Молекулярное баркодирование, анализ репертуаров Т-клеточных рецепторов и антител

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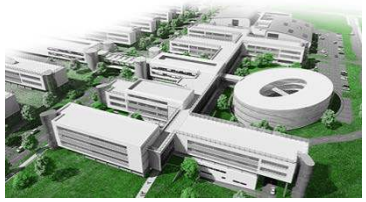
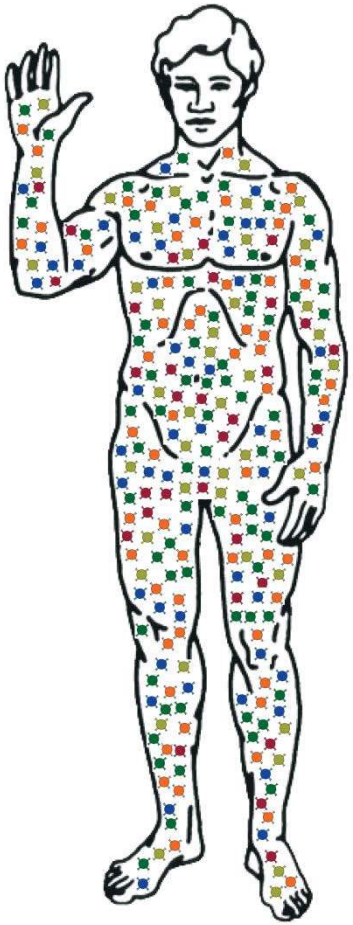
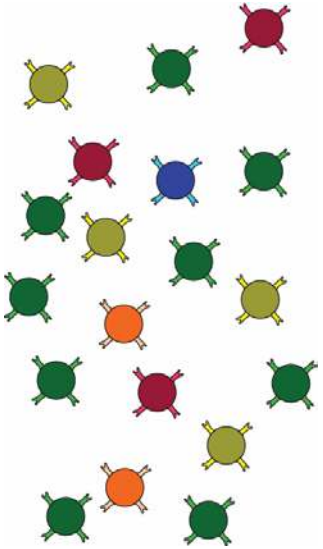


Dmitriy M. Chudakov
chudakovdm@mail.ru

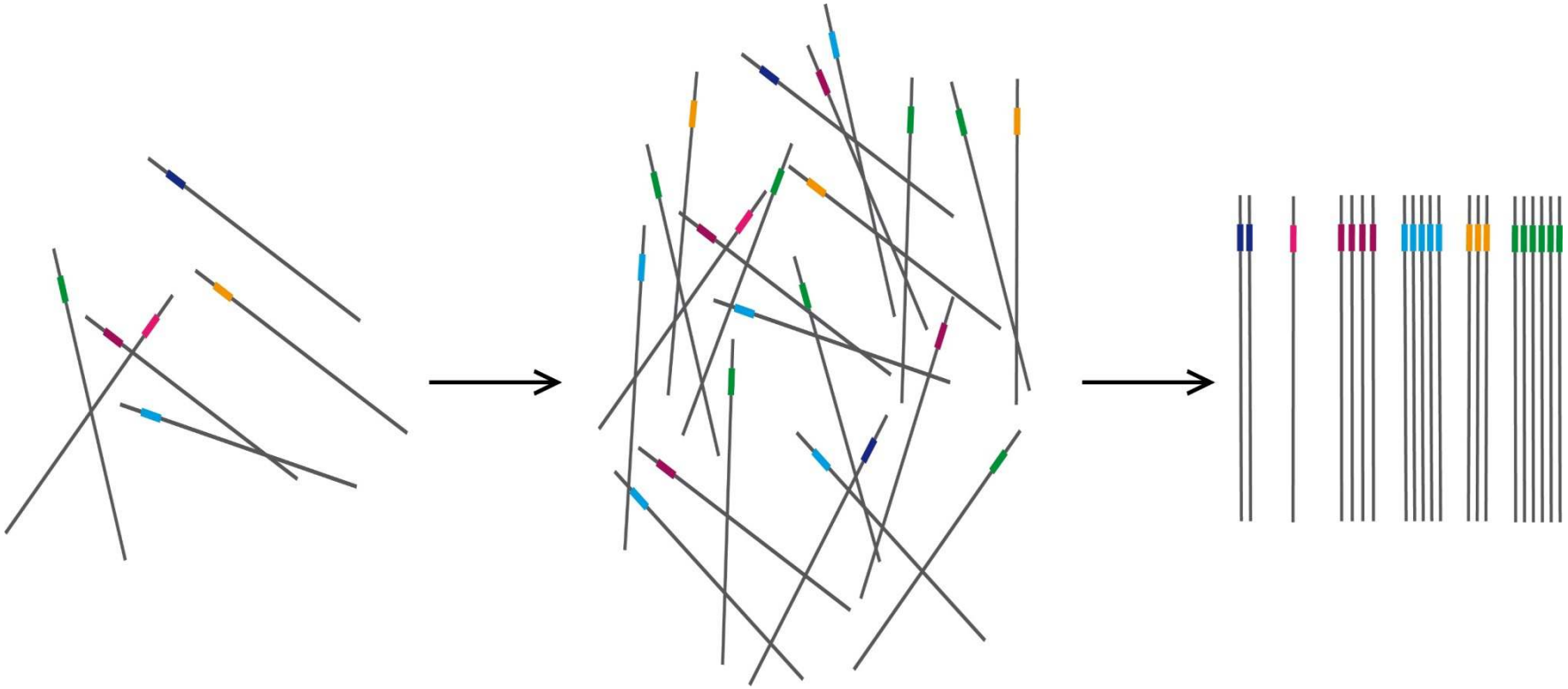
Массированное секвенирование на Illumina (почти) без ошибок: что может молекулярное баркодирование

Анализ ампликонов

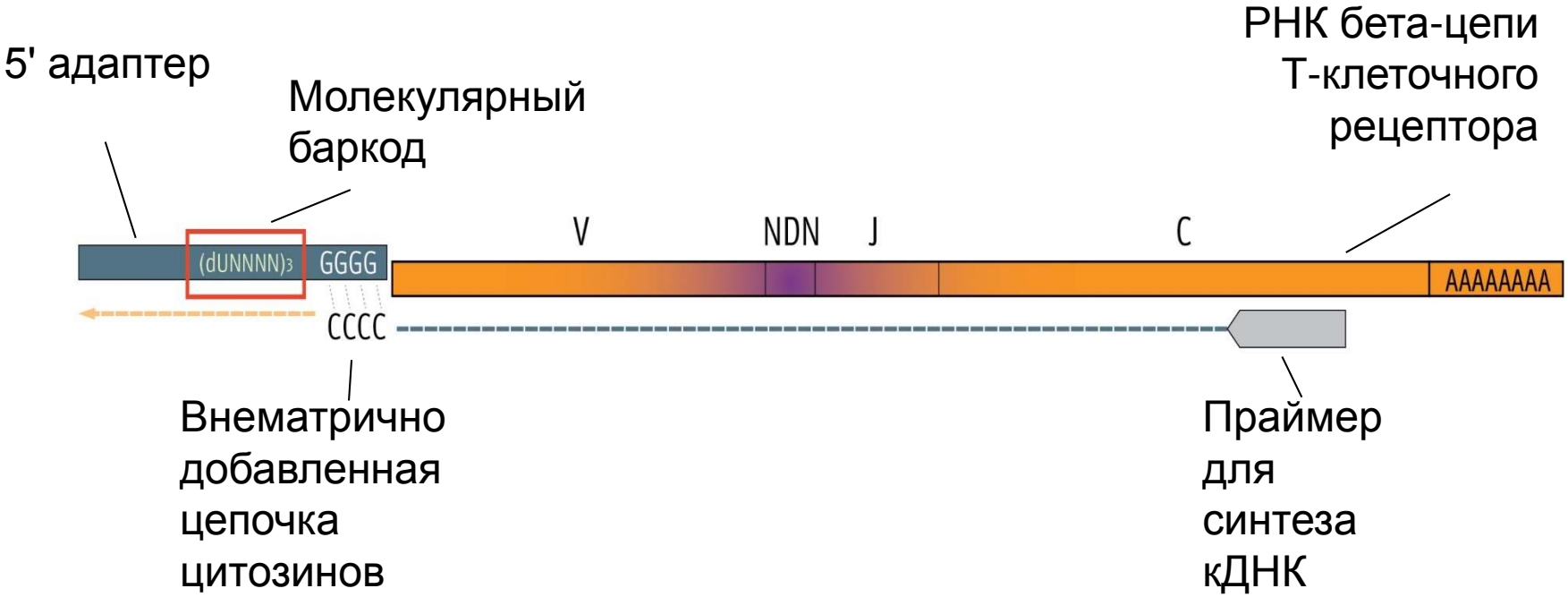




Логика молекулярного баркодирования



Молекулярное баркодирование через template switch

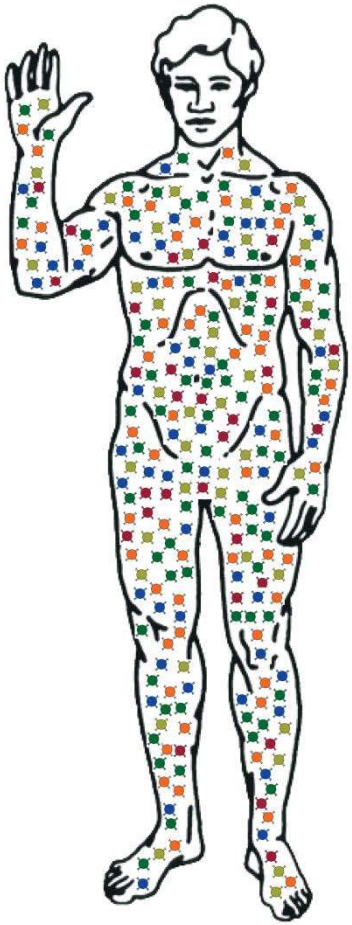


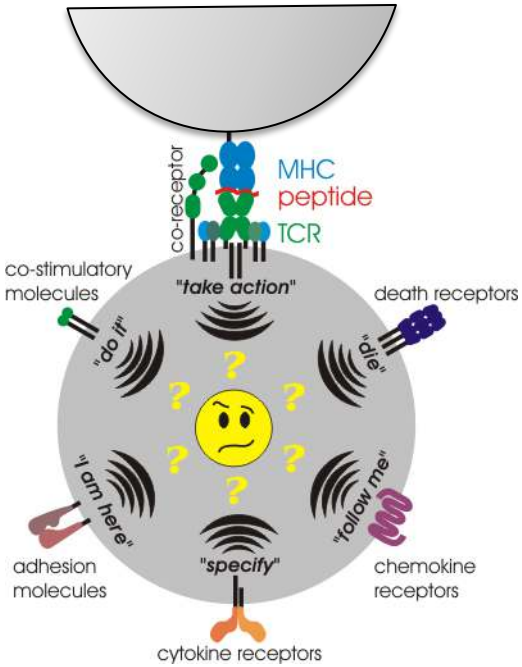
Адаптер с NNNN:

GGTA**U**CAACGCAGAG**UNNNNUNNNNUNNNNU**CTTrGrGrGrG

GGTA**U**CAACGCAGAG**UAGATUTTCAUACATU**CTTrGrGrGrG
GGTA**U**CAACGCAGAG**UCTGGUGTACUCTGGU**CTTrGrGrGrG
GGTA**U**CAACGCAGAG**UGAAAUCCGTUAGAGU**CTTrGrGrGrG

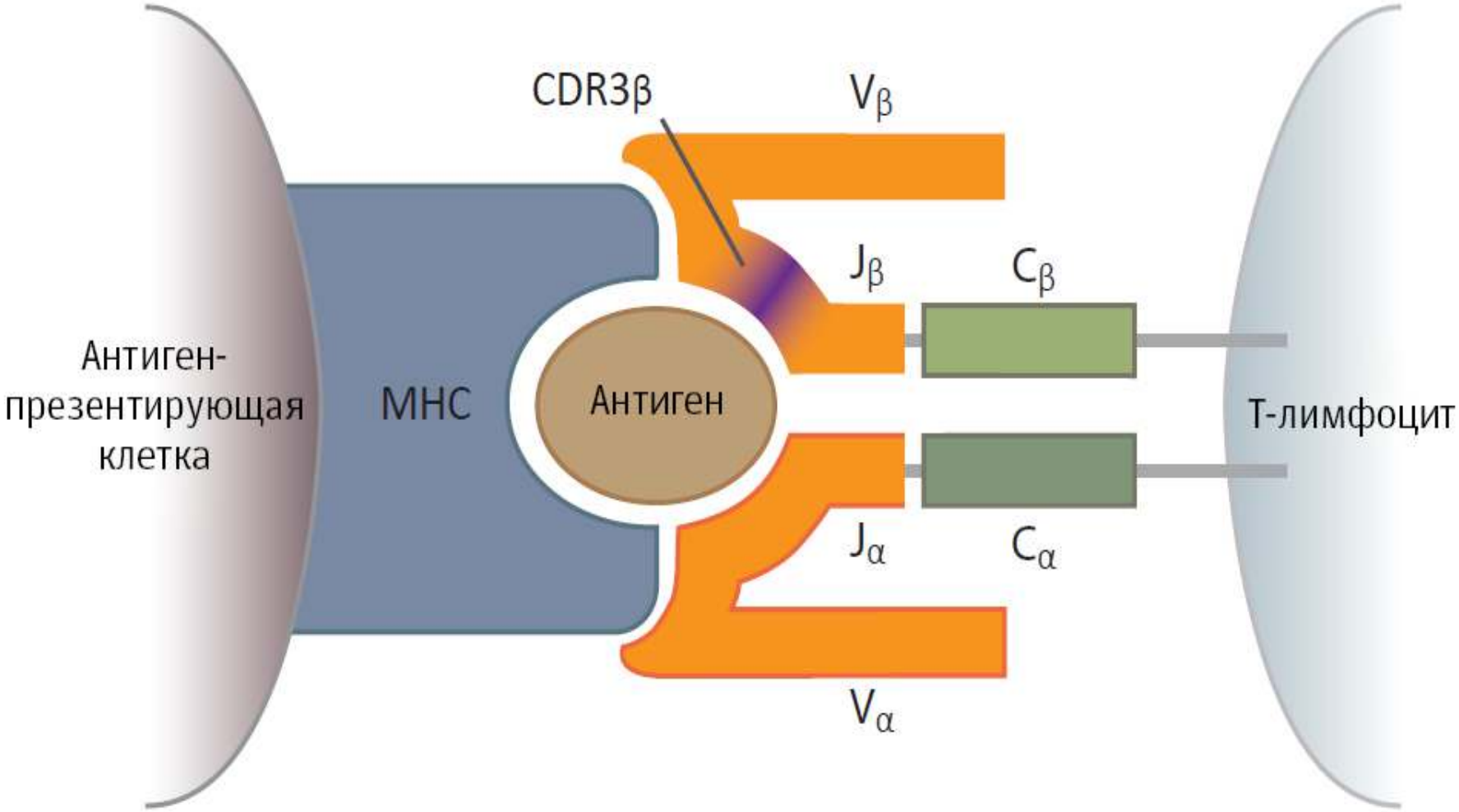
Т клетки и Т-клеточные рецепторы



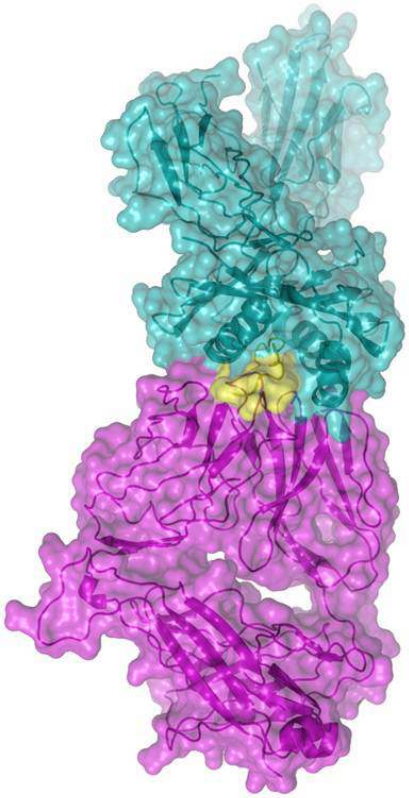


В каждом из нас – до 10^{12} Т клеток

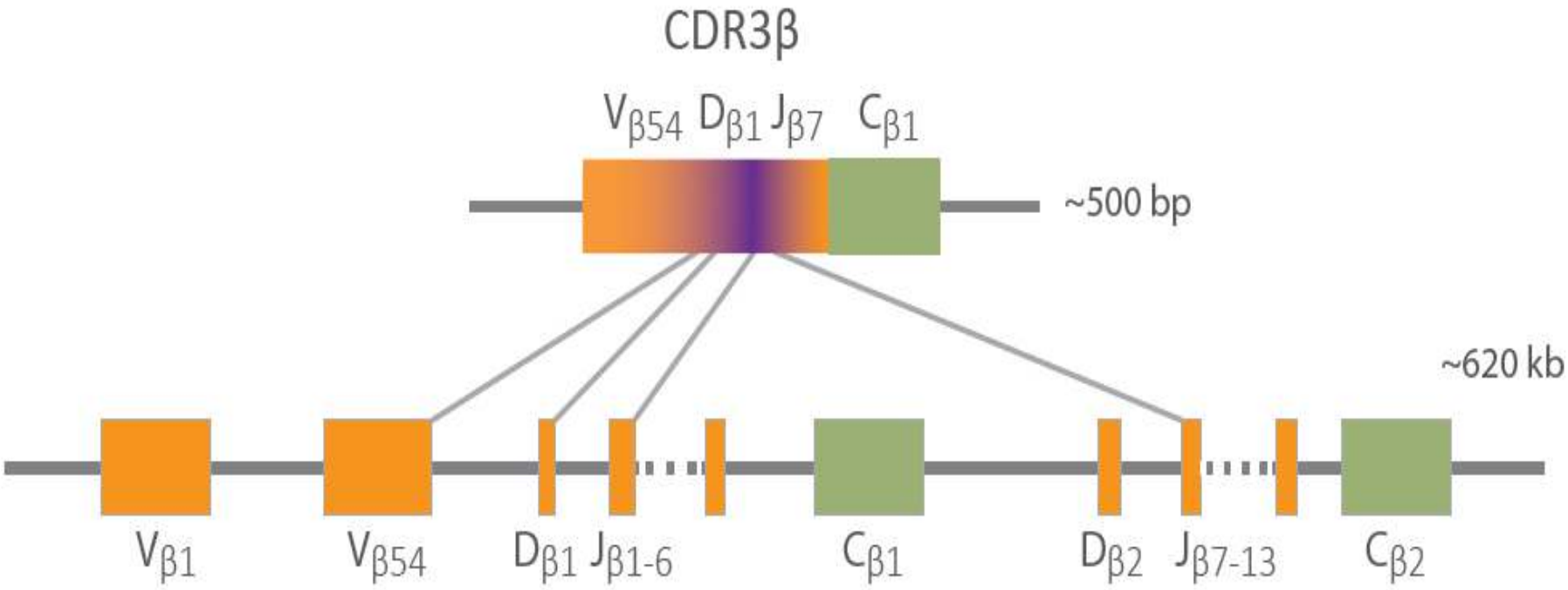
Структура αβ Т-клеточного рецептора



Специфичность Т-клеточного рецептора



Генерация разнообразия β-цепи Т-клеточного рецептора



Еще чуть сложнее

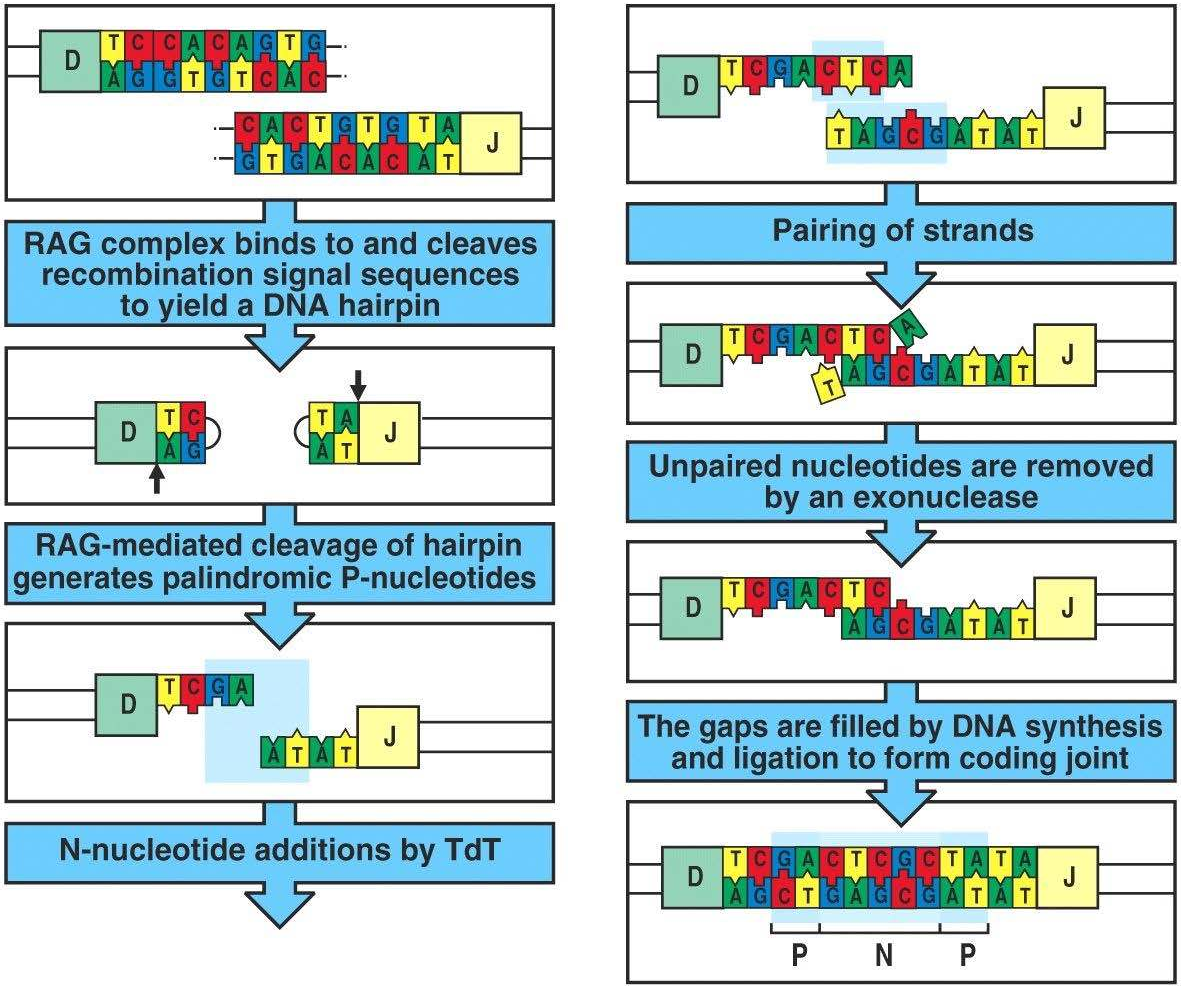
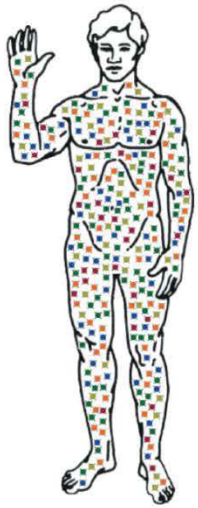
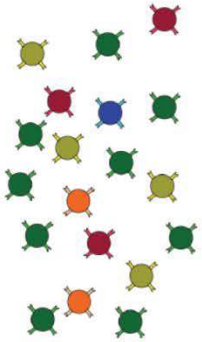
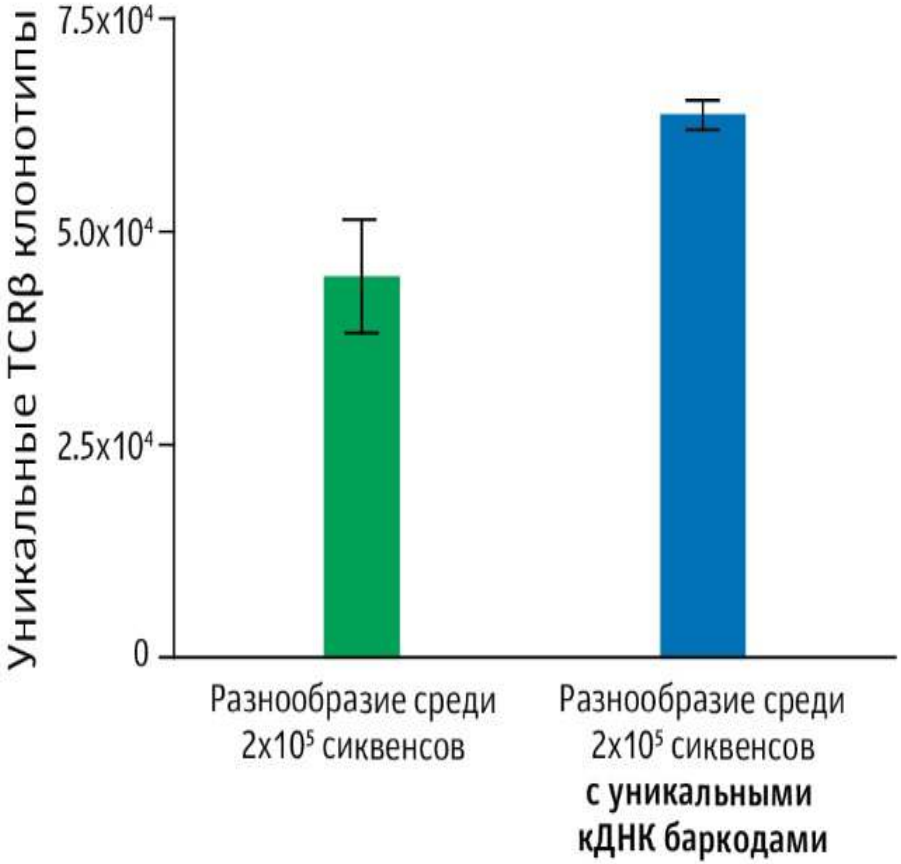


Figure 4-8 Immunobiology, 6/e. (© Garland Science 2005)

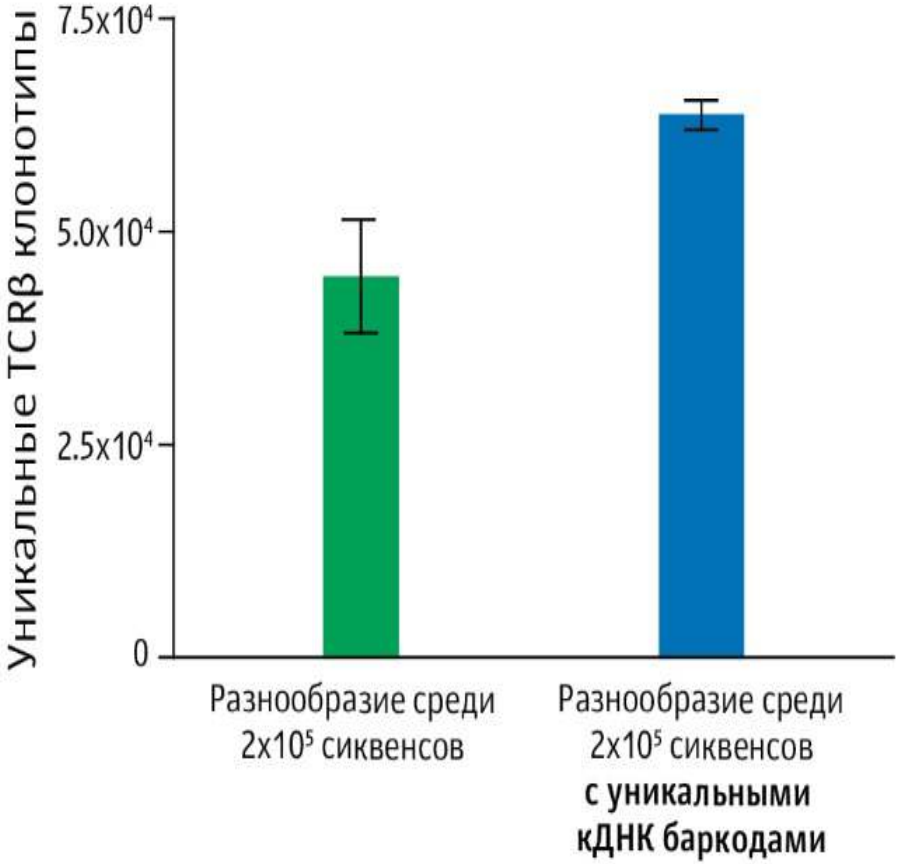


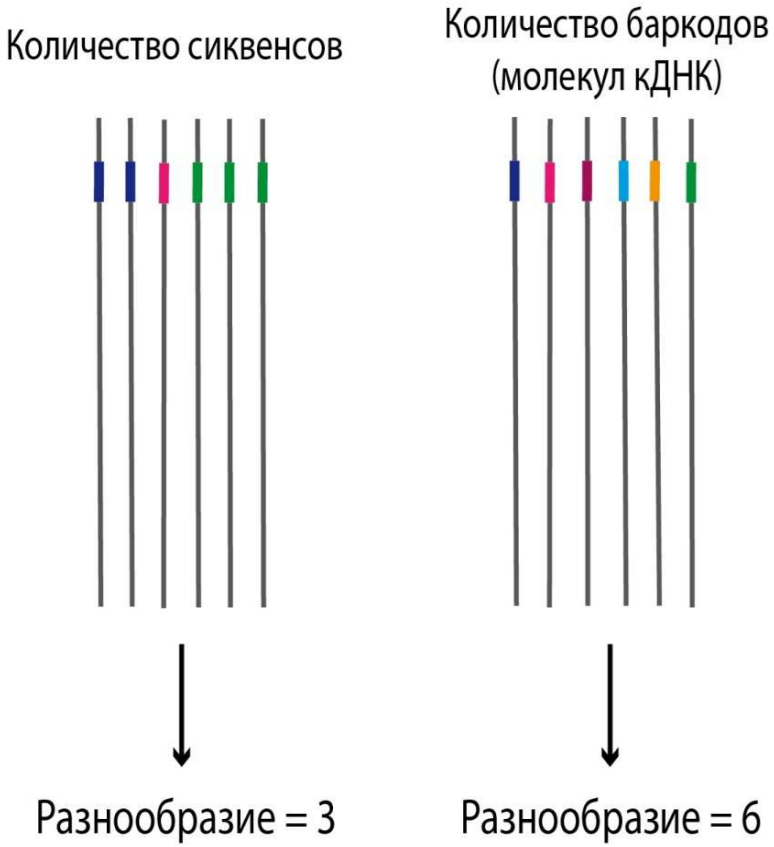


Нормировка с помощью молекулярного баркодирования

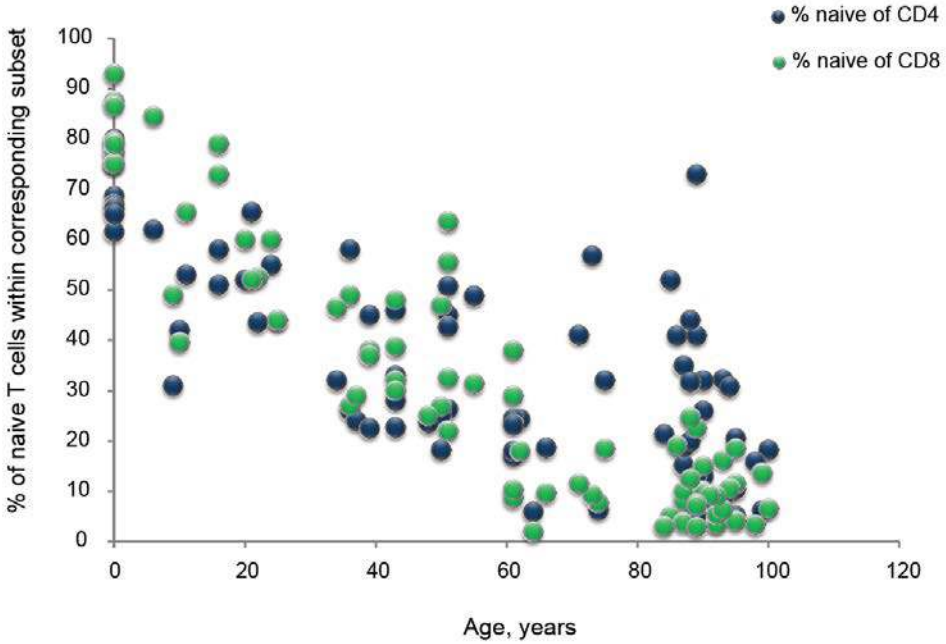


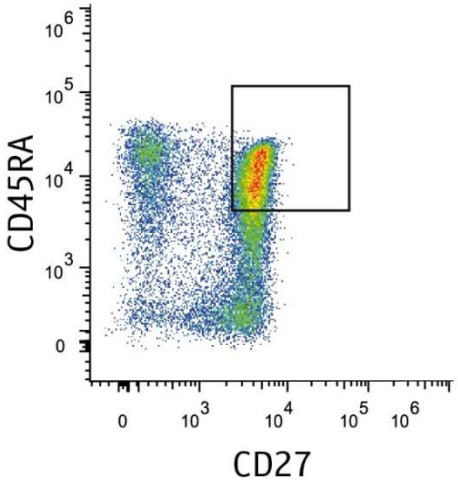
Нормировка с помощью молекулярного баркодирования





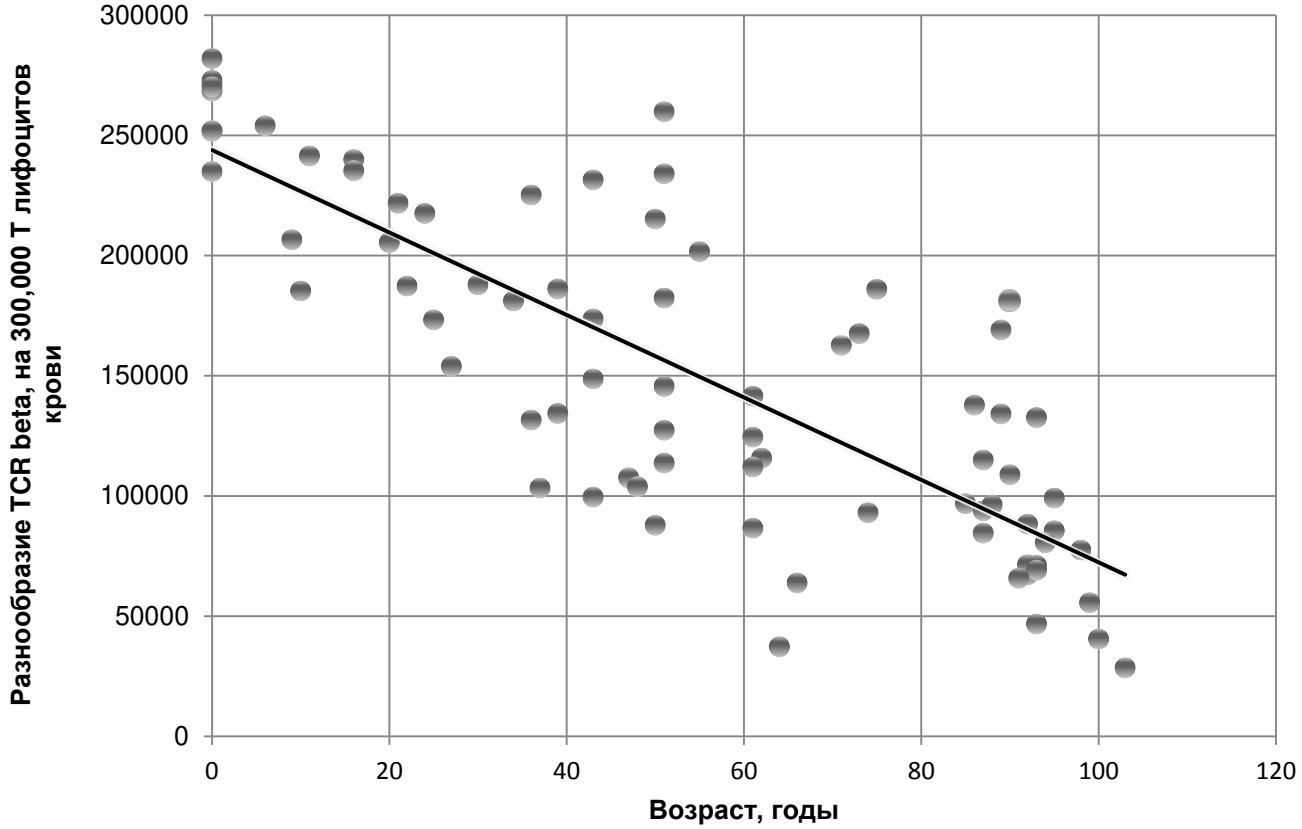
С возрастом процент наивных клеток в крови снижается



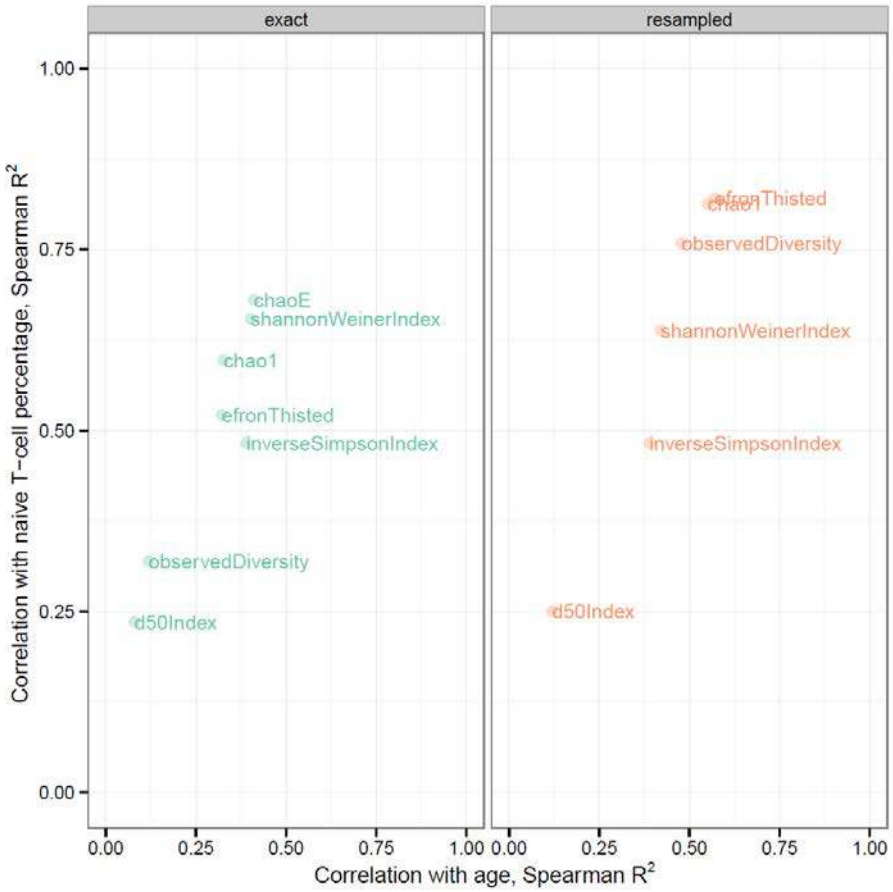


Britanova et al., J immunology, 2014

Снижается и разнообразие репертуара TCR



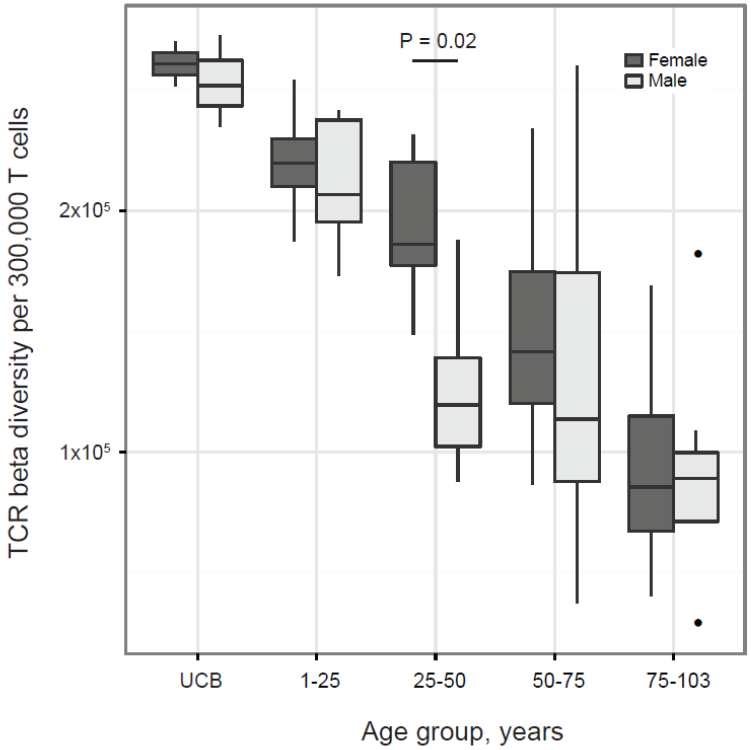
Diversity metrics

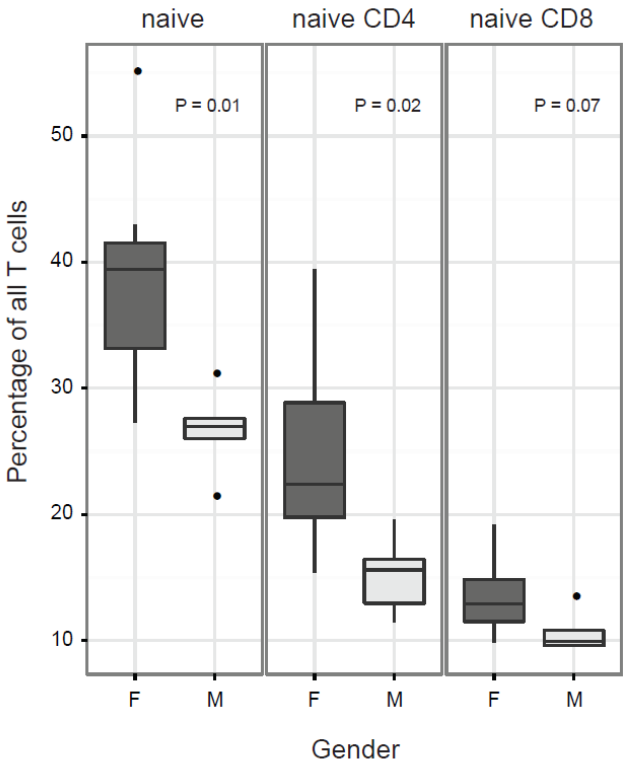


~100 humans, aging, naïve%, total blood TCRbeta diversity

Shugay et al, VDJtools, **PLOS Comp Biol**, *2015*.

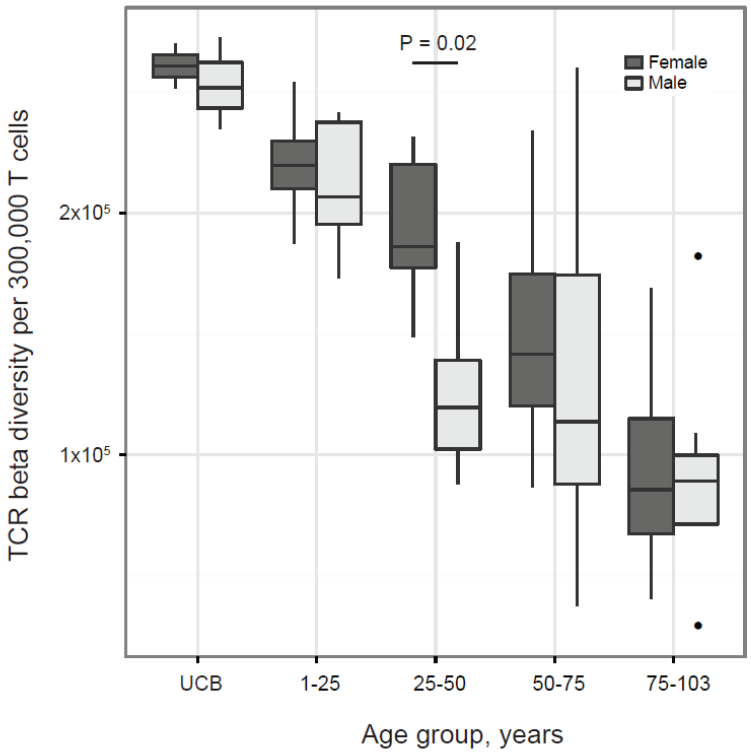
Мужики стареют быстрее

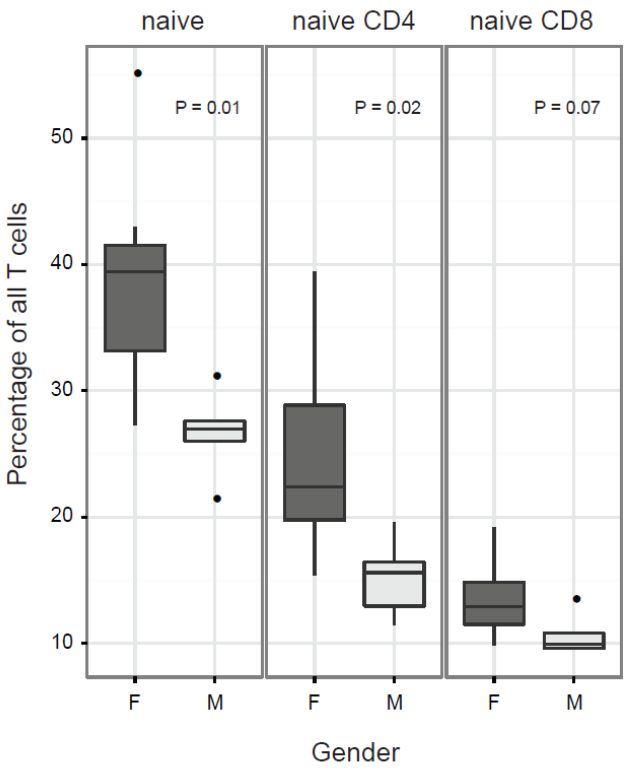




Britanova et al., ***on consideration***

Но! Старые мужики живут столько же

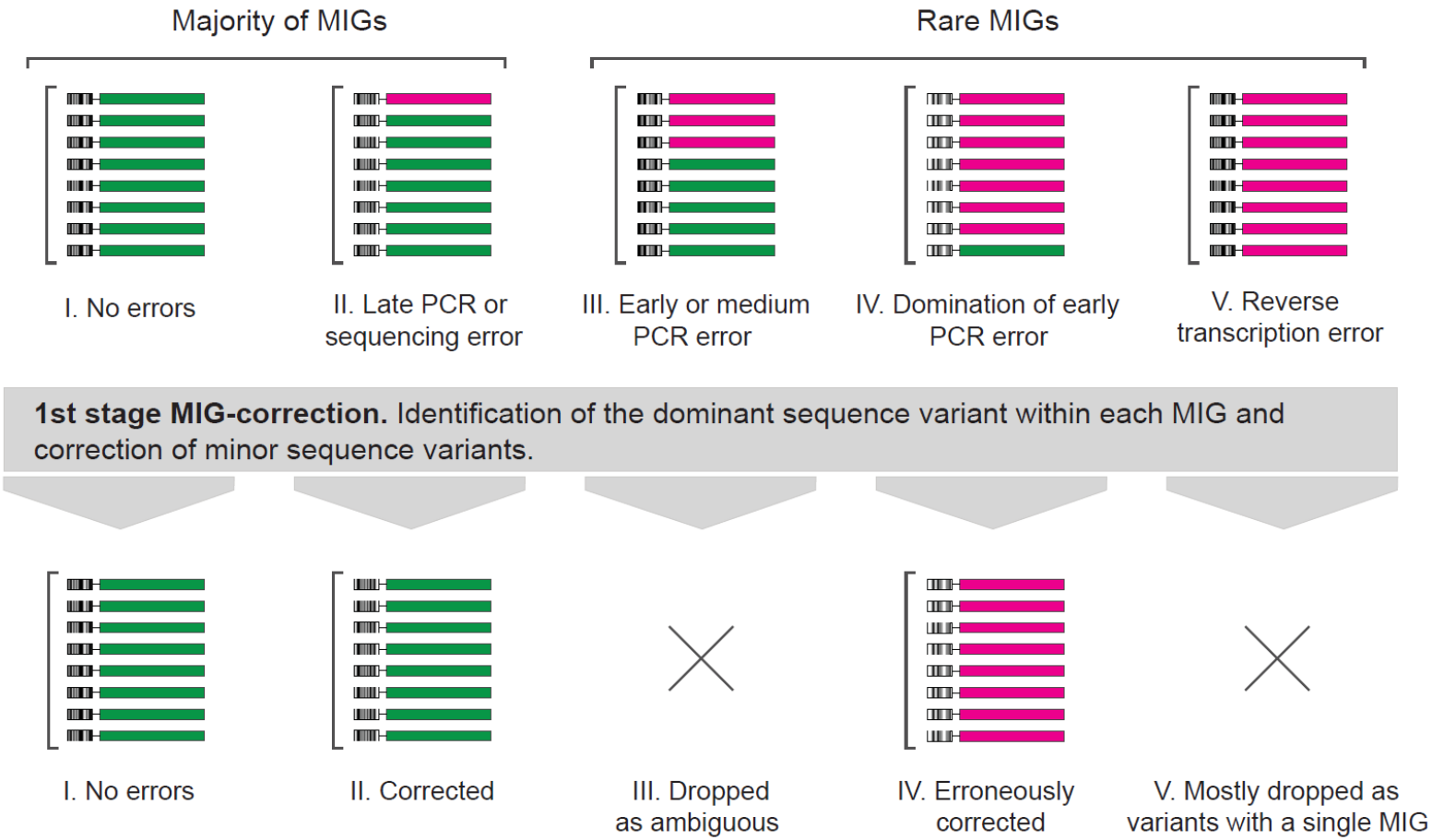




Britanova et al., ***on consideration***

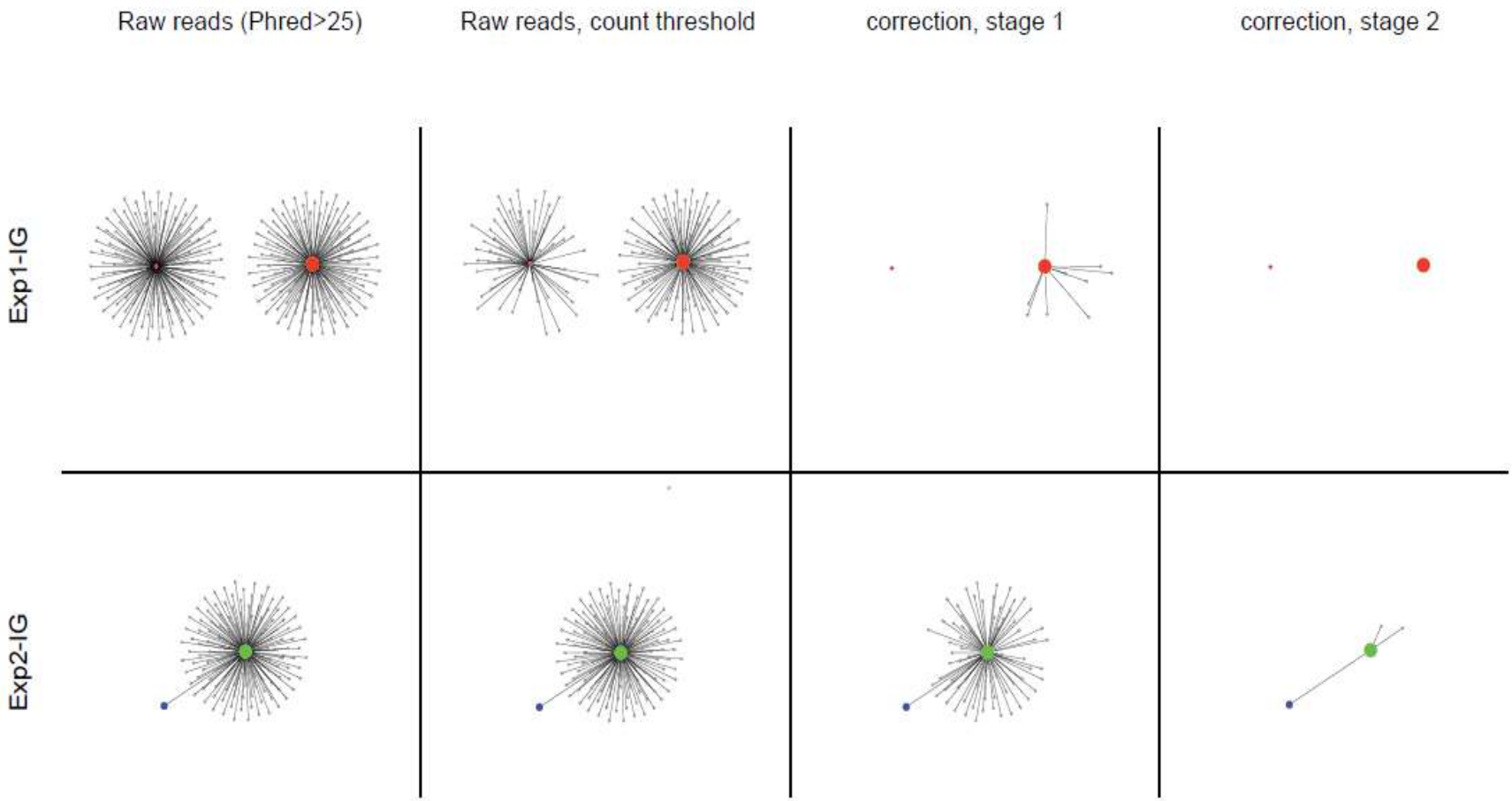
Молекулярное баркодирование и коррекция ошибок ПЦР и секвенирования

Error correction



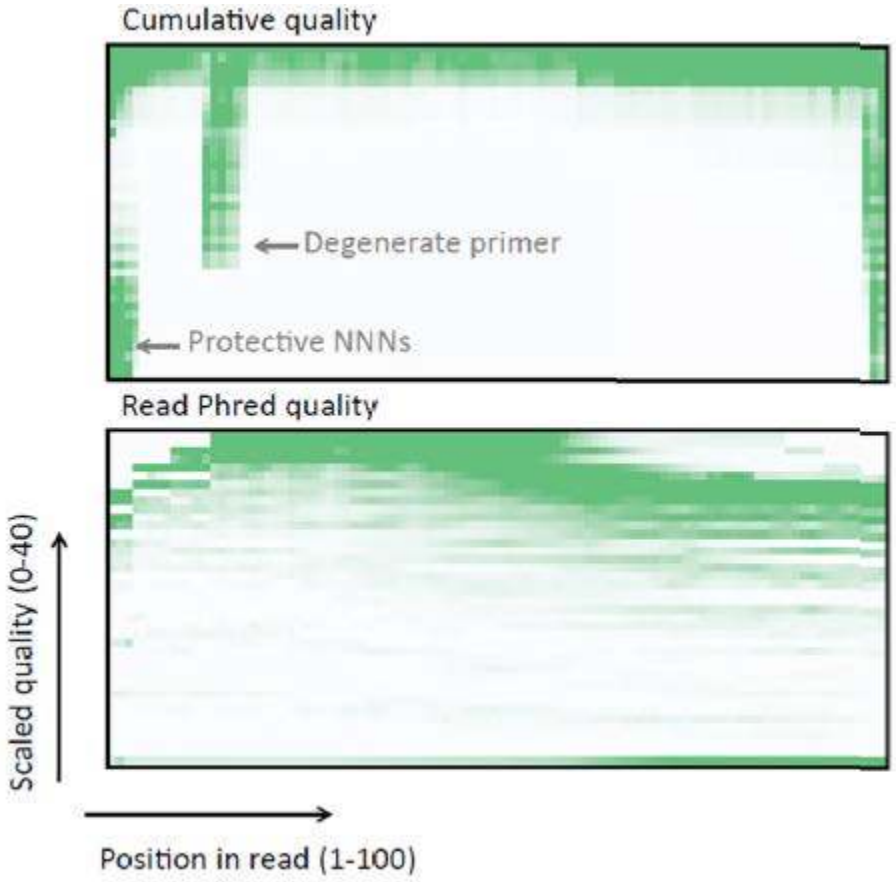
Shugay et al., MIGEC, ***Nature Methods***, 2014

Deep antibody sequencing, control clonotypes



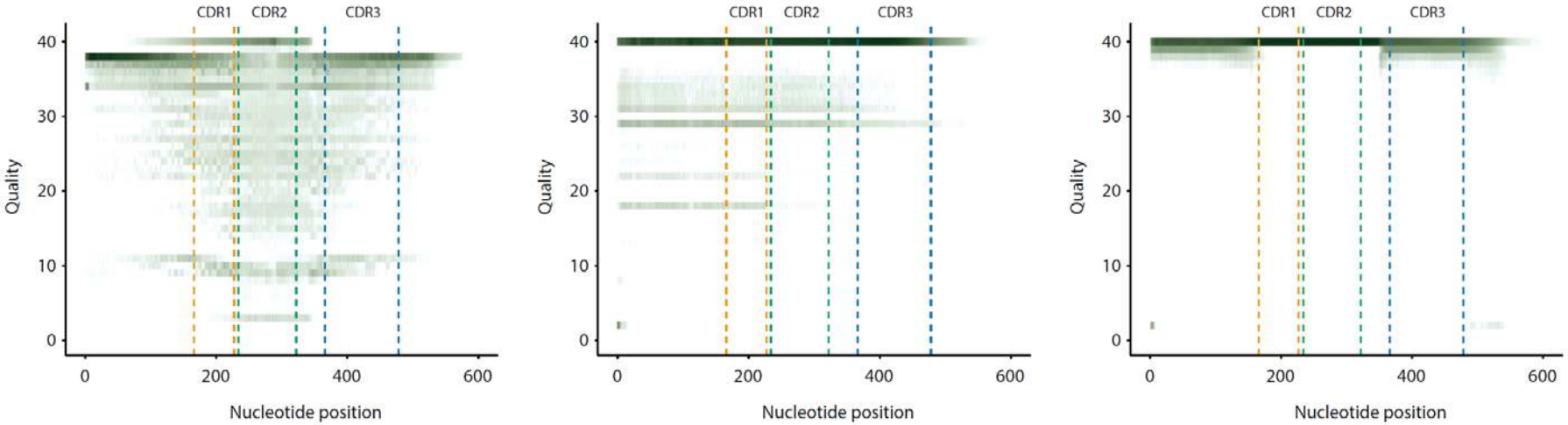
Shugay et al., MIGEC. ***Nature Methods***, 2014

Molecular barcoding: error probability



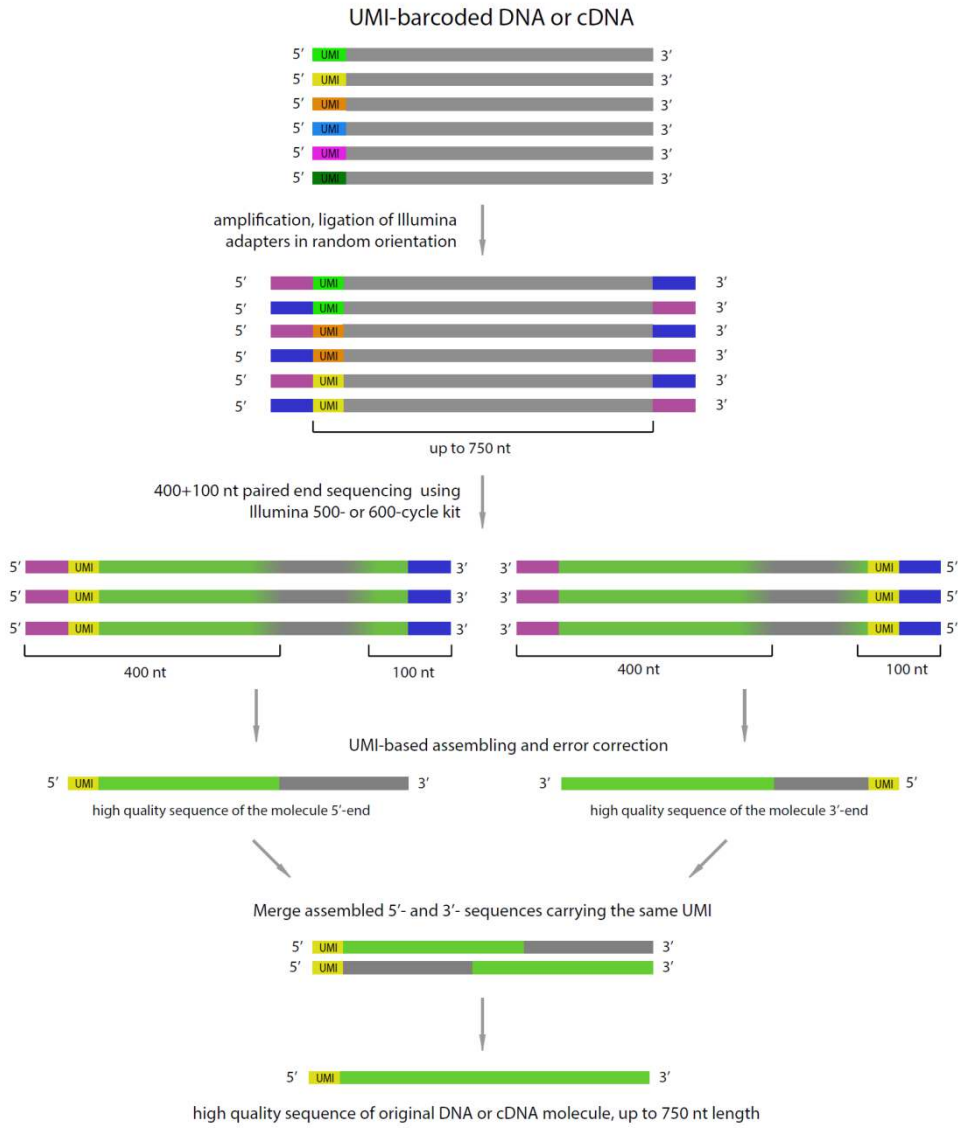
Shugay et al., ***Nature Methods***, 2014

Molecular barcoding: dramatic improvement of long range sequencing quality



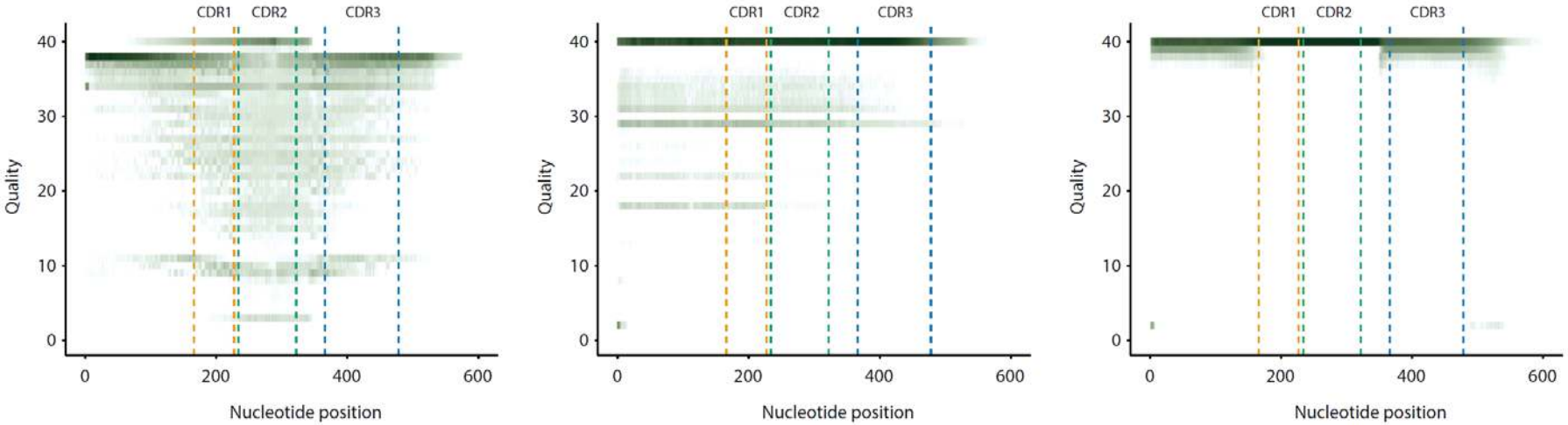
Turchaninova et al., **Nature Protocols**, 2016

750 nt length (!) nearly error-free (!) sequencing on Illumina - asymmetric logic:



Turchaninova et al., **Nature Protocols**, 2016

Molecular barcoding: dramatic improvement of long range sequencing quality

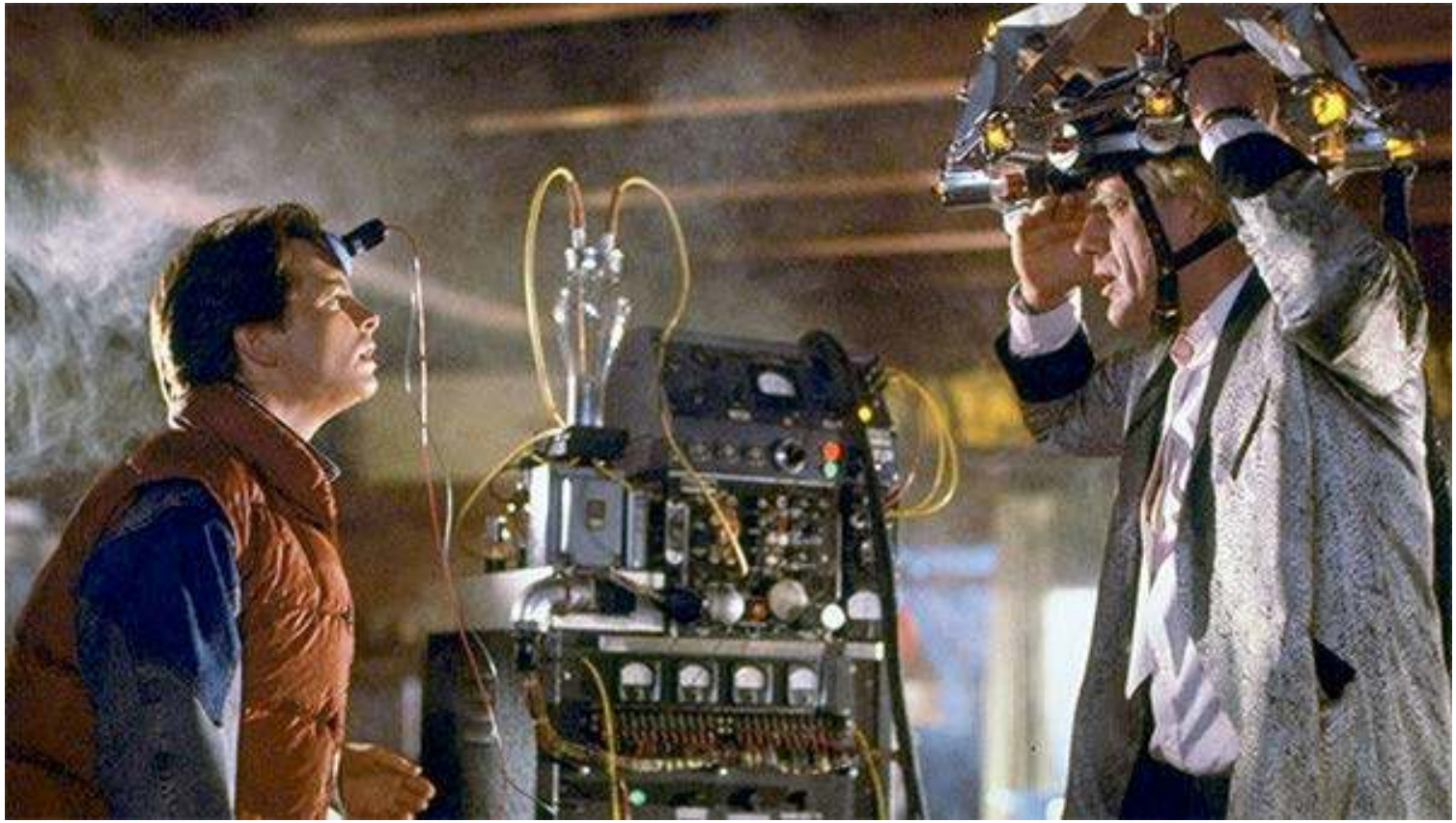


Turchaninova et al., **Nature Protocols**, 2016

With molecular barcodes

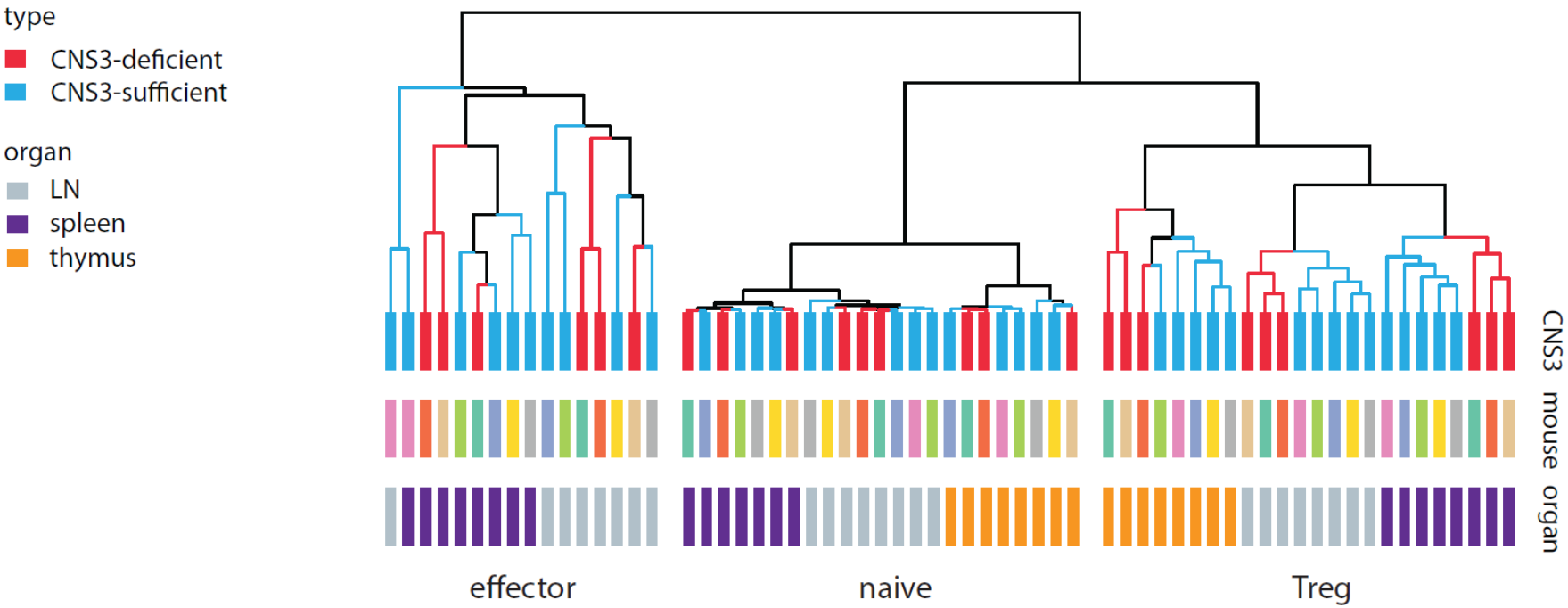
- Know the numbers
- Can normalize
- Eliminate errors
- Improve/rescue sequencing quality
- Make it longer

direct link between programmist and biologist



Некоторые штуки из жизни репертуаров Т-клеточных рецепторов

TCR repertoire distinguishes subsets and organs



Mice TCR alpha amino acid CDR3 rep; mice mutant, factor important for Tregs

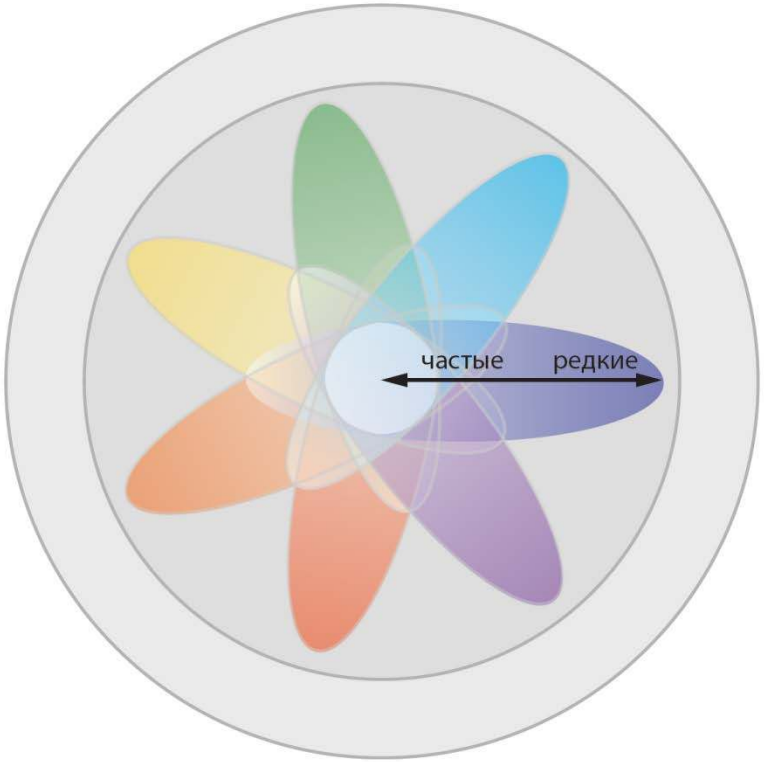
Feng Y., et al., **Nature**, *2015*.

10^{13} вариантов бета цепей Т-клеточных рецепторов может быть сгенерировано

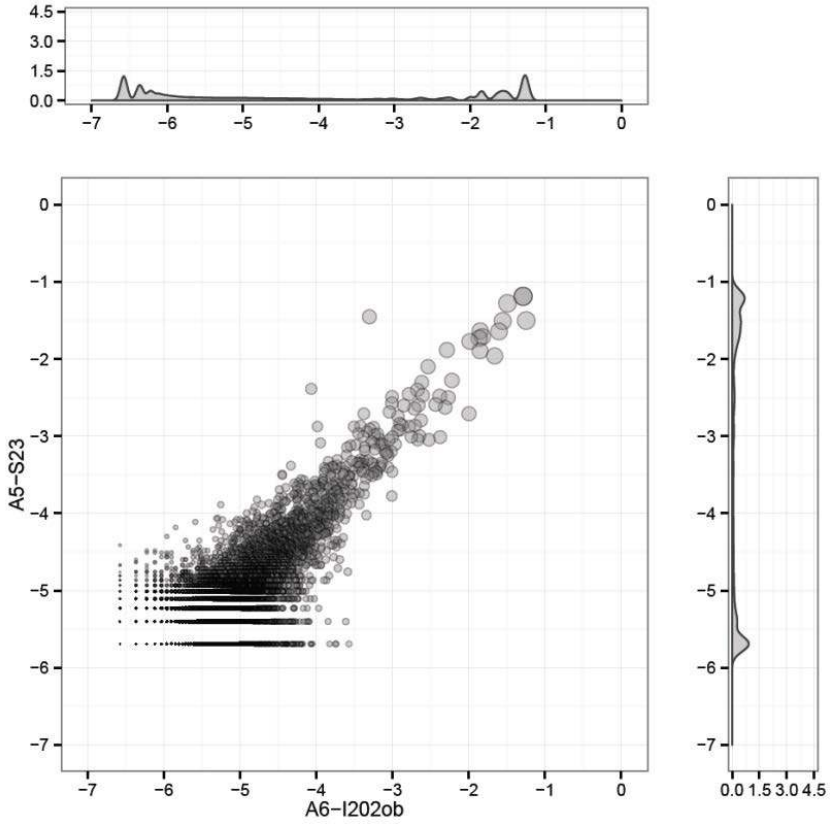
10^{11} вариантов проходит селекцию и используется в людях

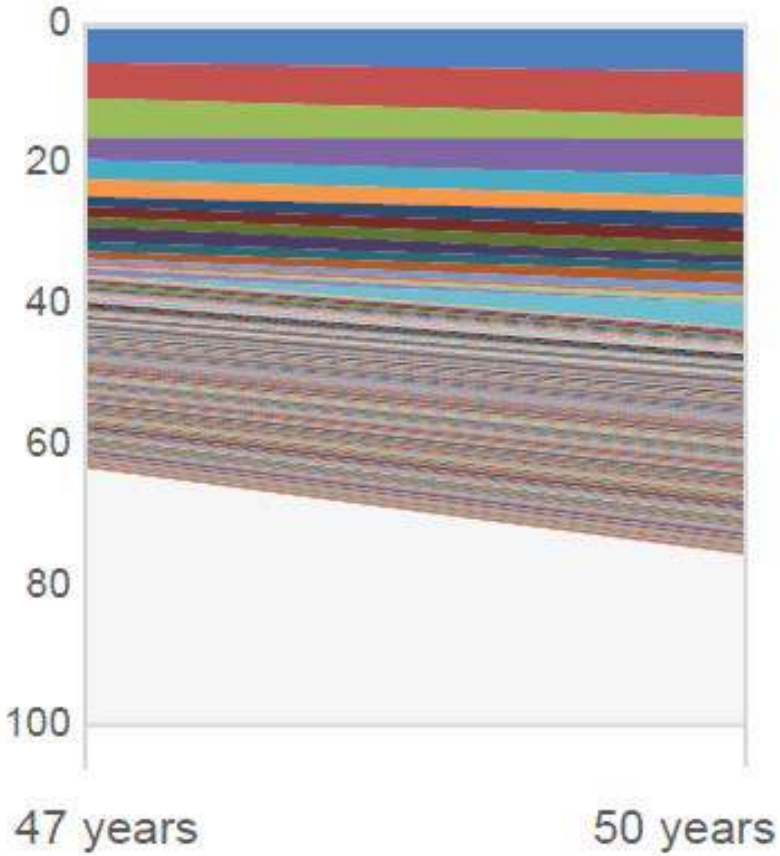
10^{8} вариантов у одного человека

до 40% одинаковых у двух людей



Stability of individual TCR repertoire for years





Britanova et al., ***J immunology 2016***

Репертуары Т клеточных рецепторов в онкологии

1. Эволюционные отношения опухоль-иммунная система – опухоль ищет дыры и способы обмана:

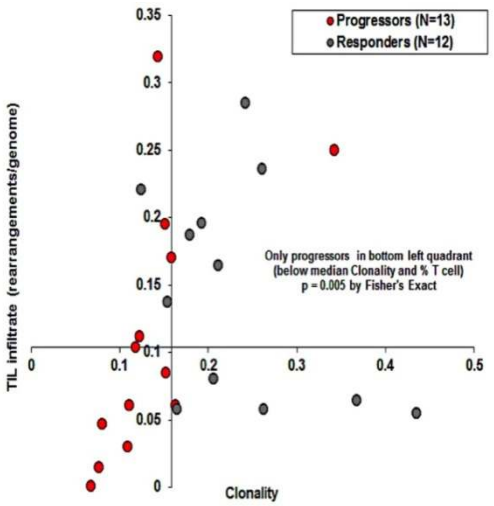
- Нарушить трафик T cells
- Нарушить метаболизм T cells
- Иммуносуппрессировать цитокинами
- Нарушить праймирование T cells
- Пригласить/превратить в Treg
- Задавить экзосомами

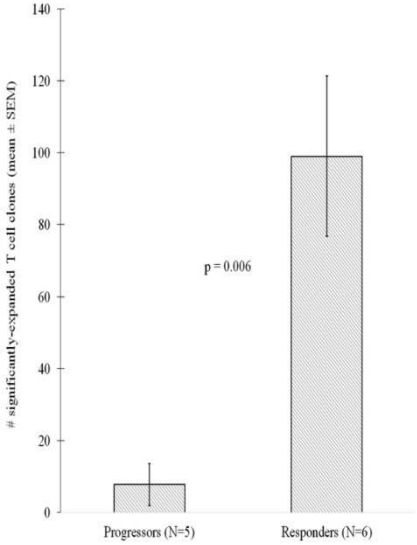
2. Иммунотерапия рулит: куча таргетов, куча испытаний, куча позитивных результатов.

3. Нужно предсказывать её эффективность, обосновывать выбор конкретной иммунотерапии, сочетания с таргетной терапией, вакцинацией, etc.

Structure of TCR repertoire

e.g. – prognosis on the anti-PD1 immunotherapy for melanoma:





Nature. 2014 November 27; 515(7528): 568–571. doi:10.1038/nature13954.

PD-1 blockade induces responses by inhibiting adaptive immune resistance

Anti-cancer TCRs for T cell therapy

- MHC-multimers - identification of T cells bearing specific TCRs
- activation marker expression following antigen stimulation
- antigen-induced proliferation
- Computational approaches – TCR repertoire + exome sequencing + prediction attempts

High-throughput identification of antigen-specific TCRs by TCR gene capture

Carsten Linnemann[1], Bianca Heemskerk[1,12], Pia Kvistborg[1,12], Roelof J C Kluin[2], Dmitriy A Bolotin[3], Xiaojing Chen[4,5], Kaspar Bresser[1], Marja Nieuwland[2], Remko Schotte[1,6], Samira Michels[1], Raquel Gomez-Eerland[1], Lorenz Jahn[7], Pleun Hombrink[7], Nicolas Legrand[6,8,11], Chengyi Jenny Shu[1], Ilgar Z Mamedov[3], Arno Velds[2], Christian U Blank[1], John B A G Haanen[1], Maria A Turchaninova[3], Ron M Kerkhoven[2], Hergen Spits[6,8,9], Sine Reker Hadrup[10], Mirjam H M Heemskerk[7], Thomas Blankenstein[4,5], Dmitriy M Chudakov[3], Gavin M Bendle[1,11,13] & Ton N M Schumacher[1,13]

nature medicine

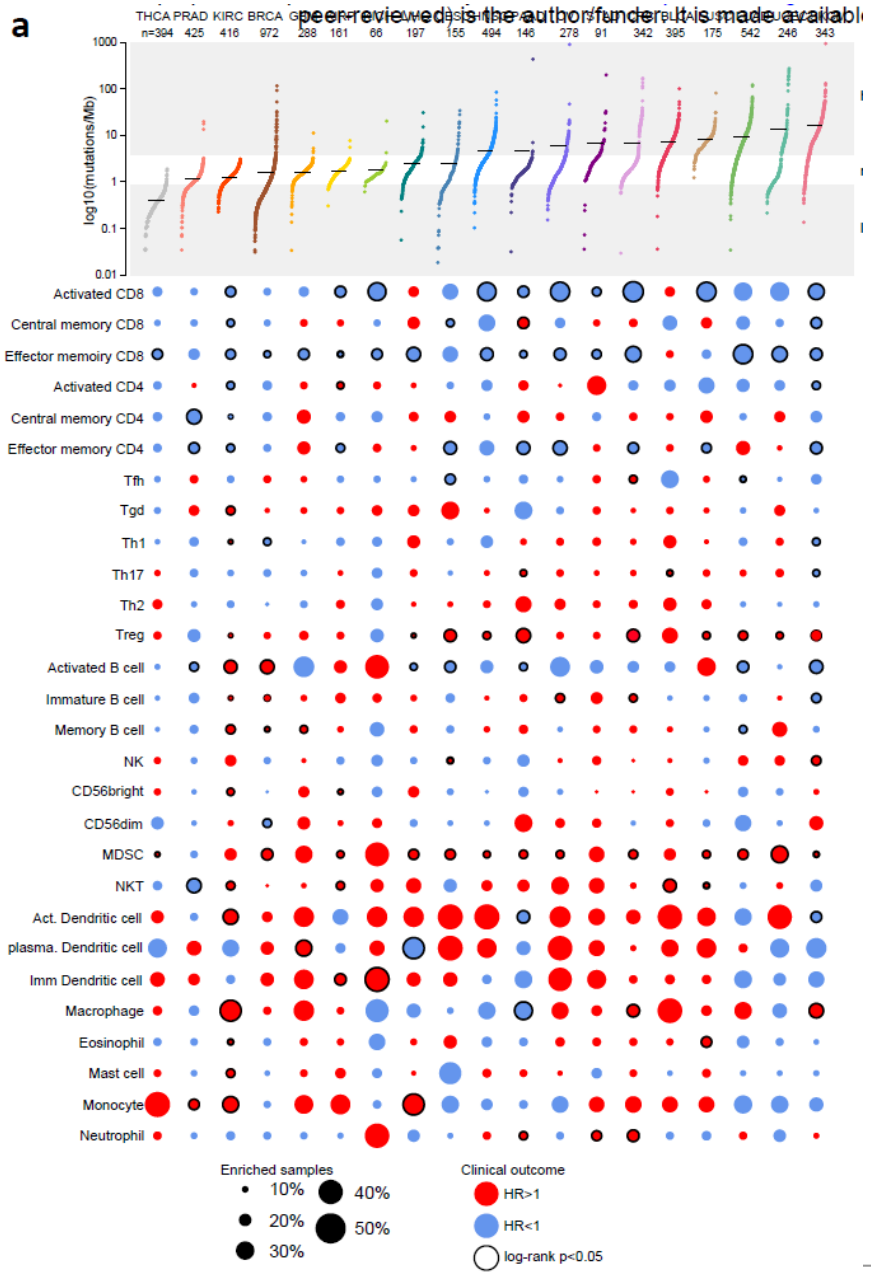
NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

Prof. Ton Schumacher

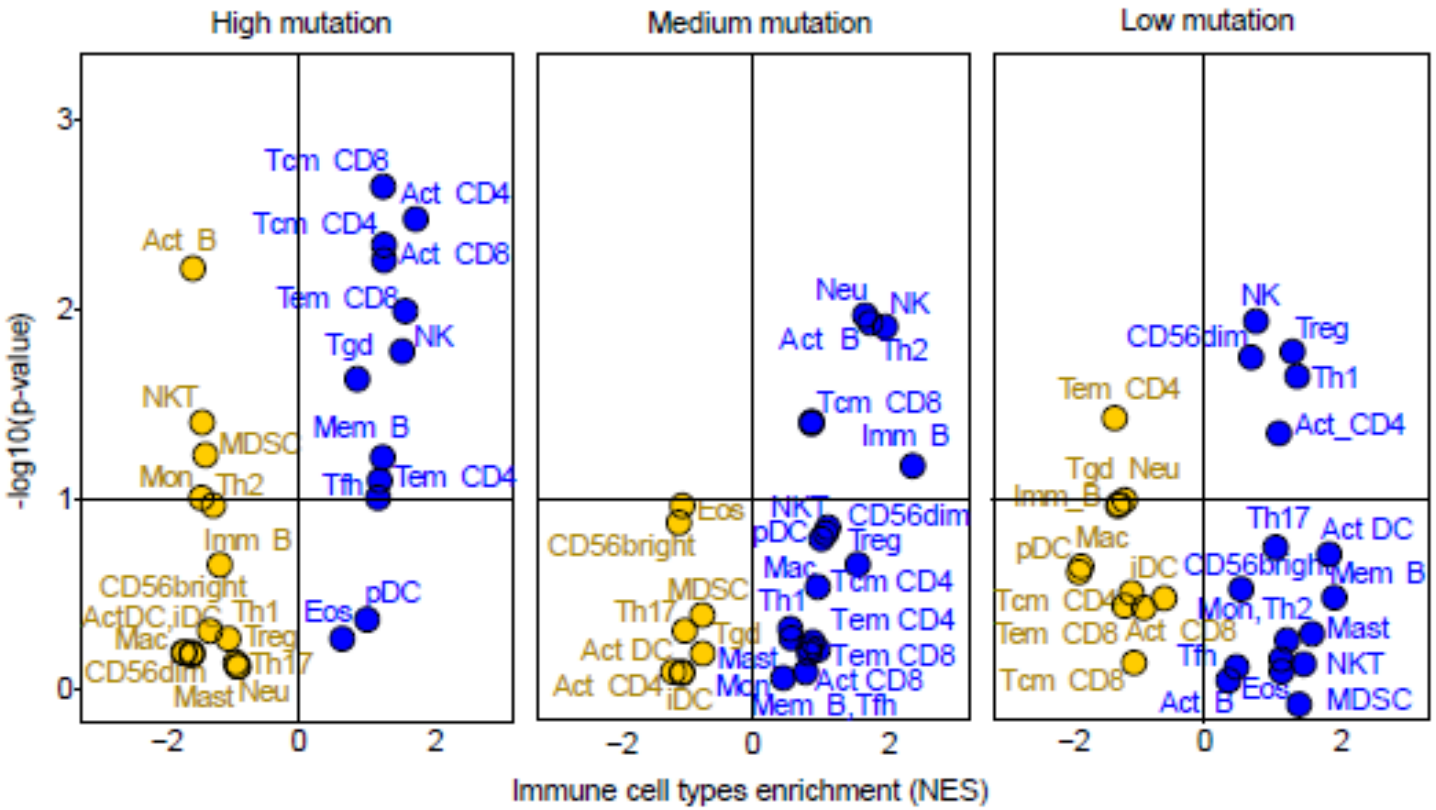
4. куча информации в RNAseq:

- представленность сабсетов иммунных клеток
- экспрессия иммуномодуляторов и проч – способы ухода от иммунной системы
- репертуар ТКР (крупные клоны)
- драйверные мутации – таргетная терапия
- HLA+неоантигены
- germline antigens
- Pathways – таргетная терапия, иммунотерапия

peer-reviewed) is the author/funder. It is made availabl

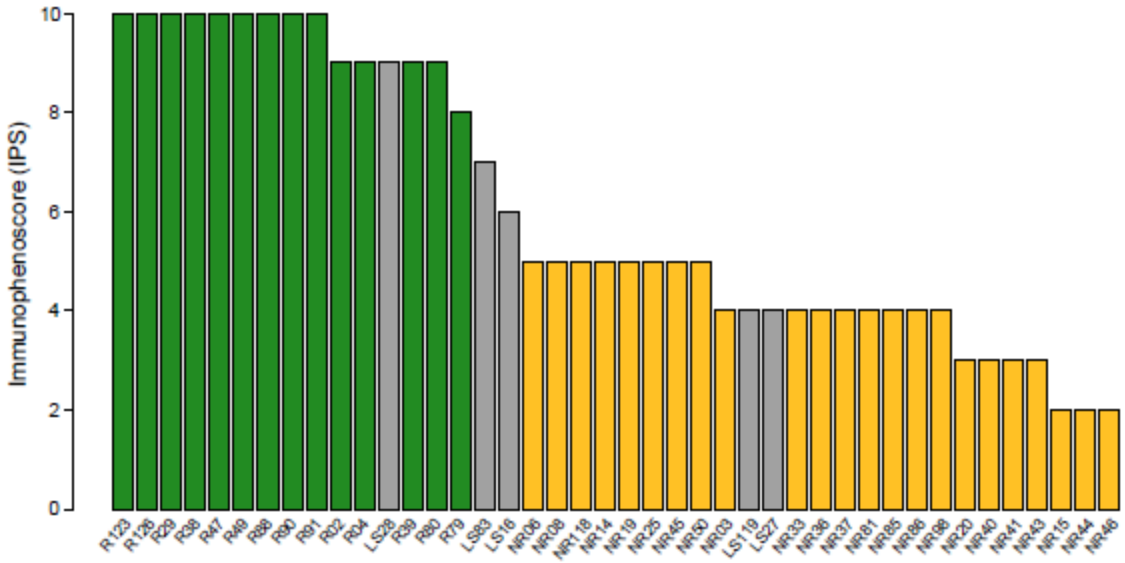


Zlatko, in press



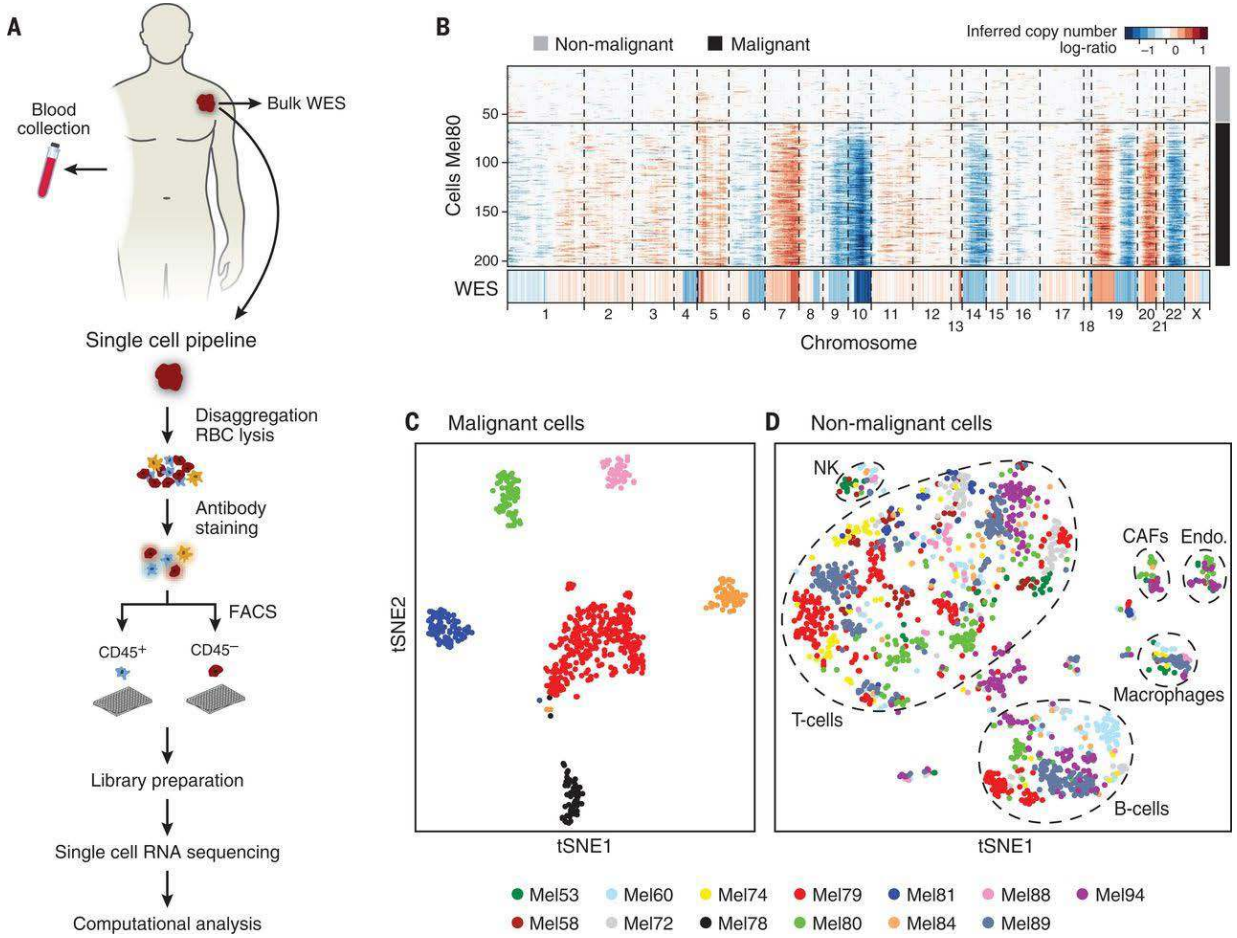
Zlatko, in press

Immunophenoscore



Zlatko, in press

Single cell transcriptomics



Science, 2016

Some key Collaborations

CEITEC
Central European Institute of Technology
BRNO | CZECH REPUBLIC

Dr. Sarka Pospisilova

Prof. Yuri Lebedev

MEMORIAL SLOAN-KETTERING CANCER CENTER
ESTABLISHED 1884

Prof. Alexander Rudensky

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

Prof. Ton Schumacher

ИНСТИТУТ ИММУНОЛОГИИ
МОСКВА

Prof. Ravshan Ataullakhanov

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

Prof. David Price

Center Rogacheva, Moscow

Dr. Mikhail Maschan

UNIVERSITY OF CAMBRIDGE

Prof. Hill Gaston