Next generation sequencing based tests regulation for accelerated innovation in precision medicine

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«Precision medicine» vs «personalized medicine»

Precision medicine is an innovative approach to tailoring disease prevention and treatment that takes into account differences in people's genes, environments, and lifestyles.

The goal of precision medicine is to target the right treatments to the right patients at the right time.

“Personalized medicine” is an older term with a meaning similar to “precision medicine.”

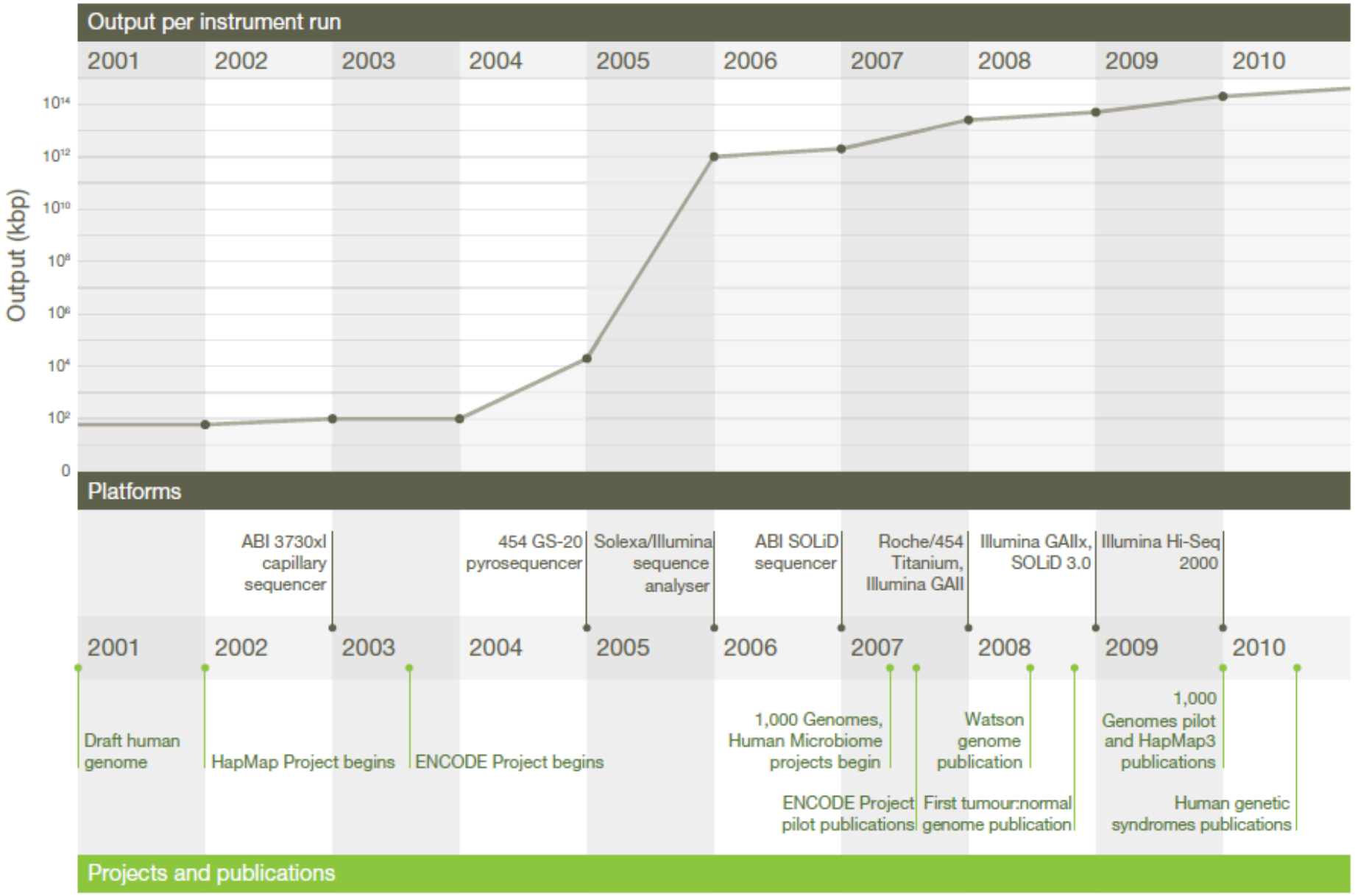
Concern that the word “personalized” could be misinterpreted to imply that treatments and preventions are being developed uniquely for each individual.

INTRODUCTION

The terms “Next generation sequencing (NGS)”, “massively parallel or deep sequencing” describe DNA sequencing technology which has revolutionized genomic research.

Using NGS an entire human genome can be sequenced within a single day.

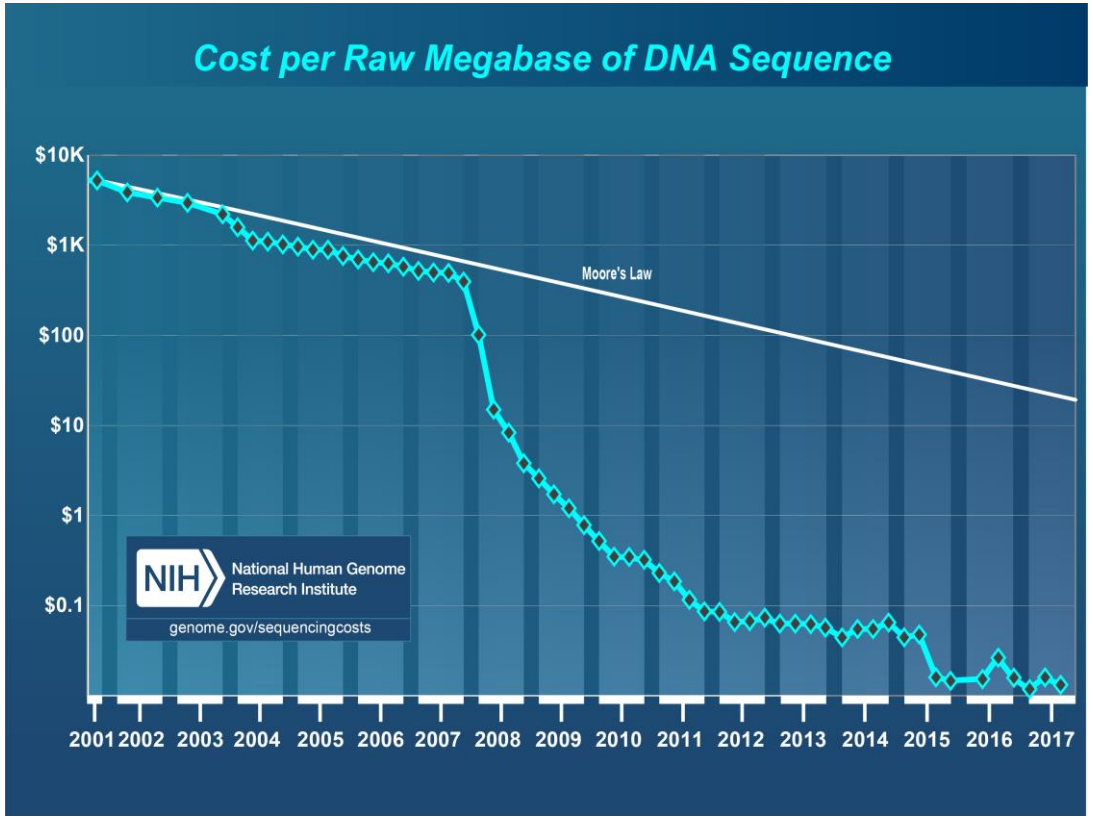
In contrast, the previous Sanger sequencing technology, used to decipher the human genome, required over a decade to deliver the final draft.

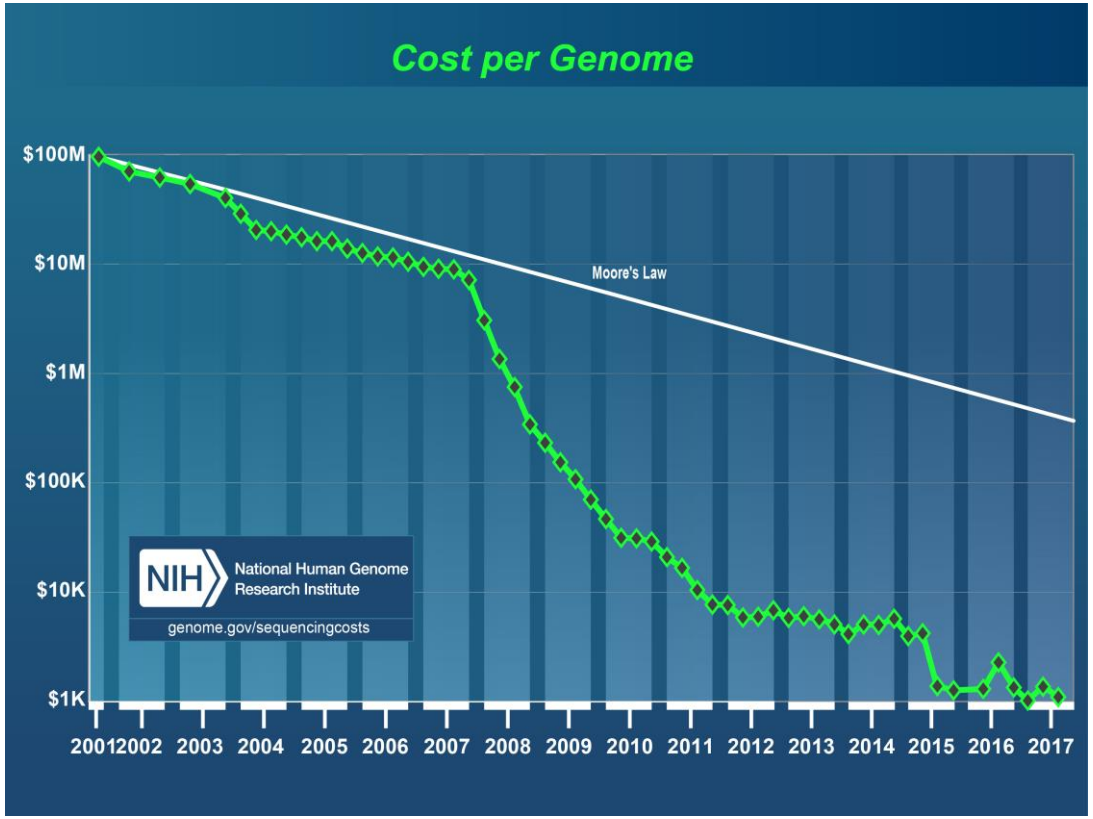


Changes in instrument capacity and the timing of major sequencing projects

Mards ER. Nature. 2011

DNA Sequencing Costs

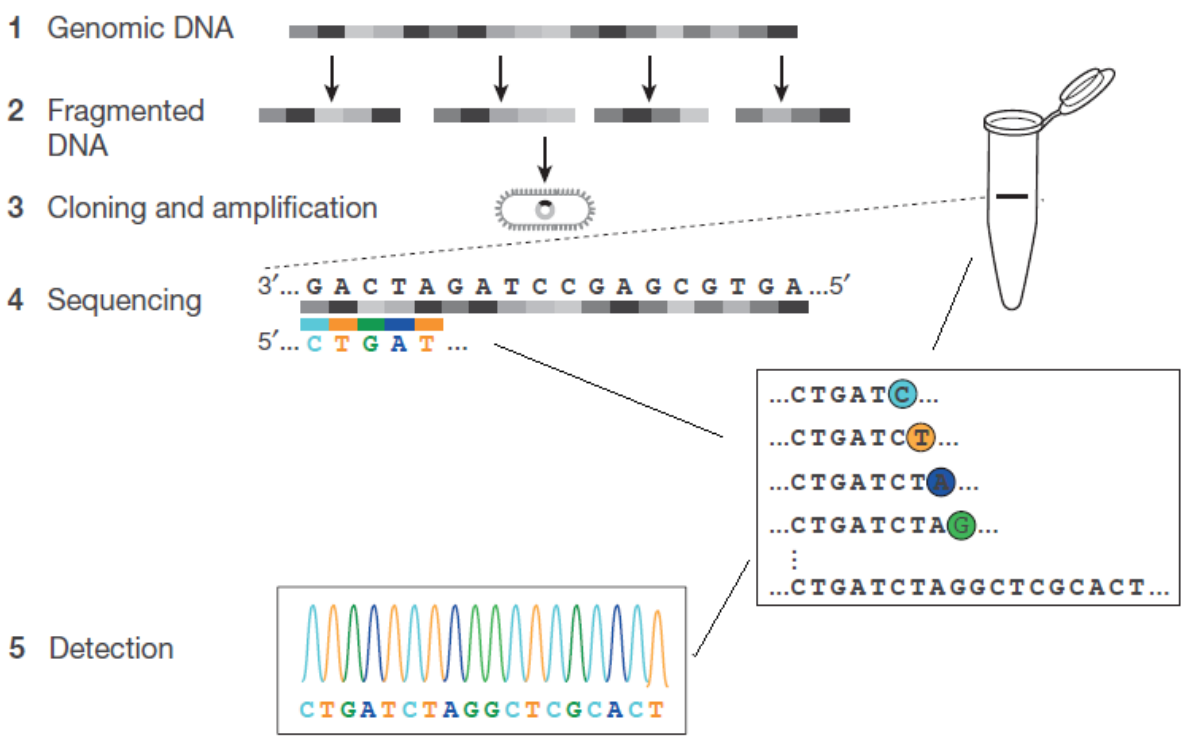




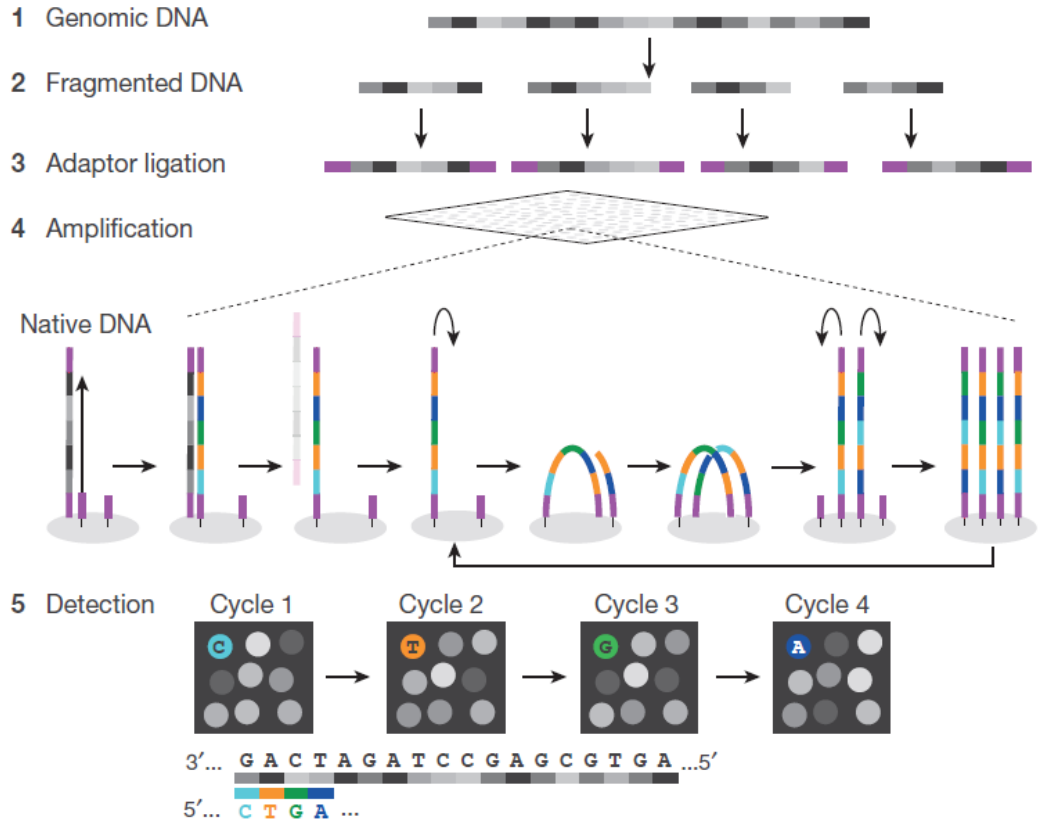
The cost of sequencing a whole genome has dropped 1000-fold

DNA sequencing technologies

First generation sequencing (Sanger)



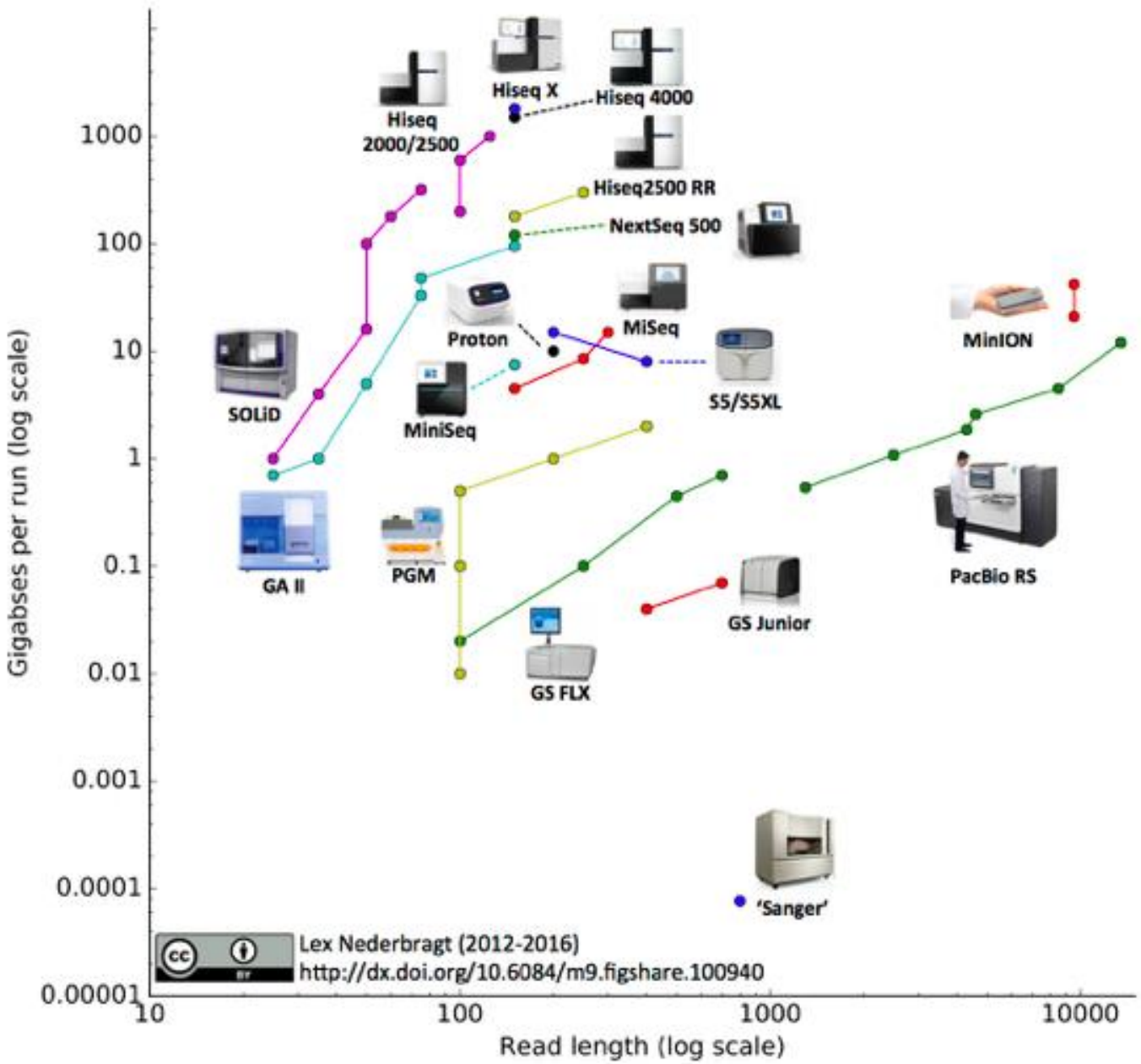
Second generation sequencing (massively parallel)



Adapted from Shendure J et al. Nature. 2017

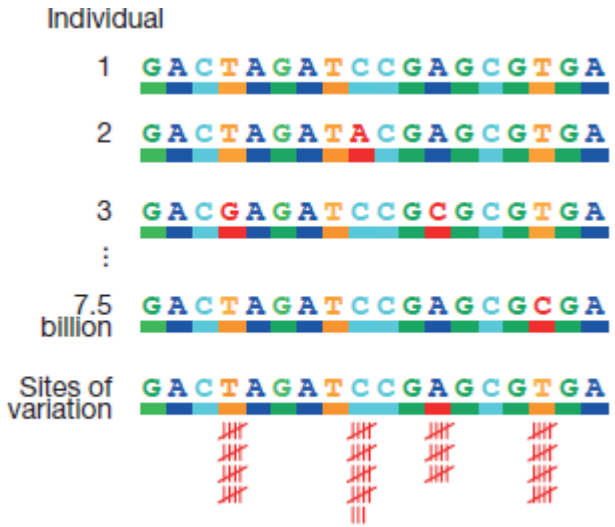
Third generation sequencing (Real-time, single molecule)?

Developments in high throughput sequencing



Clinical application of Next generation sequencing

Genome resequencing



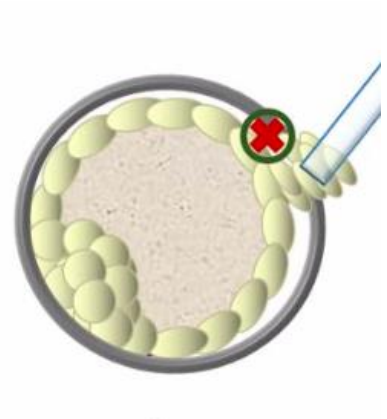
HLA typing

Cystic fibrosis

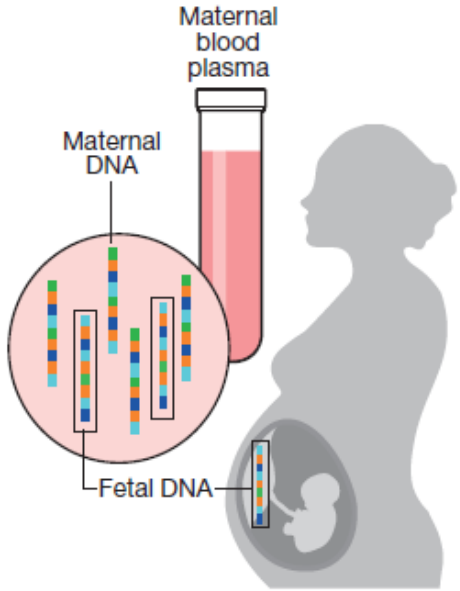
Cancer

Virus, Bacteria

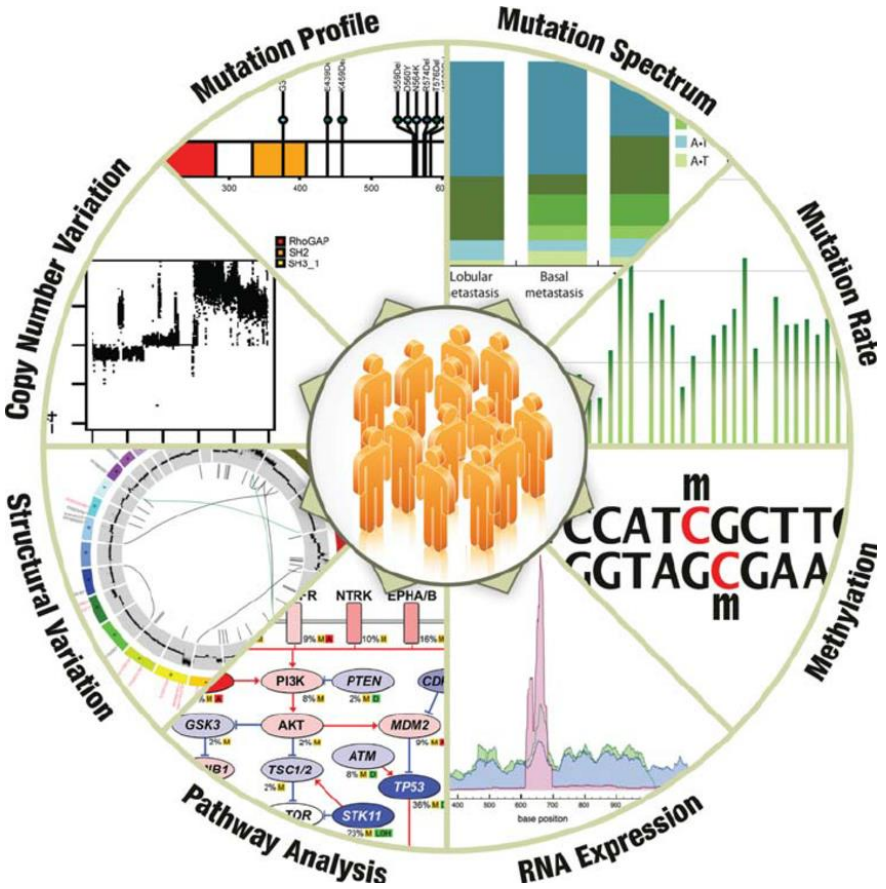
Preimplantation genetic diagnosis for monogenic diseases (PGD) and for aneuploidy testing (PGD-A)



Non-invasive prenatal testing



Landscape of cancer genomics analyses



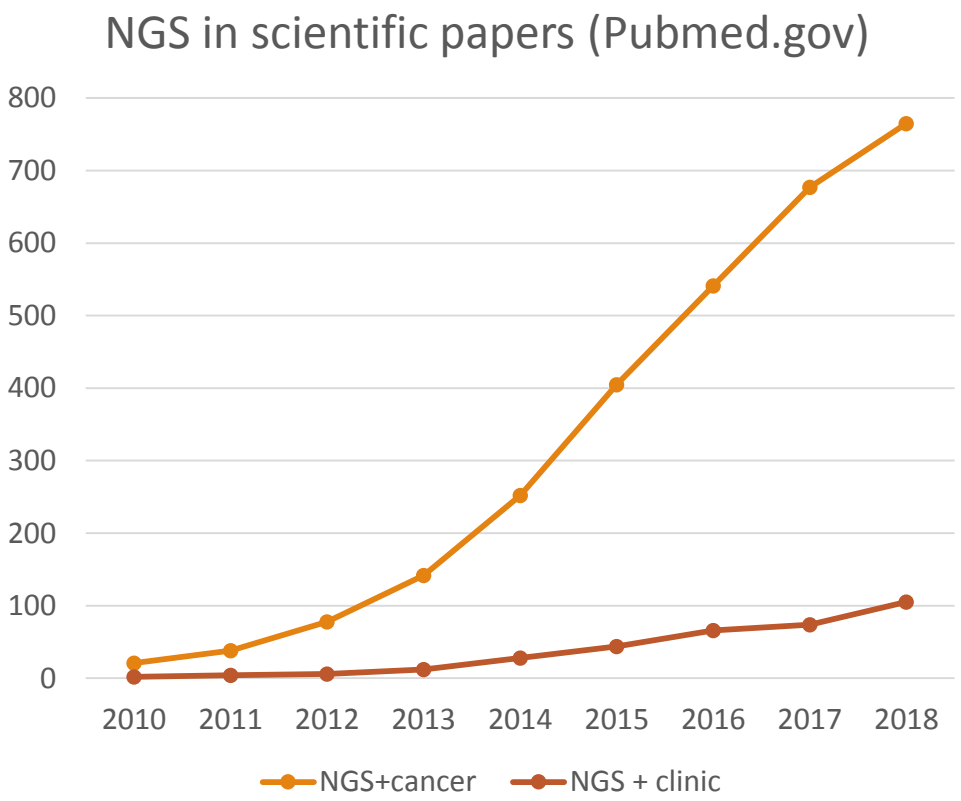
NGS data will be generated for hundreds of tumors from all major cancer types in the near future. The integrated analysis of DNA, RNA and methylation sequencing data will help elucidate all relevant genetic changes in cancers.

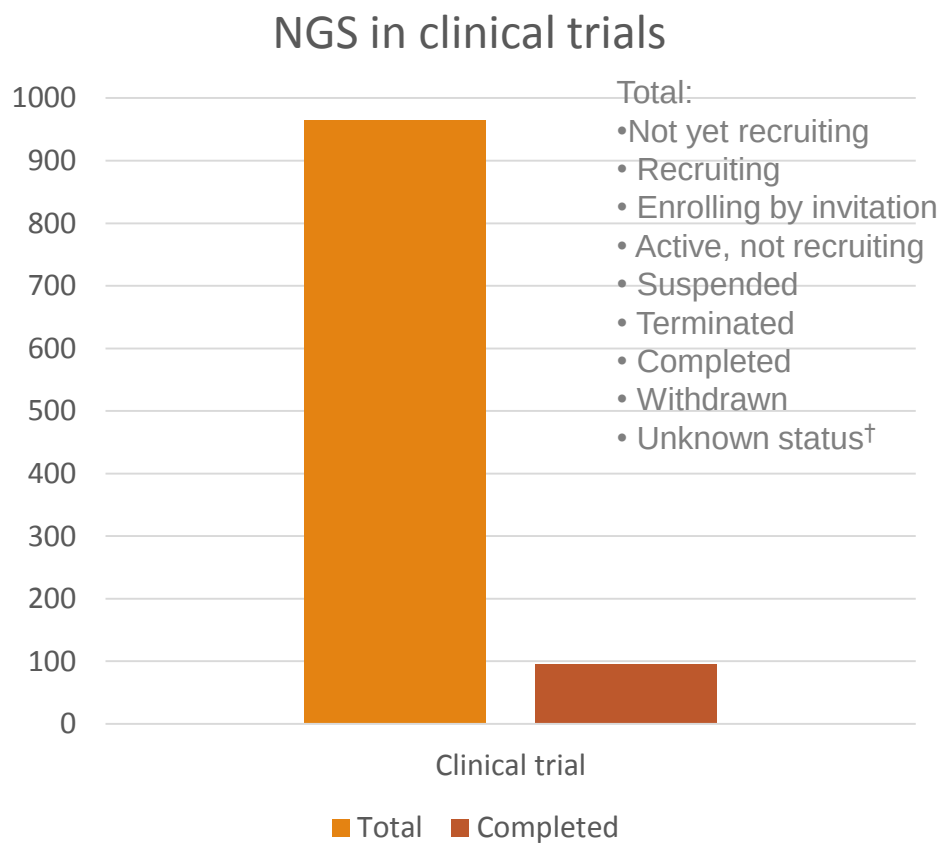
NGS for cancer:

- targeted therapies, based on the mutations present in an individual cancer
- non-invasive diagnosis or monitoring by sequencing of tumour-released circulating cells or cell-free DNA;
- Identification cancer- specific, protein- altering mutations that may serve as neoantigens for ‘personal vaccines’.

Li Ding et al. Human Molecular Genetics, 2010

NGS in scientific and clinical publications / studies





A number of genetic tests rose to more than 55,000 for over 11,000 conditions

NGS utility

Use of Next-Generation Sequencing Tests to Guide Cancer Treatment: Results From a Nationally Representative Survey of Oncologists in the United States.

Overall, 75.6% of oncologists (total 1281) reported using NGS tests to guide treatment decisions.

Of these oncologists, 34.0% used them often to guide treatment decisions for patients with advanced refractory disease,

29.1% to determine eligibility for clinical trials, and

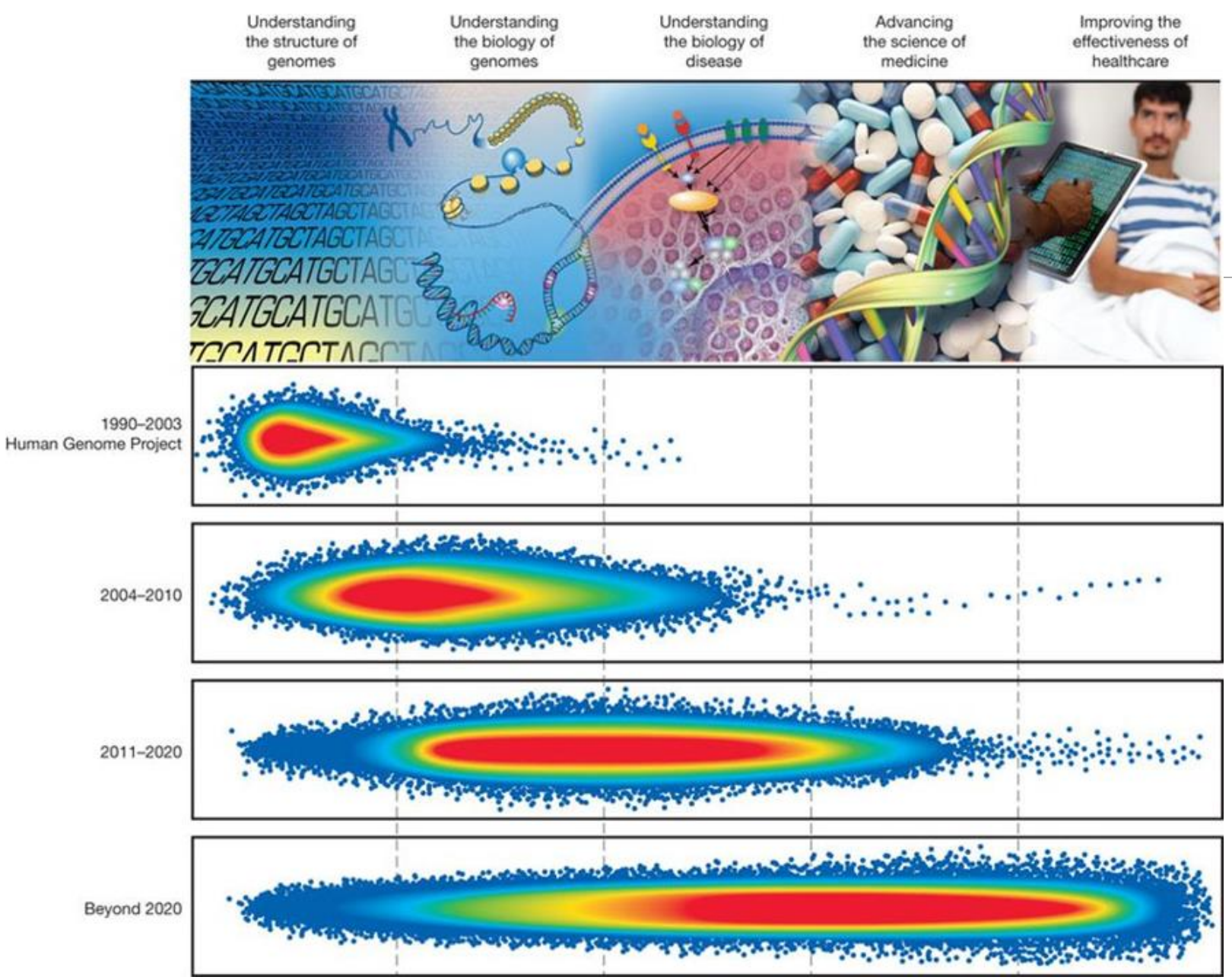
17.5% to decide on off-label use of Food and Drug Administration–approved drugs.

NGS test results informed treatment recommendations often for 26.8%, sometimes for 52.4%, and never or rarely for 20.8% of oncologists.

Oncologists younger than 50 years of age, holding a faculty appointment, having genomics training, seeing more than 50 unique patients per month, and having access to a molecular tumor board were more likely to use NGS tests.

N. Freedman, et al. (2018). Use of Next-Generation Sequencing Tests to Guide Cancer Treatment: Results From a Nationally Representative Survey of Oncologists in the United States. JCO Precision Oncology. 1-13.

Rapid adoption of NGS technology in medicine



The progression from base pairs to bedside is depicted in five sequential, overlapping domains.

Rapid adoption of NGS technology in medicine has lead to the identification and curation of novel genetic variants that promise to improve diagnostic accuracy and reduce unnecessary healthcare costs.

ED Green et al. Nature 470, 204-213 (2011) doi:10.1038/nature09764

NGS is a medical device

3.20 In Vitro Diagnostic (IVD) Medical Device: 'In Vitro Diagnostic (IVD) medical device' means a medical device, whether used alone or in combination, intended by the manufacturer for the in-vitro examination of specimens derived from the human body solely or principally to provide information **for diagnostic, monitoring** or compatibility purposes.

NOTE 1: IVD medical devices include reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles and are used, for example, for the following test purposes: diagnosis, aid to diagnosis, screening, monitoring, predisposition, prognosis, prediction, determination of physiological status.

NOTE 2: In some jurisdictions, certain IVD medical devices may be covered by other regulations.

(GHTF/SG1/N071:2012)

Examples of NGS based medical device

GENDX

NGSgo® & NGSengine for HLA typing
Complete workflow: reagents & software, Platform independent^ For HLA-A, B, C, DRB1, DQB1, DPB1, DQA1, DPA1, DRB3/4/5, G

illumina®

Praxis Extended RAS Panel
FDA-approved NGS *in vitro* diagnostic for evaluating RAS mutations in colorectal cancer to determine patient eligibility for treatment with Vectibix®

illumina®

VeriSeq NIPT Solution
Reagents, instruments, and CE-IVD marked library prep and analysis/reporting software in an automated workflow for in-lab prenatal aneuploidy screening

ThermoFisher SCIENTIFIC

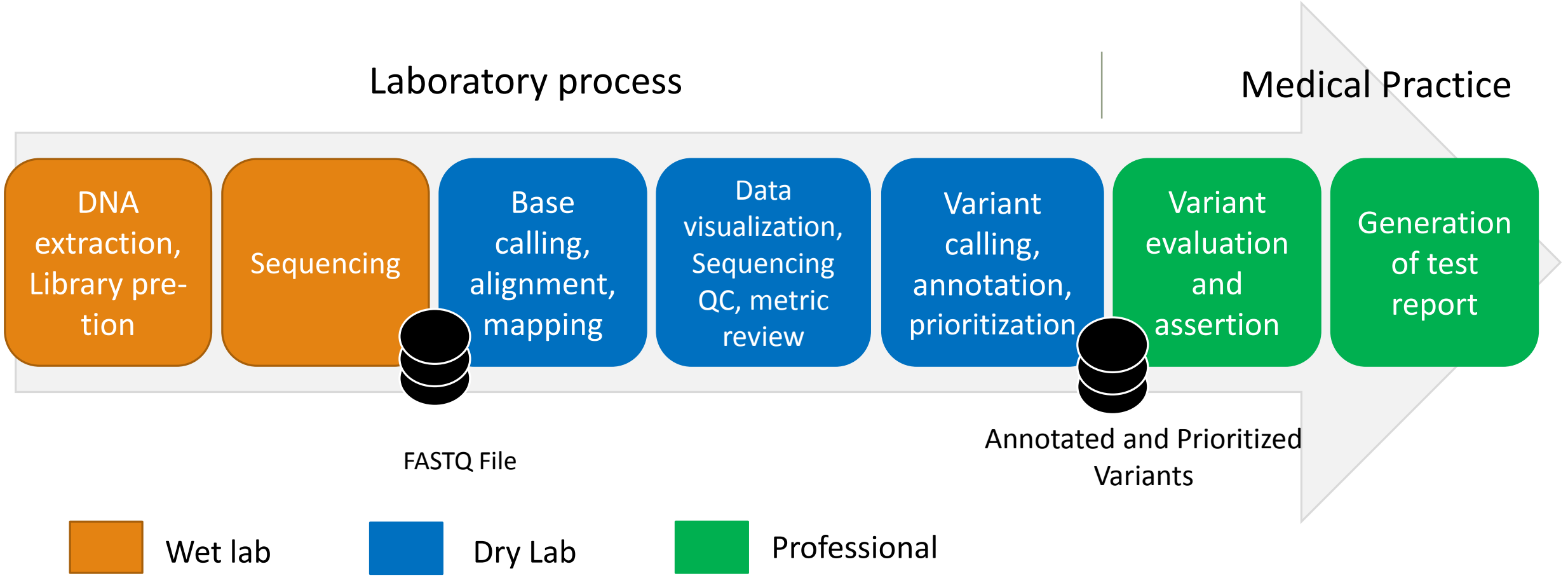
Oncomine Dx Target Test
FDA-approved TNGS in vitro diagnostic to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 from RNA from patients with non-small cell lung cancer (NSCLC)

Problem 1. Differences between NGS based tests and routine MD

While most IVDs are typically intended to detect a limited number of predefined analytes to diagnose pre-specified conditions, NGS-based tests can measure millions of analytes (i.e., bases) related to numerous conditions and have the potential to detect previously unidentified variants.

NGS-based tests often have broad intended uses, and specific variants and the nature of the clinical information that will be returned from these tests is often not known until after the test has been run.

NGS workflow



Problem 2. New metrics

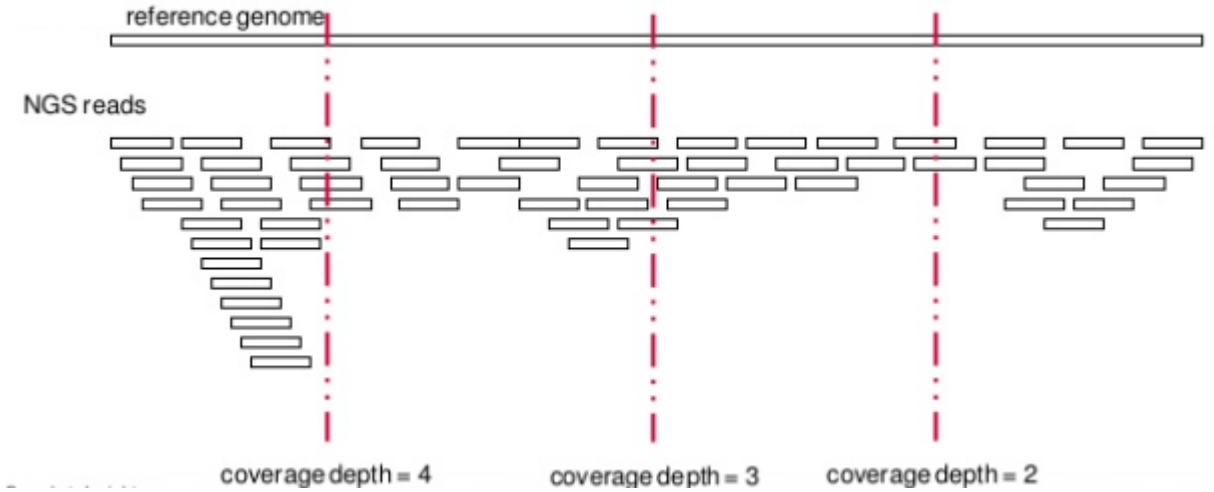
Quality Control (QC) Metrics for NGS

QC metrics	Description
Sample Quality Control:	Usually NGS experiment with high-quality DNA and/or RNA samples. However, many experiments are performed with degraded nucleic acids.
Library Quality Control:	Before sequencing most NGS libraries are checked to verify the insert size is as expected and that there are no contaminating adapter-dimers
Sequencing Quality Control:	Criteria for minimum sequencing depth and other quality metrics (% reads mapped to target regions, % of targets with specified coverage, duplication rate).
Yield:	Yield is the number of bases generated in the run.
Error Rate:	Refers to the percentage of bases called incorrectly at any one cycle.
%Q30	The percentage of bases with a quality score of 30 or higher, respectively.
Variant detection and review	Variant allele frequency, local sequencing depth and quality score
Cluster PF (%):	In Illumina clustering a single molecule should generate a single cluster with a clear signal in the base being sequenced.

NGS analysis. Sequencing depth

Depth of coverage. Per-base coverage is the average number of times a base of a genome is sequenced. The coverage depth of a genome is calculated as the number of bases of all short reads that match a genome divided by the length of this genome. It is often expressed as 1X, 2X, 3X,... (1, 2, or, 3 times coverage).

Breadth of coverage. Breadth of coverage is the percentage of bases of a reference genome that are covered with a certain depth. For example: 90% of a genome is covered at 1X depth; and still 70% is covered at 5X depth.



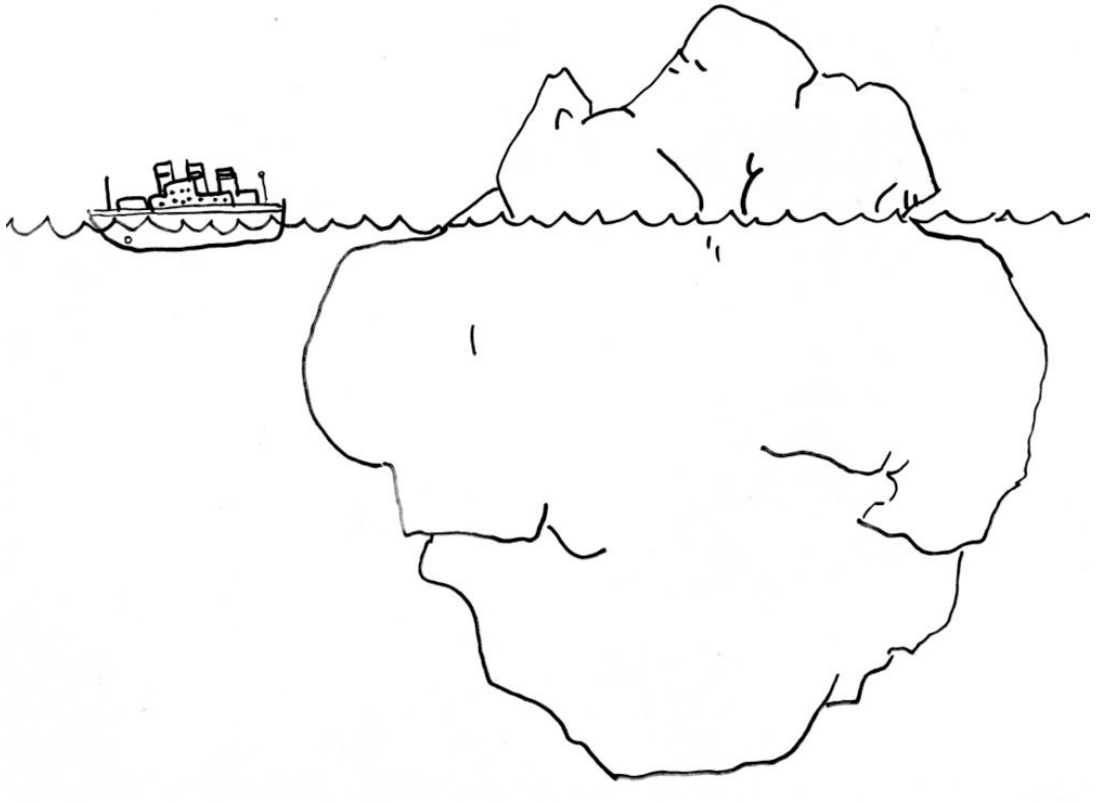
Problem 3. Database

The NGS which tends to produce raw data of size as large as 1TB per sample often poses difficulty for the information mining and reasonable interpretation. The word's current sequencing limit is evaluated to be 13 quadrillion DNA bases per year. The high volume of biological data generated through NGS across the world in research laboratories are instances of Big Data.

Recently, the NIH-financed 1000 Genomes Projects have resulted in 200 terabytes of crude sequencing information, which were submitted to the GenBank during the initial six months of operation, which is twice the extent that had been stored into all the GenBank during the last 30 years.

What about availability and safety data?

Problem 4. Validation of NGS based tests



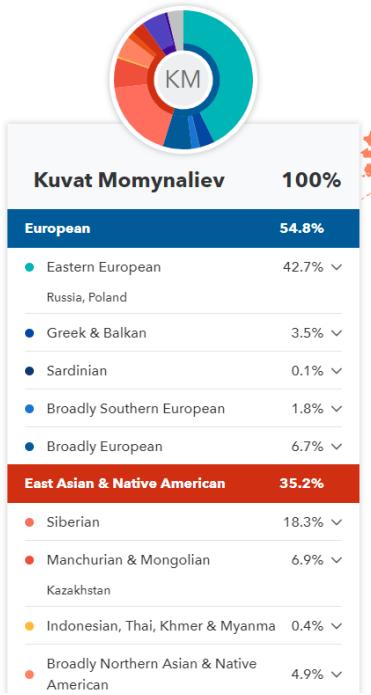
Usually, a limited number of mutations is claimed for registration, which are then validated, but it turns out that the data was obtained also for other mutations as well.

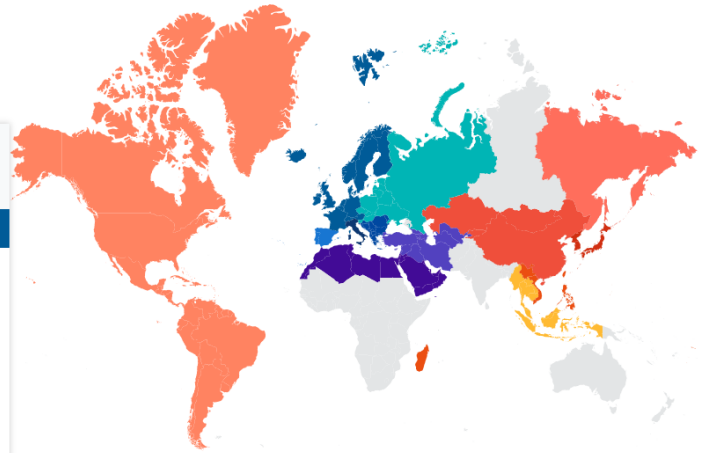
How to be in the case of the whole genome?

It is impossible to validate the entire genome using the method traditional method of comparison

New approaches?

Problem 4. Validation of NGS based tests.





genome_Kuvat_Momynaliev_v4_Full_20190305012733 — Блокнот

Файл Правка Формат Вид Справка

```
# This data file generated by 23andMe at: Tue Mar 05 01:27:33 2019
#
# This file contains raw genotype data, including data that is not used in 23andM
# This data has undergone a general quality review however only a subset of marke
# individually validated for accuracy. As such, this data is suitable only for re
# educational, and informational use and not for medical or other use.
#
# Below is a text version of your data.  Fields are TAB-separated
# Each line corresponds to a single SNP.  For each SNP, we provide its identifier
# (an rsid or an internal id), its location on the reference human genome, and th
# genotype call oriented with respect to the plus strand on the human reference s
# We are using reference human assembly build 37 (also known as Annotation Releas
# Note that it is possible that data downloaded at different times may be differe
# improvements in our ability to call genotypes. More information about these cha
# https://you.23andme.com/p/cc1f6355086428f9/tools/data/download/
#
# More information on reference human assembly builds:
# https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/
#
# rsid  chromosome      position        genotype
rs12564807      1       734462  AA
rs3131972       1       752721  AG
rs148828841     1       760998  CC
rs12124819      1       776546  AA
rs115093905     1       787173  GG
rs11240777      1       798959  AG
rs7538305       1       824398  AA
rs4970383       1       838555  CC
rs4475691       1       846808  CC
rs7537756       1       854250  AA
rs13302982      1       861808  GG
rs55678698      1       864490  CC
i6019299        1       871267  CC
```

Together with the results, I obtained data on 600 thousand of nucleotide polymorphisms.

Is this data accurate?

Problem 6: Scientific Communities and NGS Requirements

In the absence of clear and understandable requirements for the efficacy and safety of a NGS based test for in vitro diagnostics, scientific communities are trying to develop requirements and recommendations for them.

This includes developing various “assessment systems” for diagnostic tests based on NGS, such as the “diagnostic result”, which is defined as the probability that a disease-causing variant will be identified and a molecular diagnosis will be made.

Problem 6: Scientific Communities and NGS Requirements

Roy S et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Recommendation of the Association for Molecular Pathology and the College of American Pathologists. J Mol Diagn. (2018)

Jennings LJ et al. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. (2017)

Gargis AS et al. Good laboratory practice for clinical next-generation sequencing informatics pipelines. Nat Biotechnol. (2015)

Matthijs G et al. Guidelines for diagnostic next-generation sequencing. Eur J Hum Genet. (2016)

Endrullat C et al. Standardization and quality management in next-generation sequencing. Applied & Translational Genomics. (2016)

Possible, some Solutions

For timely patient access to useful genetic tests that have the potential impact on the patient therapy and decision making, regulators probably need to determine the main approaches to the design, development, and validation of NGS-based tests, as well as their applicability to various conditions.

Examples of the creation of local regulatory approaches are currently known (FDA, 2018).

It is obvious that regulatory approaches for NGS-based tests should be balanced in order to ensure adequate introduction of scientific innovations into clinical practice.