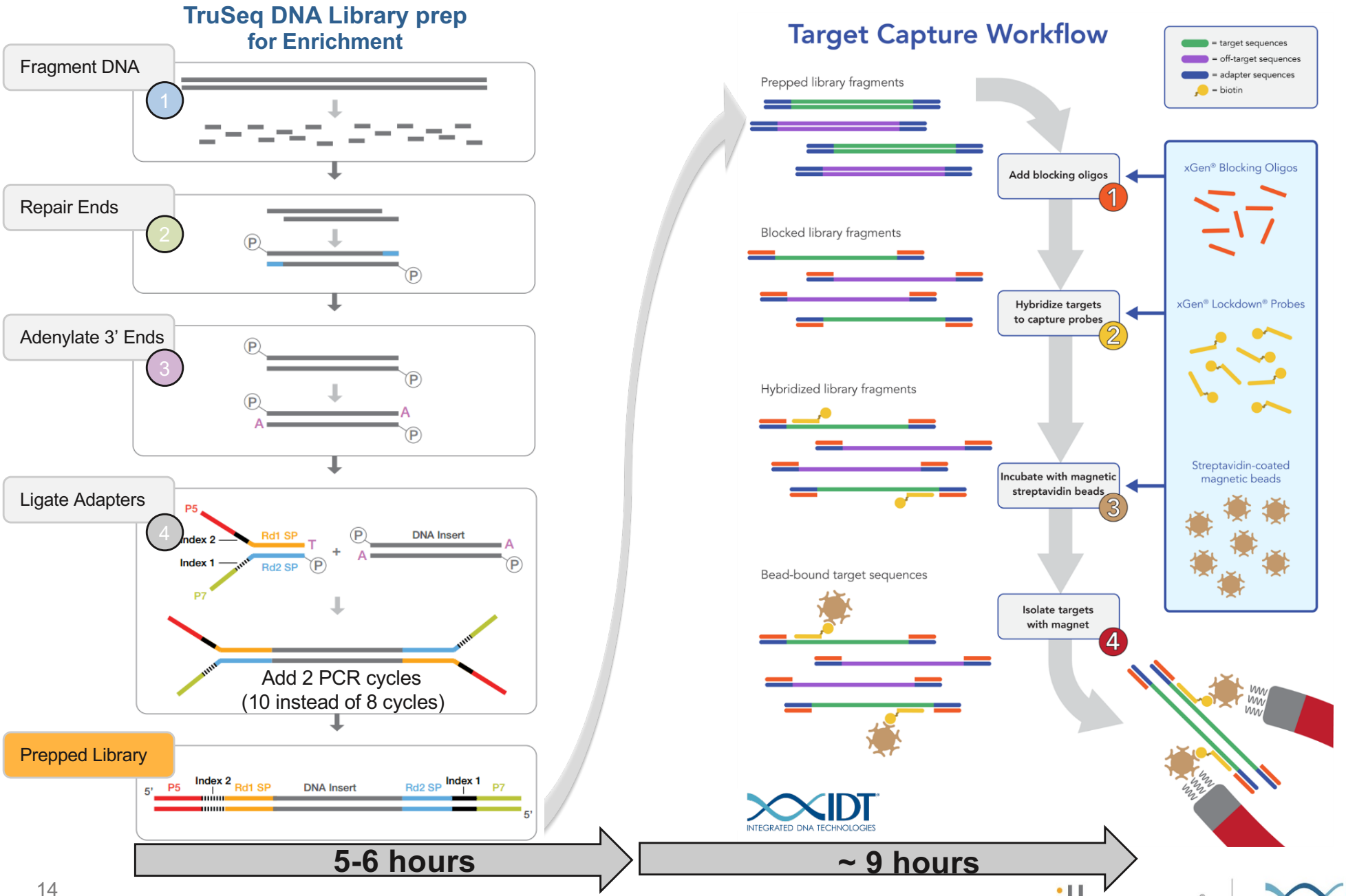

**xGen® Lockdown**

**xGen® Blocking Oligos**

*Purchase enrichment and panels from partners*

Inclusive configurations | Modular configurations

# TruSeq®-xGen® Exome Workflow



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# Efficiency Matters

*On-target efficiency reduces sequencing required*


| | TruSeq Exome (Illumina) | TruSeq - xGen Exome (Illumina-IDT) |
|---|---|---|
| % On-Target Reads | >80% | **>90%** |
| Target coverage at 20X | >90% | **>96%** |
| Mean Coverage Required | 100x | **50x** |
| Sequencing | 8 Gb | 3.5 Gb |

**Pool multiple pre-enrichment libraries to maximize throughput**
**55% fewer Gb of sequencing required***

Data generated from 23M reads (3.5Gb) on a HiSeq 2500 in Rapid Run Mode (TruSeq) or NextSeq High Output Mode (Nextera) using Enrichment 3.0 App on BaseSpace Sequence Hub. Data on file at Illumina 2017. Dataset available on BaseSpace.

*Compares requirement of 8Gb of sequencing for TruSeq Exome enrichment workflow to 3.5Gb for xGen Exome Enrichment workflow to achieve over >90% of target coverage at 20x





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# Sequence More for Less

*Load more samples per run*

NovaSeq™ S2

HiSeq® 4000

HiSeq® 2500

NextSeq®

## Exomes / Flowcell[&]

| | 8Gb (100x mean coverage) | | 4Gb (50x mean coverage) |
|---|---|---|---|
| NovaSeq™ S2 | 66* | → | 132* |
| HiSeq® 4000 | 48 | → | 96 |
| HiSeq® 2500 | 39 | → | 78 |
| NextSeq® | **6** | → | **12** |

## Price / Sample[#]

| | 8Gb (100x mean coverage) | | 4Gb (50x mean coverage) |
|---|---|---|---|
| NovaSeq™ S2 | $284 | → | $142 |
| HiSeq® 4000 | $236 | → | $118 |
| HiSeq® 2500 | $509 | → | $277 |
| NextSeq® | **$464** | → | **$232** |

& Human 48Mb exome at 100X mean coverage
# Sequencing cost only; library prep not included. Sequencing reagent cost in USD divided by number of samples.
* Pending release of individual addressable lanes

# RNA-Seq




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# Illumina's Suite of RNA Library Prep Solutions

| Total RNA-Seq | mRNA-Seq/ GEx Profiling | | Targeted Profiling | miRNA Analysis |
|---|---|---|---|---|
| **TruSeq Stranded Total RNA** | **TruSeq Stranded mRNA** | **TruSeq Stranded RNA Access** | **TruSeq Targeted RNA Expression** | **TruSeq small RNA** |
| • Coding + ncRNA<br>• Transcript-level abundance<br>• Splicing Analysis<br>• Fusion Discovery<br>• FFPE compatible<br>• Any species | • Coding RNA<br>• Transcript-level abundance<br>• Splicing Analysis<br>• Fusion Discovery | • Human Exonic RNA<br>• Transcript-level abundance<br>• Splicing Analysis<br>• Fusion Discovery<br>• FFPE Compatible | • 10s-1,000s of targets<br>• Max 384 samples<br>• Coding + ncRNA<br>• Transcript-level abundance<br>• Fusion Validation<br>• FFPE Compatible | • miRNA abundance<br>• miRNA discovery |






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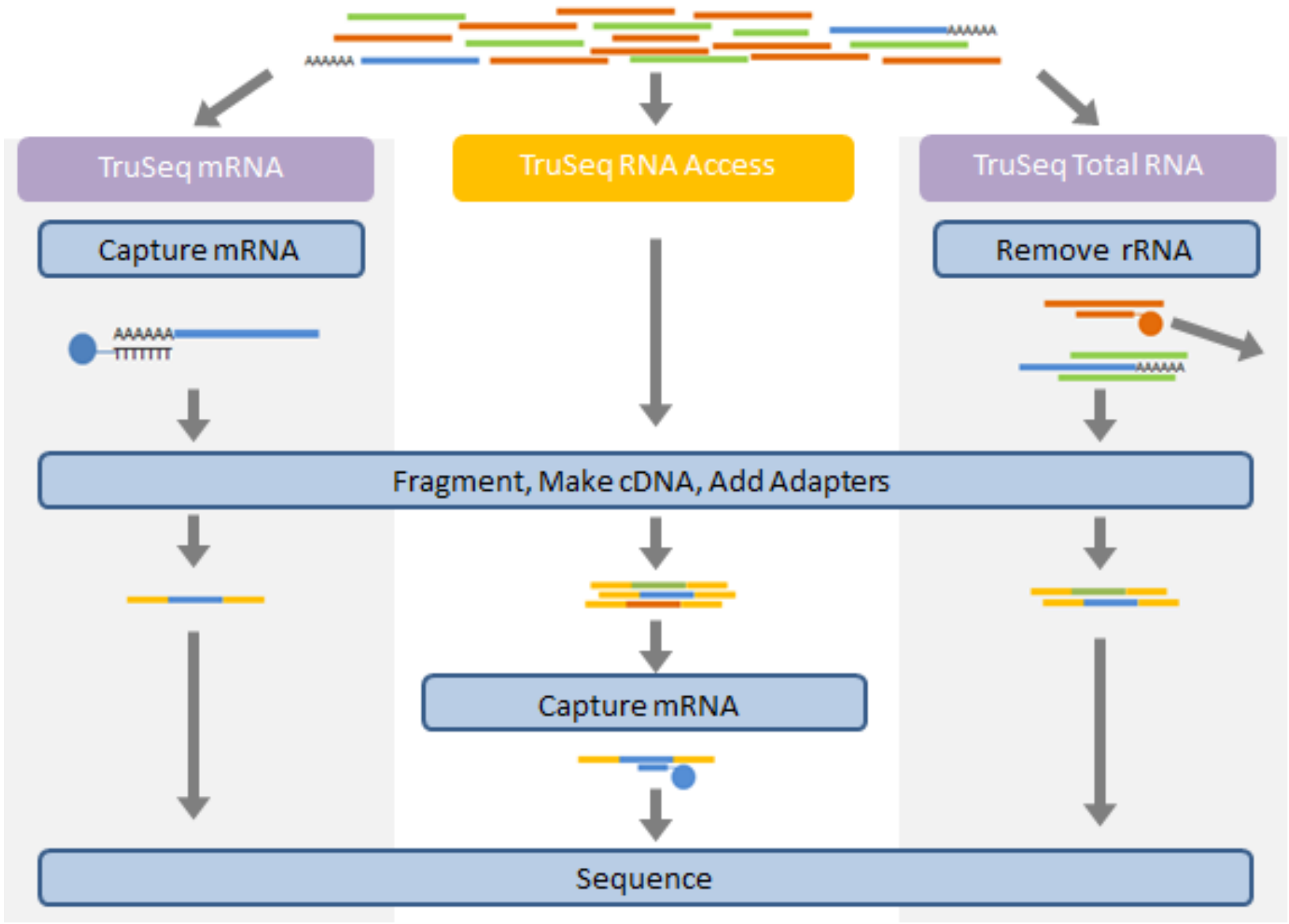
# RNA Library Prep Chemistries



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# TruSeq Stranded mRNA

## *PolyA RNA-Seq for Cost Effective Transcriptomes*

- **PolyA** pull down for quantification of mRNAs in eukaryotic samples
- Quick and **cost effective** library prep
- Maintains strand information for delineation of sense/antisense transcription
- Requires **~25M reads** for average mammalian studies


RNA Isolation | **Library Prep** | Sequencing | Data Analysis



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# TruSeq Total RNA

## *Versatile solution for discovery*

- **Ribosomal depletion allows use across many organisms** and cell types
- Provides high quality results, from low quality samples, including FFPE
- Requires **~50M reads** for average mammalian studies

Ribo-Zero depletion can target human, mouse, rat, plant, globin, gram negative and gram positive bacteria, yeast, drosophila, cow, dog, epidemiological mixes, and more!

RNA Isolation | Library Prep | Sequencing | Data Analysis



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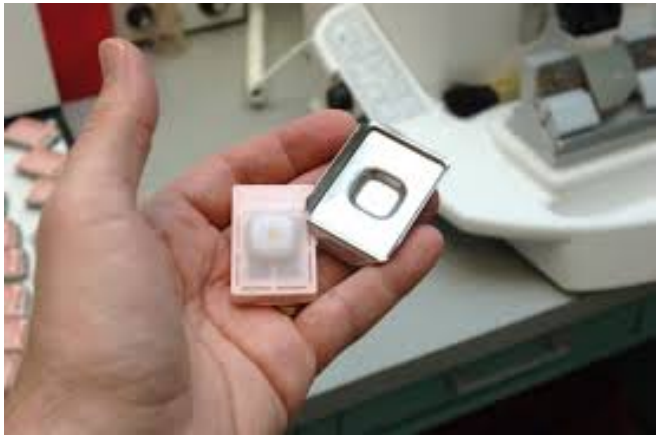
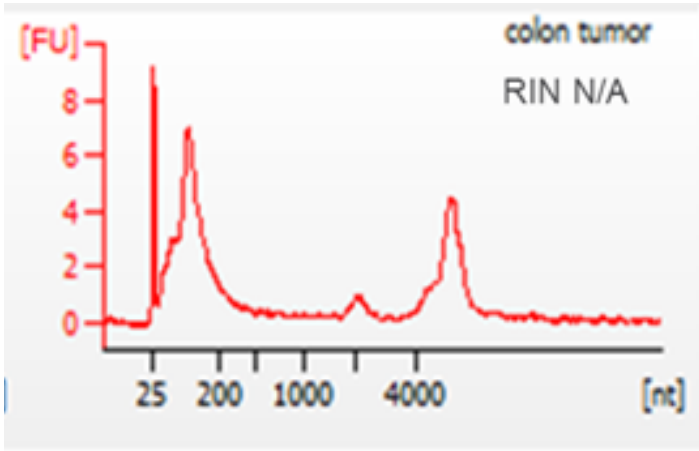
# TruSeq RNA Exome

## *Quality data from degraded and low input **human** RNA*

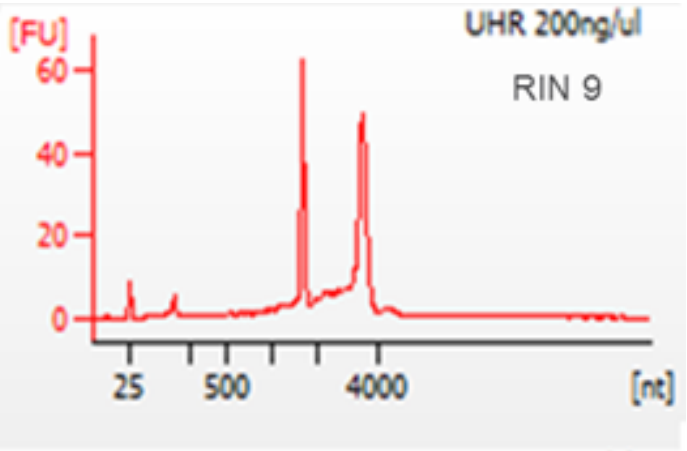
- TruSeq RNA Access enriches for the human exonic RNA from degraded/lot input samples
- Requires 10ng fresh RNA, as low as 20-100ng FFPE RNA
- Correlates with mRNA and Total RNA data



FFPE




Fresh/Frozen




RNA Isolation | **Library Prep** | Sequencing | Data Analysis



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# Hot Topic: Single Cell RNA-Seq



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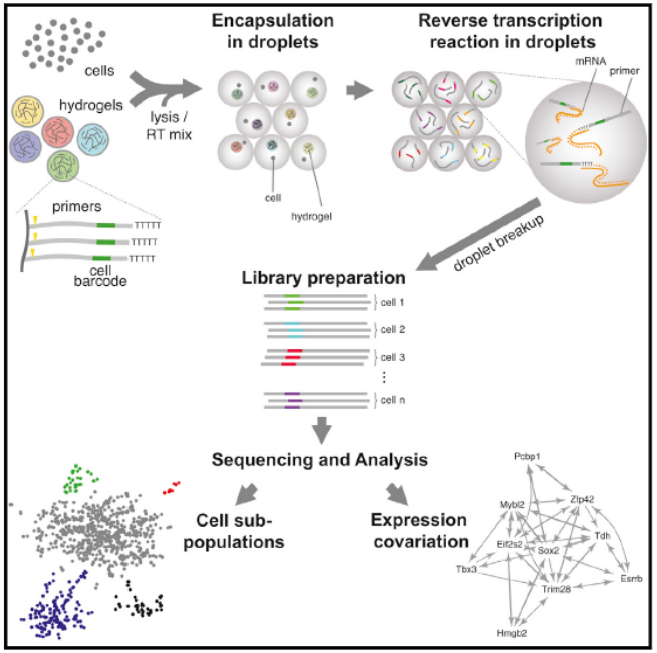
# Single Cell Analysis

## Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells

Allon M. Klein,[1,6] Linas Mazutis,[2,3,6] Ilke Akartuna,[2,6] Naren Tallapragada,[1] A
David A. Weitz,[2,*] and Marc W. Kirschner[1,*]




Klein *et. al.*, Cell, 2015

## Single Cell RNA-Sequencing of Pluripotent States Unlocks Modular Transcriptional Variation

Aleksandra A. Kolodziejczyk,[1,2,5] Jong Kyoung Kim,[1,5] Jason C.H. Tsang,[2] Tomislav Ilicic,[1,2] Johan Henriksson,[1] Kedar N. Natarajan,[1,2] Alex C. Tuck,[1,3] Xuefei Gao,[2] Marc Bühler,[3] Pentao Liu,[2] John C. Marioni,[1,2,4,*] and Sarah A. Teichmann[1,2,*]

Kolodziejczyk et al., 2015, Cell Stem Cell

## Single-cell messenger RNA sequencing reveals rare intestinal cell types

Dominic Grün, Anna Lyubimova, Lennart Kester, Kay Wiebrands, Onur Basak, Nobuo Sasaki, Hans Clevers & Alexander van Oudenaarden

Grun *et. al.*, Nature Letters, 2015

## Deep sequencing reveals cell-type-specific patterns of single-cell transcriptome variation

Hannah Dueck[1], Mugdha Khaladkar[2], Tae Kyung Kim[3,4], Jennifer M. Spaethling[3], Chantal Francis[2], Sangita Suresh[5,6], Stephen A. Fisher[2], Patrick Seale[7], Sheryl G. Beck[8], Tamas Bartfai[11], Bernhard Kuhn[5,6,9,10,12], James Eberwine[3†] and Junhyong Kim[2*†]

Dueck *et. al.*, Genome Biology, 2015

## Transcriptome *in vivo* analysis (TIVA) of spatially defined single cells in live tissue

Ditte Lovatt, Brittani K Ruble, Jaehee Lee, Hannah Dueck, Tae Kyung Kim, Stephen Fisher, Chantal Francis, Jennifer M Spaethling, John A Wolf, M Sean Grady, Alexandra V Ulyanova, Sean B Yeldell, Julianne C Griepenburg, Peter T Buckley, Junhyong Kim, Jai-Yoon Sul, Ivan J Dmochowski & James Eberwine

Lovatt *et. al.*, Nature Methods, 2014



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# Single Cell Analysis

Nat Biotechnol. 2018 Mar 28. doi: 10.1038/nbt.4103. [Epub ahead of print]

**Simultaneous single-cell profiling of lineages and cell types in the vertebrate brain.**

Raj B[1,2], Wagner DE[3], McKenna A[2,4], Pandey S[1], Klein AM[3], Shendure J[2,4,5], Gagnon JA[1,2,6], Schier AF[1,2,7,8,9,10].

Trends Cancer. 2018 Apr;4(4):264-268. doi: 10.1016/j.trecan.2018.02.003. Epub 2018 Mar 9.

**Single-Cell Transcriptomic Analysis of Tumor Heterogeneity.**

Levitin HM[1], Yuan J[1], Sims PA[2].

Elife. 2018 Mar 27;7. pii: e33105. doi: 10.7554/eLife.33105.

**Single-cell RNA-seq reveals hidden transcriptional variation in malaria parasites.**

Reid AJ[#1], Talman AM[#1], Bennett HM[#1], Gomes AR[1], Sanders MJ[1], Illingworth CJR[2], Billker O[1], Berriman M[1], Lawniczak MK[1].

Stem Cell Reports. 2018 Mar 20. pii: S2213-6711(18)30107-3. doi: 10.1016/j.stemcr.2018.03.001. [Epub ahead of print]

**Heterogeneity of Human Breast Stem and Progenitor Cells as Revealed by Transcriptional Profiling.**

Colacino JA[1], Azizi E[2], Brooks MD[2], Harouaka R[2], Fouladdel S[2], McDermott SP[2], Lee M[3], Hill D[3], Madden J[4], Boerner J[4], Cote ML[5], Sartor MA[6], Rozek LS[7], Wicha MS[8].

Nat Genet. 2018 Apr 2. doi: 10.1038/s41588-018-0089-9. [Epub ahead of print]

**Single-cell RNA sequencing identifies celltype-specific cis-eQTLs and co-expression QTLs.**

van der Wijst MGP[1], Brugge H[1], de Vries DH[1], Deelen P[1], Swertz MA[1]; LifeLines Cohort Study; BIOS Consortium, Franke L[2].



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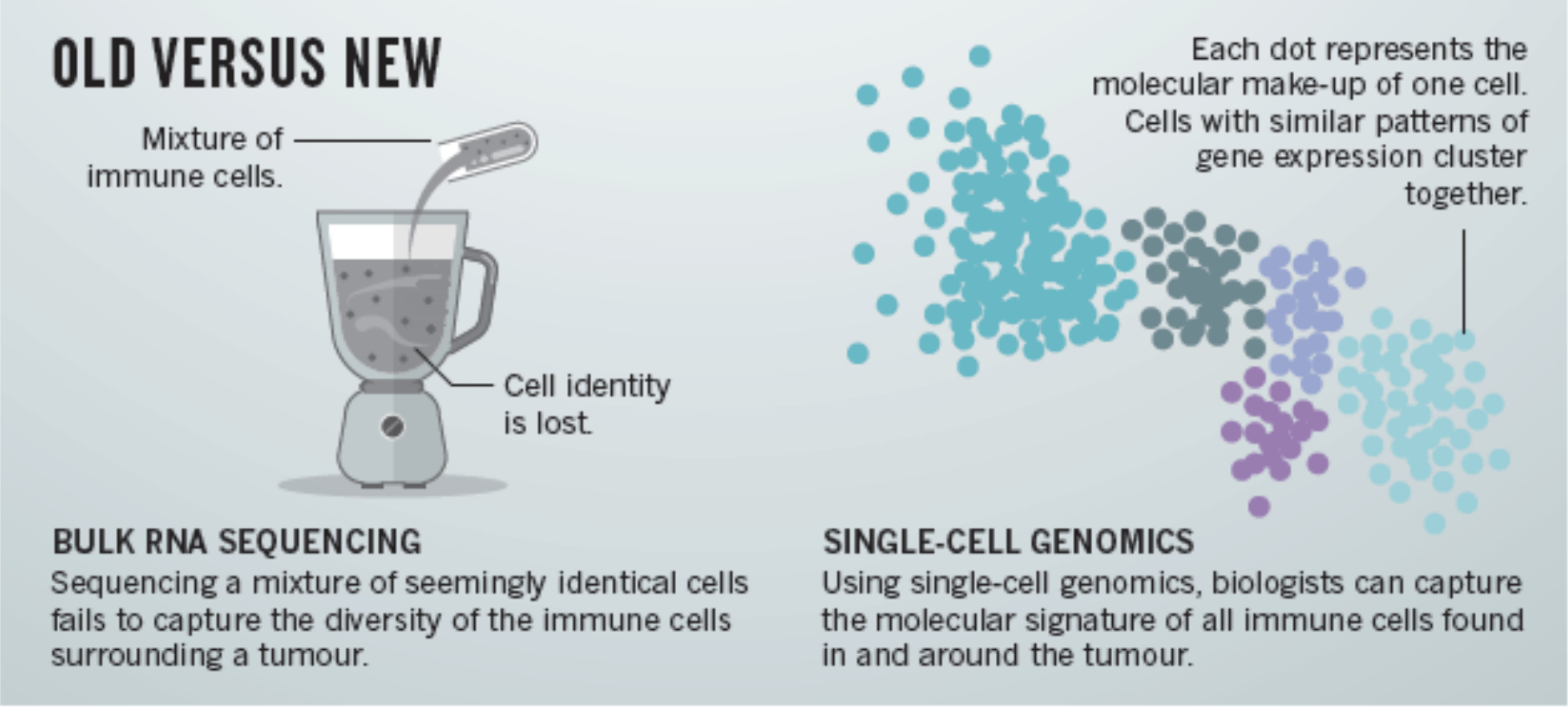
# Bulk vs Single-Cell Genomics




Claire Welsh/Nature

Amir Giladi and Ido Amit 28 | NATURE | VOL 547 | 6 JULY 2017



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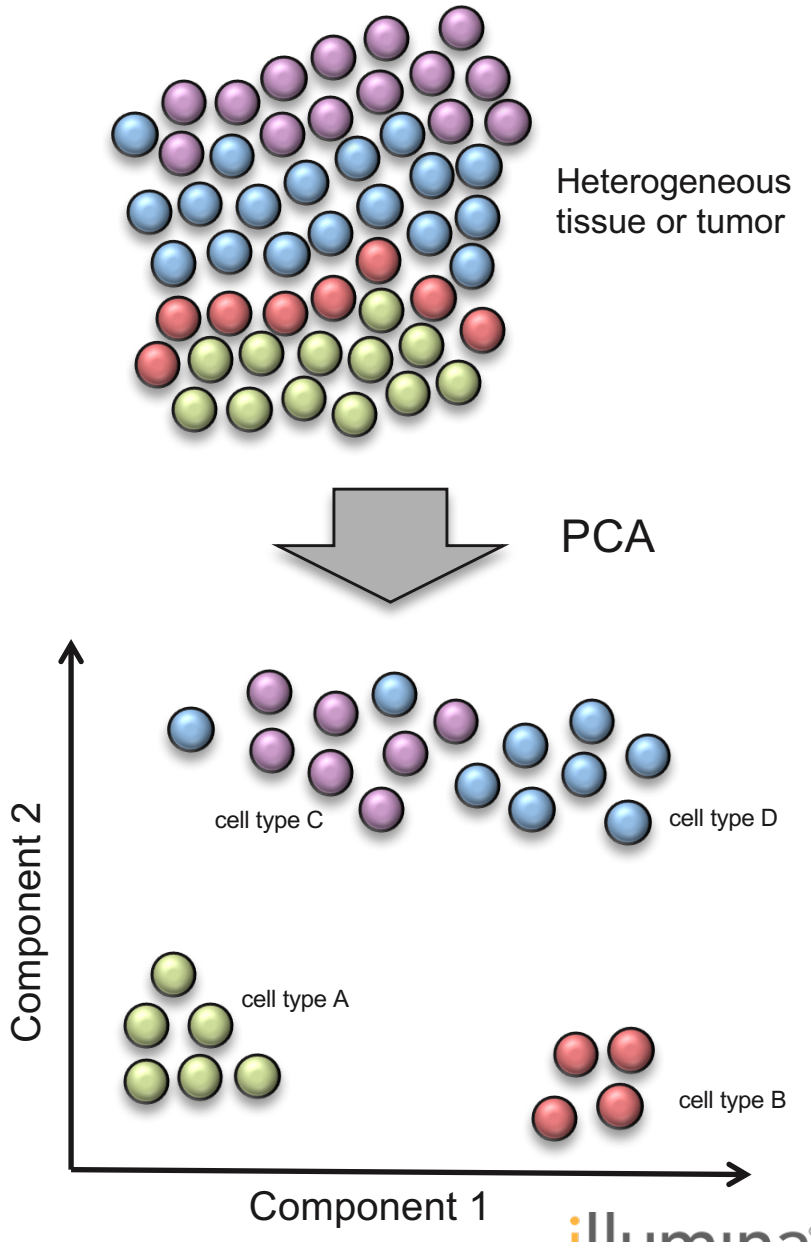
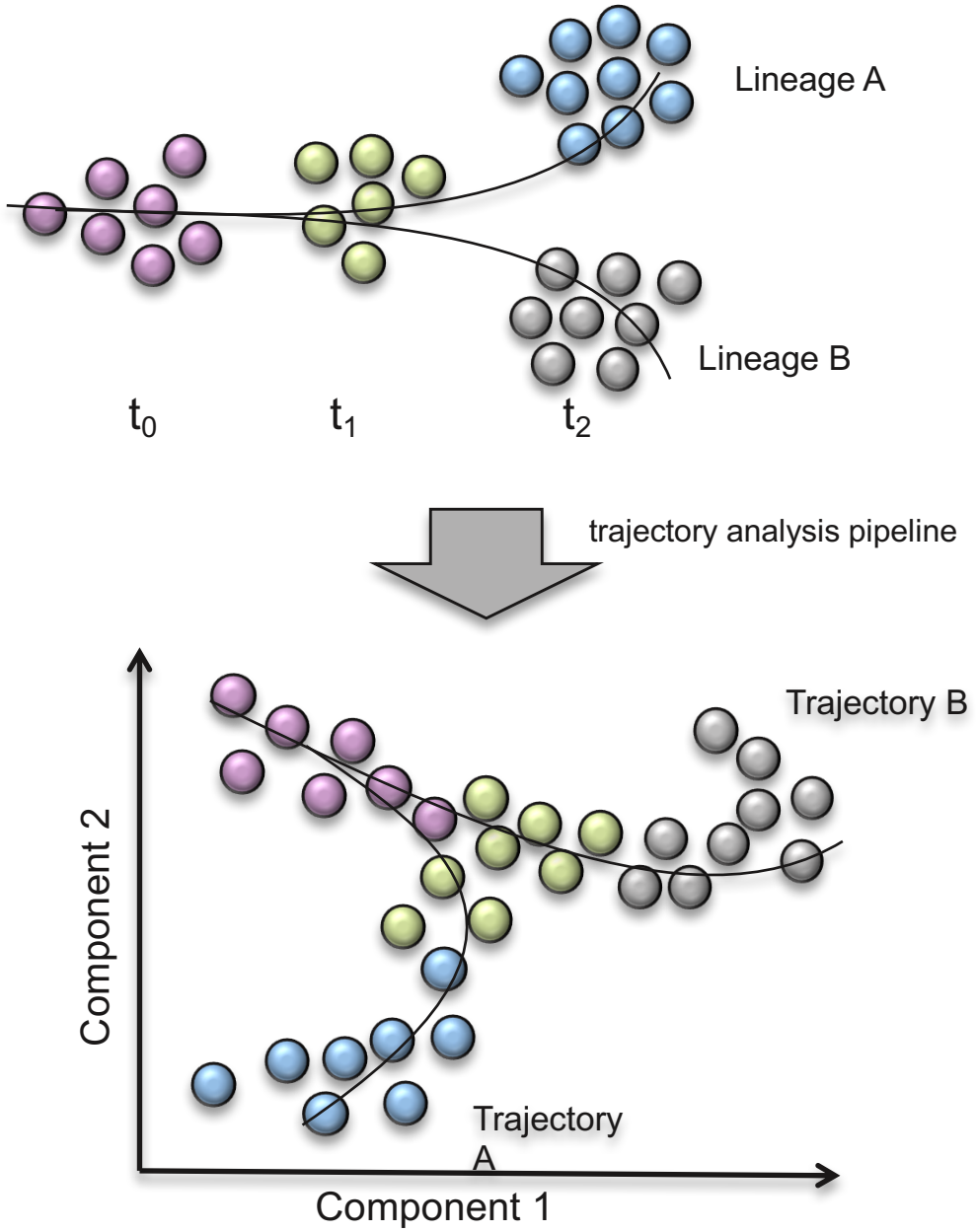
# Assessing Cell-to-Cell Heterogeneity

- Understand composition of complex cell mixtures
- Discover rare cell types
- Determine ratios of cell types within a complex tissue or tumor
- Determine specific cell types driving a disease pathology




Trapnell, *et. al. F1000 Research,* 2016

# Mapping of Cellular Trajectories




- Time series data to map cell developmental trajectories over a dynamic process
- Study of differentiation over time
- Study signal responses to external stimuli ex: drugs, heat, etc…

Trapnell, *et. al. F1000 Research,* 2016



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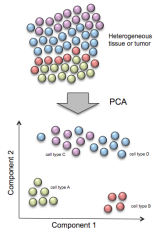
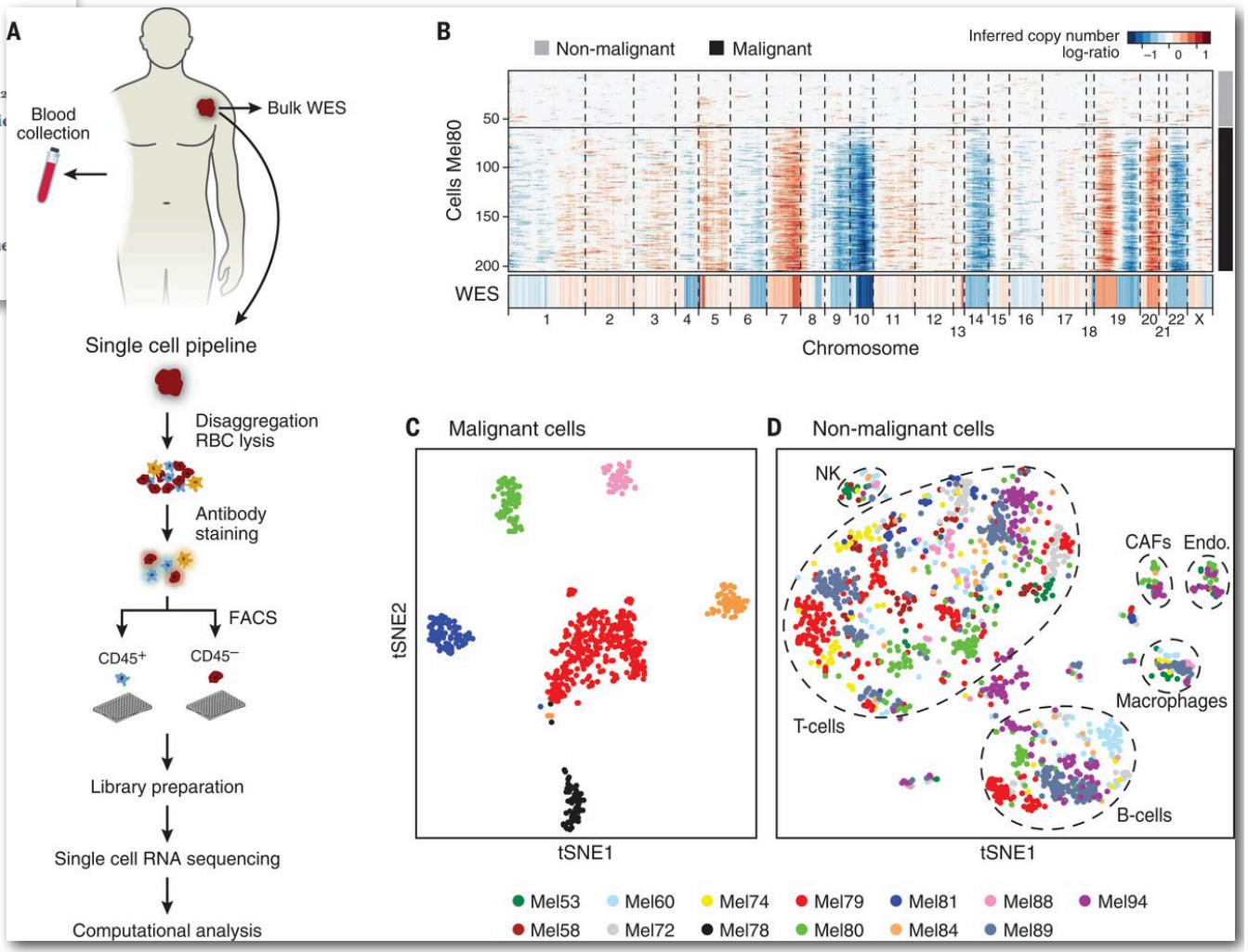
# Assessing Heterogeneity in Cancer




## Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq

Itay Tirosh,[1*] Benjamin Izar,[1,2,3*†‡] Sanjay M. Prakadan,[1,4,5,6] Marc H. Wadsworth II,[1,4,5,6] Daniel Treacy,[1] John J. Trombetta,[1] Asaf Rotem,[1,2] Christopher Rodman,[1] Christine Lian,[7] George Murphy,[7] Mohammad Fallahi-Sichani,[8] Ken Dutton-Regester,[1,2,9] Jia-Ren Lin,[10] Ofir Cohen,[1] Parin Shah,[2] Diana Lu,[1] Alex S. Genshaft,[1,4,5,6] Travis K. Hughes,[1,4,6,11] Carly G. K. Ziegler,[1,4,6,11] Samuel W. Kazer,[1,4,5,6] Aleth Gaillard,[1,4,5,6] Kellie E. Kolb,[1,4,5,6] Alexandra-Chloé Villani,[1] Cory M. Johannessen,[1] Aleksandr Y. Andreev,[1] Eliezer M. Van Allen,[1,2,3] Monica Bertagnolli,[12,13] Peter K. Sorger,[8,10,14] Ryan J. Sullivan,[15] Keith T. Flaherty,[15] Dennie T. Frederick,[15] Judit Jané-Valbuena,[15] Charles H. Yoon,[12,13†] Orit Rozenblatt-Rosen,[1†] Alex K. Shalek,[1,4,5,6,11,16†] Aviv Regev,[1,17,18†‡] Levi A. Garraway[1,2,3,14†‡]




- Used single-cell whole exome and whole transcriptome
- Cells grouped into clusters based on CNV

Tirosh *et al.*, 2016, Science



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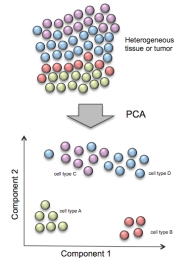
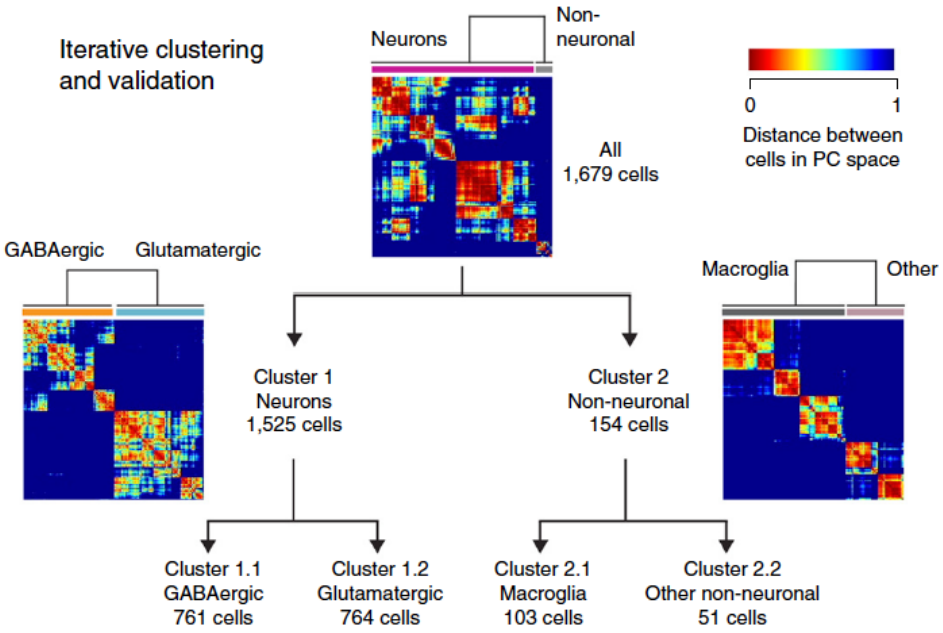
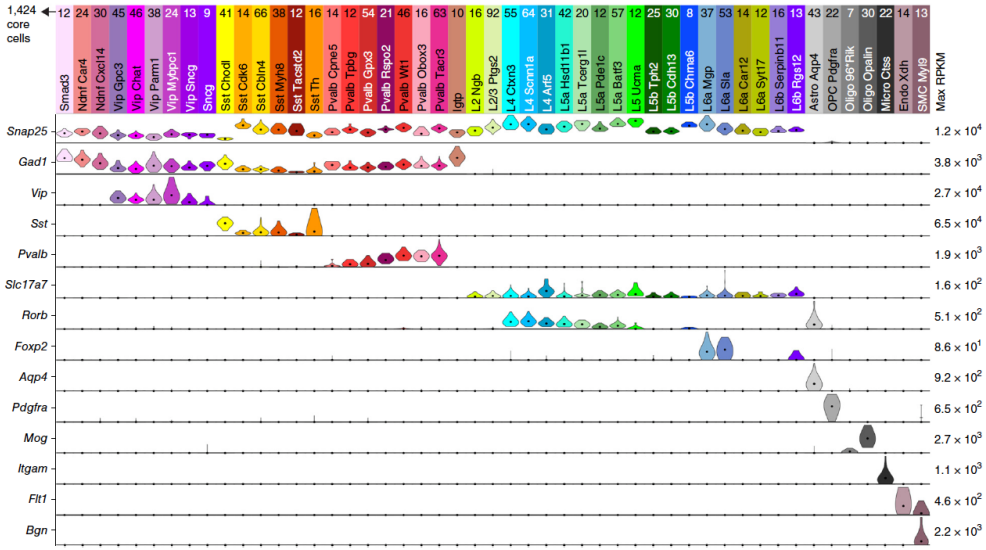
# Revealing Cellular Taxonomy




## Adult mouse cortical cell taxonomy revealed by single cell transcriptomics

Bosiljka Tasic[1,2], Vilas Menon[1,2], Thuc Nghi Nguyen[1], Tae Kyung Kim[1], Tim Jarsky[1], Zizhen Yao[1], Boaz Levi[1], Lucas T Gray[1], Staci A Sorensen[1], Tim Dolbeare[1], Darren Bertagnolli[1], Jeff Goldy[1], Nadiya Shapovalova[1], Sheana Parry[1], Changkyu Lee[1], Kimberly Smith[1], Amy Bernard[1], Linda Madisen[1], Susan M Sunkin[1], Michael Hawrylycz[1], Christof Koch[1] & Hongkui Zeng[1]




Tasic et al., 2016, Nature Neuroscience



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nature COMMUNICATIONS

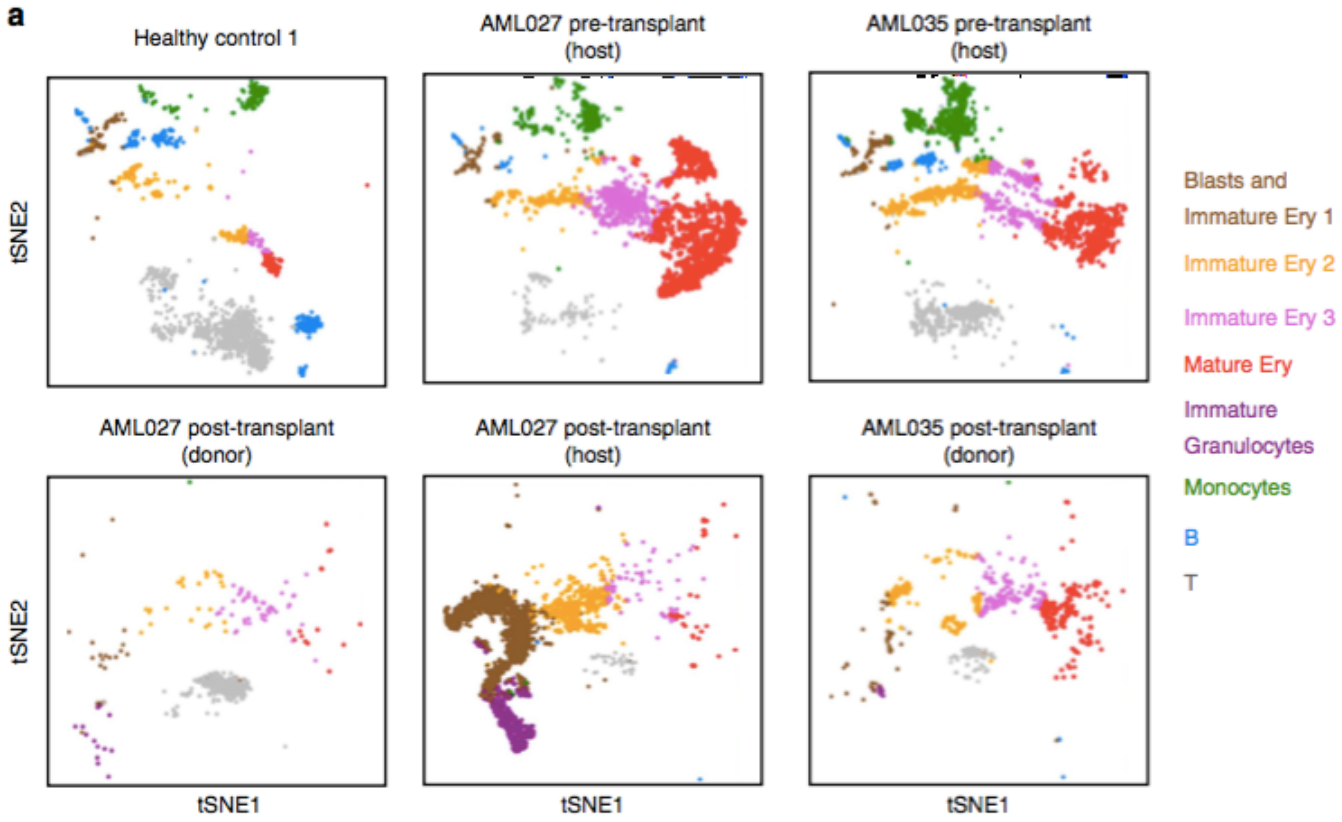
ARTICLE

Received 20 Sep 2016 | Accepted 23 Nov 2016 | Published 16 Jan 2017

DOI: 10.1038/ncomms14049 OPEN

# Massively parallel digital transcriptional profiling of single cells

Grace X.Y. Zheng[1], Jessica M. Terry[1], Phillip Belgrader[1], Paul Ryvkin[1], Zachary W. Bent[1], Ryan Wilson[1], Solongo B. Ziraldo[1], Tobias D. Wheeler[1], Geoff P. McDermott[1], Junjie Zhu[1], Mark T. Gregory[2], Joe Shuga[1], Luz Montesclaros[1], Jason G. Underwood[1,3], Donald A. Masquelier[1], Stefanie Y. Nishimura[1],



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# Hot Single-Cell Applications

- Single-Cell Pooled CRISPR Screens
- Single-Nuclei sequencing
- Single-Cell T Cell or B Cell Receptor Sequencing
- Single-Cell ATAC-seq
- Single-Cell Epitope Detection
- Single-Cell Multiplexing, Multiplet Detection, and Batch Effect
- Single-Cell Preservation methods and considerations
- SNV detection in Single Cell DNA

# Sequence Hub Cloud

## Simplifying bioinformatics

**For Research Use Only.  Not for use in diagnostic procedures.**



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# Multiple Layers of Security

- **Secure Data**
  - Data encrypted in transit, Genomic data encrypted at rest,
  - Access control, activity logging
- **Secure Employees**
  - Background checks, training on secure development
  - Training on HIPAA
- **Secure Physical Environment**
  - Built on AWS, ISO 27001 certified data centers
- **Secure Application**
  - Code reviews, penetration testing








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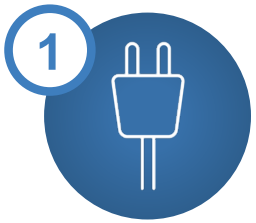
# Four Key Features




**Plug and play** with tight instrument integration


Easy **sharing and collaboration** worldwide


Simple **push-button analysis** with public and private analysis tools

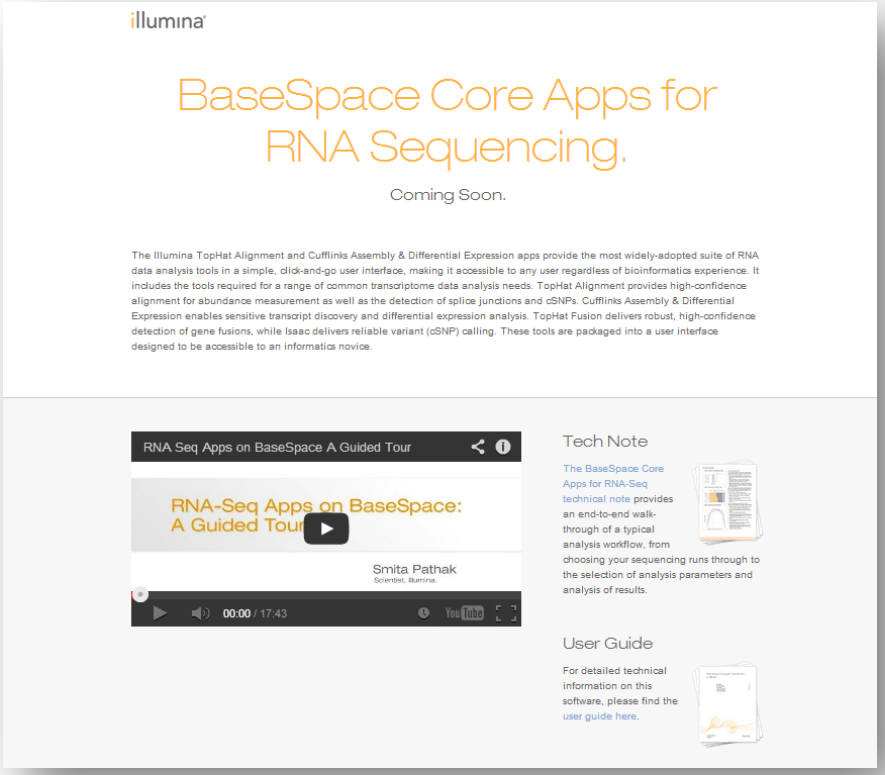

Advanced **automation and integration**

# Getting started with BaseSpace

- **Register for a free account**

**Basespace.illumina.com**

- **Import free public data in to your account**
- **Peruse an app catalog of 90+ and growing push-button analysis pipelines**




RNA Isolation | Library Prep | Sequencing | Data Analysis



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# Simple **push-button analysis** with public and private analysis tools

- Over 90 published Apps supporting all of Illumina library prep kits
  - — TruSeq Amplicon, TruSeq Targeted RNA, TruSight RNA Pan-Cancer, TruSight Tumor 15


18 Sequence Hub Labs Apps

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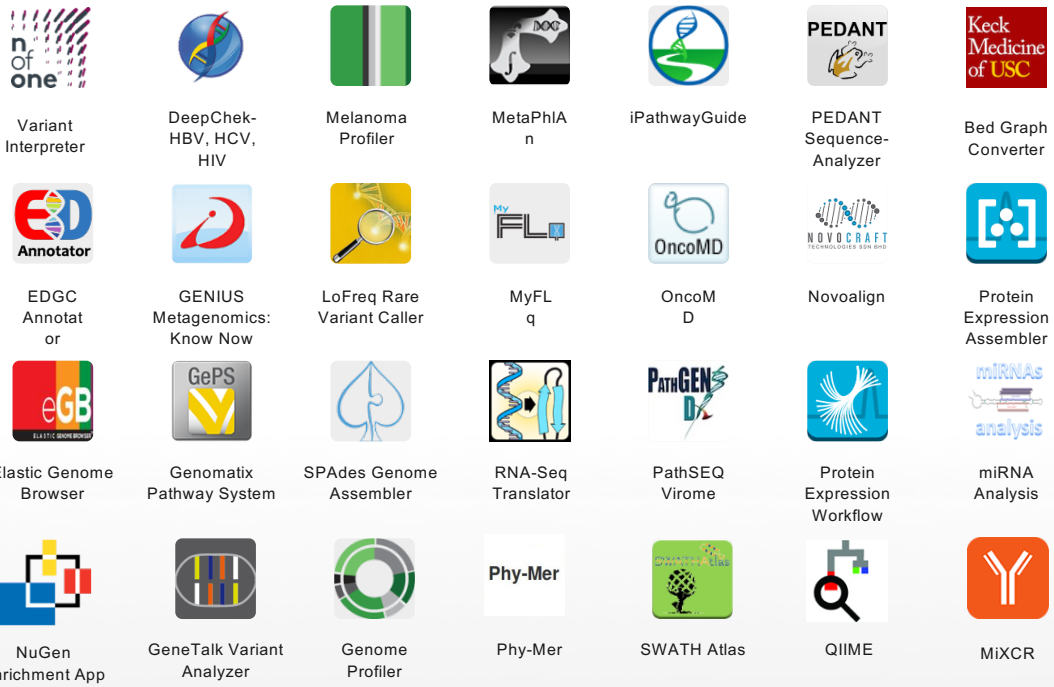
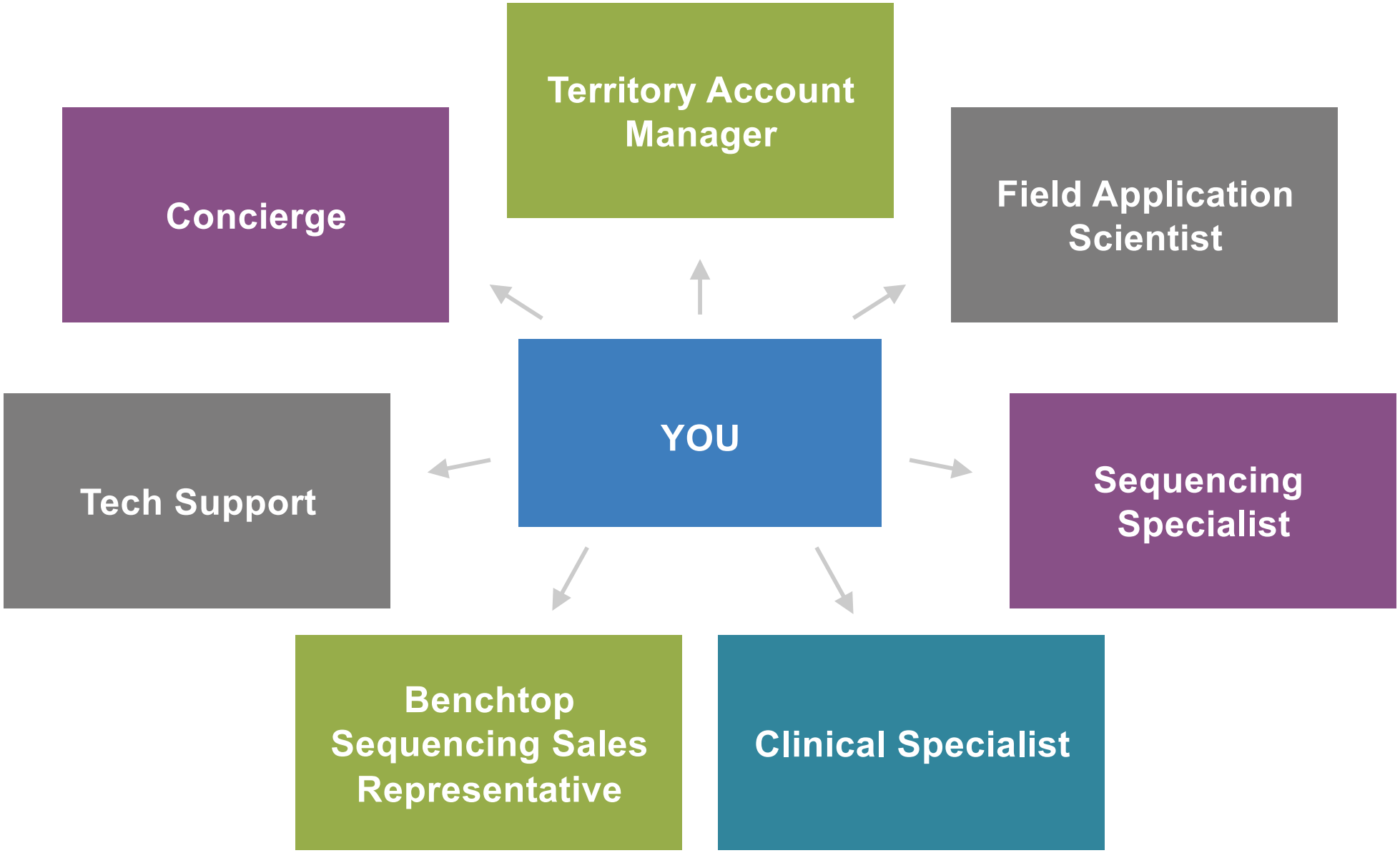



34 Third-Party Apps

# In Closing…

- **NextSeq ideal for:**
  - WGS (small to medium sized genomes, metagenomics)
  - WES
  - RNA-Seq
  - Single-Cell
- **Core lab is ready and able… now**

# We are Here for You!



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