How to Start an NGS Lab for Reproductive Health Research

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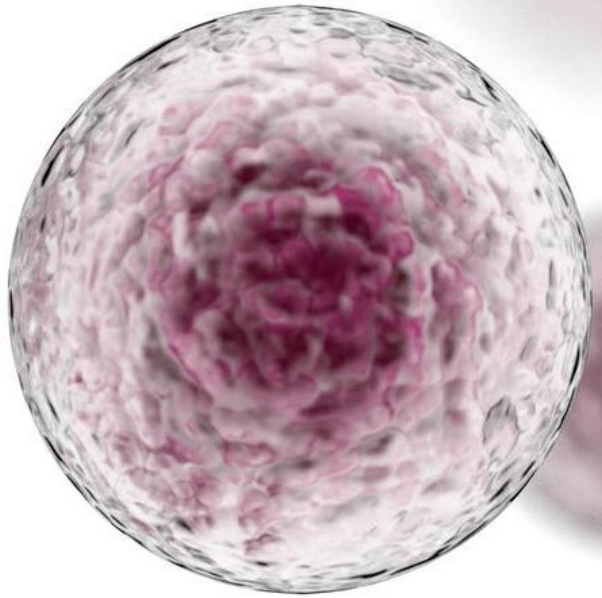
The world leader in serving science

Agenda

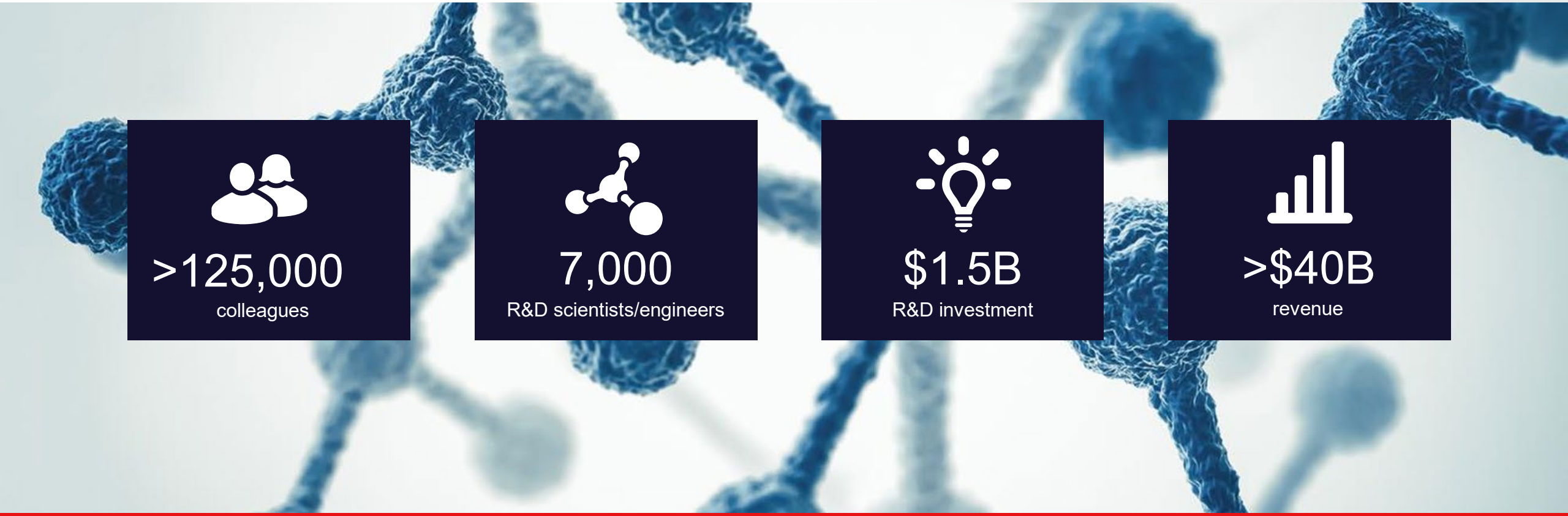
1. Introduction to Preimplantation Genetic Testing
2. PGT Technologies
3. In-house PGT-A with LP-WGS and Semiconductor Seq
4. NGS Laboratory Design
5. Quality Management and Validation



World leader in serving science

ThermoFisher SCIENTIFIC

ThermoFisher SCIENTIFIC | thermo scientific | applied biosystems | invitrogen | fisher scientific | unity lab services | patheon | PPD



>125,000
colleagues

7,000
R&D scientists/engineers

$1.5B
R&D investment

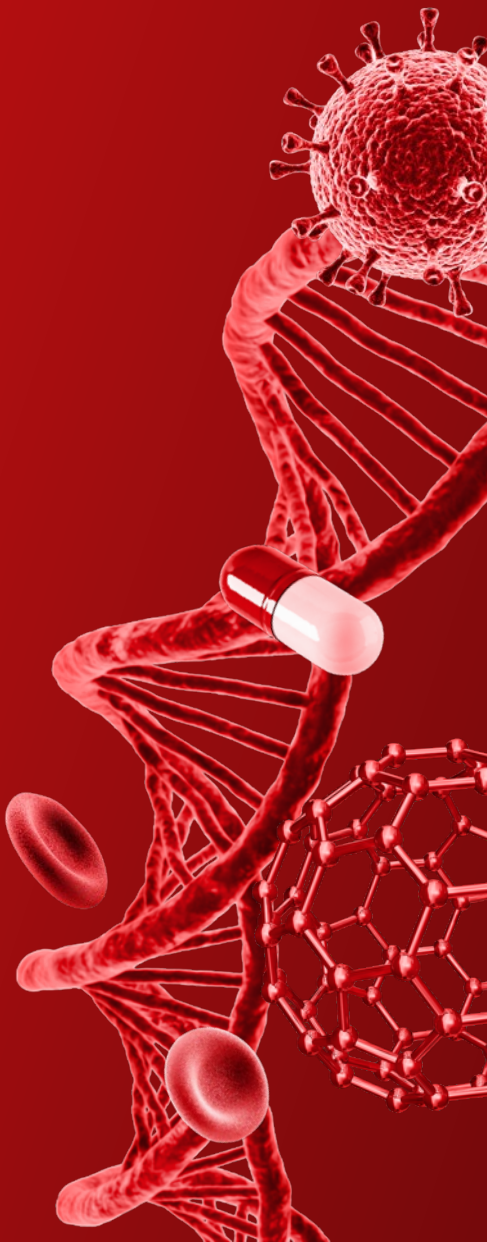
>$40B
revenue

We enable our customers to make the world healthier, cleaner and safer

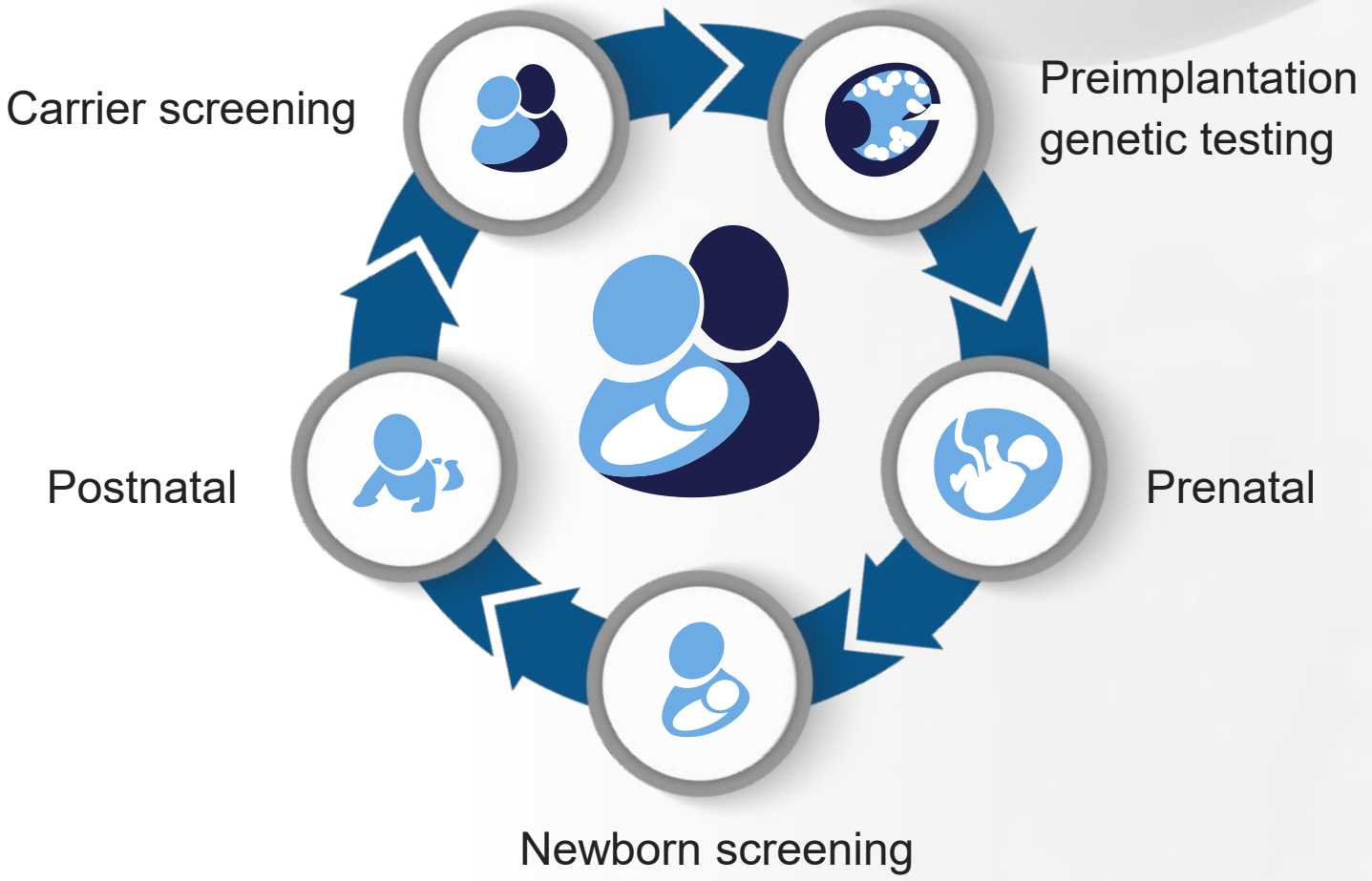
Our Mission is our purpose

We enable our customers to make the world healthier, cleaner and safer

Introduction to Preimplantation Genetic Testing



Complete portfolio of reproductive health research solutions



Design by Alain Rico

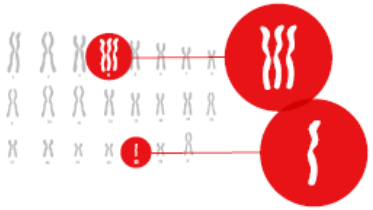
Complete portfolio of reproductive health research solutions

Design by Alain Rico

The different genetic variations

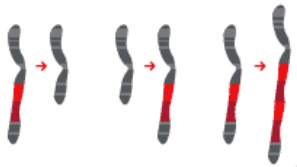
Polyploidy

Cells have more than two sets of chromosomes, a condition that is not compatible with life in humans.



Trisomy or monosomy

The number of copies of a particular chromosome is either one more (trisomy, a total of three) or one less (monosomy, a total of one) than the usual two copies. In some cases, human life is possible with these anomalies. However, they may lead to serious impairments.

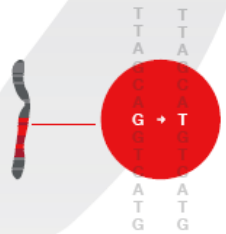


Deletion, insertion, or duplication

Parts of a chromosome are missing, inserted, or duplicated. Gene deletions or duplications lead to copy number variation (CNV).

Inversion or translocation

A part of a chromosome is either inverted or found in a different locus, sometimes even on a different chromosome.



Single-nucleotide polymorphism (SNP)

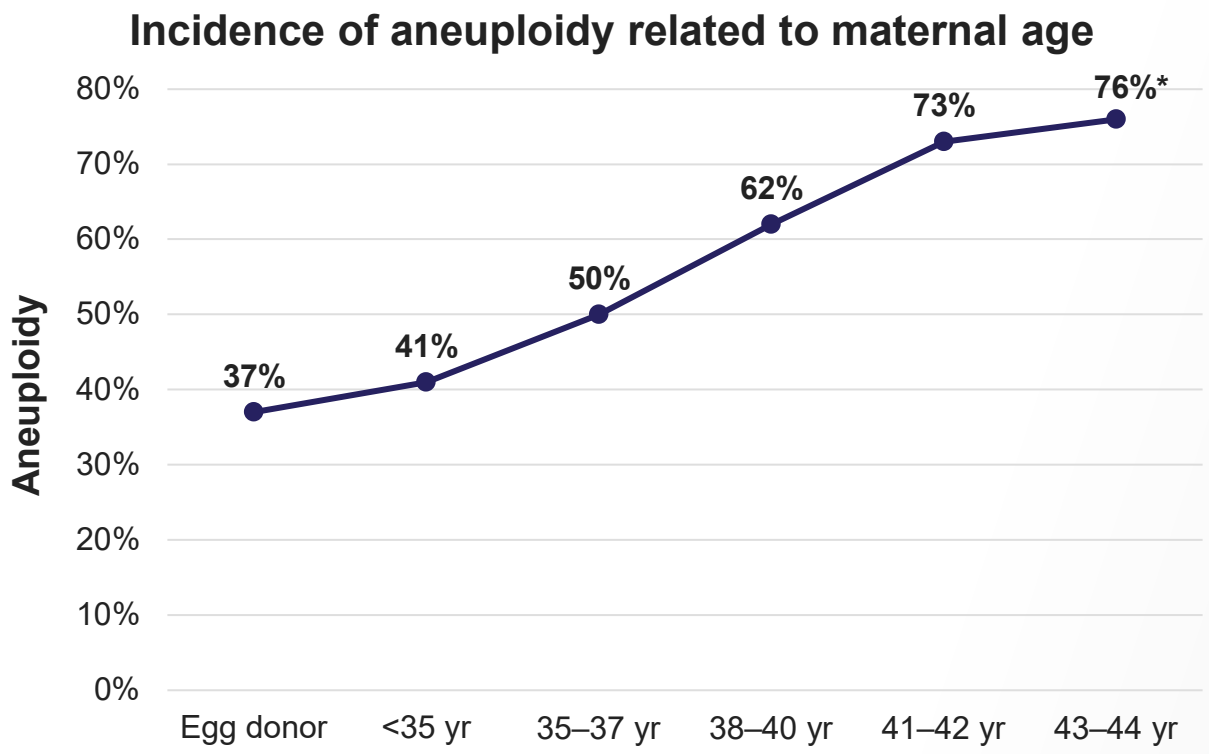
Incorporation of a change in a single nucleotide leads to a shift to another base, or its deletion or duplication. Many congenital diseases are caused by SNPs.

Different types of PGT

PGT-A	PGT-SR	PGT-M
• Preimplantation genetic testing – aneuploidy (PGT-A)	• Preimplantation genetic testing – structural rearrangement	• Preimplantation genetic testing – monogenic disease
• Analysis of chromosomal **aneuploidy**	• Analysis of **structural rearrangements**	• Analysis of **monogenic diseases**
• e.g., Trisomy 16, 22	• e.g., unbalanced translocations	• i.e., single gene disorders
• Previously referred to as PGS	• Previously referred to as PGD	• Previously referred to as PGD

Background

Aneuploidy in blastocyst embryos is common, even in young women, with only a very small percentage of aneuploid embryos surviving to birth. Most losses are due to failed implantation or miscarriage.



* ANOVA; *P* <0.05

Rubio C, Rodrigo L, Garcia-Pascual C, et al. (2019) *Biol Reprod.* doi:10.1093/biolre/ioz019.

"The prevalence of aneuploidy in human blastocysts obtained *in vitro* is between 30% and 85%."

Franasiak JM, Forman EJ, Hong KH, et al. (2014) *Fertil Steril.* doi:10.1016/j.fertnstert.2013.11.004.

"The risk of spontaneous miscarriage is between 10% and 65%."

Heffner LJ. (2004) *N Engl J Med*. doi:10.1056/NEJMp048087.

"The risk of aneuploidy in a human fetus is between 0.2% and 3%."

Hassold T and Hunt P. (2001) *Nat Rev Genet*. doi:10.1038/35066065.

PGT sampling methods

Invasive

Trophectoderm Biopsy (TE)

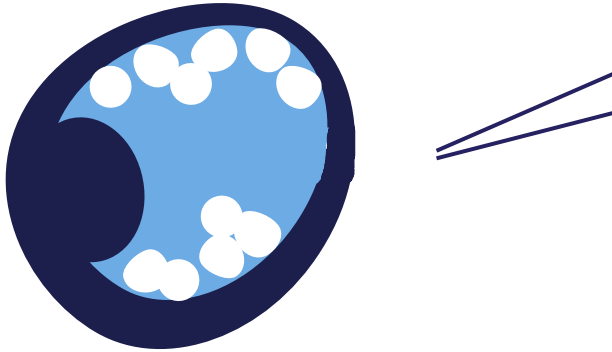
- 3-5 cells extracted through trophectoderm laser opening
- TE biopsy contains 3.9% - 15.6% of total embryo cells
- Not suitable for fragile embryos

Minimally Invasive

Blastocoele Fluid (BF)

- Probe punctures the zona pellucida to aspirates fluid from the blastocoel cavity
- Fragmented/low quality DNA
- Variable volume for sampling

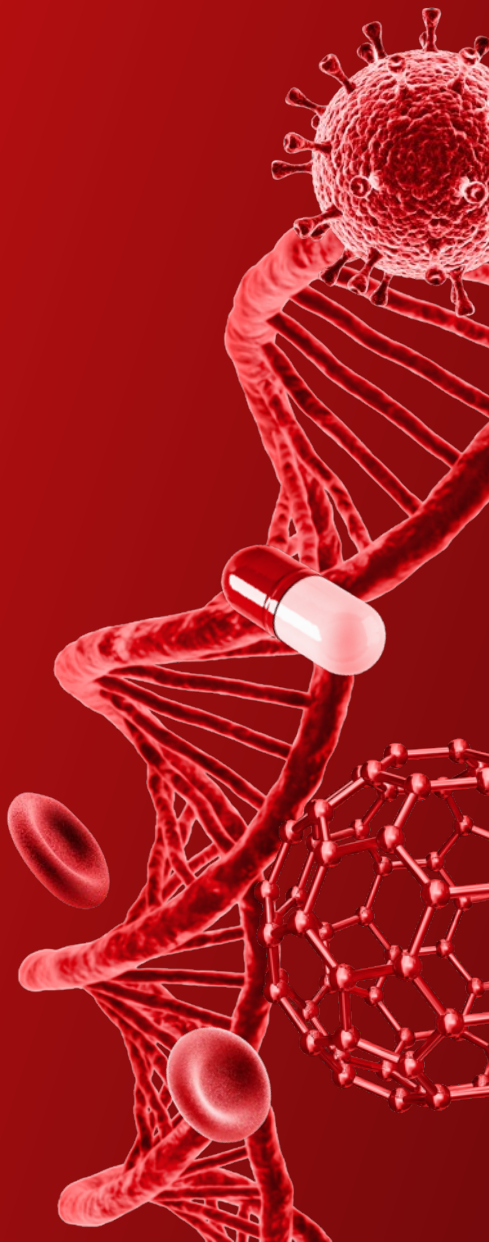
Non-Invasive



Spent Culture Media (SCM)

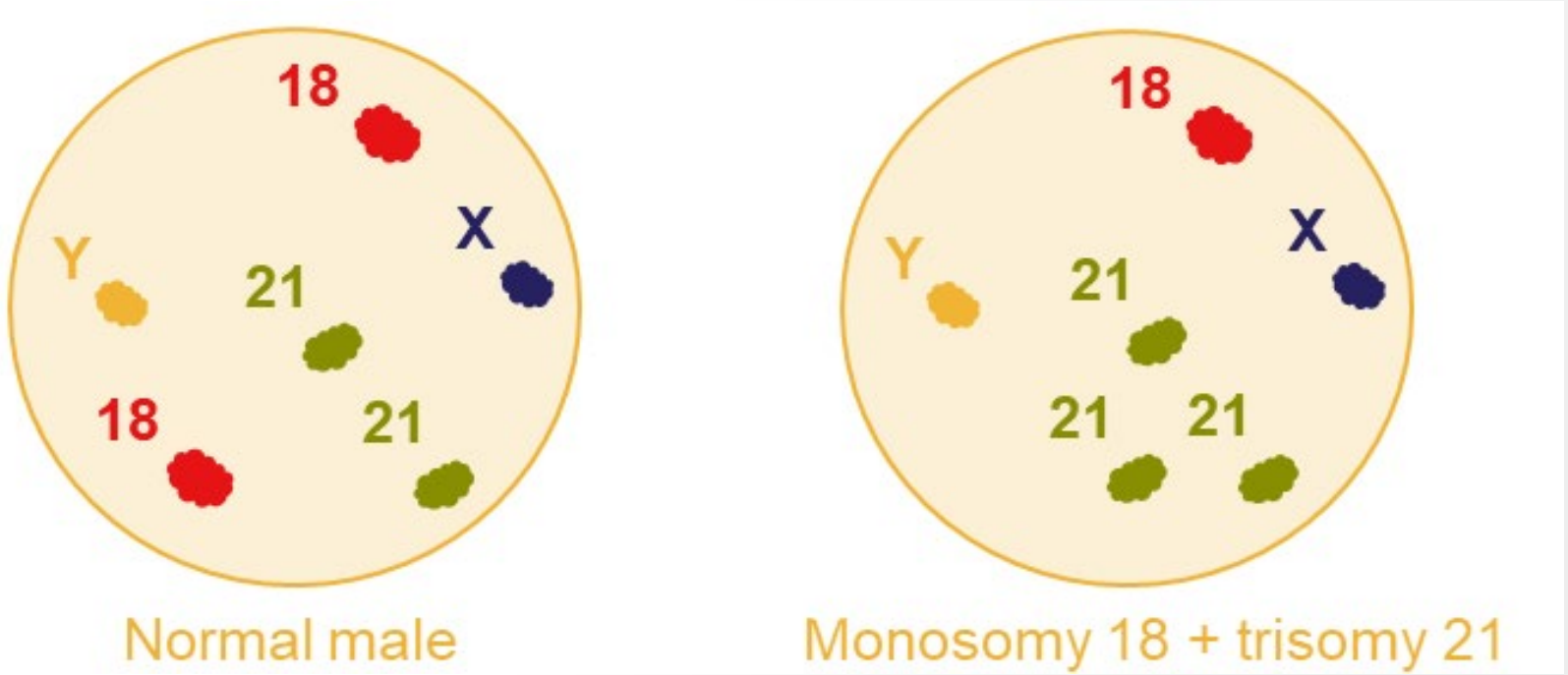
- Requires striping of cumulus cells
- Expressed DNA may not reflect entire embryo chromosome state
- Due to the small amounts of DNA, contamination can bias results

PGT Technologies



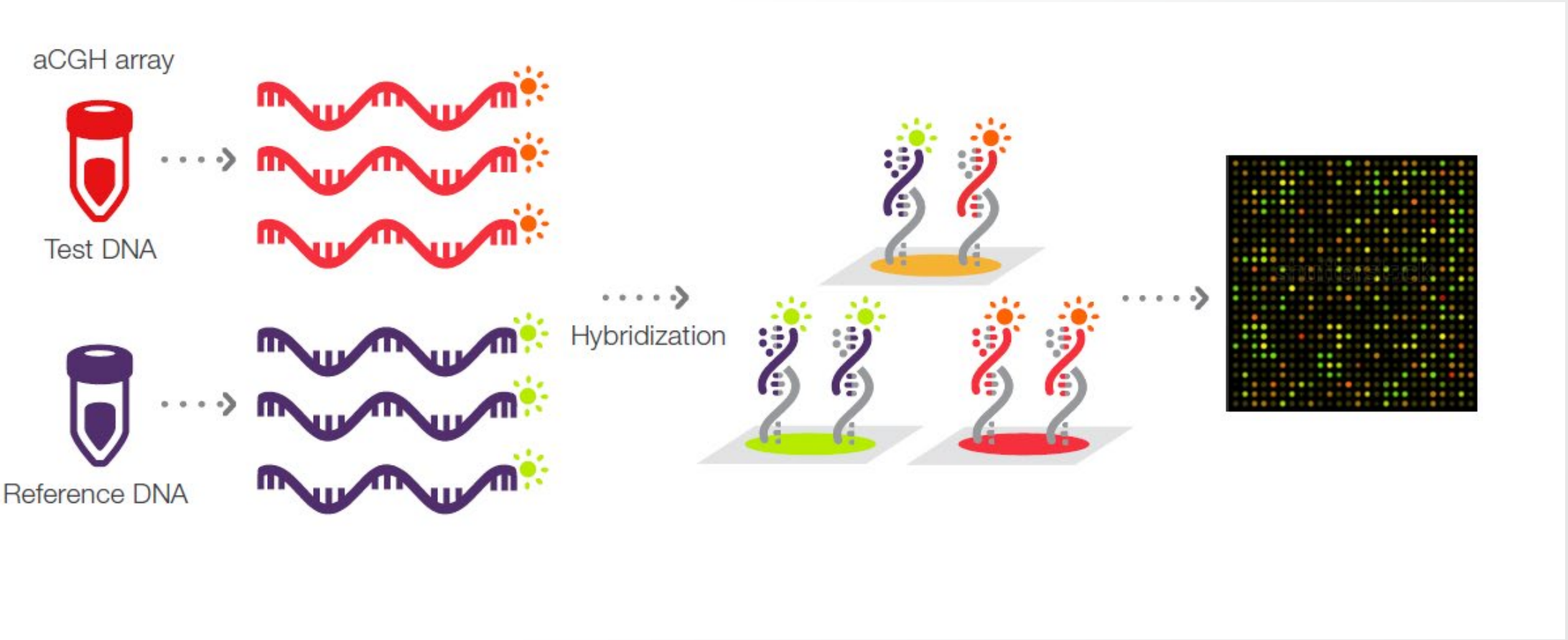
Fluorescent In Situ Hybridization

FISH



Array Comparative Genomic Hybridization

aCGH



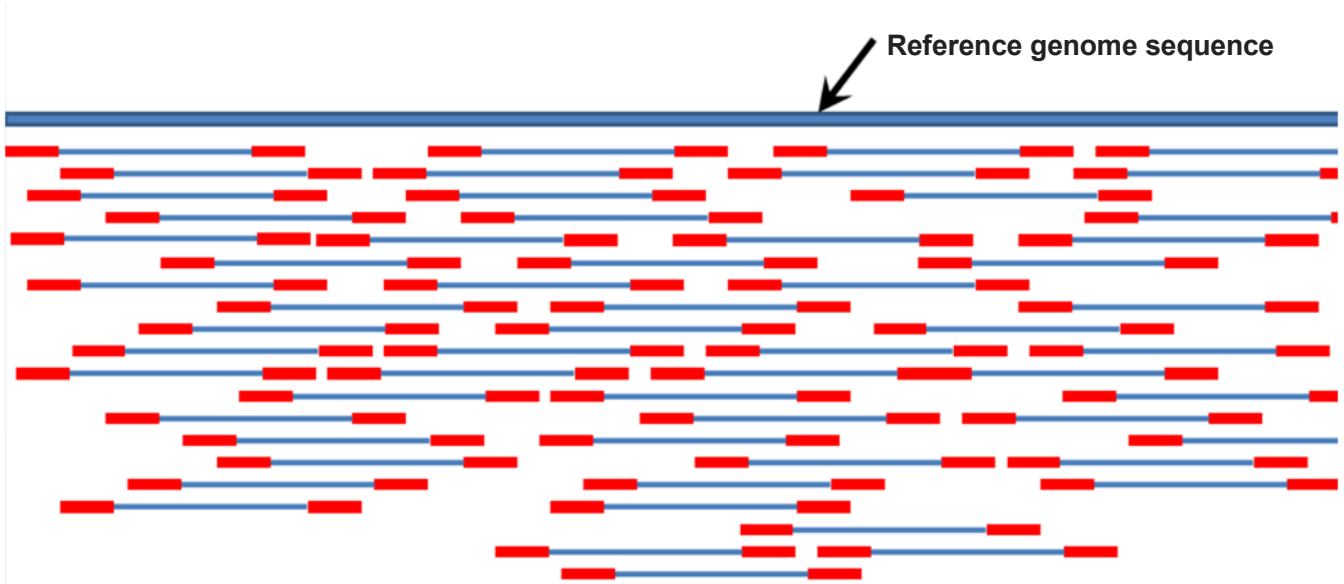
Source: Thermo Fisher Scientific Digital Asset Management tool – item EXT2267

Single Nucleotide Polymorphism Array

SNP array

Source: Thermo Fisher Scientific Digital Asset Management tool – item EXT2267

What is next-generation sequencing?



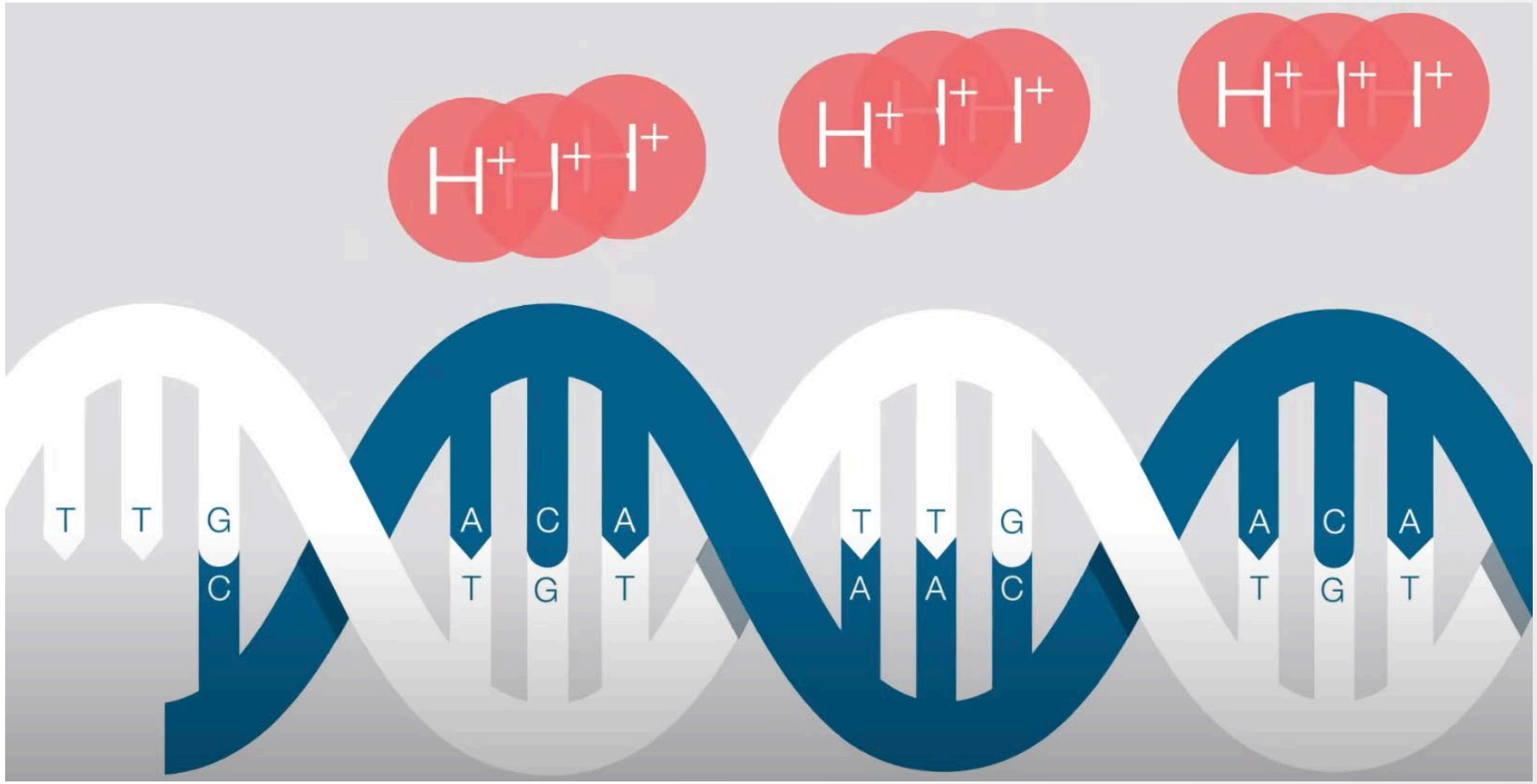
Next-generation sequencing (NGS) is a technology for determining the sequence of DNA or RNA to study genetic variation associated with diseases or other biological phenomena.

NGS is also called "**massively parallel sequencing**" because millions of DNA strands are sequenced simultaneously.

Source: Wikimedia Commons—by Suspencewl [CC0]

Semiconductor Sequencing

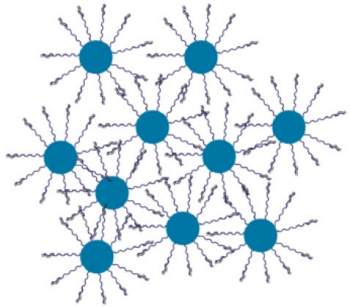
NGS



Ion Semiconductor Sequencing

ThermoFisher SCIENTIFIC

1



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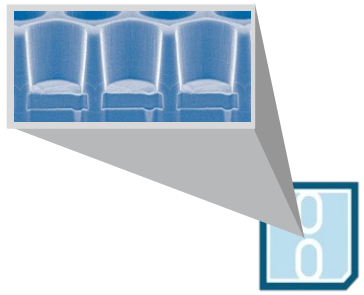

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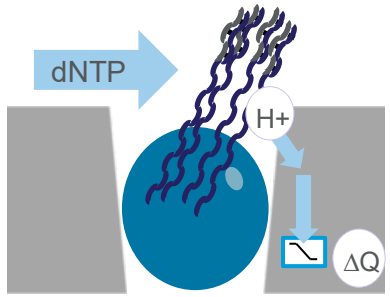

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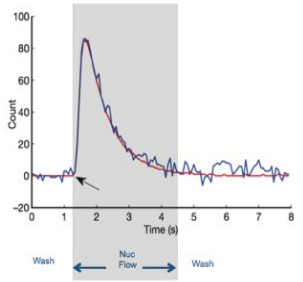


5

Template libraries are bound to beads and distributed into wells of sequencing chip

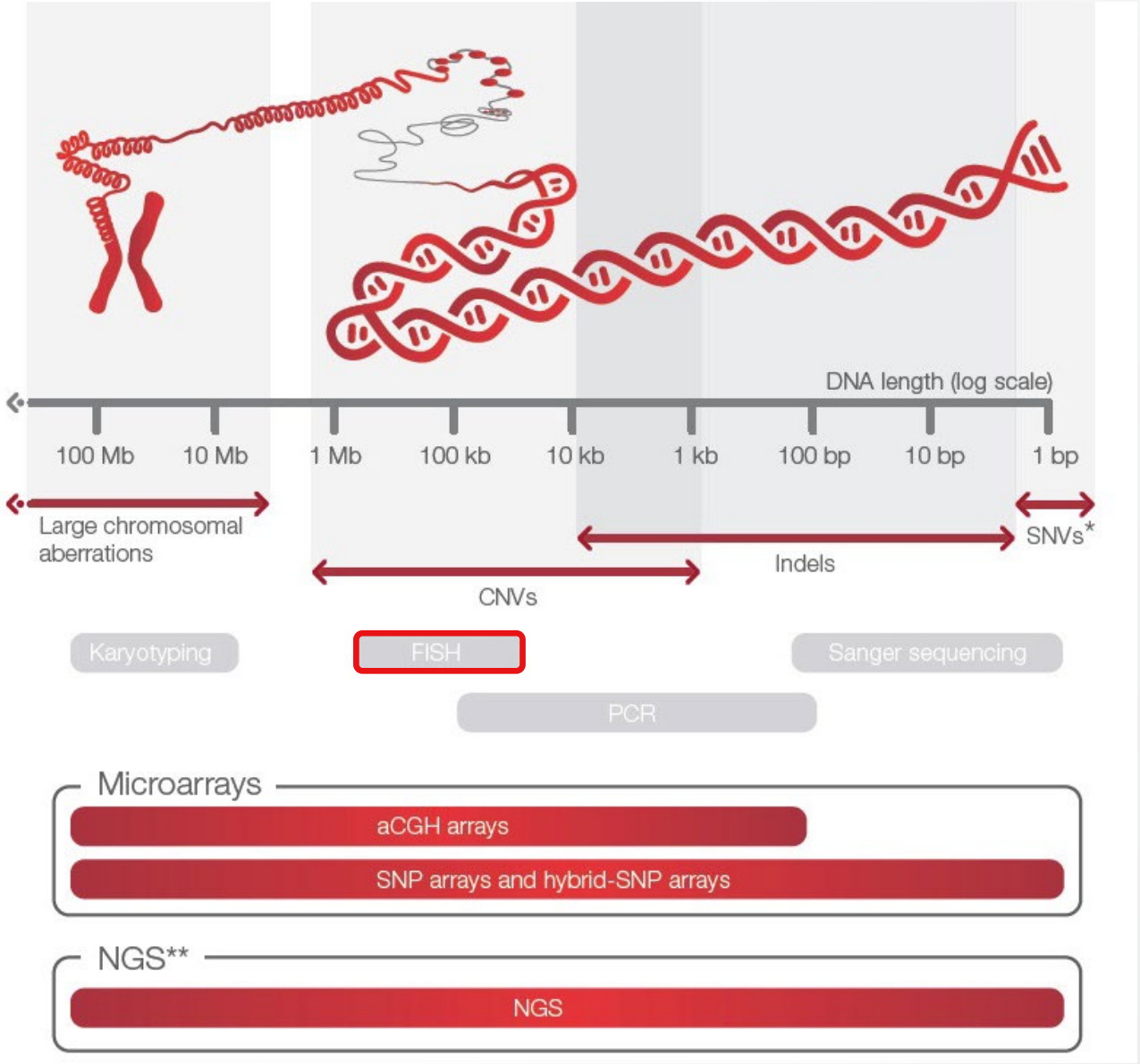
Nucleotides flow over chip one at a time to build a complementary strand

When a base is incorporated, a hydrogen ion is released, changing pH

Sequential changes in pH are detected by a sensor, building an output sequence

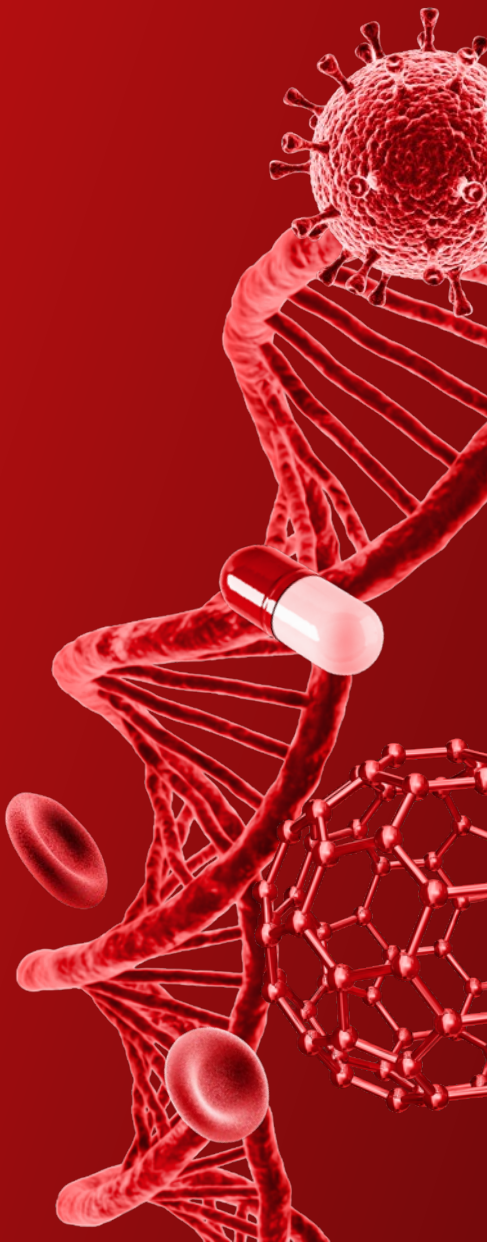
Base incorporation leads to changes in pH, which are translated into a sequence

Platforms vs. kind of events detectable



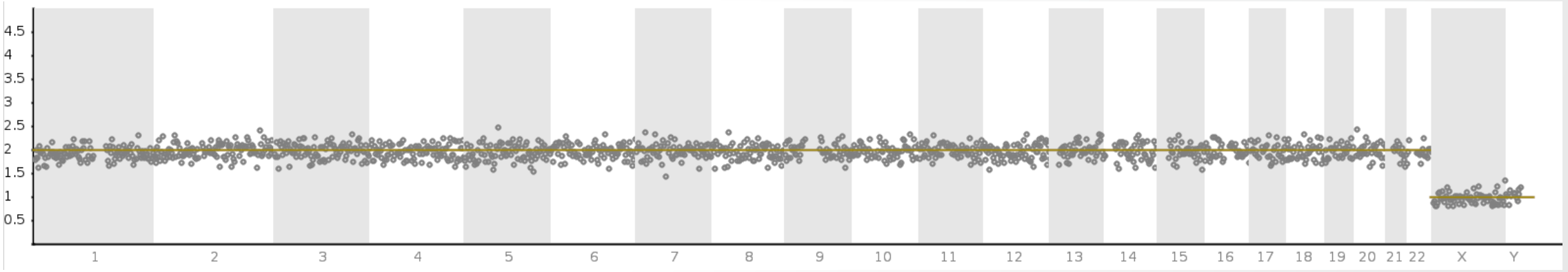
Source: EXT2089-graph-VF-10-gray-3-1280x1280

In-house PGT-A with Low-Pass Whole Genome Sequencing and Semiconductor Sequencing



What is Low-Pass Genome Sequencing

- Like whole genome sequencing (WGS) but on a **diet**
- WGS requires sufficient number reads (~360M reads*) to cover the entire genome at adequate depth to genotype
- However, to detect large chromosomal and sub-chromosomal CNVs, sequencing the genome requires much lower depth (<0.01X coverage per sample or ~100,000 reads)

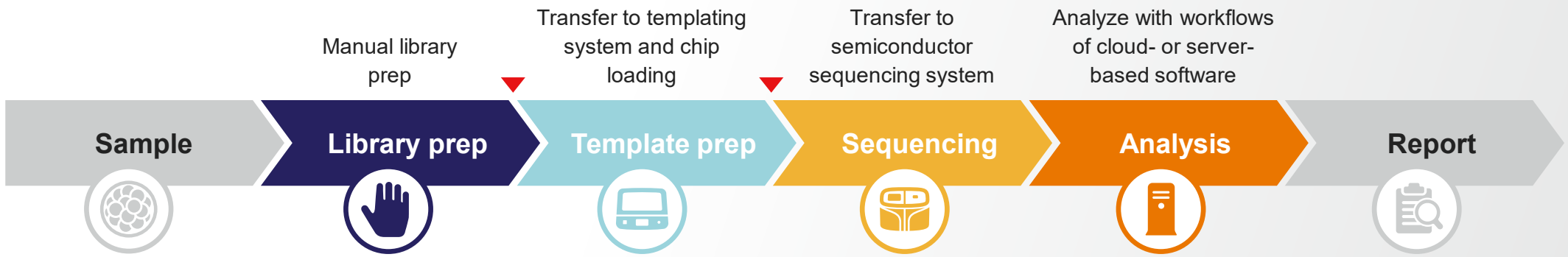


*30X coverage with 2X125 bp PE reads

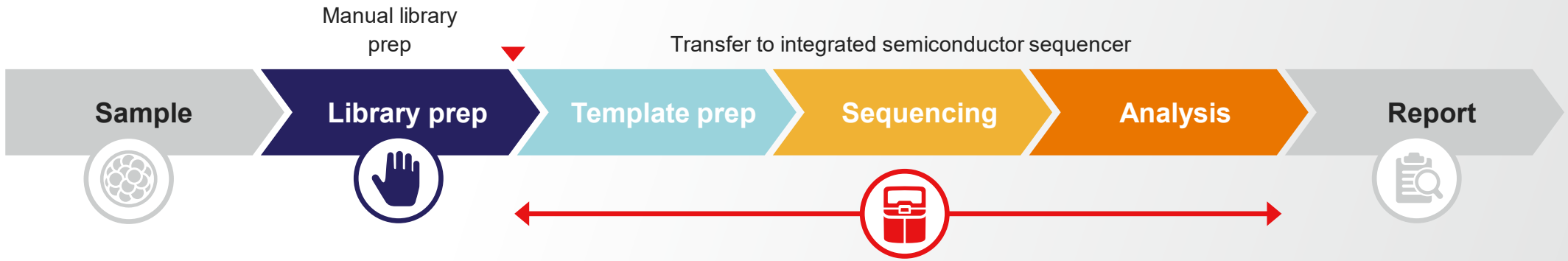
Simplified PGT-A workflow on a single instrument

Load-and-go workflow for templating, sequencing, and analysis on Integrated Semiconductor Sequencer

Workflow using Semiconductor Sequencer System

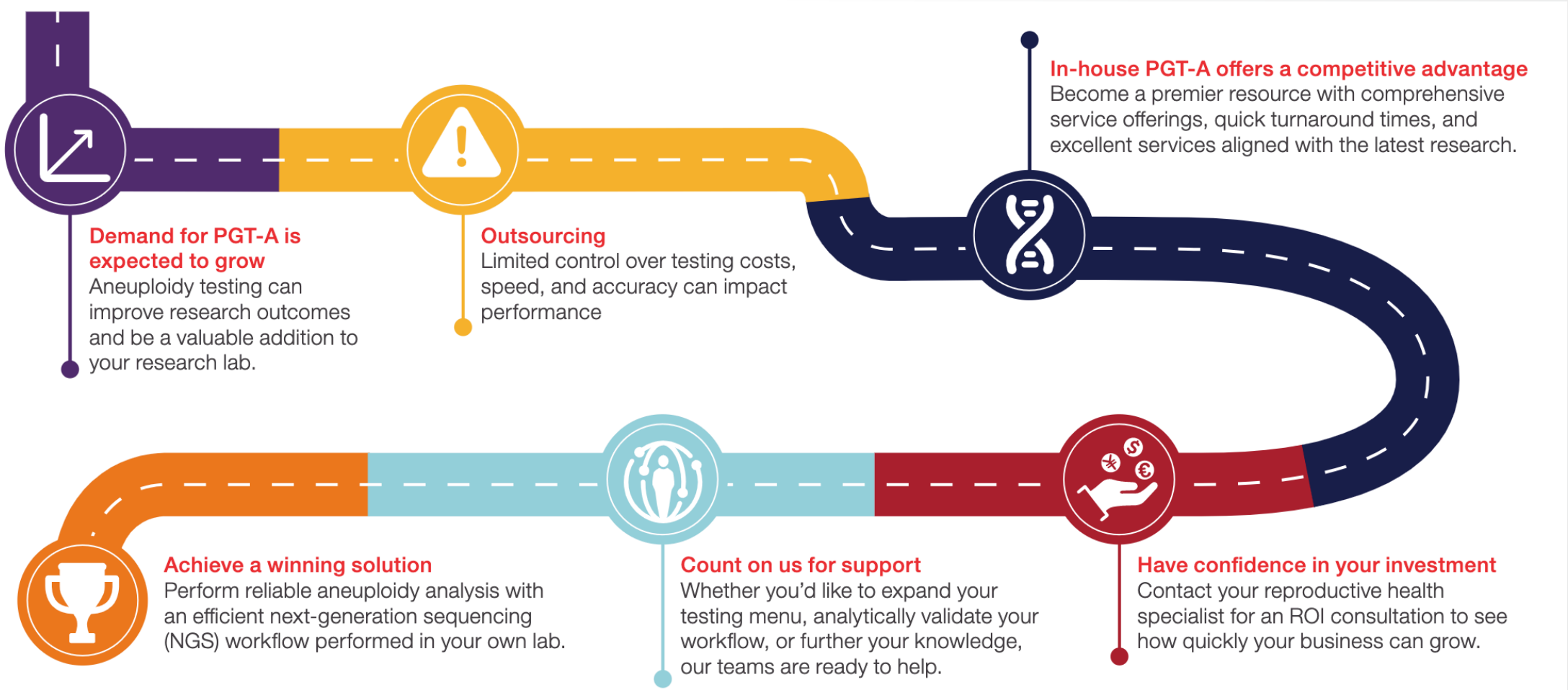


Workflow using Integrated Semiconductor Sequencer

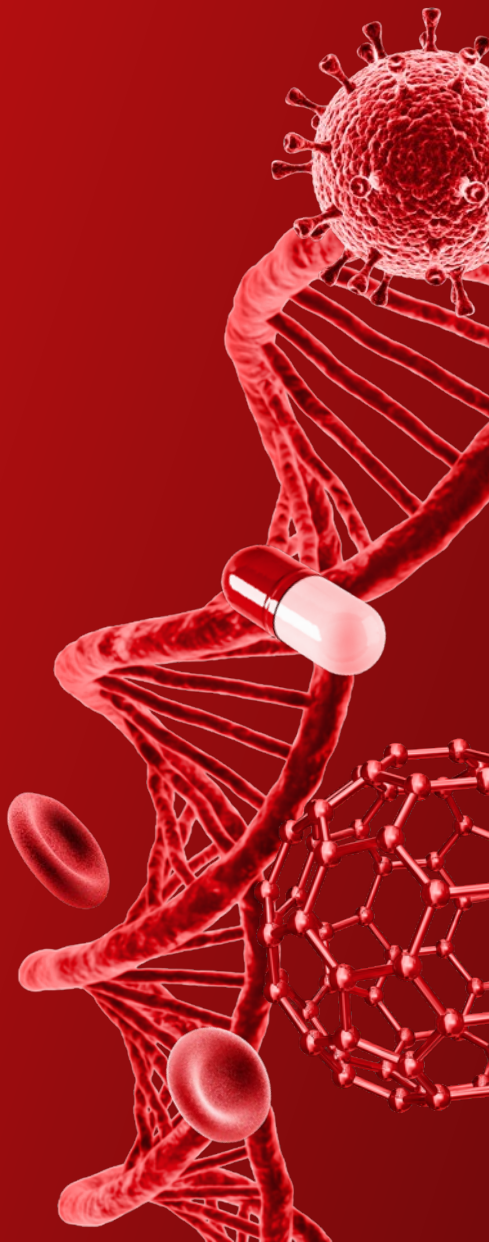


Why bringing PGT-A in-house?

Drive success

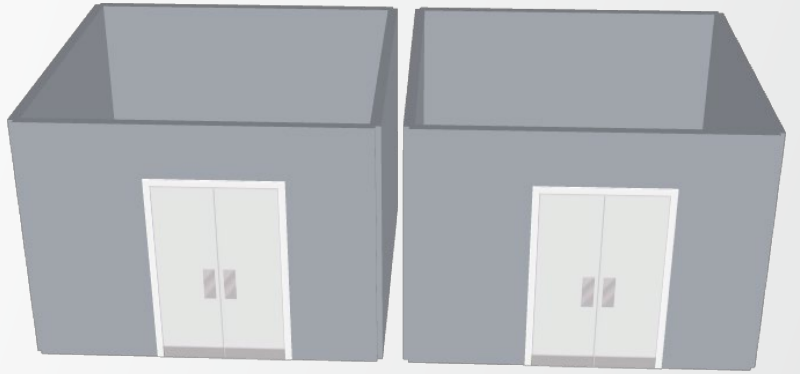


NGS Laboratory Design

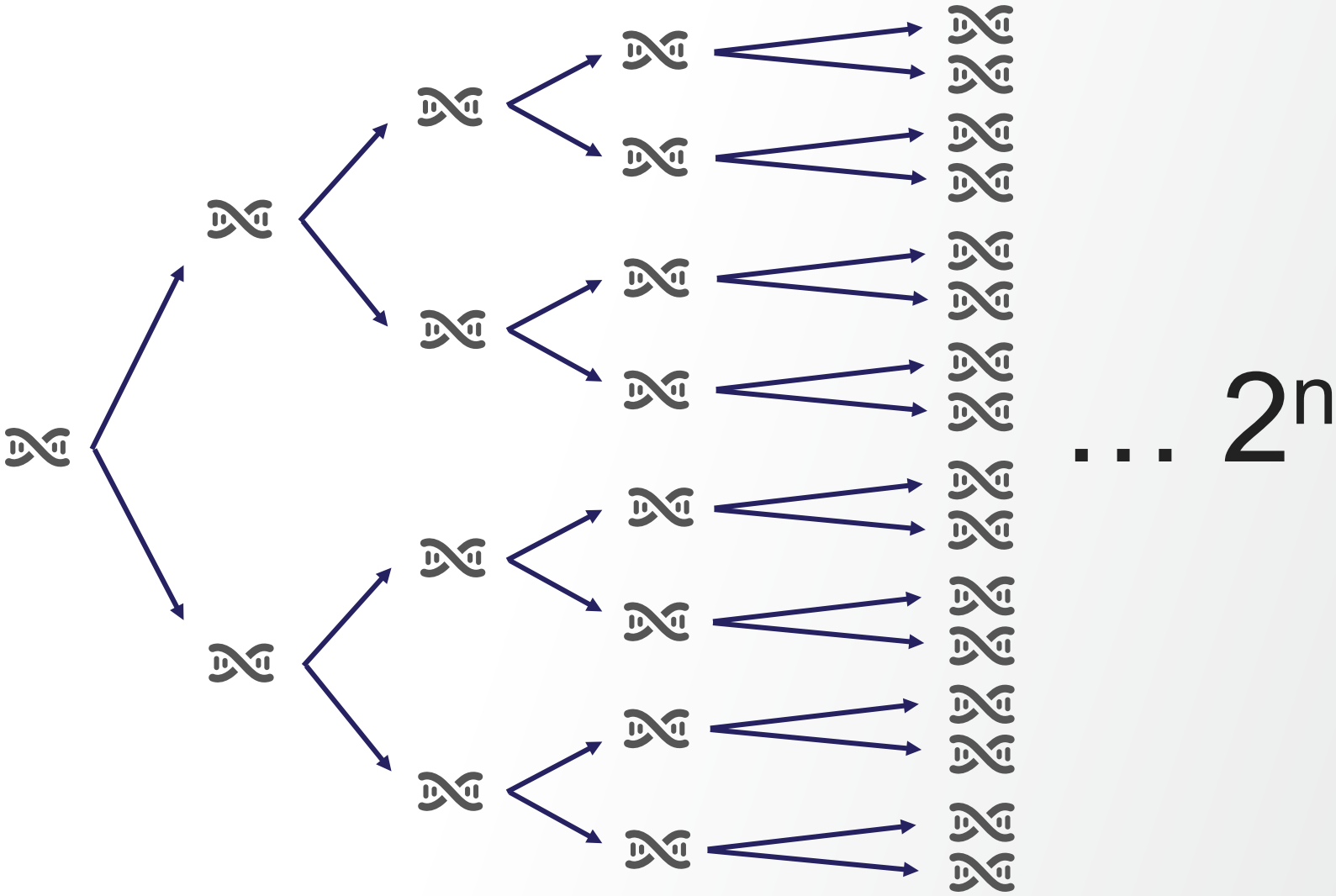


Separate genetic lab from embryology lab

Separate pre- and post-amplification steps



PCR



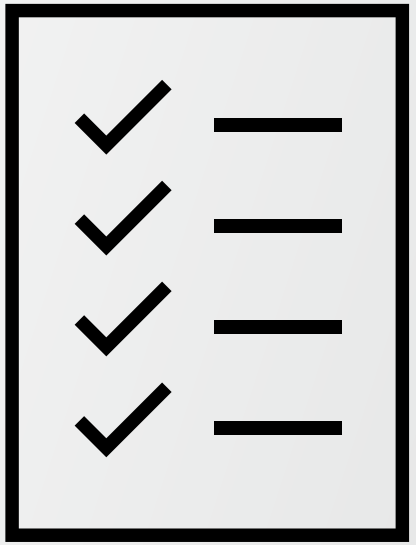
“Clean” vs. “Dirty”

Default as soon as PCR amplification is involved

- Flow hood needs to be away from post-amp workspace
- Limited amount of DNA: dedicated hood, thermal-cycler, etc
- 1-room design (if regulation allows)
- 2-room design ★

General NGS lab design guidance

Dedicate the lab to the NGS workflow



- Restrict access
- Separate changing and hand-washing rooms
- Separate material cleaning room
- Provide a safe working environment that minimises the risk of distraction, fatigue
- Minimise the release of Volatile Organic Compounds

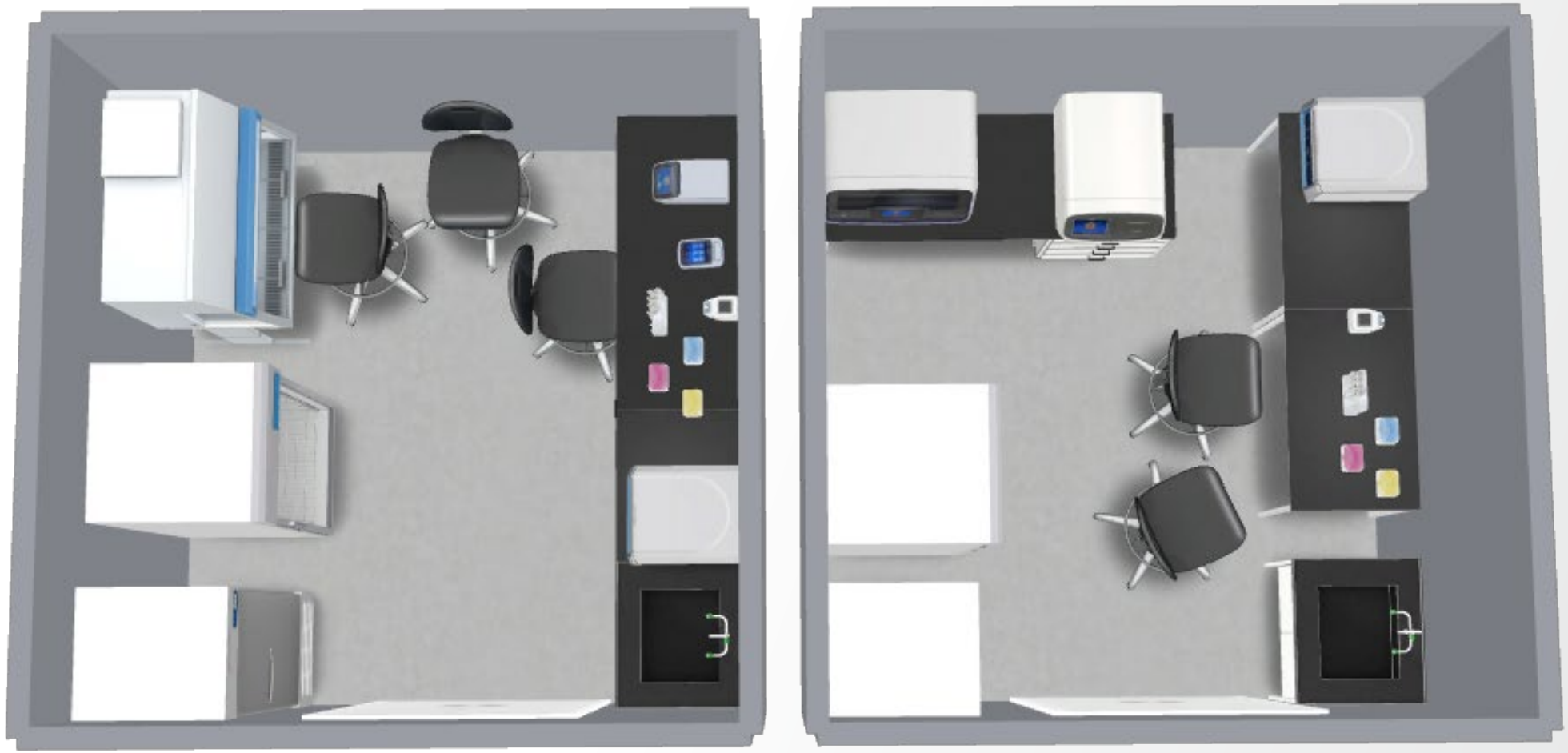
1-room PGT-A lab design

Rendering using Lab Products Virtual Tools, Thermo Fisher Scientific

1-room PGT-A lab design with integrated NGS system

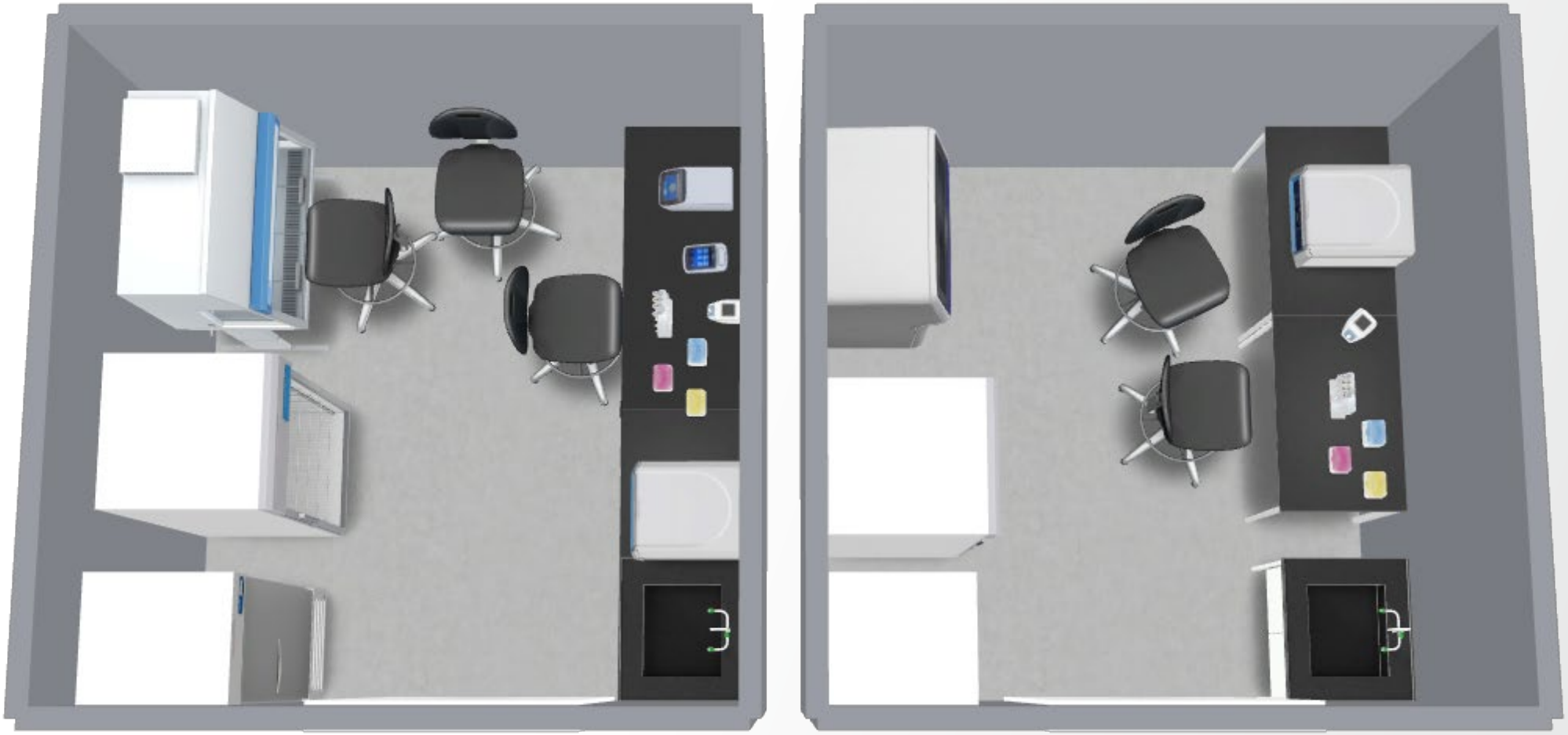
Rendering using Lab Products Virtual Tools, Thermo Fisher Scientific

2-room PGT-A lab design



Rendering using Lab Products Virtual Tools, Thermo Fisher Scientific

2-room PGT-A lab design with integrated NGS system



Rendering using Lab Products Virtual Tools, Thermo Fisher Scientific

Hood

- Minimum requirement: PCR hood with UV light for cleaning
- Recommended: laminal flow hood with UV light for cleaning
  - Flow to avoid contamination from outside
- After barcoding, post-PCR area

Cleaning and protective items

- Protective clothing including gloves and hair cover/hat (critical for niPGT-A)
- Change gloves after each step
- All plastic-ware including tips DNAse/RNAse-free
- Specific set of pipettes and tips for each step is an option
- DNA decontamination solutions, 1% bleach, or UV irradiation (254 nm/5 min, 30 min after work)
  - UV treatment before and after each workflow step
  - Cleaning with NaOH after each run

(Ancillary) Instruments

Typical molecular biology lab equipment

- Thermal cycler

- Centrifuges (with plate adapters)
- Micro/pico centrifuges
- Vortex/mixers
- Pipet with secured attachment

- Analysis server, data storage

- Uninterrupted power supply (UPS) for critical steps

- UPS and analysis servers can sit underneath a bench

Images from thermofisher.com

Benches

- Table for sequencers and ancillary must support weight (e.g., circa 300 lbs/136 kg)
- Vibrations

Do not install an equipment generating vibrations on the same deck as an instrument using pipettes or sensors

Images from fisherscientific.com

Storage

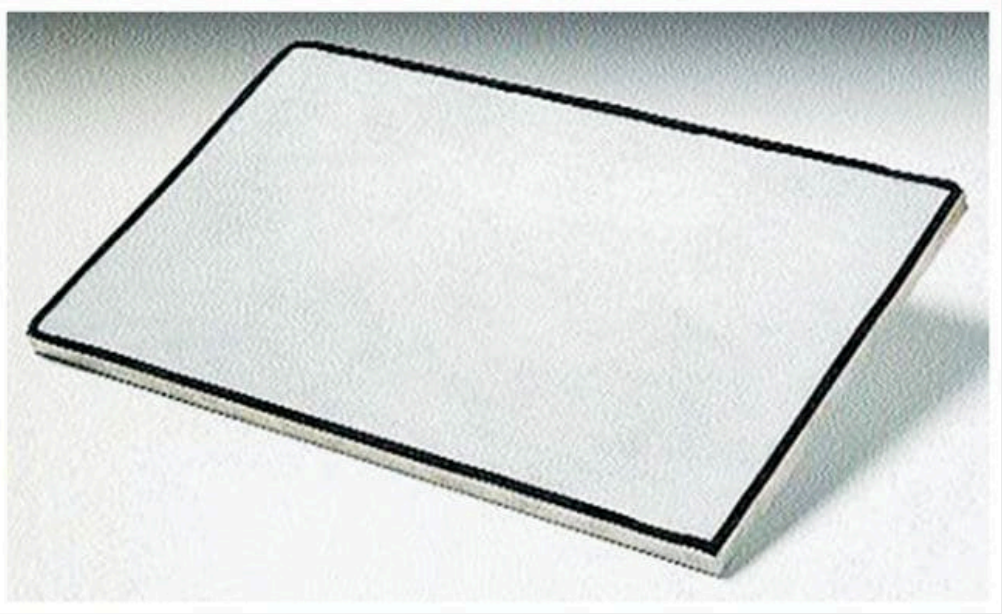
- Fridge
- Freezer
- Space to store boxes at room temperature

No shelves above instruments with vertical door and/or when service is required from the top

Images from thermofisher.com

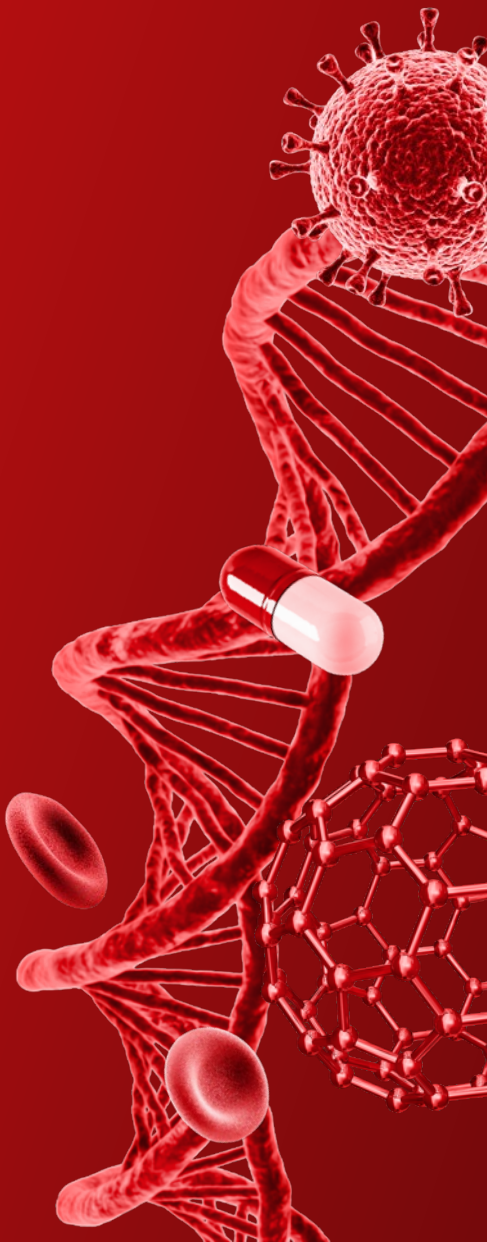
Air

- Positive pressure help to minimize air contamination
- Filters
- High-efficiency particulate air (HEPA) and volatile organic compound (VOC) control



Images from fisherscientific.com

Data Analysis and Management



Aneuploidy analysis by PGT-A

Analyze with cloud- or server-based solution

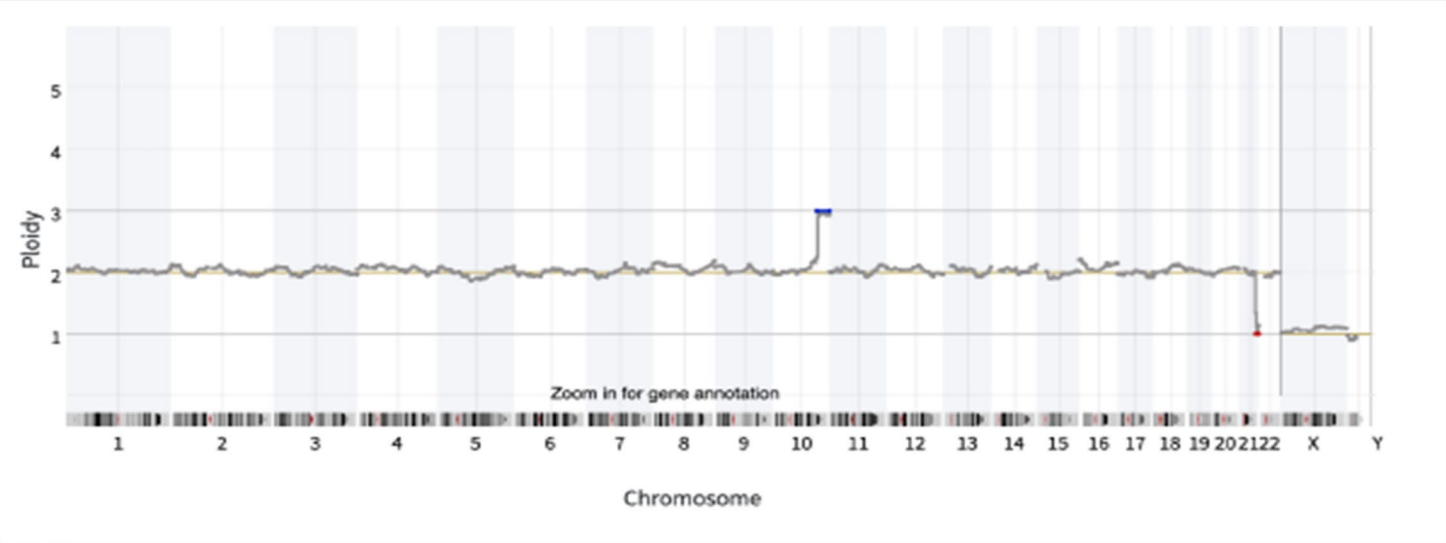
Aneuploidy
Male with loss on chr13; gain on chr14, chr15

Mosaicism
Mixture of a normal female with male (chr9 duplication, 45 Mbp) at 40%

Top three plots from data courtesy of Eurofins Genoma Group S.r.l. (Rome). Bottom plot contains data from a sample from Coriell Institute for Medical Research.

Aneuploidy analysis by PGT-A

Analysis on Integrated Semiconductor Sequencer



Test Address

Reproseq Report
ReproSeq™ PGS - GX5 - w1.0.0 - v0.0.19

Date: 22 Jul 2022

Patient		Partner		Other	
Sample First Name:	**First1**	Partner First Name:	**Partner First1**	Consultant:	**Consultant123**
Sample Last Name:	**Laste1**	Partner Last Name:	**Partner Laste1**	Referring Center:	**CenterABC**

Summary

Embryo ID	Sample Name	Gender	AN(+)	AN(-)	Triploidy	Interpretation
reproSeq_embryo 4	reproSeq_sample _004	Male		11		
reproSeq_embryo 3	reproSeq_sample _003	Female	14, 6			
reproSeq_embryo 2	reproSeq_sample _002	Female	14, 9			
reproSeq_embryo 1	reproSeq_sample _001	Male	10	21		

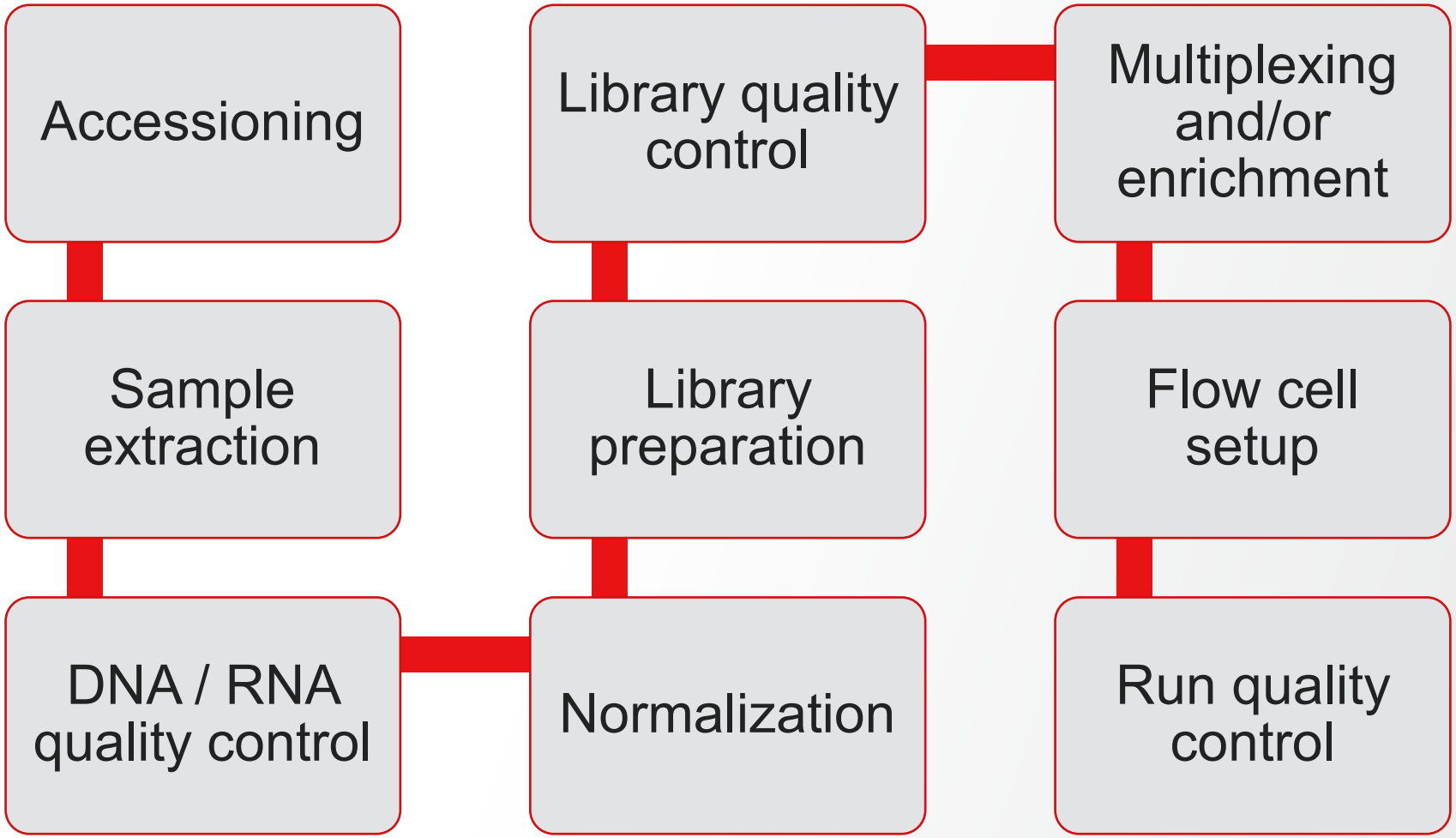
Genexus software plot and data from R&D

LIMS product capabilities

Product features for customers in NGS space

- **Connectivity with**
  - HL7 messaging
  - Data manager and sweeper
  - RestAPI and Odata API
  - Mobile application
  - Hands-free with barcode scanners and voice control
- **Automation with**
  - Liquid handler communication (uni- or bi-directional)
  - Pass / fail criteria with rules engines
  - Notifications with SMS and email push
  - Validation for barcode collisions
- **Statistical methods**
  - Analytical quality control for instrument maintenance
  - Statistical quality control for process interrogation and Westgard Sigma Rules™
  - Rscript and Python™ for additional statistical method support

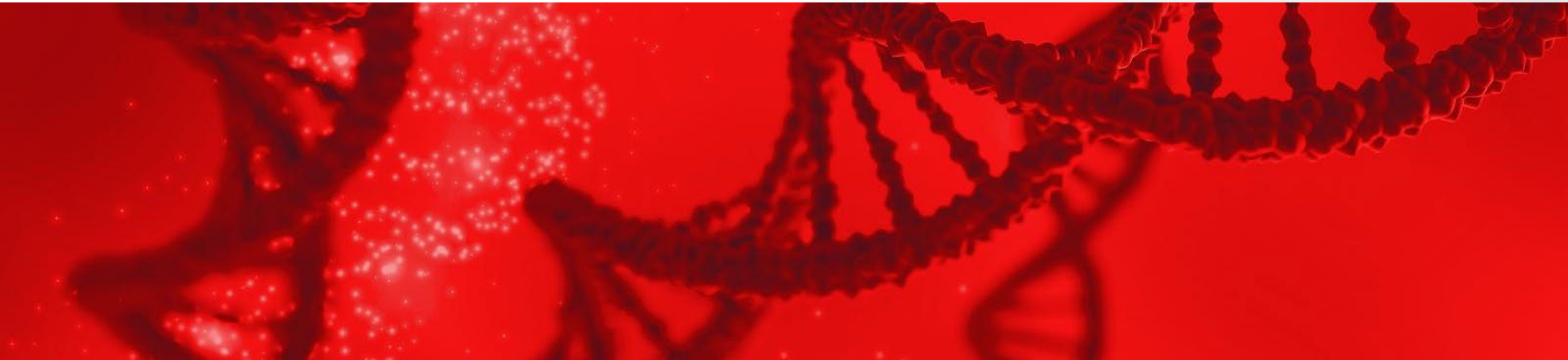
NGS workflow in LIMS



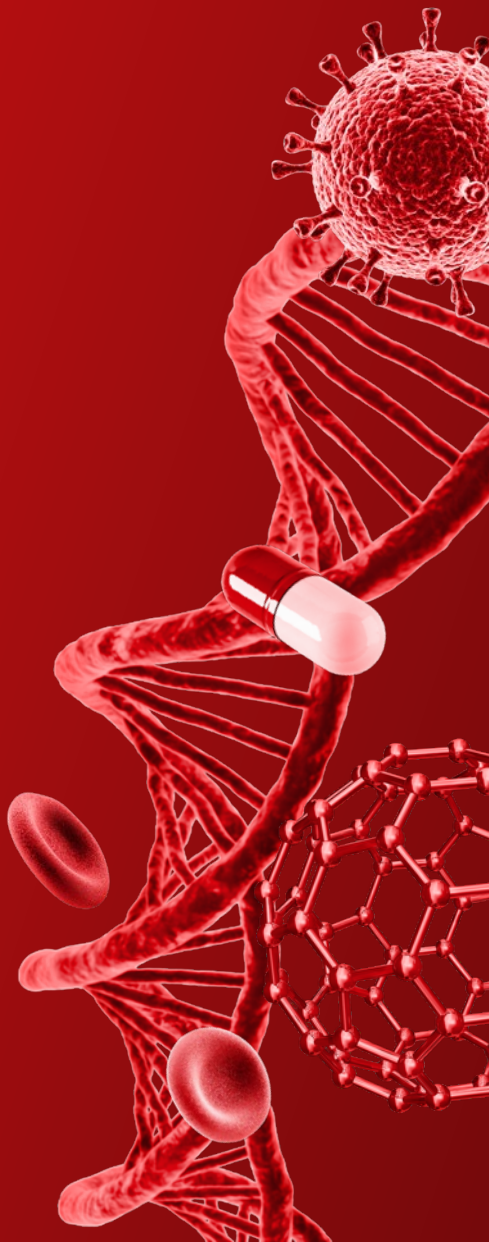
LIMS solutions add-ons

Solutions and features for customers in NGS space

- Biorepository Solution for LIMS
  - Store leftover materials
  - Managing shipping requests
  - Easy-to-configure freezers and containers for sample management
- Data Analytics Solution for LIMS
  - Pre-configured dashboards to manage lab efficiently
  - Gain valuable insights into your process with predictions and machine learning
  - Microsoft PowerBI™ connectivity to reduce “read-only” needs for leadership
- Statistical Quality Control
  - Track process for stability over time
  - Create rules to notify when controls trend towards violation
  - Quickly generate reports on process control



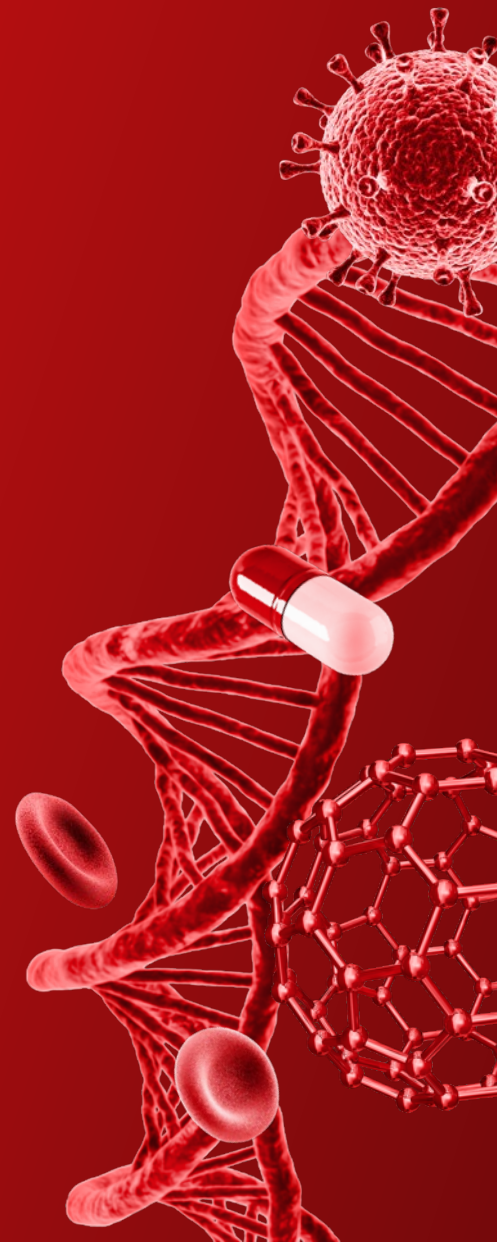
Employees



Education Background

At the bench	At the desk
• 2 years degree (Molecular) biology university level • 4 years degree or MD for CAP-accredited	• Typically, director level • PhD and/or MD
• Continuous training and knowledge validation	• Continuous training and knowledge validation

Support and Service

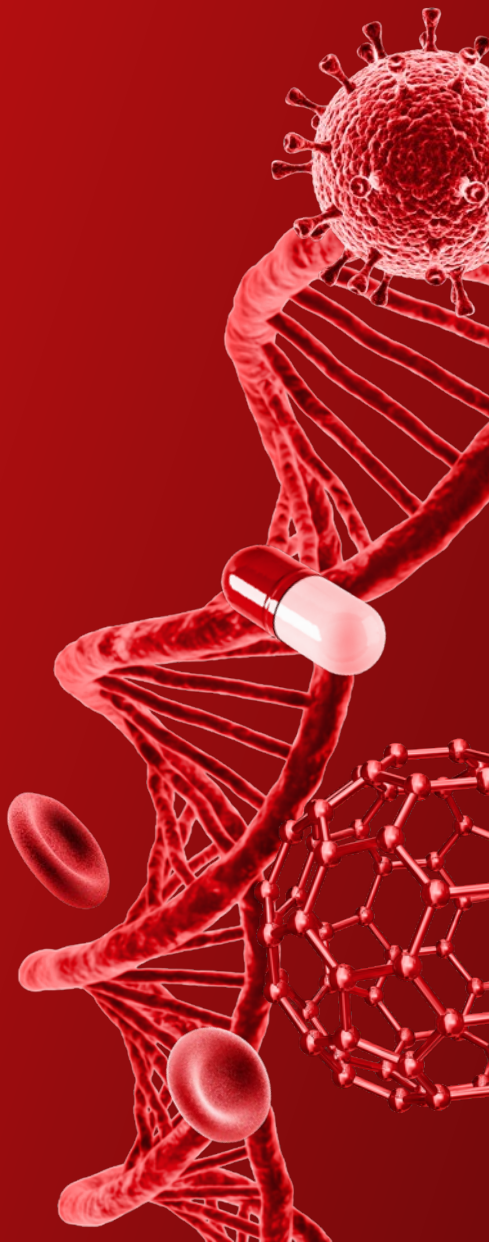


Comprehensive Support and Service

Here to help you

- Field Application Scientists
- Field Bioinformatics Scientists
- Specialized Account Consultants
- Follow-the-sun 24/5 Priority Technical Support Plans

Quality Management and Validation guidance



Quality and competence

Each lab requires a Quality Management System to guide and document lab processes.

- Each lab will require a Quality Management System (QMS) be in place
  - Standard Operating Procedure
  - Calibration and preventive maintenance (pipettes, centrifuge, etc)
  - Training
  - Competency assessment
  - Installation Qualification of instrumentation
  - Operation Qualification of workflow
  - Assay Validation

Analytical validation

The use of analytical technique, process, and procedure to develop and produce results in **documentation form** as tangible evidence, assuring that all parts within the scope of a workflow are suitable for intended use.

What is an analytical validation (AV)?

Analytical validation, also known as method or process validation, is:

- An industry-accepted process
- Tests a procedure or workflow by analytical methods
- Documents results with tangible evidence
- Proves the workflow or procedure to be suitable for the intended use

How frequently should an AV be performed?

A typical recommendation:

- Upon adding a new test to the menu
- After a major change in the workflow*
- Upon adding new tissues or targets to the test*
- Upon adding instruments/updating instruments in the workflow*

* We recommend following an established change control process with risk analysis.

Overview of Assay Validation Guidelines for PGT-A

Regulatory body 1	2	3	4	5

• Accuracy
• Precision
• Analytical sensitivity
• Analytical specificity
	• Accuracy
	• Precision
	• Reproducibility
	• Analytical sensitivity
	• Analytical specificity
		• Accuracy
		• Precision
		  • Reproducibility
		  • Repeatability
		• Analytical Sensitivity
		• Analytical Specificity
		• Limits of Detection & Quantitation
			• Accuracy
			• Precision
			  • Reproducibility
			  • Repeatability
			• Analytical sensitivity
			• Analytical specificity
				• Analytical accuracy
				• Accuracy (initial validation)
				• Analytical sensitivity
				• Precision
				• Reproducibility

Analytical validation is a requirement for accreditation and regulatory compliance

Validation plan design will vary based on specific guideline requirements

CAP Guidelines for PGT-A assays

MOL.31130 Accuracy

Evaluate sufficient characterized samples to provide a high degree of assurance a test can correctly call aneuploidies.

- **Analytical Accuracy**
  - Use of commercial controls and cell lines to assess accuracy of aneuploidy calls
  - Minimum of 20 embryo biopsies, including positive and negative for aneuplodies
  - Include as many different types of aneuploidies as possible.

MOL.31130 Analytical Sensitivity

- Evaluate by mixing aneuploid and non-aneuploid cell lines to mimic mosaicism.
  - Determines the PGT-A assay's ability to make correct calls in the presence of non-aneuploid DNA.
- **Analytical Sensitivity**
  - Use a series of dilutions to determine when an aneuploidy can be detected in presence of other cell lines.
  - E.g. 40%, 50%, 60%, etc.

CAP Guidelines for PGT-A assays (cont.)

MOL.31130 Analytical Specificity

Assessment of potential cross-reactivity with other genetic variants.

- **Analytical Specificity**
  - Assess a series of non-aneuploid cell lines to determine if test is making false anueploidy calls.

MOL.31130 Precision

The results of the same sample with repeated analyses will produce the same result within a run.

- Repeating the workflow in triplicate and analyze on the same sequencing run
- A minimum of 3 samples including cell lines and embryo biopsies.

CAP Guidelines for PGT-A assays (cont.)

MOL.31130 Reproducibility

The assay will produce the same result across multiple operators and multiple runs using the same sample.

- 2-3 different operators execute workflow independently using the same samples to ensure the results are consistent across runs and operators.
- Ensure same answer regardless of technologist or instrumentation
- All instruments require separate AVs per assay.

APV, AVR, and AV consulting service options

1 Analytical performance verification (APV)

- Comprehensive prevalidation or bridging study solution
- Custom control kit*
- Workflow guidance
- Data analysis
- Consultation with AV specialist
- APV summary template

Prevalidation or bridging study assistance

2 Analytical validation regional (AVR)

- Designed to support international accreditation and validation requirements for medical testing
  - **ISO 15189, CLIA/CAP, CLSI, or NYSDOH as guidance**
- Dedicated project management guidance*
- Workflow training, guidance, and optimization
- Template documentation
- Samples and controls*
- Data analysis
- Validation summary template

Flexible validation guidance

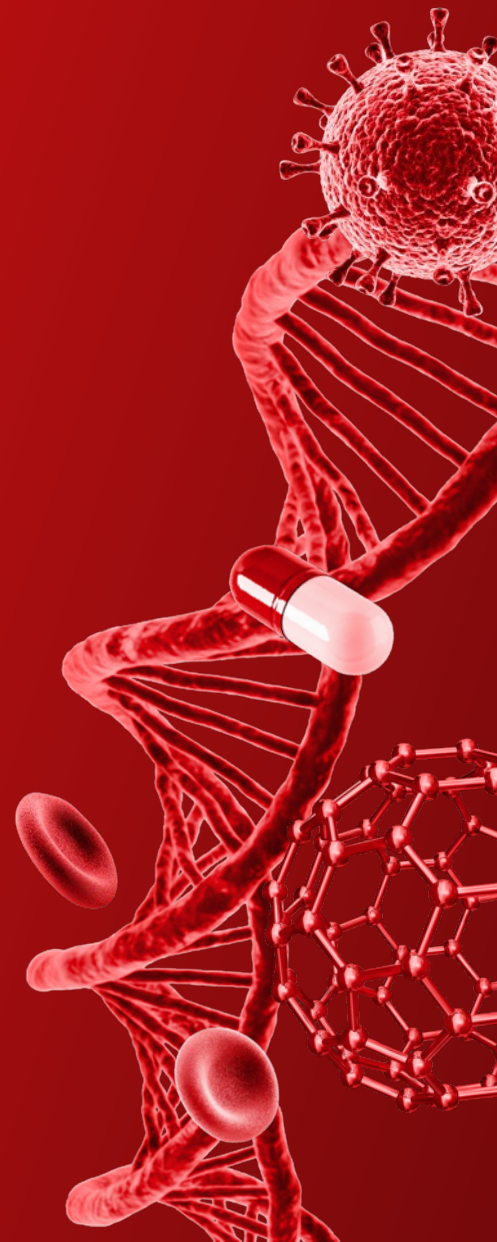
3 Analytical validation (AV)

- Designed to support US accreditation and validation requirements for medical testing
  - **CLIA/CAP, CLSI, or NYSDOH as guidance**
- Dedicated project management guidance*
- Workflow training, guidance, and optimization
- Template documentation
- Samples and controls*
- Data analysis
- Validation summary template

Complete validation solution

* For details, contact AV specialist, or professional services for business development. Provision depending on consulting service purchased.

Take Home



Bring PGT-A In-House

Uncomplicated

Follow guidance from scientific societies

Specialty support ready to help

Validation Consultation Services

One Thermo Fisher Scientific

Thank you

Learn more at thermofisher.com/pgt, thermofisher.com/av and thermofisher.com/samplemanager